Expression of Genes Involved in a Radiation-Induced Bystander Effect

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14th International Congress of Radiation Research

organized on behalf of the
International Association for Radiation Research

by the
Polish Radiation Research Society - memorial to
Maria Skłodowska-Curie

in collaboration with the
Radiation Research Society

28 August – 1 September 2011

Warszawa
Poland
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Media coverage

Exhibitors

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AINSIE: Australian Institute of Nuclear Science and Engineering
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IARR: International Association for Radiation Research
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PTChem: Polish Chemical Society
RRS: Radiation Research Society
SAR: Sociedad Argentina de Radioprotección
SFR: Swedish Society for Radiation Biology
SIRR: Italian Society for Radiation Research
WIHIE: Military Institute of Hygiene and Epidemiology (Poland)
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<tr>
<th>Hours</th>
<th>Sunday 28.08.2011</th>
<th>Monday 29.08.2011</th>
<th>Tuesday 30.08.2011</th>
<th>Wednesday 31.08.2011</th>
<th>Thursday 01.09.2011</th>
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<tr>
<td>08:00-09:00</td>
<td></td>
<td>Eye openers (EO1-EO6)</td>
<td>Eye openers (EO7-EO12)</td>
<td>Eye openers (EO13-EO17)</td>
<td>Eye openers (EO18-EO23)</td>
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<tr>
<td>09:00-10:00</td>
<td>Coffee break</td>
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<tr>
<td>10:00-11:00</td>
<td>Symposia (S1-S5)</td>
<td>Symposia (S13-S18)</td>
<td>Symposia (S19-S24)</td>
<td>Symposia (S30-S35)</td>
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<td>11:00-12:00</td>
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<tr>
<td>12:00-13:00</td>
<td>Conference lectures (CL1-CL6)</td>
<td>Conference lectures (CL7-CL12)</td>
<td>RRS award lecture (PL4)</td>
<td>Conference lectures (CL13-CL18)</td>
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<tr>
<td>13:00-14:00</td>
<td>Lunch break</td>
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<tr>
<td>14:00-15:00</td>
<td>Registration</td>
<td>Fukushima nuclear plant accident lecture (PL1)</td>
<td>Poster sessions (POS14-POS22)</td>
<td>Lecture about Marie Sklodowska-Curie (PL7)</td>
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<tr>
<td>15:00-16:00</td>
<td>Poster sessions (POS1-POS13)</td>
<td>Coffee break</td>
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<td>Coffee break</td>
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<tr>
<td>16:00-17:00</td>
<td>Coffee break</td>
<td>IARR award lecture (PL3)</td>
<td>ARR award lecture (PL5)</td>
<td>Poster sessions (POS30-POS41)</td>
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<tr>
<td>17:00-18:00</td>
<td>ICRU award lecture (PL2)</td>
<td>ICRR 2011 debate (plenary)</td>
<td>ERRS award lecture (PL6)</td>
<td>Closing ceremony</td>
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<tr>
<td>18:00-19:00</td>
<td>Opening ceremony</td>
<td>Symposia (S7-S12)</td>
<td>Free time</td>
<td>Symposia (S25-S29)</td>
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<tr>
<td>19:00-20:00</td>
<td>Welcome reception</td>
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<tr>
<td>20:00-21:00</td>
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<td>Congress gala dinner 19:30 - 24:00</td>
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<td>21:00-22:00</td>
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</table>
## Detailed program

<table>
<thead>
<tr>
<th>Start time</th>
<th><strong>Sunday 28 August 2011</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>18:00</td>
<td><strong>Opening ceremony and welcome reception (LR1 Kongresowa)</strong></td>
</tr>
</tbody>
</table>

Chair: Marek K Janiak (Poland)
Addresses: Maciej Żylicz, Advisor to Bronisław Komorowski, President of Poland, Waldemar Pawlak, Minister of Economy, Honorary Patron of ICRR2011
Hanna Gronkiewicz-Waltz, Mayor of the capital city of Warsaw
Michał Kleiber, President of the Polish Academy of Sciences
Ohtsura Niwa, President of IARR
Antonina Cebulska-Wasilewska, President of ICRR2011

Stage performance by the Polish folklore dancing group PROMNI

Welcome reception in the foyer of LR1 Kongresowa

### Acronyms:
- CL – Conference lecture
- CR – Coffee and lunch room
- EO – Eye opener
- LR – Lecture room
- MR – Meeting room
- PL – Plenary lecture
- POS – Poster session
- PR – Poster room
- S – Symposium

### Lecture rooms:
- LR1 Kongresowa
- LR2 Ratuszowa
- LR3 Skłodowska
- LR4 Warszawska
- LR5 Mikołajska
- LR6 Kruczkowski

### Poster rooms:
- PR1 Starzyński
- PR2 Broniewski

### Coffee and lunch rooms:
- CR1 Marmurowa
- CR2 Korczak

### Meeting rooms:
- MR1 Rudniew
- MR2 Rudniew annex
- MR3 – MR5 Warszawska annexes
- MR6 Puszkin

Please see rear part of the book for a layout of the rooms and an explanation of the room names.
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<tr>
<th>Start time</th>
<th>ROOM</th>
<th>LR1 Kongresowa</th>
<th>LR2 Ratuszowa</th>
<th>LR3 Skłodowska</th>
<th>LR4 Warszawska</th>
<th>LR5 Mikołajska</th>
<th>LR6 Kruczkowski</th>
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<tr>
<td>08:30</td>
<td>Eye opener lectures EO1-E06</td>
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<td>EO2. Chair: H. Tanooka M. Atkinson (Germany): The genetics of radiation-induced cancer.</td>
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<td>EO3. Chair: A. Ottolenghi E. Gudowska-Nowak (Poland): Modelling radiobiological responses: where are we?</td>
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<td>EO4. Chair: S. Chandna Y. Wang (USA): Outline the role and importance of microRNAs in cell function including response to radiation.</td>
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<td>EO5. Chair: M. Kruszewski G. Bauer (Germany): Reactive oxygen and nitrogen species involved in radiation-induced signalling/bystander effects.</td>
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<td>EO6. Chair: K. Bobrowski C. Chatgilialoglu (Italy): Chemical radiation studies related to radical-based DNA damage.</td>
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<td>09:30</td>
<td>Coffee break (CR1 Marmurowa and CR2 Korczak)</td>
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<tr>
<td>10:00</td>
<td>Symposia S1 – S6</td>
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<td></td>
<td>S1. Normal tissue effects of radiation Chair: M. Robbins, J. Williams 10:00 C. Limoli (USA): Stem Cells, Oxidative Stress, and Normal Tissue Injury. 10:25 M. Robbins (USA): Use of anti-inflammatory therapies to modulate radiation-induced late effects. 10:50 M.C. Vozenin (France): Inflammation and fibrosis 11:15 K. Fleckenstein (Germany): Hypoxia and inflammation in radiation-induced normal tissue injury.</td>
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<td>S2. Chromatin modifications and DNA damage response Chair: I. Szumiel, J. Dobrucki 10:00 J. Dobrucki (Poland): Histone methylation and heterochromatin protein 1 in DNA damage response. 10:25 T. Pandita (USA): Chromatin modifications and DNA damage response. 10:50 J.R. Morris (UK): Regulation of post-translational modifications in the double-strand break response. 11:15 M. Falk (Czech Republic): Induction, repair and misrepair of DNA double-strand breaks (DSBs) in the</td>
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<td>S3. Space research Chair: F. Cucinotta, M.A. Tabocchini 10:00 F. Cucinotta (USA): Introduction 10:10 G. Zhou (China): Different responses between G0 and exponentially growing cells exposed to ionizing radiation. 10:30 M.A. Tabocchini (Italy): DNA damage and repair after low doses of charged particles. 10:50 J. Pluth (USA): Radiation quality dependent effects on phosphorylation kinetics of proteins involved in early DNA damage response. 11:15 M.K. O’Banion (USA): Hippocampal neurogenesis and contextual fear response</td>
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<td></td>
<td>S5. RRS President's symposium - biological significance of complex DNA damage: physics, chemistry and biology of complex damage Chair: P. O’Neill 10:00 A. Ottolenghi (Italy): Modelling of DNA damage dependence on radiation quality. 10:25 M. Lomax (UK): The pros and cons of processing clustered DNA damage sites. 10:50 K. Elmoth (Sweden): Formation and consequences of complex DNA lesions using radiation with different LET. 11:15 A. Georgukias (USA): New insights into the processing of</td>
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context of higher-order chromatin structure. in C57BL/6 mice exposed to 2 Gy whole body protons. to irradiation. oxidatively induced clustered DNA lesions. Clinical applications? contaminated environments.

12:00 Conference lectures CL1 – CL6

CL1. Chair: I. Guseva Canu R. Wakeford (UK): Chernobyl effects: what do we know after 25 years?
CL2. Chair: A. Haimovitz-Friedman N. Cordes (Germany): A sticky matter: ECM and radiation cell survival.

13:00 Lunch break (CR1 Marmurowa and CR2 Korczak)

14:00 Plenary lecture PL1 (LR1 Kongresowa)

Chair: C. Streffer (Germany)
T. Ohnishi (Japan): Disaster of Fukushima-Daiichi nuclear power plant (Japan) by earthquake and tsunami and RI-pollution.

15:00 Poster sessions POS1 – POS13 (PR1 Starzyński and PR2 Broniewski)

POS1 Adaptive response; POS2 Biological dosimetry; POS3 Bystander effects; POS4 Cell signalling; POS5 Effects of radiation on inflammation and immunity; POS6 Computational/theoretical studies in radiation physics and chemistry; POS7 Radiation chemistry and space research; POS8 Radiation chemistry of bioactive compounds; POS9: Boron neutron capture therapy (BNCT); POS10 Epidemiology; POS11 Molecular imaging in diagnosis and therapy; POS12 Hadrontherapy; POS13 Interdisciplinary studies.
Authors of odd poster numbers are asked to be at their posters from 15:00 to 15:45
Authors of even poster numbers are asked to be at their posters from 15:45 to 16:30

16:30 Coffee break (CR1 Marmurowa and CR2 Korczak)

17:00 Plenary lecture PL2 (LR1 Kongresowa)

Chair: Hans Menzel (Switzerland)
Dudley Goodhead (UK): On the track to clustered damage and radiation effects. ICRU L.H. Gray award lecture.

18:00 Symposia S7 – S12

S7. Advances in combined therapies: ionizing radiation, hyperthermia and chemotherapy
Chair: E. Azzam, E.A. Rapasky
18:00 F. Wenz (Germany): Combined radio-xyz-therapy, A
S8. Environmental radiobiology
Chair: C. Mothersill, D. Oughton
18:00 M. Stuart (Canada): Adaptive responses and bystander effects in a multiple stressor context.
18:20 T.G. Hinton (France): Lessons
S9. Doses received from modern medical procedures
Chair: S. Mattsson, A. Almén
18:00 A. Almén (Sweden): Diagnostic radiology and interventional procedures.
18:30 S. Mattsson
S10. Autophagy: a double-edged sword in cellular radiation response
Chair: B.G. Wouters, D. Chan
18:00 B.G. Wouters (Canada): The unfolded protein response enables high rates of autophagy
S11. Radiation damage to biomolecules (II): nucleic acids and their constituents
Chair: M.D. Sevilla, T. Majima
18:00 T. Majima (Japan): Charge transfer in DNA.
18:30 A. Adhikary (USA): Formation of S12. Ethics of radiation protection
Chair: F. Zölzer, C. Streffer
18:00 C. Streffer (Germany): Ethical aspects and culture of radiological protection in medicine and research.
18:30 J. Lochard
<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Topic</th>
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<tbody>
<tr>
<td>19:00</td>
<td>E.A. Repasky (USA)</td>
<td>Can targeting normal, homeostatic vasomotor function by mild hyperthermia result in improved responses to radiation or chemotherapy?</td>
</tr>
<tr>
<td>18:40</td>
<td>G. Rudolfesen (Norway)</td>
<td>Learned…Lessons Lost…Observations in Radioecology 25 Years after Chernobyl.</td>
</tr>
<tr>
<td>19:00</td>
<td>D. Oughton (Norway)</td>
<td>Chernobyl birds.</td>
</tr>
<tr>
<td>18:30</td>
<td>D. Chan (USA)</td>
<td>Induction of autophagic cell death in renal cell carcinoma.</td>
</tr>
<tr>
<td>19:00</td>
<td>H. Kim (USA)</td>
<td>Radiation dose protection and mitigation by Carbamazepine (CBZ) is autophagy independent.</td>
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<tr>
<td>18:30</td>
<td>W. Bulski (Poland)</td>
<td>Nuclear medicine for diagnostics and therapy.</td>
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<tr>
<td>19:00</td>
<td>D. Hunting (UK)</td>
<td>How can we exploit the properties of low energy and hydrated electrons to improve radiotherapy?</td>
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<tr>
<td>19:00</td>
<td>B. Taebi (Netherlands)</td>
<td>Ethics of radiological protection for nuclear power production and waste management.</td>
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<td>Radiation-induced DNA sugar-phosphate backbone radicals via ionization and excitation pathways.</td>
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<td>The ethical foundation of the radiation protection system.</td>
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<tr>
<th>Start time</th>
<th>ROOM</th>
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<tr>
<td>08:30</td>
<td>LR1 Kongresowa</td>
<td>Eye opener lectures EO7-EO12</td>
<td>EO7. Chair: M. Atkinson J. Williams (USA): Animal models: the good, the bad and the useless.</td>
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<tr>
<td></td>
<td>LR3 Sklodowska</td>
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<td>EO9. Chair: W.F. Morgan O. Kovalchuk (Canada): Radiation induced epigenetic effects.</td>
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<td></td>
<td>LR4 Warszawska</td>
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<td>EO10. Chair: M. Harms-Ringdahl G. Hildebrandt (Germany): Radiation-induced cardiovascular effects.</td>
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<td>09:30</td>
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<td>Coffee break (CR1 Marmurowa and CR2 Korczak)</td>
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<td>10:00</td>
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<td>Symposia S13-S18</td>
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<td></td>
<td>S15. The use of archiving data and biological material - examples and strategies</td>
<td>Chair: B. Grosche, G.A. Thomas 10:00 P.N. Schofield (UK): The STORE data warehouse; an international infrastructure for data sharing in radiobiology. 10:30 W.F. Morgan (USA): Mining the lifespan studies of beagles exposed to radiation in utero or as juveniles. 10:50 G.A. Thomas (UK): The Chernobyl Tissue Bank – a model for integrating &quot;omics&quot; research on single blocks of tissue. 11:10 E. Douple (Japan): The use of unique archived data and biological samples by the STORE data bank.</td>
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<td>S18. Radiation-produced intermediates - basic problems</td>
<td>Chair: I. Carmichael, S. Pimbllott 10:00 S. Ptasinska (USA): DNA damage induced by fast-flowing metastable species in a cold plasma. 10:25 A. McNamara (Australia): A comparison of X-ray, proton and alpha beam track structures using Monte Carlo simulations. 10:50 R. Edge (UK): Reaction of carotenoids with free radicals and singlet oxygen. 11:15 I. Carmichael (USA): Scavengers as a mitigating strategy against radiation damage in macromolecular crystallography.</td>
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<td>13:00</td>
<td>Lunch break (CR1 Marmurowa and CR2 Korczak)</td>
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<td>14:00</td>
<td>Poster session POS14 – POS22 (PR1 Starzyński and PR2 Broniewski)</td>
<td>POS14 DNA repair; POS15 Genetic instability; POS16 Individual radiation sensitivity; POS17 Radioecology; POS18 Radiation chemistry in materials science; POS19 Radiation research and nuclear power; POS20 Combination treatments; POS21 Non-cancer effects; POS22 Heavy ions. Authors of odd poster numbers are asked to be at their posters from 14:00 to 14:45 Authors of even poster numbers are asked to be at their posters from 14:45 to 15:30</td>
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<td>15:30</td>
<td>Coffee break (CR1 Marmurowa and CR2 Korczak)</td>
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<tr>
<td>16:00</td>
<td>Plenary lecture PL3 (LR1 Kongresowa)</td>
<td>Chair: O. Niwa R. Hill (Canada): The varied faces of hypoxia in cancer: from radiation resistance to metastasis to stem cell niche. IARR H.S. Kaplan award lecture.</td>
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<tr>
<td>16:45</td>
<td>Debate (LR1 Kongresowa)</td>
<td>Chair: J. Williams, I. Turesson Debate title: This house believes that further advances in radiation oncology will come from physics rather than from radiation biology. Debaters: B. Maciejewski (Poland) and S. Bentzen (USA)</td>
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<td>19:30</td>
<td>Conference gala dinner (Warsaw Centre EXPO XXI)</td>
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**Wednesday 31 August 2011**

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<th>ROOM</th>
<th>LR1 Kongresowa</th>
<th>LR2 Ratuszowa</th>
<th>LR3 Skłodowska</th>
<th>LR4 Warszawska</th>
<th>LR5 Mikołajska</th>
<th>LR6 Kruczkowski</th>
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<td><strong>Eye opener lectures EO13 – EO17</strong></td>
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<td><strong>10:00</strong></td>
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<td><strong>Symposia S19 – S24</strong></td>
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<td>S19. Vascular endothelial cell response to radiation - a new dimension</td>
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12:00 Plenary lecture PL4 (LR1 Kongresowa)
Chair: P. O’Neill
D. Brenner (USA): Exploring the two two-edged swords. RRS G. Failla award lecture.

13:00 Lunch break (CR1 Marmurowa and CR2 Korczak)

14:00 Poster session POS23 – POS29 (PR1 Starzyński and PR2 Broniewski)
POS23 Radiation protection; POS24 Low dose effects; POS25 Stem cells; POS26 Radiation damage to biomolecules; POS27 Normal tissue damage; POS28 Microdosimetry; POS29 Physical dosimetry.
Authors of odd poster numbers are asked to be at their posters from 14:00 to 14:45
Authors of even poster numbers are asked to be at their posters from 14:45 to 15:30

15:30 Coffee break (CR1 Marmurowa and CR2 Korczak)

16:00 Plenary lecture PL5 (LR1 Kongresowa)
Chair: K. Williams
D. Hirst (UK): Nanoparticle therapy with the Midas touch. ARR Weiss award lecture.

16:45 Plenary lecture PL6 (LR1 Kongresowa)
Chair: A. Wojcik
P. Jeggo (UK): Revealing the complexity of DNA double strand break repair; the processes, the lesion and the environment. ERRS Bacq and Alexander award lecture.

17:30 Symposia S25 – S29
S25. Cell adhesion/migration in response to irradiation
Chair: M.H. Barcellos-Hoff, N. Cordes
17:30 M. Pruschy (Switzerland): Targeting the irradiation-induced proangiogenic and proinvasive phenotype.
18:00 M.H. Barcellos-Hoff (USA): Mechanisms and consequences of radiation-induced phenotypes.
18:30 N. Cordes (Germany): Should I stay or should I go? Cell

S26. Radiation research award session
Chair: P. O’Neill
17:30 M. Boerma (USA): Experimental radiation-induced heart disease: past, present and future.
18:00 A. Paun (Canada): Genetic variation in immunity alters murine response to whole thorax irradiation.

S27. Tumor hypoxia and radioresistance
Chair: B. Wouters, H. Harada
17:30 H. Harada (Japan): Molecular mechanism behind HIF-1-mediated radioresistance and postirradiation recurrence of tumors.
18:00 Brad Wouters (Canada): Novel oxygen sensitive signalling pathways and their potential as therapeutic targets.
18:30 R. Ali (USA): Imaging of hypoxia-

S28. Computational approach to understanding DNA protection by protein binding
Chair: M. Davidkova, R. Martin
17:30 M. Davidkova (Czech Republic): Effect of protein binding to direct and indirect radiation damage to DNA.
18:00 S. Ptasinska (USA): The action of amino acids on electron irradiated DNA films.
18:30 R. Martin (Australia): The

S29. Radiation chemical studies of bioactive compounds
Chair: J.L. Gębicki, T. Mukherjee
17:30 T. Mukherjee (India): Mechanistic studies on herbal drugs and their active ingredients in relation to their antioxidant and radioprotection ability.
18:00 M. Landauer (USA): Radioprotection by the soy isoflavone genistein.
18:30 A. Sikora (Poland): Novel tools in the research
| Induced radiation resistance and treatment response. | Development of new radioprotectors – DNA binding studies with methylproamine analogues. 18:30 H. Fujimoto (Japan): Structural analysis of the interaction between the Ku protein and DNA. | On antioxidants - the global profiling of ROS/RNS in cell-free and cellular systems. |
### Thursday 1 September 2011

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<tr>
<th>Time</th>
<th>ROOM</th>
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<tr>
<td>08:30</td>
<td>Eye opener lectures EO18 – EO23</td>
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<td>EO23. Chair: J. Williams V. Meineke (Germany): Planning/responding to nuclear terrorism.</td>
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<td>09:30</td>
<td>Coffee break (CR1 Marmurowa and CR2 Korczak)</td>
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<td>10:00</td>
<td>Symposia S30 – S35</td>
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|       | S30. Biological effects of low doses  
Chair: S. Salomaa, D. Averbeck  
10:00 S. Salomaa (Finland): Biological effects at low doses - European Low Dose Risk Research Strategy  
10:15 C. Badie (UK): What do we know about the mechanisms of cancer induction and how might this affect the shape of the dose-response at low doses?  
10:30 R. Wakeford (UK): The risk of cancer from low level exposure to radiation – the epidemiological evidence.  
10:45 M. Atkinson (Germany): What do we know about the factors underlying individual susceptibilities and how large is this variation?  
11:00 E. Cardis (Spain): Molecular epidemiology and low dose risk. |
|       | S31. Dynamics of repair of radiation-induced cellular DNA damage in real time  
Chair: D. Chen, A. Yasui  
10:00 A. Yasui (Japan): Repair mechanisms of DNA strand breaks identified by visualizing proteins in human cells.  
11:15 A. Asaithamby (USA): Visualization of spatio-temporal dynamics of ionizing radiation induced clustered DNA lesions. |
|       | S32. New tools in biological dosimetry  
Chair: P. Voisin, B. Thierens  
10:00 B. Thierens (Belgium): The automated micronucleus assay as a reliable biodosimetric tool for population triage in large scale radiation accidents.  
11:00 M. Drouet (France): Towards the validation of gene expression modifications as a biodosimeter.  
11:20 P. Voisin (France): Standardisation of biological dosimetry by cytogenetics: status, |
|       | S33. Countermeasures in case of accidental radiation exposure  
Chair: V. Meineke, J. Williams  
10:00 J. Williams (USA): Animal Models for countermeasure research.  
10:20 M. Drouet (France): Mesenchymal stem cell therapy for treatment of localized radiation injuries (the minipig model).  
10:50 Z. M. Ran XZ (China): Studies on hematopoietic protection and immunity adjustment in combined radiation-thermal injury.  
11:15 M. Hauer-Jensen (USA): The somatostatin antagonist, SOM 230, is a highly effective mitigator of intestinal radiation injury. |
|       | S34. Stem cells and regenerative medicine for the treatment of radiotherapy side effects  
Chair: M. Benderitter, C. Limoli  
10:00 P. Van Luijk (Netherlands): Stem cell sparing radiotherapy: a novel approach to the prevention of radiation-induced xerostomia.  
10:30 C. Guha (USA): Hepatocyte transplantation for amelioration of RILD.  
10:45 A. Ch. Jensen (Poland): Mesenchymal stem cell ameliorates severe radiation pelvic complication: clinical transfer.  
11:00 M. Drouet (France): Bone regeneration and engineering in irradiated fields.  
|       | S35. Radiation research and nuclear power  
Chair: D. Swiatla-Wojcik, A. Chmielewski  
10:00 B. Mincher (USA): Radiation chemistry effects on nuclear solvent extraction: examples from CMPO radiolysis.  
10:25 A. Chmielewski (Poland): Chemistry for the nuclear energy of the future.  
10:50 J. Wren (Canada): Radiation-induced aqueous chemistry and corrosion in nuclear reactor environments.  
11:15 D. Swiatla-Wojcik (Poland): Modelling and simulation for controlling chemistry in advanced nuclear energy systems. |
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<th>Time</th>
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<tr>
<td>11:30</td>
<td>M. Klinger</td>
<td>(Italy): Fat grafting after mastectomy and radiotherapy.</td>
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<tr>
<td>11:30</td>
<td>M. Barcellos-Hoff</td>
<td>(USA): Systems biology - where are we and where can we go?</td>
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<td>11:30</td>
<td>M. Durante</td>
<td>(Germany): Particle therapy: from the laboratory to the clinic.</td>
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<td>11:30</td>
<td>M. Mostafavi</td>
<td>(Poland): Radiation chemistry and technology of polymers: recent advances.</td>
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<td>11:30</td>
<td>Y. Dubrova</td>
<td>(UK): Genomics and the therapeutic response.</td>
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<td>11:30</td>
<td>C. West</td>
<td>(France): Regenerative medicine based on stem cell injection for radiation burn treatment.</td>
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<tr>
<td>11:30</td>
<td>J.-L. Ravanat</td>
<td>(France): Fat grafting after mastectomy and radiotherapy.</td>
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<td>12:00</td>
<td>Conference lectures CL13 – CL18</td>
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<td>12:00</td>
<td>CL13. Chair</td>
<td>H. Paretzke (USA): Systems biology - where are we and where can we go?</td>
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<td>12:00</td>
<td>CL14. Chair</td>
<td>E. Blakely (Germany): Particle therapy: from the laboratory to the clinic.</td>
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<td>12:00</td>
<td>CL15. Chair</td>
<td>M. Mostafavi (Poland): Radiation chemistry and technology of polymers: recent advances.</td>
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<td>CL17. Chair</td>
<td>C. West (France): Regenerative medicine based on stem cell injection for radiation burn treatment.</td>
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<td>12:00</td>
<td>CL18. Chair</td>
<td>B. Mincher (Sweden): Radiation-induced dissolution of spent nuclear fuel.</td>
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<tr>
<td>13:00</td>
<td>Lunch break</td>
<td>(CR1 Marmurowa and CR2 Korczak)</td>
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<tr>
<td>14:00</td>
<td>Plenary lecture PL7</td>
<td>(LR1 Kongresowa)</td>
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<td>15:00</td>
<td>Coffee break</td>
<td>(CR1 Marmurowa and CR2 Korczak)</td>
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<td>15:30</td>
<td>Poster session POS30 – POS41</td>
<td>(PR1 Starzyński and PR2 Broniewski)</td>
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<td>17:00</td>
<td>Closing ceremony</td>
<td>(LR1 Kongresowa)</td>
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**Invitation to ICRR2015 in Kyoto, Japan: Masahiro Hiraoka - President of the ICRR2015**
Abstracts of oral presentations

Monday

Eye openers EO1 - EO6

EO01. low-level radiation exposure and cancer risk. Suminori Akiba, Kagoshima University Graduate School of Medical and Dental Sciences, Japan

It is yet unclear whether chronic radiation exposure increases cancer risk as large as acute exposure does. The cancer risk of chronic radiation exposure has been examined by many epidemiological studies. Among the studies conducted in high background radiation (HBR) areas the most important is the study in Karunagappally for its high doses and dense population. A cohort study of 69,958 residents in Karunagappally accumulated 736,586 person years of observation and identified 1,379 cancer cases including 30 cases of leukemia by the end of 2005 (Nair et al 2009). Poisson regression analysis of cohort data showed no excess cancer risk from exposure to terrestrial gamma radiation. The excess relative risk (ERR) of cancer excluding leukemia, assuming a linear dose-response relationship, was estimated to be -0.13 Gy^{-1} (95% CI -0.58, 0.46). In Yangjiang area in Guangdong Province, China, another important HBR area, a cohort of 125,079 men and women was followed. The cancer mortality study accumulated 1.7 million person-years and identified 1,003 cancer deaths during the follow-up period of 1979–1995 (Sun et al. 2000 J Radiat Res). The ERR/Sv of cancer excluding leukemia was estimated to be -0.11 Gy^{-1} (95% CI -0.67, 0.69).

Also important are the studies of residents along Techa river in Ural, Russia (Krestininna et al., 2005), and of residents in building with Co-60 contaminated building materials in Taiwan (Hwang et al., 2008). The ERR estimate of solid cancer obtained from the Techa river study was 0.99 (95% CI: 0.3, 1.9), a value significantly higher than the Indian estimate (P=0.04). The Taiwanese study reported an ERR estimate of 0.53Gy^{-1} for solid cancer.

Among nuclear workers, far by the most extensive evaluation of solid cancer risk was conducted by the ‘IARC 15-country’ study (Caridis et al. 2001; Cardis et al. 2002). However, the variability of the radiation risk estimates reported by IARC 15-country study has been questioned (Wakendorf 2005; Shigematsu 2005; Lagarde 2005; McGeoghegan 2005).

In addition to reviewing those studies, we conducted meta-analyses of HBR area studies and nuclear worker studies. Our results suggest that the ERR estimate of solid cancer is unlikely to be higher than the estimate of 0.47Gy^{-1} obtained from atomic bomb survivor study.

EO02. The genetics of radiation-induced cancer. Michael Atkinson, Helmholtz Zentrum Munich, Germany

No abstract

EO03. Modeling Radiobiological responses - where are we? Ewa Gadowska-Nowak¹, S. Ritter², 1.M. Smoluchowski Institute of Physics and M. Kac Complex Systems, Research Center, Jagiellonian University, Kraków, Poland, 2: GSI, Darmstadt, Germany

Understanding of processes involved in the induction and transformation of radiation-induced biological damage is required for efficient design and improvement of methods of cancer treatment and radioprotection. The action of ionizing radiation in biological targets depends on radiation modality and results in alteration and breakage of chemical bonds, radiolysis of water, disruption of cell membrane integrity and changes in active transport – all contributing to cell death and genetic mutations. On the other hand, pattern of energy distribution and energy absorption in the critical target are responsible not only for molecular lesions in the cell but also for their impaired processing which causes erroneous DNA replication and cell division leading to the formation of chromosome aberrations.

At a micrometer scale the energy deposition of X-rays and photons is fairly uniform producing DNA damage in a stochastic manner. In contrast, the spatial distribution of the energy deposition after exposure to radiations with high linear energy transfer (LET), such as charged particles, is inhomogeneous and reflects localized accumulation of deposit energy along the center of the particle path. Differences in the radiation quality are further manifested in the clustered DNA damages following the particle radiations. By combining track structure calculations with DNA models at different genomic scales, prediction of DNA damage becomes possible and provides valuable estimate of the relationship between the initial physical events and observed biological responses.

The major objective of the presentation is to review recent advances in calculation methods and biophysical modeling aimed to provide heuristic reproduction of expected radiation damage induction. Since a precise prediction and quantification of that damage is of great importance for both radiation protection, particularly in open-space-long term manned missions, and hadrontherapy, further improvement and possible refinement of existing models will be also discussed.

References

C.K. Wang, Mutation Res. 704 175 (2010).

EO04. Outline the role and importance of microRNAs in cell function including response to radiation. Ya Wang, Emory University, USA

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at a post-transcriptional level, which is one of the fastest developing research areas in recent years. Considering that ~1% of the human genome is devoted to miRNA genes, most human genes are regulated by at least one miRNA and each miRNA has multiple miRNA targets, the potential impact of altered miRNA levels is conceivably enormous. miRNAs directly or indirectly affect almost all cell functions. Ionizing radiation is an efficient DNA damage inducer that plays an important role in promoting carcinogenesis and killing cancer cells, which are associated with multiple genetic/epigenetic changes that are directly or indirectly affected by miRNAs. The change in miRNA levels contributes to both radiation-induced carcinogenesis and cancer radiotherapy. This eye opener lecture will focus on the following aspects: 1) introduce the main mechanism by which miRNAs regulate gene expression and subsequently affect cell function; 2) describe the major effects of miRNAs on stem cells, carcinogenesis, cancer progress, etc; 3) briefly describe the miRNA changes in radiation response; 4) provide some examples concerning miRNAs as a therapeutic tool for cancer treatment including sensitizing tumors to radiation.

EO05. Reactive oxygen and nitrogen species involved in radiation-induced signalling/bystander effects. Georg Bauer, Universitätshilnabnä Ferrburg, Germany

There is an ongoing and partially controversial discussion on bystander effects in radiation biology. The term “bystander effect” thereby indicates that cells that have not been directly challenged by radiation are affected by neighbouring irradiated cells through intercellular signaling mechanisms. Part of the controversy in the field is due to the use of the same term, i.e. bystander effect, for a multitude of functionally different biological phenomena.

This talk is focused on low dose radiation-mediated bystander effects during intercellular ROS-mediated apoptosis signaling in malignant cells. Our studies are based on three defined stages of multistep oncogenesis: nontransformed, transformed (premalignant) and tumor cells. Transformed cells generate extracellular superoxide anions and thus are subject to intercellular induction of apoptosis. Tumor cells show superoxide anion generation, but are resistant to intercellular ROS signaling due to resistance controlled by membrane-associated catalase. Low dose gamma irradiation modulates apoptosis in transformed cells due to induction of a strong increase in superoxide anion generation and peroxidase release. Only a minority of cells within the population needs to be directly hit by radiation, as strong bystander effects enhance the subsequent generation of specific signaling components. Reconstitution experiments, combined with inhibitors and siRNA allowed to define distinct amplification steps involved in establishment of intercellular apoptotic signaling. Mitochondrial functions are required for the initial perception of radiation, TGF-beta seems to have a crucial function for the subsequent amplification of ROS signaling-related activities and a complex interplay of distinct reactive oxygen and nitrogen species causes the final intercellular apoptotic signaling. In tumor cells, additional singlet oxygen-dependent amplification steps are necessary to achieve apoptosis induction. These data demonstrate how low doses of radiation trigger ROS-dependent signaling reactions with amplificatory potential and thus can finally lead to a strong and specific biological effect. Low dose radiation triggered ROS-dependent signaling effects may have a substantial impact on the control of oncogenesis and a possible therapeutic potential.
Diffusible hydroxyl radicals (HO·) are known to react with DNA either by hydrogen abstraction from the 2-deoxyribose units or by addition to the base moieties. The majority of HO· attacks occur at the base moieties. However, there is growing evidence that the oxidation of 2-deoxyribose in DNA plays a critical role in the genetic toxicology of oxidative stress and inflammation. In the last decade, significant efforts have been devoted to creating a site-specific radical generating system for a better understanding of reaction mechanism.

Our studies focus on the selective generation of relevant radicals by chemical radiation methods that allow quantitative data to be obtained. It is now clear that once a certain radical is formed, it can partition among various pathways. Uninuclear processes, such as fragmentations or cyclizations, compete with bimolecular processes, such as reactions with oxygen, thiols, oxidants or reductants. Examples of the fate of C5 radical and tautomism in the guanyl radical will be presented.

Selected references:

S01. Normal tissue effects of radiation

S01-01. Stem Cells, Oxidative Stress, and Normal Tissue Injury. Charles Limoli, University of California, Irvine, USA

The redox environment impacts normal stem cell niches throughout the body. Hematopoietic, muscle, and neural stem cell compartments respond to changes in reactive oxygen (ROS) and nitrogen (RNS) species by triggering signaling networks that impact cellular proliferation, survival and differentiation. Work from many labs including our own has found that irradiation can trigger acute and chronic increases in oxidative stress. Low dose and/or protracted dose rates can elicit radioadaptive changes that have beneficial effects on proliferation and survival, while influencing the development lineage-specific cell fates. Higher doses and dose rates have been found to impede the regeneration of irradiated tissues, through the depletion and/or damage of endogenous stem cell pools, and by promoting the onset and persistence of secondary reactive processes involving oxidative stress and inflammatory cytokines. Increasing evidence suggests that these important stem cell pools are differentially protected from ROS/RNS damaging agents compared to their immediate progeny (i.e. precursor/progenitor cells) due to enhanced DNA repair, antioxidant status and reduced cell cycle activity. Thus, many of the adverse effects of irradiation on normal tissue are the consequence of damage to the rapidly expanding pool of precursor cells derived from asymmetric cell division. Irradiation of the bone marrow impairs the health of bone by promoting osteoclastogenesis (osteoclast-mediated bone resorption) and inhibiting osteoblastogenesis (osteoblast-mediated bone formation), with the net effect of reducing bone mass and structural integrity. Irradiation of the skeletal muscle impairs myogenesis (formation of muscle tissue) by damaging satellite cells (i.e. muscle stem cells) and reducing pro-proliferative levels of nitric oxide. In the brain, irradiation depletes neural stem and precursor cells and leads to persistent increases in ROS/RNS and inflammatory cytokines that inhibit neurogenesis (formation of new neurons and glia) and adversely impact cognition. In each of these foregoing cases, interventions targeted to reduce specific reactive species can ameliorate the adverse effects of radiation exposure, and points to the importance of understanding the interplay between endogenous stem cell niches and the microenvironmental redox state.

S01-02. Use of anti-inflammatory therapies to modulate radiation-induced late effects. Mike Robbins, Wake Forest School of Medicine, USA

The last twenty years or so have seen a fundamental shift in our understanding of the mechanism(s) involved in the development and progression of radiation-induced late normal tissue injury. The classic target cell kill hypothesis in which radiation injury is fixed and untreatable has been replaced by the orchestrated response hypothesis in which cells are active participants in the normal cellular response to injury that initiates an active chronic process leading to progressive late injury. Importantly, a growing body of evidence indicates that radiation-induced late effects can be modulated. Although the precise mechanism(s) involved in the development and progression of radiation-induced late effects remain unclear, there appears to be general acceptance of a primary role for acute and chronic oxidative stress/inflammation. Despite the lack of mechanistic data establishing a causal link between oxidative stress/inflammation and radiation-induced late normal tissue injury, numerous studies have shown that administering a variety of drugs, including renin-angiotensin system blockers, peroxisomal proliferator-activated receptor (PPAR) agonists, and antioxidant/antioxidant enzymes can prevent and/or ameliorate the severity of radiation-induced late effects in a variety of late responding normal tissues. Moreover, these effects appear to be selective. Agents shown to modulate late normal tissue injury do not protect tumor cells against radiation; indeed current data suggest that these agents may well also possess antitumor properties. Although these findings have yet to be successfully translated to the clinic, they have provided a firm rationale for the continued design and development of anti-inflammatory-based interventional approaches for the treatment of radiation-induced late normal tissue injury.

S01-03. Hypoxia and inflammation in radiation induced normal tissue injury. Katharina Fleckenstein, Dept. of Radiotherapy and Radiation Oncology University Medicine Mannheim University of Heidelberg, Germany

In radiotherapy for cancer normal tissue tolerance limits the dose that can be delivered. To further enhance the therapeutic index, it is inevitable to understand normal tissue response to radiation.

Advances in normal tissue radiobiology have demonstrated that radiation triggers a cascade of molecular events that begins immediately and continues to promote tissue damage. This state can be sustained for months to years after irradiation.

It is well known that a burst of reactive oxygen species (ROS) initiates these events. It is suggested that ROS induced cell damage and involvement in complex signaling as well as cytokine induction and activation can lead to (local) hypoxia and subacute/chronic inflammation. This in turn produces chronic oxidative stress. In case anti-oxidant defense mechanisms are exhausted a vicious circle produces a non-healing wound response with insufficient tissue remodeling and vascular injury. This can lead to expression of functional damage with atrophy, fibrosis and/or necrosis long after the normal tissue was irradiated. An overview of radiation induced normal tissue injury with emphasis on normal lung repair is presented.

S01-04. Inflammation and fibrosis. Marie-Catherine Vozenin, InsermU1030 /IGR, France

Normal tissue response to ionizing radiation is classically divided into an early phase characterized by acute inflammatory response thought to be one of the priming signals that trigger radiation-induced fibrosis; and a late phase characterized by chronic fibrosis. Although the molecular and cellular features of acute inflammation have been well described, the inflammatory parameters involved during the late phase are less known.

However chronic inflammatory processes involving specific paracrine mediators and cellular factors are thought to be involved in the downstream activation of fibrogenic pathways including activation of the TGF-b1/Smads pathway. For instance, pro-inflammatory mediators such as IL-6, TNF-a and MCP1 can be tightly regulated within fibrotic tissues. Amongst the cellular mediators, the polarization of both lymphocytes T-helper and macrophages (Th1 vs Th2 and M1 vs M2) can be of importance. Therefore using radiation-induced cardiac fibrosis as a model we investigated the features of chronic inflammation induced by increasing doses of irradiation (0; 0.2; 2 and 16 Gy) in two strains of mice: a wt C57Bl6 and its prone mouse C57Bl6/J. Although these findings have yet to be shown to modulate late normal tissue injury do not protect tumor cells against radiation; indeed current data suggest that these agents may well also possess antitumor properties. Although these findings have yet to be successfully translated to the clinic, they have provided a firm rationale for the continued design and development of anti-inflammatory-based interventional approaches for the treatment of radiation-induced late normal tissue injury.
through TGF-β/mad activation. Further studies are ongoing to confirm this hypothesis.

S02. Chromatin modifications and DNA damage response

S02-01. Histone methylation and heterochromatin protein 1 in DNA damage response. Jurek Dobrucki, Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, Poland

Chromatin has been shown to undergo local decondensation in response to DNA damage. Histone modifications (‘histone code’), including methylation, are important factors involved in control over chromatin relaxation and condensation. Heterochromatin protein 1 (HP1) is known to be as principal component of heterochromatin, and a factor involved in maintaining compaction of heterochromatin. HP1 binds to methylated lysine 9 on histone H3, and acts as an epigenetic regulator, and a factor involved in chromosomal translocations (PEV) and condensation. Histone deacetylation at Lys20 in HP1 results in an increased binding of HP1 to chromatin and decreases the location of repair factors in mammalian cells. It is now becoming clear that the basal levels of HP1 are critical for DNA damage repair. We will discuss how such modifications influence DNA damage repair through sensing the damage and recruitment of the factors involved in repair. Although induced histone lysine modifications have been linked to the recruitment of DNA repair factors in mammalian cells, it is now becoming clear that the basal levels of specific histone modifications are critical for DDR. We will discuss the role of the histone acetyl transferase MOF, and its specific histone acetylation at H4K16 (H4K16ac) in DDR at several stages, including DNA damage sensing and DSB repair by both non-homologous end joining and homologous recombination.

S02-02. Chromatin modifications and DNA damage response. Tej Pandita, UT Southwestern Medical Center, USA

Chromatin, the physiological packaging structure of histones and DNA, is an important determinant of protein-DNA interactions, with consequences for DNA metabolism and transcription control. Histone modifications are critical for the higher order organization of DNA as such modifications create a natural barrier against access to DNA during transcription, damage repair and recombination. There is an increasing body of evidence relating histone modifications like phosphorylation, acetylation or methylation to critical roles in the DNA damage response (DDR) and DSB repair. Following DNA damage, chromatin structure is altered by (i) ATP-dependent chromatin remodeling, (ii) incorporation of histone variants into nucleosomes and (iii) covalent histone modifications. The histone code hypothesis posits that distinct modifications manifested at specific histone tail residues serve as a chromo "barcode" that facilitates the function of repair proteins. Although induced histone lysine modifications have been linked to the recruitment of DNA repair factors in mammalian cells, it is now becoming clear that the basal levels of certain histone modification are critical for DDR. We will discuss the role of the histone acetyl transferase MOF, and its specific histone acetylation at H4K16 (H4K16ac) in DDR at several stages, including DNA damage sensing and DSB repair by both non-homologous end joining and homologous recombination.

S02-03. Regulation of post-translational modifications in the double-strand break response. Jo Morris, University of Birmingham, UK

Post translational modifications form the basis of the mammalian cell response to DNA double-strand breaks. These modifications begin with phosphorylation co-ordinated by ATM followed by a cascade of ubiquitylation events regulated by multiple E3 ubiquitin ligases. Chromatin modification by ubiquitin regulated recruitment of DNA repair mediators such as BRCA1 and 53BP1. Our own work has shown that SUMO modification is also key to the recruitment and activity of DNA repair factors. I will present our recent data that indicates that the 19S proteasome is part of the cascade and differentially regulates the DNA damage response.

S02-04. Induction, repair and misrepair of DNA double-strand breaks (DSBs) in the context of higher-order chromatin structure. Martin Falk, E. Lukňaštová, S. Kozubek, L. Štefančíková, L. Weiterová, Institute of Biophysics of Academy of Sciences of CR, Czech Republic

Repair of DSBs, the most deleterious DNA lesions for human health, is associated with a carefully regulated complex sequence of epigenetic chromatin modifications (CHMs). CHMs mediate specific interactions between repair participants as well as reorganizations of higher-order chromatin structure required for DSB processing. Although we have some knowledge about the "biochemical" aspects of DSB repair, the relationship between the chromatin structure and repair processes remains unclear.

We show that local chromatin decondensation, provoked by epigenetic modifications, is required to open damaged domains for huge repair complexes and that these changes are reverted into the original status before terminating the repair. We also demonstrate that chromatin reorganizations together with the original chromatin structure at the site of DSB and at pan-nuclear level influence the sensitivity to DSB induction, the mechanism, efficiency and fidelity of DSB repair and formation of chromosomal translocations: Using gH2AX foci as a specific DSB marker and the ImmunoFISH method in combination with high-resolution confocal microscopy, we have found that gene-poor condensed heterochromatin is less sensitive to DSB induction by sparsely ionizing g-rays than genetically active, "open" chromatin domains. Overall, correct repair in dense heterochromatin requires extensive chromatin decondensation, which is frequently followed by "protrusion" of heterochromatic DSBs into low-density chromatin "holes". Consequently, two or more DSBs may cluster inside these holes and potentially form translocations. Since "protrusion" of DSBs into a particular hole is dictated by the original chromatin structure around the DSB site, our mechanism of formation of chromosomal translocations has aspects of both the Breakage-First and Position-First hypothesis.

Acknowledgement: Supported by the Grant Agency of ASCR, project No.: IAA500040802.

References:
ORAL PRESENTATIONS

S03-03. DNA damage and repair after low doses of charged particles. Maria Antonia Tabocchini, F. Antonelli, V. Dini, G. Simone, E. Sorrentino, M. Belli, A. Campa, G. Espósito, Istituto Superiore di Sanità, Italy

Galactic cosmic radiation is characterized by the presence of high energy protons and high charge and energy (HZE) particles. Fluence rates are very small, of the order of a proton every few days and a HZE particle every month per cell nucleus, but still sufficient to deliver an average dose of few mSv per day. HZE particles, although much less abundant, are more effective than protons in damaging biological systems. It is thought this higher effectiveness is related to the larger probability to produce clustered DNA damage, in particular DNA double strand breaks (DSB) associated with other DSB or different lesions, within a localized DNA region.

Improvement of space radiation protection requires a better understanding of the radiation quality dependence of the mechanisms involved in the biological effects of charged particles, with special attention to DNA damage and repair. In this context, the limitations of physical methods in the study of DNA damage induced by low doses/fluences, has motivated the search of alternative approaches. Nowadays, immunofluorescence techniques, based on the use of antibodies against proteins involved in DNA damage response (typically g-H2AX, but also 53BP1, ATM, etc...), are the only ones capable to detect damage in single cells at doses as low as those released by one particle traversal. These techniques carry their own limitations, associated to the inability to discern DNA damages in close proximity. Also the relation between the processing of DSB and that of the fluorescent foci is still not yet completely clear. However, it is possible to estimate the DNA damage complexity by the time course the fluorescent foci persistence and, perhaps, by their morphology.

In this presentation we will review the data collected in in vitro experiments, together with their analysis and interpretation. We will describe in particular our experiments performed on primary human fibroblasts irradiated with protons, a-particles, C- and Fe-ions. Advantages and constrains related to different irradiation geometries, i.e. irradiation at small angle or perpendicular with respect to the cell plane, will be discussed.

The use of these functional techniques is providing an important boost to the study of the mechanisms associated to the radiation quality dependence of biological effects of charged particles.


High charge and high-energy (HZE) particles are a unique component of the space radiation environment and contribute significantly to the overall radiation dose received by astronauts. Previous studies in our laboratory have shown that high and low LET radiation elicit unique phosphoprotein signaling profiles. The total dose received from high LET radiation results from a combination of the track core, the high-energy delta-rays surrounding the track, and the low-energy delta-rays produced at some distance from the core particle. The contribution of the various components of high LET radiation to their biological effectiveness is poorly understood. We hypothesize that the fate of the cell and the kinetics of the signal response will be influenced by the cell type, as well as the nature and complexity of the damage.

For these studies, ions and energies were carefully chosen based on charge, energy, and LET to differentiate direct track effects from delta-ray exposures, and used to determine how radiation components affect the activation of key proteins modified in response to radiation damage. To better understand the pathways triggered in response to HZE exposure, we have quantitatively examined phospho-protein profiles of various DNA repair proteins (gamma H2AX, phospho-ATP2, phospho-SMC1) within multiple cell types at various times (0.5 to 24 h) post-assorted radiation quality exposures using flow cytometry and immunofluorescence microscopy. Flow cytometry data is ideal in that large numbers of cells can be analyzed on a single-cell basis and various statistical approaches for analysis can be applied to detect subtle differences in the low dose range. In addition, the various phases of the cell cycle can be analyzed separately to determine how cell cycle may influence the profiles obtained.

Our results indicate that phospho-protein kinetics and the magnitude of the cellular response in different cell types varies dependent upon cell type and radiation quality. Quantitative evaluation of early signaling mechanisms in the context of the quality of HZE radiation will promote better understanding of the risk associated with high LET exposure.


A major concern of deep space travel includes untoward effects of protons and heavy ions on the brain. We have previously examined the effects of low to moderate doses of whole body irradiation with gamma rays and heavy ions (4He) on adult hippocampal neurogenesis and gene expression in young C57BL/6 mice. We found a clear decline in hippocampal neurogenesis measured by both BrdU incorporation and doublecortin staining for neural precursors with doses of 1 Gy and above. In the current study we carried out similar experiments using 1 GeV/n protons at the NASA Space Radiation Laboratory, Brookhaven National Laboratories. Unanesthetized 10-12 week old male C57BL/6 mice were irradiated with 10, 20, 50, 100 or 200 cGy single dose protons and sacrificed at time points up to 12 months post-irradiation. Sham-irradiated mice served as negative controls. Specific endpoints included measures of hippocampal neurogenesis, hippocampal-dependent learning using contextual fear conditioning, and markers of neuroinflammation. We found evidence of decreased body weight gain starting at 6 months in mice receiving doses of 50 cGy and higher, as well as decreased hippocampal neurogenesis based on counts of BrdU labeled neural cells in the dentate gyrus. Despite these clear effects of radiation, we did not detect radiation-associated changes in contextual fear conditioning, a hippocampal dependent behavior. Further characterization of tissues is underway.

Supported by NASA Space Radiation Biology Program Grant NNX08BA09G.

S04. Radiation and immune response modifiers

S04-01. Defining a role for body temperature in cytokine regulation and neutrophil homeostasis following total body irradiation. Elizabeth Repasky, M. Capitano, M. Nemeth, T. Mace, P. McCarthy, Roswell Park Cancer Institute, USA

Important immunological barriers to infection, the gut mucosa and the skin, as well as the hematopoietic compartments are damaged by total body irradiation (TBI) exposures. This leaves those exposed to sufficient doses of radiation highly susceptible to infection and new strategies are needed that can safely and rapidly boost peripheral leukocyte numbers in order to prevent illness or death from microbial invaders.

We hypothesized that mildly elevating core temperature, which we and others have shown previously to alter leukocyte numbers and migration patterns, following a non-myeloablative dose of TBI, will enhance immune reconstitution through a thermally sensitive, cytokine driven bone marrow release of granulocytes to the peripheral circulation. The following observations support this hypothesis: C57BL/6 mice were given TBI (3Gy) followed 2 hours later by a mild heating (where core body temperature was elevated to 39.5°C). In mice that were heated following TBI, a significant increase in recovery rate of peripheral blood granulocytes was observed by day 8 compared to mice that received radiation alone or heating alone. In addition, G-CSF concentration was increased two fold in the serum of radiated/heated mice correlating with the increased granulocyte recovery. G-CSF production was driven by IL-
17 production in the intestine seen only when radiation was followed by heat treatment. IL-17 was later confirmed to be necessary for the thermal enhancement of neutrophil recovery when the effect of heating after radiation was lost in the IL-17R knockout mouse model. Flow analysis of the bone marrow from wild type mice revealed comparable percentages of hematopoietic stem cells in the control (63.3±2.8% of LSK were CD34-) and the radiation/heat-treated groups (62.0±1.2% of LSK were CD34-); however, there was a significantly lower percentage of hematopoietic stem cells in the bone marrow of the radiation-alone group (43.4±1.7%; p<0.003). Flow analysis revealed that increasing core body temperature following total body irradiation increased the overall percentage of granulocyte-macrophage progenitors when compared to control and radiation-alone groups (12.4±2.1% vs. 6.7±1.3% and 7.6±2.1%; p<0.025). This increase in progenitor percentages in radiated/heated mice is associated with an increase in overall number of CFUs (45±3.8) when compared to radiation alone (32±4.3). These data reveal a previously unexplored role of body temperature in regulation of hematopoiesis and marrow output following stress and may help in the development of novel, clinically applicable strategies to ameliorate the effects of TBI. Supported by NHLI 0849075 and R01 CA71599

S04-02. Cancer cells stressed by radiotherapy become targets for anti-CTLA-4 treatment. Sandra Demarra, New York University School of Medicine, USA

We have previously shown in mice models of poorly immunogenic tumors that a therapeutic strategy combining local radiotherapy (RT) with antibody targeting the co-inhibitory CTLA-4 receptor on T cells had synergistic anti-tumor effects (Demarra et al. Clin. Cancer Res. 2005, 11:728-734). The anti-tumor response was largely mediated by effector CXCXR6+ CD8 T cells. Increased T cell recruitment by RT-induced CXCL16 chemokine was required for efficient rejection of the irradiated tumor (Matsumura et al. J Immunol 2008, 181:3099-3107). RT has been shown to induce an immunogenic tumor cell death and modulate the expression of several cell surface molecules on surviving tumor cells. To determine if RT-induced changes influence the interaction between T cells and tumor cells we studied the behavior of T cells infiltrating the poorly immunogenic 4T1 tumors by intravital two-photon laser scanning microscopy (TPLSM). To visualize the endogenous anti-tumor T cells we used transgenic mice expressing green fluorescent protein (GFP) under the control of CXCXR6 promoter. To visualize tumor cells, we transduced 4T1 cells with a retroviral vector expressing the cyan fluorescent protein (CFP). We found that while both RT and anti-CTLA-4 ligation given as monotherapy increased the motility of activated CD8 T cells infiltrating 4T1 tumors the combination of RT with anti-CTLA-4 promoted increased arrest of tumor-specific T cells with tumor cells. The latter required interaction of NKG2D on CD8 T cells with its ligand retinoic acid early inducible-1 (Rae-1), which was up-regulated by radiation on the tumor cells. Blocking of Rae-1 interaction increased motility of anti-CTLA-4 treated T cells within irradiated tumors inhibiting their contact with tumor cells, and abrogated immune-mediated tumor rejection. These results demonstrate the critical role of radiation-induced NKG2D ligands for the anti-tumor effects of anti-CTLA-4 in the setting of a poorly immunogenic tumor, and provide a molecular mechanism underlying the effectiveness of combined RT and anti-CTLA-4 treatment.

S04-03. The Tipping Point for Combination Therapy: Cancer Vaccines with Radiation. James Hodge, National Cancer Institute, NIH, USA

Local radiation is an established therapy for human tumors. Radiation may also act synergistically with immunotherapy to enhance immune responses, inhibit immunosuppression, and/or alter the phenotype of tumor cells, thus rendering them more susceptible to immune-mediated killing. As monotherapies, both immunotherapy and radiation may be insufficient to eliminate tumor masses. However, following immunization with a cancer vaccine, the destruction of even a small percentage of tumor cells by radiation could result in cross-priming and presentation of tumor antigens to the immune system, thereby potentiating immune responses. This talk will discuss a) mechanisms by which many forms of radiation therapy can induce or augment antitumor immune responses, b) preclinical systems that demonstrate that immunotherapy can be effectively combined with radiation therapy, and c) current clinical trials where standard-of-care radiation therapy is being combined with immunotherapy. Capitalizing on the immunological effects induced by radiation treatment by adding potent antitumor vaccines may lead to synergistic approaches to cancer management that offer feasible, well-tolerated therapeutic options for cancer patients.

S04-04. Distinct in vivo responses of tumor-associated macrophages to irradiation. Chi-Shun Chang1, F. Celn1, S. Fu2, S. Wang1, C. Yu1, J. Hong2, 1: National Tsing Hua University, Taiwan, 2: Chang-Guang University, Taiwan

Macrophages display different phenotypes with distinct functions and can rapidly respond to environmental change. Tissues can re-orchestrate their components in response to the damages from ionizing radiation in a dose, space, and time dependent fashion. It is expected that the functions of macrophages within tumor tissues, so called tumor-associated macrophages (TAMs), will be altered in response to the spatial and temporal changes of tumor microenvironment following radiation therapy (RT). Indeed, we found that distinct subtypes of TAMs are redistributed to different locations associated with hypoxia or necrosis in 3 murine tumors, TRAMP-C1 prostate adenocarcinoma, ALT511C1 astrocytoma, and GL261 glioma, following RT. This distribution was not observed in tumors receiving chemotherapy or antiangiogenesis agent. The specific spatial distribution of sub-types of TAMs also occurs in tumors growing from pre-irradiated tissues. When this unique TAM distribution pattern in irradiated tumors is interfered by targeting specific subtype of TAMs, radiation-induced tumor growth delay is enhanced. This indicates that irradiated-tissues have distinct microenvironment in favoring the development of M2 TAMs. Our current work focuses on characterizing the changes of tumor microenvironment following irradiation, identifying the responses of TAMs to these changes, searching factors that drive TAMs to promote tumor re-growth, and defining specific subtype of TAMs responsible for this effect. (This study is supported by NHRI-EX100-9827BL, NSC99-2627-M-007-008, and NSC98-2628-B-182-002-MY3

S05. RRS President’s symposium - biological significance of complex DNA damage: physics, chemistry and biology of complex damage

S05-01. Modelling of DNA damage dependence on radiation quality. Andrea Ottolenghi1, D. Allion1, L. Giovanni Mariotti, W. Friedländ1, K. M. Prise 3, G. Schettino 3, 1: Università of Pavia, Dipartimento di Fisica Nucleare e Teorica and INFN, Italy, 2: Università di Pavia, Laboratorio Energia Nucleare Applicata & Dipartimento di Fisica Nucleare e Teorica & INFN, Pavia, Italy, 3: Università di Pavia, Dipartimento di Fisica Nucleare e Teorica & INFN, Pavia, Italy, 4: Helmholtz Zentrum München, Institute of Radiation Protection, Neuberger, Germany, 5: Center for Cancer Research and Cell Biology, Queen’s University, Belfast, UK, 6: Centre for Cancer Research and Cell Biology, Queen’s University, Belfast, UK

We report the results of an investigation on the role of radiation quality and track structures in inducing DNA damage. A theoretical investigation on the induction of DNA damage in terms of DNA fragmentation spectra is presented, together with an evaluation of RBE for different radiation qualities. To allow the study of low dose effects, preliminary results on DNA damage evaluated through γH2AX, will also be discussed. DNA fragmentation calculated using the PARTRAC code, will be presented for different radiation types (different LETs and different specific energies). Furthermore, the characteristics of these spectra, and in particular the high number of short fragments (less than 1 kb), obtained by the simulations, will be analysed and compared with available experimental data. The results of the integration between experimental and theoretical methods will be discussed.

It is known that DNA fragmentation studies require doses above a few grays and a DNA molecule free of histones and other proteins, usually obtained by a high temperature lysis condition that can elicit a temperature-induced damage response (such as the labile sites, i.e. breaks in the DNA strands induced by the high temperature level reached during the experimental procedure). For these reasons, the availability of different experimental techniques is considered important, in order to investigate the radiation DNA damage induced by low doses of irradiations of different qualities (typically in the order of fractions of a gray). The number of double strand breaks induced after irradiation could be roughly estimated (at least for low doses) counting the number of induced foci. However, also because the induction of foci is a biochemical process involving both formation and loss of foci there is never an exact correspondence between the number of DBSs and the γH2AX, even for
X-irradiation. In this work we investigated the induction and the disappearance of γ-H2AX foci after irradiation with X-rays and alpha particles. After a preliminary investigation concerning the size and the shape of the different foci, the main objective of the work was to quantify (experimentally and theoretically) the different kinetics of the phenomena and the different residual amount of foci after 24 hours of irradiation. This work was partially supported by the European Commission (FP7 EURATOM projects "EPRADASO" and "DOREMII")

S05-02. The pros and cons of processing clustered DNA damage sites. Martine Lomax¹, S. Cunniffe¹, L. Eccles¹, E. Smirnova¹, V. Shah¹, M. Greenberg², P. O’Neill¹; 1: Gray Institute for Radiation Oncology and Biology, University of Oxford, ORCRC, Roosevelt Drive, Oxford, OX3 7DQ, UK; 2: Organic and Bioorganic Chemistry, Johns Hopkins University, Baltimore, MD 21218, USA

A signature of ionizing radiation exposure is the induction of DNA clustered damaged sites, defined as two or more lesions within one to two helical turns of DNA, by passage of a single radiation track. Synthetic oligonucleotides containing DNA lesions at known positions have been used to investigate the efficiency of the base excision repair (BER) pathway to repair clustered DNA damaged sites. The repair of an abasic (AP) site is severely impaired when two 8-oxo-7,8-dihydroguanine (Go) lesions are in close proximity to an AP site on the opposing DNA strand. In addition, Go in close proximity, on the opposing strand to the long patch BER substrate tetrahydrofuran (THF-an AP site analogue) or 2-deoxyribonolactone (dl- a radiation induced oxidised AP site) can impair the efficiency of repair of the THF or dl by long patch BER. In all of these cases the lesions are repaired sequentially, thus preventing the formation of DSB. In contrast, two bistranded AP sites result in the rapid formation of double strand breaks (DSB) except when separated by up to two bases in a positive orientation, even in the presence of 8-oxoG. These results show that non-DSB clustered damaged sites compromise the base excision repair pathway leading to lifetime extension of the lesions within the cluster, compared to isolated lesions. The efficiency of mutation induction of the clustered lesions was assessed in an E.coli based assay and compared to that of the lesions in isolation. The mutation frequency of the clustered DNA damage sites that do not lead to DSB in the repair assay increased by five to ten fold compared to the mutation frequency of the isolated lesions. As the efficiency of repair of lesions within the clustered damage site is reduced the lesions have a longer half life and are thus more likely to be present at replication, leading to replication induced mutations. In addition, DSB are formed in E. coli from some clustered DNA damage sites, leading to loss of plasmid and a reduction in the transformation efficiency of E. coli. These studies give insights into how the processing of ionizing radiation induced non-DSB clustered damage can lead to mutations or DSB, and ultimately genetic instability or cell death. Steps in the BER pathway may be identified that can be exploited to increase the chances of DSB formation to increase the efficiency of the treatment of cancer by radiotherapy.

S05-03. Formation and consequences of complex DNA lesions using radiation with different LET. Kecke Elmroth, Gothenburg University/Department of Oncology, Sweden

It is now evident that high-LET radiation qualities such as alpha-particles and ions cause increased yields of double-strand breaks (DSBs) compared with low-LET radiation, as determined using methods that include measurement of correlated breaks. This is the result of clustering of lesions along the track within chromatin structure of the nucleus in eucaryotic cells, and typical RBE values for DSB induction between 1.4 and 3 have been reported. Besides prompt DSBs, another type of complex lesion is now under investigation, i.e. non-DSB clustered lesions, defined as 2 or more damages formed within 10-20 bp. The yield of bistranded non-DSB clustered lesions varies with radiation quality, and RBE values <1 have been reported. Clustered lesions are believed to contribute to mutagenic processes since the repair may be compromised, but the pathways involved in repair are not yet identified and the biological significance of this type of complex damage in intact cells is not revealed.

Here, formation and repair of prompt DSBs and non-DSB clustered lesions, induced on opposite DNA strands, after irradiation with different radiation qualities (alpha-particles, ¹²⁷I and heavy ions) are discussed, as well as some biological consequences, with special emphasis on modulating factors such as chromatin compactness and proliferation status. Normal fibroblasts at low passage number were used and DSBs and non-DSB clustered lesions were obtained by pulsed-field gel electrophoresis. In the case of high-LET irradiation fragment analysis was used to assess correlated breaks. Some of our latest findings will be presented.

S05-04. New insights into the processing of oxidatively induced clustered DNA lesions. Clinical applications? Alexandros Georgakilas, Biology Dep./East Carolina University, USA

Human beings are daily exposed to background radiation and various sources of oxidative stress. Research has focused the last decade on the effects of ionizing radiation on DNA which is considered as the key target of any radiation in the cell. Ionizing radiation and endogenous sources of cellular oxidative stress can also induce closely spaced oxidatively-induced DNA lesions called ‘clusters’ of DNA damage or Locally Multiple Damage Sites (LMDS) as first introduced by John Ward. There are limited data on the use of the DNA damage molecular assays for the detection of bistranded non-double strand break (DSB) oxidative clustered DNA lesions (OCDLs) in vitro or in vivo. Our laboratory has provided a novel adaptation of neutral single cell gel electrophoresis (Comassay) or pulsed field gel electrophoresis to measure these unique types of lesion and their repair. Thus far, our research has contributed to the identification of novel DNA repair pathways for OCDLs, as well as uncovering different strategies that these mutagenic and repair resistant lesions are processed by the cell or human tissue. By this way, meaningful insights can be discovered for the biological and clinical application of DNA damage clusters. In this presentation, I will focus on new insights and findings on the induction and processing of these types of complex DNA damage in the cells and mammalian tissues and potential clinical applications.

S06. Radiation damage to biomolecules (I): peptides, proteins, membrane lipids.


Three-dimensional structures of biological macromolecules are largely determined by crystallography: intense synchrotron produced X-ray beams of around 13keV energy are used to measure the diffraction intensities of reflections from crystals of the molecule of interest, and the structure is solved by obtaining phase information by a variety of methods. The crystals typically have between 30% and 70% solvent content, contained in channels between the macromolecules. Protein crystals at room temperature (RT) suffer radiation damage during the diffraction experiment even on a laboratory X-ray source. In the past, the required data had to be collected from several different crystals and merged together, but the intense X-ray beams from modern synchrotrons can destroy crystalline order in seconds. Over the last 20 years, the use of cryo-cooling techniques which allow X-ray data to be collected with the sample held in a stream of cooled nitrogen gas at 100K, has become the norm; at 100K crystals can withstand around 70 times the dose [1] compared with RT (depending on the dose rate [2]), and the necessary data can usually be obtained from a single crystal. However, observations of degradation of crystal diffraction with increasing radiation dose at 100K have now become commonplace at third generation synchrotrons. Researchers seek to understand the physical and chemical processes involved in this damage (reviewed in [3], which manifests itself in a number of different ways, including: changes in crystal colour, decreasing diffraction power with dose, a small but measurable linear increase in unit cell volume, and specific structural damage to covalent bonds in the amino acids of the protein molecules. Enzyme active sites seem particularly sensitive to damage, a phenomenon which potentially generates misleading information on biological mechanisms. Thus the issue of radiation damage has recently become a concern for all structural biologists.

The radiation chemistry of the processes important in a 100K protein crystal is poorly understood: current investigations and results will be presented.


S06-02. Formation and repair of protein radicals. Role of thyl radicals. Willem Koppenol, ETHZ, Switzerland
As radicals play a role in ageing and diseases, it is of considerable interest to delineate the processes that take place as soon as a radical is formed. Given their preponderance in cells, protein radicals are most likely to be formed than DNA or membrane radicals. Initiators are NO₂ and CO₂+ from the reaction of ONOO with CO₂ and HO from the Fenton reaction. Then, what is the fate of a radical randomly generated on the surface of a protein? Three paths are possible: it is repaired by the antioxidants monohydrogen ascorbate (HASc), urate or glutathione (GS), it reacts with O₂-, or it oxidises another amino acid nearby. In the case of histones around DNA, one can envisage formation of nucleotide radicals by intermolecular electron transfer to a protein radical.

Reaction of a dioxy radical with an antioxidant breaks the radical chain reaction, but produces a hydroperoxide which may later engage in deleterious reactions. By time-resolved spectroscopy - flash photolysis and pulse radiolysis - we have investigated protein repair processes. GSH generally reacts too slowly to prevent damage. In addition, the glutathione thyl radical oxidises rapidly its own a- and b-hydrogens: kₗ = 3×10⁷ s⁻¹ and kₛ = 7×10⁴ s⁻¹, with K = 0.4. We estimate that the equilibrium ratio of S- : beta : alpha-centered radicals is, very approximately, 8 : 3 : 1. Due to the reaction of C-centred radicals with O₂, the S-centred radical is less available for the well-established reaction with thiols to form disulfides and O₂·. In addition, formation of dioxy radicals leads to depletion of GSH. Hasc repairs radicals faster than GSH by a factor of 10³. The former is able to prevent intramolecular electron transfer from Tyr to Trp. Given the physiological concentrations of Hasc, repair may be competitive with dioxy formation. Similar to Hasc, urate repairs Trp and Tyr radicals in solution and in proteins with rate constants of up to 10⁷ and 10⁶ M⁻¹ s⁻¹, respectively. However, repair by urate is strongly dependent on the protein. Urate inhibited electron transfer from Tyr to Trp in chromatypsin, but not in pepsin. Both Hasc and urate reduce amino acid dioxy radicals. While reduction by Hasc is an overall 2nd-order reaction, k = 10⁷ M⁻¹ s⁻¹; that by urate is 0-order in urate k = 10⁵ s⁻¹. Both GS and urate radicals oxidise Hasc, which results in depletion of this antioxidant.

S06-03. A new biomimetic model of free radical reactivity in lipids. Branka Mihaljević1, I. Tartaro-Bujak1, C. Ferreri2, C. Chatgilialoglu1, 1- Ruder Bošković Institute, Croatia, 2- ISOF, Consiglio Nazionale delle Ricerche, Bologna, Italy

The modeling of free radical reactions under naturally occurring conditions has become a basic step in the research of fundamental mechanisms of biological processes. The far most known process regarding polysaturated fatty acids (PUFA) is the peroxidation process which is generally considered to be the major mechanism of cell injury in organisms subject to oxidative stress. A much recent review deals with the geometrical isomerisation of PUFA, evidencing that the cis double bond configuration is a inherent characteristic of membrane lipids which can be altered by radical stress. In view of the fact that these processes in lipids are the most relevant chemical processes occurring to PUFA, it is very useful to pursue model studies with the aim of having established the simplest biomimetic model of cell membrane, the micelles of linoleic acid (LH), prepared by addition of a non-ionic surfactant (TWEEN®20) and the resulting solutions were irradiated by ionizing radiation up to 400 Gy under a variety of conditions where thyl radicals are the main reactive species. The irradiation-induced peroxidation and trans isomerization in our model systems under controlled oxidation conditions have been studied. The concentrations of hydroperoxide of linoleic acid (LOOH) were determined using the spectrophotometric ferric thiocyanate method, while geometrical isomers were analyzed by GC using known conditions for the separation of cis and trans isomers.

Data on relative importance of these processes in this competitive environment have been considered. This lecture will demonstrate that hydroperoxo- and trans-PUFA can be the resulting effect of oxidative free radical conditions. While under anaerobic conditions only the cis-trans isomerization was observed, in air-equilibrated solutions a substantial amount of LOOH was produced and the cis-trans isomerization process was still observed, e.g., irradiation of 500 mikrom of LH at 400 Gy and dose rate of 4.6 Gy/s gave 20 mikrom of LOOH and 10% conversion of LH into mono-trans isomers.

The effect of micelle size will be discussed in cell culture, in order to gather data on the effect of supramolecular organization for the outcome of the two processes, and in particular, to envisage any positional preference of the two double bond.

S06-04. Fatty acid composition of muscle tissue measured in amphibians living in radiologically contaminated and non-contaminated environments. Marilynne Stuart1, C. Ferreri1, S. Kim1, A. Festa1, J. Carr1, 1- AECL, Canada 2- L.O.F. - BioFreiRadicals, Consiglio Nazionale delle Ricerche, Italy

Fatty acid composition was identified as a potential biological indicator of effects for environmental exposure to radiological contaminants. This end point was measured on muscle tissue of Mink frog (Rana septentrionalis) obtained from a radiologically contaminated pond and a non-contaminated pond. It was also measured after the frogs obtained from both ponds were exposed to a 4 Gy cobalt-60 gamma radiation dose delivered in vivo at a dose rate of about 8 GY/min). Differences in fatty acid composition were observed between contaminated and non-contaminated frogs. It was also shown that the contaminated and non-contaminated frogs responded differently to exposure to an acute high dose of cobalt-60 gamma radiation. Fatty acid composition was found to be a sensitive marker that may be useful to study and monitor biota health in environments that are radiologically contaminated.

Conference lectures CL01 - CL06

CL01. Chernobyl effects: what do we know after 25 years? Richard Wakeford, The University of Manchester, UK

On 26 April 1986, an explosion in the core of the reactor in Unit 4 of Chernobyl nuclear power station in Ukraine, and the subsequent fire in the core that burned for ten days, released radioactive material that caused heavy contamination in areas in the vicinity of the site and lesser contamination throughout much of Europe, the levels depending on wind direction and deposition from the plume that was enhanced by rain. Emergency workers received high doses of radiation, and these doses were sufficiently high for 134 workers to develop acute radiation syndrome, resulting in 28 deaths. The release to atmosphere of radioisotopes of the volatile element iodine was the major problem for populations in the worst affected areas of Ukraine, Belarus and the Russian Federation, and many children received doses to their thyroid gland of around 1 Sv or more, which has caused a major part of the 6000 cases of thyroid cancer that occurred in this highly exposed group over the 20 years since the accident, with many more cases to come if the present thyroid cancer radiation risk models are correct. Risk estimates obtained from studies of those exposed to Chernobyl radioiodine are broadly compatible the estimates obtained from children exposed to external sources of radiation. However, doses to other tissues from external irradiation from deposited radionuclides and from intakes of radioactive material such as the radioisotopes of caesium were much lower, and currently the evidence for an excess risk of other cancers is not persuasive, although there are indications of excesses of childhood leukaemia and breast cancer in the heavily contaminated areas of the former USSR. About 600 000 “liquidators” were involved in emergency and recovery work, and those working in the first year after the accident tended to receive the highest doses. Studies of these liquidators are not straightforward because of the real possibility of ascertainment bias arising from the interest in health effects in these workers, but appropriately conducted studies have provided evidence of an excess risk of leukaemia, although the interpretation of this evidence is not unequivocal. Evidence also exists for an excess of eye cataracts and blood circulatory system disease in these workers. The psychological impact of the accident is also readily apparent, especially among mothers of young children. In conclusion, the health effects of radiation exposure from the Chernobyl accident have not been as great as feared in its immediate aftermath, but the excess of thyroid cancers is substantial.

CL02. A sticky matter: ECM and radiation cell survival. Nils Cordes, Oncoray - National Center for Radiation Research in Oncology, Dresden University of Technology, Germany

Tumor therapy resistance remains a major obstacle in the fight against cancer. Aside from genetic alterations, both the microenvironment and the stroma of a malignant tumor were found to critically contribute to the acquisition and maintenance of the resistance phenotypes. During the last decade, compelling data have been accumulated showing how interactions between cells and the surrounding extracellular matrix (ECM) can hamper radiation-induced cell death. While cell adhesion receptors of the integrin family are central to this process due to their adhesion and signaling function, further signaling and adapter molecules localized at the membranous cell-ECM interaction sites, called focal adhesions, have been identified as key regulators of the cellular ORAL PRESENTATIONS

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radio-sensitivity. Prime examples of such molecules are Particularly Interesting New Cysteine-Histidine-rich 1 (PINCH1), Integrin-Linked Kinase (ILK) or Focal Adhesion Kinase (FAK). We and others demonstrated that specific targeting of these proteins essentially modulates the survival and proliferation of normal and tumor cells upon exposure to ionizing radiation. Due to frequent overexpression and/or hyperactivity of these proteins, current research aims at clarifying their role as potential cancer targets. Latest findings and methodology add emerging novel insights and facets to this scenario. For example, investigations of the repair of DNA double strand breaks (DSB) in hetero- versus euchromatin DNA regions exhibited a similar distribution of DSBs in three-dimensional (3D) ECM cell cultures and tumor xenografts in contrast to conventional 2D cell cultures. Moreover, the total number of residual DSBs is significantly lower in 3D than in 2D, an observation in line with increased clonogenic survival in 3D as well as reduced levels of lethal chromosomal aberrations. Another point of concern is the cooperative and mutual interaction in cell adhesion receptors, e.g. integrins, and transmembrane growth factor receptors/receptor tyrosine kinases (RTK). RTKs co-localize together with integrins at focal adhesion sites, which renders optimal regulation of cell functions feasible. Newest data in human head and neck squamous cell carcinoma cells suggest differential expression, phosphorylation and susceptibility to the anti-EGFR antibody Cetuximab and its potential for radiosensitization. The usage of cell culture models better reflecting physiological growth situations, a concept exemplified by multicellular spheroids, has now been complemented by ECM-based 3D cell culture models that enable molecular analysis on both single and multicellular level. Particular translational work will hopefully show whether and how our current radiobiology knowledge reflects reality and what actions can be undertaken to overcome radioresistance of tumor cells with concomitant sparing of normal tissue.

CL02. Chronic exposure, multiple stressors and other uncomfortable issues in radiation protection. Carmel Motsis, McMaster University, Canada

Ever since the acceptance that non-targeted effects (NTE) can be measured in unirradiated cells or distant progeny of irradiated cells, the question not only radiation effects paradigms such as the linear-non-threshold model (LNT), but may also have relevance to wider mechanisms in cancer biology, population ecology and evolutionary biology concerning process of selection, the transmission of heritable traits, the relevance of “social” interactions between cells, organisms and populations and the mechanism by which cells/organisms respond rapidly to environmental stress. This presentation will also argue that a key consequence of findings in NTE biology is that at any given level of organization, from gene to ecosystem – communication of stress signals and heritability of stress adaptations provide the bridges linking one hierarchical level to the next and enable the rapid propagation of change triggered by stress at one level, resulting in change at a higher (or lower?) level.

CL03. Clinical relevance of the immune response. Silvia Formenti, NYU School of Medicine and NYU Cancer Institute, USA

Advances in understanding cancer immunity have indicated that the patient’s own immune system is an important contributor to the success of classical anti-cancer therapy, both in terms of local and systemic control. Particularly, evidence is emerging that the killing effect of standard modality based therapies can be strategically directed toward an “immunogenic” cell death, converting the irradiated tumor into an individualized vaccine. The corollary provides a surprising route to controlling metastatic disease and is a paradigm shift with direct clinical implications: by effectively immunizing the patient to his/her own tumor the right cancer therapy can also prevent or delay active systemic disease. Central to this is the need to induce immune system ‘danger signals’ to convert anti-tumor activity from smoldering to high intensity. While both chemo and radiation therapy-induced cancer cell death is sensed by the host’s immune system, radiotherapy has the distinct advantage of being restricted to the tumor site. Our group has shown that local ionizing radiation therapy (RT) can be successfully combined with immunotherapy strategies to enable the immune system to reject the tumor and results in responses outside the irradiated field (abscopal effects). We demonstrated in multiple syngeneic murine models the abscopal effect of combining radiotherapy with the blockade of co-inhibitory receptor CTLA-4, or with TGF-beta blockade. Similar results could be achieved with strategies that enhance the immune response of the host, like the addition of Flt3 ligand or Toll-Like Receptor agonists. Moreover, our group has identified a preferential dose/fractionation regimen of radiation to achieve the abscopal effects.

This work has been translated to the clinic with promising results. For instance, in a trial of combining RT to a metastatic site with GMS-CSF, 11/27 patients demonstrated an objective response outside the irradiated field, and in another study in situ tumor vaccination with a TLR9 agonist and RT induced systemic anti-lymphoma clinical responses (Brody, Journal of Clinical Oncology 2010).

In conclusion, a novel application of RT has emerged as a strategic partner in the immunotherapy of established cancer.

CL04. Chronic exposures, multiple stressors and other uncomfortable issues in radiation protection. Carmel Motsis, McMaster University, Canada

CL05. Nanotechnology in cancer therapy. Haifa Shen, The Methodist Hospital Research Institute, USA

I will present our advances in the use of multistage delivery vectors (MSV) for breast cancer therapy. Successful cancer treatment demands that drugs are delivered to the right place at the right time, without causing significant collateral damage in order to achieve a sufficient therapeutic index. Many systems are being developed as intravascular carriers to enhance tissue-specific drug delivery. However, the biological barriers inside the body pose a great challenge to achieve this goal. The multi-stage vectors were designed to accomplish the simultaneous objectives of deploying molecularly targeted therapeutic payloads, while also achieving a high degree of penetration across the multiple, sequential biological barriers which adversely affect the body distribution of all agents of therapy, drugs and nano-carriers alike. These are particle-in-particle “Russian Doll” systems, with each stage designed to provide transport across a set of sequential biological barriers, and provide associated levels of targeting specificity. We have demonstrated well-controlled and highly reproducible manufacturing techniques for the MSV, and the complete, harmless, and time-tailed degradation of their components. We have extensively studied the subcellular trafficking and transport properties of the MSV and their sub-components, demonstrating in particular their ability to deploy their payload in the cell cytoplasm, where most therapeutic agents are required for therapeutic action. We have also demonstrated the ability to decorate the first-stage of the MSV with targetting moieties that improve their biodistribution. While personalized therapy is being pursued in the medical world for cancer treatment, we have achieved personalized cancer tissue-specific drug delivery with nano-vectors. The use of chemotherapy drug-containing MSV for the treatment of triple-negative breast cancer in immunodeficient mice will be demonstrated. Systemic delivery of MSV-siRNA in animal models of breast and ovarian cancers will also be discussed.

CL06. Protein radiation chemistry: one electron oxidation of methionine in peptides and proteins. Chantal Houée Levin, Université Paris Sud, France

The one-electron oxidation of peptides and proteins is relevant to many fields of Biology. A hot point is the behaviour of the methionine residues in peptides and proteins that we are currently studying by experimental and theoretical methods. A recent problem is that of the final compound coming from OH oxidation of this residue. We tried to bring new elements by using various models and quantification method. Among other results, we were able to precise the range of the one-electron reduction potentials of Methionine in various environments.

Plenary lectures 01 - 02
PL01. Disaster of Fukushima-Daiichi nuclear power plant (Japan) by earthquake and tsunami and RI-contamination. Takeo Ohnishi, Ara Medical University School of Medicine, Japan

Tohoku-Chihou-Taïheiyo-Oki earthquake (M9.0) hit Nuclear Power Plant of Tokyo Electric Power Co. (Tepco) at Fukushima-Daiichi at 14:46 on March11th, 2011. Scram was stopped by the insertion of controllers at 14:47. Though Tepco prepared tide embankments of 5.2 meter in height, the massive tsunami more than 14 meter high attacked and stopped the electric emergency systems of diesel electric machine at 15: 39. During several days after these events, hydrogen explosions were caused by over-heat at unit 1, unit 3 & unit 4. In addition, the most serious damage was caused in a fuel nuclear storage of unit 2. Extremely large amounts of radioisotopes (RI) such as iodine-131 and caesium-137 were released to air, soil, tap water, vegetables, milk and seawater around wide areas in East Japan, especially Fukushima prefecture. On Apr. 12th, this accident was classified as the same as “Level 7” of Chernobyl accident (1986) higher than “Level 5” of Three Mile Island accident (1979). Government evacuated about 100,000 people around Fukushima-Daiichi from their cities and villages. Three contractors were installing cables in the first floor and basement of the turbine building of unit 3, and standing in water that resulted in exposures of 240.8, 226.62 and 17.55 mSv to the skin on their legs. On the end of April, Tepco reported that 8 and 11 workers at Fukushima-Daiichi were exposed for 200-150 mSv and 150-100 mSv, respectively. On June 13th, it was reported that other 8 workers were exposed to more than 250 mSv, though 250 mSv is temporarily allowed dose for radiation workers in an emergency situation by Japanese authorities. On April 27th, it was reported that two non-radiation women were exposed at 17.55 mSv and 7.49 mSv, though ICRP recommends maximum 5 mSv for 3 months for women.

Tepco and Japanese government are still working for the prevention of more disasters to Tepco and government.

PL02. On the Track to Clustered Damage and Radiation Effects. Dudley Goodhead, Medical Research Council, UK

From a variety of perspectives, ionizing radiation can be regarded as an extraordinarily effective agent at bringing about biochemical changes and effects. As a consequence ionizing radiations are widely used as therapeutic agents. But also radiation is able to cause harm even at very low doses and this limits the application of radiation in diagnostic medicine and other human activities. Fortunately the levels of background radiation on earth are very small and the physical methods available to detect radiation are highly sensitive. The reason for the exquisite biological effectiveness lies in the track structures of the radiations. The insults from ionizing radiation are always in the form of highly structured tracks and these dominate the nature of the biological damage and response at all doses, from environmental background levels all the way up to the high doses applied in radiotherapy. The nature of the tracks, and the consequent initial spectra of clustered damage that they can cause, provide strong guidance for expectations of health risks at the low doses that are of most relevance in radiation protection, but which are statistically inaccessible to epidemiological studies. The guidance extends to expectations for low-dose-rate exposures and for ionizing radiations of different qualities. But straightforward guidance must be tempered by current uncertainties of the role and mechanisms of so-called ‘non-targeted’ effects of radiation at cellular and tissue levels. It will be suggested that even these effects must be largely dependent on the radiation track structures. Current research, however, is not focused on these aspects and at present the mechanisms and implications remain largely a mystery. This is an open challenge to the research community.

S07. Advances in combined therapies: ionizing radiation, hyperthermia and chemotherapy

S07-01. Combined radio-xyz-therapy. A clinical perspective. Frederik Wenz, Department of Radiation Oncology, University Medical Center Mannheim, Germany

Any novel approach in radiation oncology has to be judged by the potential increase of the therapeutic index. Radiotherapy has a well established role in certain cancer entities and more than half of all cancer patients receive radiotherapy during the course of their disease. Acceptable tumor control and normal tissue complication rates can be achieved in many tumor sites, however, some entities can still be treated only with insufficient success or intolerable side effects. Therefore, radiotherapy is more and more applied combined with other pharmaceutical or physical therapies to enhance outcome. Examples of combined radiochemotherapy and radioimmunotherapy for solid tumors but also the combination of radiotherapy and hyperthermia and other physical approaches are discussed from a clinical perspective.

S07-02. Combined therapies in treatment of bladder cancer. Zeljko Vujaskovic, Duke University Medical Center, USA

Bladder cancer is the fourth most prevalent cancer in the USA with approximately 70, 980 new cases per year resulting in 14,330 deaths. For the majority of these patients, current standard-of-care treatments will be inadequate for prevention of more disasters to Tepco and Japanese government are still working for the depression of more disasters to Tepco and Japanese government are still working for the depression of muscle invasive bladder cancer without a substantial increase in toxicity. Rationale, current status and future directions will be discussed.

S07-03. Can targeting normal, homeostatic vasomotor function by mild hyperthermia result in improved responses to radiation or chemotherapy? Elizabeth Repasky, A. Sen, M., B. Hylander, S. Evans, J. Spenyak, A. Singh, Roswell Park Cancer Institute, USA

Traditionally, most applications of hyperthermia in the cancer clinic focus heat on the tumor with limited involvement of normal surrounding tissue. In contrast, we have been interested in applying hyperthermia to surrounding normal tissues, as well as tumors, to determine whether we can exploit differences in the way normal tissues respond to elevated temperature in comparison to that of the tumor, for therapeutic gain. We hypothesize that physiologically-mediated, thermoregulatory processes in normal vasculature can be used to effectively alter the tumor microenvironment. The goal of this presentation will be to show that systemic mild heating can be used to reduce interstitial fluid pressure within several types of tumors and that this condition can enhance the efficacy of both radiation and chemotherapy.

The possibility that elevated pressures within tumors (interstitial fluid pressure or IFP) can impede delivery of therapeutics into tumors was recognized decades ago. Moreover, high IFP can increase regional hypoxia in tumors (one of the major factors limiting effective radiation therapy) by helping to collapse or compress blood vessels. We previously reported (Y. Xu et al., JH 2007) that increasing the core body temperature of mice that had human tumors did not cause a decrease in IFP in murine tumors CT26 and B16 suggesting that these tumors can create a selective and prolonged increase in tumor vessel perfusion and increase the uptake of liposomal doxorubicin. We report here on our new and ongoing studies (Sen et al., Cancer Res 71: 2011) focused on testing the effects of mild systemic heating on interstitial fluid pressure and blood flow in different tumor models and on efficacy of radiation or chemotherapy.

We observed that increasing body temperature of mice by 2 degrees does not increase tumor vascular perfusion (measured as number of perfused vessels and blood flow by laser Doppler) but also reduces tumor IFP and hypoxia at least over a 24h time period. Further, radiation treatment of murine tumors CT26 and B16-F10 and human head and neck FaDu xenografts 24 hours after mild systemic thermal stress results in significantly enhanced tumor response as compared to a control group of tumor bearing mice. In contrast, we did not observe a decrease in IFP using a typical local hyperthermia protocol using anaesthetized mice, and heating the tumor locally at 42 °C for 40 min. Newer studies are testing various combinations of mild heating and cytotoxic drugs to determine how reduced IFP can influence efficacy of chemotherapy and this work will also be presented. Study supported by the NIH/NCI grants 1 R01 CA135568 and P01 CA94045

S08. Environmental radiobiology

Ionizing radiation represents one of many “stressors” introduced by humans in the environment. Catfish lymphoblasts B (3B11) were exposed to three types of stressors: thermal (temperature), chemical (chlorine) and radiological (60Co gamma radiation). The cells were exposed to various levels of each stressor for 24 hours before dose-response curves were obtained for each individual stressor and for a combination of the stressors. These data showed that increases in temperature (in the range of 11 to 35 °C) contribute to a reduction in cell viability, an increase in growth rate, a decrease in lysosomal membrane stability and an increase in micronuclear frequency. Chlorine concentration exceeding 15 mg/L caused a decrease in cell viability, an inhibition of cell growth and a decrease in lysosomal membrane stability. Exposure to up to 250 mGy gamma radiation (at a dose rate of 5 mGy/min), did not affect the end points studied. At 500 mGy decreases in cell viability and cell growth were noted. When all the stressors were present simultaneously (35°C, 25 mg/mL and 500 mGy, respectively), decreases in cell viability, cell growth and lysosomal membrane stability were observed. Generally the increases were higher than what was expected based on the sum of the observed effects of the single stressors. However, evidences of adaptive responses were noted in a range of doses or concentrations of the radiological and chemical stressors tested. These were observed at lower levels than levels where detrimental effects were observed but at levels that are generally more environmentally relevant. Adaptive responses were also observed to take place in vivo in organisms inhabiting radiologically contaminated environments and evidence of transmissibility of adaptive responses to other organisms via bystander signals are starting to emerge. Adaptive responses and bystander effects are cellular processes that are therefore important to understand and experimental data obtained in amphibians will be presented to illustrate their importance.

S09-02. Lessons Learned….Lessons Lost….Observations in Radiocology 25 Years after Chernobyl, Tom G. Hinton1, J. Garner-Laplace2, J. T. Smith3, S. Geraskin4, 1: IRSN, France 2: University of Portsmouth, UK, 3: Russian Institute of Agricultural Radiology and Agroecology, Obninsk, Russia

Globally, the Chernobyl accident influenced the nuclear industry, government politics, and international relationships. Debatably, the accident also redirected several sub-disciplines in science: nuclear engineering, risk analyses, accident consequence management, epidemiology, and radioecology. As radioecologists, we offer our perspective on a few lessons learned; lessons previously known—but strongly reinforced; and lessons lost by the accident. During the last decade, a scarcity of quality field data perpetuates many of the controversies surrounding Chernobyl, particularly those related to environmental effects. The radioecology that focuses on the Fukushima accident will benefit from the lessons learned (and lost) at Chernobyl, as radioecology will play a key role in answering many of the questions that are of major consequences for Japanese public health. The benefits of enhanced collaboration between radiation biologists and radioecologists are stressed.

S09-03. Chernobyl birds. Geir Rudolføen, Norwegian Radiation Protection Authority, Osterås, Norway

No abstract

S09-04. Radiation effects on earthworms. Deborah Oughton, Norwegian University of Life Sciences (UMB), As, Norway

No abstract

S09. Doses received from modern medical procedures

S09-01. Diagnostic radiology and interventional procedures. Anja Almén, Swedish Radiation Safety Authority, Sweden

The number of patients exposed to ionizing radiation from different types of diagnostic and interventional X-ray procedures is increasing in most countries. The development of new technology, e.g. for computed tomography equipment, is fast. These changes influence the radiation dose to the patients. The reason for assessing radiation dose is still very high. E.g. when assessing the effects of the exposure e.g. in the form of stochastic and non-cancer effects the dose has to be known. A rough description of absorbed dose for X-ray procedures could be given. The radiation dose distribution is uneven in the body of a patient. The dose variations between patients having the same examination are large. The range between the different examinations in different hospitals is great. The levels of radiation doses for different types of organs are varying from hundreds of mGy to several thousands of mGy. Examples of such variations will be given.

The radiation dose trend over time for x-ray examinations is not unambiguous. For some examinations it is clear that there is a potential for a reduction of the radiation dose due to e.g. new detector technology. For some types of examinations also a decrease in dose has been observed. But the equipment is used differently in different clinics and the potential for reducing radiation dose could be easily erased by the local equipment set up. Totally new applications results in both relatively low and high radiation doses.

It is also important to acknowledge that generalized descriptions of radiation dose have limited use and care should be taken when such information is used in for example epidemiologic studies. This includes the use of effective dose, a mean value for a group of patients gives limited information. The effective dose presented is also sometimes calculated with rather simple conversion factors not fully taken into account the specific technique factors used for the examination. However, new technology gives increased opportunities to study radiation dose, e.g. in the form of P2V-values. The digital collection of images now present for all types of equipment and used in many countries, makes radiation dose data digitally available for an individual patient. However, still the radiation dose to the patient’s individual organs/tissues have to be assessed. The development gives opportunities to increase the available information on radiation dose and the quality of the information.

S09-02. Nuclear medicine for diagnostics and therapy. Sören Mattsson, Medical Radiation Physics, Lund University at Malmö, Sweden

This part of the symposium will discuss methods of dose estimations for radiopharmaceuticals and levels of doses received by the patients. The basic absorbed dose calculations are done in the same way for the diagnostic and therapeutic situations. Information on the activity concentration and its time variation in different organs/tissues of the body are key parameters as input in the dose calculation programs and associated geometrical models of the body and its organs and tissues. Dose variations between individuals undergoing the same investigation or treatment using the same activity of a specific radiopharmaceutical may be considerable due to differences in body size (weight), kinetics (uptake fraction, distribution and retention), which in turn depends on age, sex, disease, and type of medication. The way of administration (i.v., p.o., inhalation) also influences the doses. Information about absorbed dose gradients within individual organs (in kidneys, in brain, etc) may also be of importance. Short-range particles like Auger-electrons may create dose gradients also at the cellular level. Depending on the need of accuracy, dose estimates are carried out in different ways and expressed using various dose quantities (absorbed dose in a point, absorbed dose distributions, mean absorbed dose to an organ or tissue or to the whole body, effective dose).

Together with judgement of the diagnostic value/image quality, a dose estimate (mainly in the form of effective doses) is an often used parameter in the optimisation of diagnostic procedures for various groups of patients. For around 85% of the nuclear medicine investigations the effective doses are in the range 0.01-10 mSv. Investigations using PET and SPECT will give effective doses in the higher end of the span and organ doses up to 100 mGy. When using dose data in connection with individual risk estimates and epidemiological studies, more detailed evaluations of organ and tissue doses are needed. The use of radiopharmaceuticals for therapy also requires a detailed and patient- and tissue-specific dosimetry for dose planning for the tumour as well as for normal tissues (bone marrow, liver, kidneys, etc.) in order to guarantee the therapeutic outcome and to minimize adverse effects in normal organs and tissues (which may receive doses close to the tolerance levels being of the order of tens of Gy).

S09-03. Doses from radiotherapy. Wojciech Bulski, Maria Skłodowska-Curie Oncology Centre, Warszawa, Poland

The doses received by patients during radiotherapy are several orders of magnitude higher than the doses in diagnostic radiology and in nuclear medicine. The X-rays and Radium radiation were used for treating patients within seventy years after their discovery. The doses delivered to patients during the first decades of radiotherapy are difficult to evaluate. At the time there were no well established dosimetry systems and radiation units

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definitions. The doses were estimated on the basis of individual patient reactions, mainly skin reactions.

Brachytherapy with radium sources was a Low Dose Rate (LDR) treatment and the doses were evaluated on the basis of the published tables for typical source arrangements according to various systems (Manchester, Paris, Quimby).

The doses in modern High Dose Rate (HDR) brachytherapy vary according to tumour location. In gynecology the doses are usually 4 fractions of 7.5 Gy, 1 fraction per week. For bronchus tumours it is 144 Gy calculated in 3 weekly fractions of 7.5 Gy, for oesophagus it is 3 weekly fractions of 6 Gy. For breast cancer it is 4 fractions of 4 Gy, or a boost of 10 Gy after external beam therapy. In prostate cancer it is usually 3 fractions of 15 Gy one fraction very three weeks. In LDR prostate cancer brachytherapy with 1-25 permanent implants the total dose is 144 Gy calculated over a period of 5 T1/2 (half-live 59.34 days). In intraoperative HDR brachytherapy of soft tissue sarcomas the doses are of the order of 20 Gy. The early X-ray generators did not allow for proper beam shaping so large volumes of healthy tissues were irradiated during the attempts to hit the cancer targets. The low energy dose distributions and poor penetration of the beams resulted in high dose irradiation of the skin in attempts to hit deeply seated targets. Therefore the doses to the targets were limited. The advent of the Co-60 units and modern linear accelerators changed the scene. The high energy machines limited the skin doses and the much narrower penumbra allowed for sparing healthy tissues.

The great breakthrough in radiotherapy was the introduction of the CT technology in patient anatomy imaging and tumour delineation. It allowed for the reduction of the healthy tissue volume to be irradiated ad dose escalation to the tumour. The further developments in radiobiology leading to new schemes in dose fractionation lead to better relations between the Tumour Control Probability (TCP) and Normal Tissue Complication Probability (NTCP).

Introduction of multileaf collimators and intensity modulated radiotherapy techniques allowed for further dose escalation and better tumour control. The doses in radical radiotherapy are presently of the order of 70 Gy and over, depending on fractionation schemes. Palliative treatments may be of the order of 20 Gy in 5 daily fractions. In special techniques the doses might be different. In the Total Body Irradiation (TBI) before bone marrow transplantation requires doses of 12 Gy delivered in 6 fractions of 2 Gy, 2 fractions per day. In radiosurgery of brain tumours the doses are of the order of 16-24 Gy in a single fraction.

The trend in modern radiotherapy is to work out the optimal fractionation schemes allowing for normal tissue sparing while escalating the doses to the tumour for better local control.

S10. Autophagy: a double-edged sword in cellular radiation response

S10-01. The unfolded protein response enables high rates of autophagy and protection against metabolic stress in tumors. Bradley G. Wouters, Ontario Cancer Institute, Toronto, Canada

No abstract


Denise Chan, University of California San Francisco, USA

Kidney cancer is a largely intractable disease, resistant to standard chemotherapy and radiation therapy. It has been estimated that 75-90% of familial and sporadic renal cell carcinomas, the most common type of kidney cancer, is due to inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene. However, we set out to exploit this characteristic feature of kidney cancer for therapeutic gain. Synthetic lethal screening is one approach for targeting specific cancer-driving mutations. Classical synthetic lethality is the genetic interaction of two mutations, in which single mutation of has no effect on viability but simultaneous mutation causes cell death. We extended this concept with a chemical synthetic lethal screen of 64,000 compounds to identify small molecules that are selectively cytotoxic to RCC cells that lack functional VHL. Through this screen, we identified a small molecule, STF-62247, which induces autophagic cell death preferentially in RCCs that have mutated VHL, whereas cells expressing wild-type VHL are relatively unaffected by this compound. STF-62247 induced the formation of large vacuoles and processing of the autophagy marker LC3 in RCCs with mutated VHL. The downregulation of the essential autophagy gene, ATG9 prevented STF-62247-induced cytotoxicity. Autophagic cell death induction was independent of HIF but rather was due to increased vesicle acidification within cells lacking VHL. This method discovered a previously unrecognized interaction between loss of VHL and autophagy.

S10-03. Radiation dose protection and mitigation by Carbamazepine (CBZ) is autophagy independent. Hyun Kim1, M. E. Bernard2, A. Farkas3, M. W. Epperly2, D. Shields3, F. Houghton2, T. M. Dixon3, D. Franchiola4, X. Zhang4, H. Wang4, S. J. Greenberger5, 1: University of Pittsburgh Cancer Institute, USA, 2: University of Pittsburgh Cancer Institute, Department of Radiation Oncology, USA

Background: Cellular upregulation of autophagy may protect against radiation damage. CBZ has been shown to be a radiation protector and mitigator in vitro and in vivo. We evaluated the mechanism by which CBZ modifies radiation damage.

Materials and Methods: Mouse hematopoietic progenitor cells (32DCl3) were incubated with either lithium chloride (1 or 10 mM) or valproic acid (1, 5 or 10 mM) for 1 hour prior to or immediately after irradiation (0 – 8 Gy), and plated in clonogenic survival assay. Radiation dose modification by CBZ was evaluated by clonogenic assay in Atg5+/+ and Atg5−/− mouse embryonic fibroblasts (MEF) deficient for autophagy protein 5 (Atg5). Wild type and Atg5−/− MEFs were incubated with 10 μm CBZ for 1 hour prior to or immediately after irradiation. Antioxidant levels were quantified 6 hours after 4 Gy irradiation by a commercial kit (Northwest Life Science Specialties).

Results: Autophagy promoting drugs CBZ, lithium and valproic acid were compared. The latter two did not protect or mitigate radiation damage in vitro. 10 μm CBZ before irradiation increased the ñ from 5.4 ± 0.9 to 11.1 ± 0.2 (p = 0.0287) in Atg5+/+ MEFs and from 4.6 ± 0.7 to 16.1 ± 2.6 (p = 0.0002) in Atg5−/+ MEFs. 10 μm CBZ after irradiation increased the ñ in Atg5+/+ and Atg5−/+ MEFs to 8.8 ± 0.2 (p = 0.0119) and 9.8 ± 1.5 (p = 0.0037), respectively. Atg5−/+ cells incubated with 10 μm CBZ demonstrated a decrease in antioxidant levels independent of irradiation (50.0% decrease after 4 Gy irradiation and 84.0% decrease without irradiation). In contrast, antioxidant levels in Atg5−/+ cells were at a lower baseline but were not significantly reduced after incubation with CBZ.

Conclusion: CBZ functions as a radiation dose modifier by an autophagy independent mechanism.

Acknowledgement: This project was supported in part by NIH T32CA21885 and NIAID Center for Medical Counter Measures (CMCM) Grant 1U19 AI68021.

S11. Radiation damage to biomolecules (II): nucleic acids and their constituents

S11-01. Charge Transfer in DNA. Tetsuro Majima, Osaka University, Japan

One-electron oxidative DNA damage induced by radiation and photoradiation has been extensively studied because it leads to the formation of oxidative lesions that causes carcinogenesis and aging. It has also received attention from a therapeutic point of view since DNA is one of the potential targets of photodynamic therapy. To either suppress or to promote photosensitized DNA damage, it is important to understand the kinetic mechanisms. With this respect, we have been working on one-electron oxidation of DNA using pulse radiolysis and laser flash photolysis as well as quantitative HPLC analyses of DNA modified by photosensitizers (Sens). Upon photoradiation of Sens, charge separation between Sens and nucleobase produces Sens radical anion and nucleobase radical cation. Since G exhibits the lowest oxidation potential among the four DNA bases, G radical cation is finally formed, of which reaction with water and molecular oxygen leads to oxidative DNA damage. Before the reaction of G radical cation takes place, the positive charge transfer (CT) occurs between nucleobase. Here we report our recent studies on the CT in DNA. The positive charge migrates along DNA mainly via a series of short-range CT processes between G-C base pairs, which have relatively high HOMO levels. As such, the CT efficiency sharply decreases with the insertion of A-T base pairs between the G-C base pairs. We have previously demonstrated that the CT efficiency through DNA can be dramatically increased by using deazaadenine (Z), an analogue of A, to adjust the HOMO levels of the A-T base pairs closer to those of the G-C base pairs. The CT efficiency was also increased by replacing A bases with dianmineaurine (D). We succeeded the rapid long-range charge transfer in DNA using Z and D in the place of A. Next, we designed a functionalized DNA system in which absorbed photon energy is converted into chemical energy to form I-1 covalent bonds, where more than 100 I-1 molecules were produced per functionalized DNA. Utilizing the fact that CT kinetics through DNA is
S11-02. Formulation of radiation-induced DNA sugar-phosphate backbone radicals via ionization and excitation pathways. Amitava Adhikary, A. Kumar, M. D. Sevilla, Oakland University, USA

Sugar radicals formed in the DNA sugar-phosphate backbone are immediate precursors of radiation-induced DNA-strand breaks and may lead to cell death, mutation, and subsequent neoplastic transformation. The very high global concentration (65 to 220 mg/ml) of macromolecules (DNA, RNA, proteins etc.) in the cell nucleus makes the role of direct-type effects of radiation in cells of crucial importance. The most likely formation of these sugar radicals is via the “direct ionization” pathway of the sugar-phosphate backbone in DNA owing to the direct-type effects. However, studies employing electron spin resonance (ESR) spectroscopy and DFT theory in our laboratory have unraveled other mechanisms of DNA-sugar radical formation via direct-type effects, viz., (i) excitation of DNA base cation radicals in monomers, oligomers, and in highly polymerized DNA, (ii) dissociative electron attachment due to low energy electrons (LEE) and (iii) direct one-electron oxidation of the phosphate group in 5'-TMP provided the phosphate group in its fully conjugated base form (P=O). Sugar radical formation by base cation radicals has been found to be influenced by various factors, e.g., wavelength of the incident light, pH of the solution (i.e., the prototropic equilibria between various states of the base cation radical), length and the sequence of the oligomer, as well as the site of phosphate substitution (3' or 5') in nucleotides. In addition to forming sugar radicals, highly oxidizing excited states of one-electron oxidized guanine are produced with 405 nm light at pH 5 and below that are able to oxidize chloride ion in the surrounding solution to form ClO• via an excited state hole transfer process. The maximum amount of ClO• has been observed to be produced with ds DNA oligomers where the one-electron oxidized guanine exists in its cation radical (G•+). Form. Thus, via excited state hole transfer, the dsDNA is apparently able to protect itself from cation radical excited states by transfer of damage to the surrounding environment.

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S11-03. How can we exploit the properties of low energy and hydrated electrons to improve radiotherapy? Darel Hunting, L. Sanche, Université de Sherbrooke, UK

Low energy electrons (LEE), with energies below 20 eV, are the most abundant of the secondary species produced by the treatment of living tissue with high energy radiation (> 10^4 MeV of absorbed radiation). Our group was the first to show that LEE induce both single and double strand breaks in DNA. In addition, we have shown that electrons with an energy of zero eV can induce single strand breaks, while for the threshold for the production of double strand breaks is approximately 5 eV. LEE are efficient at producing DNA double strand breaks and the single-hit kinetics of double strand break production indicates that a single electron can induce a double strand break. Given the low energy involved, the DNA ends at the double strand break must be essentially flush rather than staggered, which renders the break more difficult to repair and more prone to mutagenic repair. Radiotherapy is often combined with chemotherapy and we have shown that the presence of low levels of cisplatin or carboplatin adducts renders the DNA much more sensitive to the formation of double strand breaks. Again, we hypothesize that these breaks must also be flush. LEE can undergo solvation and the yield of solvated electrons induced by ionizing radiation is similar to that of the hydroxyl radical. We have found that solvated electrons readily react with cisplatin adducts in DNA resulting in the rupture of the guanine-Pt bonds and in local DNA damage. Finally, solvated electrons react with modified DNA bases, such as bromouracil, a radiosensitizer. We have shown that the presence of bromouracil in a mismatched region of double stranded DNA leads to strand breaks and DNA interstrand crosslinks. In conclusion, the unique reactivity of both low energy and solvated electrons offer opportunities for improving radiotherapy and chemotherapy. This work was supported by NSERC, CIHR and the CRS.

S12. Ethics of radiation protection

S12-01. Ethical Aspects and Culture of Radiological Protection. Christian Streffer, University Clinics Essen, Germany

Standards and rules of radiation protection are based on scientific knowledge and clinical experience about radiation effects and risks as well as on judgements of values. About 50 years ago ethical approaches have already been included into the recommendations of the International Commission on Radiological Protection (ICRP) and have been further developed during the last decades. The possible risks which are caused by exposures to ionising radiation, the complexity of the problems, the uncertainties of dose estimates as well as of radiation effects in the low dose range and judgments on late effects have shown the need to reach a broad consensus in our societies for the acceptance of ionising radiation. The acceptability has a normative notion which includes the autonomy of humans, distributive justice and other ethical values. The steady development of a culture for radiological protection is necessary. The basic principles of the ethical approaches recommended by ICRP are: justification, optimisation and dose limits. These principles are valid for all situations where ionising radiations are used in medicine, research and technologies. This is also the case for occupational exposures which occur to staff in research laboratories and medical institutions as well as to the public when releases of radioactive material occur from these installations into the environment. However, there exists one exception: no dose limits have been set for patients when ionising radiations are used in diagnostics and therapies of these patients. These exposures are for the direct benefit of the patients and the physician. So far, the physician has to justify that the patient receives more good than harm under optimised conditions.

The physician has great responsibility for his knowledge steadily brought to the newest information standards about possible radiation effects especially with individuals of variable radio-sensitivities with respect to differences between species, sexes, age groups, differences between males and females, to differences of age groups including the embryo and foetus and to differences according to genetic predisposition. Some of these problems are also valid for dose limits of staff in research laboratories and medical institutions. Distributive justice, transparency and communication of risks for autonomous decisions have to be considered in this connection.

S12-02. The ethical foundation of the radiation protection system. Jacques Lochard, CEPN 28, rue de la Redoute, France

The present system of radiological protection is based on three basic principles – justification of radiation effects, optimisation of protection and application of dose limits – combining scientific knowledge from different disciplines, a set of values rooted in ethics, moral social behaviour and standards, and the experience accumulated from the day to day practice of radiation protection professionals. Because of the remaining uncertainties concerning the risk associated to radiation at low doses this system is fundamentally promoting a prudent approach for protecting the people against the detrimental effects of radiation exposure. This is done through a continuous questioning for guiding actions: 1. Are the activities generating exposure justified? 2. Is any individual exposed to a risk considered by the society as not tolerable? 3. Are all exposures maintained as low as reasonably achievable under the prevailing circumstances? The links between the scientific knowledge, the various values and the principles driving the day to day actions are not at all straightforward. Basic scientific data concerning the levels of exposure of different groups of people and the risk attributable to these exposures must be balanced with considerations concerning the tolerability of risk according to the exposure situations, the equity in the distribution of exposures within the exposed group and the socio-economics consequences of implementing protection actions. With the complexification of the science of radiation, the growing involvement of stakeholders in the development of the principles and the multiplication of the exposure situations were radiation are controllable, there is an evident challenge for maintaining the coherence of the system and particularly its ethical foundations.

The paper will consider how the values of precaution, equity and tolerability, but also biodiversity and sustainability as far as the protection of biota is concerned, which are structuring the radiation principles, find their roots in the three major families of normative ethics i.e. consequentialism, deontological and the ethic of virtue. A particular attention will be given to the “ALARA principle” which has become progressively the corner stone of the system of protection in all domains.

S12-03. Ethics of radiological protection for nuclear power production and waste management. Behnam Taeb1, S. Gardiner2, 1: Deift University of Technology, Netherlands 2: University of Washington, USA
This paper presents an ethical reflection on the ICRP’s principles of radiological protection for nuclear power production and waste management. First, we will focus on the evolution of these principles and draw a historical parallel between various periods of commercial nuclear power deployment and the changes in radiological protection philosophies. We will then scrutinize the three ethical principles of radiological protection from the perspective of justice among contemporaries and between generations, alternatively known as intragenerational and intergenerational justice. The Justification Principle has been proposed to assure that an exposure produces sufficient benefits; the Optimization Principle attempts to reasonably reduce exposure, while maximizing net benefits; and the Dose Limit has been proposed to avoid injustice that potentially arises for (groups of) individuals (ICRP publication 103). ICRP acknowledges that in assessing the acceptability of a practice, we need a normative qualitative analysis. In this paper, we present the framework of justice for such ethical analysis. We will examine whether these principles could sufficiently guarantee a just protection of all the members of different generations. Particularly in addressing the protection of distant future generations, supplementary principles will be needed in order to assure that we do not impose undue burdens on posterity.

Tuesday
Eye openers EO07 - EO12

EO07. Animal models: the good, the bad and the useless. Jacky Williams, University of Rochester Medical Center, USA

Researchers in in vivo systems contend with many questions regarding the use of animal models: how to choose the right species or strain; does age or gender matter; which tumors to use and how do they interface with the chosen model; how does the faster development and shorter lifespan of animals extrapolate to humans; most importantly, the relevance of the model to the human condition. These questions are as true in the radiation sciences as they are in any other discipline and are critical when considering the translational applications of much of the research being performed. This talk will discuss both the benefits and downsides of using in vivo models, including both large and small species, focusing on how best to use (and extrapolate) the data generated.

EO08. Advances in biological indicators of radiation exposure. Kai Rothkamm, Health Protection Agency, UK

In biological dosimetry, the effects of ionising radiation on biological materials, i.e. cells, tissues and organisms, are used to estimate the level of exposure. Many effects increase in severity or yield with increasing radiation dose. This allows the radiation dose to be determined using a calibration curve that describes the extent of the biological effect for a range of known radiation doses. Suitable indicators of exposure can be found across a wide spectrum of biological effects, ranging from early physicochemical changes of electronic states in irradiated biological and other materials through biochemical and cellular damage to complex tissue responses. Several parameters are important in determining the usefulness of a specific endpoint as a biomarker for radiation exposure. These include dose dependence, sensitivity, specificity, signal persistence, inter-individual variation, confounding factors, invasiveness of sampling and simplicity, speed, throughput and cost of sample processing and analysis methods. Other complicating exposure-related factors that need to be considered are protracted or intermittent exposures, different radiation qualities and non-uniform or internal exposures. Currently, some of the most successful and best validated quantitative biomarkers for human exposure to ionising radiation measure the extent of chromosome or DNA damage in peripheral blood cells. Other promising approaches that have recently emerged include gene expression and micro-RNA assays, protein biomarkers as well as metabolic profiling. This lecture reviews recent developments in established biodosimetry methods as well as advances in biomarker discovery and validation and discusses their characterstics using examples of simulated or real radiation accidents as well as planned medical exposures

EO09. Radiation-induced epigenetic effects. Olga Kovalchuk, University of Lethbridge, Canada

Radiation poses a threat to the exposed individuals and their progeny. It is known to cause genome instability that is linked to carcinogenesis. Radiation-induced genome instability manifests as elevated delayed and non-targeted mutation, chromosome aberration and gene expression changes. Its occurrence has been well-documented in the directly exposed cells and organisms. Yet, the mechanisms by which it arises remain obscure. We hypothesized that epigenetic alterations play leading roles in the molecular etiology of the radiation-induced genome instability. Epigenetic changes comprise cytosine DNA methylation, histone modifications and small RNA-mediated events. We will present new and compelling evidence that epigenetic changes (DNA methylation, histone modifications and microRNAome changes) are important for the molecular etiology of radiation-induced genome instability and carcinogenesis. The new model of the radiation-induced (epi)genome instability will be introduced and discussed.

EO10. Radiation-induced cardiovascular effects. Guido Hildebrandt, University of Rostock, Germany

No abstract


Perhaps the earliest ‘radiation chemistry’ paper was published by Pierre Curie and Marie Skłodowska Curie in 1899. The history of the subject is described in articles edited by Jezry Kroh (Early Developments in Radiation Chemistry, Royal Society of Chemistry, 1989). So it is easy to trace the development of the subject for the first ninety years or so, but it is much more difficult to look forward and predict where the subject might be at the end of this or the next decade. This Conference provides an opportunity to take stock of where the subject stands today in relation to past activity, and where it might go in the future. In some European countries, activity in the area has declined markedly in the last decade or so. Is there a critical mass, below which research activity in radiation chemistry is not nationally sustainable, especially if facile access to major accelerator-based radiation sources is considered essential? Has the subject simply matured, broadened and been re-labelled? Certainly the contraction in the number of specialized radiation laboratories is compensated by an increase in breadth of applications and utilization of the data by a wider group of scientists. A consequence of the continued expansion of the European Union is broader access to research funds to facilitate trans-national collaboration and research networks, and this could be a key component of regeneration of radiation chemistry in Europe. Experimental techniques have, in the main, changed little in 40 years, apart from ultrafast techniques, and improvements to routine methodology are needed. Strengths of radiation chemistry include its solid quantitative base and kinetics expertise. Collation and evaluation of data was a major asset of the subject, work generously funded by the USA’ taxpayers for decades, but such activity has declined. There are, however, new tools which can potentially replace ones no longer available. Can 21st century approaches, among the likes of Wikipedia, be developed to replace these lost or declining resources? In conclusion, radiation chemistry continues to evolve. Arguably, the potential for broadest impact is outside the field we would label as ‘radiation chemistry’. Overall, we need a Janus-like approach in maintaining the speciality whilst enhancing its impact in mainstream science.


Radiation damage of biomolecules induced by electron scattering has recently received increased attention since it was discovered that low energy electrons can effectively induce strand breaks in plasmid DNA. It is known that low energy secondary electrons are formed as abundant species in the interaction of high energy radiation with matter. The aim of our studies is to elucidate the underlying mechanisms of electron driven damage in biological compounds. For a thorough investigation of the processes on the molecular level mass spectrometry is utilized, where electron scattering from isolated biomolecules or small biomolecular complexes is investigated under high vacuum conditions. Our studies with isolated biomolecules indicate strong fragmentation of these compounds upon electron attachment which is significantly enhanced by the sensitization with halogenated or nitroaromatic compounds. For production of small biomolecular complexes we utilize the helium nanodroplet isolation technique. Thereby single molecules are embedded in the cold helium droplets and these molecules form agglomerates once
they are cooled down. This technique allows the investigation of neutral species not studied in the gas phase before. As will be shown for a few examples like nucleobases and other biomolecules, electron induced processes are partly modified by the presence of additional molecules in the environment. For example, the ring dissociation of nucleobases driven by electron attachment in the electron energy range between about 5-10 eV is completely quenched in the droplets in favor of hydrogen loss. In conclusion, helium nanodroplets provide a good opportunity to study electron induced chemistry in biomolecules on the molecular level. Their off-ambient temperatures also allow studies of astrochemically relevant species as will be discussed.

S13. New modalities for cancer treatment

S13.01. Manipulating the tumour microenvironment in combined modality therapy. Gillies McKenna, E. Fokas, N. Qayum, J. Hong Im, C. Kelly, J. Michael Brady, R. J Muschel, Gray Institute, Oxford University, UK

We have used the Class I PI3K inhibitors PI-103, GDC-0941, BKM 120 and the dual PI3K mTOR inhibitor, BEZ 235 to study effects on the tumour microenvironment. All of these drugs in appropriately selected models reduce hypoxia in tumours. Most experiments were done using xenografts of human tumours in immunosuppressed mice, but we also confirmed the effects of several of the drugs in a spontaneous murine breast carcinoma. We examined features of the tumour vasculature after inhibition of the pathway. We used Doppler ultrasound with and without microbubbles to measure rate of blood flow in the tumours. In both xenografted and transgenic tumour models, there was a striking increased blood flow. We also saw increased perfusion. These changes were apparent as soon as 3 days after initiation of treatment. Enhanced perfusion after signalling inhibition persisted until the tumours reached the maximal electrically permitted sizes.

To understand how the vasculature might have been altered to result in increased perfusion, we undertook a series of evaluations of the tumour vasculature. There was a dramatic remodelling of the tumour vessels that resulted in longer, unbranched vessels with a greater diameter than the vessels in the untreated tumours. By mathematical modelling we could show that these vascular changes would be predicted to result in increased perfusion. In addition to structural changes, the vessels also had features of greater maturity with increased pericyte coverage. We measured doxorubicin delivery by evaluation of the microscopic distribution of fluorescence as well as by HPLC analysis to demonstrate drug levels in homogonized tumours. Either measurement showed substantial increases in drug delivery that translated into synergistic enhancement of the anti-tumour effect of doxorubicin. The decreased hypoxia would also be predicted to lead to enhanced efficacy of radiation therapy. We verified this using BKM120 and BEZ-235. Irradiation of tumours (with a single dose) after treatment with either drug led to a substantial and synergistic increased growth delay. After radiation alone, oxygenation and perfusion of the tumours was indistinguishable from control, unirradiated tumours. However, radiation during treatment with the PI3K inhibitors led to decreased hypoxia that persisted throughout the experiment. The changes in hypoxia and perfusion were durable and could be observed for at least 60 days.

S13.02. Nanoparticle targeting and radiosensitization. Tatjana Paunesku, Northwestern University, USA

Titanium dioxide based nanoparticles and nanocomposites can be used to target specific cells in the whole organisms and/or specific subcellular organelles inside cells. Nanoparticle surface conjugation modifications regulate the targeting efficiency of the resultant nanocomposites. Moreover, while some nanocomposite formulations appear to show synergistic cell killing effects with irradiation treatment, this radiosensitizing effect of nanocomposites can be increased further by conjugating or even adsorbing non-targeting small molecules to the nanoparticle surface.

S13.03. Targeting tumour metabolism to improve the outcome of radiotherapy. Ian Stratford, University of Manchester, UK

One of the most fundamental metabolic alterations during malignant transformation is the increased reliance of tumour cells on glycolysis as a major energy supply. A consequence of this is the high level of lactate that is often seen in solid tumours. Recent clinical reports indicate that increased lactate in tumours is associated with a more aggressive tumour phenotype. In addition, increased lactate concentrations in the extracellular space in experimental tumours correlates with an increase in radiation resistance. The monocarboxylate transporter mct-4, removes excess lactate from tumour cells and we have demonstrated that mct-4 is a HIF-dependent gene with increased protein expression being seen in hypoxic cells. The prognostic significance of mct-4 has been evaluated in a large series of head and neck squamous cell carcinomas (HNSCC) treated with radiotherapy. In a multivariate analysis, mct-4 expression is identified as an adverse prognostic indicator and is equivalent to stage in predicting outcome. Interestingly, in the same series of patient samples, the constitutively expressed monocarboxylate transporter, mct-1, shows no relationship to outcome.

We have used siRNA to mct-4 to knock down protein levels in a panel of HNSCC cell lines. These cells are less able to survive under hypoxic conditions and further show much greater radiosensitivity in hypoxia. There is no change in the radiosensitivity of aerobic cells. Knock down of constitutively expressed mct-1 does not change the radiation response of either aerobic or hypoxic cells. In addition, we have shown that transient knock down of mct-4, but not mct-1, potentiates the activity of cisplatin and also inhibits cell migration. HNSCC cells stably transfected to contain tet-inducible shRNA to mct-4 have been prepared and the growth of these tumours in nude mice is identical to that of untransfected cells. However, when the stably transfected cells are grown as tumours to a size of 100mm^3 and mice then treated with doxycycline to induce knockdown of mct-4, a substantial inhibition of tumour growth occurs and the tumours also show a much enhanced response to radiation. These results indicate the identification and validation of mct-4 as a potential therapeutic target in HNSCC.

S13.04. Combining PARP inhibitors with radiation therapy: rationale, strategy and potential biomarkers. Anthony Chalmers, Beatson Institute for Cancer Research & Beatson West of Scotland Cancer Centre, Scotland

The risk of normal tissue toxicity frequently prevents delivery of curative doses of radiation therapy, and many radiosensitizing agents have failed to improve outcomes because they exacerbate normal tissue effects. Novel radiosensitisers must therefore be tumour specific. Poly(ADP-ribose) polymerase (PARP) is a DNA damage sensing protein with an important role in the base excision repair pathway. Potent and specific inhibitors of PARP have been tested in patients and are extremely well tolerated. PARP inhibitors exert a modest radiosensitising effect in cellular systems but have clinical potential because this effect is observed only in replicating cells. Whereas most tumours have rapid proliferation rates, some critical normal tissues such as brain and spinal cord are non-replicating. Furthermore, work in animal tumour models has shown that PARP inhibitors increase tumour control to a greater extent than predicted by cellular data.

Many tumours exhibit defects in DNA repair and/or cell cycle checkpoint signalling that may enhance the radiosensitising effects of PARP inhibition. Vasoactive effects of these compounds also promote changes in the tumour environment that increase drug delivery and may enhance radiosensitivity. The pre-clinical data predict that adding PARP inhibitors to current radiation regimes (both with and without concomitant chemotherapy) has the potential to improve outcomes. However it is likely that certain patients will derive more benefit than others, and in many cases there is likely to be an increase in acute toxicity. It is therefore of great importance to identify tumour biomarkers that can predict whether the addition of a PARP inhibitor will be beneficial. Several clinical trials combining PARP inhibitors with radiation are in development. The challenges associated with conducting such studies will be discussed, and an update on existing studies given.

S14. Non-targeted effect-its mechanism and significance

S14.01. Introduction to non-targeted effects. Munira Kadhim, Oxford Brookes University, UK

No abstract.

S14.02. Is Radiation-Induced Non-Targeted Response Relevant to Human Health. Tom Hei, Columbia University Medical Center, Center for Radiological Research, USA

Since the unequivocal demonstration of the presence of bystander mutagenesis in mammalian cells using a charged particle microbeam, there have been many reports of the extracellular effect of ionizing radiation using a variety of endpoints in cell cultures, 3D human tissue
It has been believed that the first target of radiation carcinogenesis is DNA. However, this is not proved for radiation carcinogenesis yet. We discovered that frequency of aneuploid cell was closely related to that of radiation-induced cell transformation and natural cell transformation by high-density cultivation, but gene mutation was not. Cell with p53 gene becomes tetraploid, but does not get tumorigenicity. On the other hand, cells without p53 gene function become a triploid easily, and acquires tumorigenicity. Both radiation exposure and high-density cultivation elevated the level of intracellular oxidative radicals. These radicals induced centrosome destabilization and produced cells carrying extra centrosome, which promote merotelic attachment of chromosomes by altering spindle microtubules. Unresolved merotelic attachments give rise to lagging chromosomes at anaphase. Aneuploidy was seen in high frequency in early process of cell transformation. These results strongly suggest that a main target of carcinogenesis by low dose radiation is not DNA, but is centrosome, which are the proteins to constitute chromosomal homeostasis maintenance mechanism. In addition, this route may be the same as that of natural carcinogenesis.

S14-04. Modular Systems Biology and Low Dose Radiation Responses. Francis A. Cucinotta¹, Y. Li², C. Carra³, M. Wang⁴, 1: NASA Johnson Space Center, Houston TX, USA, 2: U.S.A.R.A. Division of Life Sciences, Houston TX, USA

Systems radiation biology aims to describe tissue and cellular responses in terms of underlying molecular interactions and signaling pathways. Modular systems biology (MSB) describes the complexity of biological systems using well defined modules that represent distinct biological response pathways or sub-systems within pathways. We review methodical concepts from control theory that can be used to identify and construct well defined modules for describing complex biological processes and radiation responses. The DNA damage response and TGFbeta/Smad signaling are two important response pathways following radiation exposure that lead to a variety of cellular and tissues outcomes including apoptosis, differentiation, and the epithelial-to-mesenchymal transition (EMT). We apply the MSB approach to develop a computational description of the ATM and TGFbeta/Smad signaling pathways. Applications to low dose and dose-rate exposure to low LET radiation and space radiation are considered. Stability analysis of modular pathways and the identification of pathway signals to cellular and tissue phenotypes such as EMT will be discussed.

S14-05. Circulatory Disease from Exposure to Low-Level Ionizing Radiation and Estimates of Potential Population Risks: a Meta-Analysis of Epidemiological Studies. Mark P Little¹, T. Azzioz², D. Bazyka³, S. Bouffler⁴, E. Caridis⁵, S. Chekin⁶, V. Chumak⁷, F. Cucinotta⁸, F. de Vathaire⁹, P. Hall⁴, J. Harrison¹⁰, G. Hildebrandt¹, V. Ivanov¹, V. Khashchev¹², S. Kljusenko¹³, M. Kreuzer¹⁴, K. Orzasa¹⁵, T. Schneider¹⁶, S. Tenorio¹⁷, A. M. Thomsen¹⁸, I. Tzoulou¹⁹, R. G. Toland⁰⁰, J. R. Vainio¹, W. Vandoolaeghe¹⁰¹, R. W. Zhang¹⁰², L. Zheng¹⁰³, S. Lipshultz¹⁰⁴, 1: Radiation Epidemiology Branch, National Cancer Institute, USA, 2: Southern Urals Biophysics Institute, Chelyabinsk Region, Russia, 3: Research Center for Radiation Medicine, Kyiv, Ukraine, 4: Health Protection Agency, Centre for Radiation, Chemical and Environmental Hazards, UK, 5: Center for Research in Environmental Epidemiology (CREAL), Barcelona, Spain, 6: Medical Radiological Research Center of Russian Academy of Medical Sciences, Obninsk, Russia, 7: NASA Johnson Space Center, Houston, Texas 77058, USA, 8: Radiation Epidemiology Group, Institut Gustave Roussy, Villejuif Cedex, France, 9: Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden, 10: Department of Radiotherapy and Radiation Oncology, University of Leipzig, Germany, 11: Federal Office for Radiation Protection, Oberschleissheim, Germany, 12: Department of Epidemiology, Radiation Effects Research Foundation, Hiroshima City, Japan, 13: CEPN (Nuclear Evaluation Protection Center), Fontenay-les-Roses, France, 14: Helmholtz Zentrum München, Oberschleissheim, Germany, 15: UCL Institute of Cardiovascular Sciences & Great Ormond Street Hospital for Children, London, UK, 16: Department of Epidemiology and Biostatistics, School of Public Health, London, UK, 17: Dalton Nuclear Institute, University of Manchester, UK, 18: Department of Pediatrics, Leonard M. Miller School of Medicine, University of Miami, USA.

Based on observations in irradiated populations, the health risks of low-level exposure to ionizing radiation have been related primarily to cancer. At high radiation doses a variety of other well-established effects are observed, in particular to the cardiovascular system. The universality of the target theory of radiation carcinogenesis is DNA. However, this is not proved for radiation carcinogenesis yet. We studied the mechanisms and consequences of non-targeted effects such as bystander effects and genomic instability in bystander cells in various situations, the health risks of low levels of radiation are considered. The observations that heritable DNA alterations can be propagated to cells many generations after radiation exposure and that bystander cells exhibit genomic instability in ways similar to directly hit cells indicate that the low dose radiation response is a complex interplay of various modulating factors. In animal studies, there is evidence of out of field irradiation resulted in mutation and induction of COX-2 in both lung and mammary tissues. The potential implication of the non-targeted response in radiation induced secondary cancer will be discussed. A better understanding of the mechanism of the non-targeted effects will be invaluable to the clinical relevance of the bystander effects and ways in which the bystander phenomenon can be manipulated to increase therapeutic gain in radiotherapy.

S14-06. Highlights of the NOTE program 2006-2010. Sikso Salomaa¹, STUK: Radiation and Nuclear Safety Authority, Helsinki, Finland

The universality of the target theory of radiation-induced effects is challenged by observations of non-targeted effects such as bystander effects and genomic instability. Essential features of non-targeted effects are that they do not require direct nuclear exposure by radiation and they are particularly significant at low doses. The European NOTE project studied the mechanisms and consequences of non-targeted effects and discussed the need for a new paradigm in radiation biology. The new paradigm should cover both the classical (targeted) and the non-targeted effects. Better understanding of non-targeted effects may have important consequences for risk assessment and for consideration in radiation protection. Based on the research carried out in NOTE and other recent studies, a new paradigm is proposed that covers both the classical radiation effects that are explained by the DNA targeted effects as well as non-targeted effects that are explained by cellular signaling, epigenetic changes, and damage induced due to radiation exposure as well as the effect of tissue micro environment. This new paradigm would better describe radiation carcinogenesis. It would also facilitate the solution of the
S14-07. Expression of genes Involved in a Radiation-Induced Bystander Effect. Hayley Furlong,1 Carmel Mothersill,2 Orla Howe3; 1: Radiation and Environmental Science Centre, Focas Research Institute, Dublin Institute of Technology, Kevin St, Dublin 8; 2: Medical Physics and Applied Radiation Sciences, National Research Building, 1280 Hamilton, Ontario L8S 4K1

Introduction: The radiation induced bystander effect is relevant to carcinogenesis, it may have significant implications for risk estimation for radiation exposure. Currently the mechanisms and cellular events are the subject of intense investigation, because little is known. It is thought that the radiation induced bystander response is due to a bystander factor secreted in the medium post irradiation. However the biological nature of this factor is currently unknown, but it is thought to be a protein of some sort that may be involved in the apoptosis cascade.

Materials and Methods: HaCaT epithelial cells were used in this study and exposed to 0, 0.5 and 0.05 Gy low doses of ionising radiation (IR). The medium was harvested from the cultures post-IR and placed on recipient HaCaT cells for different time points. RNA was extracted from the cells using the TRIReagent protocol and stored for subsequent gene expression studies. Specific genes were selected for this study, in particular those involved in the apoptotic cascade. Primer design and primer optimisation methods were carried out to establish definite primer temperatures for the subsequent gene expression studies, using Real-Time PCR.

Results: Preliminary gene expression data will be shown along with primer optimisation of the specific apoptosis genes under investigation in this study. Currently a paper is in progress detailing this novel data.

Discussion: This data could contribute to the discovery of the molecular mechanisms involved in the production of a radiation induced bystander effect and will therefore have potential clinical relevance for patients undergoing radiotherapy treatment.

S15. The use of archiving data and biological material - examples and strategies

S15-01. The STORE data warehouse: an international infrastructure for data sharing in radiobiology. Paul Schofield,1 M. J Atkinson,2 M. Birchwiks3, S. Tapi3, M. Gruenberger,1 B. Grosche1; 1: University of Cambridge, UK 2: Helmholtz Centre Munich, German Research Centre for Environmental Health, Institute of Radiation Biology (IBB) 3: Federal Office for Radiation Protection, Germany

With the increasing globalisation of the biological sciences, and the move towards large complex datasets, the need to share primary data between investigators gains growing urgency. Such sharing of data not only permits its reuse and reanalysis in the light of our developing understanding, but also supports scientific accountability for published research [1–4]. The economic and ethical arguments for sharing data and other science “commons” ie those resources held in common by the community are very strong [5] and have been recognised and agreed by international funding agencies, such as the US NIH, NSF, the Wellcome Trust and the UK research councils, and policy bodies such as the OECD [6]. Many funders now insist that investigators deposit primary data into public databases and to make biological materials and genetically modified organisms available to the academic community with minimal restrictions.

The European Commission has recognised that the radiobiology community needs an infrastructure through which to deposit and share data. We have previously developed the ERA radiobiology database, a legacy database for historical international large-scale radiation exposure experiments which would otherwise have been lost [7, 8]. There is a parallel urgency to provide a repository from new primary datasets and to provide a virtual archive or directory of material resources which investigators wish to share. The response to that need is provided in the STORE data warehouse http://www.storedb.org/.

STORE provides a user-oriented database for the storage of any data type associated with a study, from text files to magnetic resonance datasets and zoomable images. It is also designed to contain links to datasets in other public databases through DBXrefs, URLs and accession numbers. Each dataset is managed by the investigator and tagged with appropriate terms to allow easy search and retrieval, but access and security can also be limited by the investigator – data can be made universally accessible or restricted to password holders. By permitting database cross-references users may provide pointers to physical archives of cells, tissues, slides etc, which may then be accessed directly through database links and web services or through emailing a specified contact.

The flexibility and agility of STORE allows it to act as a sharing and archival infrastructure for the whole radiobiology community. Access is free and available to any group worldwide and help can be provided online or through arranging a visit form the STORE team.

We hope that STORE will provide for the first time an international infrastructure for radiobiology which will encourage cooperation and increase the value of funded research around the world.

S15-04. The Use of Unique Archived Data and Biological Samples By the Radiation Effects Research Foundation, Evan Double, S. Fujiwara, Y. Kusunoki, E. Grant, Y. Kodama, N. Takashahi, H. Katayama, A. Suyama, R. Shore, Radiation Effects Research Foundation, Hiroshima and Nagasaki, Japan

This presentation reviews the archived data bases and biological samples that have been used by the Atomic Bomb Casualty Commission and its successor, the Radiation Effects Research Foundation (RERF), as a unique resource to study long-term health effects in the survivors of the atomic bombings of Hiroshima and Nagasaki and in their children. Follow-up for mortality and cancer incidence of a fixed sample of about 120,000 A-bomb survivors and control subjects (Life Span Study) was instituted in 1950, and a subset (Adult Health Study) of about 15,000 received additional morbidty surveillance based on biennial health exams begun in 1958. Questionnaires during the exams obtain information on socioeconomic, lifestyle, and other factors that may confound or modify radiation effects. The longitudinal morbidity and laboratory data are complementary for the assessment of non-cancer diseases and conditions. In addition, samples collected during the biennial exams provide stored serum (14,700 persons), urine (4,300 persons), blood cells (7,200 persons), and lymphocytes; the latter includes uncultured mononuclear cells and immortalized lymphoblastoid cell lines from 3,500 persons and stored cryogenically in liquid nitrogen. Chromosome slides are available for 20,000 persons and teeth from about 1,000 persons for electron-spin resonance radiation biodosimetry. An early autopsy program provided paraffin-embedded tissues from about 6,600 persons. In utero-exposed persons and controls (In Utero Cohort) began biennial clinical exams in 1978 (about 1,600 persons). Children of exposed and non-exposed parents who were conceived after the bombs (F2 Cohort) began clinical studies in 2002 (about 12,000 persons). For transgenerational studies, immortalized lymphoblastoid cells are cryogenically stored for 1,500 F1, from 1,000 ‘father–mother–child trios’, including about 300 F1, born to at least one parent exposed to doses greater than 1 Gy. The biosamples are a valuable resource for biochemical and mechanistic laboratory studies, making possible a wide range of studies. Examples of the use of the unique data bases and biosamples are presented along with strategies as RERF scientists attempt to elucidate radiation-associated disease mechanisms using new technologies and collaborative expertise.

S15-05. Qualitative and quantitative proteomic analysis using formalin-fixed paraffin-embedded (FFPE) tissue. Soile Tapió, O. Azimzadeh. Institute of Radiation Biology, Helmholtz Zentrum München, Germany

Radiobiological tissue archives are a valuable source for retrospective protein biomarker discovery. However, during the formalin-fixation process proteins undergo degradation and cross-linking, making conventional protein analysis challenging. We have systematically validated various extraction and separation methods of FFPE proteins using different gel-free and gel-based approaches. As a model system we have used both archival and fresh/frozen cardiac tissue of sham- and irradiated C57BL/6 mice. We show that 1D SDS-PAGE followed by Liquid Chromatography–Electrospray Tandem Mass Spectrometry (LC-ESI MS/MS) is the preferred approach for qualitative proteomic analysis (1). A number of peptides were identified in regions of the 1DE not corresponding to the expected molecular weight, indicating the presence of protein-protein complexes due to cross-linking, and protein fragmentation due to prolonged sample storage. For quantitative proteome analysis we used label-free approach (2) to detect putative molecular biomarkers of ionising radiation. The proteomics data were confirmed by complementary analysis including bioinformatics and immunoblotting. This study will facilitate the development of proteomic analysis of FFPE tissue and provide a tool for the validation in clinical archival samples of biomarkers of exposure, prognosis and disease.


S16. Induction of secondary cancers by ionizing radiation

S16-01. Understanding and Potentially Reducing Second Cancers after Radiotherapy. David Brenner,1 I. Shuryak,2 R. Sachs,2 1: Columbia University Medical Center, USA 2: University of California, Berkeley, USA

As radiotherapy patients are being treated at younger ages and are living longer, there is increasing concern about their second cancer risks. Devising coherent strategies for minimizing such cancer risks requires understanding second cancer risks at a minimum as a function of site, dose and age.

Radiotherapy acts both as an initiator of cancer, inducing pre-malignant stem cells in stem-cell niches, but also as a promoter of pre-malignant damage, by increasing the mean number of pre-existing pre-malignant stem cells per niche, which in turn is subject to post-exposure homeostatic regulation. Mechanistic models describing these processes yield second cancer predictions consistent with available epidemiological dose-dependent cancer risk models, but also enable us to understand lifetime radiation risks as a function of age at exposure. Understanding second cancer risks allows us to consider potential new strategies for second-cancer risk reduction. As an example, we consider second breast cancers after standard post-lumpectomy adjuvant whole-breast radiotherapy:

In the contralateral breast, the enhanced cancer susceptibility of breast-cancer patients dominates, with second-cancer risks (up to 15% in long-term survivors) that are genetically-independent of the primary. However in the irradiated ipsilateral breast, as well as improving primary tumour control, standard whole-breast radiotherapy largely eliminates the large genetically-independent background cancer risk, probably by killing the existing pre-malignant cells in that breast – and replaces that large background cancer risk with a smaller radiation-induced cancer risk. Thus as well as improving primary tumour control, whole-breast radiotherapy to the ipsilateral breast radiotherapy markedly reduces the independent second-cancer risk.

It follows that standard radiotherapy might logically be accompanied by concomitant lower-dose prophylactic mammary irradiation (PMI) to the contralateral breast. Because there are comparatively few background pre-malignant cells in the breast, it appears possible to kill essentially all of them with a comparatively low radiation dose (e.g., 20 Gy in 10 fractions). Thus PMI could markedly reduce the significant cancer risk in the contralateral breast, without inducing significant complications.

S16-02. The delayed genetic effects of radiotherapy – what we know and what we do not. Yuri Dubrova, Department of Genetics, University of Leicester, UK

In recent decades, improvements in treatment have produced dramatic increases in survival rates amongst cancer patients. However, along with surgery, the mainstays of cancer treatment are radiotherapy and chemotherapy, both of which are potentially genotoxic and mutagenic. This can lead to the development of secondary, treatment-related tumours. Despite the significant progress made in this area, a number of pertinent questions, particularly regarding the long-term effects of radiotherapy, still remain unanswered. For example, according to the results of recent studies the mutagenic effects of ionising radiation are not restricted to the directly exposed cells and can manifest either in the non-irradiated descendants of exposed cells (radiotherapy-induced genomic instability) or in the non-exposed neighbouring cells (bystander effect). The contribution of these delayed effects to therapy-related secondary malignancy will be discussed.

different ways over varying time periods. The cellular responses to radiation may receive. So mixtures may provoke different responses if given in different ways; and in combination it may then interact with target cells/organs in yet further and different ways. Radiation and the chemical stressor may be regarded as either a mixture with each retaining its unique properties, or a compound with new properties of its own. These may have markedly different biological effects. If the two stressors are administered at different times, the cellular responses to a SMN will be modified by the first stressor it receives. So mixtures may provoke different responses if given in different ways over varying time periods. The cellular responses to compound stressors may be equally unpredictable. Legislation needs predictability and certainty. Low dose effects are unpredictable and uncertain. The precautionary principle would suggest that if an effect cannot be predicted, then perhaps it should be legislated against, (Although it could be beneficial). Some kind of acceptable damage endpoint is needed that can be extrapolated through all life forms, environments and life stages. This presentation will discuss some of the issues and suggest approaches to develop a framework for research and regulation in this complex area.

S17-02. Developmental co-exposure to low doses of ionizing radiation and environmental toxicants during a critical period of brain development exacerbates cognitive effects. Melanie Maier1, S. Taposı2, A. Stampfı2, M. Atkinson1, 1: Institute of Radiation Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany 2: Institute of Toxicology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

Introduction: Engineered Nanoparticles (ENP) are used in increasing quantities for industrial products, foodstuffs and pharmaceuticals. In our previous studies on the effects of ENP using isolated Langendorff hearts of guinea pigs we detected an increase in heart rate and occurrence of arrhythmia. We adapted the isolated heart allowing us to dissociate direct effects of the heart from systemic inflammatory effects. The novel Langendorff portable system enables simultaneous irradiation and treatment with ENP of isolated hearts.

Method: Hearts were excised immediately and mounted in the transportable Langendorff equipment. They were perfused retrograde with oxygenated Krebs-Henseleit-Buffer that was recirculated afterwards. Hearts were irradiated with a gamma dose of 0.5 Gy and 3 Gy (Cs-137). After irradiation the particle solution was added and circulated for the next four hours. By recording the ECG of Langendorff hearts, alterations in heart rate and ECG form could easily be discovered. Guinea pig as an experimental animal model was chosen as its ECG form shares similar features with the human one.

S17-03. Portable Langendorff system for determination of effects of nanoparticles after radiation. Melanie Maier1, S. Taposı2, A. Stampfı2, M. Atkinson1, 1: Institute of Radiation Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany 2: Institute of Toxicology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

We are indirectly or directly exposed to persistent environmental toxicants as well as to ionising radiation (IR). Neurological disorders and diseases are rarely unidimensional or multifactorial and it is therefore important to integrate different environmental agents, multiple stressors, which might act synergistically to exacerbate developmental toxic effects. Several epidemiological studies indicate that exposure to environmental agents, including IR, during early human development can have deleterious effects on cognitive development in childhood. By using the mouse as an animal model we can study the effect of a single toxic agent administered directly to the neonatal animal during different stages of the rapid brain growth (BG). This animal model allows us to isolate the effects of certain environmental agents, combination of environmental stressors and to specify certain issues that can be difficult to solve in traditional developmental toxicity tests as well as in epidemiological studies. These effects are induced when the exposure occurs during a critical phase of the developmental brain development. Of special concern is that the effects after

S17-01. Multiple Stressors – Issues to Ponder. Colin Seymour, McMaster University, Canada

Radiation may interact with chemical stressors in a number of different ways; and in combination it may then interact with target cells/organs in yet further and different ways. Radiation and the chemical stressor may be regarded as either a mixture with each retaining it’s unique properties, or a compound with new properties of it’s own. These may have markedly different biological effects. If the two stressors are administered at different times, the cellular responses to a SMN will be modified by the first stressor it receives. So mixtures may provoke different responses if given in different ways over varying time periods. The cellular responses to compound stressors may be equally unpredictable. Legislation needs predictability and certainty. Low dose effects are unpredictable and uncertain. The precautionary principle would suggest that if an effect cannot be predicted, then perhaps it should be legislated against, (Although it could be beneficial). Some kind of acceptable damage endpoint is needed that can be extrapolated through all life forms, environments and life stages. This presentation will discuss some of the issues and suggest approaches to develop a framework for research and regulation in this complex area.

Grants from Programme Hospitalier de Recherche Clinique (N’AOM 06 158), la Ligue Nationale Contre le Cancer and INCA and the continued financial support from EDF is very gratefully acknowledged.


Radiotherapy is one of the major treatment modalities for cancers, about half of the patients receiving radiation alone or in combination with other therapeutic modalities. At the same time, radiation is a carcinogenic agent and therefore the issue of the induction of secondary cancers in long term survivors from radiotherapy becomes increasingly important. In this respect there has been suggested that risk predictions could be used as complementary criteria for the selection of plans in addition to the estimation of the possible deterministic effects. Most of the data on radiation carcinogenesis risk come from radiation protection studies and therefore attempts to include risk estimations into treatment planning must take into consideration the specific features of radiation treatment, such as dose levels, dose heterogeneity and fractionation. Of particular importance is the model that describes the dose response of individual patients as well as populations of patients as may be encountered in clinical studies. This presentation explores several methods for estimating the risk of cancer following radiotherapy and investigates the influences of the particular features of radiotherapy. It also deals with the confounding factors that may be reflected in the results and may therefore hamper the interpretation of epidemiological data. In particular, it reviews the importance of dose heterogeneity and the competition between cell killing and the induction of carcinogenic mutations in predicting risk for secondary cancer, and therefore the importance of dose volume histograms for risk assessments in radiotherapy. The role of cell survival, inducible repair and heterogeneity of the patient response were also taken into consideration for explaining the dose response curves observed experimentally. The findings stress the importance of taking into account the details of the clinical delivery of dose in radiotherapy for treatment plan evaluation or for retrospective analyses of the induction of secondary cancers as this would provide more reliable parameters for future risk modelling.

S17. Multiple stressors (covers humans and environmental studies)
S17-04. Epidemiology of uranium workers in France. Radiological and not-radiological exposure and its effects. Irina Guseva Canu, Institut de Radioprotection et Sûreté Nucléaire, France

Epidemiological studies have reported direct evidence of adverse health effects from protracted occupational exposure to low doses of external radiation. However, the effects of internal radiation exposure among workers are less clear.

A pilot study of French nuclear workers with potential for protracted internal exposure to low doses of uranium was launched in 2005 at the AREVA NC Pierrelatte (1960-2006) plant, one of the main uranium processing plants in France. This study aimed at assessing the feasibility of analyzing the risk of cancer and non-cancer mortality associated to occupational exposure to uranium by developing a method of exposure assessment. Exposure to uranium and other chemical pollutants handled at the plant was assessed through the plant-specific Job Exposure Matrix elaborated on the basis of expertise and individual occupational data. This study yielded original results on cancer and non-cancer mortality related to protracted low-dose uranium exposure, which suggest that effects on mortality differ by type and solubility of uranium compounds. These results should be verified on larger workers population, such as the Tracy-U cohort. This cohort is currently in construction, expecting to gather occupational biomedical and dosimetric data from about 10000 French nuclear workers.

A short summary will be given of the cohort extension to other uranium exposure assessment. Exposure to uranium and other radionuclides of low energy electrons (photo electrons and Auger electrons), similar to the absorbed dose not take into account the track structure of the ionising particle or the biological target structure, the frequency of the distribution of the ionisation cluster size in the target is likely to be an important determinant of the biological effectiveness of the radiation. Here we compare the ionisation cluster distribution of X-ray, proton and alpha beam using Monte Carlo simulations. Simulations were performed in a liquid water phantom and in the ionisation cluster distribution as a function of the distance from the beam was determined at different points along the beam trajectory. X-rays generate an abundance of low energy electrons (photo electrons and Auger electrons), similar to that associated with MeV protons or alphas, leading to an increase of the relative biological effectiveness above 1. We have obtained a theoretical comparative analysis of the ionisation cluster distributions for each particle type, with the aim of better understanding the underlying mechanism of experimental treatment plans, such as microbeam radiation therapy and targeted alpha therapy.

S18. Radiation-produced intermediates - basic problems

S18-01. DNA damage induced by fast-flowing, metastable species in a cold plasma. Sylwia Prasinska1, A. Styczyńska1, B. Bahnev2, N. J. Mason2. 1: University of Notre Dame, USA 2: The Open University, USA

Cold plasmas are increasingly being suggested as a tool for wound treatment, cosmetic surgery and dentistry areas. As part of our wider programme to study radiation damage of biomolecules we have used such a cold plasma to explore its effect on DNA molecules [1,2]. In these experiments highly purified plasmid DNA was exposed to the plasma and DNA strand breaks were determined by gel electrophoresis. Rapid degradation of supercoiled DNA is observed, up to 60% within the first ten seconds of plasma treatment, followed by a period when there was a slower accumulation of damage. The complexity of plasma-generated species, i.e. excited atoms, charged particles, electrons and UV light gives a variety of possible pathways by which DNA can be damaged. Therefore the aim of this study is to understand the interaction of particular components of the plasma with DNA.

In order to estimate the effect of plasma species on plasmid DNA, optical and electrostatic filters were placed in front of the sample. The physical and chemical properties of the plasma jet were characterized by means of optical emission (OE) spectroscopy. The obtained OE spectra have shown the presence of oxygen to uranium by developing alternative methods for the plasma jet in order to excited atoms and molecules and radicals many of which fluoresce in the UV.

We have estimated that most of the damage to DNA during plasma irradiation is due to the interaction of excited or/and reactive species, e.g. O2, He+, O and OH. The damage due to other components present in the plasma such as charged particles is restricted as a result of rapid electron recombination.

References:


The lethal damage to cells by ionising radiation is thought to be initiated by single and double strand breaks in the DNA molecule. In particular, the clustering of ionisation events in the DNA molecule appear to correlate with the formation of such strand breaks and investigating the cluster distribution within DNA segments could be highly beneficial to radiation therapy treatment planning. Since quantities, such as the absorbed dose do not take into account the track structure of the ionising particle or the biological target structure, the frequency of the distribution of the ionisation cluster size in the target is likely to be an important determinant of the biological effectiveness of the radiation. Here we compare the ionisation cluster distribution of X-ray, proton and alpha beam pencil beams at the nanometric scale, by means of Monte Carlo simulations using the simulation toolkit Geant4. The Geant4 Very Low Energy extension models were used to model the track structure determined by the low energy secondary delta electrons. Simulations were performed in a liquid water phantom and the ionisation cluster distribution as a function of the distance from the beam was determined at different points along the beam trajectory. X-rays generate an abundance of low energy electrons (photo electrons and Auger electrons), similar to that associated with MeV protons or alphas, leading to an increase of the relative biological effectiveness above 1. We have obtained a theoretical comparative analysis of the ionisation cluster distributions for each particle type, with the aim of better understanding the underlying mechanism of experimental treatment plans, such as microbeam radiation therapy and targeted alpha therapy.

S18-03. Reaction of Carotenoids with Free Radicals and Singlet Oxygen. Ruth Edge1, F. Boehm2, F. George Ruscott1. 1: The University of Manchester, UK 2: Charité-Universitätsmedizin Berlin, Germany 3: Keele University, UK

Carotenoids are responsible for the colouration of many fruits, vegetables, birds, flowers and animals. For example, beta-carotene in carrots, lycopene in tomatoes, canthaxanthin in flamingos and astaxanthin in salmon. They arise both in the reaction centre and antenna complexes of photosynthetic systems and have wide-scale commercial use as food colourants. In medicine, carotenoids have been shown to be useful in treating erythropoietic protoporphyria, age-related macular degeneration and cataracts. Epidemiology has shown that a high carotenoid intake may be able to protect against coronary heart disease and several forms of cancer. However, some clinical trials report that supplementation in the consumption of beta-carotene may lead to deleterious effects in certain sub-populations such as heavy smokers. Linked to such observation, the pro-oxidant as well as anti-oxidant roles of the carotenoids are now much discussed.

Since the quenching of free radicals and singlet oxygen by carotenoids is believed to contribute to their anti-oxidant properties and their ability to inhibit the onset of diseases, as well as their pro-oxidant properties, we have studied a wide range of carotenoid-reactive oxygen species reactions using the pump-probe techniques of pulse radiolysis and laser flash photolysis. Carotenoids are shown to be efficient singlet oxygen quenchers with lycopene exhibiting the most efficient quenching in organic solvents. However, in membrane environments there is little or no difference in the quenching efficiency between the dietary carotenoids, and their aggregation reduces the quenching efficiency.

Free radical interactions with carotenoids leads to at least three processes, electron and hydrogen atom transfer and adduct formation. The most studied is electron transfer where the carotenoid loses an electron to become a radical cation. The reactivity of such carotenoid radicals with other biomolecules has also been studied, showing how a switch from anti- to pro-oxidant behaviour may occur. These reactions are related to...
the carotenoid redox potentials with lycopene’s being the lowest allowing it to reduce/repair other carotenoid radical cations and be ‘sacrificed’ where mixtures of carotenoids are present in oxidative environments.

S18-04. Scavengers as a mitigating strategy against radiation damage in macromolecular crystalllography. Ian Carmichael, E. Garman*, 1; Notre Dame Radiation Laborotary, USA 2; University of Oxford, UK

The rate of radiation damage to macromolecular crystals (of e.g., proteins) at room temperature has previously been shown to be reduced by the use of certain radical scavengers [1]. Presumably these scavengers can intercept damage agents formed as a result of energy deposition in the media surrounding the protein. Indeed an inverse dose rate effect has been observed at room temperature in the absence of scavengers, lending support to this idea [2]. Some limited and conflicting information on the effectiveness of scavengers has been reported at cryotemperatures (typically 100 K) where most synchrotron MX beamlines currently operate.

Here the effects of sodium nitrate, an electron scavenger, are investigated at 100 K. For sodium nitrate at a concentration of 0.5 M in chicken egg white lysozyme crystals, the dose tolerance is increased by a factor of 2 as judged from the global damage parameters, and no specific structural damage to the disulfide bonds is seen until the dose is greatly in excess (>factor of 5) of the value at which damage appears in electron density maps derived from a scavenger-free crystal. In the electron diffraction maps of the electron density maps, ordered nitrate ions adjacent to the disulfide bond are seen to sequentially lose oxygen atoms, and appear to protect the disulfide bonds. The mechanisms of action of this scavenger in the crystalline environment have been postulated [3] and will be presented, and some further possibilities suggested by these results will be explored.


Conference lectures CL07 - CL12

CL07. ATM-53BP1 pathway in response to DNA double strand breaks and cancer development. Thanos Halazonetis, M. Kocylowski, A. Dereh-Oz, University of Geneva, Switzerland

We recently proposed a model for cancer development that tries to explain the presence of genomic instability and p53 mutations in human cancers. The key feature of the model is that oncogenes induce DNA replication stress, as revealed by the presence of DNA damage and genomic instability suggested by these lesions. We further test our model, we reviewed data from two recent studies that have catalogued the presence of a specific class of genomic aberrations, large deletions and insertions, in a few thousand human cancers. Analysis of the data reveals that most of the prevalent recurrent focal deletions target common fragile sites and large genes. We (and others) further showed that in various experimental systems, deletions in common fragile sites and large genes are due to the presence of DNA replication stress. Thus, taken together, these results suggest the presence of DNA replication stress in human cancers, consistent with the recently proposed oncogene-induced DNA damage model for cancer development. Interestingly, our model is also relevant in the context of induced pluripotent stem cells, whose reprogramming is dependent on oncogene expression.

The ATM-53BP1 pathway is central to the response of mammalian cells to DNA DSBs and is activated in human cancers. We have shown that recognition of DNA DSBs by 53BP1 involves binding of the Tudor domain of 53BP1 to methylated lysine residues in histones. Others have also shown that ubiquitination of histones is also important for 53BP1 recruitment to sites of DNA DSBs. We now show that localized expression of ubiquitin ligases in cell nuclei leads to recruitment of 53BP1 to chromatin bypassing factors that are otherwise necessary for 53BP1 focus formation in response to irradiation, such as histone H2AX and MDC1.

CL08. Inflammatory Responses to Radiation. William McBride, University of California, Los Angeles, USA

The link between radiation and inflammation lies in that they both generate reactive oxygen/nitrogen (ROS/RNS) species that generate pro-inflammatory states. This over-accumulation is made more complex by the fact that many forms of inflammation exist that change temporally and depend upon the tissue type and microenvironment, but many of the features of the radiation tissue damage response, including cell death, proliferation, infiltration, angiogenesis and wound healing are dictated by the inflammatory component within the lesion. Molecules released from damaged cells (DAMPS) play an important role in dictating the responses made, as do the levels of specific cytokines that orchestrate responses. Over time, radiation-induced tissue damage responses may recur especially after doses that approach tolerance, with associated inflammation. In a sense, late radiation effects can therefore be viewed as chronic inflammatory states. A corollary of the involvement of ROS/RNS is that anti-inflammatory programs are linked to anti-inflammatory cytokine involvement that dampen and attempt to control overexuberant responses.

This lecture will attempt to present the interplay of factors that are critical to radiation-induced inflammation, its role in normal tissue and tumor responses, and discuss how these may be targeted for therapeutic improvement.

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CL09. Mobile phones and health risk. Elisabeth Cardis, CREAL, Barcelona, Spain

No abstract

CL10. Hypoxia and its role in radiotherapy of tumors. Albert van der Kogel, Radboud University Nijmegen Med Ctr, Netherlands

The oxygenation status is one of the main factors that are known to modify the radiation sensitivity of cells and tissues. The first research to firmly establish the role of molecular oxygen in the fixation of free radical damage can be attributed to Harold Gray and coworkers in the 1950’s, and as of today the concept of hypoxic cells being radioresistant has become one of the dogmas of radiotherapy. However, like several other key dogmas of radiotherapy these concepts have been translated directly from in vitro cell assays to organized tissues and tumors in vivo. A more realistic view of hypoxia in tumors emerged in the 1970’s with the recognition of acute vs chronic hypoxia as well as transient hypoxia. These different forms of hypoxia clearly had a different impact on radiosensitivity, and also introduced a more dynamic image of hypoxic cells and their fate after radiation. Reoxygenation, initially simply viewed as hypoxic cells gaining access to oxygen after irradiation and death of well oxygenated cells, also was shown to have various mechanistic origins, varying in time scale from minutes to days. At a molecular level the discovery of hypoxia-inducible factor in the early 1990’s changed the emphasis from a pure physico-chemical interaction to a complex network of signaling cascades with a large impact on many cellular functions.

In this lecture the implications of the dynamics of various forms of hypoxia on modern image-guided high precision radiotherapy will be discussed.

CL11. Antioxidants: Help or Hype. Ann R. Kennedy Kennedy, University of Pennsylvania, USA

Antioxidants have been shown to mitigate or prevent various adverse biological effects caused by exposure to the types of radiation encountered during space travel as well as to those encountered on Earth. The primary types of space radiation of particular concern for the health of astronauts are protons and highly energetic, heavy, charged particles known as HZE particles. Relatively little is known about the biological effects of protons and HZE particle radiation compared to what is known about the effects of conventional/referenced radiations, such as x- or gamma rays. It is known, however, that some space radiations are equally effective as, and sometimes considerably more effective than, conventional radiations at producing biologic effects at comparable dose levels. In experimental radiation research involving cells and animal model systems, calculated relative biological effectiveness (RBE) values have been particularly high for the HZE particle radiations and some biological endpoints. Such high RBE values for HZE particles are presumably due to their high linear energy transfer (LET) radiation.

At this time, antioxidants have been shown to suppress: proton and HZE particle radiation induced oxidative stress, cytotoxicity, and malignant transformation in both in vitro and in vivo systems, cataractogenesis in vivo and several central nervous system (CNS) adverse effects. There are
some concerns about the use of antioxidants as countermeasures for space radiation induced adverse biological effects which will be discussed during this conference lecture. It is believed that antioxidants are not only in the "help" category, but can be classified as "essential" for mitigating and/or prevention of both acute and chronic adverse biological effects brought about by exposure to radiation.

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CL12. Radiolysis of sub- and supercritical water. Yosuke Katsumura, The University of Tokyo, Japan

Introduction

Radiolysis of water at elevated temperatures is a challenging subject not only in science but also in technology, especially in nuclear technology. In current nuclear power plants, water is used as a coolant and circulated in the primary circuit at around 300 °C. Coolant water is decomposed under strong radiation filed of fast neutrons and γ-rays at the reactor core and, consequently, the chemical condition of the coolant is determined predominantly by water radiolysis. Therefore, understanding of water radiolysis at elevated temperature is essential to keep the safe operation and integrity of the nuclear power plants. This is also important to the development of supercritical water-cooled reactor, in which higher than 600 °C is expected as outlet temperature. Then, understanding the radiolysis of sub- and supercritical water (> 374.1 °C and > 22.1 MPa) is important. Since the speed of reactions is accelerated at elevated temperatures, an ultrafast pulse radiolysis technique has been highly expected.

In the present work, at first, an ultrafast pulse radiolysis system has been developed for the study on the radiolysis of sub- and supercritical water is introduced. Then, experimental results and their characteristics are summarized. The characteristics of the reactions at elevated temperatures will be clarified based on the Monte Carlo simulation.

Results and Discussion

Temporary behavior of G(eaq) observed at elevated temperatures

In order to evaluate the G(eaq), both temperature dependent density change of water and shift of the absorption band of the hydrated electron should be taken into account. In addition, an assumption that the absorption coefficient at the peak of the hydrated electron is temperature independent is introduced according to recent evaluation by Elliot and Bartels, and temporal behavior at different temperatures was easily evaluated. From this measurement it is clear that the initial yields are almost temperature independent. However, decay is accelerated with increasing temperature. At room temperature, spur decay seems continuous over 6 ns but, above 300 °C, faster and larger decay completed within 1 ns is observed.

It is interesting to compare the decay of the hydrated electron in light water and heavy water. The difference of the initial yield is not appreciable but the decay in D2O is slightly slower than in H2O. This result is consistent with the observation at room temperature by Bartels. Similar behavior is kept at elevated temperatures.

Spur decay at elevated temperatures

Among the spur reactions, the following reactions would be responsible to the fast decay at elevated temperatures.

\[ e_{aq} + OH \rightarrow OH \]  
\[ e_{aq} + H \rightarrow H \]  
\[ e_{aq} + e_{aq} + 2H_2O \rightarrow H_2 + 2OH \]

The rate constants of above reactions have been investigated intensively by Elliot and Bartels and have summarized the latest data. The rate constant of the (1) is increasing smoothly and evaluated to be 3x1016 and 3.7x1016 M⁻¹s⁻¹ at room temperature and 300 °C, respectively. It was reported that the reaction (2) is increasing significantly from 2.0x1016 to 7.1x1016 M⁻¹s⁻¹ at room temperature and 300 °C, respectively. On the contrary, the reaction (3) is increasing from 6.0x1015 to 7.5x1015 M⁻¹s⁻¹ at room temperature and 150 °C, respectively, but above 150 °C the rate constant drops rapidly. It is concluded that the contribution of the reaction (2) becomes larger and larger at elevated temperatures and faster decay is attributable to the reaction (2).

Monte Carlo simulation

Monte-Carlo techniques are used to model the complex succession of events that are generated in liquid water under irradiation. The detailed description of the Monte-Carlo code IONLYS-IRI developed by Prof. Jay-Gerin group, that simulates the initial production of the various reactive species and the subsequent chemical reactions of these species, has been given previously. This has succeeded to reproduce the experimental results in supercritical water radiolysis not only with low LET radiation but also with heavy ion beams. Recently, this has been extended from room temperature to high temperatures up to 350 °C. In this simulation, the latest data set of reactions at elevated temperatures has been taken.

It was found that the Monte Carlo simulations are able to reproduce the present experimental results obtained from room temperature to 300 °C quantitatively and that the contribution of the reaction (2) becomes predominant at elevated temperatures as mentioned in a previous section. Density dependence in supercritical water at 400 °C.

It was reported that the yield of hydrated electron in supercritical water is strongly dependent on pressure, namely density based on the scavenging experiment. Therefore, it is interesting to check above by direct measurement and pressure dependent measurement has been performed at 400 °C. The direct measurement of the hydrated electron confirms the reliability of our previous evaluation that the yield of hydrated electron is density dependent; lower density, the higher yield. However, previous Monte Carlo code developed up to now seems difficult to apply the radiolysis of supercritical water because the clustering structure in the supercritical water should be taken into account. Further work is highly expected.

Acknowledgement: This work has been done in collaboration with Dr. Yusa Muraya at University of Tokyo and Dr. Mingzhang Lin at Japan Atomic Energy Agency in our research group, Prof. Mehran Mostafavi and Dr. V. de Waale in a group at Laboratory for Chemical Physics, Paris-Sud, France and Prof. Jean-Paul Jay-Gerin and Dr. Jintana Mesuangnoen in a group of University of Sherbrooke, Canada. The UNAC Division, Nuclear Professional School, especially Prof. M. Uesaka and Mr. T. Ueda, has supported this work strongly and continuously. Without their support, we could not obtain the above valuable results.

Oral presentations

PL03. The varied faces of hypoxia in cancer: from radiation resistance to metastasis to stem cell niche. (H.S. Kaplan lecture).

Richard Hill, Ontario Cancer Institute/Princess Margaret Hospital, Canada

The microenvironment of a tumour is complex involving aspects, such as hypoxia, associated with pathophysiological conditions largely driven by the vascular and lymphatic components, and interactions between the tumour cells, the stromal cells and the extracellular matrix. Our understanding of how these features of the tumour microenvironment cross-react remains quite limited but hypoxia is increasingly being investigated as a target to improve tumour response to drug treatment. This is no surprise to radiation scientists since hypoxia has long been argued to play a role in radiation response. The demonstration in the 1950’s of the effects of hypoxia on cellular radiation sensitivity led to the prediction that hypoxic cells in tumours would limit response to radiotherapy. However, the findings that hypoxic cells in tumours could reoxygenate during fractionated radiotherapy cast doubt on the importance of hypoxia. Although some studies with hyperbaric oxygen and hypoxic cell radiosensitizers supported the role of hypoxia in treatment resistance, the data was not widely regarded as convincing. It was only with the introduction of direct methods to assess levels of hypoxia in tumours in the 1990s that it was appreciated that there was a wide range of hypoxic levels in tumours and that hypoxia is heterogeneous both spatially and temporally. That tumours with high levels of hypoxia are likely to respond poorly to treatment has now become more widely accepted. However, there remains limited use of techniques to measure hypoxic levels in tumours prior to therapy or to address this issue therapeutically. Also in the 1990’s it was demonstrated that hypoxia in tumours could promote increased metastatic spread and the discovery of the HIF transcription factors led to findings that hypoxia affects the expression of many genes. Recent findings also suggest that hypoxia plays an important role in tumour growth through its effects on specific cell signaling pathways and that hypoxic cells may be particularly vulnerable to drug inhibition of specific pathways. Most recently gene expression changes associated with hypoxia have been suggested to play a role in the maintenance of a stem cell niche in tumours. Various aspects of hypoxia as it relates to tumour progression and treatment response will be discussed in the lecture.

Wednesday

Eye openers EO13 - EO17

To determine health effects of radiation in A-bomb survivors, the Radiation Effects Research Foundation has been conducting studies on the Life Span Study (LSS) population which consists of 93,000 A-bomb survivors and 27,000 controls. **Solid cancer:** The most important result of the LSS is elevation of cancer risk with increase of radiation dose. For incidence of solid cancers, it is estimated that, at age 70 following exposure at age 30, solid cancer rates increase by about 35% per Gy for men and 38% per Gy for women. The age-exposure dependent excess relative risk model of lung cancer, cigarette smoking has been found to be an important modifier. Radiation has similar effects upon first primary and second primary cancer risks. Finally, it appears that radiation-associated increases in cancer rates persist throughout life.

The in utero group exhibited dose related increase in incidence rate for solid cancers. The lifetime risk, however, may be considerably lower than for early childhood exposure. **Leukemia:** In the most recent decade of observation (1991-2000) on mortality, it was suggested that the effect of A-bomb radiation on leukemia mortality has persisted for more than five decades. In addition, significant dose-response for myelodysplastic syndromes was found in Nagasaki LSS members 40 to 60 years after radiation exposure. **Cardiovascular disease:** Mortality due to cardiovascular disease (CVD), including heart disease and stroke, has been studied in the LSS. Dose-related increase in CVD mortality in the LSS is apparent. The linear dose-response estimate for excess relative risk (0.7% per Gy) for heart disease and 9% for stroke, although the risks below about 0.5 Gy are unclear. The Adult Health Study, which is clinical follow-up program, has offered further support by showing associations of radiation exposure with alterations in CVD risk factors, including blood pressure, inflammation, and lipid metabolism. However, there are still important questions remain unanswered.

**Future perspective:** In view of the nature of the continuing increase in solid cancer risks, the LSS should continue to provide important new information on radiation exposure and solid cancer risks for another 15 to 20 years. In addition, the LSS also should continue to provide important information on non-cancer disease risks, such as CVD.

**EO14. Molecular Mechanisms of Extreme Radioreistance displayed by Insect Cells.** SUDHIR CHANDNA,1 K., S. HAMBARDE,1 A. KUMAR SINGH,1 S. KUMAR SINGH,1 J. SWAROOP KUMAR,1 V. SINGH,1 S. SUMAN2; R. KUMARSWAMY,1 B. S. DWARKANATH,2 V. JAIN3, R. KUMAR SETH4, R. P. TRIPATHI1, 1: Institute of Nuclear Medicine & Allied Sciences, India, 2: Lombardi Comprehensive Cancer Center, Georgetown University, Washington DC, USA, 3: Medical School Hannover, Hannover, Germany, 4: Gurgaon, Haryana, India 5: Department of Zoology, Univ of Delhi, Delhi, India

Introduction: Lepidopteran insect cells are an intriguing model of radiation response since these display 50-100 times higher radioresistance than mammalian cells despite having numerous homologies with the latter. Extensive studies are conducted in our laboratory for understanding the cellular responses & molecular pathways induced by radiation/ stress in these cells.

Methods: Cellular radiation/stress responses of S91 ovarian cell line derived from Spodoptera frugiperda, the Fall armyworm (order Lepidoptera; class Insecta) were studied; including DNA & cytogenetic damage, DNA repair, oxidative stress, nitrosative stress, NOS pathway, micro-RNA regulation as well as modes and mechanisms of cell death.

Results: S91 cells display excessive resistance to g-radiation doses up to 500 Gy-1000 Gy, with the iso-effect doses inducing DNA damage varying by up to 10 times. However, cell death was induced only at 1000 Gy or higher, about 100 times higher than radiation doses lethal for human cells. Mitochondrial/ calcium disturbances were detected only at death-inducing doses, leading to typical apoptosis. Mitochondrial pathway of apoptosis was also induced by actinomycin-D with a excessive sensitivity in S91 cells, and cytosolic cytochrome-c release was Bax-mediated/ mPTP-independent in nature. These cells have significantly stronger antioxidant mechanisms accompanied by a prominent absence of NO-induced NO-mediated response. Proteomic profiles indicated a stronger mitochondrial response with alterations in expression of much lesser number of proteins following irradiation. Peculiar differences in chromatin organization may also prevent DNA damage or enhance DNA repair, which is being investigated. These cells also showed dependence on certain micro-RNAs known to regulate cell death in Drosophila, indicating presence of alternate/additional mechanisms.

Conclusion: Lepidopteran insect cell radioresistance seems to have developed at important checkpoints during the stress response mechanisms, and is revealing novel features of cellular radiation response. These studies may help greatly in the development of more effective modalities for biological radioprotection.

**EO15. Combined Chemo-Radiation Therapy.** Krzysztof Składowski, Institute of Oncology, Maria Skłodowska-Curie Cancer Center, Poland

Over the last 10 years the combination of two cytotoxic agents - radiation and chemotherapy has become a standard (sometimes a “gold standard”) management of human cancer patients. Nowadays, there is a wide spectrum of neoplasms where sequential or concurrent combination of both works effectively in the clinic. Induction (neoadjuvant) chemotherapy before main loco-regional treatment based on radiation is indicated usually for advanced stage of cancer disease in head and neck, breast, oesophagus, gastro-intestinal tract, and including androgen deprivation hormonotherapy, in prostate. Concurrent chemo-radiation is effective postoperatively in high grade glioma patients, in lung cancer patients, oesophagus cancer patients, rectal cancer patients, cervix cancer patients and some pediatric neoplasms, both postop and definitively in head and neck cancer patients. The benefit coming from adjuvant chemotherapy (given in postop and/or postradiotherapy settings) is well documented in breast cancer patients, gastrointestinal cancer patients and some sarcoma patients. Also in many systemic neoplasms like lymphoma or plasmacytoma where chemotherapy remains the treatment of choice some kind of cooperation with irradiation still exists. Thus it is not only obvious that a longer follow-up period for the combination for a target of that combined treatment, but majority of it has obtained some kind of benefit compare to single modality treatment. What is the source of current big success of combined chemo-radiation if both anticancer treatment modalities have existing from more than forty years and where does it come from? The clinical point of view will be presented in eye-opener lecture as an attempt answer on these questions.

**EO16. Track structure simulations for radiation physics, chemistry and biology.** Werner Friedland, P. Kundrát, P. Jacob, Helmholtz Zentrum München, Germany

Mechanistic modelling of biological effects of ionizing radiation is an important tool for a quantitative understanding of the underlying action mechanisms. Mechanistic modelling plays a crucial role in inter- and extrapolating the data, as needed e.g. for treatment optimization in radiotherapy of cancer or for estimation of radiation risk at low doses where epidemiological data possess limited statistical power only, or under unexperienced irradiation conditions like a manned Mars mission. Moreover, it may improve the analysis and interpretation of measurements by non-linear extrapolation beyond the limits of the experimental protocol.

An established bottom-up approach for testing hypotheses on radiation action and response mechanisms is provided by Monte Carlo track structure simulations of ionizing radiation, supplemented by multi-scale models of biological structures and mechanism modelling of biological effects and consistencies of the radiation insult. In combination with simulations on relevant cellular events, intercellular signalling processes and top-down modelling of effects in tissues, organs or organisms including tumour control or cancer induction, the hierarchy of mechanistic modelling tools has the potential to allow improved predictions of treatment outcome or radiation risks.

The presentation will be focused on the PARTRAC suite of modules for track structure calculations, radiation chemistry simulation, modelling tools for DNA structures, DNA damage and DNA repair processes, and modelling approaches on intercellular signalling processes. The computational framework will be reviewed and the key results providing insight into the mechanisms of radiation action will be presented.

**EO17. Radiation effects in ionic liquids and/or ultrafast processes and the initiation of radiation damage.** James F. Wishart, Brookhaven National Lab, Upton, USA

No abstract

**S19. Vascular endothelial cell response to radiation - a new dimension**

**S19-01. Irradiated endothelium and mechanotransduction signaling.** Mohan Natarajan, UT Health science Center, USA

Endothelium is considered as the largest organ in the body disseminated into every tissue and more prone to get exposed to radiation on any situation either in occupational, therapeutic, diagnostic or accidental. Being the major source of the paracrine effectors, endothelial damage is a
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heralding event in radiation toxicity on nearby and distant tissue. There is a rapid progress in radiobiological effects on the multi-functional activity of vascular endothelium. However, mechanistic approach, in general, studied on in vitro static cell culture experiments. The in vitro static cultures may limit the understanding of the combined effect of other supporting factors that occur in the \textit{in vivo} micro- and macro-environment. Therefore it is important to understand the contribution of supporting factors such as extracellular matrix and local environment in which the cells/tissues reside; in particular with endothelial cells, where the cells are constantly undergoing different types of shear stress due to continuous blood flow. Cardiovascular biology has clearly established that the structural and functional capacity of endothelium is different with the flow shear stress. We considered all the above issues and looked at the endothelial response to radiation with a new perspective. Human primary aortic endothelial cells were subjected to shear stress (16 dynes/cm$^2$) using an \textit{in vitro} parallel plate flow system that simulate the physiological hemodynamic flow shear stress. The cells were then exposed to $^{137}$Cs gamma rays to a total dose of 2 or 10 Gy at a dose rate of 1 Gy/min and were subjected further to flow shear stress after radiation exposure for an additional 4 h. The changes at the molecular and biochemical functions were compared with static cultures. Contribution of machanotransduction signaling associated with flow shear stress in the outcome of radiation response is discussed in this presentation.

S19-02. Vascular-mediated normal tissue injury – where are we and where are we going? Martin Hauer-Jensen, University of Arkansas for Medical Sciences, USA

The risk of injury to normal tissues continues to limit the cancer cure rates that can be achieved with radiation therapy. While killing of target cells is the primary determinant of radiation toxicity, damage to the microvascular endothelium substantially impacts both early and delayed toxicity in many organ systems. Past strategies aimed at preventing or mitigating radiation-induced microvascular injury relied mainly on various anticoagulants or antiplatelet agents. However, recent insight into crosstalk between the coagulation and inflammatory systems; into mechanisms underlying thrombin’s cellular, receptor-mediated properties; and into the molecular basis of interactions between endothelial cells and other cell types provide opportunities for development of molecules or functions that ameliorate radiation-induced vascular injury in animal models with little or no effect on blood clotting. Examples include preservation or restoration of the thrombomodulin/protein C system, blockade of cellular thrombin receptors, or strategies to prevent uncoupling of endothelial nitric oxide synthase. This lecture provides an overview of the mechanisms by which radiation causes endothelial dysfunction and discusses how novel vasculo-protective interventions may ameliorate radiation-induced oxidative and nitrosative stress, endothelial dysfunction, and tissue toxicity.

S19-03. Adhesiveness of aortic endothelium is increased in response to high LET radiation. Dennis F. Kucik, S. Khaled, K. B. Garcia, X. Wu, T. Yu, S. Babitz, University of Alabama at Birmingham, USA

Exposure to radiation from a variety of terrestrial sources is associated with increased risk of heart disease and stroke. We recently demonstrated that $^{56}$Fe irradiation accelerates atherosclerotic plaque development in the apoE mouse model. The purpose of the current study was to investigate the mechanism at the cellular level. Since radiation also induces vascular inflammation, a possible mechanism for exacerbation of atherosclerosis is an increase in the adhesiveness of vascular endothelial cells, triggering pro-atherogenic accumulation of leukocytes. Human aortic endothelial cells (HAECs) were grown as monolayers and exposed to 0 to 5 Gy 600 MeV $^{56}$Fe, followed by measurement of adhesiveness under physiological shear stress using a flow chamber adhesion assay. Twenty-four hours after irradiation, HAEC adhesiveness for leukocytes was increased. Because the adhesion molecule VCAM-1 is critical in both development of atherosclerosis and endothelial cell-leukocyte adhesion, we then investigated the role of the endothelial VCAM-1 and its leukocyte receptor, the integrin $\alpha_{4}\beta_{1}$. Measurement of endothelial adhesion molecule expression demonstrated that radiation-induced adhesiveness increases were not due to an increase in expression of VCAM-1 on the endothelial cell surface. Instead, antibody blockade of $\alpha_{4}$ on leukocytes abolished the radiation-induced adhesiveness, suggesting involvement of the VCAM-1/$\alpha_{4}\beta_{1}$ receptor-ligand pair in the mechanism. Since the leukocyte integrin $\alpha_{4}$ can be activated by chemokines presented on the endothelial cell surface, the effect of pertussis toxin (PTX), an inhibitor of G-protein coupled activity, was tested. PTX specifically inhibited radiation-induced adhesiveness, with no significant effect on non-irradiated cells. Therefore, high-LET radiation can induce increased adhesiveness of aortic endothelial cells through chemokine-dependent signaling from endothelial cells to leukocytes, even in the absence of increased expression of the adhesion molecules involved.

S19-04. Targeting vascular endothelium for radiosensitization and tumor cure. Adriana Haimovitz-Friedman, Memorial Sloan-Kettering Cancer Center, USA

While there is significant interest in combining anti-angiogenesis therapy with conventional anti-cancer treatment, clinical trials have as of yet yielded limited therapeutic gain, mainly because mechanisms of anti-angiogenic therapy remain to a large extent unknown. Currently, anti-angiogenic tumor therapy is conceptualized to either normalize” dysfunctional tumor vasculature, or to prevent recruitment of circulating endothelial precursors into the tumor. An alternate biological, restricted to delivery of anti-angiogenics immediately prior to single dose radiotherapy (radiosurgery), is provided in the present study. Genetic data indicate an acute wave of ceramide-mediated endothelial apoptosis, initiated by acid sphingomyelinase (ASMase), regulate tumor stem cell response to single dose radiotherapy, obligatory for tumor cure. Here we show VEGF prevented radiation-induced ASMase activation in cultured endothelium, occurring within minutes after radiation exposure, consequently repressing apoptosis, an event reversible with exogenous C16 ceramide. Anti-VEGF antibody-enhanced ceramide generation and apoptosis. In vivo, MCA/129 fibrosarcoma tumors were implanted in asmsav+/- mice or asmsa-/- littermates and irradiated in the presence or absence of anti-VEGFR2 DC101 or anti-VEGFR G6-31 antibodies. These anti-angiogenic agents, only if delivered immediately prior to single dose radiotherapy, de-repressed radiation-induced ASMase activation, synergistically increasing the endothelial apoptotic component of tumor response and tumor cure. Anti-angiogenic radiosensitization was abrogated in tumors implanted in asmsa-/- mice that provide apoptosis-resistant vasculature, or in wild-type littermates pre-treated with anti-ceramide antibody, indicating that ceramide is necessary for this effect. Understanding the temporal sequencing of anti-angiogenic drugs and radiation enables optimized radiosensitization and design of innovative radiosurgery clinical trials.

S20. What, if anything have omics technologies taught us about radiation effects/risks?

S20-01. Influence of omics research on radiation paradigms and risk: past and future. Antonio Brooks, Pacific Northwest National Laboratory, USA

Radiation standards have been based primarily on human epidemiology data. It is well established that high doses of radiation produce a dose related increase in cancer frequency. However, it is not possible to detect a significant increase in radiation induced cancer in the low dose region or to determine the shape of the dose-response relationship in this region. This makes it necessary to develop models that can be used to predict the cancer risk in the low dose region. The current model that is used by regulatory bodies is based on DNA damage and suggests that since DNA damage increases linearly with dose that mutations and cancer will also increase as the same function. Thus, each and every ionization is postulated to produce an increase in cancer risk. To help models with a scientific basis and to decrease the uncertainty associated with the risk estimates in the low dose region studies have been conducted to define the mechanisms of action in this region and to use these data to help define the shape of the dose-response relationships. Key to this research is to determine if molecular and cellular systems can respond to low doses of radiation and see if the responses are the same following exposures at high and low doses. Many research programs are in place to address these questions including the DOE Low Dose Radiation Research Program. This presentation will review the data produced by this program over the past 10 years using modern biology and technology. The focus of the presentation will be to demonstrate that this research has had a marked impact on the paradigms used in radiation biology and the relationship between radiation exposure and DNA alterations, many other biological responses. It will also evaluate the usefulness of the modern molecular biology to guide the regulatory community in evaluating the shape and slopes of the dose-response relationships in the low dose region. Finally, presentation will address the question: Can cellular and molecular data have an impact on regulatory standards or the perception of radiation damage and risk?
Research supported by USDOE low dose program under a contract to PNPNL to Dr. Brooks.

S20-02. Insight into the bystander effect from functional genomics. Sally Amundson¹, S. A. Amundson², 1: Columbia University Medical Center, USA 2: Center for Radiological Research, Columbia University Medical Center, USA

The radiation bystander effect is a potentially important component of the overall biological response of tissues and organisms to ionizing radiation, but the signaling mechanisms responsible for communication between irradiated and non-irradiated bystander cells are not fully understood. It has become abundantly clear over the past decade that such complex responses cannot be understood solely through deterministic studies of individual molecules or simplified pathways. We have used whole genome gene expression as measured by microarray, coupled with gene ontology and network analyses, and a novel approach to time series analysis, to delve beneath the surface of responses to radiation in both directly hit and bystander cells and tissues. Such work has elucidated signaling pathways and transcription factor networks involved in transmitting and responding to the bystander signal, including NF-kappaB and AKT- GSK3B-CTNNB1, as well as suggesting other potential regulatory mechanisms, such as epigenetic control of the response to radiation through KDM5B and HDACs.

S20-03. What ‘OMICS’ can and cannot tell us about DNA damage response and DNA repair in embryonic stem cells? Peter Stambronn, University of Cincinnati College of Medicine, USA

The “OMICS” technologies have revolutionized our approaches to numerous biological questions, including cellular mechanisms involved in responses to radiation and/or DNA damage. Current high throughput technologies are likely to overlook one or more subsets of mechanisms. One example is the manner by which mouse embryonic stem cells protect the integrity of their genomes. Although most proteins in the G1/S checkpoint are present, both major DNA double strand break (DSB) response pathway are compromised. P53 does not translocate to the nucleus in these cells after radiation, and CHEK 2 is associated with centrosomes and is not available to phosphorylate some of its substrates. As a result, the G1/S checkpoint does not function, cells with damaged DNA transit to the S phase where the damaged DNA is replicated, the damage exacerbated and the cells propelled to apoptosis. The net result is that the ES cell population has cells with pristine DNA. A second example is the DSB repair pathway utilized by mouse ES cells compared with somatic cells. Somatic cells primarily utilize error-prone non-homologous end joining (NHEJ) whereas the ES cells utilize high fidelity homologous recombination (HR)-mediated repair. When induced to differentiate, pathway utilization is switched. Regulation of protein abundance of some of the proteins involved in HR repair appears to be at neither the level of transcription nor of protein translation but rather of posttranslational translation, a mechanism that would not be detected by current “OMICS” methodologies.

S20-04. What have ‘omics studies taught us about health risks? Marianne Sowa, Pacific Northwest National Lab, USA

There is a wealth of data available on DNA damage associated with exposures to ionizing radiation and the subsequent cellular responses. Indeed, much of radiation research has focused on these initial insults and induced responses, particularly DNA repair, cell signaling pathways, cell cycle checkpoint control, mutation induction, chromosomal rearrangements, transformation and apoptosis etc. While many of these endpoints correlate with exposure dose, few, if any, provide substantive information on human health risk(s) associated with radiation exposure. We will discuss recent advances in high throughput ‘omics technologies to evaluate what they have taught us about health risk(s) to humans associated with exposure to ionizing radiation.

S21. Theragnostic radiotherapy

S21-01. The perspectives of TPMCC (Therapeutic Personalized Multimodality cancer Care) in combined treatment for cancer of the head and neck. Rafal Suwiński, Center of Oncology M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland

Recent developments in cancer imaging, computerized treatment planning and real-time verification procedures resulted in widespread shift from obsolete 2-dimensional radiotherapy towards CT/MRI/PEt guided treatments. Three-dimensional (3D) radiotherapy conform the isodose distribution to spatial dimensions of the tumor and dose-limiting structures. Modern therapeutic units allow accounting for interfraction and intrafraction tumor motion, resulting in conformal image-guided 4D radiotherapy (spatial changes of the tumor in time represent 4-th dimension). Such technological advances, does not, however, put a ceiling on further developments in conformal radiotherapy. Current interest of research focuses over conformation of combined cancer therapy (that incorporates surgery, radiotherapy, chemotherapy and/or targeted drugs) to individual biological characteristics of the tumor. It will result in TPMCC (Therapeutic Personalized Multimodality cancer Care) that accounts not only for spatial extent of the tumor but also for its biological uniqueness. Individual characteristics of the tumor can be increasingly well defined based on the analysis of molecular signatures. We have contributed to these attempts by analyzing the expression of selected genes in relation to the outcome of accelerated postoperative radiotherapy for head and neck. We were able to define molecular prognostic of squamous cell cancer of the head and neck that predicted the benefit from treatment acceleration. By contrast, another subgroup of the patients, with different molecular profile, did not benefit from accelerated treatment, but developed increased acute mucosal reactions. Our results support the studies that demonstrate the potential of molecular profiles to individualize combined treatments for head and neck cancer, enhancing the rationales for TPMCC (Therapeutic Personalized Multimodality cancer Care).

S21-02. Feasibility and Safety of reducing the irradiation dose in regions of active neurogenesis for prophylactic cranial irradiation in patients with small-cell lung cancer. Rafael Tarnawski, L. Michalecki, Cancer Center and Institute of Oncology Gliwice Branch, Poland

Purpose/Objective: Prophylactic cranial irradiation (PCI) is performed on patients with limited or extensive small-cell lung cancer to reduce brain metastases and prolong survival. PCI may induce neurocognitive impairment. We tested the feasibility of reducing irradiation doses to neural stem cell (NSC) regions while maintaining prescribed doses to the planned target volume (PTV). We report the initial number and localization of brain metastases in group of patient after PCI with dose reduction in region of active neurogenesis (subgranular and subventricular zones).

Materials and Methods: Irradiation plans utilizing intensity-modulated radiotherapy (IMRT), helical TomoTherapy, and RapidArc for 10 consecutive lung cancer patients were evaluated. The dose distribution, volume histograms, and dose homogeneity indexes were analyzed. Planned and actual dose distributions were compared by dosimetric analysis. A group of 31 patients with limited disease stage small cell lung cancer qualified for PCI entered current study. We used Eclipse 8.6 (Varian Medical System) treatment planning system to prepare 5-9 fields IMRT plans. Patients were irradiated using LINAC Varian 23EX with IGBT during each irradiation. All plans were prepared in order to achieve homogenous dose distribution of 30 Gy with 2 Gy per faction (conventional PCI) in brain excluding NSC. The most important restriction was to reduce the radiation dose to 15-20 Gy in the NSC compartment, which is located in the middle of PTV.

Results: Both helical tomotherapy and LINAC-based IMRT reduced the radiation dose to the NSC regions by approximately 45% while maintaining the full dose to the rest of brain. From the initial number of 31 patients, 23 patients received PCI with protection of NSC. During the observation time 9 patients had progressive disease outside brain. The follow-up MRI revealed brain metastases in 3 patients. One patients had multiple (30) metastases, two other had single metastases in temporal and frontal lobe outside the region of reduced dose. Conclusion: Protecting the regions of active neurogenesis is technically feasible. The median follow-up time is short, but the reduction of radiation dose in NSC compartment did not seem to compromise local control in brain. We did not observe any metastases only in region of reduced dose.

S21-03. Radiotherapy of painful vertebral hemangiomas. Leszek Miszczuk, A. Tukiendorf ², 1: Cancer Center and Institute of Oncology Gliwice Branch, Poland 2: Cardiff Research Consortium; UK

Background: In autopsy examinations, the prevalence of vertebral hemangiomas is present in 12% of the whole population, but, fortunately, only 1% of them is painful. A lot of treatment modalities are used as surgery, alcohol ablation, percutaneous vertebroplasty, balloon kyphoplasty and finally, radiotherapy, which seems to be less iatrogenic, easier and cheaper method than others.
Purpose: An evaluation of dose-response relationship and an attempt to define predictive factors of painful vertebral hemangiomas radiotherapy. Material and Methods: The analyzed material comprises group of 101 patients (137 painful vertebral hemangioma irradiations). Delivered fraction dose (fd) varied from 2 to 15 Gy (123 cases were fractionated and 14 treated with radiosurgery), and total dose (TD) from 8 to 30 Gy (111 cases irradiated with fd of 2 Gy to 24 Gy). The pain relief, changes in analgesic requirements and reossification were evaluated. Results: We defined a pain level decrease in percent of primary pain level decrease. Means of pain relief 1, 6, 12, 18 months after radiotherapy were 60.5%, 65.4%, 68.3%, 78.4% respectively. Percentage of patients with no need for analgesics and patients using tramadol were 39%, 40%, 44% 57% and 20%, 17%, 22%, 11% in these times. The percentage of patients with complete pain relief changed from 36/48% 1 month, to 64/22% 1.5 year after radiotherapy. No impact of radiotherapy on reossification was found. We found the significant positive impact of fd and TD increase for analgesics uptake reduction and pain relief. We found following positive predictive factors: female gender, older age, better PS, bigger Hb concentration, shorter symptoms duration and lower analgesics uptake before radiotherapy.

Conclusions: The obtained results permit to form the conclusion that radiotherapy is an effective method of painful vertebral hemangiomas treatment and that positive result of treatment is correlated with the increase of fd and TD. The positive predictive factors were found: female gender, older age, better PS, increased Hb concentration, shorter symptoms duration and lower analgesics uptake before radiotherapy.


Background. The results of gastric cancer treatment are still poor. In many institution surgery follows by chemoradiotherapy is considered to be the gold-standard treatment. Recent II phase studies have suggested that preoperative chemoradiotherapy followed by surgery may result in improved survival. Methods. In prospective III phase trial, a total of 119 patients with operable gastric cancer, after stratification by weight loss, tumor location, T-stage, and N-stage were randomized to receive preoperative chemoradiotherapy followed by operation and chemotherapy (group A) versus standard treatment (group B) which consisted of surgery with adjuvant chemoradiotherapy. In both arms, the stomach or stomach bad and regional lymph nodes were irradiated to total dose of 45 Gy in 25 fractions. Patients received concurrent chemotherapy (5-FU 325mg/m² d1-5 and d29-33). In the group A the patients underwent surgery within 6 weeks after radiochemotheraphy. The surgical procedure was total or partial gastrectomy depending on D1, D2 extention of tumour. The adjuvant chemoradiation consisted of four -5 days cycles of 5-FU (375mg/m²/day) and LV (20mg/m²/day).

Results. The resectability rate was higher in the group A than in the group B. The difference was 16% (87% vs. 71%). R0 resection rate was also higher in the neoadjuvant treatment. The histopathological examination showed that mean tumor volume in the group A was seven times smaller than in the group B (10m³ vs. 70cm³). In the group A the pCR was observed in 21% of patients. The 1- and 2-year overall survival rates of the neoadjuvant group and the adjuvant group were 69% and 63%, 57% and 40% (p=0.1), respectively. The 2-year locoregional control rate was 90% and 71% in the group A and in the group B (p=0.022), respectively. Mild vomiting, diarrhea and leucopenia were most frequent toxicities. In the group A and in the group B, leucopenia G3 or G4 occurred in 5% and 7% , respectively. Preoperative chemoradiotherapy was not associated with more surgical complications.

Conclusion. The neoadjuvant chemoradiotherapy compared to the standard treatment increased locoregional control without higher incidence of toxicity, but not significantly improved overall survival.

S21-05. Does Accelerated Radiotherapy Reflect Radiobiology? 15 year experience with CAIR (7 days irradiation). Krzysztof Składowski, M. Hutnik, A. Wygoda, B. Maciejewski, Institute of Oncology, Maria Skłodowska-Curie Cancer Center, Gliwice, Poland

Background. Definitive accelerated radiotherapy (DART) with no compromise of total dose has the level I evidence-based positive impact on local tumor control for head and neck cancer patients. Gliwice experiences with Continuous Accelerated Irradiation (CAIR) utilizing 7 fractions in 7 days a week coming from phase III studies have shown several clinical outcomes which could be relevant to radiation biology.

Material and Methods. Over the last 15 years 450 patients has been collecting in the trials where CAIR fractionation system (fraction-dose was given once-a-day, regularly at 24 hour interval, including Saturdays and Sundays) was compared with: 1 – Conventional Fractionation (CF: 5 fractions in 5 days a week, from Monday to Friday) or 2 - Concomitant Boost Fractionation (CBF: 7 fractions given in 5 days, once-a-day at Mondays, Wednesdays and Thursdays, and twice-a-day with 8 hour interval at Tuesdays and Fridays).

Results. Our outcomes related to radiation biology concern both tumour cure and mucosial toxicity. The first trial has shown an extraordinary gain of CAIR for both tumor control and patient survival compared to CF. Moreover a lot of grade 4 mucosal toxicity leading to high rate of consequential late effect was recognised as a result of impaired recovery of normal mucosa and it has suggested weekend breaks could be detrimental for the outcome because of the timing of tumour/mucosa clonogen accelerated repopulation (CAR). Therefore dose per fraction has been lowered to 1.8Gy and next trial has undergone where weekend-in fractionation (CAIR) was randomized with weekend-off one (CBF). Those two DART schedules had to have identical overall treatment times as the main trial assumption was no difference in tumor cure effectiveness, what has observed indeed. Because the only difference was the timing of dose delivery. We hypothesized that different biological mechanisms have been mainly depressed in radiation response – sublethal damage repair (SLDR in CBF and CAIR in CAF and they have been possibly reflected in the clinic as a difference in distribution of types of primary-tumor failure, time to local recurrence and rate of mucositis. The results of efficacy on the dose per fraction in our clinical trials showed that preoperative chemoradiotherapy followed by surgery may result in improved survival.

Purpose: To evaluate the efficacy of pain management and the impact on quality of life for patients undergoing continuous accelerated irradiation (CAIR) for primary cancer local control. The efficacy of pain management and the impact on quality of life were assessed using validated measures and qualitative interviews. The study was conducted at a single institution in Poland.

S22. Nanotechnology targeting DNA damage and repair pathways: advances in the clinic

S22-01. Targeting the epidermal growth factor receptor to augment head and neck tumor radioresistance. Eddy Yang, S. Nowsheen, T. Cooper, J. A. Bonner, University of Alabama-Birmingham, USA

Purpose: Overexpression/amplification of the epidermal growth factor receptor (EGFR) is a hallmark of head and neck cancers and confers increased resistance and inferior survival rates. Despite targeted agents against EGFR, such as cetuximab, almost half of treated patients fail this therapy. PARP inhibitors (PARPi) have gained recent attention due to their unique selectivity in killing homologous recombination (HR)-deficient tumors while maintaining minimal toxicity in normal tissues. As EGFR inhibition has been reported to alter cellular DNA repair capacity, we investigated whether cetuximab could induce a transient DNA repair deficit and subsequently augment cyotoxicity with the PARPi ABB-888 in head and neck cancer.

Methods: The head and neck cancer cell lines UM-SCC1, UM-SCC6, and FaDu were used in this study. Kinetics of DNA damage and repair were assessed by immunofluorescence staining as well as cleaved caspase 3 and 9 levels. Cell cycle analysis was performed via flow cytometry.

Results: Cetuximab increases gH2AX foci, which are well established markers for DNA double strand breaks (DSBs), in head and neck tumor cells. This coincides with reduced DSB-repair as indicated by attenuation of IR-induced Rad51 and DNA-PK foci, which are well characterized in situ markers of the homologous recombination (HR) and non-
homologous end-joining (NHEJ) DNA repair pathways, respectively. Importantly, cetuximab augments cytotoxicity with the PARP ABT-888 both in vitro and in vivo, through a mechanism involving persistent DNA damage and subsequent activation of the intrinsic pathway of apoptosis. The observed effects are not due to cell cycle redistribution.

Conclusions: By generating a DSB repair deficiency, cetuximab can render head and neck tumors susceptible to PARP inhibition. The combination of PARP and the PARP inhibitor ABT-888 can trigger a more effective and innovative treatment strategy to enhance therapeutic ratio and improve outcomes in head and neck cancer patients. Furthermore, this strategy may also be feasible for other EGFR overexpressing tumors, including lung and brain cancers.

S22-02. Re-engineering the DNA double strand break machinery for therapeutic purposes. William Dyvan1, S. Meiler1, M. Porteus2, G. Bao1, S. Li1, H. Xiong2, R. J. Lee3, J. Pandey1, D. Goyal1, Z. Chen1, M. Wade1, Z. Cao1, 1: Georgia Health Sciences University, USA, 2: Stanford University School of Medicine, 3: Georgia Institute of Technology, 4: Wuhan University School of Medicine 5: Ohio State University, USA

The ability to influence processes that occur within and around double-strand break (DSB) repair foci would be useful in a variety of clinical settings, ranging from radiation therapy to the development of safe approaches for homologous recombination-mediated gene correction in patients. We hypothesized that receptor-mediated endocytosis would allow delivery macromolecular agents or other engineered nanomaterials to sites of DSB repair thus modifying or redirecting the activity of the repair machinery. We discuss three examples: (i) delivery of folate-conjugated single chain antibody variable fragment (scFv) directed against the DNA-PK catalytic subunit. The scFv colocalized with its target in the cell nuclei and sensitized tumor cells to radiation (P<0.001 based on an F test). Sensitization enhancement ratios based on mean inhibitory dose were 1.92 ± 0.42 for KB cells and 1.63 ± 0.13 for NCI-H292 cells. (ii) Delivery of transferrin-conjugated zinc finger nucleases (ZFNs) to stimulate targeted gene correction by homologous recombination. ZFNs were efficiently internalized and promoted correction of a generic model recessive disorder in mouse somatic cells. Delivery of ZFNs as proteins, as an alternative to the use of ZFN-encoding viral vectors, affords greater spatiotemporal control, remarkably reduces long-term patient risk, and in preliminary work, achieved correction efficiencies comparable to vector-based methods. Current work focuses on co-delivery of agents to direct the repair machinery toward productive homologous recombination, rather than nonproductive end joining repair. (iii) Delivery of a transferrin-conjugated nanocomplex assembled around a streptavidin hub. The nanocomplex, unlike the simple protein conjugates, was retained in cytoplasmic lysosomes. Gold nanoparticles will be presented along with future considerations to allow the optimisation of gold nanoparticles as radiosensitizing agents.

S23. New developments in radiation dosimetry

S23-01. DOSIMETRY TECHNIQUES TO SUPPORT LONG-TERM HEALTH RISK STUDIES. Steven Simon, National Cancer Institute, National Institutes of Health, USA

Radiation dosimetry for the purpose of supporting long-term health risk (epidemiologic) studies differs in some important respects from dosimetry performed for compliance determination, radiation safety, and medical treatments. The requirements of radiation epidemiologic studies are due, in part, to their purpose as a research tool to quantify the risk of cancer and other health effects following exposure to ionizing radiation. In the context of supporting health risk studies, there are several important criteria that dose estimates must meet including: be estimated on an individual basis for moderately large-sized groups, be estimated for specific organs of interest, be unbiased (i.e., as close to the true dose as possible), be estimated from historical and sometimes incomplete data, have best precision possible (minimal uncertainty), have the uncertainty fully disclosed by setting realistic confidence bounds in which the true dose is believed to lie, use of appropriate dose units, and when possible, verification by independent techniques, e.g., biodosimetry. Unlike situations in radiation protection where doses are often conservatively estimated, the goal of dosimetry for epidemiologic studies is an accurate estimation of organ dose to many individuals in a defined cohort. To accomplish that, many types of data are required, often well beyond the kind of information collected for compliance radiation determination or for medical dosimetry. The success of dosimetry for epidemiologic studies depends on using a variety of tools including reliable exposure-assessment models that can predict realistic doses given appropriate input data, person-specific information, e.g., age, gender, and body size, surrogate measurements of the exposure that include environmental measurements, film-badge measurements or other personal dosimeter data, and biodosimetric measurements used for validation of estimates. The goal of this presentation is to review the unique requirements of dose estimation for epidemiologic studies in order to improve our understanding and appreciation of epidemiologic findings and to discuss recent developments in this field.

S23-02. Developments in dosimetry methods in radiotherapy. Pawel Kukolowicz, Cancer Center-Institute Warsaw, Poland
The prerequisite for safe implementation of all procedures in radiotherapy and radiology is to know or at least to estimate the absorbed dose delivered to a patient. Continuous development of treatment and diagnostic procedures requires advanced dose measurement techniques, especially when the dosimetric error may cause either a high incidence of complications or an unacceptably low tumour cure rate. Therefore in this presentation I will focus mostly on the new dosimetry methods used in radiotherapy. I will then follow with a discussion on the new dosimetry developments in radiology.

Recently a huge effort has been made in development of dosimetry techniques applied to Intensity Modulated Radiation Therapy (IMRT) and radiosurgery. The dose distribution obtained in IMRT and radiosurgery is characterized by a high dose gradient in the treated volume. The high dose gradient region demands the measurements to be performed with detectors of a high-spatial resolution. The absolute measurements are usually performed with the cylindrical ionization chambers of a small effective volume. The chambers of effective volume smaller than 0.01 cc are commercially available (Standard Imaging/Exxrad Hemispherical, Planar and Cylindrical). The relative point dose measurements (sometimes absolute measurements as well) are carried out with semiconductor detectors. The diode detectors have extremely small active volumes and high sensitivity to radiation, which is of special interest in dynamic treatment methods. However, their response varies with orientation and the relatively high atomic number of detectors causes a greater sensitivity to the low-energy photons. Alternatively a solid state diamond detector may be used, which is almost a soft-tissue equivalent, and exhibits a small directional dependence.

Not only has the high dose gradient characterized both treatment methods, but the dose delivery is often a dynamic process where the incident fluence and intensity are varying during the treatment. Therefore, measurement methods of calculated dose distributions are limited to integrating dosimetric techniques. Currently commercially available methods for 2D integrating dosimetry are radiographic and radiochromic films, computed radiography, diode and ionization chamber arrays. Radiochromic films and arrays of diodes and ionization chambers are fairly new products in the field of dosimetry. Radiochromic film is almost a perfect detector as it is nearly tissue-equivalent and does not require post irradiation processing (self-developing monomer) for generating the optical density response. However, there are still several pitfalls of using films for a variety of dosimetric measurements. The most important is a limited accuracy due to the lack of specialized scanners for reading out a light transmission and variations in film sensitivity of the same batch. Detector arrays used for dose measurements across the beam represent very popular tools available for routine clinical QA. However, their most important drawback is their small spatial resolution (5 to 10 mm spacing). On the upside, this type of systems is simple and easy to maintain.

In-vivo dosimetry is an important part of a conventional radiotherapy process. However, application of the verification methods in IMRT and radiosurgery is limited to using Electronic Portal Imaging Devices (EPIDs). EPIDs have been used for pre-treatment and in-vivo verification. Although measurements performed with EPIDs require a well calibrated set of corrections they provide important real-time information especially through new and promising transient dosimetry. It is expected that further development of EPID’s dosimetry will continue and resolve the existing issues.

Dosimetry techniques described so far are 1D or 2D methods. Truly comprehensive dosimetric verification of the modern radiotherapy requires 3D dosimetry. Recently investigated new 3D dosimetry systems can be divided into three categories such as polycrylamide gels, Frickel gels, and radiochromic plastics. Application of all of these is rather complex and requires a dose read-out using a magnetic resonance imaging or an optical-computed tomography. Dose measurements are of special importance in image-guided techniques. Recently a MOSFET detectors have been tested for image-guided radiotherapy. The overall uncertainty of the system was determined to be approximately 2% and it is expected to be improved as development continues. However, the ease of use makes it suitable for estimating the dose resulting from image-guided procedures.

### ORAL PRESENTATIONS

#### S23-04. Developments and new trends for radiation accident dosimetry with biological samples and personal items.

This presentation is an overview of recent developments and new trends in the field of physical and biophysical methods considered for radiation accident dosimetry and triage.

In all events of uncontrolled exposure of individuals to ionizing radiation when data from conventional personal dosimetry do not exist (persons from public exposed to orphan or lost radiation source, nuclear and radiological terrorism, absence of dosimeter, radiotherapy or interventional radiology accidents, etc.), it is desirable to have several complementary methods for determining accurately the dose distribution in victim’s organism or for identifying people that need medical cares and additional dose assessment in case of large scale events. Gold method does not exist and the choice of the most appropriate is dependent on cases. Over the last 30 years, bones and tooth enamel measured by Electron Paramagnetic Resonance (EPR) spectroscopy were frequently used as bio indicators of external exposure. EPR remains very pertinent and complementary to biological dosimetry, especially when irradiations are localized or highly heterogeneous, because it can give the absorbed dose in one or several locations in the victim’s organism. Nevertheless, since it requires an invasive sampling, its applicability has been limited. In order to overcome this difficulty and in the light of large scale events implying new needs in terms of measurement capacity for triage, new approaches are investigated.

Since tooth enamel is one of the most sensitive materials for EPR, some new ways to measure it were investigated: it consists in measuring teeth directly in the mouth with EPR at low microwave frequency or in decreasing mass of biopsies (from 100 down to 2 mg) by using high frequency EPR. Some alternative materials measured with conventional EPR are also foresee and are illustrated in this presentation with a specific focus on nails and LCD glass sheet from mobile phones.

In addition, luminescence techniques are also considered with materials from mobile phones (LCD glass sheet, chips, and electronic components) or enamel (ex vivo and in vivo).

We will be giving further insight into these new methodologies and discuss advantages and disadvantages, possible capacity as well as complementarity with biological analysis methods.

#### S24. Radiation chemistry in materials science

Micromachining of polymeric materials are very attractive technology for biomedical micro electro mechanical system (Bio-MEMS) applications, e.g., flow cytometers, biological assays, and nano-scale size filters. However, hand fabrication limits the ability of current devices to be effectively reduced in size. For example, in the case of perfluorinated polymers such as PTFE, crosslinked PTFE (RX-PTFE), there was no suitable solvent for chemical etching to perform wet bulk micromachining, thus micromachining of these polymers had been considerably difficult. In the previous study, the micro-fabrication of RX-PTFE was carried out by synchrotron radiation (SR) direct photo-etching technique. It was found that the fine micro-structure with high aspect ratio could be fabricated. However, a high accuracy nanometer-scale mask is very expensive. In order to realize applications for micromachining of polymeric materials with high resolution, at least three orders higher than those for microwave polymers such as PTFE, crosslinked PTFE (RX-PTFE), biodegradable polymers and aromatic polymers were about two ~ three orders higher than those for perfluorinated polymers, biodegradable polymers and aromatic polymers show 5~6× 10⁻ⁱ⁸ m³/ions cm⁻², 7~9× 10⁻¹⁸ m³/ions cm⁻² respectively. It was found that the etching rates for perfluorinated polymers were about two ~ three orders higher than those for biodegradable polymers and aromatic polymers. Seventeen years ago; Failla’s legacy of synergistic interactions between physicists and biologists has remained its central theme ever since. The second two interactions with scientists who have pulled off this trick, especially Rainer Sachs and Eric Hall. Our field is in many ways dominated by two different two-edged swords. The first, of course, is that radiation can both cause cancer and cure cancer. The second, no less important, is that both physics and biology are needed to understand either of these phenomena. Broadly speaking, the same radiobiological mechanisms dominate both radiotherapy and radiation carcinogenesis. At radiotherapeutic doses, while cell killing mechanisms are diverse and the details complex, the consequences of chromosome aberration play a central role. The similar fractionation patterns shown in radiotherapeutic response and in chromosome aberration formation are perhaps the strongest arguments here. At low radiation doses, induction of chromosome translocations is central, at least for leukemogenesis. And here lie the similarities, because dicentric and translocations are produced by almost exactly the same complex mechanisms. The consequence of these parallel mechanisms is that, even in these days of super-specialization, it is possible for a radiation scientist to contribute meaningfully both to low-dose radiation risk estimation and to improvements in radiation therapy. My own career has been blessed with interactions with scientists who have pulled off this trick, especially Rainer Sachs and Eric Hall. The second two-edged sword in our field relates to physics and biology. Their interactions have been central to both low-dose radiation risk estimation and to radiation therapy. Again I have been extraordinarily fortunate to have worked for most of my career at the Center for Radiological Research, founded by Gioacchino Failla almost a century ago, Failla’s legacy of synergistic interactions between physicists and biologists has remained its central theme ever since. To date it’s fair to say that physics has dominated radiation therapy, and biology has dominated low-dose radiation risk estimation. Moving forward, it seems likely that these roles may finally be reversed. One can easily see physics playing an increasing role in low-dose radiation risk estimation, through technologies such as single-particle microbeams - and one can certainly expect that biology will finally move to its long-anticipated position at center stage in radiation oncology.
S25. Cell adhesion/migration in response to irradiation

S25-01. Targeting the irradiation-induced proangiogenic and proinvasive phenotype. Martin Pruschy, P. Hollenstein-Furmanova, C. Rohrer Bley, K. Orłowski, C. Shen, A. Broggni-Tenzer, University Hospital Zurich, Switzerland

Ionizing radiation (IR) affects multiple cellular components, which induce a multilayered tumor stress response. Para- and autocrine factors are released into the tumor microenvironment in response to irradiation and treatment-activated intracellular stress responses. During the time course of a fractionated radiation regimen they modulate thereby the tumor microenvironment and these processes co-determine the treatment sensitivity of the tumor and eventually treatment outcome. Using a panel of genetically defined tumor cells, derived from different tumor entities, we analyzed the expression and secretion of biologically active factors in response to IR. Such IR-activated modulators of tumor angiogenesis and tumor cell migration eventually represent interesting novel targets for combined treatment approaches. With a specific focus on microtubule stabilizing agents (MSA) we investigated deregulation of important components of the tumor microenvironment by the combined treatment modality of IR with the clinically relevant MSA patupilone. In a lung carcinoma tumor model, IR alone induced hyperangiogenic and proinvasive effects, but hyperangiogenicity was counteracting effect of patupilone was observed when IR was combined with patupilone, on the level of HIF-1α protein stability, VEGF-expression and VEGF-secretion, but only in patupilone-sensitive and not in patupilone-resistant tumor cell lines. Patupilone and IR thereby dysregulated hypoxia-induced tumor angiogenesis, contributing to the potency of this promising combined treatment modality. IR also enhanced the enzymatic activity of secreted matrix metalloproteinases and increased the invasive capacity of several tumor cells. Interestingly low dose patupilone-pretreatment also counteracted specifically IR-induced enhanced MMP activity and patupilone pretreatment completely abrogated this effect.

S25-02. Mechanisms and Consequences of Radiation-Induced Phenotypes. Mary Helen Barcellos-Hoff, New York University School of Medicine , USA

Ionizing radiation is a complete carcinogen, able to both initiate and promote carcinogenesis. While mechanisms leading to initiation, i.e. DNA damage, repair, and mutation, are widely studied, there is growing interest in the contribution of radiation-induced and radiation-stimulated phenotypes that contribute to radiation health effects, like carcinogenesis. Radiation-induced phenotypes are encompassed within a class of radiation responses called non-targeted effects (NTE) that are often observed in the progeny of irradiated cells or at delayed times. An important feature of NTE is switch-like dose dependence, i.e. effects are elicited in vitro by relatively low doses. Non-malignant human mammary epithelial cells exposed to a single exposure (3-200cGy) of either low or high LET radiation are primed to undergo TGFβ mediated epithelial mesenchymal transition (EMT), characterized by loss of junctional complexes, increased motility and greater phenotypic plasticity. Transition through EMT is also increased by IR- induced cell invasion. This effect is mainly regulated by the secretion of the corresponding tissue inhibitors of metalloproteinases (TIMPs). These results indicate that multiple proangiogenic and proinvasive factors are specifically induced by ionizing radiation. Combined treatment modalities of IR with pharmacological agents, which prevent the secretion of these factors, represent a promising treatment approach.

S25-03. Should I stay or should I go? Cell migration after irradiation. Nils Cordes, OncoRay - National Center for Radiation Research in Oncology, Dresden University of Technology, Germany

Whether radiation exposure attenuates or stimulates cell migration and invasion is still under debate. The prime model for such investigations is glioblastoma. This hard to treat and highly aggressive tumor type infiltrates the surrounding normal tissue in a destructive manner. Arguments in favor of radiogenic tumor cell migration/invasion have been exclusively generated in preclinical in vitro and in vivo systems. Moreover, tumor heterogeneity and differentiation are large and do not allow a reasonable interpretation with regard to tumor cell migration and invasion. Further vagueness exists for the distribution
of tumor cells within the tissue at the pretreatment stage. Despite these clinically relevant factors, our molecular knowledge on how tumor cells migrate is in its infancy as underscored by elegant work on ameboid single cell and group cell invasion that even questions the central role of integrin cell adhesion receptors and matrix metalloproteases (MMP) in theses processes. Here, we show data on glioblastoma cell migration, invasion and survival upon exposure to X-rays. Both migration and invasion of tested cells (U138MG, A172, LN229, LN18) either remain unaffected or are effectively hampered by irradiation. Mechanistically, we hypothesize that radiogenic induction of beta1 and beta3 integrins on the cell surface results in local anchorage supported by overwhelming MMP activation and matrix degradation by MMP-2 and -9. To block basal or enhance radiation-dependent decrease of migration and invasion, monoclonal anti-integrin antibodies alone or in combination with MMP inhibitors were effectively applied. Orthotopic tumor cell models, 3D cell cultures and clinically applicable high-resolution imaging on the single cell level seem to be required to reach clarification of this debate and gain further in-depth information about the process of tumor cell migration.

S26. Radiation research award session

S26-01. Experimental radiation-induced heart disease: past, present and future. Marjan Boerma, University of Arkansas for Medical Sciences, USA

Radiation-induced heart disease (RIHD) is a serious side effect of radiotherapy of intrathoracic and chest wall tumors. The threshold dose for development of clinically significant RIHD has recently been shown to be lower than previously assumed. Therefore, research into mechanisms of RIHD has gained substantial momentum. RIHD becomes clinically apparent after a latent time of on average ten years after radiation exposure, but this time can be shorter or longer depending on radiation dose, volume exposed, and additional cardiovascular risk factors. Chronic manifestations of RIHD include accelerated atherosclerosis, myocardial fibrosis and valve abnormalities. There is no method to prevent or reverse RIHD once it occurs. We use a combination of pharmacological and genetic animal models to perform mechanistic studies into RIHD. This presentation will give an overview of our experimental models and present some recent results with regard to the role of certain molecular pathways, such as the kallikrein-kinin system and the epidermal growth factor receptor pathway. The long-term goal of this work is to identify targets by which we may intervene in RIHD, thereby enhancing the efficacy and safety of thoracic radiotherapy.

S26-02. Genetic variation in immunity alters murine response to whole thorax irradiation. Alexandra Panu, C. Haston, McGill University, Canada

Thoracic cavity radiotherapy is limited by the development of alveolitis and fibrosing alveolitis in a susceptible subpopulation of treated patients. To identify genes which influence the lung response to radiation we combined an assessment of a genomewide single nucleotide polymorphism (SNP) and haplotype association evaluation of inbred strain response (fibrosing alveolitis in 27 mouse strains) with prior linkage (loci on chromosomes 1.6,7.9 and 17) and gene expression data. Mice were exposed to 18 Gy whole thorax irradiation and the alveolitis and fibrosis phenotypes were determined. In the inbred strain panel, nine strains developed significant fibrosis (>4.5% of lung), with the remainder succumbing to alveolitis only or alveolitis with minimal fibrosis (<2% of lung). Genome wide SNP analysis identified 10 loci as significantly associated with radiation-induced fibrotic lung disease (p<8.41*10^-10). The most significant SNP lies in a conserved non-coding region downstream of Cadm1 located in the linkage interval on chromosome 9. The polymorphism potentially disrupts a cis regulatory element, as the cell adhesion molecule gene shows differential expression between fibrotic and alveolitis-prone strains in our panel. Within previously identified loci other genes containing SNPs associated with fibrosis include Slamf6 P16 and Cika which function in immune responses. Combining genomic approaches identified variation within specific immunity genes as associated with the fibrotic lung response to thoracic irradiation in mice. Knowledge of genetic factors predisposing to lung disease in mice could eventually permit pre-treatment identification of radiation-sensitive or resistant patients which would assist in defining patient specific radiotherapy.

S27. Tumor hypoxia and radioreistance

S27-01. Molecular mechanism behind HIF-1-mediated radioreistance and postirradiation recurrence of tumors. Hiroshi Harada1, M. Hiraoka1, 1: Career-Path Promotion Unit, Kyoto University, Japan 2: Kyoto University Graduate School of Medicine, Japan

Tumor recurrence frequently occurs after radiation therapy. Hypoxia-inducible factor 1 (HIF-1) has been strongly associated with radioreistance and post-irradiation recurrence of tumors, but underlying mechanisms have not been fully elucidated. The purpose of this study is to elucidate the mechanism underlying HIF-1-mediated radioreistance and recurrence of malignant tumors. In the present study, we monitored HIF-1 activity in a subcutaneous tumor xenograft with a novel SHRE-ODD-luc reporter gene, which expresses ODD-luciferase fusion protein under the regulation of a HIF-1-dependent SHRE promoter. In addition, Applying a Cre-ER72loxP-dependent site-specific recombination system, we established a unique strategy to tag hypoxic tumor cells with luciferase proteins and performed cell tracking experiments to analyze the fate of the tagged cells during and after radiation treatment. The optical imaging experiments revealed that 1) radiation up-regulates intratumor HIF-1 activity and induces VEGF expression, 2) VEGF protects endothelial cells from cytotoxic effect of radiation, and 3) the radio-protected tumor blood vessels supply oxygen and nutrients to tumor cells and assure tumor growth. In addition to such an indirect function of HIF-1 in tumor radioreistance, the cell tracking experiments clearly showed that hypoxic tumor cells predominantly survive radiation therapy, migrate to tumor blood vessels in HIF-1-dependent manner, and mainly cause recurrent tumors after radiation therapy. Our findings provide rational basis for combining radiation therapy with hypoxia- and HIF-1-targeting therapies.

S27-02. Novel oxygen sensitive signalling pathways and their potential as therapeutic targets. Bradley G. Wouters, Ontario Cancer Institute, Toronto, Canada

No abstract

S27-03. Imaging of Hypoxia-Induced Radiation Resistance and Treatment Response. Rehan Ali, E. Graves, A. Giaccia, Stanford University, USA

AIM: With the advent of methods for noninvasively imaging hypoxia, there is significant interest in applying this technology towards prospectively identifying hypoxic tumors and tailoring treatments to their specific radiosensitivities. We used hypoxia PET to interrogate murine tumor models, and followed their response to a variety of radiotherapy regimens with a molecular imaging methods.

METHODS: 18F-EFD was synthesized using a modified version of the published synthetic protocol. Mice bearing subcutaneous, orthotopic, or spontaneous tumors of the lung, head and neck, or pancreas were injected with 200 uCi of EFD and imaged using microPET at three hours post-injection. Radiotherapy was delivered in between one and four daily fractions to total doses between 10 and 40 Gy, using a collimated single field irradiator. Tumor response was measured in terms of change in size by daily caliper measurements and weekly x-ray computed tomography (CT) imaging, and in terms of functional response by periodic fluorescence imaging (FDG) and EFD PET as well as bioluminescence imaging (BLI) and fluorescence imaging using an angiogenesis-specific integrin-binding peptide probe.

RESULTS: Associations between initial EFD uptake and radiotherapy response as measured by change in tumor size were noted for single fraction treatments, while multifraction treatments demonstrated no significant differences in outcome between tumors with high and low initial EFD uptake. A variety of responses to radiotherapy were noted amongst the measurement strategies employed, including an inflammatory response evident by FDG PET and fluctuating oxygenation post-treatment.

CONCLUSIONS: Hypoxia imaging is significantly associated with radiotherapy response in preclinical model systems. Prospective evaluation of these findings as well as clinical translation are now in progress.

S28. Computational approach to understanding DNA protection by protein binding

S28-01. Effect of protein binding to direct and indirect radiation damage to DNA. M. Kier, N. Mar, M. Čepič, M. Špokoný-Maurizi, 1: Nuclear Physics Institute ASCR, Czech Republic 2: Centre de Biophysique Moléculaire CNRS, France
Binding of proteins to their cognate DNA modulates radiation damage of both partners. Theoretical simulation can provide a realistic estimation of yields and distributions of radiation damages in complex cellular targets such as DNA complexes with proteins. It can be therefore used as a tool to elucidate the role of protein interaction in processes of radiation damage to DNA.

Theoretical model RADAMOL, based on Monte Carlo technique has been developed to simulate direct and indirect radiation damage to biological target represented by its atomicistic conformation. Time-space evolution of charged particle tracks is followed from initial energy deposition by charged particle in water up to the end of chemical stage with non-homogeneous distribution of radical species (10^5’s). Direct ionizations of DNA or surrounding bound water molecules lead to electron and hole generation followed by charge migration and localization in DNA. Indirect damage comprises lesions formed by chemical reactions of deoxyribose, nucleobases and amino-acids with OH-, e^- and H-radical species. The spectrum of simple and complex damages in the target is achieved.

Using RADAMOL, radiation damage of free DNA lac operator and DNA with bound lac repressor protein has been calculated for electrons, protons and alpha particles. The effect of lac repressor binding on overall DNA damage will be demonstrated. The contribution of direct and indirect radiation action to the spectrum of simple and complex DNA damage will be discussed with regard to radiation track structure and protein binding to DNA.

S28-02. The action of amino acids on electron irradiated DNA films. Sylwia Ptasińska1, L. Sanchez1, 1: University of Notre Dame, USA 2: Groupe en Sciences des Radiations, Département de Médecine Nucléaire et de Radiobiologie, Faculté Médecine, Université de Sherbrooke, J1H 5N4, Sherbrooke, Québec, Canada

The damage to DNA induced by ionizing radiation can occur through many different reactions, e.g. electron transfer or radical attack. The type of damage, as well the amount of damage induced depends upon the presence of other molecular components in close proximity to DNA, particularly the proteins.

In these experiments a short chain of single stranded DNA with and without of amino acids was exposed to very low energy electrons, ~1eV, and total fragmentation yield was detected. It has been suggested that such an increase in DNA damage is mostly due to the formation of H radicals produced via dissociative electron attachment to glycine [2]. At higher ratios of amino acid/nucleotide, glycine and arginine appear to shield DNA from the direct action of these low energy electrons. However, the level of radioprotection of DNA is higher in the case of arginine. This could be related to the stronger intermolecular reactions between arginine and nucleotides than in the case of glycine and nucleotides.

In order to get further insight into the role of amino acids on DNA the binding energies for amino acid nucleobase/nucleotide pairs were calculated by applying forcefield theories. From computational modeling of the interactions (i.e. van der Waals, electrostatic and hydrogen bonds) between these amino acids and DNA units the binding energies obtained are higher for arginine than glycine.

References:

S28-03. The Development of New Radioprotectors – DNA Binding Studies with Methylproamine Analogues. Roger Martin1, C. Skene2, J. White3, P. Lobachevsky3, 1: Peter MacCallum Cancer Centre, Australia 2: Bio21 Institute, University of Melbourne, Australia 3: Bio21 Institute, University of Melbourne, Australia

Methylproamine was the first lead compound in a programme aimed at developing new DNA binding radioprotectors for protection of normal tissues in radiotherapy patients by topical application. Although a potent radioprotector, methylproamine has an innate cytotoxicity imposing a narrow concentration range between radioprotection and cytotoxicity. Our lead optimisation program, involving the design and synthesis of over 150 analogues of methylproamine, has identified new lead compounds that are both less cytotoxic than methylproamine, but without compromise of radioprotective activity. Indeed, some of the analogues are substantially more active than methylproamine. Starting from the basic structural features of methylproamine, namely the methyl piperazine ring, the link to two benzimidazoles and a substituted phenyl ring, the analogues reflect modification in one or more of these four features. The DNA binding of many analogues has been studied by a spectrophotometric method involving the use of oligodeoxynucleotides with a defined minor groove binding site (namely GATTCC). For a subset of analogues the binding studies have extended to oligodeoxynucleotides without the minor groove binding site. The binding measurements have been supplemented with docking calculations using Autodock4, developed at the Scripps Institute. Analogues in which one or both benzimidazoles are replaced with other heterocycles often have increased cytotoxicity, associated with a switch of the predominant binding mode from minor groove to apparent intercalation. This is usually associated with a profound decrease in radioprotective activity suggesting that minor groove binding is an important requirement for radioprotection. This conclusion is also supported by the low radioprotective activity of analogues with benzimidazole substituents that prevent approach of the ligand to the minor groove. However it also clear from the structure-activity studies, that DNA binding by no means the sole determinant of radioprotective activity. Our working hypothesis, which is supported by early pulse radiolysis studies, is that DNA bound ligands reduce transient radiation-induced oxidising species on DNA. This hypothesised mechanism implies electron donation from the ligand into the DNA and movement along DNA to the lesion.

S28-04. Structural analysis of the interaction between the Ku protein and DNA. Hirofumi Fujimoto1, K. Saito1, K. Tsudhida1, H. Maekawa1, N. Hondo1, T. Sakurai1, T. Kotulic Buntar1, T. Nematoto1, M. Pinak1, H. Oke1, M. Koike2, M. Higuchi2, 1: National Institute of Infectious Diseases, Japan, 2: Japan Atomic Energy Agency, 3: University of the Ryukyus, 4: Research Organization for Information Science and Technology, 5: National Institute of Radiological Sciences, Japan

Ku is an abundant nuclear protein that plays an important role in repairing double-strand breaks in DNA, which arise from exposure to ionizing radiation or chemical agents. Human Ku is composed of two homologous subunits, Ku70 and Ku80, and contains a ring structure that can encircle DNA. Several lysine residues on Ku70 have been reported as a target of acetylation that controls the interaction with other proteins and nucleotides. Three lysine residues inside the ring of Ku70 (K317, K331 and K338) are acetylated in vivo. The physiological significance of acetylation on those lysine residues inside the ring is still unclear, but the mutation analysis demonstrated that the ability of Ku to bind DNA was suppressed in vitro.

In this study, we examined the acetylation effect of those three lysine residues on the affinity of Ku with DNA applying computer simulation techniques. Experimentally, the effects of acetylation have been studied using recombinant mutants in which lysine residues are substituted with glutamine as a mimic of acetylated lysine (KQ mutant), or with arginine as a mimic of nonacetylated lysine (KR mutant). To design experimentally verifiable molecular models, we replaced three lysine residues on the wild type Ku70 ring with arginine, glutamine and acetyl lysine independently.

Comparing with the wild type Ku, the binding free energy was reduced in the KQ mutant while the KR mutant had no effect, which is consistent with previous experimental results. Unexpectedly, the binding energy between Ku and DNA was maintained at almost the same level as in the wild type protein despite full acetylation of the lysine residues. These results suggest that acetylation of the lysine residues in the Ku70 ring did not remarkably reduce the affinity of Ku for DNA, although those residues have been acetylated in in vivo studies. In addition to lysine residues inside the Ku ring, the in vivo acetylation of lysine residues has been reported at the C-terminal interdomain region of Ku70. The acetylation at this region is thought to control the interaction between Ku and Bax, a pro-apoptotic factor that associates with mitochondria-dependent apoptosis. In our Ku-DNA complex models, those lysine residues are likely to interact with DNA and/or Ku80. Possible mechanisms will be discussed.

S29. Radiation chemical studies of bioactive compounds

S29-01. Mechanistic studies on herbal drugs and their active ingredients in relation to their antioxidant and radioprotection ability. TULSI MUKHERJEE, Bhabha Atomic Research Centre, India
Antioxidant research in Chemistry Group, BARC started with an idea to show that physico-chemical methods are very useful in understanding the efficacy and mechanism for the reaction of the biologically important radicals with natural and/or synthetic organics. With this view several state of the art facilities have been developed in this group during last decades. The physicochemical parameters like redox potential, reactivity, half-life, chemical transformation and stable end products determine the positive and adverse effects of the test molecules in use. Results from these studies are expected to provide a clear view of the electron transfer processes that occur both in free radical induced damage as well as repair by antioxidants. The kinetic parameters e.g., formation and decay rate constants predict the efficacy of an antioxidant and its fate after the reaction. Radioprotection is another very important area of research being pursued in our group. Several natural and synthetic molecules are in different levels of studies. Here again radiation chemical and radiation biological research are being carried out in hand. New molecules are being synthesized for testing their antioxidant as well as radioprotective action and the mechanism of their chemical activity.

In this article starting with the description of the facilities available, discussion will also be continued on herbal extracts and their active ingredients as their efficacy in antioxidant and radioprotective effects. The systems chosen for discussions will be Indian herbal extracts such as triphala extracts, chevvetol from beelit leaves, curcumin from turmeric, methoxyphenols from Indianaliacholin, dehydrogingerdione from ginger, bakuchiol from Poria corylifolia, barberine and jetorhizin from Tinospora corylifolia and few selected selenium compounds.

S29.02. Radioprotection by the soy isoflavone genistein. Michael Piotrowski, J. Joseph Normandie and V. A. Landauer, Armed Forces Radiobiology Research Institute, Uniformed Services University, USA

Radioprotective agents are chemical compounds that are administered prior to irradiation and provide protection from ionizing radiation-induced injury. They have applications for use in clinical oncology, space travel, emergency responders, and military scenarios. The ideal radioprotector would be nontoxic, increase survival, could be stored without refrigeration, have a long shelf-life, and would not degrade performance. The use of the soy isoflavone genistein as a radioprotective agent using adult CD2F1 male mice will be discussed. A single subcutaneous (SC) or intramuscular (IM) injection of genistein provided optimal radioprotection when administered 24 h before a total body irradiation dose (LD90/30) of 50 Gy gamma radiation. There was a significant increase in 30-day survival for animals receiving genistein, with the optimal dose being 200 mg/kg. Genistein also resulted in significant increases in survival when administered by oral gavage for two to six days prior to irradiation. The improvement in genistein-induced radioprotection was related to accelerated neutrophil and platelet recovery, resulting from earlier and more pronounced multilineage, hematopoietic progenitor cell reconstitution in the femoral marrow compartment. Protection of the bone marrow was also associated with a genistein-induced transient pause in the cell cycle where hematopoietic stem cells remained in the Go quiescent phase. Genistein also induced increases in serum levels of granulocyte colony-stimulating factor (G-CSF) 4-24 h after injection. In sublethally irradiated mice, an accelerated rate of body weight recovery was observed in genistein-treated mice. The genistein doses used did not result in any adverse behavioral or histopathological effects when evaluated in normal nonirradiated animals. These results demonstrate that genistein at nontoxic doses is an effective radioprotective agent when administered parenterally or orally to mice.

S29.03. Novel tools in the research on antioxidants - the global profiling of ROS/RNS in cell-free and cellular systems. Tomas Scholz, GSI, Darmstadt, Germany, Gisela Kalyanaraman, NIH, Bethesda, USA

Ionizing radiation causes genomic instability by DNA damage. Consequently, a sophisticated signal transduction network has evolved to sense DNA damage and respond appropriately. DNA damage triggers the activation of cell cycle arrest pathways and the induction of several DNA repair processes. In the case of irreparable damage, different responses occur which result in cell death by senescence, apoptosis, autophagy or by a classical radiation-induced mitotic cell death. This presentation will review the interactions between cell death pathways and molecular responses with respect to the radiation damage.

EO20. Radiation and DNA lesions: when quality matters. Gisela Taucher-Scholz, GSI, Darmstadt, Germany

In contrast to gamma- or X-rays, high- linear energy transfer (LET) charged particle radiation induces highly localized DNA damage. The spatially correlated lesions induced include damaged bases as well as...
Single- and double-strand breaks (DSBs) in close proximity. The resulting quality of the complex lesions produced is the basis of an increased biological effectiveness, which is of advantage in carbon ion tumor therapy but of concern during space travel. This session will address the molecular consequences of spatially localized DNA damage. Energy deposition by ionizing radiation occurs via secondary electrons independent of radiation quality. Biophysical modelling indicates that the spatial distribution of ionizing events is the critical feature determining different biological effects due to lesion clustering. Studies using plasmid DNA, cells or tissue have provided data on the induction and repair of clustered DNA damage, without directly reflecting biological effectiveness. Recently, using repair proteins as surrogate markers for DNA lesions, ion-induced damage tracks have proved useful for the visualization of induction and processing of DNA damage at the single-cell level. Immunofluorescence microscopy or live cell imaging techniques have thus revealed the spatiotemporal dynamics of repair proteins. A remarkable positional stability of DSBs became apparent, independent of radiation quality. However, differences in the quality of the DNA damage induced are related to impaired and/or error prone repair.

Besides lesion quality, chromatin structure has recently emerged as a factor influencing the damage response. Although the subcellular localization of damage does not reduce the accessibility to repair, chromatin complexity indicates a requirement for additional repair factors. In heterochromatin regions that are rich in repetitive DNA sequences, the processing of damage from highly condensed to lower density euchromatic areas may be a conserved mechanism to prevent potentially deleterious repair events.

E021. Health effects of exposure to radiation from residential radioactive building materials. Ping-Kun Zhou, Beijing Institute of Radiation Medicine, China

A series of case-control studies have been conducted and reported in world-wide on the health effects of radiation exposure from increased level of residential radon in houses as well as the building materials containing naturally occurring radionuclides as (40)K, (232)Th, and (238)U and their progeny, or cobalt-60-contaminated buildings. The exposure levels in most of these reported populations were within several mSV per year. These low levels of exposure to radioactive building materials resulted in a small excess risks in some reports, and no obvious effects on health, especially on clinical outcome in others. It has been demonstrated a certainly increased lung cancer risk in a high residential radon exposure area in Gansu Province, China. However, there has not found any increased cancer risk associated with the high levels of natural radiation in a high background radiation area (HBRA) of Yangjiang in China. The average annual effective dose is about 6.4 mSv in this HBRA. On the contrary, the mortality of all cancers in HBRA was generally lower than that in control area, but not significant statistically. An increased cytogenetic aberration was found in HBRA population as compared with the control population. The abnormal chromosomal changes or micronucleus was also reported in others cases living in houses with high level of exposure to radioactive building materials. It is likely that the cytogenetic change from genomic lesion is a sensitive index to low dose irradiation. Here the health effects have also been reviewed on a number of studies on the residents ever living in the cobalt-60-contaminated buildings.

E022. Optimising drugs that target redox pathways. Bob Anderson, University of Auckland, New Zealand

Oxygen plays a pivotal role in inhibiting drug metabolism by reductases which function via a one-electron reduction pathway. Use is made being of this redox pathway in the development of bioreductive produgs to selectively release cytotoxins to kill hypoxic cancer cells, which are resistant to front line chemotherapy drugs that function on aerobic cells in cycle. The problem of hypoxic tumour cells resistance to treatments was discovered by Gray and colleagues some 60 years ago, where they showed increased cell kill by radiation in the presence of oxygen compared to cells irradiated in anoxia. The discovery spawned much interest in the search for compounds which could penetrate into hypoxic regions of tumours and mimic the effect of oxygen. Advances were made in the development of the drug formaldimide as radiosensitizer, through the quantification of their redox properties but culminated with, in the main, unsuccessful clinical trials using nitromidazoles in the 1970-1980s. Part of this failure was due to the inherent redox-related normal tissue toxicity of these drugs, possible through futile redox cycling with oxygen, which restricted usable doses of these drugs to less than optimum levels to exhibit radiosensitisation with fractionated radiotherapy. The emergence of regimens of stereotactic ablative radiotherapy, where a few tightly focussed large radiation doses are combined with a radiosensitizer, is giving fresh impetus to the improved use of radiosensitizers in the treatment of solid tumours. Whereas bioreductive produgs do also exhibit cytotoxicity towards cancer cells, these cytotoxins can be designed to be released to attack hypoxic cells, which should result in a high therapeutic index. In this presentation, the pathways by which different classes of cytotoxins are released or unmasked are discussed together with examples from our laboratory on how classes of redox active drugs can be optimised for killing not only cancer cells but can be targeted to killing invasive micro-organisms.

E023. Planning/responding to radiological or nuclear terrorism. Viktor Meneke, Bundeswehr Institute of Radiobiology, Germany

Latest since the terrorist events of 9/11 risk perception in the field of potential radiological or even nuclear scenarios has dramatically changed. In earlier times, especially in the face of cold war threat scenarios, knowledge, education and training to cope with the consequences of a nuclear attack had primarily been specific expertise of military institutions, now this special knowledge has also to be made available to a broader community of emergency planers, physicians as well as first responders. This fact means to discuss intriguing facts of unimaginable and sensitive scenarios in a wider community. Bearing attention to this circumstances diverse perceptions and thus precautionary planning activities can be observed in different countries around the world. These differences in the perceived risks result in different levels of preparation. Planning for a dirty bomb scenario implies preparedness to handle both conventional injuries due to the explosive device as well as a potential contamination of patients and wounds. It is estimated that in case of such an event the number of victims will be in the range of 10-50. It has to be taken into account that in most countries the medical health system (first responders and hospital level) have not been adequately trained to handle contaminated persons. The surgical care for patients with incorporated radioactive shrapnels moreover remains a challenge. Psychological effects for the public pose the most important difficulties.

In case of a nuclear scenario planning and response has to take into account thousands of direct and indirect affected victims, including thermal, blast and combined injuries. The latter scenario implies preparedness on a total different level as compared to a small scale dirty bomb scenario. In a nuclear scenario handling of an indescribably high number of victims shifts the perspective from the cure of individual patients to a pure mass casualty processing and the respective means and tools, such as triage, stockpiling, allocation of scarce resources will turn out as the limiting factors. Specific aspects of both scenarios and resulting implications for response planning will be addressed. The important role of the radiotherapy community in this context will be discussed.

S30. Biological effects of low doses

S30-01. Biological effects at low doses - European Low Dose Risk Research Strategy. Sisko Salomaa, STUK- Radiation and Nuclear Safety Authority, Helsinki, Finland

Although much is known about the quantitative effects of exposure to ionising radiation, considerable uncertainties and divergent views remain about the health effects at low doses. In 2009, the European High Level and Expert Group (HLEG) identified key priority questions to be addressed by a joint strategic research agenda and initiated the establishment of a European Research Platform, called MELODI (Multidisciplinary European Low Dose Research Initiative). In 2010, a Network of Excellence called DoReMi was launched by the Euratom FP7 programme. DoReMi will act as an operational tool for the development of the MELODI platform during the next six years. The joint programme for research focuses on the areas identified by the HLEG as the most promising in terms of addressing/resolving the key policy questions, namely: the shape of dose response curve for cancer, individual susceptibilities and non-cancer effects. Radiation quality, tissue sensitivities and internal exposures will be addressed as cross cutting themes within the three main research areas. DoReMi defines low doses as those of 0.1 mGy or less and medium doses as 0.1 Gy - 1 Gy for low LET radiations. Low and medium dose rates are defined as 0.1 Gyh or less for low LET radiations. For high-LET radiations, dose and dose-rates of interest are lower, e.g. for alpha particles by an order of magnitude. The DoReMi Network of Excellence has received funding from the European Atomic Energy Community’s Seventh Framework Programme (FP7/2007-2011) under grant agreement n° 249689.
S30.02. What do we know about the mechanisms of cancer induction and how might this affect the shape of the dose-response at low doses. Christophe Badjie, Health Protection Agency, UK

Cancer is a common disease in human populations, particularly where infectious diseases do not contribute significantly to premature deaths. Huge efforts have been made in worldwide research with the causes and treatment of cancer. However we still do not have a comprehensive understanding of the mechanisms of cancer induction. Radiation protection depends primarily on epidemiological studies of exposed populations to establish health risk estimates. However direct estimates of cancer risk are not available below around 100 mSv. Below this level risks are estimated by linear extrapolation from risks at higher doses. The validity of linear extrapolation is supported by biophysical evidence on the linearity of radiation damage induction in DNA with dose and knowledge that mutations and large scale genetic alterations are associated with cancers. Basic cancer research has now led to the development of a more complete understanding of the fundamental characteristics of cancer. This includes characteristics of the cancer cells themselves and the tissue environment in which they proliferate. This talk will provide an overview of the effects that radiation has on the processes known to contribute to carcinogenesis and what is known about relevant dose-response relationships.

S30.03. The Risk of Cancer from Low Level Exposure to Radiation – the Epidemiological Evidence. Richard Wakeford, Dalton Nuclear Institute, The University of Manchester, UK

The great majority of epidemiological evidence for an increased risk of cancer following exposure to ionizing radiation comes from studies of those exposed acutely to high doses and high dose-rates, such as the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki in 1945. Risk models based on these studies assume that the excess risk of cancer is linear at low doses and low dose-rates, and that there is no threshold dose below which an excess risk is absent. However, the radiation-induced risk at low doses (<100 mSv) is small, such that it is difficult to definitively detect the predicted excess risk against the background noise of statistical fluctuations in the baseline rates. Nonetheless, direct evidence for a radiation-related risk at low doses does exist. Studies of those exposed to medical diagnostic radiation have indicated an excess risk following doses of ~10 mGy of X-rays: breast cancer among those receiving fluoroscopic examinations during treatment for tuberculosis or the X-ray examination of scoliosis patients, and a raised risk of childhood cancer following obstetric radiography. Studies of paediatric CT scans should show a raised risk of childhood leukaemia if our risk models are right. One of the most promising fields of research is the study of nuclear industry workers. The early workers were exposed to levels of radiation that would not be acceptable today and many received moderate doses (>100 mSv), but accumulated over decades as a series of small doses. These workers are now reaching the end of their lifespan, and so informative data are growing, leading to studies with reasonable power to detect health effects. The Third Analysis of the UK National Registry for Radiation Workers found statistically significant positive trends with cumulative recorded dose for leukaemia and for all cancers other than leukaemia, and similar studies in other countries should also provide interesting findings of considerable relevance to the low dose risk of cancer.

S30.04. What do we know about the factors underlying individual susceptibilities and how large is this variation? Michael Atkinson Helmholtz Zentrum München, Germany

No abstract

S30.05. Molecular epidemiology and low dose risk. Elisabeth Cardis CREAL, Barcelona, Spain

No abstract

S30.06. What do we know about the mechanisms of non-cancer effects at low and moderate doses? Jean-Rene Jourdain, IRSN, France

No abstract


The Radiation Effects Research Foundation has investigated the late health effects of the atomic-bomb radiation in the survivors (Life Span Study). Exposure to radiation was calculated based on information about location and shielding at the time of the bombings that was collected in 1950s and uses the latest version of the dosimetry system, DS02. Outcomes include death and cause of death, and records of cancer patients by their national population registry and death certificates from all areas of Japan. During the period 1950-2003, 58% of the 86,611 cohort members who were in the cities at the time of the bombing and have a dose estimate have died. The risk of all-cause mortality significantly increased in relation to radiation dose. For solid cancers the additive radiation risk (i.e., excess cancer cases per 104 person-years per Gy) continues to increase throughout life, with a linear dose-response relationship. The sex-averaged excess relative risk per Gy (ERR/Gy) was 0.42 (95% confidence interval: 0.32, 0.53) for all solid cancer at age of 70 years after exposure at age 30 based on a linear model. For each decade decrease in age at exposure, the risk increased by about 29% (95%CI: 17%, 41%). The estimated lowest dose range with a significant ERR for all solid cancer was 0 to 0.20 Gy and zero dose was the best estimate of the threshold. The risk of cancer mortality significantly increased for most major sites including stomach, lung, liver, colon, breast, gallbladder, esophagus, bladder, and ovary, whereas the rectum, pancreas, uterus, prostate, and kidney parenchyma did not have statistically increased risks. An increased risk of non-neoplastic diseases including the circulatory (ERR/Gy=0.11, 95% CI: 0.05, 0.17), respiratory (0.21, 95% CI: 0.10, 0.33), and digestive system (0.11, 95% CI: -0.01, 0.24) was observed, but whether these are cause relationships remains further investigation. There was no evidence of a radiation effect for infectious or external causes of death. These findings are consistent with the previous reports and provide increased precision in risk estimates. Further study should continue to clarify additional aspects, particularly the effects of radiation exposure at young ages. 80% of those who were exposed have died. The survival of 20 years or younger are still alive at the end of period for this analyses.

S31.01. Repair mechanisms of DNA strand breaks identified by visualizing proteins in human cells. Akira Yasui, IDAC, Tohoku University, Japan

DNA repair in living cell requires detection of DNA damage and recruitment of DNA repair proteins to the site of the damage in chromatin. To understand this process within cell, we have developed methods for local production of various types of DNA damage within the cell. We produced DNA single-strand breaks (SSB) by UV-irradiation through porous filter to human cell expressing UV damage endonuclease (UVDE) (1) and by laser micro-irradiation (2). Using these methods we have firstly characterized SSB repair process and identified novel proteins required for the activation of poly(ADP-ribose) polymerase PARP (3). For non-homologous end joining (NHEJ) of DNA double-strand breaks (DSB), we established a cell line with multiple restriction sites to visualize proteins involved in NHEJ at DSB under microscope and identified the chromatin-remodeling complex, CHRAC, which is required for the accumulation of Ku proteins at DSB in a damage-induced manner (4). Thus, the visualization of the DNA damage response is a powerful tool for reconstituting the repair process within cell. Recent progresses in this field will be presented and discussed.


S31.02. STUDY OF EARLY DNA DAMAGE RESPONSES AFTER CHARGED PARTICLE IRRADIATION BY BEAMLINE MICROSCOPY. Burkhard Jakob, GSI Biophysik, Darmstadt, Germany

Approaches to visualise DNA lesion processing substantially contribute to the understanding of the DNA damage response organisation. Charged particle irradiation has emerged as a tool to generate discrete sites of subnuclear damage by means of extremely localised dose deposition, thus facilitating the spatiotemporal analysis of repair even at low doses. We developed a remote controlled beamline microscope capable of measuring the very early cellular responses after low energy ion irradiation. Recently, we could demonstrate that the device can also successfully be used at the high energy branch (1GeV/u) of the University of Manchester, UK, and Darmstadt, Germany. As a major application, beamline microscopy allows determining the real time recruitment kinetics of fluorescently tagged repair proteins after irradiation with charged particles. The classification into fast recruited and slow breaks identified by biophysical evidence on the linearity of radiation damage induction in DNA with dose and knowledge that mutations and large scale genetic alterations are associated with cancers. Basic cancer research has now led to the development of a more complete understanding of the fundamental characteristics of cancer. This includes characteristics of the cancer cells themselves and the tissue environment in which they proliferate. This talk will provide an overview of the effects that radiation has on the processes known to contribute to carcinogenesis and what is known about relevant dose-response relationships.
proteins like DNA-PK or XRCC1 or slower ones like 53BP1 in combination with molecular dependencies helps to establish the hierarchical organisation of damage recognition and subsequent repair events. Interestingly, XRCC1 shows similar recruitment constants, but significant differences in the retention times for eu- and heterochromatin compartments. Complementary to the recruitment of repair proteins we started to examine their binding characteristics at the damaged sites in relation to the damage density and complexity by FRAP analysis. Immunocytochemical analysis of radiation induced foci provides information about the mobility of DSBs. By evaluation of the displacement of GFP-53BP1 foci in live cells up to 12 hours post-irradiation, we detected a slow mobility of damaged chromatin sites both after particle or X-irradiation similar to that of undamaged nuclei. Our data indicate that the repair of high LET radiation induced DSBs in mammalian cells is not coupled to an increased motional activity of lesions enhancing the probability of translocations. The occasional appearance of cluster formation of radiation-induced foci may represent a higher mobility of chromatin along the ion trajectory. These observations support the hypothesis that spatial proximity of DNA breaks is required for the formation of radiation-induced chromosomal exchanges. DSB stability thus might be a strategy to prevent cancer formation.

S31-03. Movement of DNA double-strand breaks. Jacob Atten, T. Borovski, J. Stap, K. Franken, P. Krawczyk, University of Amsterdam, Netherlands

DNA double-strand breaks (DSBs) can efficiently kill cancer cells, but they can also produce unwanted chromosome rearrangements (CRs) when DNA ends from different DSBs are erroneously joined. Movement of DSB-containing chromatin domains might facilitate these DSB interactions and promote formation of CRs. We present microscopy data on the movement of DSBs produced by X-rays and alpha particles in fixed and in living mammalian cells. We will also report on the influence of agents that affect higher-order chromatin organization. The results will be discussed in the context of the mobility of non-damaged chromatin, on a time-scale relevant for DSB repair.

S31-04. Visualization of Spatio-Temporal Dynamics of Ionizing Radiation Induced Clustered DNA Lesions. Aroumougame Asarathabh, D. J. Chen, Molecular Radiation Biology, Department of Radiation Oncology, University of Texas Southwestern Medical Center at Dallas, USA

Cellular approaches, including indirect immunostaining, in combination with in vitro assay systems have significantly improved our understanding of induction and repair of different DNA damages in response to ionizing radiation (IR); however, some of the challenging questions in the study of IR induced DNA damages cannot yet be answered. We have established a new approach that allow us to directly monitor dynamics of DNA repair factors in response to gamma- and high-charged and energy particles irradiation at single cell levels. 53BP1 fused to yellow fluorescent protein (YFP-53BP1) as a DNA double-strand break (DSB) surrogate marker let us to follow single DSB from induction to repair. Using this approach we found that the DSBs induced by very low g-radiation doses (5 mGy) were repaired with efficiency similar to repair of DSBs induced at higher doses. Live cell imaging also revealed that the YFP-53BP1 foci are dynamic structures: 53BP1 rapidly and reversibly interacted at these DSB sites, and the time frame of recruitment and affinity of 53BP1 for DSB sites were indistinguishable between low and high doses, providing mechanistic evidence for the similar DSB repair after low- and high-dose radiation. In addition, using a combination of live cell and immunostaining approach, we found that high-LET IR induced clustered DNA lesions are refractory to repair and it is because of the unique spatial distribution of different types of DNA lesions within the clustered damages, but not the physical location of these damages within the sub-nuclear domains, determined the cellular ability to repair the damage. Further, this approach allowed us to directly measure the repair capabilities of cells grown in three-dimensional Matrigel culture, and we found that the IR induced DSBs repair is significantly attenuated in cells grown in three-dimensional Matrigel culture as compared with monolayer cultures. Thus, with this unique approach we can explore the spatial dynamics of IR induced DNA lesions in cells but also can find answers to some of the challenging questions in the study of IR induced DNA damages.

S32. New tools in biological dosimetry

S32-01. The automated micronucleus assay as a reliable biodosimetric tool for population triage in large scale radiation accidents. Hubert Thierens1, A. Vrl2, H. Romm3, U. Oestreicher4, S. Baron5, K. Rothkamm3, E. Ainsbury1, S. Sommer2, C. Benike1, A. Wojcik1, 1: University Ghent, Belgium, 2: Bundesamt für Strahlenschutz, Germany, 3: Health Protection Agency, UK, 4: Institute of Nuclear Chemistry and Technology, Poland, 5: Institut für Radiobiologie der Bundeswehr, Germany, 6: Stockholm University, Sweden

Introduction: Mass casualty scenarios of radiation accidents require high throughput techniques of biological dosimetry for population triage to identify individuals for whom clinical treatment is indicated. To this end the micronucleus assay with automated scoring of micronuclei by computerized image analysis is a very suitable technique. Within the MULTIBIODOSE EU FP7 project a network of biodosimetry laboratories with expertise in automated micronucleus scoring is installed.

Results: Within the network the Metasysytems MSearch image analysis system is used by all partners for automated scoring of micronuclei. A standardized protocol adapted for the automated micronucleus assay was set up based on DAPI nuclear staining. Classifier settings for automated analysis with the Metafer system were optimized. This protocol with classifier settings was applied to blood samples irradiated in vitro and distributed by UGhent to all participating laboratories. All partners of MULTIBIODOSE performed a completely automated analysis and a semi-automated scoring with off-line inspection of the image gallery of binucleated cells containing micronuclei. Preliminary statistical analysis of the data points to a satisfactory agreement among the laboratories. To obtain reliable results in the low dose range (<1 Gy) the semi-automated scoring is indicated. To this end reference image galleries with sets of binucleated cells are distributed among partners. At the moment common dose response curves for acute and protracted total body dose and acute partial body exposure are determined.

Conclusions: The results obtained up to now within the MULTIBIODOSE project are very promising for application of the automated micronucleus assay as a high throughput technique for biodosimetry in large scale accidents by a network of collaborating laboratories throughout Europe.


In the event of a large scale radiological emergency biological dosimetry is an essential tool that can provide timely assessment of radiation exposure to the general population and enable the identification of those exposed people, who should receive immediate medical treatment. A number of biodosimetric tools are potentially available, but they must be adapted and tested for a large-scale emergency scenario. These methods differ in their specificity and sensitivity to radiation, the stability of signal and speed of performance. A large scale radiological emergency can take different forms. Based on the emergency scenario different biodosimetric tools should be applied so that the dosimetric information can be made available with optimal speed and precision.

The aim of this multi-disciplinary collaborative project is to analyse a variety of biodosimetric tools and adapt them to different mass casualty scenarios. The following biodosimetric tools will be established, improved and/or validated: the dicentric assay, the micronucleus assay, the gamma-H2AX assay, the skin speckle assay and the blood serum protein expression assay. In addition EPR/OSL dosimetry in components of pocket electronic devises is investigated. The assays were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario as well as speed of performance.

The project involves the key European partners with extensive experience in biological dosimetry. Training is carried out and automation and commercialisation pursued. An operational guide will be developed and disseminated among emergency preparedness and radiation protection organisations.

The final deliverable of this project will be establishment of a biodosimetric network that is fully functional and ready to respond in
case of a mass casualty situation. Thus, the project will strengthen the European security capabilities by achieving tangible results. The project is funded by the FP7 Security program. URL: http://www.multibiodose.eu.

S32.03. BOOSTER: Bio-dosimetric Tools for triage to Responders. Mehdi Gmar, CEA LIST, France

The BOOSTER Project addresses the requirement under FP7 Security Topic of Bio-dosimetric tools to manage radiological casualties (SEC-2009-4.3.2). The BOOSTER Project is a capability project designed to research and develop new bio-dosimetric tools in order to quickly evaluate the level of exposure of potential casualties, determine by appropriate sensors its consequences and allow an efficient triage of exposed people. These bio-dosimetric tools will be integrated to a useful and usable toolbox along with a prognostic toolkit based on radiation sensors. These approaches will allow an effective management of the situation. At the end of the project, civil protection operators and emergency services will be trained and commercial exploitation potentialities defined.

S32.04. Towards the validation of gene expression modifications as a biodosimeter. Gaetan Gruel, I. NOSEL, A. VAURDoux, IRSN, France

A worsening of the accidental hazards linked to the use of ionizing radiation is currently being observed for 4 reasons. First, the increasing need for radiation sources in numerous industrial applications (food sterilization, construction, engineering…) leads to an increasing probability of abnormal/unsuitable use, storage or loss of the sources. Second, advances in nuclear medicine generate new protocols and tools that are more efficient but also much more complex to carry out, and increase the risk of accidental overexposure. Third, the possibility of a terrorist attack using radiological or nuclear devices has to be taken into account. And finally, recent events in Fukushima (Japan) highlight the risks of mass exposure in case of nuclear power plant accidents. All these issues could lead to the accidental exposure of one to several thousand individuals not wearing dosimeters. The dicentric assay is the current standard for radiation biodosimetry, but several time limitations arise in the particular situation of mass casualties. In addition to the time required for the stimulation of cell division, manual dicentrics scoring take a lot of time. This highlights the need to establish new biomarkers and approaches to biodosimetry in order to be able to assign the individuals with significant exposure to appropriate medical care and to manage the long term medical follow-up. Many alternatives have been proposed among which gene expression profiling seems particularly promising. For around ten years, microarrays have been suggested for the diagnosis of ionizing radiation exposure. Technological and methodological advances in genomics have led to the potential discovery of many radiation-responsive biomarkers in peripheral blood cells. Indeed, changes in gene expressions can be induced and detected by peripheral blood lymphocytes with exposures to ionizing radiation. Moreover, the gene expression bioassay seems sensitive even at low radiation doses. However, the use of microarray analysis raises new challenges for both experimental and statistical methodologies. We will present an overview of the promising use of microarrays in biodosimetry and the associated problems.

S32.05. Standardisation of biological dosimetry by cytogenetics: status, advantages and limitations. Philippe Voisin*, Institut de RadioProtection et Sûreté Nucléaire, France

The wide use of radioactive sources and X-rays, for medical, industrial, agricultural, research and military purposes increases the risk of overexposure of workers and population. In addition, the recent Fukushima catastrophe reminds us how critical the management of such mass casualties is. Biological dosimetry, based on radio-induced chromosomal aberrations, mainly dicentrics, has become a routine component of the accidental dose assessment. In order to standardize biological dosimetry use, several ISO standards were developed by the ISO WG18 “biological dosimetry” to address the critical aspects of dicentric assay*. The first publication of 19238 ISO standard in 2004 provides for expertise, minimum requirement for experimental processes, quality assurance and quality control programmes, and evaluation of performance. Another 21243 ISO standard published in 2008 was intended to define performance criteria for cytotgenetic triage. The described approach included pre-planning, reagent stockpiling, sample handling, sample processing, networking, and modification of the ISO 19238 scoring criteria. Advantages given by these standards are: First, their application ensures quality of practice and high confidence level. Second, it allows evaluation of results between laboratories, particularly for an international collaboration or intercomparison. Finally, each new laboratory should get from this standard most useful information to perform dicentrics assay in the best experimental and reproducible conditions. There is also some limitation. While the quality system is a natural way for any R&D activity, the application of such standard is time consuming because all the processes must be checked for deviation and this checking is required regularly. It is especially true when a specific ISO standard is used for supplementing classical accreditation process which follows more general 17025:2000 ISO standard. For this purpose, the 19238 ISO standard was heavily updated by more detailed description of the experimental and statistical steps for satisfying the accreditation requirements and this implementation come into force probably next year. Another ISO standard is drafting on micronuclei assay in expertise and population triage and future project concerns automation in cytogenetics dosimetry.

*And the members of the ISO Working Group 18 on biological dosimetry.

S33. Countermeasures in case of accidental radiation exposure

S33.01. Animal models in countermeasures research. Jacky Williams, University of Rochester Medical Center, USA

As the awareness of a terrorist threat from a nuclear or radiological event has increased in the past few years, a greater focus has been placed on the development of countermeasures to both the acute and long-term consequences of radiation injury. However, the large numbers of researchers who have now found themselves in this field have found that they are caught in a minefield of regulations since there is no appropriate human population that can be used for basic clinical trials. This has placed a greater burden on model development since such models now need to demonstrate mechanism of injury as well as countermeasure efficacy. In addition, since such agents are being tested in a “healthy” population, there is less apparent therapeutic application for such agents and, therefore, limited pharmaceutical backing, often a requisite when bringing a drug through to clinical use. This talk will discuss some of the current models that are being used in ongoing research, as highlighted in recent consensus workshops, as well as some of the legal and regulatory aspects regarding the use of countermeasures/drugs in patients following accidental or terrorist-related radiation injury.

S33.02. Mesenchymal stem cell therapy for treatment of localized radiation injuries: the minipig model. Michel Droquet1, F. Fabien1, R. Diane1, S. Harry2, M. Viktor3, A. Diane1, 1: IRBA-CRSSA, France 2: Bundeswehr Institute of Radiobiology, Germany

Cutaneous radiation syndrome (CRS) is the delayed consequence of localized skin exposure to high doses of ionizing radiation (IR). New therapeutic management of IR-burned patients has suggested the benefit of local cellular therapy using mesenchymal stem cell injections in favour of wound healing and pain control. Here we examined in a large animal model the therapeutic potential of adipose tissue-derived stem cells (ASCs) to prevent or cure CRS. Göttingen minipigs were locally irradiated using a 60Co gamma source at the dose of 50 Gy and grafted with autologous ASCs (n=5) or PBS injected (n=8). Multipotent adult stem cells were cultivated in MEMa with 10% fetal calf serum and basic fibroblast growth factor (2ng./mL.) and then were intradermally injected four times from days 25 to 135 (50x104 each time). All controls exhibited a clinical evolution similar to human after a latency phase of several weeks: early erythema, hair loss, dry and moist desquamation, leading to final necrosis. Immunohistomorphology revealed severe skin damages and rhabdomyolysis in the muscle tissue below the entry area. In ASC-grafted minipigs, the clearing of the damaged epidermis appeared earlier than in non-grafted animals and an ultimate wound healing was observed in four out of five animals (day 130±28, complete in two of them). A final strong hyperproliferative activity was even observed in one case leading to sumnumeros cell layers in the entry area. Q-dot labelling confirmed that grafted ASCs accumulated at the dermis/subcutis barrier in which they attracted numerous immune cells which resulted in transient pro-angiogenic activity. Globally this study suggests that local injection of ASCs may represent useful tools to be incorporated in therapeutic strategies to mitigate CRS.

S33.03. Studies on hematopoietic protection and immunity adjustment in combined radiation-thermal injury. Xin-ze Ran, C. Shi,
Background and Purpose: Combined radiation-thermal injury (CRTI) mainly occurs under the circumstances of nuclear explosion, nuclear accident, depleted uranium or nuclear terrorism attacks. This article discusses several key points in CRTI management, including hematopoietic protection and reconstruction, infection control and immune adjustment.

Materials and Methods: Acute radiation sicknesses were created by y-ray irradiation on rats, mice, and dogs with a 10Co source. TBSA (total body surface area) third-degree thermal injury (confirmed by pathology) were inflicted on the back skin of animals either by immersion in 60°C thermal injury for 7 seconds in mice under the anesthetized conditions. Combined injury was established by the combination of the above two simple injuries.

Main results: We have developed effective therapies targeting the different key features of CRTI spectively. After CRTI, recombinant human interleukin-3 (rhIL-3) treatment could remarkably reduce the quantity of bacterial translocation, increase S IgA (secretory Immunoglobulin A) content in mucus membrane and the number of plasma cells that excrete IgA in intrinsic layer of the intestine in comparison to single thermal injury or radiation injury. Chitosan wove pVlTR03 (Human recombinant phylaxin) and GLP2 (Glucagons peptide 2) nanoparticles could increase the average survival time of animals inflicted with whole body irradiation (10Gy) combined with 15% third-degree thermal injury. After treatment by cervical sympathetic ganglia block (SB), number of peripheral blood lymphocytes, erythrocytes and blood platelet production of serum inflammatory cells factors TNF-a, IL-1b and IL-6 declined, thus making death rate of animal 30 d after thermal injury lowered by 40%. If excision of eschar and auto-skyn grafting were adopted, spontaneous endosmosis rate of animal thymocyte, amount of formed reflection, declining degree of thymocytes and spleen cells would be less than that in control group, and time of recovery would start earlier. It was found that both interleukin-3 (IL-3) and reconstructed granulocyte-monocytes monoclonal colony stimulating factor (rhGM-CSF) have good effect on the recovery of hematopoietic function caused by combined radiation- thermal injury. The blood serum and peritoneal lavage fluid from rats with thermal injury and CRTI had shown stimulating effects on the proliferation of bone marrow CFU-E, BFU-E and CFU-GM colonies in vitro.

Conclusion: The pathogenesis of CRTI is extremely complicated and the treatment is very difficult. The management strategy for combined injury should refer to the successful experiences learned from single injury and develop comprehensive therapeutics. The key features include protection and reconstruction of haematogenesis, infection control and immunity modulation, and improvement of enteric epithelium repair. This study was supported by the National Key Basic Research and Development Plan of China (“973” Project, No.2005CB522605), the National Natural Science Foundation of China (No.30770642) and the Key Scientific Research Project of “Eleventh Five-Year Plan” of PLA (No.06G076).

S33-04. The somatostatin analog, SOM 230, is a highly effective mitigator of intestinal radiation injury, Martin Hauer-Jensen, University of Arkansas for Medical Sciences, Little Rock, USA

Development of effective non-toxic medical countermeasures for use in radiological and nuclear emergency situations is urgently needed. While some progress has been made in the post-exposure management of radiation-induced bone marrow injury, the management of GI radiation toxicity remains symptomatic and underdeveloped.

The novel somatostatin analog, SOM230, can substantially reduce lethality, structural bowel injury, post-radiation mucosal permeability, and bacterial translocation in mice exposed to total body irradiation. The mechanism by which SOM230 mitigates radiation injury is related to inhibition of exocrine pancreatic secretion, but may also involve interleukin 12 and CXCL-4. Remarkably, SOM230 retains its potent lethality-protecting properties even when administration begins 48+ hours after TBI, a realistic time-frame in a mass casualty situation. Thus, SOM230 is an effective radiation mitigator, providing an opportunity to protect/rescue the gut mucosa long after the initial damage has occurred. Because of its low toxicity and ease of administration, SOM230 is uniquely suited as an enteroprotective agent in first responders, rescue workers, and cleanup workers in radiation accidents or terrorism situations; in military operations in radiation-contaminated fields; as well as in the post-irradiation mass exposure mitigation setting.

S34. Stem cells and regenerative medicine for the treatment of radiotherapy side effects

S34-01. Stem cell sparing radiotherapy: a novel approach to the prevention of radiation-induced xerostomia, Peter van Luijk1, R. Coppe1. 1. Dept. Radiation Oncology, University Medical Center Groningen, University of Groningen, Department of Radiation and Stress Cell Biology, department of Cell Biology, University Medical Center Groningen, University of Groningen, Netherlands

Many factors play a role in the response of tissues to irradiation, but ultimately the ability of stem cells to reconstitute functional cells determines the onset and the severity of radiation side-effects. Dry mouth syndrome resulting from radiation-induced salivary dysfunction is a major problem. To determine the role of stem cells and their localization within the salivary gland in the response to radiation, we performed a number of studies in mice, rats and patients.

First we showed that transplantation of cells that express stem cell markers and show stem cell properties can rescue salivary gland function in mice. This indicates that the salivary gland stem cell is a target for salivary dysfunction after irradiation and opens new opportunities for the prevention of salivary dysfunction by stem cell treatment.

Subsequently in the mouse, rat and human parotid gland we determined the distribution of cells expressing the stem cell marker c-kit and found that these cells were located in exclusively in ducts. More intense c-kit expression in larger ducts suggested that these cells are predominantly located in the major ducts. To test this, the rat parotid gland was split in a central part containing the major ducts and the outer parts, dispersed and cultured as stem cell containing salispheres. Indeed significantly more spheres could be grown from the central than from the outer parts of the gland, suggestive of a higher number of more stem cells in the center of the parotid gland. High-precision proton irradiation of this central part in vivo indeed resulted in excessive reduction of saliva production. In contrast minimal effects were observed after irradiating of the other parts of the gland.

Excitingly, in 36 patients treated in the British Columbia Cancer Agency, dose to the crano-ventral extension of the gland containing the major ducts was most predictive of a survival rate of saliva production after radiotherapy. In conclusion, the response of the parotid gland to radiation critically depends on the dose to its stem cells, located predominantly in the major ducts. These findings indicate new opportunities to prevent radiotherapy-induced salivary dysfunction by e.g. specific avoidance of the region containing the glands or transplantation of salivary gland stem cells.

S34-02. Cognitive restoration after cytotoxic cancer treatments, Charles L. Limoli, M. M. Acharya, L. Christie, V. K. Parihar, Department of Radiation Oncology, University of California, USA

Systemic chemotherapy and cranial radiotherapy are routinely used to control the growth of primary and metastatic lesions throughout the body. While beneficial, these cytotoxic treatments often lead to cognitive decrements that adversely impact the quality of life of millions of cancer survivors. The unintended consequences of cancer treatments on cognition can in part be caused by the inhibition of neurogenesis. Radiation and chemotherapy-induced reductions in neurogenesis are likely due to the depletion of neural stem cells, and in conjunction with elevated oxidative stress and inflammation contribute to the unintended consequences of cranial irradiation. Many factors play a role in the response of tissues to irradiation, but ultimately the ability of stem cells to reconstitute functional cells determines the onset and the severity of radiation side-effects. To address these long-standing clinical problems, we have explored the use of stem cells as therapeutic agents to combat radiation-induced cognitive decline. Athlymic rats subjected to cranial irradiation were transplanted with human embryonic (hESC) or neural stem cells (hNSCs) to determine if they could provide some remediation of the adverse effects of cranial irradiation. Results show that after acute, whole-brain irradiation (10 Gy) animals transplanted with stem cells showed significant improvement in cognitive function 4 months after irradiation, compared to irradiated animals not engrafted with stem cells. At each post-irradiation time point, significant survival and differentiation along CNS lineages was found, suggesting that cognitive improvement was in part dependent on the presence of engrafted cells. These data demonstrate that stem cells have the potential to aid long-term protection from the adverse consequences of cranial irradiation on cognition, and additional evidence will be presented showing that such benefits are due to functional cell replacement.
S34-03. Hepatocyte transplantation ameliorates radiation induced liver damage. Chandan Guha, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA

Purpose: Radiation therapy (RT) is ineffective in the treatment of abdominal malignancies because of potential lethal injury to internal organs, such as, liver, kidney and intestine. Although bone marrow transplantation has been used to rescue marrow failure post-chemo/RT, cell therapy has never been used to salvage toxicities of solid organs. We developed a rodent model of liver failure, following treatment of F344 rats with partial hepatectomy (PH) and whole liver RT. In this model, hepatic irradiation inhibited liver regeneration and induced liver sinusoidal endothelial cell (LSEC) death, perivenous hepatocellular steatosis and atrophy and mortality from radiation-induced liver disease (RILD). We hypothesized that loss of irradiated LSEC and hepatocytes could be replaced by intraportal/intrasplenic transplantation of autologous or allogeneic LSEC and hepatocytes, resulting in engraftment and selective repopulation of the donor cells, thereby providing metabolic support and ameliorating RILD.

Experimental procedure: Dipetideleptidase IV (DPPIV)-deficient, F344 rats and C57Bl/6 mice were administered HIR (0, 5, 10, 15, 30 and 50 Gy) with or without 68% PH (simulating liver resection in liver cancer patients). One to seven days after HIR, 1-5 x 10^7 DPPIV-proficient hepatocytes or LSEC were transplanted via intrasplenic or intraportal injection. Animals were observed for survival and representative animals were sacrificed for histopathological evaluation at various time points. DPPIV histochrome was performed to identify and evaluate donor cell engraftment and repopulation. Blood was collected for liver function test. Results: Animals receiving PH+RT+ hepatocyte transplantation (HT), had a significant improvement of survival with amelioration of the histopathological manifestations of RILD. Cell transplantation ameliorated the extensive bile duct proliferation and ductular fibrosis that were noted 12-16 weeks after PH+RT. Compared to unirradiated controls, HIR augmented the engraftment of transplanted LSEC and hepatocytes (p<0.01) with progressive repopulation of the irradiated anterior liver lobes by transplanted LSEC and hepatocytes. There was near-total (80-95%) replacement of the irradiated host parenchyma and the endothelium by the donor cells within 2 - 6 months. In animals receiving cell transplantation, liver function was normal and liver histopathology showed no evidence of RILD.

Conclusion: Transplantation of hepatocytes and LSEC can ameliorate RILD in rodents and mouse. This raises the possibility of salvaging cell transplantation therapy to rescue liver function in liver cancer patients treated with high dose chemoradiation therapy.

S34-04. Mesenchymal stem cell ameliorates severe radiation pelvic disease: clinical transfer. Alain Chapel, Institut de Radioprotection et de Sûreté Nucléaire, France

No abstract

S34-05. Bone regeneration and engineering in irradiated fields. Olivier Malard1, F. Jégoux1, P. Weiss2, 1: 1 National Institute for Health and Medical Research, INSERM Unit 791, Centre for Osteoarticular and Dental Tissue Engineering (LJOAD), Rennes University Hospital, Department of Head and Neck Surgery, France 2: National Institute for Health and Medical Research, INSERM Unit 791, Centre for Osteoarticular and Dental Tissue Engineering (LJOAD), Rennes University Hospital, Department of Head and Neck Surgery, France

Introduction. Head and neck cancers are the fourth cancer in the world and its mortality rate is near 50%. The locations most frequently involve the oral cavity. These cancers are treated in most cases by a combination of radiation and surgery. Mandible bone and dento-skeletal consequences affect orofacial function (swallowing, eating, talking), and provide aesthetic, psychological features that significantly distress the quality of life of these patients.

Experimental research for irradiated bone reconstruction. Two thirds of these tumors are diagnosed at an advanced stage III or IV with local involvement to the mandible. Surgical excision requires segmental resection of the mandible. Post-surgical rehabilitation is challenging, partly due to radiation induced healing difficulties impeding surgical reconstruction. Moreover, mandibular irradiation osteoradionecrosis can occur as a life-threatening complication. Heavy surgical process, i.e., free bone flaps, used for post-operative reconstruction are not always achievable, due to poor vessels quality or poor patient general status.

After conducting several studies to reach a reproducible animal model of irradiated bone, we studied the behaviour of conventional synthetic bone substitutes (calcium-phosphate ceramics biomaterial) in irradiated bone implantations. Real effectiveness was gradually demonstrated when using the combination of biphasic calcium phosphates and total bone marrow. The addition of different osteogenic medium cultured induced mesenchymal stem cells (MSC) replacing or associated to bone marrow has also been widely evaluated in different studies. The MSC association always provided pore outcomes compared to total bone marrow in irradiated areas.

As part of a Phase I clinical study, 10 patients with osteoradionecrosis are currently being treated by the association of MBCP granules with complete autologous marrow. The results of the primary and clinical studies are presented.

Bibliography

S34-06. Regenerative medicine based on stem cell injection for radiation burn treatment. Radia TAMARAT, INSTITUT DE RADIOPROTECTION ET DE SÛRETÉ NUCÉLAIRE, FRANCE

No abstract

S34-07. Fat grafting after mastectomy and radiotherapy. Marco Klinger1, F. Caviggioni, D. Forcellini, V. Vinci, L. Maione, 1: Università degli Studi di Milano, IRCCS Istituto Clinico Humanitas, Italy

Mastectomy with axillary dissection and radiotherapy is still today one common procedures in oncologic surgery. During the years, many different surgical and conservative technique have been proposed for post-radiotherapy scar treatment. Fat grafting is today used for aesthetic and functional surgery, to treat burns’ scars, post-traumatic scars or to correct post-surgical scars. The adipose tissue harvested and processed with Coleman’s technique is injected by sharp, 0,1-0,2mm cannulas at the dermal-hypodermal junction of post-radiotherapy scar areas. After 3-6 months follow-up, clinical and histological features show a skin quality and thickness improvement; moreover lipolifting decreases pain and functional impediment. Unfortunately a condition of neuropathic pain, named Post-Mastectomy Pain Syndrome (PMPS) can appear after mastectomy and radiotherapy. Fat grafting can improve pain control in this group of patients. It is a safe, low-invasive and rapid surgical procedure. Our results suggest its effectiveness for therapeutical approach of PMPS and radiodamaged tissues.

S35. Radiation research and nuclear power

S35-01. Radiation chemistry effects on nuclear solvent extraction: Examples from CMPO radiolysis, Bruce Mincher1, B. J. Mincher2, S. P. Mezyk3, G. Elia3, G. S. Groeneveld4, 1: Idaho National Laboratory, USA 2: California State University Long Beach, USA

Aqueous reprocessing of used nuclear fuel begins with its dissolution in nitric acid followed by the solvent extraction of desired metals. Solvent extraction formulations must be robust toward radiolytic degradation in an irradiated mixed organic/acidic aqueous environment. In the USA, the compound octylphenyldisobutylcarbamoylmethylphosphine oxide (CMPO) has long been proposed for the extraction of the trivalent actinides. A mechanistic understanding of the radiation chemistry of this ligand requires the use of pulse radiolysis to identify the pertinent transient reactive species, and steady state radiolysis combined with analytical chemistry to identify ultimate products. Common scavenging reactions can be invoked to limit the suite of radicals produced to the most important ones as OH, •H and •NO2. Since the extraction process is biphasic, organic phase radical reactions must also be considered.
We have developed pulse radiolysis techniques for the production and measurement of \( \text{NO}_3^- \) radical kinetics in both the aqueous and organic phases. The bimolecular rate constant for the reaction of the \( \text{NO}_3^- \) radical with \( \text{CMPO} \) was estimated at \( 8.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \) in t-butanol, slower than in water (1.28 ± 0.13) \( \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \). However, the same trend was not seen for the diaminic actride extraction ligand dimethyl dioctyl heptyloxymalonamide (DMDHOEMA). Moreover, model compound measurements showed that aliphatic species reacted faster in the organic phase, but could only be measured at low reagent concentrations. The reactivity of different species could be defined by the concentration of reagent ions and the solvent. The radiation chemistry of \( \text{CMPO} \) has also been investigated using steady state radiolysis by g and a sources. A G-value of -0.14 mmol J\(^{-1} \) was found for irradiations of 0.1 M \( \text{CMPO} \) in dodecane, in the absence of the acidic aqueous phase. This was constant across a range of g-dose rates, and also for g-irradiation using a 5 MeV\( ^+ \) He-ion beam. Electrospray ionization-mass spectrometry (ESI-MS) techniques were also developed for the detection of the stable products of these g and He-ion beam irradiated samples.

S35-02. CHEMISTRY FOR THE NUCLEAR ENERGY OF THE FUTURE. Andrzej G. Chmielewski, Institute of Nuclear Chemistry and Technology & Warsaw University of Technology, Poland

Chemistry – radiochemistry, radiation chemistry and nuclear chemical engineering play a very important role in the nuclear power development. Even presently developed technology for its application has yet to reach its full potential. Improvements are needed and proposed. These developments concern all stages of the technology; front end, reactor operation (coolant chemistry and installation components decontamination, noble gas release control), back end of fuel cycle, etc. Chemistry for a partitioning and a transmutation is a new challenge for the chemists and chemical engineers. The IV generation of the nuclear reactors cannot be developed without chemical solutions for fuel fabrication, radiation - coolants interaction phenomena understanding and spent fuel/waste treatment technologies elaboration. Radiochemical analytical methods are fundamental for radioecological monitoring of radionuclides of natural and anthropological origin. This paper addresses just a few subjects and is not detailed overview of the field; however illustrate a role of chemistry for a safe and economical nuclear power development. This year being so important anniversary of the science development breakthrough is also a year of the terrible disaster in Japan devastated by earthquake and tsunami, Fukushima Nuclear Power Plant was seriously damaged but survived this apocalypses, however the sequence of the accident has illustrated importance of radiation- and radio- chemistry on the safe operation and shut down of nuclear reactor and decontamination of wastes originated.

S35-03. Radiation-Induced Aqueous Chemistry and Corrosion in Nuclear Reactor Environments. Jungsook Wren, the University of Western Ontario, Canada

Understanding the factors that can affect the corrosion of reactor materials is important for the design, operation and maintenance of nuclear power reactors. Water radiolysis generates both oxidizing and reducing species whose net interactions with the alloys used in reactor construction are not well characterized. Corrosion products (crusts) are a mixture of solid oxides formed by a variety of wear and dissolution/precipitation processes. They are a particular problem in nuclear plants where neutron activation of material passing through the reactor core can generate hazardous radionuclides.

To develop a fundamental understanding of impact of radiation on aqueous chemistry and consequent materials degradation, we are carrying out studies in three main areas: (1) water radiolysis kinetics under continuous, long-term irradiation conditions, with dissolved species present including corrosion product metal ions (iron, nickel, cobalt, chromium) and nitrogen-containing species (from nitrate/nitrite to ammonia), (2) radiation-induced corrosion of reactor materials (carbon steel, stainless steel, Stellites) with the purpose of developing a corrosion kinetics model, and (3) radiation-induced colloid formation from dissolved metal ions, and the impact of this process on the net solubility and transport of the metal ions. An integrated, multi-disciplinary approach is taken that combines experimental investigation of both integrated and separate effects tests with extensive combined chemical kinetics, interfacial and mass transport model analysis. We employ a range of experimental techniques to follow the aqueous chemistry, electrochemical measurements to measure the corrosion kinetics, and sophisticated in-situ and ex-situ analyses of metal-metal oxide surfaces that are studied. Our findings from our work illustrates the important impact that long-term radiolysis has on reactor coolant chemistry and materials performance will be presented.

S35-04. Modelling and simulation for controlling chemistry in advanced nuclear energy systems. Dorota Swiatłza-Wojcik, Technical University of Lodz, Poland

Stability, safety and efficiency of nuclear energy systems considerably depend on our knowledge of the mechanisms governing degradation of materials. Novel designs and development of new materials require understanding of radiation-induced chemical effects in nuclear fuel, coolant, and at solid/liquid interfaces. In water-cooled reactors the coolant passing through the core is exposed to a mixed flux of fast neutrons, gamma rays, and alpha particles. Irradiation of liquid water initially results in formation of short-living dry and hydrated electrons, H\text{atoms}, OH\text{ radicals, H}^\text{+} \text{ and OH}^- ions and stable molecular species \text{H}_2\text{O}_2 and \text{H}_2. Consecutive reactions of radical, ionic and molecular species, including water molecules, constitute a set of more than 40 reactions describing the radiolysis of water. As a result of these reactions transient species decay with production of reactive H\text{O}_2 and O\text{2}. Strong oxidants formed in the water coolant are responsible for corrosion and degradation of reactor constructive elements and materials of associated pipe systems. Challenging for the future nuclear energy systems is to control chemistry of the systems exposed to high radiation and high temperature fields. Modelling closely integrated with measurements provide qualitative and quantitative insights into the radiation-induced chemical effects and mechanistic models. A multiscale view is a long standing tradition in radiation biology: the three ‘R’s’ of biophysics, repair, repopulation and re-oxygenation, describe how cellular, tissue and organismal radiation responses contribute to efficacy of radiotherapy. Key differences between systems biology and prevailing analytical paradigms is the emphasis on networks versus components, distributed versus hierarchical consequences, and redundancy versus uniqueness. Just as DNA damage elicits a dramatic transition in signaling within a cell, irradiated cells activate specific networks, distinct from those of the unirradiated cells. Thus, the tissue response to damage is the integration of different cell types generating dynamic, multilayered networks that are coordinated by endocrine, autocrine and paracrine signals. Recent studies will be used to illustrate systems biology approaches to network analysis, cellular responses and tissue effects following radiation that restore homeostasis or initiate pathology.

Conference lectures CL13 - CL18

CL13. Systems biology - where are we and where can we go? Mary Helen Barcellos-Hoff, New York University School of Medicine, USA

A major challenge in radiation biology is to understand how cellular responses to radiation are integrated in a multicellular context. Systems biology addresses problems of organization and tackles phenomena not resolvable into local events by using multiscale data to build descriptive and mechanistic biological models. A multiscale view is a long standing tradition in radiation biology: the three ‘R’s’ of biophysics, repair, repopulation and re-oxygenation, describe how cellular, tissue and organismal radiation responses contribute to efficacy of radiotherapy. Key differences between systems biology and prevailing analytical paradigms is the emphasis on networks versus components, distributed versus hierarchical consequences, and redundancy versus uniqueness. Just as DNA damage elicits a dramatic transition in signaling within a cell, irradiated cells activate specific networks, distinct from those of the unirradiated cells. Thus, the tissue response to damage is the integration of different cell types generating dynamic, multilayered networks that are coordinated by endocrine, autocrine and paracrine signals. Recent studies will be used to illustrate systems biology approaches to network analysis, cellular responses and tissue effects following radiation that restore homeostasis or initiate pathology.

CL14. Particle therapy: from the laboratory to the clinic. Marco Durante, GSI, Germany

Radiotherapy is one of the most common and effective therapy for cancer. Generally, patients are treated with X-rays produced at electron accelerators. The use of high-energy charged particles was proposed several years ago, both for their physical and radiobiological advantages compared to X-rays. Particle therapy is now becoming an emerging technique in radiotherapy. Protons and carbon ions have been used for treating many different solid cancers, and several new centers with large accelerators are under constructions. However, there is currently an open debate on the cost/benefit ratio of this technique. The Biophysics group at GSI had in the past years an intense activity in the \text{12}C\text{ion} therapy pilot project. The treatment of tumours at the base of the skull and brain was extremely successfully, using the technique of raster scanning. The therapy project paved the way to ion therapy in Europe, leading to the
construction and planning of several clinical accelerator facilities. However, several research issues are still open in this field, for instance: predicting RBE of different particles in the tumours and in the normal tissue; treatment of moving targets; impact of genetic background on sensitivity; effects on angiogenesis and metastatic potential; fractionation; hypoxia. This talk identifies the open research questions and shows some recent data recently obtained in some of these fields.

CL15. Radiation chemistry and technology of polymers: recent advances. Piotr Ulanski, Technical University of Lodz, Poland

Polymer processing is one of the fields of radiation chemistry that reached the stage of commercial application (cable and tire industries, sterilization of medical products). Definitely, these technologies will be further developed and improved. In this talk an attempt will be made to identify several fields of activity related to radiation chemistry of polymers, where current progress allows to expect new applications in research and technology.

While so far irradiation was perceived as a rather “rough”, non-selective tool for initializing polymerization and grafting, this is changing now with the advent of radiation-induced reversible addition-fragmentation chain transfer (RAFT) polymerization, which should allow synthesizing polymers of precisely controlled properties. Processing of natural polymers is expected to gain momentum after discoveries on radiation cross-linking of polysaccharides. Similarly, radiation cross-linking of PTFE should yield materials combining excellent chemical resistance and mechanical properties. Progress is expected also in the field of polymeric biomaterials, where some radiation technologies have been already commercialized (hip joints). Hydrogel would dressings and many more are being elaborated. Radiation chemistry can also offer some help in solving problems related to basic studies in general polymer chemistry. In this talk some directions of radiation-related studies will be indicated which may lead to significant progress in determining rate constants of elementary reaction steps, studying propagation-depropagation equilibria and intramolecular reactions.

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6) US patent 4,871,490

CL16. Genomics and the therapeutic response. Catharine West, University of Manchester, UK

Genetic variation is commonly reported as single nucleotide polymorphisms (SNPs). The ~10 million SNPs in the human genome underlie differences in inherited traits between individuals and populations. Given the evidence that radiosensitivity is an inherited phenotype, SNPs are likely to explain much of the variation in radiosensitivity between individuals. Therefore, an increasing number of studies have looked at SNPs in candidate genes to try and predict a patient’s likelihood of developing toxicity following radiotherapy. Most studies have failed to replicate. The ability to detect genuine SNPs associated with radiosensitivity/radiotherapy toxicity is related to the effect of individual SNPs (for most extremely small) and the frequency of the less common/minor allele. Common SNPs with large effect sizes could be found with a small number of cases but knowledge from studies of other traits and diseases suggest they are unlikely to occur. Individual SNPs appear to confer only very small effects/risks. Genome wide association studies (GWAS) are now widespread for other traits and are finding confirmed variants. The results of the first GWAS in radiogenomics were published in 2010. The establishment of a Radiogenomics Consortium in 2009 should provide a route for carrying out replication and larger studies. A consortium approach is essential for radiogenomics because of the large number of cases – many thousands of samples from a range of populations but also to gather for each patient data on possible non-genetic risk factors (e.g. age, smoking history, radiation dose, radiation volume, co-morbid conditions) and radiotherapy toxicity.

CL17. Radiation-induced DNA damage in mammalian cells - novel types of lesions. Jean-Luc Ravatan, CEA Grenoble, France

Reactions of reactive oxygen species, and among them the highly genotoxic hydroxyl radical (HO•), with DNA constituents have been extensively studied during the last three decades. An almost complete radiation-induced decomposition pathway of the different DNA constituents is now available. In addition, the development of highly sensitive and specific analytical tools, such as HPLC coupled through electrospray ionization to tandem mass spectrometry has allowed the detection of the identified DNA lesions in dsDNA, subsequently to DNA hydrolysis, and sometime at the cellular level following DNA extraction. Recent results have highlighted the importance of the formation of complex DNA lesions. Formation of such damage is initiated by a single ionization event generating so-called tandem lesions constituted of two (at least) adjacent modifications. Indeed, a complex DNA lesion consisting in a strand break, together with an interstrand crosslink has been identified and its formation involves as the initial event HO•-mediated hydrogen abstraction at C4’ of the deoxyribose moiety. Through a complex cascade of events the generated C4’ radical gives rise to a conjugated adduct and to another one which reacts with a cytosine base located on the complementary strand.

In addition, it has been shown recently that most of HO•-mediated DNA oxidation generates a significant (50%) of 8-oxo-7,8-dihydro-2’- deoxyguanosine (8-oxodGua) containing tandem lesions. Importance of such tandem lesions is suggested by the role played by peroxide radicals that could react with adjacent DNA bases.

Indirectly such observations indicate that decomposition of initially produced base (or sugar) radicals may be influenced by their local environment, and thus, the decomposition reactions observed at the nucleoside level may be different, at least partly, in comparison to those in dsDNA. Tandem DNA lesions being partly refractory to repair by DNA glycosylases, the biological consequences of such damage should be evaluated and this will require additional investigations.

One of the key issues in the safety assessment of a future geological repository for spent nuclear fuel is the rate of fuel dissolution in the event of groundwater intrusion. This is believed to be governed by the dissolution of the fuel matrix, UO2. Under normal groundwater conditions at the depth of a deep repository, the solubility of UO2 is very low and the fuel matrix is assumed to act as a barrier. However, the inherent radioactivity of the fuel itself will cause radiolysis of the groundwater (in the event of a canister failure) producing highly reactive radicals and molecular species. These species will alter the conditions significantly. The mechanism and kinetics of oxidative dissolution of UO2 have been studied in detail for several decades. The rate of oxidative dissolution depends on the dose rate which is directly related to the specific activity of the spent nuclear fuel. On the basis of experimental findings and numerical simulations, a relatively simple model describing the rate of spent nuclear fuel dissolution under various conditions has been presented.

In recent years, it has become obvious that the solid phase structure and composition (depending on fuel burn-up) have very strong influence on the reactivity of the fuel surface towards aqueous radionuclides products. For simple UO2 powder H2O2 mainly reacts by oxidation of UO2 while for more complex pellet materials where UO2 is doped with rare earth oxides (a model for fission products) H2O2 mainly reacts by surface catalyzed decomposition not leading to oxidation of UO2. During the last decade, the inhibiting effect of H2 on radiation induced dissolution of spent nuclear fuel has been extensively studied and discussed. The discrepancies between different experimental studies and numerical simulations have caused significant confusion. The inhibition can partly be explained by a noble metal catalyzed redox process and partly in terms of hydroxyl radical (OH•) scavenging by the metal surface.

These issues will be discussed in detail in the presentation.

Plenary lecture 07
PL07. Maria Sklodowska-Curie, Hélène Langevin-Joliot, Paris, France
No abstract
Monday

POS01 Adaptive response

POS01-01. Effects of ionizing radiation on bacteria cells. Nadezhda Kudryasheva1, M. Alexandrova2, T. Rozhko1, 1: Inst of Biophysics SB RAS, Russian Federation, 2: Siberian Federal University, Russian Federation

Marine luminous bacteria serve as a convenient model for studying effects of ionizing radiation on living organism. These bacteria are widely used as ecological bioassay for more than forty years. The bioluminescent (BL) biosassays are traditionally applied for monitoring of chemical toxicity, and not long ago we used them for the first time to monitor radiation toxicity in solutions of alpha- and beta-radiouclides. Main testing physiological parameter of the biosassays is BL intensity.

The purpose of the work was to study chronic effects of radionuclides on glowing of luminous bacteria Photobacterium Phosphoreum. Effects of model solutions of alpha-emitting nuclide Am-241 and beta-emitting nuclide tritium were studied. The bacteria were grown in nutrient media with addition of Am-241 (up to 7 kBq/L), H3-labeled aminoacid valine, or tritiated water (up to 100 MBq/L). The Am-241 inhibited bacterial growth at all activities of the nutrient media. The tritium increased bacterial growth at activity <30 MBq/L, and inhibited it at >30 MBq/L. Bacteria were sampled at exponential and stationary stages of growth; BL kinetics of the samples was studied and compared with that of a control (nonradioactive) sample. Three stages were found in BL kinetics of the radioactive samples: (1) absence of the effect, (2) BL activation, and (3) BL inhibition. The BL activation reached 1000-2000%; it was attributed to hormesis phenomenon. No linearity in radioactivity-BL intensity dependences was found. All three BL kinetics stages were found in solutions of both Am-241 and tritium, i.e. the response of the cells was unified. Accumulation of Am-241 and tritium in cells and DNA was determined.

Role of peroxides (as secondary products of ionizing radiation in water) in the effects radionuclides on luminous bacteria and their enzymatic reactions was studied. Peroxides were found in to be effective in Am-241 solutions and were not – in tritium solutions.

POS01-02. Prevention and Therapy of Diabetic Complications by LDR: An innovative approach. Lu Cai, University of Louisville, USA

Induction of hormesis and adaptive response by low-dose radiation (LDR) has been extensively indicated. Adaptive response induced by LDR was not only resistant to damage caused by subsequently high-dose radiation, but also cross resistant to other non-radiation challenges, including diseases-related oxidative stress. Oxidative stress is a major cause for diabetes and its complications; we have investigated whether LDR has potential for the prevention or therapy of diabetic complications. Using type 1 diabetic animal models that was induced with streptozocin in rats and mice, we have demonstrated that LDR at dose levels of 25 – 75 mGy can afford a significant preventive effect on diabetes-induced renal dysfunction along a prevention of renal inflammation, oxidative damage and remodeling. The preventive effect of LDR on diabetic complications were also mirrored by its significant prevention of diabetes-induced cardiac and testicular oxidative damage and structure changes, along with a significant increase in serum and organ’s antioxidant levels.

Furthermore, we also demonstrated the significant acceleration of diabetic wound healing by exposure to LDR in the diabetic rats at 2 months after diabetes onset, which was found to be predominantly mediated by LDR’s stimulation of bone marrow stem cell proliferation and peripheral mobilization. Therefore, these studies demonstrate for the first time that LDR has a great potential for the prevention and even the therapy of diabetic complications.

POS01-03. Detection of novel human miRNAs responding to ionizing irradiation. Nan Ding, X. Wu, J. He, L. Chang, W. Hu, G. Zhou, Institute of Modern Physics, Chinese Academy of Sciences, China

Purpose: Up to now, more than 1048 human miRNAs have been identified. However, the recognition of new human miRNAs becomes more and more difficult. Ionizing radiation, such as X-rays, g-rays, and heavy ion beams, causes cellular damages, signal transduction, DNA repair, cell cycle checkpoints, and apoptosis, in which many miRNAs take part. Therefore, we hypothesize that some miRNAs which rarely express under normal conditions may increase their expression upon irradiation.

Materials and methods: Total RNAs of HeLa cells were isolated 1 h after exposure to 2 Gy of X-rays, and total small RNAs were enriched by using PAGE, then sequenced by using Solexa technology.

Results: Finally, 421 kinds of known miRNAs and 337 kinds of unknown sequences were identified, among which 11 novel miRNAs were characterized by bioinformatic ways and verified by qRT-PCR. Furthermore, putative targets of these miRNAs were predicted by TargetScan software and compared with known proteins down-regulated by radiation. It was confirmed that some of the targets of these novel miRNAs were radiation-related proteins.

Conclusion: These results imply that these 11 novel miRNAs are radiation-related miRNAs. This study reveals a new way to find novel miRNAs.

Keywords: microRNA, ionizing radiation, Solexa, qRT-PCR

POS01-04. Implications of whole body radiation exposure for prostate carcinogenesis in the TRAMP mouse model. Mark Lawrence1, B. Blyth1, R. Ormsby1, E. Bezak2, W. Tilley2, P. Sykes3, 1: Flinders University and Medical Centre, Australia, 2: Royal Adelaide Hospital 3: Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide, Australia

Increased tumour latency and reduced tumour frequency following low dose irradiation provides in vivo evidence for radiation adaptive responses. Although adaptive responses have been observed for haematological malignancies, there is little in vivo evidence available for epithelial carcinogenesis, such as in prostate cancer. Little is known regarding the susceptibility of the prostate to radiation-induced carcinogenesis. The TRAMP transgenic mouse, with 100% prostate cancer incidence and reproducible progression, provides an opportunity to investigate the implications of high and low dose radiation exposures.

We hypothesise that low dose radiation (10-100 mGy) will inhibit TRAMP prostate tumourigenesis, and that a high dose (2 Gy) will accelerate tumour progression. Endpoints include time-to-palpable-tumour, tumour size/weight, and histopathology grade. We have also developed high-throughput analysis techniques for immunofluorescent staining of a cellular proliferation marker, transgene expression, DNA repair foci and apoptosis in prostate sections.

There was no change in proliferation or transgene expression in any of the lobes (ventral, dorsal-lateral or anterior) in TRAMP mice 6 weeks after irradiation with 2 Gy compared with sham-irradiated controls. Irradiated mice had higher relative prostate weights, implicating a response during the 6 week period (e.g. proliferation, inflammation or apoptosis). However, there was ultimately no difference in time-to-palpable-tumour between the groups.

In low dose experiments, preliminary data show no change in proliferation in any of the lobes of the prostate 3 days following 50 mGy X-rays.

On-going studies are exploring the long term effects of high and low dose radiation exposure in the TRAMP model, using both single and multiple irradiations.

Research funded by The Prostate Cancer Foundation of Australia and the Low Dose Radiation Research Program, Biological and Environmental Research, US Dept of Energy, DE-FG02-05ER64104.

POS01-05. Enhanced phosphate solubilization by radiation induced mutants of Pantoea. Young-Keun Lee, S. Murugesan, Korea Atomic Energy Research Institute, South Korea

Three mineral phosphate solubilizing bacteria where isolated from rhizosphere soil samples of common bean and weed plants. 16S rDNA analysis indicated that the isolate P2 and P3 are closely related to Pantoea dispersa while isolate P4 is closely related to Pantoea terrae. Isolates P2 and P3 recorded 468.42µg/ml and 407.45µg/ml of tricalcium phosphate solubilization (TCP) solubilization respectively on 5 days incubation. Isolate P4 recorded the TCP solubilization of 215.84µg/ml and the pH was dropped to 4.44 on 24h incubation. Further incubation of P4 sharply decreased the available P to 28.94µg/ml and pH level was raised to 6.32. Gamma radiation induced mutagenesis was carried out at LD99 dose of the wild type strains. A total of 14 mutant clones with enhanced MPS activity and 4 clones with decreased activity were selected based on solubilization index (SI). Mutant P2-1 recorded the highest P-solubilizing potential among any other wild or mutant clones by releasing 504.21µg/ml of phosphorous i.e. 35% higher than
its wild type by the end of day 5. Comparative evaluation of TCP solubilization by wild type isolates of Pantoea and their mutants, led to selection of three MPS namely P2-1, P3-2 and P3-4 with a potential to release > 471.67µg/ml of phosphorous from TCP. These expressing mutant clones are considered as suitable candidates for biofertilizer.


Mitochondria are known to play an essential role in radiation response. However, the exact mechanism underlying mitochondria-initiated pro- or anti-apoptotic pathways in low dose radiation induced adaptive response remain elusive. We have reported that Cyclin D1 up-regulated by NF-κappaB is involved in low dose radiation induced adaptive response (Ahmed, et al., Oncogene 27:6738-6748, 2008). The present study tested the hypothesis that Cyclin D1/CDK4 complex function as a key factor delivering the adaptive signaling to mitochondria to induce an anti-apoptotic response in irradiated cells. We found that Cyclin D1/CDK4 was present in the mitochondria of human skin keratinocytes and other mammalian cells. Cyclin D1/CDK4 mitochondrial translocation was significantly enhanced in a certain time following exposure to low dose range of ionizing radiation (10–100 cGy x-ray). Cyclin D1/CDK4 was able to directly interact with mitochondrial antioxidant MnSOD and affect MnSOD activity, which contributes to the adaptive radiosensitivity in cells with reduced ROS level and sustained maintenance of mitochondria membrane potential. These results provide the evidence that cell cycle regulator Cyclin D1/CDK4 is able to regulate mitochondrial function via direct interaction with mitochondrial antioxidants in low dose radiation-induced adaptive resistance. Data elucidating the mechanisms related to Cyclin D1/CDK4/MnSOD pathway in mouse and human cells and tissues will be presented.

**POS01-07.** RADIATION HORMESIS AND RADIOADAPTIVE RESPONSE IN DROSOPHILA MELANOGASTER FLYS WITH DIFFERENT GENETIC BACKGROUNDS: THE ROLE OF CELLULAR STRESS-RESISTANCE MECHANISMS. Alexey Moskalev, E. Plusmina, M. Shaposhnikov, Institute of biology of RAS, Russian Federation

The purpose of this work is to investigate the role of cellular stress-resistance mechanisms in the low-dose irradiation effects on Drosophila melanogaster lifespan. In males and females with the wild type Canton-S genotype the chronic low dose irradiation (4 5 Gy/year; 4 Gy) induced the hormetic effect and radiation adaptive response to acute irradiation (30 Gy). The hoesmsis and radiosensitive responses were observed in flies with mutations in autophagy genes (atg7, atg8a) but absent in flies with mutations in genome stability genes, such as ATM, ATR, PCNA, XPC, BLM and p53 homologs, and heat shock protein 70. The obtained results demonstrate the essential role of cellular stress-resistance genes in hormesis and radiation adaptive response of the whole organism.

**POS01-08.** On the molecular mechanisms for radioadaptive responses. Mitsuru Nenoi, G. Vares, B. Wang, National Institute of Radiological Sciences, Japan

When pregnant ICR mice were irradiated with high doses of X-rays (4-5 Gy) on day 12 after fertilization (E12), three only living fetuses (out of 12) remained per dam on E18 which exhibited widespread malformations. However, when the mice were pre-exposed to low dose of X-rays (0.3 Gy) on E11, the number of living fetuses significantly increased while the fraction of malformed fetuses dropped. In order to reveal the molecular mechanisms underlying this radioadaptive response (RAR), we analyzed the modulation of gene expression in irradiated and non-irradiated tissues. Using DNA microarrays, several RAR-related target genes (such as Tead3 and Cacna1a) were identified, and their functional involvement in RAR was confirmed by RNA interference experiments using cultured fetal limb bud cells. Meanwhile, we also investigated RAR against the mutagenic effects of high LET radiation in cultured lymphoblastoid cells. The cells were exposed to 0.02 Gy or 0.1 Gy priming X-rays or high-LET heavy-ion radiation (C-ions at 20 keV/µm or 40 keV/µm and Ne-ions at 150 keV/µm), followed six hours later by a 1 Gy challenging dose of heavy-ion radiation. Reduced mutation frequencies at the HPRT gene locus were observed in cells pre-exposed to priming radiation, compared to cells exposed to challenging dose alone. Analysis of the H2AX kinases in irradiated cells indicated that increased DSB repair rates were observed in adapted cells, suggesting the functional involvement of an efficient DSB repair mechanism in RAR. The implication of molecular mechanisms underlying RAR in low-dose radiation risk will be discussed.


As humans are exposed to a variety of chemical agents as well as radiation, health effects of radiation should be evaluated in combination with chemicals. To explore possible modification of genotoxic effects of radiation by chemicals, we examined modulating effects of N-methyl-N’-nitro-N’-nitrosoguanidine (MNNG), a direct-acting methylating agent, against genotoxicity of γ-radiation. Human lymphoblastoid TK6 cell and the murine malignant T cell derivative, i.e., 1T1 cells, were treated with MNNG for 24 h before they were exposed to γ-irradiation at a dose of 1.0 Gy, and the resulting genotoxicity was examined. In TK6 cells, the pretreatments with MNNG at low doses suppressed frequencies of the thymidine kinase (TK) gene mutation and micronucleus (MN) formation induced by γ-irradiation and thus the dose responses of TK and MN assays were U-shaped along with the pretreatment doses of MNNG. In contrast, the genotoxic effects of MNNG and γ-irradiation were additive in MT1 cells and the frequencies of TK mutations and MN induction increased along with the doses of MNNG. Apoptosis induced by γ-irradiation was suppressed by pretreatments with MNNG at a low dose in TK6 cells, but not in MT1 cells. The expression of p53 was induced and cell cycle was delayed at G2/M phase in TK6, but not in MT1 cells, by the treatments with MNNG. These results suggest that pretreatments of MNNG at low doses suppress genotoxicity of γ-irradiation in human cells and also that mismatch repair proteins are involved in the apparent adaptive responses.

**POS01-10.** Disorders of Lipid Metabolism in a Gamma-Irradiated Model. Changhyun Roh, M. Park, H. Park, U. Jung, S. Jo, Korea Atomic Energy Research Institute, South Korea

Even though the risk of degenerative diseases by radiation has been reported, the main degeneracy during exposure to radiation is poorly understood. Exposure to ionizing radiation such as gamma-rays is one of the methods used to stress specific model system. Biological responses to ionizing radiation are complex processes. The chemical and physical reactions induced by ionizing radiation are cumulative in living organisms with developed mechanisms. Ionizing radiation effects have been reported for various biological responses including growth arrest, oncogenesis, mutation, chromosomal instability and so on. Recently, we reported the degeneracy of adipocytes during exposure to radiation (Jo et al., IJRB, 2011, 87(3), 302–310, Patent, Korea/2010-0037873). Furthermore, we showed the fat inhibition by orlistat administration in a gamma-irradiated mouse model. (Roh et al, EJILST, 2010, 112, 1384–1388). We suggest that gamma-irradiation in animal model might result in fat accumulation and orlistat treatment as an anti-obesity agent inhibits the accumulated fat in C57BL/6 mice. In this study, we showed that gamma-irradiation could trigger a biological response like the fat accumulation of gonadal white adipose tissue (WAT), mesenteric (mWAT), and retroperitoneal (pWAT) in mice. To induce fat accumulation through gamma-irradiation, 2-month-old female C57BL/6 mice were irradiated at 0.5 Gy (0.11 Gy/min, 10 times), 1 Gy (1.1 Gy/min, 5 times), and 5 Gy (1.1 Gy/min, 1 time), and raised for an additional 6 months. The white adipose tissue (wWAT, mWAT, and pWAT) of the gamma-irradiation mice weighed significantly higher than what was seen in normal mice, indicating that gamma-irradiation induced the fat accumulation in the adipose tissue. In this regard, gamma-irradiated mice could be evaluated as a useful model for the development of anti-obesity drugs. Here, we present our observation of the triggering of fat accumulation in the adipose tissue induced by ionizing radiation. [This study was supported by the Nuclear R&D Program of MEST (Grant No. 2007-00091)]
POSTER PRESENTATIONS

Keywords: Ionizing radiation, lipid metabolism, white adipose tissue, fat accumulation

POS01-11. Influence of the overexpression of DNA repair and damage response genes (mnk, BRC2A, spnB, and Hus1) on Drosophila melanogaster whole organism radiosensitivity. Mikhail Shaposhnikov, A. Moskalev, Institute of Biology of Komi Scientific Center of the Ural Branch of the RAS, Russian Federation

DNA repair and damage response genes play crucial role in the cell radiosensitivity, however the effect of overexpression of these genes on the whole organism radiosensitivity was not studied. The objective of our research was to investigate the role of DNA repair and damage response genes overexpression in the whole organism radiation response in Drosophila melanogaster.

Effects of conditional ubiquitous overexpression of mnk, BRC2A, spnB, and Hus1 on the radiosensitivity to 30 Gy of acute irradiation were studied with the Drosophila melanogaster imagos. To investigate the possible role of genes in radioadaptive response overexpression was generated 72 h before acute irradiation, using the miPiristone inducible GeneSwitch GAL4 system. The effect of irradiation on the age dynamics of imago mortality has been analyzed. The obtained results will be presented and interpreted from a point of view of mnk, BRC2A, spnB, and Hus1 roles in Drosophila melanogaster whole organism radioadaptive response.

POS01-12. Plants from Chernobyl zone are tolerant to DNA damaging agents. Galina Shevchenko1, A. Talalaiiev2, J. Dooman1, 1: Institute of Botany, NAS Ukraine, Ukraine 2: Institute of Botany, NAS Ukraine 3: Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK

Despite of the impact of radiation, active vegetation in the Chernobyl area during the recent years proves that plants can adapt to chronic radiation. Few investigations have been conducted on plants from the Chernobyl zone in respect of DNA maintenance and stabilization. One of the immediate targets of radiation is the genetic material, DNA, which can be irreparably damaged. Radiation is known to cause a great number of DNA lesions ranging from single base damage to DNA single-strand breaks (SSBs) and DNA double-strand breaks (DSBs). Our investigations show that Arabidopsis thaliana plants from the Chernobyl zone can tolerate chemical (radiomimetic, heavy metals) and physical (UV irradiation) DNA damaging agents much better than control plants from non-polluted zones. Some DNA damage repair genes are up-regulated, suggesting that mechanisms of DNA repair promote the genome stability under these conditions. It is known that plants have evolved several DNA repair pathways. We continue the investigations on gene expression from different DNA repair pathways (homologous recombination (HR), non-homologous end-joining (NHEJ), photoreactivation, base excision repair, etc) known to control the plants to get deeper insight on the mechanism of plant tolerance.

POS01-13. The roles of protein kinase Ca in radioadaptive response with hydrogen peroxide. Akira Tachibana, S. Saotome, A. Murase, H. Tauchi, Ibaraki University, Japan

Radioadaptive response is the acquirement of cellular resistance to ionizing radiation by prior exposure to low dose. It has been suggested that protein kinase C (PKC) is involved in the induction of radioadaptive response. PKC is a family of protein kinase, which plays an important role in cellular signal transduction, and nine isoforms have been identified. Although one of the isoforms, PKCa, was found to be activated by low dose of X-rays, it is still obscure which isoform of PKC is responsible for radioadaptive response. To make it clear if PKCa is involved in radioadaptive response, we made it clear if PKCa is involved in radioadaptive response, we examined adaptive response induced by the treatment with low dose of 0.5 Gy X-rays could significantly reduce the mortality from the high challenging irradiations with accelerated carbon or silicon particles, but not iron particles. In the present work, induction of AR by the priming dose of 0.5 Gy X-rays in combination with a challenging dose of 5.5 Gy with accelerated neon ions was further investigated in the same system with the same endpoint. Results showed that the priming dose of 0.5 Gy X-rays could markedly reduce the mortality from the challenging dose of 5.5 Gy neon ions. Taking together, the priming dose of 0.5 Gy X-rays could induce AR against the lethality caused by the challenging irradiations from carbon, neon and silicon, but iron particles, of which the LET values of about 15, 30 and 55, and 200 keV/micrometer, respectively. It is suggested that AR could be induced by priming low LET X-rays in combination with subsequent challenging high LET irradiations from certain kinds of accelerated heavy ions. The successful induction of AR would possibly be an event relating to the LET value of the heavy ion particle of the challenging irradiations. These findings would provide a new insight into the study on radiation-induced AR in vivo.


Low LET radiation-induced adaptive response (AR) against the detrimental effects from high LET radiation has not been well studied in vivo. Using 30-day survival in mice after challenging irradiations as the index, we previously demonstrated that a priming dose of 0.5 Gy X-rays could significantly reduce the mortality from the high challenging irradiations with accelerated carbon or silicon particles, but not iron particles. In the present work, induction of AR by the priming dose of 0.5 Gy X-rays in combination with a challenging dose of 5.5 Gy with accelerated neon ions was further investigated in the same system with the same endpoint. Results showed that the priming dose of 0.5 Gy X-rays could markedly reduce the mortality from the challenging dose of 5.5 Gy neon ions. Taking together, the priming dose of 0.5 Gy X-rays could induce AR against the lethality caused by the challenging irradiations from carbon, neon and silicon, but iron particles, of which the LET values of about 15, 30 and 55, and 200 keV/micrometer, respectively. It is suggested that AR could be induced by priming low LET X-rays in combination with subsequent challenging high LET irradiations from certain kinds of accelerated heavy ions. The successful induction of AR would possibly be an event relating to the LET value of the heavy ion particle of the challenging irradiations. These findings would provide a new insight into the study on radiation-induced AR in vivo.

POS01-15. Adaptive Response in a thyroid cell line after treatment with Tc-99m, Re-188 or X-rays. Maria Wendisch, R. Freudenberg, R. Runge, G. Wunderlich, J. Kotzerke, Universitätssklinikum Carl Gustav Carus, TU Dresden/ Klinikum und Poliklinik für Nuklearmedizin, Germany

Purpose: Radioadaptive response describes the phenomena of decreased radiotoxicity after high dose irradiation subsequent to a low dose pre-treatment. The results of this study should provide an indication if nuclear medicine therapy could be influenced by previously diagnostic investigation. Previous studies described radiation-induced adaptive response after exposure of cells with extracellular sources. In this study, we analysed adaptive response after irradiation with the radionuclides Tc-99m and Re-188 and therefore cellular radionuclide-uptake in comparison to extracellular irradiation with X-rays.

Materials and methods: The thyroid rat cell line PC3 C13 was exposed 4 hrs after different adapting doses of 0, 0.01, 0.05 or 0.5 Gy to a high dose up to 15 Gy Re-188, Tc-99m or X-rays (dependent on radionuclide and biological assay). Clonogenic cell survival was detected by colony forming assay and DNA-damage was measured by alkaline comet assay. In addition, intracellular radionuclide uptake was quantified.

Results: Adaptive response was observed after treatment with beta emitter Re-188 or X-rays. PC3 C13 cells exhibited both increased clonogenic survival and decreased DNA-damage when pre-irradiated with a low dose of Re-188 or X-rays before exposure to a later challenging dose.

Cells incubated with Tc-99m indicated no adaptive response. After pre-irradiation doses of 0, 0.01, 0.05 or 0.5 Gy PC3 C13 cells showed no differences in DNA-damage but a reduced survival dependent on pre-irradiation dose.

Conclusions: In this study we analysed the adaptive response after irradiation with the radionuclides Re-188 and Tc-99m in comparison to X-rays. These results suggest that adaptive response exists after treatment and cellular uptake of Re-188 but not of Tc-99m. Therefore, adaptation of the index, we previously demonstrated that a priming dose of 0.5 Gy X-rays could significantly reduce the mortality from the high challenging irradiations with accelerated carbon or silicon particles, but not iron particles. In the present work, induction of AR by the priming dose of 0.5 Gy X-rays in combination with a challenging dose of 5.5 Gy with accelerated neon ions was further investigated in the same system with the same endpoint. Results showed that the priming dose of 0.5 Gy X-rays could markedly reduce the mortality from the challenging dose of 5.5 Gy neon ions. Taking together, the priming dose of 0.5 Gy X-rays could induce AR against the lethality caused by the challenging irradiations from carbon, neon and silicon, but iron particles, of which the LET values of about 15, 30 and 55, and 200 keV/micrometer, respectively. It is suggested that AR could be induced by priming low LET X-rays in combination with subsequent challenging high LET irradiations from certain kinds of accelerated heavy ions. The successful induction of AR would possibly be an event relating to the LET value of the heavy ion particle of the challenging irradiations. These findings would provide a new insight into the study on radiation-induced AR in vivo.

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The Translationally Controlled Tumor Protein (TCTP) is a highly conserved protein that is widely expressed in all eukaryotic organisms. Although TCTP has been implicated in various cellular processes for survival, its role in radiation effects is poorly understood. We show that TCTP is significantly increased in nuclei of γ-irradiated human fibroblasts in an ATM-dependent manner, and knockdown of TCTP expression by siRNA approach abolished the low dose γ-ray-induced adaptive response against chromosomal damage, which highlighted a role for TCTP in DNA repair. Co-immunoprecipitation with anti-TCTP antibodies in cell nuclei extracts and analysis by Q-TOF tandem mass spectrometry revealed several TCTP-interacting proteins that are involved in important DNA damage sensing and repair processes. Further co-immunoprecipitation and immunoblotting experiments confirmed the physical interaction of TCTP and Ku70/86, suggesting the potential role of TCTP in non-homologous end joining (NHEJ). Knockdown of TCTP expression inhibited the DNA binding activity of both Ku70 and Ku86 by more than 50% compared to scramble siRNA-transfected control group after γ-ray exposure. In chromatin isolated from human cells, TCTP was found to be physically interacting with ATM and to exist in a complex with γH2AX, in agreement with its distinct localization with the foci of the DNA damage marker proteins γH2AX, 53BP1 and P-ATM. Ongoing studies are focusing on the co-localization and interactions of TCTP with Ku and other important proteins implicated in DNA repair and repair. Collectively, our studies reveal that TCTP may be a critical chaperone in multiple DNA damage sensing and repair processes.

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University of Melbourne Dept of Obstetrics & Gynaecology, Australia, 2: Health Protection Agency Centre for Radiation, Chemical and Environmental Hazards, UK, 3: Gray Institute for Radiation Oncology and Biology, University of Oxford, UK, 4: Ludwig Institute of Cancer Research, UK

Purpose: The biological effects of MRT are not well understood, and are complicated by differences in measuring the MRT doses delivered to the tissues. Film and semiconductor dosimetry as well as Monte Carlo methods struggle to provide accurate estimates of dose profiles and peak-to-valley dose ratios at the position of the targeted and traversed tissues whose biological responses to MRT determine treatment outcome. H2AX biodosimetry: The aim of this study was to investigate whether techniques could be applied to MRT-irradiated mouse skin in order to estimate the 'valley' dose between adjacent microbeams.

Materials & H2AX analysis allowed microbeams to be methods: traced and DNA damage foci to be quantified in valleys between beams following MRT treatment of fibroblast cultures and murine skin. Foci levels in cells located in valleys were compared with calibration curves using known broadbeam synchrotron X-ray doses to generate spatial dose profiles.

Results: We calculated peak-to-valley dose ratios of 30-40 for cell cultures and approximately 60 for murine skin, consistent with the range obtained with conventional dosimetry methods.

Conclusions: Gamma-H2AX is a sensitive and quantitative biomarker for radiation exposure which may be employed to visualise, quantify and validate dose distributions delivered to tissues in microbeam radiotherapy regimens. This biological dose mapping approach could find several applications both in optimising MRT or other radiotherapeutic treatments and in estimating localised doses following accidental radiation exposure using skin punch biopsies.

POS02-05. Radiation metabolomics shows species conservation of urinary biomarkers following ionising radiation exposure in the rabbit. Albert J. Hornacek Jr., S. Sumant, R. Kumar, M. Maniar, J. B Tyburski, K. Datta, 1: Lombardi Comprehensive Cancer Center, USA 2: Georgetown University Medical Center 3: Onconova Therapeutics 4: Georgetown University Medical Center, USA

BACKGROUND: Catastrophic events occurring in the past ten years—both natural and man-made, abroad and domestic—have directed concern toward the potential for mass human casualty scenarios following large-scale radiological events. Regardless of whether from compromises to nuclear power reactors or acts of terrorism, the need for expanding and enhancing our capacity for dealing with large numbers of radiological exposures at one time is pressing. Current technologies for determining whether and to what extent individuals have been exposed are slow, labor-intensive, of limited scalability, and difficult to deploy in the field. In this study we address the limitations in biodosimetry defining the use of urine small molecule response to total body irradiation in the rabbit.

OBJECTIVE: We endeavored to define the rabbit urine small molecule response to radiation as a function of both known radiation biomarkers from other animal models and novel candidate biomarkers specific for the rabbit. METHODS: Spot urine samples were collected before and up to 8 hours after exposure to 9 Gy gamma radiation delivered at 1 Gy per minute from a cesium source. Samples were analyzed by Ultra-Performance® Liquid Chromatography (UPLC) coupled to Time-of-Flight Mass Spectrometry (TOFMS). Aligned total-ion mass chromatograms were used to identify candidate biomarkers by principal components analysis, and tandem MS with authentic standards was used for confirmation. RESULTS: Principal components analysis revealed dramatic shifts in the urine metabolome of rabbits post irradiation, as evidenced by clear separation in component one scores. These changes were in part a function of up-regulated excretion of several known urine radiation biomarkers with up to 28-fold higher concentrations post exposure. In addition a novel, up-regulated candidate radiation biomarker was identified. CONCLUSION: Our results provide evidence for conservation of urinary radiation biomarkers in a non-radiant animal model. Defining the rabbit urinary metabolome changes is a necessary first step in developing this non-radiant animal model for radiation biodosimetry research and testing of radioprotectors. These results will also inform ongoing efforts to identify urinary radiation biomarkers in humans.

POS02-06. Improvement of the automated chromosome aberration finding and counting system for the biological dosimetry. Akira Furukawa, National Institute of Radiological Sciences, Japan

The biological dose estimation technique, such as chromosome aberration counting has been required to process large number of sample preparations at low dose radiation exposure, or at large number of people who wants the exposure test, such as Fukushima case. The automated chromosome aberration detecting and counting system from the images of microscope was developed before, but the software of that system was made for expensive workstation, therefore the system was very difficult to become popular, and the operator of the system was required much skill in information technology. Then, we translated the program of the system from the workstation to a personal computer (PC). The PC was using recent Ubuntu operating system, a distribution of Linux. This enabled extra low cost and user friendliness even ordinary biologists with no special knowledge of computers.

The performance of the PC system was measured by same images as the old workstation version. The resulted speed was more than three times higher, depending on how fast machine used, than old one.


Chromosomal aberrations in peripheral blood lymphocytes have been validated as biomarkers of cancer risk, and cytogenetic damage can therefore be used to characterize excess health risk incurred by individual crewmembers after their respective missions. Traditional risk assessment models are based on epidemiological data obtained on Earth in cohorts exposed predominantly to acute doses of gamma-rays, and extrapolation to the space environment is highly problematic, involving very large uncertainties. Cytogenetic damage could play a key role in reducing uncertainty in risk estimation because it is incurred in the space environment, and is assessed in specimens from the astronauts themselves.

Cytogenetic damage has been assessed in blood lymphocytes from more than 30 astronauts before and after they participated in long-duration space missions of 3 months or more on the International Space Station. Chromosome damage was assessed using fluorescence in situ hybridization whole chromosome analysis techniques. For all individuals, the frequency of chromosome damage measured within a month of return from space was higher than it was before flight, and biodosimetry estimates were within the range expected from physical dosimetry.

Follow-up analyses have been performed on most of the astronauts at intervals ranging from around 6 months to many years after flight, and the cytogenetic effects of repeat long-duration missions have so far been assessed in four individuals. Relative cytogenetic risk factors were estimated from astronaut biodosimetry data using the quantitative approach derived from the European Study Group on Cytogenetic Biomarkers and Health database. Astronauts were categorized into low, medium, or high tertiles according to their yield of chromosome damage. Age-adjusted tertile rankings were used to estimate cancer risk, and results were compared with values obtained using traditional modeling approaches. Individual tertile rankings increased after space flight, and analysis of follow-up samples indicated that the tertile rankings of more than 50% of the individuals assessed so far remained in the high category. Crewmembers who shift and remain in the high category are projected to have increased lifetime cancer risk.

POS02-08. DOSE-RESPONSE CURVES BY DICENTRICS ANALYSIS AND MICRONUCLEUS TEST FOR BIOLOGICAL DOSIMETRY IN RADIATION ACCIDENTS. Valeria Hadjidjevova1, R. Hristova1, P. Atanasova2, L. Popova1, A. Staynova1, S. Deleva1, 1: National Centre of Radiobiology and Radiation Protection, Bulgaria 2: Radiobiology and Radiation Protection, Bulgaria

The frequencies of dicentrics and micronuclei in human peripheral blood lymphocytes are the most frequently used biomarkers for the purposes of biological dosimetry in case of ionizing radiation exposure. Dose-response relationships are established for chromosomal aberrations and micronuclei induced in human peripheral lymphocytes after in vitro gamma irradiation. Peripheral blood samples of 7 different donors were used. The blood irradiation was performed with 137Cs, gamma-rays, at dose-
rate 0.87 Gy/min., using different doses: 0.0, 0.05, 0.1; 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3.0 Gy. Chromosomal aberrations assay and cytchalasin-B micronucleus test were applied. In addition, fluorescence in situ hybridization technique with pancentromeric DNA probe was applied for differential detection of centromere positive and centromere negative micronuclei. Linear-quadratic dose response relationship was established based on dicentric and ring chromosomes yield. The relationship can be described by the following equation: Y = 0.001 + 0.027*D + 0.025*D², where (Y) = dicentric and ring chromosomes yield, (D) = radiation dose obtained.

In vitro 137Cs radiation exposure showed a linear-quadratic relationship for the yield of micronuclei as well. Dose-effect curve established for the total number of radiation induced micronuclei can be described by the following equation: Y = 20500 + 39.596*D + 17.010*D². Linear-quadratic relationship also, was obtained for the yield of centromere negative micronuclei, although a high degree of variability among different donors. Excel software was established for calculation of the received dose by using these equations, as a whole body equivalent dose acute irradiation.

Results and conclusion: Reference biodosimeters such as the dicentric chromosome assay may not distinguish PBI from TBI when equivalent whole-body doses are similar and the time of exposure is sufficient for peripheral blood homogenization. Such confounding situations need more discriminating biodosimeters. In our study, as expected, the shielding of 50% bone marrow prevented aplasia to happen and hematologic parameters contributed to discriminate HBI and TBI. Moreover, some parameters may be discriminant as dosimetric markers such as neutrophils to lymphocytes ratio, creatine kinase and citrullin levels, and mitochondrial DNA. Platelet count and F1t-3 lignid levels may be good markers of injury.

Both early and more delayed assessment of clinical and biological status is required for reliable discrimination. Further work is in progress, in particular through ongoing international collaborations, for comprehensive interpretation.
In conclusion, γH2AX analysis of irradiated lymphocytes enables rapid and accurate dose estimations for at least 24 hours post exposure while foci levels remain elevated for several days after irradiation to at least 0.5 Gy of X-rays. Dispersion analysis of foci/Intensity distributions helps determine partial body doses and the irradiated fraction size in cases of non-uniform exposures.

POSTER PRESENTATIONS

POSTER 2-12: Studies on Application of DNA Repair Competence Assay as a Screening Test in Biological Dosimetry. Justyna Miszczyk1, A. Panek2, Z. Drag3, Z. Rudek1, A. Cebulskas-Wasilewska1, 1: Radiation and Environmental Biology Department, Institute of Nuclear Physics, Poland 2: Institute of Sociology, Jagiellonian University, Poland

In case of accidental mass exposure to ionisation radiation there is a strong need to establish possible effects in people in a very short time. It seems that it would be beneficial to establish the method that would allow rapidly to screen and stratify their radiation population in order to find individuals at the highest risk for biological retrospective dosimetry with chromosome aberrations (CA) technique.

The aim of presented study was to find out whether the DNA repair competence test with the application of SCGE (single cell gel electrophoresis) technique, known also as the Comet Assay, and X-ray challenging dose can be used for biological dosimetry purposes.

To simulate in vivo accidental radiation exposure conditions, blood samples and isolated lymphocytes were irradiated with various doses from 0 to 4 Gy of X-rays. Then blood samples underwent culturing for classic (CA) and molecular (FISH) cytogenetic procedures, while isolated lymphocytes after 1 hr of incubation, were irradiated another time with a challenging dose of 2 Gy. The DNA damage was detected with the alkaline version of comet assay before, then immediately after first and second irradiations and subsequently again after period allowing cells to proceed with fast DNA damage repair (40 min of incubation).

There was a linear-quadratic dose-response curve (R2 = 0.99 and R2 = 0.89 for TDNA and TM parameters respectively) after the first irradiation. DNA damage detected by Comet parameters revealed a linear correlation with frequency of the stable (translocations by FISH) and unstable chromosome aberrations (CA) (R2 = 0.92 and R2 = 0.91 for TDNA and TM parameters respectively). Linear-quadratic relationship was also obtained after additional challenging dose (R2 = 0.98 and R2 = 0.99 for TDNA and TM respectively). To estimate the DNA damage repair, we also investigated the residual DNA damage (RD) after various doses of radiation. There was a linear-quadratic relationship between RD and applied doses (R2 = 0.91 and R2 = 0.90 for TDNA and TM parameters respectively) and linear correlation between frequencies of the unstable chromosome aberrations (dicentrics and rings) (R2 = 0.88 and R2 = 0.81 for TDNA and TM respectively).

Obtained results revealed a high association between results of the DNA repair competence assay and molecular and classic cytogenetic biomarkers of cancer risk, that confirm a possible application for biological dosimetry as a rapid biomonitor, that could be applied to identify the most exposed members in the population at risk.

Acknowledgments: The research was partially supported by grants: ("Studies on individual susceptibility to radiation-induced damages, DNA repair and instability of human chromosomes in lymphocytes of patients diagnosed or treated with radiotherapy" (0296/B/PO1/2008/35) and the National Atomic Energy Agency in Poland ("Development of measurement procedures and creation of the position for rapid diagnosis of retrospective dosimetry" (ZP No. 11/2008)).

POSTER 2-13: Optimization of calcyl cucumber A-induced PCC assay for chromosome aberration studies. Tomisato Miura1, A. Nakata2, M. A. Yoshida2, W. F. Blakely1, 1: Department of Biomedical Sciences, Hiroshi University Graduate School of Health Sciences, Japan 2: Department of Radiation Biology, Institute of Radiation Emergency Medicine (IEM/HU), Hiroshi University, Japan 3: Armed Forces Radiobiology Research Institute (AFRRI), USA

Background: Calyculin A-induced premature chromosome condensation (PCC) assay is a simple and useful method to assess both cell-cycle progression and the induction of non-reciprocal sister-chromatid exchanges in binucleate cells in interphase (non mitotic cell cycle-phase). Treatment with CA (50 nM) for 60 min, which is typically used in these studies, however, results in the induction of fuzzy compactness and shortened length chromosomes.

Methods: In this study, an optimization of calcyl cucumber A exposure on chromosome morphology and PCC induction frequency was investigated using a human peripheral blood lymphocyte (PBL) ex vivo irradiation (60 Co-rays; ~0.6 Gy/min; 0 to 30 Gy) model. Morphological criteria for assessment of chromosome compactness and length were established to evaluate calcyl cucumber A effects.

Results: Treatment with calcyl cucumber A (50 nM) for 15- and 30-min resulted in 11.3- and 9.9-fold increases in the frequency of G2/M-PCC cells with extended length chromosomes compared to the 60-min treated group over a broad dose range (0 to 20 Gy), respectively. The G2/M-PCC scoring index per PCC in 15- and 50-min treated groups was increased by 1.9 and 1.8 compared to the 60-min treated group over to 20 Gy, respectively. The G2/M-PCC efficiency of 30-min treated group was highest in the three conditions (i.e., 15-, 30-, and 60-min treatment) of calcyl cucumber A exposure.

Conclusion: Calcyl cucumber A treatment for 30 minutes before the 48-h harvest of mitogen-stimulated human PBL is optimum for the formation of suitable chromosome morphology necessary to assess structural chromosome aberrations induced by exposure to radiation using the chemical induced-PCC assay.

POSTER 2-14: Hematological changes as prognostic indicators of survival in irradiated minipigs. Maria Moroni, E. Lombardini, R. Salmon, M. Kazemzadeh, V. Nagy, M. H. Whitnall, Armed Forces Radiobiology Res Inst, USA

For advanced development of radiation countermeasures, the FDA’s Animal Efficacy Rule highlights the need to develop alternative large animal models. The rule specifies that the pathophysiology of the disease in the animal model must be well-characterized and must reflect that in humans. So far, manifestations of the acute radiation syndrome (ARS) have been characterized extensively in two large animal models, non-human primates (NHP) and canines; we are evaluating the suitability of the minipig as an additional model. We previously showed the Gottingen minipig manifests hematopoietic ARS phases and symptoms similar to those observed in canines, NHP, and humans. We establish here the LD50/30 radiation dose (bilateral whole-body gamma, 0.6 Gy/min), and we show that at this dose the time of nadir and the duration of cytopenia resemble those observed for NHP and canines, and mimick closely the kinetics of blood cell depletion and recovery in human patients assigned to the hematopoietic category H3 (METREPOL severity classification). No signs of GI damage in terms of diarrhea or shortening of villi were observed. Duration of thrombocytopenia, platelet counts at selected time points and the C-Reactive Protein-to-platelet ratio were significant predictive signs of whether or not animals would survive. The ratios between neutrophils, lymphocytes and platelets were significantly correlated with exposure to irradiation at specific time intervals. We conclude that the Gottingen minipig is a valid model to study the hematopoietic syndrome of the ARS. Supported by an award from NIAID to MW.

POSTER 2-15: Optimization of the protein phosphatase-inhibitors (okadaic acid) treatment in premature chromosome condensation (PCC)-ring method for biodosimetry of accidental high dose exposure. Aikumi NAKATA1, Y. SATOT2, K. SHIBUTAN2, H. ICHIKAWA3, T. MIURA2, M. A. Yoshida1, 1: Department of Radiation Biology, Institute of Radiation Emergency Medicine (IEM/HU), Hiroshi University, Japan 2: Graduate School of Health Sciences, Hiroshi University, Japan 3: Armed Forces Radiobiology Research Institute (AFRRI), USA

The dicentric chromosome is used as the most reliable biological marker for dose estimation in the cases of accidental exposure by the radiation. However, is difficult to apply the conventional dicentric-metaphase assay in the case of high dose exposure to ionizing radiation over the lethal dose. Because, such high dose exposure to the radiation induces the mitotic delay and apoptosis resulting in poor mitotic index. In order to estimate accurately the radiation dose in high dose exposure, the PCC (premature chromosome condensation) method which induces the DNA condensation in the interphase cell resulting in the chromosome formation has been developed and a ring chromosome is recommended as chromosome aberration used for biodosimetry in the cases of high dose radiation exposure. Although protein phosphatase inhibitors such as okadaic acid and calcyl cucumber A can induce PCC in interphase and metaphase chromosome aberrations in cells while in interphase (non mitotic cell cycle-phase). Treatment with CA (50 nM) for 60 min, which is typically used in these studies, however, results in the induction of fuzzy compactness and shortened length chromosomes.
Dynamics of apoptosis in γ-irradiated B human lymphocytes. Zuzana Sinkorová 1, L. Zarybníčka 1, L. Matosková 1, A. Tichý 1, J. Pechgl 1, J. Sinkora 2, J. Vavrova 2, 1: University of Defence, Faculty of Health Sciences, Czech Republic 2: Becton Dickinson, Prague, Czech Republic

Introduction: The dynamics of apoptosis progression has been studied in human PBMC irradiated with selected doses (0.5, 1, 2, 4, 6, 8 and 10 Gy) of γ-rays and subsequently cultivated for 16 hr in vitro. A dose-dependent increase and decrease of shrunk and intact cells, respectively, has been observed in the range of 0.5 - 4 Gy while higher doses have not affected the relative numbers of intact and apoptotic cells. The relative number of early apoptotic cells did not change within the dose range studied. Immunophenotyping of intact and very early apoptotic cells have indicated that the remaining intact cell represent the most convenient population for radioresistance studies in vitro. The CD21+CD27+ subset of peripheral B-cells has been confirmed as a useful biodosimetric marker in vitro. Materials and Methods: Peripheral blood mononuclear cells (PBMC) from ten healthy volunteer donors were sham-treated or irradiated with the doses of 0.5; 1; 2; 4; 6; 8 and 10 Gy and cultivated 16 hr in humidified atmosphere at 37°C. Than the cells were harvested and stained with monoclonal antibodies: CD21/APC, CD27/PEDy590, CD38/PEDy747 and Annexin V-FTIC in parallel. Acquisition and analysis were performed using the Summit (Beckman Coulter) software. Results: We have identified three major populations of PBMC based on their size and Annexin V binding and compared their relative numbers in irradiated and subsequently cultivated (16hr) samples. We have documented the relative increase in numbers of the shrunk cells with the high Annex V binding capacity with an increasing irradiation dose. This has been paralleled by a decrease of the proportion of intact, Annexin-negative cells. The proportion of the Annexin V normally sized PBMC population has not changed with an increasing irradiation dose. At the earliest recognizable stage detectable by surface phosphatidylinositol surface, Annexin V expression is not affected and no non-specific binding of monoclonal antibodies due to the loss of cell surface integrity can be detected. As apoptosis progresses and the cells bind more and more Annexin V, surface marker expression decreases. No reliable biodosimetric information has been contained in the CD38+-expression, whilst the combination of CD21 as a surface marker of a major peripheral B cell subset with CD27 has revealed a biodosimetric potential of the CD21+CD27+ B-cell subset. Conclusion: The ratio of intact, Annexin V-negative cells to the proportion of shrunk cells has proven to be very promising. We have confirmed that the B cell subpopulation and its CD21+CD27+ subset may represent promising biodosimetric markers.

POSTER PRESENTATIONS

PoS02-16. NH/NIAD Radiation/Nuclear Medical Countermeasures Research and Development Program. Navneet Ramakrishnan, M. Norman, A. DiCarlo, D. Cassatt, F. Macchiarini, L. Davies, B. Madiment, National Institute of Allergy and Infectious Diseases, USA

The NIH/NIAD medical countermeasures program develops devices and drugs to diagnose, mitigate and treat acute and delayed radiation injury to several physiological systems that can result from accidental, intentional radiation exposure and/or radiation therapy. It is a basic and translational research program with a strong emphasis on product development. Several grants, contracts and interagency agreements are funded through this program. Significant advances have already been made in the development of medical countermeasures for the mitigation/treatment of radiation-induced hematopoietic, gastrointestinal, lung and skin injuries. Several high-throughput biodosimetry techniques and devices are being developed for prompt and accurate individual radiation dose assessment. These include a high-throughput, robotics-controlled automated image acquisition system capable of analyzing 30,000 samples per day; 2) a biodosimetry tool with a fully integrated biochip and an integrated microfluidic cartridge that can perform whole-blood microarrays for radiation-injury-specific gene expression signatures; 3) a portable biodosimeter based on radiation-induced metabolomics expression signatures; and 4) a portable EPR dosimeter using nails, hair and tooth. The projects are conducting basic research to identify biomarkers of radiation damage as well as developing devices and treatment strategies, and translate basic knowledge to medical products for public use.

PoS02-17. Dynamics of apoptosis in γ-irradiated B human lymphocytes. Yumiko Suto1, M. Akiyama2, M. Hirai1, M. Yukii2, T. Tominaiga2, F. Nakayama2, T. Suzuki3, N. Sugaura4, M. Akashi5, 1: Research Center for Radiation Emergency Medicine, National Institute of Radiological Sciences, Japan, 2: Tokyo Nuclear Services Co., Ltd., 3: Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, Japan

In the conventional diconntric chromosome assay (DCA), the linear-quadratic dose response equation has been established for doses less than 6 Gy. At higher doses, DCA does not provide accurate dose response relationship. An early mitotic delay can be observed at high irradiation dose. This has been paralleled by a decrease of the proportion of intact, Annexin V binding cells. The proportion of Annexin V binding cells decreases. The proportion of Annexin V binding cells decreases. This early detectable apoptotic stage progresses and the cells bind more and more Annexin V, surface marker expression decreases. No reliable biodosimetric information has been contained in the CD38+-expression, whilst the combination of CD21 as a surface marker of a major peripheral B cell subset with CD27 has revealed a biodosimetric potential of the CD21+CD27+ B-cell subset. Conclusion: The ratio of intact, Annexin V-negative cells to the proportion of shrunk cells has proven to be very promising. We have confirmed that the B cell subpopulation and its CD21+CD27+ subset may represent promising biodosimetric markers.

PoS02-18. Induction and persistence of cytogenetic damage in cultured peripheral blood lymphocytes following 15 Gy gamma-irradiation. Narayani Tominaga1, T. Suzuki2, N. Sugaura3, M. Akashi4, 1: Research Center for Radiation Emergency Medicine, National Institute of Radiological Sciences, Japan, 2: Tokyo Nuclear Services Co., Ltd., 3: Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, Japan

Peripheral blood samples were exposed to 15 Gy gamma-ray (15Co source, 0.5 Gy/min). Isolated lymphocytes were cultured in RPMI1640 medium supplemented with 20% fetal bovine serum, 2% phytohaemagglutinin and 30 mM bromodeoxyuridine (BrdU). Cells were harvested at 48 h, 50 h, 52 h, 54 h, 56 h, 72 h and 96 h after culture initiation. For the 72 h sample, multicolor fluorescence in situ hybridization (M-FISH) was conducted to analyze complex chromosome aberrations. The first mitotic peak appeared at 52-54 h. Interestingly, cells containing aberrant chromosomes with as many as 8-10 centromeres were observed. Average dicentric equivalent count per cell ranged from 9.0 to 9.5 in 48 h – 56 h samples. In the 72 h sample, 20% of the dividing cells were in the 2nd metaphase. It should be noted that 70% of the 2nd metaphase cells were tetraploid cells including endoreduplicated cells. The high degree of polyplody noted in our material suggests that irradiation may exert an effect on the replication process, in addition to the structural damage. M-FISH analyses revealed that in certain cells, aberrant chromosomes were accurately replicated in the polyploidization process. In the 96 h sample, cells at the third metaphase with octaploid chromosome were found. In conclusion, a certain population of peripheral blood lymphocytes was found to transit to the second and even third mitoses after high-dose in vitro irradiation, persisting with severe chromosome aberrations. The occurrence of polyploidization and endoreduplication following high-dose irradiation described here validated the reported fact that after high dose radiotherapy, tetraploid cells were observed in the circulating blood of patients.


To clarify the biological effects of space environment, especially space radiation, a proposal of "Rad Gene" were performed as the first life science experiment with two human lymphoblastoid cell lines bearing wild-type p53 gene (wt53) and mutated p53 gene (mp53) in an International Space Station at Kibo module for 133 days. We scheduled four projects: (1) DNA damage induced by space radiations including the high linear energy transfer (LET) particles was detected as a track of phospho-H2AX foci in the nuclei of these frozen cells. (2) To examine the biological effects of microgravity and space radiations on gene and protein expression in the bone-marrow stage hematopoietic cells grown under microgravity and 1 gravity in ISS, and on ground for 8 days and analyzed by DNA and protein arrays. (3) p53-Dependant
regulated genes were analyzed in the cultured cells after spaceflight at frozen state exposed to space radiations. (4) To clarify the effects of space radiations on the radio-adaptive response, the space flown cells at frozen state were cultured, and then exposed to challenging X-ray irradiation doses. All of the radio-adaptive responses of cell killing, apoptosis, chromosomal aberrations and mutations were found only in wp53 cells, but not in the mp53 cells. It is expected that data from the space experiments will be helpful in designing physical and biological protection from the deleterious effects of space radiations during long term stays in space.

POS02-20. A new system for analyzing the ionizing irradiation-induced chromosome abnormalities. Satoshi Tasitato1, L. Shi', k. Fujoka2, M. Ohtaki3, 1: Hiroshima University, Japan 2: Hiroshima University, Japan

Analysis of chromosome aberrations in human peripheral blood lymphocytes is the most established method for the biological dosimetry in radiation emergency medicine. Analysis of metaphase spreads with Giemsa staining, however, requires skilled personnel because of difficulty to identify abnormal chromosomes. Fluorescence in situ hybridization (FISH) analysis of abnormal chromosomes makes it easy to identify chromosome abnormalities, but requires aging for more than two days. Here, we established a FISH analysis using telomere and centromere PNA probes to perform an analysis of abnormal chromosomes in the irradiated peripheral blood lymphocyte without aging. Good agreement on the percentage of metaphases with dicentric chromosome after irradiation from 0 Gy to 15 Gy was observed between conventional Giemsa staining and FISH analysis. The percentage of metaphase with tricentric or multicentric chromosomes from lymphocytes irradiated at high dose (≥8Gy) detected by FISH was higher than that by Giemsa staining analysis. This could be due to the difficulty to precisely identify multiple centromeres in Giemsa stained chromosomes. The FISH analysis using centromere/telomere PNA probes may become a easy and reliable method for the biological dosimetry in radiation emergency medicine.


BACKGROUND: Ongoing efforts involving radiation metabolomics in mice, rats, and more recently in rabbits have yielded a number of urine ions that serve as markers in assessing exposure to ionizing radiation. The ultimate goal of these efforts is to have a panel of human urine metabolites that can be measured with field-deployable instruments in the event of nuclear catastrophe or hostile use of radiological weapons. Currently, biodosimetry technology for determining absorbed dose of ionizing radiation exposures is limited. Moreover, animal model studies thus far have focused on male subjects for simplicity’s sake. OBJECTIVE: We determined whether gender modifies the urine small molecule profile responses to radiation exposure in rats. METHODS: Twenty-four-hour urines were collected from age-matched adult male and female rats before and at 24, 48, and 72 h after doses of total body gamma radiation ranging from 0.5 to 10 Gy. In addition, a 6-h urine was collected from a subset of rats immediately after exposure. All samples were analyzed by Ultra-Performance® Liquid Chromatography coupled to Time-of-Flight Mass Spectrometry in both positive and negative electrospray ionization modes. Principal components analysis (PCA) was used to explore differences between the radiation response in female versus male urine samples, and known as well as unknown radiation-responsive ions were examined. RESULTS: Scores from PCA revealed that at sublethal doses, gender exerts a stronger differentiating effect than does radiation exposure and that the exposure diminishes the gender differences in urine metabolite profiles. Inspection of concentrations of known urinary radiation biomarkers revealed that the magnitude of change in excretion of some ions following exposure was either greater or lesser in females compared with males. Finally, pre exposure concentrations of some radioreponsive ions were different, depending on gender. CONCLUSION: As expected, gender is a key determinant in urine metabolite profiling, and evidence from radiation metabolomics suggests that gender modifies the urinary responses to radiation exposure. These results highlight the importance of determining gender-specific ranges for normal and exposure-associated concentrations of radiation biomarkers in urine and other biofluids.

POSTER PRESENTATIONS

POS02-22. Comparison of γH2AX foci and micronuclei induced in lymphocytes of patients treated with IMRT, IMAT or RapidArc therapy for prostate cancer. Joke Werbrouck1, P. Ost2, G. De Meerleer2, A. Vriel1, H. Thiers2, 1: Ghent University, Belgium 2: Ghent University Hospital, Belgium

Introduction: The induction of secondary malignancies by contemporary radiotherapy (RT) techniques used for treatment of a patient’s primary tumour is a matter of debate. Modern rotational RT techniques such as Intensity Modulated Arc Therapy (IMAT) and RapidArc are hypothesized to increase the cancer risk compared to Intensity Modulated Radiation Therapy (IMRT) because of exposure of larger volumes of healthy tissue to low radiation doses. We investigated whether rotational techniques are associated with a higher risk for the development of secondary cancers compared to IMRT by measuring the IR induced double-strand-breaks (DSB) and the resulting chromosome damage in lymphocytes of prostate cancer patients. Moreover, the association between the number of γH2AX foci and micronuclei (MN) induced by RT was examined.

Experimental procedures: The foci and MN assay were performed to determine the IR induced DSB and chromosomal damage in 19 prostate cancer patients treated with IMRT (n=12), IMAT (n=4) or RapidArc (n=8). Two blood samples were taken before and after the 1st fraction of RT. Following the 3rd fraction, an additional blood sample was taken for the MN assay. Foci and MN were scored semi-automatically using the Metafer system (MetaSystems). The equivalent total body dose (D_{eq}) with exclusion of the tumour volume was calculated for each patient based on the treatment planning (DVH).

Summary: Patients treated with IMAT (highest mean D_{eq}), showed the highest level of foci after the 1st fraction and MN after the 3rd fraction. The difference in IR induced foci is not that clear for the IMRT and RapidArc patients despite a somewhat higher D_{eq} after RapidArc therapy. The latter induced more MN compared to IMRT. For the complete population, the IR induced foci and MN show respectively a linear and linear-quadratic response as a function of D_{eq}. A linear-quadratic fit to MN versus foci data showed satisfactory correlation (R^2 = 0.89). In vivo foci and MN yields as a function of the calculated D_{eq} were higher than expected from the in vitro dose response. This can be explained by the contribution of scattered radiation to the whole body exposure, not taken into account in the D_{eq} calculations.

Conclusion: IMAT shows the highest levels of both biomarkers, RT induced MN versus foci shows a good linear-quadratic correlation.

POS02-23. The optimisation of a finger-prick blood collection method for the gamma-H2AX assay: potential application in population triage. Joanna Kwiecinska1, Alejandra Zlocki1, Marcin Kruszewski1, Institute of Nuclear Chemistry and Technology, Poland

Accurate methods for measuring the biological effects of radiation are critical for estimating the health risk from radiation exposure. The gamma-H2AX foci scoring assay seems to be the most sensitive to ionizing radiation. The aim of our study was to optimize a finger-prick blood collection method for the automated gamma-H2AX assay in relation to various blood storage conditions.

Peripheral whole blood was collected from five healthy volunteers. For each donor, blood samples were irradiated with 250 kV X-rays (0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2, 3, 4 Gy). After irradiation, one set of samples was incubated at 37°C for 30 min. Three others sets of samples were incubated at 0°C, 4°C and 37°C for 24 h, followed by 30 min incubation at 37°C. Blood samples (50 μl) were mixed with 900 μl of medium and loaded into eppendorf tubes containing 100 μl of Histopaque 1077. The samples were spun two times at 2000 rcf for 3 min. The cells were spotted onto slides with a cytospin at 490 rcf for 10 min, fixed in methanol for 5 min and permeabilized/blocked in KCMT buffer (120 mM KCl, 20 mM NaCl, 10 mM Trition, 1 mM EDTA) containing 2 % BSA and 10 % low fat milk for 1 h at RT. The cells were incubated with anti H2AX γ antibody (1:1000 in PBS), washed in PBS and incubated with FITC-conjugated goat anti-mouse secondary antibody for 45 min at RT. Slides were mounted with DAPI Vectashield and analyzed with an automated image acquisition and analysis system Metafer (Metasystems, Germany).

Our results revealed that the number of gamma-H2AX foci was linearly associated with the radiation dose. The number of gamma-H2AX foci obtained for samples scored 30 min after irradiation was 68
Biosimetric studies are concerned with the ionizing irradiation and with its effect on cells and organisms, in general. New methods focused on the assessment of doses absorbed by organisms are on the top of interest and new ways for dose estimate still have been searched. It is generally known that radiation interacting with cells induces DNA breaks which may result in the apoptosis induction. So detection and measuring an apoptotic cells rate can correlate with the absorbed dose of irradiation. The mitochondrial permeability transition which is connected with the loss of mitochondrial membrane potential (MMP) belongs to the important sign of cell apoptosis. This early apoptosis period can be detected by the lipophilic dye JC-1 belonging to a group of mitochondrial potential sensors. This cationic dye exhibits potential-dependent accumulation in mitochondria, where forms red fluorescent J-aggregates. The downturn of mitochondrial transmembrane electrical potential is followed by JC-1 change into cytosol green monomeric form.

In our study we targeted on the radiosensitive response of porcine peripheral blood lymphocytes isolated from whole blood by histopaque system (Sigma). The cell suspension was irradiated by 0, 2, 4, 6, 8, and 10 Gy, respectively, and changes of MMP were analyzed in a time frequency of 4, 8, and 24 hours, respectively, after irradiation. Before that lymphocytes were immunophenotyped by specific monoclonal antibodies which allow us to distinguish CD3(+)CD4(+) and CD3(+)CD8(+) T cells. Then the mitochondrial membrane sensor JC-1 (Probes) was added at the final concentration 5 µmol/l, cells were washed by phosphate buffer and analyzed by the CyAn ADP flow cytometer (Dako Cytomation).

The apoptosis inducing cells (low potential cells) were detected as green (525 nm) fluorescence intensity increasing and red (590 nm) green (525 nm) fluorescence intensity increasing and red (590 nm) absorption. The detection of MMP changes seems to be suitable and hopeful tool in biodosimetric studies which could acquire wide applications within this field.

Conclusion: In vitro gene expression analysis in human PBL based on whole human DNA-microarrays allowed identifying a rather small set of radiation dose predictive and radiation-specific genes with a high potential for biodosimetric applications in vivo. The detection and measuring an apoptotic cells rate can correlate with the absorbed dose of irradiation. The mitochondrial permeability transition which is connected with the loss of mitochondrial membrane potential (MMP) belongs to the important sign of cell apoptosis. This early apoptosis period can be detected by the lipophilic dye JC-1 belonging to a group of mitochondrial potential sensors. This cationic dye exhibits potential-dependent accumulation in mitochondria, where forms red fluorescent J-aggregates. The downturn of mitochondrial transmembrane electrical potential is followed by JC-1 change into cytosol green monomeric form. In our study we targeted on the radiosensitive response of porcine peripheral blood lymphocytes isolated from whole blood by histopaque system (Sigma). The cell suspension was irradiated by 0, 2, 4, 6, 8, and 10 Gy, respectively, and changes of MMP were analyzed in a time frequency of 4, 8, and 24 hours, respectively, after irradiation. Before that lymphocytes were immunophenotyped by specific monoclonal antibodies which allow us to distinguish CD3(+)CD4(+) and CD3(+)CD8(+) T cells. Then the mitochondrial membrane sensor JC-1 (Probes) was added at the final concentration 5 µmol/l, cells were washed by phosphate buffer and analyzed by the CyAn ADP flow cytometer (Dako Cytomation). The apoptosis inducing cells (low potential cells) were detected as green (525 nm) fluorescence intensity increasing and red (590 nm) intensity decreasing cell population. Our results show that the number of low potential lymphocytes increased with the dose-dependent manner as so as with the time after irradiation. The detection of MMP changes seems to be suitable and hopeful tool in biodosimetric studies which could acquire wide applications within this field.

This work was supported by grant number WND-PO2G.01.03.01-14-054/09.

POSTER PRESENTATIONS

POS02-24. MITOCHONDRIAL MEMBRANE POTENTIAL ANALYSE WITHIN THE FRAME OF BIOSIMETRIC STUDIES. Lenka Zarybucká1, Z. Sinkorova2, J. Krocová1, J. Sinkora2, 1: Faculty of Military Health Sciences, University of Defence, Czech Republic 2: Becton Dickinson, Prague, Czech Republic

Biosimetric studies are concerned with the ionizing irradiation and with its effect on cells and organisms, in general. New methods focused on the assessment of doses absorbed by organisms are on the top of interest and new ways for dose estimate still have been searched. It is generally known that radiation interacting with cells induces DNA breaks which may result in the apoptosis induction. So detection and measuring an apoptotic cells rate can correlate with the absorbed dose of irradiation. The mitochondrial permeability transition which is connected with the loss of mitochondrial membrane potential (MMP) belongs to the important sign of cell apoptosis. This early apoptosis period can be detected by the lipophilic dye JC-1 belonging to a group of mitochondrial potential sensors. This cationic dye exhibits potential-dependent accumulation in mitochondria, where forms red fluorescent J-aggregates. The downturn of mitochondrial transmembrane electrical potential is followed by JC-1 change into cytosol green monomeric form.

In our study we targeted on the radiosensitive response of porcine peripheral blood lymphocytes isolated from whole blood by histopaque system (Sigma). The cell suspension was irradiated by 0, 2, 4, 6, 8, and 10 Gy, respectively, and changes of MMP were analyzed in a time frequency of 4, 8, and 24 hours, respectively, after irradiation. Before that lymphocytes were immunophenotyped by specific monoclonal antibodies which allow us to distinguish CD3(+)CD4(+) and CD3(+)CD8(+) T cells. Then the mitochondrial membrane sensor JC-1 (Probes) was added at the final concentration 5 µmol/l, cells were washed by phosphate buffer and analyzed by the CyAn ADP flow cytometer (Dako Cytomation). The apoptosis inducing cells (low potential cells) were detected as green (525 nm) fluorescence intensity increasing and red (590 nm) intensity decreasing cell population. Our results show that the number of low potential lymphocytes increased with the dose-dependent manner as so as with the time after irradiation. The detection of MMP changes seems to be suitable and hopeful tool in biodosimetric studies which could acquire wide applications within this field.

This work was supported by grant number WND-PO2G.01.03.01-14-054/09.

POS02-25. Enhanced frequency of micronuclei in lymphocytes from current as opposed to former uranium miners. Friedo Zölzer1, Z. Freitinger-Skalicky1, R. Havránková1, Z. Hon1, L. Navrátil1, J. Rosina2, J. Škopěk1, 1: University of South Bohemia, Czech Republic 2: Czech Technical University in Prague, Czech Republic

Micronuclei can be used as markers of past radiation exposure, but few pertinent studies have dealt with alpha radiation. Here we report on micronuclei in lymphocytes from uranium miners, comparing some that are currently active and others that retired 15 – 20 years ago. Their radiation exposure is assumed to come mainly from radon and its decay products in the breathing air at the work place.

Current miners showed a greater micronucleus frequency than former miners. This can be attributed to their recent radiation exposure, while the smaller frequency in the former miners probably results from the disappearance of potentially micronuclei containing lymphocytes from the peripheral blood, which is known to occur with a half-life in the order of a year.

For current miners there is a significant correlation between micronucleus frequency and effective dose received over the last 12 months. The dose at which a doubling of the micronucleus frequency is observed is around 10 mSv. This is a much smaller dose than would usually be expected to be detectable with this test, and raises a number of questions about the induction of micronuclei by alpha radiation from radon and its decay products. These investigations have been supported by the Czech Ministry of Education, Youth and Sports within the framework of the National Research Program II (NPV II, project 2B080001).

POS02-26. Identification of radiation-specific gene expression changes in human PBL after ex vivo irradiation suitable for biodosimetric applications. Katja Knop1, S. Boldt2, O.Wolkenhauer2, R. Krehuber2, 1: Department of Safety and Radiation Protection, Forschungszentrum Jülich, Germany 2: Department of Computer Science, Systems Biology and Bioinformatics Group, University of Rostock, Germany

Introduction: In case of a large-scale radiation accident with involvement of individuals without physical dosimeters it is important to identify individuals who have received a moderate to high radiation dose to ensure proper medical care. As current methods are time-consuming, a fast and reliable method based on gene expression alterations is developed.

Methods: Human blood from 6 healthy donors (3 male and 3 female, belonging to 3 different age classes) was irradiated ex vivo with 0, 0.5, 1, 2 and 4 Gy (γ-rays, Cs-137). Peripheral blood lymphocytes (PBL) were isolated and subjected to ionizing radiation (6, 24 and 48 h for 0, 0.5, 1, 2 and 4 Gy). PBL were irradiated in 1 ml of peripheral blood, which was kept in a non-nutrient medium for 24 hours at 37°C. The peripheral blood samples were then incubated for 6 hours in a non-nutrient medium containing 25 and 200 µg/ml Paracetamol (Paracetamol) and 0.1 and 0.4 µg/ml Mitomycin C (Mitomycin C) at 37°C. The culture medium was added to the irradiated PBL at 100 µl/ml. The cells were then harvested, and gene expression was accordingly analyzed.

Results: By a p-value and fold-change driven gene selection 16 genes were identified allowing a radiation dose prediction accuracy of 96% independently on the time-point post irradiation up to 48 h. For 6 predictive genes qRT-PCR measurements based on pooled and non-pooled irradiated samples additionally validated the observed radiation-induced gene expression alterations. Furthermore, qRT-PCR analysis showed that the strong up-regulation of these genes is highly radiation-specific as the up-regulation after irradiation was much more pronounced when compared to exposure with Paracetamol or Mitomycin C. Protein expression analysis showed only for two genes a weak correlation between gene and protein expression after irradiation.

Conclusion: In vitro gene expression analysis in human PBL based on whole human DNA-microarray data allowed identifying a rather small set of radiation dose predictive and radiation-specific genes with a high potential for biodosimetric applications in vivo.

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POS3 Bystander effects

POS03-01. Bystander response to DNA-incorporated I-125 in human breast cancer cell cultures depends on cell line. John Akadugu, E. Azzam, R. Howell, UMDNJ New Jersey Medical School, USA

This study uses a three-dimensional cell culture model to investigate bystander cell killing in human breast cancer cells (MCF-7, MDA-MB-231) treated with 125I-labeled 5-iodo-2'-deoxyuridine (125IEdU). MCF-7 and MDA-MB-231 cells were derived from clinical effusions taken from women with invasive ductal carcinoma and adenocarcinoma, respectively. They respectively form metastatic xenografts in mice in an estrogen-dependent and independent manner. In the present study, these cells were cultured three-dimensional in a Cytomatix® carbon scaffold. Cultures were pulse-labeled for 3 h with 125IEdU to selectively irradiate a minor fraction of cells, and simultaneously co-pulse-labeled with 0.04 mM 5-ethylidy-2'-deoxyuridine (EdU) to identify the radiolabeled cells using Click-IT® EdU and flow cytometry. After thorough washing, the cultures were incubated for 4 h. The cells were then harvested, serially diluted, and seeded for colony formation. Aliquots of cells were subjected to flow cytometry to determine the percentage of cells labeled with 125IEdU/EdU. Additional aliquots were used to determine the mean ^ 2 activity per labeled cell. Two experiments were conducted for each cell line. The percentage of labeled cells was...
about 15% and 10% for MCF-7 and MDA cultures, respectively. This created irradiation conditions wherein the cross-dose to unlabeled cells was very small relative to the self-dose to labeled cells. The surviving fraction relative to EDU-treated controls was plotted as a function of mean absorbed dose to the labeled cells and the data were least squares fitted to a two-component exponential function. Resulting survival curves indicated substantial killing of MCF-7 bystanders, however, no significant bystander killing of MDA-MB-231 cells was observed. These results confirm the capability of ³²P-IdU to induce bystander cell killing and suggest that the bystander response is dependent upon characteristics of the cell line.

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We investigated the role of gap junction intercellular communication (GJIC) in determining the responses of human cells to different types of ionizing radiation that differ in their LET properties. We focused on the communication of signaling events among irradiated cells and between irradiated and bystander cells. Confluent cell cultures were exposed to isosurvival doses of protons (LET 0.2 keV/μm), gamma rays (LET 0.9 keV/μm), X rays (LET 2 keV/μm), carbon ions (LET 76 keV/μm), alpha particles (LET 122 keV/μm) or iron ions (LET 103 keV/μm) under conditions wherein all cells are traversed by radiation tracks. Clonogenic survival and micronucleus formation were assayed within minutes or longer incubation periods after irradiation. As expected, carbon ions, alpha particles and iron ions were more effective than protons, gamma rays and X rays at inducing cell killing. Holding gamma-proton and X-irradiated cells in the confluent state for several hours promoted increased survival and decreased micronucleus formation. However, maintaining cells irradiated with alpha particles, carbon ions or iron ions in the confluent state for various times prior to subculture was associated with persistent DNA damage and decreased survival. Inhibiting gap junction communication with 18-glycerorythretic acid, or knockdown of connexin43, promoted against the toxic effects during confluent holding. Together, the data indicated that GJIC amplifies the toxic effects of high-LET radiations after irradiation, but has minimal effects on the net effect of low LET radiations.

Using microbeam irradiation systems, we have also investigated the role of GJIC in modulating DNA damage in confluent irradiated cells under conditions by which 0.2% of cells in the population was targeted with X rays (LET 6 keV/μm) or carbon ions (LET 103 keV/μm). The results show that the propagation of stressful effects from irradiated bystander cells depends on LET and dose, and that GJIC promotes the propagation of stressful effects in bystander cells exposed to high LET radiations. We conclude that radiation quality affects GJIC-mediated propagation of stressful effects between irradiated cells and between irradiated and bystander cells. Characterizing the nature of the communicated molecules would have translational implication in radiotherapy and radioprotection.

POS03-03. Responses of bladder transitional cell carcinoma cell lines to modulated radiation fields. Malgorzata Bilk, K. Butterworth, K. M. Prise, K. McCloskey, CCRCB, Queens University Belfast, UK

Introduction: Bladder cancer is the second most common urological malignancy. Conventional treatment involves surgical resection and radiation therapy. Cell signalling between differentially irradiated cell populations within the target tumour volume is likely to influence both therapeutic outcome and adverse effects.

Purpose: "To investigate responses of bladder cancer cell lines to uniform and non-uniform radiation fields."

Study design, materials and methods: Bladder urethelial cancer cell lines, T24 and HT1376 were investigated along with a DU145 prostate cancer cell line used as a positive control. Clonogenic cell survival assays were performed to quantify survival responses to uniform and non-uniform field radiation using 223 kVp X-rays. Non-uniform fields were produced by shielding 50% of the cell culture flask. DNA double-stranded breaks were analysed by gamma-H2AX immunohistochemistry.

Results: HT1376 cells were less sensitive to radiation induced cell killing in comparison to T24 or DU145 cells for uniform exposures. Cell survival was determined for the exposed and shielded regions of the flask in non-uniform fields and compared to 50% survival response in uniform field exposures. In T24 cells (n=3), survival in the shielded region was lower than predicted from the scattered radiation dose and saturated at 30% at a scattered dose of 0.06 Gy. Cell survival for DU145 (n=3) in the shielded region was also significantly lower and dropped to 50% at the dose of 0.24 Gy. These responses were prevented by physical inhibition of cell-cell communication and indicate a role for bystander signalling. HT1376 cells (n=3) showed no significant changes in cell survival within the shielded region.

Conclusion: The data shows that the modulated radiation exposures impact on survival and DNA damage responses. This was related to the underlying radiosensitivity of the cells to direct irradiation with the most radiosensitive cell type showing minimal impact from a modulated beam.

POS03-04. The cross-talk between adaptive, bystander and long-term effects of space radiation: the role of intercellular communication. Manuela Buonanno, S. M deToledo, E. I Azzam, UMDNJ-NJMS, USA

Exposure of mammalian cells to low dose/low fluence ionizing radiation has been shown to elicit phenomena such as genomic instability, bystander effects and adaptive responses that impact the net biological outcome of the exposure. Here we show that the manifestation of these phenomena and their cross-talk in normal human cells exposed to space radiation depends on the linear energy transfer (LET) of the radiation and involves a prominent role for gap-junction communication.

Stressful effects were observed in bystander AG1522 cells that were co-cultured with AG1522 cells exposed to high but not low LET radiation. Further, these effects persisted in progeny cells for at least 20 generations. Relative to control, the progeny of bystander cells that had been in co-culture with 1 GeV/u iron ions (LET ˜151 keV/μm) exhibited a reduced cloning efficiency that was associated with higher levels of micronuclei, protein oxidation, lipid peroxidation, and decreased activity of metabolic and antioxidant enzymes. In C3H 10T1/2 mouse embryo fibroblasts, the effect in bystander cells resulted in an increase in the spontaneous frequency of neoplastic transformation. These stressful effects were not observed when irradiated and bystander cells were co-cultured in the presence of gap junction inhibitors, or if the irradiated cells were targeted with 1 GeV proton-bystander cell pairs (LET ˜0.24 keV/μm).

In deep space, most cells in an astronaut’s body are likely to be traversed by a proton before being hit by a high charge and high energy (HZE) particle. When AG1522 human cells were exposed to 20 Gy from 50 MeV or 1 GeV protons, they were protected from the clastogenic effects of a subsequent dose of 50 Gy from 1 GeV/u iron ions. Further, exposure to low dose proton irradiation propagated signaling events that protected bystander cells from the damaging effects of iron ions. The protective effect was transient and decayed by 24 h. Collectively, the data highlight the significance of radiation quality and intercellular communication in determining low dose/low fluence radiation effects, which may contribute in the assessment of the health risks of low dose radiation exposure.

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POS03-05. Determining the impact of area, dose and dose-rate on out-of-field cell survival and radiation induced bystander responses following exposure to intensity-modulated radiation fields. Karl Butterworth, Queens University Belfast, UK

Intensity-modulated radiation therapy (IMRT) is an advanced radiotherapy approach in which highly modulated fields are used to achieve dose conformity across a target tumor volume. Recent data from our laboratory has shown significant alterations in cell survival occurring out-of-field which cannot be accounted for on the basis of scatter (LET 151 keV/μm) alone. The aim of this study was to determine the impact of area, dose and dose-rate on out-of-field cell survival responses following exposure to intensity-modulated radiation fields.

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Cell survival was determined by clonogenic assay in human prostate cancer (DU145) and transformed fibroblast (AG0-1522) cells following exposure to different field configurations delivered using a X-Ray 225 kV X-ray generator. Uniform survival responses were compared to in- and out-of-field responses in which 25 – 75% of the cell population was shielded. Dose delivered to the out-of-field region was varied from 1.6 – 37.2 % of that delivered to the in-field region using different levels of brass shielding. The impact of dose rate on response was determined for 0.2 – 4 Gy / min for uniform and modulated exposures.

Survival responses showed little dependence on dose rate and area in- and out-of-field with a trend towards increased survival with decreased in-field area and decreased survival with decreased out-of-field area. Directly irradiated cells were shown to scale in proportion to dose delivered to the in-field region and also local dose delivered out-of-field. Mathematical modelling of these findings has shown survival response to be highly dependent on dose delivered in- and out-of-field but not on area or dose rate.

These data provide further insight into the radiobiological parameters impacting on cell survival following exposure to modulated irradiation fields highlighting the need for refinement of existing radiobiological models to incorporate non-targeted effects and modulated dose distributions. This work is supported by Cancer Research UK (Grant C1513 / A7047).

**POSO3-06. Bystander and Direct Effects Induced by Carbon Ions in AG01522 Normal Human Primary Fibroblasts as Revealed by Micronuclei Induction**, Alessandro Campa, F. Antonelli, G. Esposito, V. Dini, G. Simone, M. Belli, M. Antonella Tabocchini, Istituto Superiore di Sanità, Italy

This study investigated on the ability of medium taken from cultures of AG01522 normal human primary fibroblasts irradiated with C-ions to induce a response in non irradiated AG01522 cells (medium-mediated bystander effect). The C-ion beam of the Superconducting Cyclotron radiobiology facility at the INFN-Laboratori Nazionali del Sud (LNS, Catania, Italy) was used, with E about 45 MeV/u at the cell entrance, corresponding to LET about 49 keV/µm. The cell response was measured as chromosomal damage observed by the micronuclei (MN) formation in prophleashelled cells using the cytogenesis block assay. The damage induced in directly C-ion irradiated AG01522 cells was firstly investigated, using graded doses of 0.1, 0.25 and 0.5 Gy. The MN induction was almost linear in this dose range. For bystander effect measurements, the medium was taken from cell cultures irradiated with C-ions at doses of 0.1 or 0.5 Gy, 1 or 5 h after irradiation, filtered and added to the non irradiated (“bystander”) cells. A bystander effect around 40 % was observable only on addition of conditioned medium taken 5 h after irradiation, with no significant difference between the two doses used (0.1 or 0.5 Gy). However, a large variability was observed between different experiments.

To study the involvement of ROS and RNS mediators of the bystander signaling, DMSO and c-PTIO (scavengers of ROS and NO, respectively) were added to the culture 1 h before irradiation. Comparison with sham-irradiated cells in the presence and in the absence of the scavengers showed that their presence tends to weaken the bystander effect observed after addition of the 5 h conditioned medium, but the data do not allow to conclude whether this reduction is statistically significant. The effect of these compounds was also tested in directly irradiated cells. They decreased the response by about 20% (DMSO) and 30% (c-PTIO), indicating that an indirect action, mediated by ROS and/or NO species, may contribute to the chromosomal damage induced by C-ions. Even if the results point to a protection of these scavengers in both direct and bystander effect, elucidating the specific role of ROS and NO for each of these effects needs further investigation.

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**POSO3-07. The role of ER stress in radiation-induced bystander effects**, Jennifer Fazzari, McMaster University, Canada

To address possible mediators of radiation induced bystander effects the Endoplasmic Reticulum (ER) and its stress response pathways are of interest due to the sensitive homeostatic environment of the organelle that is prone to physiological perturbations such as hypoxia, oxidative stress, abnormal ER calcium content and mutation. Notably, the ER stress response has also been attributed to radiosensitivity and radioresistance. Due to the role of reactive oxygen species and calcium fluxes in bystander signal production and response, the ER proves to be an organelle of interest in further understanding of this process. An initial response to ER stress is activation of the Unfolded Protein Response (UPR), triggered by misfolded proteins accumulating at the ER. The UPR signals through 3 stress sensors located in the ER membrane: ATF6, PERK and IRE1. Upon stress-induced activation, these sensors mediate a signal transduction cascade that activates various survival pathways as well as autophagy or apoptosis. IRE1 is one pro-apoptotic pathway that has been independently associated with the bystander effect yet many exist in which a correlation has yet to be studied. The goal is to elucidate whether the bystander effect is mediated by the ER stress signaling cascade through immuno-detection of ER-stress related proteins in directly irradiated cells and those exposed to irradiated cell conditioned media (ICCM) relative to cultures treated with known ER stress inducers thapsigargin or tunicamycin. Of primary concern is the detection of one isoform of XBP1, a highly active transcription factor involved in upregulation of a variety of UPR-related genes and those linked to cell signaling and DNA damage pathways. Comparative markers of ER stress such as GRP78, eIF2, CHOP, ATF4 and caspases can also be used to generate a preliminary expression profile that can be followed in subsequent investigation using a variety of techniques such as RT-PCR. In addition to giving insight into bystander signal production and response, involvement of the ER-stress response in radiation induced bystander effects will bring to light the possibility of tissue-specific bystander responses as mediated by established tissue specific ER stress activity.

**POSO3-08. Synchrotron microbeams of normal rat brain increased the expression of proteins known to be present in glioma in bystander rat brain hemisphere**, Cristian Fernandez1, E. Schuëltke2, R. Smith1, E. Bräuer-Kirsch3, J. Laisse3, J. Wang3, C. Seymour4, C. Mothersill4, 1: McMaster University, Canada 2: University of Freiburg 3: European Synchrotron Radiation Facility 4: Institut für Pathologie der Universität Bern 5: McMaster Regional Centre for Mass Spectrometry, Canada

Microbeam radiation treatment (MRT) is a pre-clinical type of radiosurgery developed for brain tumor treatment. MRT uses high flux synchrotron x-rays delivered as an array of parallel microbeams in high doses of irradiation to tumors in fractions of seconds. Some evidence suggests that MRT gives better clinical results than homogenous field of radiotherapy but the mechanism is unknown. The aim of this study was to investigate whether MRT of normal rat brain could produce an adverse or protective effect in non-irradiated tissue by analyzing the induction of bystander associated proteins. Healthy adult wistar male rats were anesthesiated and exposed to either 35 or 350 Gy MRT or to homogenous field of radiation to the right brain hemisphere, Then brain and bladder were dissected after 4, 8 or 12 hours, and samples for proteomics and bystander clonogenic assay were taken. Clongenic survival of the reporter HPVG cells showed that bystander effects occurred in both the non-irradiated hemisphere and bladder tissue.

Proteomics studies showed that the bystander-associated proteins were characterized by A, an increase in the expression of NADH dehydrogenase ubiquinone and Glial fibrillary acid proteins, which are known to be present in gliomas. B, a reduction of the Prohibitin protein, which is thought to be a tumor suppressor in humans. C, an increase on the expression of Heat shock cognate 71KDa protein, which is known to be involved in the disassembly of clathrin-coated vesicles and D, a decrease on the expression of Tubulin alpha-1A chain protein, which is known to be one of the major components of microtubules. The results suggest that the MRT irradiation of unilateral normal rat brain hemisphere produces bystander signals that may induce tumoral transformation of non-irradiated brain cells. Further studies should be done to identify how proteins C and D are involved in the transformation process. Immunohistochemistry studies are in progress to reinforce our findings.

**POSO3-09. High Charge, High Energy (HZE) particle-induced bystander effects: Contribution of secondary particles?**, Geraldine Gonon1, J. Groetzo2, M. Fromm1, E. I. Azzam1, 1: Department of Radiology, New Jersey Medical School, Cancer Center, USA 2: Laboratoire de Chimie Physique et Rayonnements Alain Chambaudet (LCPR-AC). UMR CEA EdF 3220, Université de Strasbourg, France 3: Laboratoire de Chimie Physique et Rayonnements Alain
The induction of stressful effects in bystander cells from cell populations exposed to high charge and high energy (HZE) particles is relevant to radiotherapy and to estimates of the health risks of space radiation. We investigated the kinetics of expression of stress markers in density-inhibited normal human AG1522 fibroblast cultures exposed to doses as low as 0.2 Gy from 1 GeV/u iron ions (LET = 0.151 keV/µm) or 600 MeV/u silicon ions (LET ~ 51 keV/µm) wherein 1-3% of the cells would be targeted through the nucleus. Western analyses showed greater increases than expected in the level of phospho-TP53 (serine 15) and phospho-ERK1/2 as early as 15 min after exposure. Significant increases in the levels of HSF1 and p21WAF1 could be observed by 1 to 3 h after irradiation. Although the levels of these proteins decreased at 6h and 24h after exposure, they were higher than control. Large increases in oxidative stress as judged by protein carbonylation and lipid peroxidation was also observed 24h after irradiation, suggesting perturbations in oxidative metabolism. As well, these stressful effects were also detected when the irradiated cell populations were subcultured to lower density immediately after the exposure. To distinguish irradiated cells from bystander cells, we developed glass-bottomed dishes that incorporate a 100 µm CR-39 solid state nuclear track detector bonded to the bottom edges of the culture surface. The lid of the dishes can be sealed, thus enabling irradiation in the presence of growth medium and adequate control of pH. After 12 μ460 cells, more growth was observed with N-24h after incubation with CM resulted in complete delayed than A549 cells when exposed to N-24h cells were generated by the presence of irradiated cells in a dose- and cell number-dependent manner. The effective effect of the bystander cells and H460 (hH460) cells were generated by the presence of irradiated cells in a dose- and cell number-dependent fashion. Part of the effect could be mediated by CM from irradiated cultures indicating the involvement of a transferrable factor. Thus a bystander effect seems to contribute to radiation-induced inactivation in a dose-dependent fashion after high single doses. The mechanism by which clonogenic proliferation was inhibited has not yet been identified. We are currently studying cytokine secretion by irradiated cells into the conditioned medium.

**POSTER PRESENTATIONS**

**POS03-10. Radiation-induced bystander effects in normoxic and hypoxic conditions in human lung cancer cells.** Seema Gupta, S. Tubin, M. Ahmed, University of Miami, USA

**Purpose:** Many cell lines that respire anaerobically do not show cytotoxic bystander effects (BE) following irradiation. Further, there is no existing data on the acute hypoxia (H)-induced BE. The purpose of this study was to investigate the status of radiation-induced BE in normoxic (N) and hypoxic conditions as well as acute H-induced BE. Methods: Lung cancer cell lines, A549 and H460 were exposed either to real oxygen (100% O2) or cells irradiated with 2 Gy at 3% O2 for 12h. Cells were plated at low density and incubated for 24h with fresh medium and then irradiated with 2 Gy. Cells were harvested 24h later and cells were plated at low density and incubated for 24h. The second group of H460 cells both N- and H-irradiated to 2-Gy and H460 cells were generated by continuous exposure of the cells to H. Irradiated H460 cells or challenge resistant cells (100%) were irradiated H460 irradiated for another 24h. Western analysis showed the phosphorylation of HSF1 and p21WAF1 could be observed by 1 to 3 h after irradiation. Although the levels of these proteins decreased at 6h and 24h after exposure, they were higher than control. Large increases in oxidative stress as judged by protein carbonylation and lipid peroxidation was also observed 24h after incubation, suggesting perturbations in oxidative metabolism. As well, these stressful effects were also detected when the irradiated cell populations were subcultured to lower density immediately after the exposure. To distinguish irradiated cells from bystander cells, we developed glass-bottomed dishes that incorporate a 100 µm CR-39 solid state nuclear track detector bonded to the bottom edges of the culture surface. The lid of the dishes can be sealed, thus enabling irradiation in the presence of growth medium and adequate control of pH. After 12 μ460 cells, more growth was observed with N-24h after incubation with CM resulted in complete delayed than A549 cells when exposed to N-24h cells were generated by the presence of irradiated cells in a dose- and cell number-dependent fashion. Part of the effect could be mediated by CM from irradiated cultures indicating the involvement of a transferrable factor. Thus a bystander effect seems to contribute to radiation-induced inactivation in a dose-dependent fashion after high single doses. The mechanism by which clonogenic proliferation was inhibited has not yet been identified. We are currently studying cytokine secretion by irradiated cells into the conditioned medium.

**POS03-12. Stimulation of intercellular induction of apoptosis in transformed cells at very low doses: the role of radiation quality, cell type and the local environment.** Mark Hill1, A. B. Abdelrazzak1, G. Bauer2, P. O'Neill3, 1: Gray Institute for Radiation Oncology & Biology, University of Oxford, UK 2: Institute of Medical Microbiology and Hygiene, University of Freiburg, Germany

Intercellular signalling plays and important role in the progression of transformed cell to a tumour. In order to characterise the underlying mechanism and how they are perturbed following exposure to ionising radiation, a well defined model system of intercellular induction of apoptosis was used where neighbouring normal cells are selectively eliminated. Cell lines in which clonogenic proliferation was inhibited has not yet been identified. We are currently studying cytokine secretion by irradiated cells into the conditioned medium.

**Results:** The surviving fraction (SF) of MCF7 cells irradiated with 12 Gy was decreased 8.5-fold (95% c.i. 4.4-16.3; P<0.0001; n=12) when the cell number was decreased 1 to 5 x 10^4 per flasks. Furthermore, colony formation of unirradiated cells co-cultured with irradiated (2 Gy) feeder cells was reduced by up to 28% [c.i. 22-34%; P<0.0001; n=3] when the number of feeder cells was increased (5-50 x 10^4 per flasks). Similarly, conditioned medium (CM) collected over 24h from cells cultured irradiated with 5-15 Gy inhibited colony formation of recipient unirradiated cells to a similar extent. Interestingly, cells treated with CM in this way showed no significant effect on the apoptotic fraction or cell cycle distribution. The inhibitory effect of conditioned medium from irradiated cells was confirmed for MDA-MB-231 and HUVEC.

Conclusions: The clonogenic activity of irradiated or unirradiated cells was influenced by the presence of irradiated cells in a dose- and cell number-dependent manner. Part of the effect could be mediated by CM from irradiated cultures indicating the involvement of a transferrable factor. Thus a bystander effect seems to contribute to radiation-induced inactivation in a dose-dependent fashion after high single doses. The mechanism by which clonogenic proliferation was inhibited has not yet been identified. We are currently studying cytokine secretion by irradiated cells into the conditioned medium.
cells are irradiated. The generality of the IIA response was explored using combinations of fibroblast and epithelial transformed and non-transformed cells. Although apoptosis was observed in both sets of transformed cells as a result of intercellular signalling, significant differences were observed in the perturbation of the response when co-cultured with non-transformed cells with and without exposure to radiation.

POS03-13. Modulation of mitochondrial membrane potential in bystander effect. Genro Kashino1, Y. Tamariz2, J. Kumagar1, M. Watanabe2, 1: Oita University, Japan 2: Kyoto University 3: Nagoya University, Japan

The mechanism of radiation induced bystander effect has been topic recently. In mammalian organism, the function of mitochondria is very important for the production of energy in each cell. Therefore, we focused on the changes of mitochondrial functions by bystander effect through the secreted factors in the present study. In normal human fibroblast cells (IMR-90) and human glioma cells (U373MG), the mitochondrial membrane potentials were detected by JC-1 analysis, and were reduced by the secreted factors from irradiated cells. Also, increased levels of reactive oxygen species (ROS), which is thought to be super oxide radical in mitochondria, were detected by MitoSOX analysis. The increases of ROS were thought to be leading to the production of slow releasing long lived radicals (SRLRs), which is thought to be a cause of mutagenesis (Koyama et al Mutat Res 1998).

The treatment of secreted factors from irradiated cells resulted in the induction of gene mutation at HPRT locus. These results suggest that secreted factors from irradiated cells can lead to mutagenesis through the change of mitochondrial function.

POS03-14. Effect of nicotine on the radiation-induced bystander response of the HPV-G cell line. Hedieh Katal Mohsena, C. Seymour, C. Mothersill, McMaster University, Canada

Radiation-induced bystander effects (RIBE) even though well accepted, have poorly understood mechanisms although inter-cellular signalling is clearly important. Previous work suggested cells might be harnessing existing neurotransmitters present in trace amounts in serum to signal between irradiated and non-irradiated cells. This study is therefore aimed to investigate the effect of nicotine on production of RIBE. Nicotine, a chemical found in tobacco and tomato plants, was chosen because of its widespread use and its ability to act on nicotinic acetylcholine receptors by imitating the action of neurotransmitter acetylcholine. Additionally, recent studies have revealed that low doses of nicotine have therapeutic effects in a number of neurodegenerative diseases. Cells were irradiated with X-rays doses of 0.1, 0.5, 1, 2, 3, and 5 Gy while being exposed to different concentrations of nicotine ranging from zero to 10 μM. Cells were either left to form colonies or irradiated cell conditioned medium was harvested and transferred to recipient cells. The effects were determined using the standard clonogenic assay. The results suggest nicotine is radioprotective. It was shown that concentration of 100 nM increased cell survival for all radiation doses. Results of the bystander experiment clearly showed an elevation in the number of colonies in range of 10-100 nM for different doses. This elevation was best observed in radiation dose of 1 Gy. The results of the present study suggest that low concentrations of nicotine protect directly irradiated cells and also enhance RIBE in HPV-G transfected human keratinocyte cells. It is not clear what the mechanism of protection is but inhibition of apoptosis is being considered.

POS03-15. Investigation of bystander responses in CHO-K1 cells irradiated by 12C ions. Urszula Kaźmierczak1, D. Banaś, M. Bogowicz2, J. Brażewicz2, J. Buraczewska1, J. Choiński, M. Czerekowski, M. Dąbrowska, A. Korman, M. Wojewódzka, A. Wójcik, M. Wrzesień1, 1: Institute of Experimental Physics, University of Warsaw, ul. Hoża 69, 00-681 Warsaw, Poland, 2: Institute of Physics, Jan Kochanowski University, ul. Świętokrzyska 15, 25-406 Kielce, Poland, 3: Institute of Nuclear Chemistry and Technology, ul. Dorodka 16, 03-019 Warsaw, Poland, 4: Heavy Ion Laboratory, University of Warsaw, ul. Pasteura 5A, 02-093 Warsaw, Poland, 5: The Andrzeja Sołtan Institute for Nuclear Studies, 05-400 Otwock-Swiern, Poland, 6: Institute of Biology, Jan Kochanowski University, ul. Świętokrzyska 15, 25-406 Kielce, Poland, 7: GMT Department, Stockholm University, Sweden, 8: Faculty of Physics and Applied Informatics, University of Lodz, Poland

Understanding heavy ion radiation-induced bystander responses may provide useful insights into potential therapeutic approaches for the treatment of human cancers. The aim of this study was to investigate the bystander effect in Chinese hamster ovary cells (CHO-K1) exposed to 4 Gy of high LET 12C ions (17 MeV), delivered from the cyclotron in Heavy Ion Laboratory of The Warsaw University. Immediately after irradiation, cells were transferred into transwell culture insert dishes to enable a co-culture of irradiated and non-irradiated cells. For the clonogenic survival assay, bystander cells were seeded at 150 cells/well. Irradiated cells were plated on membrane of insert. For the micronucleus assay, bystander and directly irradiated cells were plated at 125 x 10^3 cells/insert/well. All data presented in our study are representative of four separate experiments in six repetitions.

Table 1. Effects of high LET 12C ions irradiation on the frequency of micronuclei (MN) and clonogenic survival of directly and bystander CHO-K1 cells. [MNC-monomucleated cells; BNC-binucleated cells; CBPI-Cytokinesis-Block Proliferation Index; SF-Survival fraction; PE-plateing efficiency]. At least 1000 cells were examined per point.

<table>
<thead>
<tr>
<th>Micronucleus assay</th>
<th>MNC without MN</th>
<th>MNC with MN</th>
<th>BNC</th>
<th>CBPI</th>
<th>MN/1000 BNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated cells</td>
<td>0.8±0.2</td>
<td>0.0±0.0</td>
<td>0.65</td>
<td>2±0.4</td>
<td></td>
</tr>
<tr>
<td>Bystander cells</td>
<td>0.18±0.01</td>
<td>0.03±0.004</td>
<td>1.65</td>
<td>2±0.4</td>
<td></td>
</tr>
<tr>
<td>Control cells</td>
<td>0.13±0.01</td>
<td>0.03±0.004</td>
<td>1.91</td>
<td>16±6</td>
<td></td>
</tr>
</tbody>
</table>

As presented in Table 1, we observed a significant increase in the frequency of MN in BNC as well as an increased number of MNC and BNC with MN in directly irradiated cells, but not in bystander cells, as compared to control cells. These results were confirmed by the clonogenic survival results. The SF in directly irradiated cells was significantly reduced as compared to control cells. However, the SF in bystander cells was not affected, irrespective of a density of irradiated cells.

To conclude, no bystander effect was found under the experimental conditions used in our study. However, a relationship between the number of BNC without MN and the SF in clonogenic assay was observed.


The purpose of this study was to investigate the role of genetic heterogeneity in the ATM (Ataxia telangiectasia mutated) gene with respect to the individual variation of targeted and non-targeted responses to ionizing radiation.

Cell lines carrying ATM+/+ , ATM-/-, or ATM-/- genotypes were irradiated with 0, 0.01, 0.1, 1, or 2 Gy of X-rays (100 kV). Immediately after the irradiation the cells were co-cultured with non-irradiated cells (bystander cells) for 1 h or 20 h. Cell viability assay and chromosomal aberration analysis were performed for both irradiated and bystander cells.

In the irradiated cells, decreased cell viability was observed especially with doses of 1 and 2 Gy in each cell line, most clearly in the ATM-/- genotype. In the bystander cells, no difference was seen in the viability of ATM+/+, ATM-/-, or ATM-/- cell lines at any dose. Chromosome-type aberrations showed a clear dose response in irradiated ATM+/+, ATM+/-, and ATM-/- cells at 1 h and 20 h co-culture. No dose effect was observed for the chromatid-type aberrations in ATM+/+ and ATM+/- cells whereas for the ATM-/- cell line chromatid aberrations increased with irradiation dose. In the bystander cells after 1 h co-culture, the yield of chromosome-type aberrations remained at constant level over all doses within each of the studied ATM cell lines. However, it was higher in the ATM-/- cell line. After 20 h of co-culture, the yield of chromosome-type aberrations was very modest in all bystander ATM cell lines. An increase of cells with chromatid-type aberrations was observed at 0.1 Gy in bystander ATM+/- cells after 1 h co-culture. At 20 h of co-culture, this increase was no longer observable.

Conclusively, the response of the irradiated cells for both cell viability and chromosomal aberrations were dose-related and reflected the
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number of functional ATM alleles in each cell line. Further, no bystander effect was observed when applying the described experimental settings.

**POS03-17. X-RAY AND UVC-INDUCED BYSTANDER EFFECTS IN PLANTS.** Igor Kovalchuk1, F. Zemp2, V. Titov3, 1: University of Lethbridge, Canada 2: University of Calgary, Canada

Plants are capable of rapidly reprogramming patterns of gene expression, allowing fast acclimation and adaptation in response to specific environmental conditions. This ability depends on various signalling molecules operating within a plant and even between plants. Our previous experiments (Kovalchuk et al., Nature, 2003; Boyko et al., Nature, 2010, Bolognesi et al., 2010, PLoS ONE) showed that exposure to stress results in systemic increase in homologous recombination frequency (HRF). We named this signal ‘systemic recombination signal’ or SRS. Although the nature of the signal was unknown, we hypothesized that plants are able to communicate this signal through phloem and through air.

For the experiment, we used transgenic Arabidopsis thaliana plants carrying in the genome luciferase gene serving as a substrate for homologous recombination. Cells in which recombination events took place are visualized in CCD luciferase camera after application of luciferine. Recombination events then are scored and recombination frequency calculated. In the experimental set-up we planted groups of plants in Petri dish and covered one group with either aluminium or lead cover and irradiated the second group with either UVC (7,000 ergs) or X-ray (5 Gy). To test whether signal is communicated through media or through air, we used Petri dish with 3% height divider that separate the media but not the air exchange. In another set of experiments we placed two pots, one with irradiated and one with non-irradiated plants in sealed plastic bag for 4 days and scored recombination frequency in 7 days.

We found that both groups of plants, irradiated and non-irradiated grown in Petri dish had higher recombination frequency. Moreover, we found that both groups of plants grown in divided Petri dish also had higher recombination frequency. This suggested that the signal leading to increase in HRF is indeed airborne. The experiment with plastic bags also showed the increase in HRF in both groups of plants. We conclude that irradiated plants emit signals that could promote additional rearrangements in plant genome. This bystander effect could be one of the mechanisms of induced evolution, since increase frequency of rearrangements could potentially lead to diversification of genome composition in the progeny.

**POS03-18. Induction of long-lived radicals in bystander effects for point mutation.** Jun Kumaga1, K. Mioki1, G. Kashino2, M. Watanabe2, 1: Nagoya University, Japan 2: Oita University 3: Kyoto University, Japan

Irradiating cells induces bystander effects that have been recognized that non-irradiated cells received some bystander factors from irradiated cells via gap junctions and/or culture medium express biological responses similar to radiation biological effects such as mutation, chromosomal aberration, and so on. In this study culture medium with CHO cells in a culture flask was X- or γ-ray irradiated, and then the medium was transferred to other culture flasks in which non-irradiated CHO cells were plated. It is expected that irradiated cells as donor of soluble bystander factor were introduced to each flask through a membrane filter, and the flasks were stored in the incubator for 6 or 24 h. After the incubation, the recipient cells were harvested and moved into a Suprasil quartz tube for ESR measurement. ESR spectra of recipient cells were recorded by JEOL JES-RE1X EPR spectrometer in Nagoya University. For the measurement of an ESR spectrum of the cells at 77 K. When the bystander medium was treated to the donor cells for 6 h, no significant increase as +10% (p < 0.001) can be detected in the recipient cells. It should be noted that the significant increase in the levels of super oxide can be found in recipient cells at 6 h after the medium transfer from the flask of 4 Gy irradiated donor cells, so that increase in the levels of LLRs may be slowly induced after the increase in the levels of super oxide. Significant differences can be observed between treated cells with non-irradiated bystander medium. It could be speculated LLRs may be slightly produced in the recipient cells near confluent condition. Addition of ascorbate (AA: 1 mM) at the medium transfer and treated for 24 h to the recipient cells reduced the levels of LLRs as control level, however, that of N-acetylcyesteine (0.5 mM; NAC) did not. These phenomena of LLRs completely related to the induction of point mutation. AA suppressed the induction of point mutation by the medium transfer, but NAC did not. These results indicate that LLRs induced in the recipient cells are likely to be responsible for mutation induction.

**POS03-19. Mechanistic model of bystander effects accounting for non-linear release of signals by irradiated cells and non-linear response to signals by reporter cells.** Pavel Kundra, W. Friedland, Helmholtz Zentrum München, Institute of Radiation Protection, Germany

Several modelling approaches have been proposed to derive details on the mechanisms of radiation-induced bystander effects, e.g. the signal range or the size of targets whose hits by radiation initiate signal emission. The models generally assume that individual signal molecules act on bystander cells independently, yielding an exponential dependence of the effect on signal level. However, undiluted irradiated cell-conditioned medium (ICCM) reduced the survival of HPV-G reporter cells to 60 % while twice diluted ICCM did not affect their survival at all (Ryan et al 2008 Radiat Res 169: 188-196). These non-linear effects indicate the need for refining the existing models.

Modelling studies will be presented that consider the release of signals and the response to signals explicitly as two separate processes. The observed response of cells to signals has been represented by sigmoid functions, which often result from complex signalling networks. This suggests that bystander signals trigger intracellular signalling pathways in reporter cells, consistent with indications for cytokines serving as the signals. To infer information on signal release by irradiated cells, dose-dependent bystander data (Liu et al 2007 Radiat Res 168: 627-630) have been compared with set of alternative target scenarios evaluated by the Monte Carlo PARTRAC code. A distribution of targets comparable with the numbers and sizes of mitochondria, implicated in bystander effects, have been found compatible with the data. The data can be also interpreted by scenarios with only a small fraction of cells activated by radiation and emitting primary signals that trigger secondary signalling by neighbour cells. In recent experiments on mutation induction, bystander effects vanished upon 4-fold ICCM dilution but persisted even after 30-fold reduction in donor cell numbers (Zhang et al 2009 Mutat Res 671: 20-25). This indicates that scenarios with signal amplification at low and inhibition at high signal levels are more realistic than cell-autonomous ones assumed in existing bystander models. A model accounting for such interplay of positive and negative feedbacks, known in cytokine signalling, will be presented.

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**POS03-20. Cell death signalling pathways in directly irradiated cells and bystander cells.** Fiona Lyng1, F. Lyng1, K. Kumar Jella2, A. Garcia1, R. Moriarty1, B. McClean1, 1: Dublin Institute of Technology, Ireland 2: St Luke’s Hospital, Ireland

Although considerable evidence exists for radiation induced bystander effects, the mechanisms involved are still unclear and there are very few studies which directly compare direct and bystander effects. The aim of this study was to compare directly irradiated or treated with irradiated cell conditioned medium (ICCM).
Human keratinocyte (HaCaT) cells were irradiated with low doses of gamma radiation (0.005Gy, 0.05Gy and 0.5Gy) using a Cobalt-60 teletherapy unit. After irradiation, the cells were incubated for one hour and medium was harvested and filtered and added to recipient HaCaT cells which were not irradiated. Both the directly irradiated cells and the cells exposed to ICCM were incubated for 24-72 hours after treatment. Apoptosis and necrosis levels were measured using a Vybrant apotosis kit with YO-PRO-1 and propidium iodide fluorescence staining using MMP2 antibody was performed to detect mitochondrial cell death. Cell cycle phases were also analysed using flow cytometry. To further investigate the signalling pathways in bystander cells, reactive oxygen species and nitric oxide were analysed in real time after the addition of ICCM using time lapse fluorescence microscopy (30 min over 24 hours). Caspase activation was also measured after the addition of ICCM using the Apatox assay. Early apoptosis was found in directly irradiated cells and in cells exposed to ICCM after 24 hours and necrosis was found after 48 hours of treatment. Mitotic cell death was found in directly irradiated cells and in cells exposed to ICCM after 72 hours of treatment. Reactive oxygen species and nitric oxide signalling was observed in real time up to 24 hours after addition of ICCM. Caspase activation was observed after addition of ICCM. Radiation induced bystander effects may have importance in low dose radiation protection and in health risk assessment.

**POS03-21. Bystander treatment with X-ray microbeams suppresses spontaneous mutation.** Munetoshi MAEDA1, M. TOMITA1, H. MATSUMOTO2, N. USAMI3, K. KOBAYASHI4,1: Radiation Safety Research Center, Central Research Institute of Electric Power Industry (CRIEPI), Japan 2: Division of Oncology, Biomedical Imaging Research Center, University of Fukui 3: Photon Factory, High Energy Accelerator Research Organization (KEK) 4: Research Service Office, High Energy Accelerator Research Organization (KEK), Japan

Microbeam irradiation system is a powerful tool to elucidate the mechanisms underlying the radiation-induced bystander responses. Using a synchrontron X-ray microbeam irradiation system developed at the Photon Factory, KEK, we recently demonstrated that the induction of cell death, which occurred in irradiated cells and also in bystander cells, is modified by sites of energy deposition within cells. As the next step, we started the investigation to elucidate the relationship between energy deposited sites and the dose-responses of mutagenesis in bystander cells. We irradiated five nuclei of V79 cells with 10 µm × 10 µm square 5.35 keV X-ray beams, and then measured the mutation frequency in bystander cells with HPRT mutagenesis assay. The mutation frequency at null dose, 2.6x10⁻⁶ (spontaneous level) decreased to 5.3x10⁻⁷ at around 1 Gy (absorbed dose in nucleus), but at higher doses, the mutation frequency was then returned to the spontaneous level. Biphasic type of dose responses was observed in the case of bystander cells, which was not reported in our previous report. Similarity of these behaviors in dose-response may suggest the correlation in the mechanism between enhancement of bystander cell death and the suppression of mutagenesis in bystander cells. Oxidative damage of nucleotides within DNA or precursor pools caused by reactive oxygen species (ROS) is thought to play an important role in spontaneous mutagenesis. It has been reported that intracellular ROS persistently increase the fraction of genetically unstable cells and the clones of those cells do secrete factors that contribute to the perpetuation of the unstable phenotype. Cells are known to have a mechanism to suppress spontaneous mutagenesis in order to keep low mutated fraction. Bystander responses might suppress the secretion of instability-inducing factors and/or elevate the antioxidant ability in the bystander cells. It is known that exposure to nitric oxide (NO) causes mitochondrial degeneration and subsequent cell death in cells having low antioxidative ability. Unstable cells in the population might be selectively killed by bystander treatment since NO is the major mediator of bystander cell death, and hence the suppression of spontaneous mutagenesis could be observed in bystander cell population.

**POS03-22. Bystander Effect in Tumor Cells Produced By Iodine-125 Labeled.** Omar Mamlouk, P. Balagununorthoy, K. Wang, S. James Adelstein, A. I. Kassis, Harvard Medical School, USA

The bystander effect, a phenomenon elicited by irradiated cells, describes the responses and stimuli observed in neighboring cells that are not directly exposed to radiation. Earlier studies from this laboratory demonstrated that tumor cells labeled with Auger-electron emitting radionuclides ¹²⁵I and ¹³¹I can either suppress or augment the growth of neighboring cells (inhibitory and stimulatory bystander effects respectively). Here we have investigated the ability of human lymphocytes labeled with a lethal dose of 5x10²⁻¹²/2-deoxyuridine (¹²⁵I/¹³¹I) to exert bystander effect in unlabelled human adenocarcinoma colon cancer LS174T cells in vitro. Mononuclear cells were isolated from human blood by Ficoll overlay. Non-adenhert lymphocytes were separated from adherent monocytes and cultured in physiologic medium (Eagle’s memorial) to stimulate growth/DNA synthesis. After 48 hours, ¹²⁵I/¹³¹I was added and the incubation continued for another 48 hours to allow the dividing lymphocytes to incorporate ¹²⁵I/¹³¹I in their nuclear DNA. Human colon LS174T adenocarcinoma cells were co-incubated with ¹²⁵I-labeled 1:3 (I/LS174T) or 1:1 (I/LS174T) ratio co-cultures. The proliferation of the unlabeled LS174T cells was assessed after 5 days. Results showed that the co-incubation of ¹²⁵I-L with LS174T cells (1:1 ratio) leads to a significant reduction in the proliferation of the tumor cells (56±3.5%). No growth inhibition was detected when lymphocytes (unlabelled PHA stimulation) were used. When LS174T cells were cultured in the presence of supernatant media obtained from these co-incubations, a similar decrease in the tumor cell growth (52±1,3 %) was found. Since (i) ¹²⁵I is covalently bound to nuclear DNA, and (ii) >98% of the Auger electrons emitted during ¹²⁵I decay deposit their energy within 0.5 µm, the observed reduction in the proliferation of LS174T cells in presence of LS174T lymphocytes or media obtained from such co-incubations can only be attributed to an inhibitory bystander effect, probably produced by factor(s) released by the dying ¹²⁵I-labeled lymphocytes.

**POS03-23. Investigation of signaling perturbation in cells exposed at low doses of ionizing radiation.** Luca Marrioti1, C. Donata1, D. Alloni2, G. Babini3, D. Volpi1, A. Ottolenghi4: 1: University of Pavia, Dipartimento di Fisica Nucleare & INFN, Italy 2: University of Pavia, Dipartimento di Fisica Nucleare, INFN, LENA 3: University of Pavia, Dipartimento di Fisica Nucleare, CRUK/MRC Gray Institute for Radiation Oncology & Biology, University of Oxford, UK

Radiation induced bystander effects, either protective or adverse, have been identified in a variety of cells and for different endpoints. They are thought to arise from communication between cells through direct cell–cell contacts and via transmissible molecules secreted into the medium by targeted cells (1). It is likely that multiple pathways are involved in bystander phenomenon, and different cell types respond differently to bystander signaling; multiple signaling cascades involving both an initiating event and downstream signaling steps are necessary to mediate the bystander process (2). As a result, the bystander effects occur in a wide range of spatial scales (e.g. in vitro cell culture, tissues and in vivo animals) and temporal dynamics (e.g. from few minutes to several days after irradiation).

Our group investigated medium-mediated damage mechanism in AG122 cells after low dose ionizing irradiation. In particular, a systematic investigation of the release dynamics of signaling molecules for different radiation doses have been performed. An evaluation of the cytokine levels (IL-6, IL-8, IL-33 (2), TNF-α and TGF-β) in the medium of cells irradiated with different radiation doses and types was performed. The ELISA assay in Western Blot. Also Nitric Oxide levels, evaluated by its oxidized products (nitrite and nitrate) were investigated for different experimental conditions and after exposure to different doses of gamma rays.

Finally, we conclude the investigation studying the molecules involved in the perturbed signaling, looking at the actual precursor mechanisms responsible for a modulated release of these proteins in the medium. Recently, Hui and colleagues (3) illustrated a unifying model of the signaling pathways underlying radiation-induced bystander effects where NF-KB (one of the transcription factors related to cytokine expression) plays a key role. This protein regulates the expression of signaling proteins (e.g., cytokines). Cilcoxogenase-2 (COX-2) and Nitric Oxide Synthase (NOS) On the basis of these findings, also NF-KB and the COX-2 dynamics were investigated, as a function of radiation doses.

4 This work was partially supported by the European Commission (EC Contract FP7 EURATOM project ‘EPIRADBIO’ and ‘DOREMIT’)

**POS03-24. Bystander Effect Induced by Decay of Iodine-125 in Cytoplasm of Mammalian Cells.** Gautam Maulik, P.
The bystander effect, originating from irradiated cells, describes the biological responses of neighboring cells that are not targeted directly by the radiation assault. Previously, we had shown that decay of the Auger electron emitter iodine-125 (>98%) of the emitted electrons have a range <0.5 µm) within the nuclei of human adenocarcinoma colon cancer LS174T cells induces an inhibitory bystander effect. When LS174T cells were g-irradiated (dose deposited in the cytoplasm and nuclei), no such effects were seen. To assess the possible role of cytoplasmic irradiation, we have radiolabeled the mitochondrial dye Rhodamine 123 (123I-Rh) in the presence of CoCl2 and Chloramphenicol T and Chang H2AX foci were g-irradiated ataxia telangiectasia mutated (ATM) foci were not observed at 5 h (20 mGy) after 48 h of co-culture, 81% of the initial numbers of phosphorylated ATM foci remained. These findings suggest that DSB by the radiation-induced bystander effect persist for long periods, whereas DSB induced by direct radiation effects are repaired relatively quickly.

Decrease of DNA Damage and Cell cycle in Bystander Cells Cultured in Medium obtained from Human Intestinal Epithelial cells Labeled with Tritiated Thymidine. Badri N. Pandey, M. Ali, A. Kumar, Bhabha Atomic Research Centre, India Radiation induced bystander effect is manifestation of effects in neighboring cells, which may be either damaging or protective in nature. Diffusible factors play significant role in underlying bystander mechanism in irradiated cells. In present study, we have investigated effect of conditioned medium (CM) obtained from the culture of 125I-labeled human normal intestinal epithelial (INT407) cells on bystander INT407 cells. Cells labeled with 125I-thymidine showed higher DNA double strand break measured in terms of gamma H2AX foci, which gets attenuated after 15 h suggesting activation of adaptive response at longer periods. In further experiments, INT407 cells cultured in CM showed higher proliferation and plating efficiency than respective controls, which were correlated with modulation in expression of cell cycle regulating proteins (CDK-2 and p53). In order to understand the radio-sensitivity of bystander cells, INT407 cells cultured in CM were gamma-irradiated (0.5 Gy) followed by measurement of gamma H2AX foci. Our results showed that radiation-induced gamma H2AX foci/cell were found to be lower in cells cultured in CM than respective controls suggesting lower double strand break in gamma-irradiated bystander cells in CM. Our studies using microarray gene expression analysis showed that bystander cells cultured in CM showed upregulation of genes associated with DNA repair (ALKB) and cell proliferation (CDC45). Interestingly, the upregulation of CDK5 genes observed in bystander cells seems to be associated with regulation in calcium signaling, which may be further linked to upregulation of bradykinin receptor. However, the downregulation of proton transporting ATPase and adenylyte cyclase genes suggest modification in proton transport and a-cAMP signaling in bystander cells. These results provide novel understanding of protective bystander mechanism from tritium irradiated human intestinal cells involving DNA damage and cell cycle pathways.

Varying numbers of 125I-Rh-labeled cells were then co-cultured with unlabeled LS174T tumor cells (4x10^6) in six-well plates. Dead LS174T cells (freeze-thawed 3x) were used as ‘spacers’ so that the total number of cells plated was always the same (8x10^5 per well). Under these experimental conditions, any increase in cell number is consequent to the growth of the unlabeled LS174T, as the lethally 125I-Rh-labeled LS174T cells do not proliferate.

The results of our studies demonstrate that the decay of 125I within mitochondria retards the growth of neighboring unlabeled tumor cells growing in vitro. This inhibitory bystander effect is significantly less when the ratio of the 125I-labeled cells to that of unlabeled cells was lower (1:1 vs. 0.5:1). Somewhat unexpectedly, the reduction in cell proliferation was quantitatively similar to that seen when the tumor cells were co-incubated with DNA-incorporated 125I-UdR. To our knowledge, this is the first report of an 125I-labeled DNA induced inhibitory bystander effect when the decay of an Auger electron emitter occurs within the cytoplasm of a cell.

In the recent years, it was reported that DSB induced in primary human fibroblasts by 1 mGy of X-irradiation remain unrepaired for many days. The dose of 1-10 mGy can typically be delivered with diagnostic X-ray exposure and represents the range of dose exposed by individuals every year due to environmental background radiation. This report therefore shows that homeostasis in living organisms exposed to very low radiation doses may not be maintained. However, neither the mechanism through which unrepaired DSB are caused by very low radiation doses nor their biological implications are clear. Our previous study suggested that the DNA double-strand breaks (DSB) induced by very low X-ray doses is largely due to bystander effects. Thus, we hypothesized that DSB resulting from the radiation-induced bystander effects might not be repaired. The aim of this study was to verify whether DSB created by radiation-induced bystander effects are likely to be repaired. We examined the generation of DSB by enumeration of phosphorylated ataxia telangiectasia mutated (ATM) foci, which are correlated with DSB repair, in normal human fibroblast cells (MRC-5) after X-irradiation at doses ranging from 1 to 1000 mGy. At 24 h after irradiation, 100% (1.2 mGy), 58% (20 mGy), 12% (200 mGy) and 8.5% (1000 mGy) of the initial number of phosphorylated ATM foci were detected. The number of phosphorylated ATM foci in MRC-5 treated with lindane, an inhibitor of radiation-induced bystander effects, prior to X-irradiation was assessed; phosphorylated ATM foci were not observed at 5 h (20 mGy) or 24 h (200 mGy) post-irradiation. We also counted the number of phosphorylated ATM foci in MRC-5 with 20 mGy-irradiated MRC-5. After 48 h of co-culture, 81% of the initial numbers of phosphorylated ATM foci remained. These findings suggest that DSB by the radiation-induced bystander effect persist for long periods, whereas DSB induced by direct radiation effects are repaired relatively quickly.

Modulation of DNA Damage and Cell cycle in Bystander Cells Cultured in Medium obtained from Human Intestinal Epithelial cells Labeled with Tritiated Thymidine. Badri N. Pandey, M. Ali, A. Kumar, Bhabha Atomic Research Centre, India Radiation induced bystander effect is manifestation of effects in neighboring cells, which may be either damaging or protective in nature. Diffusible factors play significant role in underlying bystander mechanism in irradiated cells. In present study, we have investigated effect of conditioned medium (CM) obtained from the culture of 125I-labeled human normal intestinal epithelial (INT407) cells on bystander INT407 cells. Cells labeled with 125I-thymidine showed higher DNA double strand break measured in terms of gamma H2AX foci, which gets attenuated after 15 h suggesting activation of adaptive response at longer periods. In further experiments, INT407 cells cultured in CM showed higher proliferation and plating efficiency than respective controls, which were correlated with modulation in expression of cell cycle regulating proteins (CDK-2 and p53). In order to understand the radio-sensitivity of bystander cells, INT407 cells cultured in CM were gamma-irradiated (0.5 Gy) followed by measurement of gamma H2AX foci. Our results showed that radiation-induced gamma H2AX foci/cell were found to be lower in cells cultured in CM than respective controls suggesting lower double strand break in gamma-irradiated bystander cells in CM. Our studies using microarray gene expression analysis showed that bystander cells cultured in CM showed upregulation of genes associated with DNA repair (ALKB) and cell proliferation (CDC45). Interestingly, the upregulation of CDK5 genes observed in bystander cells seems to be associated with regulation in calcium signaling, which may be further linked to upregulation of bradykinin receptor. However, the downregulation of proton transporting ATPase and adenylyte cyclase genes suggest modification in proton transport and a-cAMP signaling in bystander cells. These results provide novel understanding of protective bystander mechanism from tritium irradiated human intestinal cells involving DNA damage and cell cycle pathways.
POSTER PRESENTATIONS

Objectives: We investigated in HCT 116 cells targeted with either internalizing (cytoplasmic localization) or non-internalizing (cell surface localization) 125I-labeled monoclonal antibodies (125I-mAbs), the relationship between mean nucleus absorbed dose and biological effects.

Methods: p53 (+/+) and p53 (-/-) HCT 116 carcinoma cell lines were incubated for 2 days with increasing activities (0–4MBq/mL) of either internalizing (anti-HER1) or non-internalizing (anti-CEA) 125I-mAbs. These non-internalized antibodies were calculated using the MIRD cellular formalism and cell survival was determined by a standard clonogenic assay. DNA damage, namely single and double strand breaks together with alkali-labile sites (SSB + DSB + AL5) was measured using the standard alkaline comet technique and chromatid aberrations were assessed using the H-TIG technique and the ATBE was induced by fibroblasts exposed to a temperature range (ATBE).

Results: The ATBE was induced by fibroblasts targeted with 125I-mAbs, but not by non-internalizing 125I-mAbs. However, similar toxicity was observed for the two targetings, demonstrating the highest toxicity per Gy of 125I-mAbs that preferentially target the cell membrane. Comparesable levels of DNA damage detected by the alkaline comet method and micronucleus in both cultures were also produced for 125I-labeled mAbs, regardless of whatever the p53 status. DNA damage was efficiently detected in the p53+ HCT 116 cells, as demonstrated by p53 and p21 activation and consequent induction of apoptosis, suggesting that the toxicity of non internalizing 125I-mAbs is not due to a defect in DNA damage detection. Moreover, cell-cycle analysis showed that cells exposed to 125I-mAbs were blocked in G2/M phase. Medium transfer experiments confirmed the involvement of bystander effects for both types of 125I-mAbs.

Conclusions: These results suggest that the biological response of cells targeted with 125I-mAbs is not fully correlated with the mean nucleus absorbed radiation dose and that bystander effects could be involved in the observed cytotoxicity.

POSO03-29. Thermal injury produces reactive oxygen species effecting surrounding non-heated cells. Martin Parschke, R. Anderson, D. Wellman, Center for Photomedicine/Harvard Medical School, USA

Previously, we described the active thermal bystander effect (ATBE). DNA damage and cell death occurs in surrounding, non-heated cells by only sharing the medium with heat exposed ones. The ATBE is an active process induced by viable heat stressed cells. The purpose of this study was to investigate the involvement of reactive oxygen species (ROS).

Human foreskin fibroblasts (HFF1) were heat exposed at different temperatures (37-54 °C) for 10 min, and co-cultured for up to 72 h with non-heated cells. Any cell-to-cell contact and heat diffusion was excluded by the experimental set up (transwell system). Cell viability was measured by MTT assay, DNA damage and apoptosis were analyzed morphologically after fluorescence staining of the DNA with DAPI. ROS levels were quantified either by PeroxOQuant assay (for medium) or by fluorescence probe DCFDA (for intercellular cytoplasm).

The ATBE was induced by fibroblasts exposed to a temperature range of 44 to 54 °C with a maximum at 46 °C. The ROS level was increased in both heated (2Fold) and non-heated, bystander cells (2.5Fold). The ROS level in heated cells peaked 30 min after heat exposure at 48 °C. Bystander fibroblasts showed an increase ROS level with a maximum at 46 °C 1 h after heat exposure. Assessment of the ROS level in the medium also revealed an increase with a maximum at around 46 °C 1 h after heat exposure. In both cell subpopulations as well as in the medium the ROS level returned to background level 3 h after heat exposure.

Our data suggest that heat induced early ROS formation plays a key role in the ATBE effect. As the ATBE is expected to have a role in (fractional) laser treatment, burn trauma and cancer treatment, further in vivo research is warranted related to ROS.


Radiation bystander effects are observed in non-directly irradiated cells that were in contact with, or received soluble signals from irradiated cells. These non-targeted effects include induction of DNA damage and alterations in cell fate (i.e., apoptosis, differentiation, senescence or proliferation), and neoplastic transformation (induced using an aerobic chamber), and have been mainly observed in in vitro systems. Until recently, evidence that radiation-associated bystander responses are effectual in vivo has been limited, and the relevance of radiation bystander responses for cancer risk has been uncertain. Results from our laboratory have provided the first direct evidence that bystander responses contributes to cancer risk in mice CNS, with drastic acceleration of medulloblastoma in radiosensitive Ptch1+/− mice irradiated with shielded brains. We have also recently shown that gap junction intercellular communication (GJIC) is critical for transmission of oncogenic radiation damage to the non-targeted cerebellum, and that a mechanism involving ATP release and upregulation of Cx43, the major GJIC constituent, regulates transduction of oncogenic damage to unirradiated tissues. However, the spatial and temporal dependence of bystander responses, and the dose-effect relationship remains a poorly understood issue in the in vivo context.

To investigate these issues, Ptch1+/− mice were irradiated with different doses (1, 2, 3 and 10 Gy) of X-rays adopting different shielding geometries to protect approximately two thirds of the mouse body. Genetic damage was measured in bystander cerebellum at different times after irradiation, while tumor development was monitored in lifetime groups. Groups of Ptch1+/− mice were also whole-body exposed to X rays (1, 2 and 3 Gy) and the same end-points were evaluated. Results obtained show a clear dose and spatial dependence of in vivo radiation bystander signaling in mouse CNS. These results may help understanding the mechanisms of bystander signaling and tumorigenesis in non-exposed organs and tissues.

POSO03-31. Radiation-induced bystander effects in hypoxia. RAJALAKSHMI SRINATH1, R. ASUR1, L. KOMMURU2, S. SHARMA1, R. GRIFFIN1; 1: UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES, USA 2: UAMS, USA

Bystander effects refer to the occurrence of damage to cells that have not been directly exposed to radiation but are near cells that were exposed and/or are affected by factors transmitted from the irradiated cells. We have previously observed the occurrence of substantial bystander effects following exposure to a single dose of 10 Gy. Few studies have evaluated the occurrence of radiation-induced bystander effects under hypoxic conditions known to exist in tumors in vivo. Here, we studied bystander killing in mouse head and neck squamous carcinoma (SCCIVII) cells under hypoxic conditions (induced using an aerobic chamber), using the media transfer protocol. Aerobic SCCVII cells exposed to 10 Gy and evaluated for clonogenic survival demonstrated a 96% decrease in cell survival, while directly irradiated hypoxic cells exhibited only 50% decrease in survival, suggesting expected radio-protection. Subsequently, aerobic and hypoxic SCCVII cells were exposed to 10 Gy and returned to the incubator for 4 hours following which the media from irradiated (donor) cells was transferred to aerobic or hypoxic unirradiated bystander (recipient) cells. The cells were then cultured under aerobic conditions for colony formation. The clonogenic survival of bystander cells, when both the donor and recipient cells were cultured under aerobic conditions, was found to be about 25% less than the sham-treated controls. Hypoxic bystander cells receiving media from aerobic irradiated donor cells exhibited only about 8% decrease, whereas only a 1% decrease in survival was observed when both the donor and recipient cells were cultured under hypoxic conditions. We observed a substantial increase in bystander cell survival under hypoxic conditions. The oxygenation state of the donor cells appears to be very important in eliciting a bystander response, as evident from the almost complete abolishment of bystander effects when hypoxic donor cells were used. These studies have evaluated the occurrence of bystander cell killing when hypoxic donor cells were used. These experiments suggest that the oxygenation state of the cells is important in eliciting a bystander response. Understanding the role of hypoxia in bystander effects will help us optimize clinical effectiveness of radiation therapy, especially in the case of spatially fractionated regimens where there are large volumes of tumor that could be considered as bystander cells.
INTRODUCTION: A central paradigm in radiation biology has been that only cells “hit” by a track of radiation would be affected to induce radiobiological consequences, and cells “not hit” should not be. However, it recently has been challenged by so called non-targeted effects, such as genomic instability, adaptive response and bystander effect, and such radiation-induced non-targeted effects may have important implications for low-dose-radiation induced biological effects. In this study we have examined cellular responses, such as bystander effect and radio-adaptive response, in normal human cells induced by low-fluence-proton irradiation using proton microbeams.

MATERIALS AND METHODS: We have investigated that the mutation frequency at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus, which was detected with measuring 6-thioguanine resistant clones, in normal human skin fibroblasts induced by the 200kV, X-ray challenging dose was lower at 0.15 times in cells pre-treated with low-dose-rate neutrons (1mSv/8h) emitted from 129Am-Be source as a priming dose than in untreated control cells. Furthermore, mutation frequency was returned to the control level when using a specific inhibitor of gap-junction mediated cell-cell communication (40µM lindane). Here we set up the hypothesis that recoiled protons emitted by the interaction between primary neutrons and surroundings near irradiated cells. Furthermore, mutation frequency was returned to the control level when using a specific inhibitor of gap-junction mediated bystander effect. To prove the hypothesis around 1.5% cells of total cell population were irradiated with single 3.4meV proton using the microbeam irradiation system, Single Particle Irradiation system to Cell (SPICE) in National Institute of Radiological Sciences (NIRS) before irradiating the X-ray challenging dose.

RESULTS: The data of the hprt mutation induction clearly showed that the X-ray induced mutation frequency was suppressed in cells pre-treated with proton microbeams and returned to the control level when using a gap junction inhibitor.

CONCLUSION: The result suggests that neutron-induced adaptive response is caused by protons and gap junction mediated bystander effect plays an important role to induce such cellular response.

POSTER PRESENTATIONS


An educational program designed to provide a “pipeline” of researchers interested in the use and development of microbeam facilities for research in biology, radiation biology, and radiation physics will be described. The rationale for the development of a training course and educational tools will be discussed and how these are particularly relevant for the development and training of microbeam facilities users in the US and abroad. The program has been designed to give interested physicists and biologists a thorough and hands-on introduction to microbeam technologies. At the completion of the course it was observed in both cell lines used in this study that the X-ray-induced bystander response is dose dependent, and that NO is the chief initiator/mediator of this effect. Since the secretion of NO depends on the p53 status, importance of p53 in the X-ray-induced bystander response will be discussed.

POS03-35. Bleomycin and ionising radiation – induced bystander effects in two fibroblast cell lines. Diana Savu, Cosmin Mustaciosu, Sorin Berea, Ileana Petcu, Horia Hulubei National Institute of Physics and Nuclear Engineering – PIN, Bucharest, Romania

Purpose: Bystander effects were examined in V79 and L929 fibroblasts in two different situations: (i) treatment with a chemotherapeutic agent, bleomycin and (ii) exposure to low doses of gamma irradiation.

Materials and methods: The cells were exposed to different concentrations (less than 60µg/ml) of bleomycin and to two doses (0.35 Gy and 0.55 Gy) of gamma irradiation delivered with low dose rates (less than 22 mSv/h). The medium transfer methodology was used to analyse the in vitro bystander effects. The bystander response was studied by using as end-points the following: viability at 24 h and 5 days, DNA damage measured by means of micronucleus assay, clonogenic survival fraction and apoptosis.

Results: The results showed that the treatment with bleomycin induced an increased DNA damage in both cell lines and the increasing is proportional to the concentration used. The bystander population of V79 and L929 fibroblasts by bleomycin frequency is also elevated but it did not depend on the concentration of bleomycin. The level of micronuclei is lower in bystander cells than in the cells exposed to bleomycin. The clonogenic surviving fraction confirms the bystander effect induced by bleomycin. Bystander response induced by gamma irradiation was observed in both cell lines used in this study at the level of micronucleus formation. Higher frequency of micronuclei was observed in bystander cells at both irradiation doses.

Conclusion: Both bleomycin and gamma irradiation induced bystander response reflected in increased DNA damage. The bystander effect is a hallmark of irradiated cells and can be seen as a responsible for the bystander effect. Further work will address the mechanisms involved in transmission of the toxic signal from treated to bystander cells.
POS4 Cell signalling

POS04-01. Targeted studies of the mechanism of radiation-induced response in a 3D tissue model after localized irradiation. Anna Acheva, M. Ghtia, G. Schettino, K. Prise, Queen's University Belfast, UK

The current work is aiming to investigate the mechanisms driving the effects of radiation using in vitro 3D tissue models. Of particular interest for radiotherapy is the DNA damage and the cell signaling that it triggers in the healthy tissue surrounding treated areas. For evaluating radiation-induced effects, we are using a conventional broad beam X-ray source (225 kVp) and lead shielding. Additionally, we use a novel gallium arsenide microcollimator (30 kVp) that allows localized exposures in 50-100 μm lines on the 3D cultures. Radiation effect was estimated by measuring DNA damage induction (53BP1 foci), their repair kinetics and changes in proliferation and differentiation independently on both the exposed and shielded part of the 3D model.

Induction of 53BP1 foci was observed in the exposed part of the sample (with dose-dependent pattern) as well as in the shielded areas of the 3D culture although in minor quantity (factor of 10 less). Damage from the 30 kVp microcollimator exposure was persistent up to 24h post irradiation. In long term incubation of the 3D skin model (7 days), we noticed significant increase of terminal differentiation expressed as elevation of the late differentiation marker Filaggrin and decrease of the early marker Cytokeratin 1 in both the exposed and shielded areas. We also tested for the involvement of the main Gap function (G3) protein Cx43 in the cell to cell communication from irradiated to shielded regions as well as the role of the pro-inflammatory enzyme COX-2 in the signal transduction. Dose-dependent up-regulation of Cx43 after direct irradiation suggests involvement of GJ in the cell signaling with COX-2 expression levels significantly higher than control levels 4 h after exposure to 2 Gy. This enzyme is probably responsible for the generation of bystander signals from the targeted cells. This was supported from data of phenotype and differentiation markers rescue after treatment of the 3D cultures with the COX-2 inhibitor sc236.

In conclusion, experiments using 3D samples clearly indicate how radiation effect is not limited to the exposed areas but signaling through GJ induces DNA damage and increased differentiation in the surrounding areas. Moreover, data indicates that inhibition of COX-2 reduces the side effects and could be used to improve the radiotherapy.

POS04-02. Nuclear Calcium Buffering Sensitizes Head and Neck Squamous Cell Carcinoma to Radiotherapy, Lídia Andrade1, J. Marques Geraldo2, A. Miranda Catarina2, C. Bruno Armond2, A. Franco Paes Leme2, S. Yokoo1, A. Beatriz Ribeiro de Queiróz2, R. Ribeiro Resende2, M. Cristina Fonseca Castelubér2, D. Lammens3, Maria Almeida4, R. Kroon Campos2, C. Renato Machado5, M. Andrade Rajão6, E. Maria de Souza Fagundes6, C. Leonar Zani2, O. Assis Martins Filho2, M. de Fátima Leite1, 1: Centro de Pesquisas René Rachou, Brazil; 2: Instituto de Radioterapia São Francisco, 3: Laboratório Nacional de Biociências, 4: Laboratório de Química de proteínas e Nanobiotecnologia Universidade Federal de São João Del Rei, 5: Laboratório de Sinalização de Cálcio Departamento de Fisiologia e Biofísica Universidade Federal de Minas Gerais, 6: Universidade Federal de Minas Gerais, 7: Pontifícia Universidade Católica de Minas Gerais, 8: Departamento de Bioquímica e Imunologia Universidade Federal de Minas Gerais, 9: Departamento de Fisiologia e Biofísica Universidade Federal de Minas Gerais

Calcium (Ca2+) signals in the nucleus have been demonstrated to play a crucial role in the proliferation of cancer cells. The aim of this study was to investigate whether nuclear Ca2+ buffering could enhance the antitumor effect of clinical doses of X-Rays on Head and Neck Squamous Cell Carcinoma (HNSCC). For that we developed an experimental protocol that simulated clinical radiotherapy and prevented bystander effects of irradiation. HNSCC cell line (A431) was submitted to the following treatments: X-Rays irradiation (10 Gy), using fractionated doses of 2 Gy daily, during five consecutive days, or association of previously described treatment with nuclear Ca2+ buffering. Twenty four hours before starting the irradiation protocol, cells were infected with an adenovirus vector that is capable of buffering Ca2+ in the nucleus. Cell proliferation was measured directly by cell counting, while cell cycle phases were analyzed by flow-cytometry. Nuclear and mitochondrial DNA lesions were detected by qPCR. Growth factor and metalloproteinasese expression were investigated by immunofluorescence, real-time PCR, and western blot. Survival fraction was evaluated by clonogenic assay. We found 4 ± 0.3% of A431 cell death following 10Gy fractionated irradiation protocol, indicating radiosensitivity. Upon nuclear Ca2+ buffering, A431 cell proliferation decreased compared to control condition. No effect on the Human Gingival Fibroblasts proliferation was observed upon nuclear Ca2+ buffering, demonstrating selectivity of the treatment towards cancer cells. Cell cycle analysis showed that association of X-Rays with nuclear Ca2+ buffering increased the percentage of A431 cells that remained at G0/M with no apparent nuclear/mitochondrial DNA damage. However, X-Rays increased expression of the radiosensitivity-related biomarker ADAM-17, with further activation of EGFR, effects that were reverted upon association of X-Rays with nuclear Ca2+ bufferring. Moreover, the association therapy decreased to 99 ± 0.4% the survival fraction of the A431 cells, and allowed to reduce the X-Rays irradiation to approximately 50% of the cumulative dose. Together, these findings contribute to the development of novel radiotherapy strategies to head and neck tumors allowing reducing X-Rays irradiation exposure.

POS04-03 β-catenin contributes to Bcl-w induced invasiveness in glioma U251 cells by inducing MMP-2 expression. In HWA BAE1, W. Sang Lee2, C. Hyun Joo2, J. Hye Kwon1, 1: Korea Institute of Radiological & Medical Sciences, South Korea 2: Korea Institute of Radiological & Medical Sciences 3: Sookmyung Women's University, South Korea

We have demonstrated previously that Bcl-w enhances the invasiveness of gastric cancer cells, particularly in those evidencing infiltrative morphology, by inducing MMP-2, uPA, and FAK expression via phosphomostide 3-kinase (PI3K), Akt, and S1p. Similarly, Bcl-w expression in glioma multiforme has been confidently associated with invading populations of cancer cells. This study demonstrates that the translocation of β-catenon the nucleus enhances Bcl-w induced invasiveness by expressing target genes, such as MMP-2, via the PI3K-Akt-β-catenin pathway in glioblastoma U251 cells. These findings significantly improve our understandings of the Bcl-w induced signaling processes in cancer cells.

POS04-04. Post-translational nuclear-import of NFKB mediates radiation induced TNFα dependent secondary signaling feed back and regulates tumor invasion and metastasis transcriptome. Joseph D Balthazar, J. Veeraraghavan, M. Natarajan, T. S. Herman, N. Aravindan, University of Oklahoma Health Sciences Center, Oklahoma, OK, USA

Ascertaining functional-specific orchestration of NFKB in response to IR may throw light on molecular blue print that underlies induced neuroblastoma (NB) relapse and metastasis. Accordingly, we investigated whether muting IR-triggered post-translational nuclear import of NFκB attenuates TNFα dependent secondary signaling feed back and attenuates IR-altered NB invasion and metastasis transcription. SH-SY5Y, IMR-32, SNK-N-MC cells, exposed to 2Gy and allowed to incubate further for 1hr or 24h, were treated with SN50. The cells were examined for NFKB DNA binding activity (EMSA), transactivation potential (QPCR) and intercellular secretion of TNFα (ELISA). IR triggered NFKB-TNFα transcriptional feed back was examined using luciferase reporter assay. Alterations in tumor invasion and metastasis transcriptome were assessed using QPCR profiling and selectively validated with immunoblotting. IR significantly induced NFKB DNA binding activity and this induction is completely and persistently (up to 72) suppressed by SN50, a specific inhibitor of nuclear import. Consistently, we observed a significant reduction in IR induced invasion and intercellular secretory activity of TNFα with both 1h and 24h post-IR SN50 treatment. Conversely, blocking TNF receptor resulted in sustained inhibition of NFKB but not AP-1 or SPI-1. Luc promoter assay revealed a significant reduction in IR induced NFKB transcription after blocking TNF receptor. Blocking post-translational nuclear import of NFKB profoundly inhibited IR-induced tumor invasion and metastasis transcriptome (Table 1.). Immunoblotting for MMP2, PYK-2, SPA-1, Dnmt3b and Ask-1 validates the role of post-translational NFKB in IR regulated invasion/metastasis signaling. Together, these data demonstrates that IR induced secondary phase (post-translational) NFKB activation mediates TNFα-dependent second
signaling and further implies that IR induced NFB in cells that survive after treatment regulates tumor invasion and metastasis signaling.

**POSTER PRESENTATIONS**

**POS04-05. The Role of STAT-3 in Cetuximab-Induced DNA Damage**

James A. Bonner, E. S. Yang, H. Q. Trumnell, S. Nowsheen, C. D. Willey, K. P. Rausch, The University of Alabama at Birmingham, USA

Objective: Cetuximab is a monoclonal antibody that inhibits activation of EGFR. Cetuximab induces apoptotic and anti-proliferative effects that have been previously shown to correlate with DNA damage. Since the Signal Transducer and Activator of Transcription-3 (STAT-3) is believed to exert an apoptotic protective effect, we hypothesized that depletion of STAT-3 may augment cetuximab-induced apoptosis and DNA damage in head and neck cancer cells.

Material and Methods: Human head and neck squamous carcinoma cells (UM-SCC-5) were transfected with short hairpin RNA (shRNA) against STAT-3 (STAT3-2.4 and 2.9 cells). A mutated form of this shRNA was transfected for a control (NEG4.17 cells). These techniques were modified from previously published methodology (Bonner J, et al., Radiotherapy and Oncology, 92:339 – 344, 2009). Radiosensitivity was assessed by a standard colony formation assay. Proliferation was assessed by daily cell counts following treatment and apoptosis was assessed by an annexin V-FITC assay. The alkaline comet assay was used to assess DNA damage. Results: The STAT-3 knockdown cells demonstrated greater radiosensitivity compared to control cells compared to STAT3-2.9 cells) demonstrated enhanced radiosensitivity compared to control cells (NEG4.17 cells, which correlated with increased apoptosis. Cetuximab resulted in greater radiosensitization in the STAT-3 knockdown cells compared to STAT3-4.17 cells. Also, the STAT-3 knockdown cells demonstrated decreased proliferation with cetuximab treatments compared to control cells (NEG4.17). The increased cetuximab sensitivity of the STAT-3 knockdown cells correlated with increased apoptosis and DNA damage compared to control cells (NEG4.17). Cetuximab treatments resulted in cell cycle distributions that were not different in the three cell lines (STAT3-2.4, STAT3-2.9 and NEG4.17 cells). Conclusions: STAT-3 knockdown led to greater cetuximab-induced anti-proliferative effects and increased apoptosis and DNA damage.

**POS04-06. Radiation induced NF-kB signaling cascade study in mammalian cells by improved detection systems.**

Arif Chishti, eutches Zentrum für Luft- und Raumfahrt, Germany

Ionizing radiation induces many signaling pathways, NF-kB is one of the most important transcription factors that respond to changes in the environment of a mammalian cell. NF-kB plays a key role not only in inflammation but also in radiation response mechanisms in response to DNA damage, such as regulation of (anti-)apoptosis, and carcinogenesis. Our previous studies showed that densely ionizing radiation (high ions with high linear energy transfer, LET) is more effective in inducing biological damage than sparsely ionizing radiation (low LET). Therefore it is important to understand radiation induced NF-kB signaling cascade. The current study aims to improve the assay system by use of the red fluorescent protein tdTomato and its destabilized variant DDDtdTomato. Newly constructed mammalian cell based reporter assays are very effective tools for studying radiation response signaling pathways. Recent studies demonstrated that radiation treatment of recombinant human embryonic kidney cell lines (HEK 293 transfected with NF-kB promoter-reporter constructs) is associated with activation of NF-kB gene expression under the control of the DNA damage sensing protein ATM. NF-kB signaling in mammalian cells is monitored by such reporter assays using different fluorescent proteins as reporter components. In conclusion, results showed accelerated heavy ions with different qualities and X-rays to have the potential to activate NF-kB in human cell. Our inhibitory analyses confirm that radiation induced NF-kB activation is ATM-dependent.

**POS04-07. Prostate tumor radioprotection modulated through CXC-chemokine signalling and loss of functional PTEN.**

Jonathan Coulter, Queen’s University Belfast, UK

Background: We have previously shown that exposure of prostate cancer cells to chemical DNA-damaging agents can modulate the expression of CXC-chemokines and their receptors. We therefore designed a series of experiments to determine whether attempts to modulate radiation (IR) similarly potentiated CXC-chemokine signalling, and to determine how the resultant effects of this signalling modulated the sensitivity of the cells to IR. Furthermore, we investigated how loss of PTEN function, a common and early-onset genetic event in prostate cancer may affect the response to IR.

Methods: IR-transcriptional events that regulate CXC-chemokine gene expression were analysed by luciferase reporter assays in both PTEN wt and knockout DU145 and 22Rv1 cells. Downstream readouts of CXCL5 and the associated receptors (CXCXR1 and CXCXR2) were characterised by both qRT-PCR and western blot. Colony forming assays were used to determine the effect of altered IR dose and cell viability following monotherapeutic, fractionated and hypofractionated radiation doses.

Results: PTEN deficient LNCaP and PC3 cells maximally induced a respective 12 fold and 8 fold upregulation of NF-kB, while a 3 fold increase in AP-1 transcription factor acted as the dominant means of CXC-chemokine signalling in PTEN wild type cells. Irrespective of PTEN status CXCL5 mRNA transcript levels were upregulated in all cells following exposure to 2 Gy. However, PTEN null cells exhibited a stronger and prolonged increase in CXCL8 mRNA correlating with a concurrent increase in CXCL8 receptor expression. Inhibition of CXCXR1 and CXCXR2 expression using siRNA significantly (p<0.01) sensitised PC3 cells to IR induced toxicity, demonstrating that inhibited activation of the CXCL8 signalling cascade may result potent radiosensitisation. Further data shows that suppression of CXC-chemokine signalling could repress stress-induced NFkB activation and anti-apoptotic gene expression, providing a mechanism for increased sensitivity to radiation.

Conclusions: Exposure to IR potently induces CXCL8 activating transcription factor NF-kB in PTEN deficient prostate cancer cells. Used in combination with current radiation treatment regimes, small molecule inhibitors of the CXC-chemokine signaling cascade could potentially act as novel radiosensitising agents.

**POS04-08. Ménage à Trois: PINCH1, PP1alpha and Akt1 as critical signaling axis for cellular radio- and chemoresistance.**

Erika Eike, S. Hellige, D. Menendez, C. Knot, C. Cordes, 1: Oncoray - National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Dresden, Germany 2: Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany 3: Department of Radiation Oncology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Dresden, Germany

Upon exposure to irradiation or cytotoxic drugs, the cellular survival is enhanced by cell-extracellular matrix (ECM) interactions. Several molecules of the multi-protein focal adhesion complexes are involved in pro-survival signaling. Besides the well known role of integrins, the main receptors for ECM, Focal Adhesion Kinase and Integrin-Linked Kinase in the cellular radiation response, the LIM-only focal adhesion protein PINCH1, important for linking integrins and growth-factor receptors, might be critically involved in tumor cell resistance to radiation and chemotherapeutics. Colony formation assays, Western blotting, phosphatase assays, Akt activity assays, site directed mutagenesis and in vivo tumor control probability assays were performed. PINCH1 knockout or knockdown significantly increased the cellular radio- and chemosensitivity in vitro and in vivo correlating with a reduced phosphorylation and kinase activity of Akt1 and Akt1 downstream targets. By immunoprecipitation and mass spectrometry, we could identify onclin as Akt1 regulator and the domain of PINCH1 (PP1) as binding partner of both PINCH1 and Akt1. Mutation of the putative PP1 binding motif, i.e. KFVEF, at the 5th IIM domain of
PINCH1 resulted in a strong reduction of PKcs binding to PINCH1. In parallel, we observed enhanced PKcs phosphatase activity and Akt1 dephosphorylation, indicating an inhibitory effect of PINCH1 on PKcs phosphatase activity. Our data show that PINCH1 is a new regulator of Akt1 and an essential determinant of radio- and chemoresistance. Further studies are underway to evaluate the potential of PINCH1 as promising tumor target in cancer therapy.

POS04-09. Irradiation results in up-regulation of phosphorylated Ras-related antigen 1 (Fra-1) in Glioblastoma Multiforme (GBM) cells. Denise Gibo, W. Debinski, Brain Tumor Center of Excellence, Wake Forest School of Medicine, USA

Ras-related antigen 1 (Fra-1), an AP-1 transcripotion factor, plays an important role in neovascularization of solid tumors, including glioblastoma multiforme (GBM). Fra-1 is controlled, among others, by oncogenic signaling of epidermal growth factor receptor (EGFR) and its mutated, constitutively activated form, EGFRVIII, in GBM cells. Fra-1 is a phosphorylated factor which transactivates JunB with which it preferentially makes AP-1 pairs in GBM cells. In the current study, we irradiated GBM cells over-expressing Fra-1 with doses ranging from 1 to 9 Gy (137 Cs) and monitored cells for up to 72 hrs, because tumor radiation is a mainstay of GBM therapy. We found that immunoreactive Fra-1 increased with time and dose of radiation. Interestingly, the higher the dose of radiation the more of a shift towards higher molecular weight bands of Fra-1 was observed. This was suggestive of an increase in phosphorylation of Fra-1, which was confirmed directly by using both phosphatase and Na3VO4, a phosphatase inhibitor. This increase in Fra-1 phosphorylation was not prevented by 5-acetamido-2',7'-diamino-20H-crown-6, although we could detect augmented levels of ROS in irradiated GBM cells. GBM cells irradiation also caused an increase in immunoreactive JunB and its phosphorylation status. Furthermore, in clonogenic cell killing assay 2 Gy already had an effect on GBM cells. Adding Flurbiprofen to irradiated cells, an anti-inflammatory drug of potential Fra-1 suppressing activity resulted in more potent killing effect of radiation and the levels of immunoreactive/phosphorylated Fra-1 surprisingly were also elevated. Thus, we found an increase in the phosphorylation state of Fra-1 and JunB in response to stress of irradiation. This was independent of the reactive oxygen species formation and may be related, at least in part, to well-described EGFR activation in response to irradiation. Adding Flurbiprofen only exacerbates cell killing using irradiation with the levels of Fra-1 increased as well. It is plausible that Fra-1 response to irradiation contributes to some of undesirable effects.

POS04-10. Involvement of DNA repair pathways in DAG-lactone radiosensitization of human LNCaP cells. Carla Hajj1, D. Galvin1, J. Truman1, L. Shenker2, R. Kolesnick2, Z. Fuku3, A.1, T. Thin1, 1: Memorial Sloan-Kettering Cancer Center, USA 2: Memorial Sloan-Kettering Cancer Center, USA

Purpose: To assess whether diacylglycerol-lactone (DAG-lactone) radiosensitization is mediated by ATM down-regulation and its effects on DNA repair pathways. Background: We previously demonstrated that pre-treatment of human androgen-dependent (LNCaP) and androgen-sensitive (CWR22-Rv1) prostate cancer cell lines with either 12-O-tetradecanoylphorbol 13-acetate (TPA, a known protein kinase C activator), or DAG-lactone radiosensitized these cells. This effect was mediated by down-regulation of ATM protein levels, thus de-repressing the enzyme ceramide synthase (CerS), generation of ceramide and apoptosis. Experimental procedures: LNCaP and CWR22-Rv1 cells were pretreated for 16 hr with 10μM DAG-lactone and irradiated with 20 or 40Gy. γ-H2AX and BRCA1 foci formation at 1 hr and 8 hr post-irradiation were scored. Results: Pre-treatment with DAG-lactone significantly enhanced ATM down-regulation at 1 hr (81.7% vs 51.9% at 20Gy) and 8 hr (70.8% vs 48% at 20Gy), respectively in LNCaP cells. Immunofluorescent staining for γ-H2AX demonstrated a dose-dependent increase in γ-H2AX foci formation at 20Gy and 40Gy for both 1hr and 8hr time points. Our data also shows that 40Gy alone produces the same amount of DNA double strand breaks (dsbs; as measured by TUNEL assay (8 hr)) as 20Gy+DAG-lactone does not induce apoptosis in LNCaP cells, while 20Gy+DAG-lactone does, indicating that ATM down-regulation is necessary for the apoptotic response. Both BRCA1 and DNA-PKcs foci were rapidly induced by radiation at 1 hr and peaked at 3 hr post treatment in the presence or absence of DAG-lactone, in both cell lines, with no significant difference between the treatments, indicating that while both HR and NHEJ are active in these cells DAG-lactone radiosensitizing effects are not mediated through these repair pathways. While apoptotic degradation is associated with γ-H2AX phosphorylation, in LNCaP cells these events would be expected at 24-48 hr. Therefore the 1hr observed effect is most likely to be associated with apoptotic DNA degradation and most likely result from DNA DSBs. Conclusions: These data suggest that unrepaired or misrepaired DNA DSB trigger post-transcriptional activation of CerS via a mechanism that is under investigation.

POS04-11. Phosphorylation and stabilization of c-Myc by NEMO renders cells resistant to ionizing radiation through upregulation of gamma-GCS. Young-Hoon Han, B. Kim, S. Kwak, J. Yang, Korea Institute of Radiological & Medical Sciences, South Korea

The transcription factor c-Myc has been previously shown to be phosphorylated and stabilized by NEMO through direct interaction in nucleus. NEMO induced upregulation of c-Myc target protein, g-glutamy1-cysteine synthetase (g-GCS), leading to an increase of intracellular glutathione (GSH) level and simultaneous enhancement of radio- and chemoresistance. NEMO enhanced c-Myc recruitment to g-GCS promoters, and c-Myc was essential for NEMO-mediated g-GCS upregulation. The phosphorylation and stabilization of c-Myc by NEMO rendered cells more resistant to ionizing radiation (IR). Thus, the interaction between NEMO and c-Myc may be targeted for the development of strategy to overcome the resistance to radiotherapy.

POS04-12. TOPORS acts as a novel E3 ligase for H2AX. CHA SOON KIM, K. Moon Seong, S. Young Nam, J. Young Kim, K. Hee Yang, Y. Jin, KHNP/Radiation Research Institute, South Korea

Eukaryotic DNA is organized into nucleosomes and higher order chromatin structure, which plays an important role in the regulation of many nuclear processes including DNA repair. Especially, H2AX plays an important role in chromatin reorganization implicated in DNA repair and apoptosis under various DNA damaging stresses, which induce the post-translational modifications of H2AX protein including phosphorylation, acetylation and ubiquitination. These modifications of H2AX protein are mediated by cellular interacting proteins. In a previous screen for H2AX interacting proteins, Topors proteins were found using in vitro translated proteins in pool. In this study, the interaction between two proteins was confirmed with GST pull-down assay and immunoprecipitation with mammalian cell extracts exposed to diverse DNA damaging stresses such as ionizing radiation, doxorubicin, camptothecin and H2O2. The cellular localization of Topors proteins was also examined by immunofluorescence assay. To elucidate the E3 ligase activity for H2AX as a substrate, ubiquitination and sumoylation were analyzed in both in vitro and in vivo. We found that Topors interacted with H2AX, and had effect on the protein stability of H2AX. We also found a novel ubiquitin E3 ligase for H2AX protein. Therefore, these biochemical data propose that Topors is a novel E3 ubiquitin ligase interacting with H2AX protein and mediate the post-translational modification involved in protein stability. [This work was supported by Grant No. E11NS06 from KHNPR and Grant No. 2011T100100303 from the MKE, Republic of Korea.]

POS04-13. Knockdown of TGF-beta activated kinase 1 (TAK1) promotes cell death induced by ionizing radiation. Takashi Kondo, Y. Furusawa, H. Sakurai, Q. Li Zhao, I. Saiki, Univ. of Toyama, Japan

TGF-beta activated kinase 1 (TAK1), one of the MAPK kinase (MAP3K) which has been widely accepted as a key kinase activating NF-κB and several MAP3Ks, is upstream of the pathways corresponding to a variety of cellular functions in response to stresses including DNA damage. To our knowledge, however, there is no experimental study assessing the contribution of TAK1 in cell sensitivity to ionizing radiation (IR) and IR-induced cell death. In this study, we examined whether and how cellular sensitivities to IR are modulated by TAK1 in cultured human cells. Stable knockdown of TAK1 in HeLa S3 cells resulted in higher loss of clonogenicity when cells were irradiated with 5 Gy. Though phosphorylation levels of ATM, Chk1, and Chk2 were not affected by TAK1 depletion, slight decrease at 1 hr (2088) as 40Gy observed in TAK1 cells was loss in TAK1 but not in parental cells, reflecting phosphorylation of TAK1 in cell cycle regulation apart from the classical pathway. In addition, TAK1 depleted cells underwent SubG1 phase more predominantly than parental cells did after release from checkpoints, implying that the defective regulation of cell cycle in absence of TAK1 might be responsible in part for the increased radiosensitivity. Global gene expression analyses by using GeneChip system revealed that genes
involved with cell cycle were differentially regulated by IR in these two cell lines. In addition, bioinformatics analyses by using Ingenuity Pathway Analysis software also identified a different genetic network associated with cell cycle regulation. Our results suggest that TAK1 plays a significant role in cell survival after irradiation probably through supplemental regulation of cell cycle progressions in a manner independent of the classical pathway mediated by checkpoint kinases. This study is the collaboration with Dr. Wei Z.-L., China Med. Univ.

**POSTER PRESENTATIONS**

**POS04-14. Mechanism of radiation-induced arrest of global protein synthesis in C3H mouse macrophages.** Yoshiksa Kubota¹, S. Takashashi², ¹: National Institute of Radiological Sciences, Japan 2: Research Reactor Institute, Kyoto University, Japan

Previously we reported radiation-induced apoptosis in macrophages of C3H mice but not other strains of mice. The depletion of McI-1, an anti-apoptotic Bcl-2 family protein known to have a very short turnover time, through radiation-induced arrest of global protein synthesis was identified as a major factor for the apoptosis. In the present study, the mechanism by which global protein synthesis was suppressed by irradiation was elucidated. Phosphorylation of the alpha-subunit of eukaryotic initiation factor 2 (eIF2alpha) is a well documented mechanism of downregulating protein synthesis under a variety of stressful conditions. Western blot analysis using phospho-eIF2alpha antibody to detect phosphorylation at serine 51 showed the increased phosphorylation of eIF2alpha in a dose dependent manner in irradiated macrophages of C3H mice, but not B6 mice. Four kinases have been identified as eIF2alpha kinases, PKR, PERK, GCN2 and IRE1 are activated by viral infection, endoplasmic reticulum stress, amino acid deprivation and hemin deficiency, respectively. The antibodies to detect the activation-specific phosphorylation site for PKR, PERK and GCN2 were available and used in Western blot analysis. Phospho-PKR and phospho-PERK antibody showed no change of phosphorylation at the activation-specific site by irradiation. On the other hand, phosphorylation of GCN2 at Throneine 898 was markedly increased by irradiation in macrophages of C3H mice, but not B6 mice. GCN2 is known to be activated through the binding of non-aminocylated tRNA to Histidyl-tRNA Synthetase-related domain located in C-terminal region of GCN2 molecule. Whether radiation induces amino acid deprivation and/or increment of uncharged tRNA in C3H mouse macrophages is under investigation.

**POS04-15. The microRNA machinery is dispensable for cell survival after gamma-ray irradiation in human primary keratinocytes.** Jérôme Lamartine, J. Nicolas, University Lyon 1-CNRS Centre de Génétique et de Physiologie Moléculaire et Cellulaire, France

Understanding the mechanisms of cutaneous radiosensitivity is an important issue since skin is the most exposed organ to ionizing radiations and among the most sensitive. Recent publications describe microRNAs (miRNAs), a group of short non-coding RNAs that negatively regulate gene expression, as potential modulators of cellular response to ionizing radiation (IR), both in vitro and in vivo in various cell types and tissues. However, in epidermal cells, the involvement of the miRNAs machinery in the cellular response to IR remains to be clarified. To address this question, we settled up an expression study of miRNAs in irradiated epidermal keratinocytes. We analyzed the results of global miRNA profiling, performed using microfluidic system of qPCR assay, which permit to assess the expression of almost 800 annotated miRNAs in proliferative, post-terminating and differentiated keratinocytes mimicking the various layers of human epidermis. These cells were irradiated at 0.5 Gy or 6 Gy and miRNAs were extracted 3 or 24 hours after irradiation. Surprisingly, very few of them were significantly up- or down regulated in independent cultures of primary keratinocytes, whatever the dose, the time post-irradiation or the differentiation status of irradiated cells. These data might indicate that miRNAs do not respond to ionizing radiations in epidermal cells and do not play a major role in the cellular response to IR. To go further into this hypothesis, we transfected primary keratinocytes targeting DICER1 or AGO2, two crucial components of the miRNA pathway, in cultured keratinocytes prior to irradiation. We didn’t observe any modification of short-term cell survival when miRNA-mediated gene silencing was abrogated. Altogether, our results indicate that the miRNA machinery is not necessary to ensure the survival of irradiated human keratinocytes.

**POS04-16. The role of radiation-induced cell death and radiation resistance in non small cell lung cancer (NSCLC).** Martin Lawlor, Queens university belfast, UK

Radiation can kill cells by several death pathways, including apoptosis. Recently, there has been evidence to suggest radiation may induce alternative modalities of cell death, such as autophagy. However there is a debate on the potential cytoprotective nature of autophagy and its relationship to apoptosis. This study aims to investigate the significance of autophagy induction post irradiation and its relationship to apoptosis. Human NSCLC lines were used, H460 p53+/+H1299 p53− and a normal human bronchial epithelial cell strain were irradiated in a dose range of 1-10Gy. Clonogenic analysis quantified radiosensitivities and western blotting analysis followed key expression patterns. A series of autophagy (3-MA) and apoptotic inhibitors (Z-VAD-fmk) were used in conjunction with radiation and flow cytometry was used to measure levels of apoptosis. A GFP-LC3 construct was used to measure induction of autophagy. In parallel radioresistant phenotypes of H460/H1299 cells were generated through hyperfractionation regimes (2Gy x 30) totalling a net dose of 60Gy. Clonogenic analysis of both cell lines, with treatment of the pan-caspase inhibitor Z-VAD-fmk, revealed a decrease in radiation-induced cell survival. Western analysis showed attenuation of caspase 3 cleavage and PARP cleavage after Z-VAD-fmk treatment. PI cell cycle analysis revealed cell death levels in 10Gy irradiated cells remained constant, even with Z-VAD-fmk treatment and using annexin V/PI stain. Z-VAD-fmk treatment showed a trend of decreased apoptosis. High background levels of the autophagy marker LC3-B II was seen in the more radioresistant H1299 cells. However, H460’s expressed a dose-dependant increase in LC3-II levels with Z-VAD-fmk treatment. H460 resistant clones generated from fractionated radiation exposures yielded an increase in radioresistance but this was not so for the p53− more radioresistant H1299 cells. The present data suggests that apoptosis may not have a significant role in radiation induced cell death in NSCLC and that the relationship between radiation and apoptosis may be more complex through autophagy activation. The generation of a radioresistant cell line provides an exciting model to investigate the possible mechanisms of increased resistance and how this may affect the mechanisms of cell death in NSCLC.

**POS04-17. Phosphorylation of CLK2 by AKT activation promotes cell survival to ionizing radiation.** Seon Young Nam, H. Sun Park, J. Kim, K. Hee Yang, C. Soon Kim, Y. Jin, Radiation Health Research Institute, South Korea

Irradiation regulates cell survival by activating a variety of signaling cascades, including the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. Our previous study showed that the activation of AKT is associated with cell protection against ionizing radiation. Given the diverse roles of the AKT pathway in the cellular response to ionizing radiation, it is important to identify new physiological substrates of activated AKT and to elucidate their biological functions. In the present study, we identified CDC-like kinase 2 (CLK2) as a new substrate of AKT activation and elucidated its role in cellular response to ionizing radiation. AKT directly binds to and phosphorylates CLK2 on serine 34 and threonine 127, in vitro and in vivo. CLK2 phosphorylation was detected in HeLa cells overexpressing active AKT. In addition, we demonstrated that ionizing radiation induces CLK2 phosphorylation via AKT activation. In contrast, the suppression of endogenous AKT expression by siRNA inhibited CLK2 phosphorylation in response to 2 Gy of γ-ray or insulin. Furthermore, we examined the effect of CLK2 on the survival of irradiated CCD-18L1a cells overexpressing Myc-CLK2. CLK2 overexpression significantly increased cell growth and inhibited cell death induced by 2 Gy. The role of CLK2 in cell survival to ionizing radiation was dependent on the phosphorylation of serine 34 and threonine 127. Our results suggest that AKT activation controls cell survival to ionizing radiation by phosphorylating CLK2, revealing an important regulatory mechanism required for promoting cell survival.

**POS04-18. Role of the transcription factor C/EBPdelta in ionizing radiation response.** Snehalata Pawar¹, R. Pathak², J. Wang³, E. Sterneck², M. Hauer-Jensen¹, ¹: University of Arkansas for Medical Sciences, USA 2: National Cancer Institute, USA

Background: Exposure of cells or tissues to ionizing radiation (IR) leads to activation of inflammation, programmed cell death and
fibrotic responses. Knowledge of the signaling pathways activated by IR is vital to understand and treat normal tissue injury, a common side-effect of radiotherapy of tumors. The transcription factor CCAAT-enhancer binding protein delta (CEBPd; Cebpd) is a cytokine-induced, acute phase response protein, with apoptotic and tumor-suppressor-like properties. Mouse embryonic fibroblasts (MEFs) derived from Cebpd-null mice display increased chromosomal instability, increased aneuploidy, and loss of contact inhibition. We have recently shown that the Cebpd-null MEFs display high levels of gamma-H2AX foci as a measure of DNA damage. The current studies begin to address the role of CEBPdelta in response to ionizing radiation using in vitro cell systems.

Methods: GEO datasets from NCBI were examined for correlations of CEBPd with IR. MEFS from Cebpd wild type (WT) & knockout (KO) mice were subjected to ionizing radiation dose of 5Gy and changes in cell cycle were monitored by FACS analysis. The human dermal fibroblasts GM03349 were exposed to increasing doses of IR (0 to 10Gy) and the kinetics of CEBPdeltax expression at RNA and protein levels were examined by harvesting cells at 1, 2, 4, 6 and 24h. The effects of the p53 activator, nutlin on CEBPd expression was examined in human dermal fibroblasts.

Results: Analysis of GEO database revealed that CEBPD is an early-response gene induced by IR and is transiently induced in an ATM-dependent manner in response to IR. Cebpd WT mels showed increased growth arrest whereas the Cebpd KO mels showed increased apoptosis in response to IR. CEBPD is an IR-inducible gene and is regulated by p53.

Conclusions: These studies begin to elucidate the mechanistic role of CEBPdeltax in IR response and may provide a novel target that can be modulated to protect normal cells and sensitize tumor cells to radiation.

POS04-19. Silver nanoparticles suppress antioxidant system through inhibition of AMPK-FOXO3 Pathway. Mei Jing Piao1, R. Zhang2, K. Cheon m3, A. Daeul Kim1, J. Choi1, J. Won Hyun1, 1: School of Medicine, Jeju National University, South Korea 2: Faculty of Environmental Engineering, University of Seoul, South Korea

Reactive oxygen species (ROS) and oxidative stress are generated by ionizing radiation, silver nanoparticles (AgNPs), and chemicals. Recently, we reported that AgNPs induced oxidative cell damage via mitochondria-derived apoptosis. The antioxidant enzymes such as catalase and glutathione peroxidase have been reported to directly protect against oxidative stress. In this study, we examined the role of antioxidant systems on AgNPs-induced intracellular ROS levels in human Chang liver cells and the molecular mechanisms involved. We observed that N-acetylcysteine, a potent antioxidant, significantly reduced intracellular ROS levels induced by AgNPs. Additionally, AgNPs decreased antioxidant enzyme expressions, which are mediated by AMP-activated protein kinase (AMPK)-forkhead transcription factor (FOXO3) pathway. However, our results suggest increased induction of ROS levels and suppression of antioxidant enzyme expressions by AgNPs increased cytotoxicity through inhibition of the AMPK-FOXO3 pathway.

POS04-20. Ionizing radiation-induced-microcephaly depends on dissociation of apical structure during embryonic development of the mouse cerebral cortex. Mikio Shimada1, J. N. Pulvers2, F. Matsuzaki3, T. Matsumoto4, K. Komatsu1, 1: Radiation Biology Center, Kyoto University, Japan 2: Laboratory for Cell Asymmetry, Center for Developmental Biology, Japan

Embryonic exposure to radiation increased the risk of microcephaly and mental retardation. It is known that radiation induced apoptosis of cerebral cortex progenitor and neural stem cells result in formation of small size brain. However, response to radiation such as DNA damage, cell cycle checkpoint and apical surface structural maintenance of cerebral cortex remain unclear. In this study, we demonstrated responses to radiation induced DNA double strand breaks and G2/M cell cycle checkpoint. 1h after irradiation, G2/M checkpoint increased in neural progenitor and stem cells and then 4h after cells recovery from checkpoint arrest. However, neural basal progenitors increased and ZO-1 and g-tubulin, which are apical structural proteins of cerebral cortex, dissociated from apical surface after irradiation. Furthermore, after irradiation, apical neural progenitor and stem cells have large foci of g-H2AX, meanwhile basal progenitor and stem cells have small foci of g-H2AX. Our finding suggest that Apical neural progenitor and stem cells means M phase cells are sensitive to radiation and lead to dissociation of structure of apical surface and microcephaly.


Radiomimetics are agents that attenuate radiation injury when administered after irradiation. We discovered that lysophosphatidic acid (LPA) a lipid mediator/second messenger rescues apoptotically condemned cells. LPA protects IEC-6 intestinal epithelial cells in vitro and intestinal crypts in vivo from radiation-induced apoptosis. Using LPA receptor KO mice and cells that lack LPA receptors we established that the LPA2 receptor subtype is required for the antiapoptotic mechanism in vitro and in vivo. Through its C-terminal PDZ and LIM-binding motifs LPA2 forms agonist-dependent macromolecular signaling complexes. LPA2 interacts with the proapoptotic protein Siva-1 and targets it for proteasomal degradation, which in turn arrests DNA-damage-activated apoptosis. Full activation of the NFkB and ERK1/2 prosurvival pathway requires formation of an LPA-LPA2 complex that activates KEAP1, and TRIP-6 ternary macromolecular complex. These signals inhibit the intrinsic mitochondrial apoptosis pathway. Furthermore, transfection of the LPA2 receptor into mouse embryonic fibroblasts derived from Lpa1/2 double knockout mice restores LPA-dependent radioprotection. Conditioned medium from gamma-irradiated U937 cells induces bystander apoptosis in irradiated IEC-6 cultures and LPA2-transfected MEFs. We applied in silico drug discovery techniques of non-lipid LPA2 agonists. We found a novel LPA2-selective agonist GR977143 that protects IEC-6 cells from radiation- and chemotherapy-induced apoptosis. GR977143 also increases the survival of mice exposed to lethal levels of radiation. Supported by AI08405.
differences could be partly due to CRT-DAMC mediated alterations in damage responses linked to changes in protein acetylation. These results suggest that cadetrelinc plays a role in the acetylation of proteins, altering pro-survival and pro-death signaling, which also influences DAMC induced alterations in radiation response of cells.

POS05-01. Repression of Natural Killer Cells in C57BL/6 Mice Following Low - Dose Gamma Irradiation. Laura Bannister1, R. Mantha1, Y. Devantier2, M. Blinkova3, D. Klokow1, R. Lance1; 1: Atomic Energy Canada Ltd., Canada 2: AECL, Canada

Exposures of mice to low doses of X or γ irradiation have been associated with reduced cancer rate and increased tumour latency. Immune stimulation is a potential mechanism for the anti-neoplastic effects of low-dose radiation (LDR). One cell type of interest is the Natural Killer (NK) cell, a sub-class of lymphocytes that act as a first line of defence against virus infection or tumour invasion. NK cells mediate lysis of target cells without previous sensitization or a requirement for major histo-compatibility restriction, and are mediators of anti-tumour immunity, and an important source of immunoregulatory molecules, such as IFN-γ. NK cells are relatively resistant to high-dose radiation in comparison with other lymphocytes, resulting in their short-term selective enrichment in lymphoid tissues and blood following irradiation. Chronic or single exposure to low-dose radiation has also been shown to increase NK cell numbers and/or cytotoxicity, and functional studies have associated stimulated NK cytotoxicity with supressed tumour metastasis in mice following irradiation. In this study, splenic NK cell percentages and cytotoxicity against lymphoma cells was measured in C57BL/6 mice 4 and 7 days following low-dose or acute high-dose total body γ irradiation. Relative NK numbers and cytotoxicity following 2 Gy total body irradiation (TBI) were similar to those of un-irradiated control mice. In contrast, we observed a decrease in both NK cytotoxicity and relative cell number following 10 mGy and 100 mGy TBI, with effects more notably dose 4 days following radiation exposure (relative NK cell percentages and cytotoxicity values were decreased to 35% and 36% of control values, respectively). Overall, our results indicate a repression in relative numbers of spleen NK cells following low-dose g radiation. One possibility is that NK cells were released by the spleen following irradiation to provide an increased level of anti-tumor activity following low dose radiation. This work was undertaken as part of the NOTE IP 034665 (F6R8), Euratom specific programme for research and training on nuclear energy, 6th FP of the EC.


The quest for reliable and unique biomarkers of radiation exposure is an ongoing challenge in the field of radiation biology. Once identified, they will provide an invaluable tool for both clinicians wanting to monitor radiation exposure, values at later time points similar to those from non-irradiated mice, and thus also not contribute a useful index to long-term impaired function. HSP that compromise their multi-lineage repopulating potential mechanism for the anti-neoplastic effects of low-dose radiation (LDR). One cell type of interest is the Natural Killer (NK) cell, a sub-class of lymphocytes that act as a first line of defence against virus infection or tumour invasion. NK cells mediate lysis of target cells without previous sensitization or a requirement for major histo-compatibility restriction, and are mediators of anti-tumour immunity, and an important source of immunoregulatory molecules, such as IFN-γ. NK cells are relatively resistant to high-dose radiation in comparison with other lymphocytes, resulting in their short-term selective enrichment in lymphoid tissues and blood following irradiation. Chronic or single exposure to low-dose radiation has also been shown to increase NK cell numbers and/or cytotoxicity, and functional studies have associated stimulated NK cytotoxicity with suppressed tumour metastasis in mice following irradiation. In this study, splenic NK cell percentages and cytotoxicity against lymphoma cells was measured in C57BL/6 mice 4 and 7 days following low-dose or acute high-dose total body γ irradiation. Relative NK numbers and cytotoxicity following 2 Gy total body irradiation (TBI) were similar to those of un-irradiated control mice. In contrast, we observed a decrease in both NK cytotoxicity and relative cell number following 10 mGy and 100 mGy TBI, with effects more notably dose 4 days following radiation exposure (relative NK cell percentages and cytotoxicity values were decreased to 35% and 36% of control values, respectively). Overall, our results indicate a repression in relative numbers of spleen NK cells following low-dose g radiation. One possibility is that NK cells were released by the spleen following irradiation to provide an increased level of anti-tumor activity following low dose radiation. This work was undertaken as part of the NOTE IP 034665 (F6R8), Euratom specific programme for research and training on nuclear energy, 6th FP of the EC.

POS05-03. Contribution of Radiation-Induced Hematopoietic Stem Cell Damage to Long-Term Immune Suppression in Mice Surviving Lethal Doses of Ionizing Radiation Exposure. Hai Lin Chua1, A. Plent1, C. Sampson1, M. Joshi1, T. J. MacVittie1, C. M. Orschell1; 1: Indiana University School of Medicine, USA 2: University of Maryland School of Medicine, USA

Residual bone marrow damage (RBMD) persists for years following exposure to radiation and is characterized by decreased self-renewal potential of hematopoietic stem cells (HSC). Prolonged immune suppression following radiation exposure has been postulated to be due to radiation-induced thymic dysfunction. The aim of the current study was to determine the relationship of RBMD and impaired HSC function to immune dysfunction following exposure. To this end, C57Bl/6 mice were exposed to 800cGy total body irradiation (TBI, 33°C, 62cGy/min, LD0) and survived and analysed at various time points up to 10.5 months post-TBI for hematopoietic function. Compared to non-irradiated age-matched controls, irradiated mice exhibited decreased peripheral lymphocyte counts at all time points examined, with normal levels of neutrophils. When assessed in competitive transplantation assays, 150 purified, sorted c-Kit+Sca+Linage-CD150+ cells (KSL CD150+), isolated from TBI mice at all time points post-exposure, were found to contain <2% of the repopulating potential of similar cells isolated from non-TBI age-matched controls. Of interest, recipients of KSL CD150+ cells from TBI mice were also severely deficient in donor lymphoid reconstitution, exhibiting values of T-cell CD4+ and CD8+ subsets, and B cells of 50% or less compared to mice transplanted with similar cells from non-TBI donors. Results of experiments assessing HSC homing potential, progenitor potential, chemotaxis to SDF-1α, chemorepulsion, and null NK activity, and marrow osteoblast content were in agreement in TBI and non-TBI mice up to 10.5mo post-TBI, indicating that none of these parameters is directly responsible for radiation-induced HSC dysfunction. While ROS content of KSL CD150+ cells from irradiated mice was increased at 1.5mo post-exposure, values at later time points were similar to those from non-irradiated mice, and thus also not contributing to long-term impaired HSC function. In summary, these data suggest that the long-term immune suppression observed following potentially lethal TBI may be due to direct effects on HSC that compromise their multi-lineage repopulating potential. [Supported by NIAID, contract # HHSN266200500043C].

POS05-04. Radiochemotherapy induces a favourable tumour infiltrating inflammatory cell composition in Head and Neck Cancer. Luitpold Distel1, T. Marriana1, B. Maik2, F. Rainer2, L. Dorota1, 1: Strahlenklinik, University of Erlangen, Germany 2: Pathologie, University of Erlangen, Germany

Tumour infiltrating inflammatory cells (TIC) have been shown to be prognostically relevant in numerous tumour types. Especially low counts of regulatory T cells (Treg) and high numbers of cytotoxic T cells (CTL) are implicated to improve the clinical outcome. However, it is unclear how TIC are influenced by radiochemotherapy (RCT). The present study was designed to investigate the effect of a radiochemotherapy on intratumoral inflammatory cells. 58 cases of head and neck squamous cell carcinoma (HNSCC) were treated by surgery after neoadjuvant radiochemotherapy. Paraffin-embedded biopsies of untreated primary tumours and tumour resections after radiochemotherapy were processed into tissue microarrays. Numbers of T cell sub types (CD3+, CD8+ CTL, Granzyme B’, CD25+, FoxP3+ Treg), B cells (CD20+), monocytes (CD68+) and dendritic cells (CD1a+) were quantified in immunohistochemical stainings. All inflammatory cell types, except for CD1a+ DC predominated following an RN event. The cutaneous radiation reaction is an important and often limiting factor for radiation therapy and as the skin is the first line of defense for exposure, it is an ideal target for biomarker investigation. In the current study, we have employed a commercially available in vitro human skin model (MatTek EpiDerm FT) to examine multi-cellular dermal radiation exposure. Significant research has highlighted the involvement of the inflammatory response following radiation exposure, particularly within the pathophysiology of the cutaneous reaction; therefore, we hypothesized that alterations in the cytokine cascade may result and thus may represent indices of exposure. Cytokine profiles of the culture media were assessed following skin insert irradiation to evaluate possible markers of radiation exposure in a multi-cellular context. Tissues were allowed to equilibrate overnight at 37°C following delivery. On Day 0 the skin inserts were exposed to various doses (0, 2, 4, 8, 12, 16 Gy) of γ radiation (Co-60) and maintained in culture for 96 hrs. Tissue viability was assessed on a daily basis with a fluorescent metabolic activity assay (alamarBlue) to ensure viability was not compromised. Culture media was sampled for cytokine analysis at 24 hr intervals and analyzed using the Bioplex system which allows for the simultaneous analysis of 17 cytokines. Preliminary results suggest that several of the analyzed cytokines have an altered expression following radiation exposure (IL-6, IL-8, MCP-1, G-CSF and IFN-γ). These findings suggest that variations in cytokine and chemokine mediator expression may prove to be indices of radiation exposure, however inflammatory signaling cascades are complex and further studies are required to validate the robustness of the radiation response.
GranzymeB+), macrophages and CD1+ DC especially with regard to the intraepithelial compartment. In cases with complete regression of the tumour after neoadjuvant radiotherapy, the counts of stromal CD1+ DC was significantly higher than in cases with incomplete response. The dramatic decrease of Treg and the comparatively stable CTL counts strongly increased the CD8/FOXp3 and the Granzyme B/FOXp3 ratio (factor 2.5 and 3, respectively). All TIC or TIC-ratios are driven by the RCT in a direction which is supposed to be prognostic. The only exception was the high number of macrophages and its association with a worse prognosis.

In conclusion radiotherapy decreases the tumour infiltrating inflammatory cells and simultaneously evokes the cell composition to a more favourable pattern.

POS050-05. Immunological markers of radiation-induced late effects. Diana Dubner1, M. Portas2, G. Cristina1, S. Michelin1. 1: Nuclear Regulatory Authority, Argentina 2: Hospital de Quemados de Buenos Aires, Brasil

Up to now there is no established parameters for the follow-up of delayed radiation injuries observed in victims of radiation accidents. Persistent inflammation has been associated with late toxicity and pathological radiation fibrosis and it is well known that cellular adhesion molecules (CAMs) play a key role in the inflammatory process generation.

The following study was conducted to examine the response of the immune system in the inflammatory reactions through the expression of CAMs (ICAM1 and β1-integrin) on granulocytes, lymphocytes and monocytes, as well as changes in subpopulations of lymphocytes in patients with skin injuries after radiotherapy or interventional fluoroscopy procedures.

Were included in this research protocol twenty patients that showed reactions in tissues (skin, subcutaneous cellular tissue, muscle, etc.) by early and late toxicity due to medical exposures to ionizing radiation, graded according to the RTOG / EORTC system. In the samples, taken along the follow-up period, the expression of adhesion molecules was measured by staining with FITC-conjugated monoclonal mouse anti-human antibodies and lymphocyte subsets in whole blood were determined by Tritest (Becton Dickinson). Flow cytometry was performed on a Becton Dickinson FACS Caliber cytometer.

A significant increase in the expression of β1-integrin on lymphocytes (7.35±0.269 vs 3.56±0.68, p<0.05) was observed. It correlated with the severity of the damage. A similar trend was noted on monocytes. The analysis of the relationship CD4+/CD8+ lymphocyte subsets revealed a downward trend in the most serious cases. These parameters, in combination with other inflammatory indicators could be used as potential follow-up markers of the chronic radio-induced inflammation process just as its response to therapeutic treatments.

Key Words: Chronic inflammation- Cutaneous radiation syndrome-Lymphocyte β1-integrin

POS050-06. Neonatal Irradiation Sensitizes Mice to Adult Pulmonary Inflammatory Challenges. Jacob Finkelstein1, C. Manning2, C. Johnston3, I. Williams1. 1: University of Rochester School of Medicine, USA 2: University of Rochester School of Medicine, USA

Rationale: Significant differences exist between the physiology of the immature, neonatal lung compared to the adult which may affect acute and late response to irradiation. Identifying these differences would be critical in developing successful mitigation strategies for this special population. Our current hypothesis suggests that radiation during this critical period is likely to alter developmental processes resulting in long-term consequences including altered susceptibility to secondary inflammatory challenge. Methods: C57BL/J mice, 4 days of age, received total body irradiation of 5.0 Gy. Mice were examined 3, 6 or 11 months post irradiation (PI). At these times mice were either challenged with inhaled LPS and examined 24 or 72 hours later or were intranasally infected with 120 HAU of influenza A virus. Body weight and survival were monitored. Pulmonary responsiveness was determined by lavage and histological examination. Analysis of epithelial and inflammatory markers and gene expression were done by immunohistochemical and mRNA analysis. Results: Irradiated neonatal mice exposed to inhaled LPS as adults and examined 24 or 72 hours later exhibited an augmented inflammatory response and a delayed ability to resolve the inflammatory response associated with this challenge. Following influenza infection, irradiated animals lost significantly more weight at 6 and 11 months post-irradiation and had reduced survival compared to the unirradiated but infected controls with a greater effect seen at 11 month postradiation. Irradiated animals had a reduced percent of lymphocytes recruited to the lung following infection compared to shams. Conclusions: These results demonstrate that early life radiation injury may affect the lung’s response to a subsequent challenge and further suggests that this effect may be dependent on the time post-irradiation that the challenge occurs.

POS050-07. Effects of radiation on B-precursor cells and related signal transduction. Deping Han, J. Ma, M. Zhang, S. Zhang, P. Okunieff, L. Zhang, University of Florida Shands Cancer Center, USA

Purpose: The transition of common lymphoid progenitor cells (CLP) to pro-B cells to pre-B cells represents the expansion of B-cell development. Our purpose is to reveal the effects of IR on this key process of functional B cells maturation and the related signal transduction.

Methods: NIH Swiss male mice were exposed to 5- or 10-Gy subcutaneous body irradiation (one leg out of the irradiated field). The bone marrow (BM) and spleen were harvested at different time points after ionizing radiation (IR) exposure, and then the percentages of common lymphoid progenitor cells (CLP) (Ter119+CD-IgM-β2M+), pro-B cells (CD19+IgM-β2M+), and pre-B cells (CD19+IgM+CD25+) were examined with antibody staining followed by flow cytometry analysis. The expression levels of signal transduction molecules STAT3 and STAT5b, which play very important roles in promoting pro-B cells development, were determined with Western blotting.

Results: While the total numbers of lymphoid cells at different stages were greatly reduced by IR, the surviving cells maintained the transition from CLP to pro-B and then to pre-B with a clear time pattern. 1) The percentage of CLP cells was significantly up-regulated within 1 week after IR, but there was no difference between the IR control 2, 3, 4, or 6 weeks after 5 Gy and 10 Gy as compared to no-IR mice. 2) The percentage of pro-B cells was increased significantly from 1-2 weeks after IR, but there was no change after 3 or 4 weeks. 3) The percentage of pre-B cells was decreased significantly 1 week and 2 weeks after sub-TBI, but increased dramatically 3-4 weeks after IR. 4) In murine spleens, the percentage of pro-B cells was increased significantly 1-2 weeks after IR followed by an increase in the percentage of pre-B cells 3 weeks after IR. 5) Western blot results showed that the expression of STAT3 and STAT5b in both BM and spleen was increased significantly 1 week and 2 weeks after 5-Gy and 10-Gy sub-TBI, but there were no evident changes in other time points.

Conclusion: These data indicate that 1-2 weeks after IR the development and maturation of common lymphoid progenitor cells “speeds up.” Two weeks is the key time point for the transition from pro-B cells to pre-B cells. These transitions reflect the functional processes that are preserved in the residual radio-resistant cells to restore the immunity that has been greatly damaged by IR.

POS050-08. Immune Response and Microbiome Change by Ionizing Radiation. Jae-Hoon Jeong, M. Kim, M. Sun Kim, Y. Kyoung Jeong, J. Young Lee, Korea Institute of Radiological and Medical Sciences, South Korea

Purpose of this study: By evaluating the importance of intestinal microbial population during radiation exposure, we aimed to provide feasibility that radioprotection could be enhanced by regulating microbiome and anti-host interaction.

Materials and Methods: Female C57BL/6 mice were irradiated using a cobalt-60 γ-radiation source. Microbial population was analyzed by metagenomic methods using pyrosequencing and CLcommunity programs. Host immune cells were analyzed by FACS and cytokine gene expressions by realtime PCR.

Results: Abdominal irradiation caused an increase of Firmicutes with a concomitant decrease of Bacteroidetes in cecal microbial population. Although Tenericutes was few before irradiation, it became a large segment of the population. After abdominal irradiation, macrophage population was increased and then the level of CD4+ T cells were elevated in colonic lamina propria immune cell. In contrast, the number of B cells was decreased dramatically after irradiation in spleen. Although immune cell population was not changed
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significantly by probiotics or antibiotics supplementation, antibiotics treatment changed the expression of pro-inflammatory cytokine genes in colon tissue after irradiation. Conclusions: Based on the microbial population study after abdominal irradiation, identification and control of the target microbial population could provide a new strategy to overcome the side-effect of irradiation. More specific approaches to control the microbiome and host interaction should be investigated.

POS050-09. Identification of cytokines mediating abscopal bone marrow suppression and acute death in mice exposed to a lethal dose abdominal irradiation (AI). Dan Jia, P. Corry, C. Fu, University of Arkansas for Medical Sciences, USA

We have shown that a lethal dose of AI elicits an abscopal suppression of the bone marrow (BM) in mice. This BM suppression is accompanied by a rapid elevation of systemic oxidative stress and followed by animal death overlapping in time with the “gastrointestinal (GI) distress”. Daily injection of the antioxidant N-acetyl-cysteine (NAC) or a single dose of BM transplantation (BMT) correct the suppressed BM and increased animal survival rate as determined previously. The GI tract hosts the largest lymphoid tissue in the body and produces a wide range of cytokines, many of which can trigger and sustain oxidative stress. The present study was aimed to uncover the GI tract-mediated than that of effectors T cells, and acute death. Young (10-12 weeks old) male C57BL/6 mice were subjected to a single dose (0 or 20 Gy) X-ray AI. Some animals were treated with either NAC or BMT. Sera were collected at post-AI day 3 (D3) and D6, and BM and jejunum homogenates obtained at post-AI D6. The samples were analyzed for cytokine content. A ratio of the experiment-to-control values greater than 2-fold in either direction was considered a true call. Of the 106 analytes tested, 32 and 40 were up-regulated by AI in D3 and D6 sera, respectively, while 7 and 12 were down-regulated. Time wise, 13 analytes were up-regulated and 3 were down-regulated by AI in both sera. Furthermore, 9 analytes showed changes in the same direction in all three tested tissue types. Finally, NAC and BMT each corrected the serum content of 29 analytes altered by AI, and shared 15 responsive analytes in common. Out of the 15 analytes, 4 were up-regulated and one down-regulated by AI in both sera. Our results demonstrate that AI elicits systemic changes in cytokine composition. Among these responding cytokines we have identified five (i.e. gp130, G-CSF, OPG, TNF-alpha and Resistin) that are crucial for BM hematopoietic and mesenchymal precursor viability and functions and responsible for AI-induced abscopal BM suppression and acute death. Moreover, that both NAC rescue and BMT rescue act upon a common set of responsive cytokines is consistent with our earlier finding that NAC rescue is accompanied by an improved BM cellularity. These results support our notion that the mitigation effects of NAC is partially through BM restoration.

POS050-10. Irradiation induced changes in the quantity and function of regulatory T cells. Katalin Lumniczky, A. Balogh, E. Persa, A. Benedek, G. Sáfrány, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Hungary

Regulatory T cells (Treg) play a key role in maintaining peripheral immune tolerance, by which they can interfere with the development of an efficient antimicrobial immune response. Given the importance of radiotherapy in the cure of malignant diseases, it is a basic question to clarify how ionizing radiation influences Treg pool and function.

The aim of the study was to evaluate radiation induced quantitative and functional changes in the splenic Treg pool as well as mechanisms responsible for Treg radiation response. Mice were total body irradiated with 2 Gy and splenic cellular fractions analyzed at different time points following irradiation. A 1.6 fold increase in the proportion of Treg cells within the total CD4+ cells was seen as soon as 1 day after irradiation, pointing to an increased radiosensitivity of the Treg population. Foxp3+ Treg cells were less prone to irradiation induced apoptosis than Foxp3- effector T cells, as shown by TUNEL assay. Also, the proliferation index of Treg cells (as evaluated by their Ki67 positivity) was not more pronounced than that of effector cells. Irradiation induced a moderate upregulation of the CTLA4 antigen on the Treg cell surface showing a spontaneous activation of Treg cells in the irradiated animals. However, the degree of Treg cell activation induced by non-specific activation stimuli was impaired by irradiation, and the regulatory capacity of Treg cells, which was evident both through their interaction with effector T cells and also with dendritic cells. IL-10 and TGF-beta RNA expression levels were moderately changed in the Treg cells of irradiated animals but were significantly increased in the effector T cells.

In conclusion, we showed that Treg cells are more radioresistant than effector T cells, which leads to their enrichment within the spleen CD4+ pool of irradiated animals. A reduction in their apoptotic potential and an increase in their proliferation index are responsible for this enrichment. Functionally, Treg cells from irradiated animals are impaired, which is evident both in their reduced suppressive capacity and also in their altered response to activation stimuli.

POS050-11. Whole lung irradiated animals infected with influenza a virus demonstrate increased mortality, reduced pulmonary function and accelerated development of radiation-induced late lung injury. Casey M. Manning, C. K. Reed, C. J. Johnston, B. Paige Lawrence, J. P. Willison, J. N. Finkelstein, University of Rochester, USA

Pulmonary fibrosis is a major complication arising from radiation exposure to the lungs. In general, pathological changes do not appear until months following irradiation; however, studies have demonstrated persistent molecular changes in the lung environment that arise immediately following irradiation. We hypothesized that a secondary challenge to the lung, given prior to the development of radiation-induced lung injury, may exacerbate radiation effects. In the present study, C57BL/6 mice were intranasally infected with influenza a virus 10 weeks post- sham or 15 Gy whole lung irradiation. Mock-infected controls from each irradiated group were given PBS. Body weight and survival were monitored after infection for 42 days. Respiratory rates were taken prior to infection and at 3, 7, 10, 14, 21, 28, 35 and 42 days post-infection. Compliance and resistance measurements were taken to assess pulmonary function prior to infection, and at 14 and 42 days post-infection. At these times post-infection, the lung was inflammation-fixed and processed for histological analysis. Irradiated animals demonstrated increased mortality following infection and failed to recover body weight lost. Both sham and irradiated animals demonstrated a reduced respiratory rate throughout the course of infection, which returned to baseline levels at day 14. However, by day 21 post-infection, respiratory rates in the infected irradiated animals increased significantly compared to all other groups. At day 42 post-infection, this increase was still evident. No significant difference in lung compliance and resistance between groups was observed at day 14 post-infection, but at day 42 post-infection, infected irradiated animals had reduced compliance and increased resistance compared to both the infected sham animals and the mock-infected irradiated animals. The observed changes in breathing rate and the reduction in compliance in infected irradiated animals are consistent with pulmonary function changes observed with pulmonary fibrosis. From this data, it is concluded that thoracic irradiation can increase severity of a subsequent pulmonary infection and additionally, virus infection following irradiation can accelerate the development of radiation-induced late lung injury.


Irradiation radiation induces pneumonitis in rats at 42-70 days and nephropathy at later times. We examined the effect of age and gender on survival and function of the lungs and kidneys after total body irradiation (TBI) followed by a syngeneic bone marrow transplant (BMT). Hypothesis: Susceptibility to radiation pneumonitis and nephropathy in rats is dependent on age and gender. Experiments: WAG/RijF(B/C) were irradiated at 5 weeks of age (young) with 11 and 12 Gy TBI. Older rats (juvenile adults) at 8 weeks (males) or 10 weeks (females) were treated with 11 Gy TBI. Rats received BMT immediately after TBI. Lung and kidney functions were measured by breathing rates through pneumonitis and blood urea nitrogen (BUN) at 120 days respectively. Morbid rats were euthanized according to IACUC guidelines. Animal care and dosimetry were done by core facilities at MCW. Results: 1) Only 65% of juvenile adult females survived through pneumonitis at 70 days after 11 Gy TBI. Breathing rates increased from 136.4 (mean)±9.85 (95% confidence interval) to 216.74±23.22 breaths/min indicating lung injury, and returned to baseline by 76 days. These females suffered a second phase of morbidity due to radiation pneumonitis between 120-150 days with no survivors after
that time. The BUN increased from baseline (19-24 mg/dl) to 131 (108-157) mg/dl at 120 days.

2) Young females (n=12) survived (100%) through pneumonitis after 11 Gy with no increase in breathing rates. The BUN was 164 (110-244) mg/dl at 120 days, which was not different from the juvenile adult females.

3) All male rats (n=5 at 5 weeks and n=18 at 8 weeks) survived pneumonitis up to 70 days with no increase in breathing rates after 11 Gy. Juvenile males survived over 120 days with a BUN of 145 (101-207) mg/dl at 120 days while >70% of young males were moribund from nephropathy by 100 days. A higher dose of 12 Gy also did not induce radiation pneumonitis in young males.

Conclusions: 1) Juvenile adult females developed radiation pneumonitis after 11 Gy TBI while young females, young males or juvenile adult males were not affected.

2) In contrast to pneumonitis, young males were more susceptible to radiation nephropathy than juvenile adults. Radiation nephropathy was comparable in either juvenile adult or young females.

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**POS05-13.** The co-activation of EBV and JCV virus is associated with chromosomal instability and higher radiation sensitivity in peripheral blood lymphocytes in Hodgkin lymphoma patients—Two poor clinical outcome. Radhia M'kacher1, M. Ricou1, G. Shim1, D. Bureau1, L. Andreoletti2, W. Knowles1, T. Girinsky1, , Alain Bernheim1, D. Violot1, C. Parmentier1, J. Bourhis1, S. Koscielny1, P. Carde1, L. Sabater1, 1: CEA, France, 2: Laboratory of Virology IRB53/EAS3798 Reims, 3: Enteric, Respiratory and Neurological Virus Laboratory, Specialist and reference microbiology division, Health protection agency, Colindale, London, UK, 4: Institut Gustave Roussy, 5: Institut Gustave Roussy, France

Background: B cells are potential sites for latency and reactivation of DNA herpesvirus Epstein-Barr virus (EBV) and polomavirus (JCV).

We investigated the JCV and EBV status in peripheral blood lymphocytes (PBL) and their potential link with chromosomal abnormalities and increased radiation sensitivity in 100 Hodgkin lymphoma (HL) patients and 100 B-cell non Hodgkin lymphoma (B-NHL) patients.

Patients and methods: JCV and EBV were assessed by PCR. FISH technique was used to localize viral infection and chromosomal abnormalities in peripheral blood lymphocytes before and after treatment and during follow-up (10 years). Radiation sensitivity was assessed before treatment. Serological studies were assessed using the haemagglutination inhibition assay. The influence of viral infection, chromosomal instability, and radiation sensitivity on freedom-from-progression (FFP) was investigated in HL patients.

Results: The overall rate of seropositivity for JCV was 86.2% in HL and 63.3% in B-NHL compared to 63% in 98 healthy blood donors (P<10^-4). PCR product sequencing of PBL identified JCV in 42% of circulating lymphocytes in HL patients only. FISH analysis revealed the presence of cells with a high JCV genome copy number (associated with the presence of rogue cells) exhibited multiplex and complex chromosomal aberrations, which increased from 15% before treatment to 52% (P<10^-7) after treatment. The proportion of chromosomal aberrations and complex chromosomal rearrangement (CCR) was larger in patients with activated JCV or EBV, especially in patients with co-activation of EBV and JCV. The co-activation of JCV and EBV was independent of known prognostic parameters, and were associated with a shorter FFP (JCV and EBV co-activation P<0.001) and the presence of rogue cells (P=0.002).

Conclusion: In HL, JCV activation and chromosomal instability have been identified in PBL, and were associated with a more dismal prognosis, especially with additional infection with EBV. Prospective longitudinal clinical studies should be launched to confirm our findings, and to determine the role of human JCV and EBV in the relapses and in the malignancy itself in HL.

**POS05-14. Combined radiation and burn injury elevates the pulmonary inflammatory response. Jessica Remus1, C. Deburghgraevae, M. Bird1, M. Hauer-Jensen2, E. Kovač3, 1: Loyola University Medical Center, USA 2: University of Arkansas for Medical Sciences, USA

Events such as a nuclear meltdown accident, nuclear attack, or medical radiotherapy all have potential for intentional or unintentional dissemination of radioactive material and resultant radiation injuries. Radiation injury frequently occurs in combination with another form of trauma, most often burns. Thus far, combined injury studies have focused on skin wound healing and damage to the gut. Since both radiation exposure and remote burn have pulmonary consequences, here we examined the early effects of combined injury on the lung. C57BL/6 mice were subjected to either total body irradiation followed by a concurrent 40 Gy b exposure on the back using a 7.5 mm diameter circular Sr-90 applicator. Control groups were untreated, TBI alone, and b alone. Clinical effects of radiation exposure were characterized by radiation skin toxicity scoring, transpidermal water loss (TEWL), and wound healing. Skin was harvested on days 4, 7, 14, 21, and 60 days post-injury for immunohistochemical staining. Wound healing, epidermal and dermal thickness, and cellularity were quantified using digital and multispectral imaging analyses.

Results: Skin changes were only visible at b irradiation sites. TBI + b resulted in more severe burns (skin toxicity score = 3.5 vs. 2.5) and higher levels of TEWL (38 g/hm² vs 20 g/hm²) compared to b alone. The highest level of TEWL preceded the worst skin score. The TBI + b burns were larger and healed more slowly (~ 5 days longer) compared to b alone. Hematoxylin & Eosin (H&E) analyses showed significant alterations in epidermal and dermal thickness and cellularularity across all injury models compared to untreated controls (p<0.03). Interestingly, fluctuations in cellularity were similar in skin sites and non-burn sites, suggesting a systemic response to radiation injury. Sustained mast cell and neutrophil infiltrates, at levels higher than control groups, were observed in TBI + b skin at 60 days post-injury.

Conclusions: Combined TBI + b radiation injury disrupts the skin barrier, impairs wound healing, and alters inflammatory infiltrate. Individuals with pre-existing skin barrier defects may be susceptible to severe radiation skin toxicity. Impaired barrier function and inflammatory response in the skin may increase the burden of radiation injury, systemic dissemination of radioactive material, and resultant radiation illness in other organ systems. Support: Center for Medical Countermeasures against Radiation Program (NIAID-U19-AI091036)
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POSO5-16. Long-lasting impairment of mucosal barrier function in the post-irradiation period impairs mucosal recovery and results in a prolonged GI syndrome. Terez Shea-Donohue1, L. Notari1, S. Yan1, A. Zhao1, T. Vanyusyl1, A. Bennett1, A. Ward2, G. Tudor2, C. Booth2, A. Fasano1, A. Farese1, T. MacVittie1, 1: University of Maryland School of Medicine, USA, 2: Epistem Ltd, USA

We established a model in which rhesus macaques (NHP), exposed to lethal doses of partial-body irradiation (PBI) within the acute GI-ARS range and sparing – 5% of bone marrow (PBI/BM5), would survive both the GI-ARS and lethal heme (H)-ARS allowing full evaluation of GI recovery kinetics in survivors. NHP (n=32) were exposed to 100Gy PBI/BM5 and euthanized at specific time points between 30 and 190 days post irradiation. These studies resulted in the identification of a prolonged GI syndrome, which was characterized by continued loss of weight and intermittent GI symptoms (e.g. diarrhea, dehydration) despite normal food intake. The aim of the current study was to investigate the mechanisms responsible for this novel syndrome. A key observation was a persistent reduction in mucosal barrier function measured by transepithelial electrical resistance of jejunal mucosa. From day 35 post-PBI/BM5, there was a significant up-regulation of the pro-inflammatory cytokines, IFN-g and IL-17A. Mucosal healing in the post irradiation period is dependent on a number of factors including sparing of stem cell crypts, migration of cells up the villus, and maturation of these cells into appropriate phenotypes. Coincident with the persistent inflammation in the post-PBE/BM5 period was an early and long-lasting impairment of mucosal restitution; assessed microscopically, including reduced numbers of crypts and failure to regenerate intact villi, which are critical for nutrient absorption. This was paralleled by significant down-regulation of the SGLT1, the most abundantly expressed glucosetransporter in the small intestine. This reduction likely contributes to the continued loss of weight observed in the post irradiation period. After 100 days in the post-PBI/BM5 period, there was a significant downregulation in the expression of Lgr5, a putative stem cell marker. These data indicate that a threshold dose of 10Gy induces long-lasting inability of the intestinal mucosa to recover. The 10Gy PBI/BM5 irradiation appeared to initiate a persistent decrease in mucosal barrier function and inflammation leading to gradual reduction in stem cell function. These findings have important therapeutic implications for the design of medical countermeasures. Supported by NIH/NIAID # RC1 AI 78520 and HHSN272201000046C.

POSO5-17. Therapeutic applications of tumor-specific T lymphocytes (CTL) induced by tumor irradiation. Tsuguhide Takeshima1, D. Wakita1, H. Kitamura1, T. Nishimura1, H. Shirato1, 1: Department of Radiation Medicine, Graduate School of Medicine, Hokkaido University, Japan

Radiation therapy is one of the primary treatment modalities for cancer as well as chemotherapy and surgical therapy. The main mechanisms of the tumor reduction after radiotherapy has been considered by damaging the tumor DNA. However, we found that tumor-specific cytotoxic T lymphocytes (CTL), which were induced in the draining lymph node (DLN) and tumor tissue of tumor-bearing mice, play a crucial role for radiation-induced tumor-growth inhibition. Here, we showed that radiation-induced CTL could be expanded ex vivo in the presence of IL-2, and could be used for adoptive tumor immunotherapy. CD8+OVA-Tetramer+ T cells in DLN and tumor mass 5 days after tumor irradiation. These cells were isolated from the cells in DLN or tumor and propagated for 7 days in the presence of IL-2 and IL-12. When the expanded radiation-induced CTL were infused into EG7-bearing mice, all mice were completely cured from tumor. We are now investigating whether small numbers of radiation-induced CTL could eradicate tumor by combination with OVA-specific Th1 cells. Our results will provide a new strategy for adoptive tumor-immunotherapy.


Purpose: From the treatment of retinoblastoma to survivors of past and present nuclear events in Japan, radiation induced retinopathy is a profoundly grim and debilitating condition leading to the comprehensive/progressive loss of vision. Leukocyte-Endothelial cell (EC) interactions are an essential component in early presentation of radiation retinopathy. This study aims to construct an in vitro model under physiological shear stress conditions to investigate the acute mechanistic, expressive and structural pathophysiology of the leukocyte–EC interface in response to direct high-dose irradiation.

Methods: Human primary retinal endothelial cells (RECs) were cultured to passage 2 and reseeded to 90-95% confluency onto 75x38mm glass slides. RECs were irradiated at a single dose of 30 Gy from a 137Cs source for 9.9 minutes (dose rate = 3 Gy/min) and incubated for 2 hours. A parallel-plate chamber and continuous flow loop were used to establish constant laminar shear stress ranging from 0.8-2.0 dyn/cm2 using U937 leukocytes in culture medium. Videos were recorded at 6 designated fields (Ø 20nm) and analyzed for 2 hours using phase-contrast microscopy. Cells entering the field were monitored for adhesion events. Confocal microscopy of fixed non-IR- and IR RECs at 2, 4 and 72 hours was also conducted using P-selectin and ICAM-1 cell-surface antibodies.

Results: Non-IR RECs exposed to U937 leukocytes under continuous flow show very few adhesion events. After 30 Gy exposure IR RECs adhesion was nearly 4 times that of non-IR RECs (p < 0.05). RECs 2 hours post-IR had increased rolling/tethering and firm adhesion. Confocal images of IR-RECs confirm an up-regulation of P-selectin at 4 hours and ICAM-1 at 24 hours compared to non-IR controls.

Conclusion: Significant changes to adhesion characteristics in our system occur almost immediately post-IR. Our in vitro model enables real-time analysis and has proven to be a vital preliminary step in assessing the detrimental consequences of adhesion-mediated endothelial cell injury and proliferation of radiation retinopathy. Using this paradigm enables us to effectively assess risk factors and causalities associated with direct exposure as well as screen/investigate potential agents aimed at combating IR-induced proliferative retinopathy in both clinical and environmental settings.

POSO5-19. The effects of ionizing radiation on pattern recognition receptors. Hironori Yoshino, I. Kashiwakura, Hiroasaki University Graduate School of Health Sciences, Japan

The immune system is composed of innate and adaptive immunity. Antigen presenting cells (APCs), such as dendritic cells and macrophages, serve as a link between innate and adaptive immunity. APCs express pattern recognition receptors (PRRs) which recognize molecular patterns present in pathogenic organisms (PAMPs). Toll-like receptor (TLR) and retinoic-acid-inducible gene-1 (RIG-I) are well-studied PRRs that play important roles in anti-bacterial or anti-viral immunity. The activation of APCs through PRRs is required for the induction of adaptive immune responses. However, it remains unknown whether ionizing radiation affects PRRs. The effects of ionizing radiation on the expression of PRRs and the response against PAMPs were herein investigated using THP1 cells (human acute monocytic leukemia cells). THP1 expressed TLR2 and TLR4 which are receptors for peptidoglycan and lipopolysaccharide (LPS), respectively. Those expressions after X-irradiation (1-5 Gy) were higher in irradiated cells than in non-irradiated cells. The response against LPS was estimated by the induction of tumor necrosis factor-a (TNF-alpha). The TNF-alpha positive cells were higher in irradiated-THP1 (2%) than in non-irradiated cells (0.5%). To investigate the effects of ionizing radiation on PRRs in detail, THP1 cells were treated with phospho-tyrosine protein 13kinase (PTPK) in order to differentiate into macrophage-like cells, and then the similar experiments were performed. In contrast to the results of THP1, the expression of TLR4 of PMA-treated THP1 after irradiation was lower in irradiated cells than in non-irradiated cells. Although TNF-alpha positive cells of PMA-stimulation was higher in PMA-treated THP1 (40%), no significant difference in the response of LPS was observed between non-irradiated and irradiated conditions. We next investigated the expression of RIG-I which recognizes double-strand RNA. Although non- or PMA-treated THP1 did not express RIG-I, RIG-I was expressed in PMA-treated THP1 after LPS stimulation. No significant effects of irradiation on the RIG-I expression in PMA-treated THP1 after LPS stimulation was observed. In conclusion, this
study demonstrated that ionizing radiation affects PRRs expression and the response of PRRs to PAMPs, but these effects depend on the cell types and differentiation state.

**POS6 Computational/theoretical studies in radiation physics and chemistry**

**POS6-01. Heavy Ion Track Structure Simulations in Liquid Water and Bone.** Michael Dingfelder, M. Dingfelder, I. G. Jorjishvili, East Carolina University, USA

Monte Carlo (MC) track structure simulations of charged particles provide valuable information on the action of radiation on matter with biological and medical outcomes. These MC codes require reliable interaction cross sections for all charged particles with the material under consideration. In current MC codes liquid water serves as surrogate of soft tissue and is studied well. However, it is often the only material available in track structure codes. Interaction cross sections for other materials are obtained from the water cross sections using density scaling, which is approximate. Calcium is of special interest since it is the most abundant and the heaviest component of bone and needed in studies of radiation effects in the bone/bone-marrow environment. Interaction cross sections of charged particles with matter are commonly calculated within the framework of the plane-wave Born approximation. This method requires detailed knowledge of the dielectric response function (DF) of the material. We have determined the optical oscillator strength of metallic calcium (i.e., the DF in the optical limit) from experimental and theoretical information and have modeled the full DF using Gaussian functions. We have calculated total and energy differential interaction cross sections for electron and proton impact on calcium and are currently evaluating the results. For this purpose we have modeled and calculated the mean free path and stopping power of silicon using the same models. The optical oscillator strength of silicon is taken from the literature. We evaluate our results with comparison to calculations for electron impact derived from the same optical data and using simple optical data models, which are available in the literature.

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**POS6-02. A critical targets model of cell survival at high doses per fraction.** Marek Fatyga, Virginia Commonwealth University, USA

The purpose of this work is to develop a practical model which extends Biologically Equivalent Dose (BED) formalism to hypofractionated treatments. Hypofractionation is becoming increasingly popular for practical reasons and because of hoped for therapeutic advantages. There is a fairly universal agreement that Linear Quadratic model overpredicts cell kill at high doses delivered in a single fraction. Most published models which aim to remedy this problem introduce a transition dose which separates the region of validity of the LQ model from a region described by a new mathematical formulation, both the transition dose and the new formulation derived from cell survival curves. These models have a drawback of introducing a fairly large number of adjustable parameters which often complicates modeling of clinical outcome. This paper presents a relatively simple, mechanistic model of cell survival which adds one new adjustable parameter, called repair probability, to the existing parameters of the LQ model and computes universal survival curves for all doses. The model postulates that cell DNA has an unknown number of “critical targets” which cause cell death if damaged and not repaired by the DNA repair mechanism. The universal survival curve is described by three adjustable parameters: number of critical targets, expected number of single strand breaks (SSB) in a critical target per unit of dose, and a probability to repair SSB in a critical target. We show that the Taylor expansion of the universal curve to small doses recovers LQ behavior, expansion of the universal curve to small doses recovers LQ behavior, mathematical properties of Poisson probability distribution under conditions of damage saturation within critical targets.

**POS6-03. The Stochastic Markov Model of Cellular Response to Radiation.** Krzysztof Wojciech Fomalski1, L. Dobrzyński2, M. Janiak3, 1: The A. Soltan Institute for Nuclear Studies, Poland 2: The Andrzej Soltan Institute for Nuclear Studies 3: Military Institute of Hygiene and Epidemiology, Poland

An entirely new mathematical dose response model is presented. A fully stochastic model based on the Markov process and the tree of probabilities is used to describe responses to ionizing radiation of a group of cells. The dozens of input parameters are used, like probability of cell death, multiplication, mutation etc. and radiation dose per one generation of cells. All cells, analyzed case by case, are subjected to stochastic tree of probabilities. It results in the new generation of cells, where each cell can be healthy, mutated or cancerous. The model do not use tendentious dose dependent function but clear stochastic approach based on Markov process. The results show that overlapping of many linear processes results in a threshold, and, generally, a non-linear response. Similar results have been experimentally observed and reported in many papers. The presented model permits the inclusion of adaptive responses and bystander effects that can lead to hormetic effects. Essentially, all known biological effects can be reproduced by the model.

**POS6-04. Dose Calculation in Radiation Treatment Using an Artificial Neural Network.** Yong Nam Kim1, J. Bak2, J. Hong Park2, S. Ho Park3, K. Jeong4, S. Kon Kim1, 1: Kangwon National University Hospital, South Korea 2: Chung-Ang University Medical Center 3: Asian Medical Center, South Korea

Dose calculations which are crucial for radiotherapy treatment planning systems require accuracy and speed, simultaneously. The conventional radiotherapy treatment planning dose algorithms are rapid but lack precision. Monte Carlo methods are time consuming but the most accurate. This study intended to apply neural network theory to develop a real-time computing algorithm for dose calculation for radiation treatment. In order to assess the feasibility of using neural network, a neural network algorithm was constructed and compared with the results obtained with Monte Carlo calculations. From the investigation of basic characteristic of learning algorithm of neural network, it is noted that the steep dose gradient in the penumbra region causes the inefficient learning performance and propagate the error in to the whole region of radiation field. In order to search for a method to solve the severe inhomogeneity of dose distribution, the Levensberg-Marquardt method was considered introducing the second order differentiation of dose distribution as the feedback to the weight of the hidden unit of neural network. The improvement was noticeable in estimating dose distribution, however it could not provide a decisive solution. A novel method was contined introducing the intermediate tuning stage in which the weight of hidden layer unit is firstly optimized considering an analytic function with less steep gradient as a dummy target/output and transferred to the original target dose distribution. It provided the successful escapement from the local minima. Except for the penumbra region, the error was less than 5%. By using the dose data measured by 1.5cm interval, the dose distribution was successfully calculated by 0.5cm mesh size. It is indicated that the feasibility of using neural network was verified. For achieving ultimate goal, the further studies should be performed to solve the problem of severe inhomogeneity of dose distribution in the human body.

**POS6-05. Optimization of the positions and irradiation times of a brachytherapy source using the genetic algorithm.** Ali Asghar Mowlavi1, M. Zibandel-Gorji2, S. Mohammadi3, 1: ICTP, TrIL, Trieste-Italy & 2. Physics Department of Sabsevar Tarbat Moallem University, Sabzevar-Iran, Iran 2: Physics Department of Payamnour University of Tehran 3: Physics Department of Payamnour University of Mashhad.

**Introduction:** As it is well known, improving in treatment planning of brachytherapy is very important. In this research, we have introduced a simple method for this purpose.

**Material and Method:** We have applied the genetic algorithm to find the proper positions and irradiation times of a $^{125}$I brachytherapy source to deliver a desired dose to the border of the tumor in two dimensions. Results: The proper positions and irradiation times of the source have been found for some shape of the tumors like cycle or square border.
Conclusion: Our results in two dimension surface are interesting, and guide us to extend this method to real shape of tumors in three dimensions.

**POSTER PRESENTATIONS**

**POS06-06. New modeling approaches to investigate cell signaling in radiation response.** Ianik Plante, NASA Johnson Space Center, USA

Ionizing radiation damages individual cells and tissues leading to harmful biological effects. Among many radiation-induced lesions, DNA double-strand breaks (DSB) are considered the key precursors of most early and late effects [1] leading to direct mutation or aberrant signal transduction processes. In response to damage, a flow of information is communicated to cells not directly hit by the radiation through signal transduction pathways [2]. Non-targeted effects (NTE), which includes bystander effects and genomic instability in the progeny of irradiated cells and tissues, may be particularly important for space radiation risk assessment [1], because astronauts are exposed to a low fluence of heavy ions and only a small fraction of cells are traversed by an ion. NTE may also have important consequences clinical radiotherapy [3]. In the recent years, new simulation tools and modeling approaches have become available to study the tissue response to radiation. The simulation of signal transduction pathways require many elements such as detailed track structure calculations, a tissue or cell culture model, knowledge of biochemical pathways and Brownian Dynamics (BD) propagators of the signaling molecules in their micro-environment. Recently, the Monte-Carlo simulation code of radiation track structure RITRACKS was used for micro and nano-dosimetry calculations [4]. RITRACKS will be used to calculate the fraction of cells traversed by an ion and delta-rays and the energy deposited in cells in a tissue model. RITRACKS also simulates the formation of chemical species by the radiolysis of water [5], notably the OH• radical. This molecule is implicated in DNA damage and in the activation of the transforming growth factor beta (TGFβ), a signaling molecule involved in NTE. BD algorithms for a particle near a membrane comprising receptors were also developed and will be used to simulate trajectories of signaling molecules in the micro-environment and characterize autocrine and paracrine cell communication and signal transduction. References:


**POS06-07. The contribution of non-targeted effects in HZE cancer risk: in silico model.** Jonathan Tang1, D. Nguyen2, J. Mao3, M. Helen Barcellos-Hoff, S. Costes1, 1: Berkeley Lab, USA 2: NYU Medical School, USA

Our main objective is to define the efficiency and physiological context in which high LET radiation increases epithelial cancer risk. The impact of radiation non-targeted effects (NTE) in promoting epithelial cells initiated by targeted cancer is poorly understood. We previously introduced a computational biology model of the mouse mammary gland using an agent-based formalism. Agents are autonomous software objects that have defined rules or mathematical equations that determine how they behave and interact with each other and their local environment. Our in silico mammary gland consists of interacting agents simulating the different lineages of mammary epithelial cells, from mammary stem cells (MaSC) to fully differentiated alveolar or luminal cells. We used this model to analyze the complex interplay between apoptosis, proliferation, and polarization that leads to morphogenesis and homeostasis in vitro (Tang et al., Integr. Biol., 2013).

Our model can now simulate tumor incidence observed in a p53 null transplanted mammary chimera in which either the host, or the donor epithelium, or both were irradiated. We first modeled ductal morphogenesis and maintenance of transplanted p53 null epithelial cells into a non irradiated cleared fat pad. P53 null cells are genotypically unstable and they were modeled by allowing each epithelial agents undergoing division to produce mutant progeny which would acquire different phenotypes. Different groups of genes necessary for agents' functions could be mutated this way. The size of each of these functional targets was tuned so that in silico tumors (i.e. duct diameters ≥ 2 mm) incidence and latency matched experimental data (i.e. 65% tumor incidence 1 year after transplant - Nguyen et al., Cancer Cell, 2011).

In agreement with previous gene expression data for low-LET, HZE also induced MaSC activity 1 to 4 weeks post-irradiation. When using such information in the model, we predicted a reduce latency for radiation-induced tumors. This prediction has already been observed for low-LET but remained to be confirmed for HZE. Overall, by compartmentalizing the radiation response into a target tissue (epithelium) and a non-target tissue (stroma) inside a cellular in silico model, we are starting to predict in a mechanistic manner cancer risk for a variety of radiation qualities.

**POS7 Radiation chemistry and space research**

**POS7-01. Linear energy transfer dependence of Nuclear Factor κB activation.** Christa Baumstark-Khan1, C. E. Hellweg2, C. Schmitz2, F. Lau1, M. M. Meier1, J. Testard2, T. Berger1, G. Reitz2, 1: DLR Institute of Aerospace Medicine, Germany 2: Laboratoire d’Accueil en Radiochimie et biologie with les Ions Accélérés, GANIL, France

Purpose. Risk assessment of radiation exposure during long-term space missions requires the knowledge of the relative biologic effectiveness (RBE) of space radiation components. Few data on gene transcription activation (e.g. CDKN1A) by different heavy ions are available, suggesting a dependence on linear energy transfer. The transcription factor Nuclear factor κB (NF-κB) is involved in the regulation of cellular survival, immune responses and inflammation, resulting in eminent importance in cancerogenesis. Therefore, NF-κB activation by accelerated heavy ions of different LET was correlated to survival. Materials and Methods. NF-κB-dependent gene induction after exposure to heavy ions was detected in stably transfected human 293 cells (HEK-pNF-κB-d2EGFP/Neo cells), using the destabilized Enhanced Green Fluorescent Protein (d2EGFP) as reporter. Results. Argon (LET 272 keV/µm) and neon ions (LET 91 keV/µm) had the highest potential to activate NF-κB, resulting in a RBE of 8.9 in comparison to 150 kV X-rays. In the LET range of 91–272 keV/µm, two particle traversals per cell nucleus are sufficient to activate NF-κB. The RBE for survival also reached its maximum in this LET range, with a maximum value of 2. Conclusions. NF-κB enhancement of survival of cells hit by heavy ions in the LET range of 91–272 keV/µm and should therefore be considered for risk assessment of radiation exposure during space travel.

**POS7-02. The Effects of Proton Radiation on the Prothrombin and Partial Thromboplastin Times of Irradiated Ferrets.** Gabriel Krigsfeld, J. K. Sanzari, A. R. Kennedy, University of Pennsylvania, USA

Protons with an energy spectrum ranging from 10-1000 MeV are a type of space radiation released during a solar particle event (SPE). The doses of radiation that could be received by astronauts from SPE radiation can be sufficiently large to cause symptoms of the acute radiation syndrome. The planned exploration class missions have augmented the risk of astronaut exposure to SPE radiation. Of interest are the effects of space radiation on the cardiovascular system. Studies are currently being performed to evaluate the acute effects of exposure to SPE-like proton radiation during the first 24 hours post-irradiation. Previous studies have shown that endothelial cell exposure to radiation can increase expression/activity of several genes/proteins involved in maintaining blood hemostasis. Therefore, we hypothesized that SPE radiation could induce coagulation disorders in animals and astronauts, in an acute manner, shortly after radiation exposure. In these studies, ferrets were exposed to SPE-like proton radiation and the effects of the extrinsic and intrinsic coagulation pathways were measured using a prothrombin time (PT) assay and an activated partial thromboplastin time (aPTT) assay, respectively. Ferrets were exposed to total body irradiation, using 110 MeV protons at doses of 0 (sham), 25, 100, or 200 cGy, at a high dose rate (HDR) of 50 cGy/min or a low dose rate (LDR) of 50 cGy/hour. At 4-8 hrs post-irradiation, plasma was isolated from blood samples and analyzed by the PT and aPTT assays. We found that plasma isolated from ferrets exposed to SPE-like proton radiation had clotting deficiencies indicative of coagulopathy. Exposure to proton doses at both the HDR and LDR led to increased PT clotting times, ranging from 21.81 seconds (measurement before proton radiation exposure) to 26.79 seconds (measurement after proton radiation exposure) (p < 0.05). In contrast, the aPTT was affected solely by LDR exposures and resulted in a 20-30% elongation in clotting time, from 31.91s to 41.58s (p <0.05). In
Solar radiation events (SPE) are unpredictable with varying intensity, dose rate and duration. With future space mission time expected to increase, the adverse biological effects from SPE radiation have become an area of interest in the space community. The dose rate of SPE radiation to the blood forming organs can vary from 10 to 50 cGy/hr (Hu, S., et al., Health Physics, 2009, April; 96(4):465-76). To investigate the biological effect of dose rate on the white blood cell (WBC) count, mice were exposed to proton radiation at doses of 50, 75 or 100 cGy at a high (3000 cGy/hr) or low (17 cGy/hr) dose rate. 6 week old ICR mice were housed under standard husbandry conditions and fed a normal rodent chow diet with ad lib access to food and water prior to irradiation at the NASA Space Radiation Laboratory. To deliver a homogeneous dose distribution, mice were irradiated with 8 different proton energies from 30.63 to 74.52 MeV at a dose rate of 3000 cGy/hr or 17 cGy/hr at doses of 50, 75 or 100 cGy. At 24 hours after the completion of the radiation exposure, eight mice irradiated at each radiation dose were sacrificed and blood was collected for complete blood cell analysis. All protocols in the experiment were approved by the Institutional Animal Care and Use Committees of the University of Pennsylvania and the Brookhaven National Laboratory. At 24 hours, the absolute WBC counts of mice exposed to 50, 75 or 100 cGy at either dose rate were significantly lower than the WBC counts of sham-irradiated control mice (p < 0.001). The WBC counts of mice at the high and low dose rates were not significantly different from each other, at all doses investigated. Analysis of total WBC count, absolute neutrophil, lymphocyte, and monocyte counts revealed no statistically significant differences between the two dose rates, at all doses examined. These data demonstrate a significant decrease in WBC counts after proton irradiation at high or low dose rate, in the dose range investigated. The data also show that the same biological effects can be observed in WBCs following proton irradiation administered at either a high (3000 cGy/hr) or low dose rate, in the dose range investigated.

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**POSTER PRESENTATIONS**

**POSTER PRESENTATIONS**

**POSTER 04: ACUTE RESPONSE TO SPACE RADIATION: PRODOMAL SYNDROME IN FERRETS.**

Experiments have been performed at the Loma Linda University Medical Center (CA) using either a 60Co source or 110- or 120-MeV protons. Ferrets are videotaped during the irradiations and for another 3-7 hours afterward in a separate facility. Non-irradiated animals are observed for 7 hours under identical conditions used for the irradiation runs. Recorded data are rendered into videos and the animals observed for retching and vomiting and other behaviors keyed by an observer into the AFRRRI Incident Response Program (IRP; linked to EXCEL) for individual and group analyses.

The key parameters used for analysis are: 1) proportion of animals in a group retching or vomiting or both, 2) the mean number of retches or vomits or both in a group, 3) the mean latency to the first retching or vomiting event, and 4) the mean duration of the prodromal period.

With 10-14 ferrets per group, the irradiations have been programmed to establish an RBE for the prodromal response in ferrets for gamma- and 110/120-MeV proton- radiations. Data analyses demonstrate that the RBE for emesis for both high dose rate (0.5 Gy/min) and low dose rate (0.5 Gy/hr) protons is approximately 1.0. Data also show that there is a dose-rate sparing effect for proton-induced emesis.

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**POSTER 08: RADIATION CHEMISTRY OF BIOACTIVE COMPOUNDS**

**POSTER 08: RADIATION CHEMISTRY OF BIOACTIVE COMPOUNDS**

**POSTER 08-01. INFLUENCE OF GAMMA RADIATION ON CHESTNUT (CASTANEA SATIVA MILL) VITAMIN E CONTENT.**

**POSTER 08-01. INFLUENCE OF GAMMA RADIATION ON CHESTNUT (CASTANEA SATIVA MILL) VITAMIN E CONTENT.**

Chestnut (Castanea sativa Mill) is a valuable source of vitamin E. The chestnut has been shown to be a functional food because of its high vitamin E content. vitamin E has anti-inflammatory activity, being promising alternatives to drugs used as cyclooxygenase inhibitors, a key enzyme in the inflammatory process. g-Tocopherol represents 95% of the vitamin E in chestnuts, which could give to this fruit a functional food classification [6].

**Acknowledgements:** This work is funded by the NSBRI Center of Acute Radiation Research (CARR) grant (#RE01801) via the Henry M. Jackson Foundation and LLU/NASA Cooperative Agreement NNX08AP21G. The NSBRI is funded through NASA NCC 9-58. The views presented here do not represent those of DoD or AFRRRI/USUHS.
The irradiations were performed in a experimental equipment with four 57Co sources, with a total activity of 305 TBq (8.233 kCi) in November 2009, after calibration with a standard Fricke dosimeter as described in a previous study [17]. 1: Institute of Applied Radiation. After irradiation geometry dose rate estimation the samples were divided in five groups to be exposed to different radiation doses: 0 (control), 0.25, 0.50, 1.00 and 3.00 kGy. Tocopherols contents were analysed by High Performance Liquid Chromatography (HPLC) coupled to fluorescence detection. The results showed a protective effect in g-tocopherol levels among storage, for the different doses of irradiation, compared to the non-irradiated samples, where this component decreased. Concerning chemical parameters, the gamma-irradiation treatment of this food product could be a promising process to increase its shelf life, avoiding anctioxidative compounds, as is observed for g-tocopherol, an isoform of the most natural powerful antioxidant: vitamin E.

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References:


Ascorbic acid (AsA) readily undergoes two consecutive, yet reversible, one-electron oxidation processes to form the ascorbate radical as an intermediate. Ascorbate radical has its unpaired electron in a highly delocalized π-representation for many biological structures. Ascorbic acid (AsA) readily undergoes two consecutive, yet reversible, one-electron oxidation processes to form the ascorbate radical as an intermediate. Ascorbate radical has its unpaired electron in a highly delocalized π-system, and hence it is a relatively unreactive free radical. These properties make AsA a superior biological antioxidant. In vitro AsA protects cells from oxidative stress by scavenging free radicals and recycling other antioxidants, such as vitamin E. The activity of ascorbic acid strongly depends on the character of the medium in which the oxidation process occurs. AsA is a powerful reducing agent in aqueous solutions, while this property is much less evident in non-aqueous media. In micellar surfactant systems the both type of media are combined and therefore these systems are considered as simplified models of biological structures – biological membranes. Micellar systems are also representative for many nutritional, pharmaceutical and cosmetic products and hence the behavior of AsA in such systems is of scientific and practical interest. In this study we observed the formation and decay of ascorbyl radical in aqueous and micellar systems, both direct and reverse. Pulse radiolysis was applied to generate oxidizing radicals of different reduction potentials, such as OH-, HOCl, •NO2, N2O5. The characteristic absorption band of ascorbyl radical with two peaks around 300 and 360 nm was observed in all systems investigated. It was also the case for the micellar systems stabilized with non-ionic Igepal, where we have observed specific interaction between surfactant molecule and AsA, also confirmed by us in NMR studies. Only little effect of surfactants, both anionic and non-ionic, on the formation and decay of ascorbyl radical was observed in aqueous solutions. However, in reverse micelles stabilized by AOT or Igepal CO-520 the decay could be separated into two steps, fast and slow, which we have ascribed to the intra- and intermicellar processes, respectively. We connect this feature with an interaction between AsA and surfactant molecules as mentioned above.

POS08-03. Enhancement of radiosensitivity by topoisomerase II inhibitor, amrubcin and amrubcinol, in human lung adenocarcinoma A549 cells and kinetics of apoptosis and necrosis induction. Sachiko Hayashi1, S. Hayashi1, M. Hatashita, H. Shioura1, A. Hayashi1, Y. Tanaka1, 1: University of Fukui, Japan 2: The Wakasawan Energy Research Center 3: Takarazuka City Hospital, Japan

The effects of amrubcin (AMR) and its active metabolite, amrubcinol (AMROH), on the sensitivity of a human lung adenocarcinoma A549 cells to ionizing radiation were investigated at 48 and 72 hours after administration of AMROH, 2.5 (g/ml) or AMROH (0.02 (g/ml), and were shown to be approximately parallel to treatment with irradiation alone. The initial shoulder shape portion of the survival curve for radiation alone, indicating the repair of sublethal damage (SLDR), was reduced as compared to that for sequential combined treatment with AMR or AMROH. Sequential treatments with AMR or AMROH prior to ionizing radiation resulted in an additive radio-enhancement effect that reduced not only survival, but also the shoulder width. Fractionated irradiation with 2 Gy per fraction with A549 cells was carried out in vitro similar to that commonly performed in clinical radiotherapy and the radio-resistance of the cells was shown to be inhibited by AMR and AMROH. Similar to AMR and AMROH, adriamycin (ADM) and etoposide (VP-16) are DNA topoisomerase II inhibitors. The effects of those 4 agents on cells that received X-ray irradiation were compared and all of the agents exhibited comparable radio-enhancement effects. The induction of apoptosis was investigated at 48 and 72 hours after administration of AMROH, radiation or combined treatment, and apoptosis was not significantly induced after any of those treatments. We also examined the induction of necrosis, and found that incidence of necrosis following combined treatment was approximately 2 times higher than that with either of the single treatments.
puls radiotherapy and flash photolysis of quinoxaline-derivatives in various solvents.

References

POSO8-05. Alpha-tocopherol and the regulation of lipid peroxidation in murine tissues in norm and under acute irradiation. Natalia Khirutsova, Emanuel Institute of Biochemical Physics, Russian Academy of Science, Moscow, Russian Federation

The effect of the acute X-rays at sublethal dose of 5 Gy on the lipid composition in the murine tissues was investigated. The content of α-tocopherol in lipids was measured and compared with different phospholipid fractions content. A single injection peros of sunflower oil was performed before irradiation as to investigate a case of an increased level of α-tocopherol content in mice organs. Changes of the interrelations between α-tocopherol content and parameters of the physicochemical lipid peroxidation regulatory system for liver, spleen and erythrocytes were revealed within one month after the sunflower injection and the acute ionizing irradiation, and an inverse correlation between the ratio of the phosphatidyl choline to phosphatidyl ethanolamine (PC/PE) and α-tocopherol content in different mice organs was observed. In the liver lipid composition the fractions which are most strongly affected by sunflower injection and acute irradiation are revealed along with a steady state level of such parameters as a molar ratio of [sterols] / [phospholipids] and relative content of phosphatidyl choline for the sunflower injection influence, the ratio of PC/PE the for the acute irradiation effect.

POSO8-06. DNA strand breaks and base damage as target lesions for radioprotection by methylproamine and its analogues. Pavel Lobachevsky1, C. Skene2, J. White1, J. Ventura1, R. Martin1, 1: Peter MacCallum Cancer Centre, Australia 2: Bio21 Institute, University of Melbourne, Australia

Methylproamine is a DNA-binding radioprotector that shares common benzenimidazole structure with fluorescent DNA Hoechst dyes. Radioprotective properties of methylproamine have been demonstrated in cell culture using such endpoints as clonogenic survival and induction of the phosphorylated histone H2AX foci that are considered as markers DNA double strand breaks. In vivo radiotherapy studies have established that DNA-associated methylproamine undergoes oxidation following exposure to ionising radiation involving electron transfer over the distance of a few base pairs from the ligand to oxidised DNA species. It is hypothesised that electron donation from methylproamine to transient DNA lesions represents one of the mechanisms of radioprotection by methylproamine. Considering that complex DNA lesions that include breaks and base damage in opposite DNA strands are events that are responsible for cytotoxic and genotoxic consequences of the exposure to ionising radiation, in the present study we have attempted to establish the ability of methylproamine to protect DNA in solution from initial radiochemical lesions that result in formation of DNA breaks and base damage. We used the plasmid DNA model that allows measurement of DNA single strand breaks (SSB) by quantitating the conversion between supercoiled and open circle relaxed plasmid forms. The combination of this SSB assay with the treatment of irradiated plasmid with formamidopyrimidine-DNA N-glycosylase (FPG) that recognises oxidised purines and converts them to SSB was exploited as an endpoint for DNA base damage measurement. To prevent formation of up to 96% of base damage that is revealed by FPG treatment. The extent of this radioprotection correlated with the fraction of DNA binding sites occupied by the ligand rather than with the concentration of the free ligand thus indicating the role of the DNA bound radioprotector in the reduction of radiation induced base damage.

In vivo contribution of the structural stability of the FGF1 protein to its radioprotective effect remains unknown. This study evaluated and compared the protective activity of FGF1, FGFC and FGF1 mutants in the absence of heparin. Each FGF was administered intraperitoneally to BALB/c mice 24 h before or after total body irradiation (TBI). The surviving numbers of crypts per cross-section were determined in the jejunum 3.5 days after TBI. TUNEL assay was performed on paraffin-embedded sections of the jejunum to evaluate apoptosis 24 h after TBI. In the case of administration 24 h before irradiation, both Q40P/S47I/H93G and FGFC had the strongest effect on crypt survival among these FGFs. The radioprotective effect of Q40P was between that of FGF1 and Q40P/S47I/H93G. However, Q40P/S47I increased crypt survival to almost the same extent as FGF1, although it was more stable than Q40P. In addition, Q40P/S47I/H93G and FGFC were effective in promoting crypt survival even when it was administered 24 h after TBI at a dose of 10, 11, or 12 Gy, whereas FGF1 was not effective. Q40P/S47I/H93G or FGFC post-treatment significantly promoted BrdU incorporation into crypts and increased crypt density, resulting in more epithelial differentiation. In contrast, the number of apoptotic cells in Q40P/S47I/H93G or FGFC-treated mice decreased to almost the same level as that in FGF1-treated mice. These findings showed that Q40P/S47I/H93G enhanced radioprotective activity in vivo.
vivo to the same extent as FGFR. In contrast, the level of stability of FGFI did not correlate with that of its protective effect on radiation intestinal damage. Therefore, not only stability but also other conditions might contribute to the radioprotective effect.

POS08-09. Radiosensitization by celastrol is mediated by modification of antioxidant thiol molecules. Bo-Jeong Pyun1, H. Ran Seo2, W. Duck Seo1, B. Won Lee3, Y. Bae Jin2, K. Hun Park2, E. Seo2, Y. S. Jeon4. 1: Ewha Womans University/College of Pharmacy & Division of Life Science & Pharmaceuticals, South Korea 2: Division of Radiation Effects, Korea Institute of Radiological and Medical Sciences 3: Department of Functional Crop, National Institute of Crop Science, Rural Development Administration 4: Division of Applied Life Science (BK21 program), EB-NCRC, Institute of Agriculture and Life Science, South Korea

The radiosensitizing effects of naturally occurring triterpenes were investigated in human lung cancer cells. Several quinone methide-containing triterpenes (QMTs) enhanced the cytotoxic effect of ionizing radiation (IR) and of these QMTs, celastrol (CE) had the greatest enhancing effect on IR-induced cell death in vitro. Additionally, the quinone methide moiety of CE was shown to be essential for CE-mediated radiosensitization; in contrast, dihydrocelastrol (DHCE), does not contain this moiety. Reactive oxygen species (ROS) production by IR was augmented in a combination with CE, which was responsible for CE-mediated radiosensitization. CE induced the thiol reactivity and inhibited the activities of antioxidant molecules, such as thioredoxin reductase and glutathione. In vivo, nude mouse xenografting data also revealed that tumor growth delay was greater in mice treated with CE plus IR compared with those treated with CE or IR alone. When DHCE, instead of CE, was combined with IR, tumor growth delay was similar to that in IR alone-treated mice. These results demonstrate that CE synergistically enhances the effects of IR and suggest the novel antinecancer therapeutic use of CE in combination with radiation therapy.

POS08-10. Somatostatin receptor 2 is involved in the intestinal protective effect of SOM230 after radiation. Wenze Wang1, P. G. Bijlsma1, S. Garg1, H. A. Schmid2, M. Hauer-Jensen1. 1: Division of Radiation Health, University of Arkansas for Medical Sciences, USA 2: Division of Radiation Health, University of Arkansas for Medical Sciences, Little Rock, AR 3: Novartis Institutes for BioMedical Research, Basel, Switzerland

Background: SOM230, a novel somatostatin analog with broad affinity to four (sst1, sstr2, sstr3, and sstr5) of the five somatostatin receptors (sst) potentialy mitigates total body irradiation (TBI)-induced lethality in mice by inhibiting exocrine pancreatic enzyme secretion, thus preventing “auto-digestion” of the intestine. However, the specific receptor involvement for this action has not been studied. Methods: Sstr mRNA levels and protein expression were measured in cultured rat AR42J pancreatic acinar cells by real-time PCR and Western blot, respectively. The effect of SOM230 in trypsin secretion was compared in AR42J cells transfected with sstr2 siRNA and control siRNA. The effect of SOM230 on intestinal proteolytic activity in vivo was measured using a protease fluorescence detection kit, and the preservation of intestinal permeability and morphology after radiation was assessed in C57BL6 mice. Results: AR42J pancreatic acinar cells mainly expressed sstr2 mRNA, with the order of expression being sstr2a > sstr2b > sstr4 > sstr5 > sstr3. Normalized to sstr3, the relative expression levels were 156.1 for sstr2a, 21.5 for sstr2b, 10.2 for sstr4, 3.1 for sstr5, and 1.9 for sstr1. SOM230 strongly inhibited trypsin secretion by AR42J cells. Knocking down sstr2, as evidenced by mRNA and protein expression levels, blocked the inhibitory action of SOM230 on trypsin secretion. SOM230 administration in vivo was associated with decreased intestinal proteolytic activity, and, when administration was started 24 h after TBI, ameliorated post-TBI increase in intestinal permeability and protected the morphology of the intestinal mucosa. Conclusions: While further studies are needed to investigate the possible involvement of sstr1, sstr3, and/or sstr5, these results strongly suggest that sstr2 plays a major role in mediating SOM230’s inhibitory effect on exocrine pancreatic secretion. Therefore, sstr2 is likely important for the mitigating effects of SOM230 on TBI-induced lethality.

POSTER PRESENTATIONS

POS09 Boron neutron capture therapy (BNCT)

POS09-01. Pharmacokinetics and micro-distribution of boronophenylalanine in animal model of head and neck squamous cell carcinoma. Pong-In Chou1, Y. Lin2, H. Chung2, J. Peer2, S. Wang3. 1: Institute of Nuclear Engineering and Science, National Tsing Hua University 2: Department of Nuclear Medicine, Taipei Veterans General Hospital, Taiwan 3: Institute for Nuclear Physics, Vienna, Austria

Purpose: The success of boron neutron capture therapy (BNCT) is highly dependent on the amount and distribution of boron in the tumor, and on the ratio of the amount of born in the tumor to that in normal tissue. The purpose of this study was to investigate the boron micro-distribution in the tumor region, and the biodistribution and pharmacokinetics of boronophenylalanine (BPA) for BNCT in nude mouse xenograft model of head and neck cancers. Material and methods: A total of 1x106 human oral squamous cell carcinoma cells (SAS cells) in 100 μl PBS were inoculated subcutaneously into the foreleg of five-week-old male nude mice. When the tumors reached a suitable size, the mice were intravenously injected with 400 mg/kg BPA. At 0,15, 30, 45 and 60 min following BPA injection, tumor, blood and normal organs were collected, respectively. Boron concentrations were measured by ICP-AES analysis. The micro-distribution of boron in tumor regions was investigated by u-track observation. Results: A 78% decrease of the boron concentration in blood was observed at 15 min after BPA injection. The tumor-to-normal and tumor-to-blood boron ratios were 1.59 and 2.43, respectively after 60 min of BPA injection. Boron concentration in the tumor region depended on the size (volume) and growth conditions of the tumor. ICP-AES analysis revealed higher boron concentrations in the smaller tumors. Notably, the alpha track distribution in the small tumor was more uniform than that in the large tumor. A low alpha track density in the central necrotic region of the large tumor was observed. The boron concentration in the pancreas reached to 79.85 μg/g, which was markedly exceeds that in blood and other organs.

Conclusions: Although the tumor-to-blood boron concentration ratio is the key parameter that is used in calculating the radiation dose during BNCT, the homogenous distribution of boron in the tumor region supposedly should be used to determine for the therapeutic efficacy of BNCT. Furthermore, when BPA is used as the boron drug for BNCT in cases of abdominal cancer, the pancreas dose should be considered.

POS09-02. The Boron Neutron Capture Therapy (BNCT) Project at the research reactor TRIGA Mainz, Germany, Gabriele Hampel1, C. Schütz2, C. Gruenwald2, D. Iffland2, J. Kritz1, P. Langguth1, C. Brochhausen1, J. Krickpark1, P. Kudejova1, K. Appelmann1, R. Moss1, N. Bassler1, M. Ziegner1, M. Blackner1, P. Sharpe4, H. Palmins5, G. Otto6, 1: Institute for Nuclear Chemistry, University of Mainz, Germany, 2: Department of Pharmaceutical and Toxicology, University of Mainz, Germany, 4: Department of Pathology, University of Mainz, Germany, 5: Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II), TU München, Garching, Germany, 6: Joint Research Centre (JRC) of the European Commission, Petten, Netherlands, 7: University of Aarhus, Nørre Ringade, Aarhus C, Denmark, Denmark, 8: Molecular Medicine, Health & Environment Department, Austrian Institute of Technology, Austria, 9: National Physical Laboratory, Teddington Middlesex, UK, 10: Department of Hepatobiliary, Pancreatic and Transplantation Surgery, University of Mainz, Germany

At the University of Mainz, Germany, a research group is carrying out fundamental research on boron neutron capture therapy (BNCT) applications towards the treatment of liver tumours. Neutron irradiations take place at the TRIGA Mark II reactor, biological, clinical research and surgery takes place at the University and its Hospital of Mainz. Both are situated in close vicinity to each other, which is an ideal situation for BNCT treatment. Within the last years, research on computational modelling for the irradiation facility, tissue and blood analysis, radiation biology, dosimetry and surgery has been performed. A preclinical trial has been started on patients suffering from liver metastases of colorectal carcinoma. Prior to normal liver resection, patients are infused intravenously with 200 mg/kg body weight of p-
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borono-phenylalanine-fucrute (BPA-F) complex, for two hours until the moment of resection. During infusion, blood samples are taken to provide pharmacokinetic data. Liver samples from tumour and tumour-free tissue are taken from different locations in the removed liver specimen. The remaining part of the specimen is then irradiated under conditions identical to those planned for BNCT treatment. For tissue analysis, both Quantitative Neutron Capture Radiography (QNCR) and Prompt Gamma Activation Analysis (PGAA) are used. For QNCR, CR-39 films overlaid with tissue slices containing 10B are irradiated at the TRIGA in Mainz. Etching and analysis, in cooperation with the pathologist, are performed afterwards. PGAA is performed at Petten and Garching. PGAA and inductively coupled plasma mass spectrometry (ICP-MS) are applied to determine the boron content in the blood samples. The results received with the different methods and at the different facilities show a good agreement. Furthermore, the blood concentration curves reflect very well the course of surgery. For the described experiments, as well as for the later treatment, a reliable dosimetry system is developed. Therefore, alanine pellets are suitable for dose measurements in a mixed neutron – gamma field.

POS09-03. Linearity tests of Gafchromic EBT2 film irradiated in an epithermal neutron beam. Ming-Chen Hsiao1, W. Chen2, Y. Liu2, H. Liu1, S. Jiang3: 1: Institute of Nuclear Engineering and Science, National Tsing Hua University, Taiwan 2: Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu 30013, Taiwan 3: Nuclear Science and Technology Development Center, National Tsing Hua University, Taiwan

Gafchromic EBT2 film belongs to radiochromic detectors, which is commonly applied in conventional radiotherapy dosimetry to provide 2D dose information. EBT2 film is, however, relatively much less used in neutron/gamma-ray mixed field dosimetry such as in boron neutron capture therapy (BNCT). Low cost and easy use of the film make it an ideal candidate for 2D dose measurement in BNCT. To establish the ability of using EBT2 film in mixed field dosimetry, its linearity response in the mixed field is certainly of our interests.

The film was calibrated by a 46Co beam (20 – 1,500 Gy) and a 6-MV LINAC (20 – 4,000 Gy). The dose range of the performed calibration in the 6-MV LINAC was raised to 4,000 Gy due to the possible high dose delivery given by the mixed field which does not commonly have in conventional therapy. The calibration curves were then used to obtain the photon-equivalent dose (γ-Gy) in the linearity test. To test the response linearity, the EBT2 film was irradiated in the BNCT epithermal neutron beam at the Tsing Hua Open-pool Reactor at 1.2 MW. To obtain different dose deliveries to the film, the film was cut into small square pieces and positioned in a 21 x 21 x 21 cm3 acrylic phantom. The beam opening was 2.5 cm in diameter. For the diameter of collimated field, two beam opening sizes were considered: 2.5 cm and 7.5 cm. The setup of EBT2 film for irradiation. Different irradiation times were performed to extend the dose observation range under a reliable monitoring of fission chamber detectors. All the measured responses were converted into pixel and optical density (OD) values and were then normalized according to the monitor readings. The calibration results were obtained in red and green wave lengths respectively as OD and pixel values, but only the red component was used in the linearity experiments. From the results, EBT2 film presented large responses to neutrons and therefore the epithermal neutron beam can easily deliver a dose over 4,000 γ-Gy to the film (30 min irradiation at 2-cm depth can give >5,000 γ-Gy). The linearity of EBT2 film, however, is only good before 1,500 γ-Gy (both OD and pixel modes). After 800 γ-Gy, the values obtained from OD and pixel calibration curves turned to clearly deviate from each other. This work has showed us a better insight into the linearity response and the functional range of the EBT2 film in the mixed field; it also reveals the fact that EBT2 film has large responses to neutrons.

POS09-04. Extension collimator design for head-and-neck cancer treatment using BNCT. Yu-Shiang Huang1, Y. Liu1, Y. Hsueh Liu2: 1: National Institute for Nuclear Science and Technology Development, National Tsing Hua University, Taiwan 2: National Tsing Hua University, Taiwan

Accurate positioning and field size control are the major items of radiotherapy. In boron neutron capture therapy (BNCT) for head-and-neck (H&N) cancer, extension collimator can be used to assist patient positioning. In this study, a preliminary design study was performed to assess different extension collimator designs for H&N cancer treatment performed at the Tsing Hua Open-pool Reactor (THOR).

The length of extension collimator affects the accommodation space of patients and patient pose holding. The moderation of field size and material can reduce fast neutron dose and protect normal tissues outside the field. Since the extension collimator is mechanically attached to the surface of beam exit, heavy material may cause installation problem. Hence, polyethylene (PE) is considered as a proper choice due to its physical density of 0.94 g/cm3 and good performance in neutron moderation. PE is consequently used as the major construction material while functional materials like Li2O, Bi, and Pb are used as collocation. MCNP5 was used to perform sensitivity study on geometric factors. Dose distributions inside and outside the collimator were calculated with the results received with the different methods and at the different facilities show a good agreement. Furthermore, the blood concentration curves reflect very well the course of surgery. For the described experiments, as well as for the later treatment, a reliable dosimetry system is developed. Therefore, alanine pellets are suitable for dose measurements in a mixed neutron – gamma field.

POS09-05. A study on the photon and electron response functions of Epxradin TE(TE) and Mg(Ar) ionization chambers. Yi-Chan Lin1, Y. Liu2, S. Nivaart1, Y. Chen1, S. Jiang2, W. Chou1: 1: Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Taiwan 2: Nuclear Science and Technology Development Center, National Tsing Hua University 3: Institute for Energy, Joint Research Centre 4: Department of Engineering and System Science, National Tsing Hua University 5: Institute of Nuclear Engineering and Science, National Tsing Hua University, Taiwan

The purpose of this study is to evaluate the photon and electron response functions of two commercially available ionization chambers, denoted as TE(TE) and Mg(Ar), employed in an epithermal neutron beam. Neutron fields used for radiology and radiotherapy always contain accompanying by photons. These photons and following electrons can create large dose delivery at the exit of the collimator. The photon energy bellow 0.1 MeV and similar response for above 0.2 MeV were considered. The response functions of the chambers to incident photons and electrons were obtained using the Monte Carlo code, MCNP5. The MCNP5 model included an epithermal neutron beam in front of collimator, which was generated by a 100 kW gadolinium filtered reactor. Photon and electron response functions were then calculated by Monte Carlo simulation. The simulation results were compared with data measured in experiments. The results showed that a length of 15 – 25 cm is good for patient positioning when considering the actual shoulder sizes. However, a longer collimator results in a weaker beam output. For the wall thickness, 1-cm can provide sufficient moderation to reduce fast neutron dose while not causing too many capture 2.2-MeV gamma rays. Note that, a thicker wall will not result in a higher epithermal neutron flux. For the diameter of collimated field, collimators can have ~2% higher epithermal neutron flux at the beam exit center. Nevertheless, the field size should be larger than the size of target tumor. PE combined with Bi or Pb have no significant improvement with regards to beam flux and background dose, but the total weight of collimator becomes much heavier. PE combined with Bi or Pb decrease gamma-ray dose around the collimator and this combination is considered as the best solution when the construction cost is not a major issue.

POS09-06. Virtual determination of 2D boron neutron capture reaction rate by indirect neutron radiography and neutron activation analysis for BNCT. Yuan-Hao Liu, Y. Huang, National Tsing Hua University, Taiwan
Boron dose is the main therapeutic dose of boron neutron capture therapy (BNCT) which can be determined by estimating the number of boron neutron capture reactions (BNCRs) occurring in the region of interest. Hence, BNCRs determination becomes an important issue. Nowadays, the estimation of BNCRs and its 2D distribution are commonly done by using solid-state nuclear track detectors. However, it needs a certain B concentration in the target, which is not always practical for small laboratory irradiations, such as BNCT. Since BNCR does not result in radioactive products, it is not suitable for INR. Nevertheless, in a hydrogen-rich phantom, BNCRs are mostly induced by thermal neutrons. As a result, it is reasonable to assume that an activation converter whose radioactivity comes mainly from thermal neutrons can be used to reproduce the relative BNCR rate distribution. To find out proper converter materials, MCNP5 was used to calculate the relative 2D reaction rate distribution of 10B and several different materials along the central axis of a 21 x 21 x 21 cm PMMA phantom irradiated in the epithermal neutron beam of Tsing Hua Open pool-Reactor. The used converters’ size is 0.0125 x 15 x 20 cm. The tested materials were: 23Na, 27Al, 63Cu, 11In, 107Ag, and 117Au. The calculated results tell that 23Na, 27Al, and 117Au can perfectly reproduce the relative BNCR rate distribution within a difference of 1% throughout the whole depth, while 63Cu and 11In can have the same effect after 1 cm depth. As to 107Ag, 11In, and 117Au, they are concluded to be not suitable for our objective due to their large resonance peaks near 0.5 eV. Therefore, INR can be used to conveniently determine the 2D BNCR distribution with properly selected converter materials.

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**POS09-08**. Investigation of the neutron beam of an accelerator-based BNCT facility by means of a Bonner sphere spectrometer, A. di Fulvio1, F. Trenor1, E. Esposito1, R. Bedogni1, 1: University of Pisa, The Faculty of Engineering, Department of Nuclear Engineering, Nuclear Production, Italy 2: The faculty of Engineering, Department of Mechanical Engineering, Nuclear Production 3: -LNL, Legnaro National Laboratory, Viale dell’Universita 2, 35020, Legnaro (Padova), Italy 4: INFN-LNF, Frascati National Laboratory, Via E. Fermi 40, I-00044 Frascati, (Roma, Italy)

one of the major problems raised by the BNCT technique is to obtain necessary neutron beam in terms of intensity and energy spectrum. In this work the Bonner Sphere Spectrometer (BSS) is applied to determine the energy neutron fluence distribution produced by a 9Be(p,xn) reaction using a 5 MeV proton beam. This neutron source is developed with the SPES-BNCT project of the Legnaro National Laboratory, INFN, of the Italian National Institute of Nuclear Physics (INFN), where an accelerator-based, high-flux thermal neutron beam facility is being developed for the experimental treatment of extended skin melanoma. The spectrometer consists of a set of polyethylene spheres with a neutron sensitive 6LiF(Eu) scintillator. While other types of spectrometer are currently being used for measurements in the 100keV-5 MeV energy region, the BSS will provide a good response also for low energy neutrons (below 100 keV). Different angles of irradiation(0,40,80 and 120deg) were considered. All measurements were performed under \(\pi\)(uncollided and collided) radiation conditions. An evaluation of the response matrix for the BSS was carried out by using the MCNPX code. The determination of neutron spectrum from the set of counts was done by FRUIT unfolding code and also different unfolding codes.

**POS09-09**. Ionization effects of charged particles produced in BNCT field, Saeed Mohammadi, Payame Noor University, Iran

The ionization effects of charged particles produced in neutron interactions for Boron Neutron Capture Therapy are considered here using SRIM Monte Carlo Code. The estimated values of these effects in a Plexiglas acrylic phantom are shown to agree well with the available experimental values in high Boron concentration areas. As expected, the ionization effects from lithium and alpha particles are significant. In the low Boron areas, proton ionization makes an important contribution and its effect on healthy tissue should not be ignored.

**POS09-10**. Physical Neutron Dosimetry for the University of Pavia Thermal Neutron Source for BNCT Radiobiological Research, David Nigg1, D. W. Nigg2, N. Protti3, S. Stella3, S. Bottorff2, M. Pizzocaro1, R. Bedogni1, 1: University of Pavia, The Faculty of Engineering, Department of Mechanical Engineering, Nuclear Production, Italy 2: INFN-LNF, Frascati National Laboratory, Via E. Fermi 40, I-00044 Frascati, (Roma, Italy), 2: University of Pavia 3: University of Pavia 4: Idaho National Laboratory USA

The Idaho National Laboratory and the University of Pavia are collaborating in the field of medical neutron dosimetry specific to Neutron Capture Therapy (NCT) applications. This effort resides within a larger framework of computational and experimental dosimetric intercomparisons of several thermal neutron sources used for preclinical NCT radiobiology research worldwide. Recognizing the importance of accurate and reproducible physical beam dosimetry as an essential tool for combination of preclinical and clinical results from different facilities, we have conducted an experimental characterization of the neutronic performance of the thermal neutron source at the University of Pavia TRIGA research reactor facility. The characterization methodology is based on neutron activation spectrometry coupled with rigorous least-squares-based spectral deconvolution procedures to produce the desired neutron flux, adjusted dosemetry reaction rates, and corresponding radiobiological dosimetric information. Results show that the Pavia neutron source is well thermalized, with a Cadmium Ratio of approximately 80 and a thermal flux of approximately 1 x 10^10 n cm^-2 s or greater, depending on the specific irradiation location. The submitted poster will discuss further details of the application of advanced modeling and simulation approaches for computational dosimetry of neutron sources, the use of neutron activation spectrometry for experimental validation of research reactor based neutron beams, and possible experiments, and the specific results obtained for the TRIGA facility at Pavia.
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POS09-11. Effect of elapsed time after injection of Boric Acid on 10B absorption in different regions of adult male rat’s brain: An alpha autodigraphy study. Ali Pazarzadeh1, S. Goodarzi2, B. Farnie3, N. Khojasteh Baghban4, 1: Science and Research Branch, Islamic Azad University, Iran 2: Nuclear Engineering Department, Science & Research Branch, Islamic Azad University, Tehran, Iran 3: Laboratory of Basic Science & Neuroscience, Basic Science Dept, Faculty of Allied Medicine, Cellular & Molecular Research Center, Tehran University of Medical Science (Pardis-e-Hemmat), Tehran, Iran

Boron Neutron Capture Therapy is a modality for cancer treatment based on the thermal neutron capture by 10B in the tissue. Distribution of 11B in different parts of normal and affected brain is still unknown. In this study, we determined the distribution of boron in different regions of the adult rat normal brain. Adult Wistar male rats divided into two groups including control and trial. Trial group received intraperitoneal shots of 2ml of neutral boron compound. 2, 4 and 6 hours after injection, animals were killed and their brains were removed. Coronal sections of 20µm thickness of forebrain, midbrain and hindbrain were prepared, sandwiched between two pieces of polycarbonates. The samples were irradiated in the thermal neutron field and then polycarbonate foils were etched. Alpha tracks on the polycarbonates were detected and studied. The plots of 11B distribution versus irradiation times of brain were analyzed statistically. Significant differences in 11B distribution in three regions were seen with the highest concentration in the forebrain at 4 hours after boron carrier injection. Awareness of 11B distribution in forebrain may help therapists use more effective treatment planning, including neutron flux level and the optimum neutron irradiation time after boron carrier injection.


In May 2010, the operation of Kyoto University Reactor (KUR) was restarted, which was suspended during four years for the change from the high-enriched uranium fuel to the low-enriched one. At the same time, boron neutron capture therapy (BNCT) using the Heavy Water Neutron Irradiation Facility (HWNIF) installed in KUR, was restarted. At this facility, thermal neutron irradiation is performed for superficial tumours, and epi-thermal neutron irradiation is done for deep-seated tumours, in the same manner as before. In December 2009, the Cyclotron-Based Epi-thermal Neutron Source (C-BENS) for BNCT was completed in our institute. In the near future, BNCT will be started using C-BENS. C-BENS is specialized for epi-thermal neutron irradiation, and the characteristics for neutron energy spectra are different from those of KUR or HWNIF. Thus, BNCT will be performed in our institute, using the neutron irradiation fields with the various beam characteristics. For the exact evaluation of BNCT therapeutic effects, the biological dose estimation consistent among the irradiation fields is important. A study on dose estimation from the viewpoints of energy transfer to biological tissue is reported.

An analytical estimation was performed for the interactions occurred in biological tissue due to the neutron irradiation with incidental gamma rays, and for the charged particles generated due to the interactions. The estimation was done for four neutron irradiation fields such as three irradiation modes of thermal, mixed and epi-thermal at KUR-HWNIF, and the C-BENS epi-thermal neutron field. The reaction cross-sections were derived from the nuclear data library “JENDL 3.3” for neutrons, and the data by Storm et al. for gamma rays. It was assumed that the biological tissue contained H (11.1% in terms of weight), C (12.7%), N (2.0%) and O (74.2%). It was confirmed that the scattering reaction of hydrogen was the main source of protons for the wider energy region in the epi-thermal neutron irradiation, from the energy spectra of the protons generated in the biological tissue under free-in-air irradiation. In the irradiation fields with the more incidental thermal neutrons, the number of protons originated from the (n,p) reactions of nitrogen exceeded the number of protons originated from the hydrogen reaction, nearly at 0.5 MeV.

The number of high-energy protons originated from the hydrogen reaction in the C-BENS field, was larger than that in the epi-thermal neutron irradiation mode of KUR-HWNIF. For the protons originated from the (n,p) reactions of nitrogen and oxygen, the yields for the higher energy were larger. Furthermore, the protons originated from the (n,p) reaction of carbon, whose threshold energy is larger, were not a little generated. Also for the alpha particles originated from the (n,α) reactions of carbon, nitrogen and oxygen, the yields for the higher energy were larger.

For the charged particles generated due to the interactions of gamma ray and the biological tissue, it was confirmed that the electrons originated from the Compton scattering were the main component. As the main energy range of the gamma rays incident in the BNCT neutron irradiation fields is from a few MeV to almost 10 MeV, the electrons from a few keV to almost 10 MeV are generated. Especially, it was confirmed that most of the generated electrons were the electrons originated from the Compton scattering of oxygen.

The more detailed estimation will be performed for the energy spectrum of the proton of below 1 MeV, whose contribution to the absorbed dose and biological effectiveness is thought to be larger. Also, the estimation for the high-energy protons which effect over some cells will be performed. Moreover, the characteristic comparison will be tried among the irradiation fields from the more micro viewpoints, using the data obtained from the in-vitro experiments, etc..


BNCT was proposed for the treatment of multifocal, non-resectable, bilobar colorectal liver metastases that do not respond to chemotherapy. The aim of the present study was to systematically assess tumor control and potential toxicity of BNCT in an experimental model of liver metastases in BDIX rats inoculated in the liver with syngeneic colon cancer cells to produce subcapsular tumor nodules that simulate liver metastases but are more amenable to follow-up. BNCT studies were performed at the RA-3 thermal neutron facility with a lithium-6 enriched carbon steel shield. Dosimetric calculations were based on BPA biodistribution studies. Tumor-bearing animals were divided into 3 groups, i.e. BPA-BNCT. BNCT mediated by boronophenylalanine (BPA) at a dose of 46.5 mg B/kg, Beam only: exposure to the same thermal neutron fluence range as the BPA-BNCT group and Sham: exposed to matched manipulation, but not irradiated. Total physical dose to tumor with BPA-BNCT was: (A) 9 ± 1 Gy, (B) 13 ± 3 Gy or (C) 17 ± 1 Gy. Normal liver received 6 to 10 Gy. Tumor nodule surface was measured pre-irradiation. At 3 weeks the animals were sacrificed for histological analysis and to evaluate tumor surface and weight. An additional set of animals was used to establish mean tumor nodule weight before irradiation. Clinical signs and body weight were monitored regularly. At 3 weeks post-irradiation, the tumor surface area post-treatment/pre-treatment ratio was 5 ± 3 for Sham, 3 ± 2 for Beam only, 1.2 ± 1.0 for BPA-BNCT (A), 0.5 ± 0.2 for BPA-BNCT (B), and 0.4 ± 0.1 for BPA-BNCT (C). Tumor nodule weight pre-treatment was 56 ± 39 mg. Three weeks post-irradiation tumor nodule weight rose to 346 ± 302 mg for Sham and to 140 ± 106 mg for Beam only but fell to 24 ± 24 mg for BPA-BNCT (A), to 19 ± 16 for BPA-BNCT (B) and to 5 ± 2 for BPA-BNCT (C). For both endpoints, the differences between the BPA-BNCT groups and the Beam only and Sham groups were statistically significant (ANOVA). No ostensible clinical, macroscopic or histological signs of toxicity were observed in any of the groups, with exception of the BPA-BNCT (C) group that exhibited moderate skin and intestine toxicity. Conclusion: BPA-BNCT induced significant partial remission of experimental tumor nodules in the liver 3 weeks post-irradiation, with no ostensible normal tissue toxicity up to a physical dose of 13 ± 3 Gy to tumor.

POS09-14. Induction of DNA double strand breaks in CHO/K1 and xrs-5 cells irradiated with mixed neutron and gamma-rays for BNCT. SENTARO TAKAHASHI1, K. Okumura2, Y. Kinashi2, K. Ono2, Y. Kubota1, R. Okayasu1, 1: Research Reactor Institute, Kyoto University, Japan 2: Research Reactor Institute of Kyoto University 3: National Institute of Radiological Sciences, Japan
In the boron neutron capture therapy (BNCT), normal as well as tumor cells are exposed to a mixed radiation field (thermal, epithermal and fast neutrons, and gamma-rays). However, little is known about the biological effects of such radiation exposures as used for BNCT. Here, the relative biological effectiveness (RBE), and the dose and dose rate effectiveness factor (DDREF) for the mixed irradiation used for BNCT in Kyoto University Research Reactor (KUR), were investigated.

CHO/K1 and xrs-5 cells were irradiated at the KUR irradiation field for BNCT. The average physical dose rates of thermal (<0.5eV), epithermal (0.5eV-100keV), fast (>10keV) neutrons, and gamma-rays were 10.0, 1.1, 7.4, and 20.5 mgY/min, respectively, when the reactor was operated at 1MW. When operated at 5MW, the dose rates became approximately 11 times higher than those at 1MW. As a reference, radiation, Co-60 gamma-ray was used at the same dose rate as the mixed irradiation. The cells were assayed for conventional colony formation, and DNA double strand breaks (DSBs) were detected by immune-staining using gamma-H2AX and 53BP1 antibodies.

The RBE for cell survival by colony formation were 3.2 and 1.3 for CHO/K1 and xrs-5 cells, respectively, when the cell were irradiated with the mixed radiation field at 1MW. The number of gamma-H2AX and 53BP1 foci 1hr-post-irradiation were similar for the mixed radiation and the reference gamma-ray, but the size of foci seemed to be different, indicating neutrons may have induced a different type of DNA-DSB. There was no significant difference in cell survival levels between the mixed irradiation conditions with 1 and 5 MW, suggesting no significant DDREF in this dose range.

**POS09-15. Overview of cyclotron-based epithermal neutron source for boron neutron capture therapy.** Hiroki Tanaka, Kyoto University Research Reactor Institute, Japan

**[Purpose]** At Kyoto University Research Reactor Institute, more than 330 patients without other treatment option have been treated by BNCT up to the present. The effectiveness of BNCT for treating not only malignant melanoma, and brain tumor but also recurrent head and neck tumor, mesotheloma, and liver cancer has been demonstrated. On the other hand, in order to realize BNCT as the approved medical treatment, we have been developed and installed new accelerator-based neutron source on December 2008. On March 2009, the sufficient intensity of neutron flux for clinical trial was successfully obtained. In this presentation, we introduce overview of cyclotron-based epithermal neutron source (C-BENS).

**[Methods]** C-BENS consists of a cyclotron accelerator producing protons with the energy of 30 MeV, beam transport system, moderator system, gamma-ray shielding, collimator and irradiation bed. The moderator system consists of two kinds of components. One is the moderator such as iron and lead for reducing the energy of high energy neutron emitted from Be(p,n) reaction. The other is filter such as aluminum and calcium fluoride penetrating the several tens keV neutrons. To simulate beam characteristics, irradiation test using a water phantom was performed. A water phantom was set in front of collimator with the aperture size of 10 cm in diameter. To determine thermal neutron distribution in a water phantom, gold wire and gold wire covered with cadmium tube were used. After the irradiation, the activation of gold wire was measured by was measured by high purity Ge detector.

**[Results]** The intensity of thermal neutron flux at the depth of 20 mm was 1.1×10^9 (neutrons/cm^2/s). The dose distribution assumed brain tumor with ratio of boron concentration between tumor and normal brain of 3.5 was evaluated. RBE factors for nitrogen and hydrogen were 3.0 and 2.5, respectively. CBE factors for tumor and normal brain were 3.8 and 1.3, respectively. Irradiation time was 45 minutes with maximum dose for normal brain of 12 Gy-eq. C-BENS can treat tumor located up to 6 cm with equivalent dose of 30 Gy- eq.

**[Conclusion]** The enough intensity for BNCT treatment using C-BENS was experimentally confirmed by water phantom measurements. C-BENS can treat brain tumor within one hour. Now, preparation to start clinical trial is progressing.

**POS09-16. TE(TE) Ionization Chamber Simulations with Various Monte Carlo Codes in Gamma-ray fields.** Shu-Wei Wu¹, Y. Liu², Y. Lin¹, Y. Chen¹, S. Nievaart¹, S. Jiang², 1: Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Taiwan 2: Nuclear Science and Technology Development Center, National Tsing Hua University 3: Department of Engineering and System Science, National Tsing Hua University 4: Institute for Energy, Joint Research Centre, European Commission, Petten, The Netherlands 5: Institute of Nuclear Engineering and Science, National Tsing Hua University, Taiwan

For high energy radiotherapy (> 6 MeV), hardon therapy and boron neutron capture therapy, these modern radiotherapies encounter the same challenge that mixed field dosimetry must be considered, including neutrons, gamma rays and other particles. In mixed field dosimetry, the tissue equivalent ionization chamber, denoted as TE(TE), is commonly used to determine the total absorbed dose. The trustiness of the measured dose highly depends on the correctness of the chamber response functions to different incident radiations, which is fundamental to the mixed field dosimetry. Hence, this study aims to first investigate the TE(TE) chamber response function to photons with energies from 20 keV to 20 MeV with regards to the wide photon energy range which mixed fields commonly have. Normally, a chamber response is obtained by using standard sources such as ^60Co and 137Cs. However, it is not practical for high energy machines due to the lack of suitable calibration beams. Thus, Monte Carlo (MC) method becomes important and necessary. Many MC codes have been developed for radiation transport calculation. It is accordingly our interest to study the response functions by using MC method and by different codes. MC codes EGSnrc, MCNP5, FLUKA, and Geant4 were used. To simplify the comparison, the chamber geometry was ideally modeled as a sphere with a radius of 4 cm. The total cell survival levels between the mixed irradiation conditions with 1 and 5 MW, suggesting no significant DDREF in this dose range.

**POS010-01. Mortality Analysis in Chernobyl Clean-up Workers from Latvia.** Jelena Reste¹, N. Kurjane¹, T. Zvagulie², M. Egîtlie³, J. Čirle³, N. Gahruševa¹, 1: Riga Stradins University Institute of Occupational Safety and Environmental Health, Latvia 2: Centre of Occupational and Radiological Medicine, Paula Stradins Clinical University Hospital, Latvia

25 years have passed since Chernobyl nuclear power plant (CNPP) accident. About 6000 of Latvian males, mainly 20-40 years old, participated in accident-clean-up works in 1986-1991 for 3 to 6 months. After returning to Latvia they have been living in non-contaminated area. The aim of our study was to assess mortality indicators by age and cause of death of CNPP clean-up workers from Latvia comparing with general Latvian male population. Materials and methods: Latvian State Register for persons exposed to ionizing radiation in Chernobyl and Latvian State Population Register data were used for calculations. Detailed mortality analysis for time period 1999-2009 was made. Standardized mortality ratio (SMR) and 95% confidence intervals (CI) were estimated using indirect age adjustment. Results: Totally 954 from 5906 CNPP clean-up workers have died from 1988 till 2010. Clean-up workers’ mortality rate gradually increased from 0.2 per 1000 in 1988 to 18.6 per 1000 in 2009. In 2009 in CNPP workers cardiovascular diseases as death cause occupied the first place (43.0% of all deaths), oncological diseases – the second (25.3%) and exogenous reasons – the third (12.7%). About the same order is in general Latvian male population. Total SMR in CNPP clean-up workers in time period 1999-2009 was very similar to Latvian male population (SMR 1.00, 95% CI (0.93-1.07)), but in age group 45-54 years it was higher than in control group (SMR 1.14, 95% CI (1.03-1.27)), otherwise in age group 60-69 years it was lower (SMR 0.71, 95% CI (0.59-0.85)). Mortality due to oncological diseases in CNPP workers was slightly lower (SMR 0.91, 95% CI
(0.76-1.08)), but due to exogenous reasons higher - SMR 1.15, 95% CI (0.96-1.36). Total mortality due to cardiovascular diseases in CNPP workers was slightly lower (SMR 0.94, 95% CI (0.84-1.06)), especially in age group 55-69 years (SMR 0.79, 95% CI (0.66-0.94)), but it was insignificantly higher in age group 40-49 years (SMR 1.21, 95% CI (0.95-1.51)) compared with Latvian male population. Conclusions: Total mortality structure of CNPP clean-up workers in last five years reflects similarity with Latvian male indicators with slight tendency to reduce mortality rates in group 40-54 years that may be explained by early ageing. In age group after 55 years mortality rate is lower that is attributable to regular health observation.

POSI0-02. Searching Better Functional Form to Fit Dose-Risk Function. Yutaka Hamaoka, Keio University, Japan

Poisson like functional form is assumed to fit ERR models. Examination of a A-bomb survivor data indicates mean and variance of incident rate is far apart that cause over dispersion. We found the data has more zeros than Poisson process assumes that leads to application of Negative Binomial model. We found NBID fit better than Poisson model.


It is well know that radiation exposure has increased risk of leukemia incidence among atomic bomb survivors. The purpose of this study is to investigate and update the leukemia incidence for the survivors. During the period 1950 to 2001, a total of 1,215 lympho-hematopoietic malignancies were identified among the Life Span Study (LSS) cohort members in Hiroshima and Nagasaki, Japan. About 40% of these were leukemias and a similar proportion was identified as non-Hodgkin lymphoma. This study applied Poisson regression methods to investigate the excess risks, as well as the shapes of radiation dose response curves, for total leukemia (excluding chronic lymphocytic leukemia and adult T-cell leukemia) and leukemia subtypes. The results indicate that there is a non-linear dose response relationship with total leukemia that varies markedly with time and age at exposure. This non-linearity is largely driven by acute myeloid leukemia (AML). Although the leukemia excess risks generally declined with attained age or time since exposure, there is evidence that the radiation-associated excess leukemia risk, especially for AML, has persisted throughout the follow-up period.

POSI0-04. Radiation risk of lung, liver, bone and connective tissue cancer incidence in Mayak PA workers. Elena Labutina, I. Kuznetsova, N. Koshurnikova, Southern Urals Biophysics Institute, Russian Federation

Results of the first analysis of the lung, liver, bone and connective tissue cancer incidence experience of the Mayak PA workers are presented. For each of these cancer sites radiation risk estimates have been used to estimate risks associated with external exposure up until 31.12.2004. By 31 December 2004 there were 458, 52 and 34 registered cases in lung, liver and bone and connective tissue cancers respectively. The excess relative risk (ERR) model has been used to estimate risks associated with external γ- and internal α- incorporated Pu⁹²³⁹ doses. Lung cancer risks were found to be significantly related to Pu⁹²³⁹ doses, ERR/Gy=9.05 (95% CI: 5.89; 13.46) for males aged 60, with the dose-response best described by a linear model. The ERR/Gy decreased significantly with attained age (p=0.01). For adenocarcinoma cancer the ERR/Gy associated with Pu⁹²³⁹ dose was 10 times greater than for squamous cell carcinoma cancer: 30.42 (95% CI: 15.43; 66.59) versus 2.95 (95% CI: 0.32; 7.84).

For liver cancer the dose-response for Pu⁹²³⁹ doses was best described by a linear-quadratic model, ERR/Gy=2.53 (95% CI: 1.30; 4.82) for all ages, and in a lower dose range ERR/Gy=1.46 (95% CI: 0.60; 3.34) for attained age of less than 55 years. For bone and connective tissue cancer the dose-response model was not studied because lack of variation in Pu dose. For hemangiosarcoma 7 out of 8 cases were diagnosed in workers who had accumulated high α-doses while for cholangiocarcinomas all 6 cases were accounted for by workers who had accumulated a Pu dose of less than 0.5 Gy.

For bone and connective tissue cancer the dose-response model was not studied because of the small number of cases. Among workers not monitored for Pu a significantly high risk was found RR=8.15 (95% CI: 2.20; 26.94) for workers employed in the most hazardous workplaces during the early years of operation of Mayak PA.

Conclusions: We tested the hypothesis that a non-linear dose response best describes the ERR/Gy for both lung and bone cancer. We used a wide range of ERR/Gy values and found that the ERR/Gy value with the highest statistical significance, 9.05 (95% CI: 5.89; 13.46), is riskier than the ERR/Gy value calculated for these cancers in previous studies. We conclude that the ERR/Gy value of 9.05 (95% CI: 5.89; 13.46) should be used in calculations of the radiation risk associated with both lung and bone cancer.

Acknowledgements: Keith Binks and Michael Gillies.

POSI0-05. Effects of lifestyle on the mortality in Nagasaki A-bomb survivors. Mariko Mine, K. YOKOTA, Y. SHIBATA, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Japan

We examined the effects of lifestyle on the mortality in Nagasaki A-bomb survivors on the basis of the mail-survey results collected from 35,035 Nagasaki A-bomb survivors in 2003. In items in the questionnaire included distance of the exposed place from the hypocenter, smoking, drinking, exercise, health checkup and mental health conditions. We dichotomized the distance from the hypocenter: less than 2 km, and 2 km or over. Mental health conditions were assessed by 12-item version of General Health Questionnaire. We excluded from the analysis 21,649 survivors who were aged less than 65 years at the survey, 5,560 who were not directly exposed, and 11 who dropped out leaving no information on the date of dropout during the period from 1 April 2003 to 31 March 2010. Among 20,815 remained, we further excluded 7,764 whose responses were incomplete. In 13,051 survivors finally remaining for the analysis, 2,555 died from 1 April 2003 through 31 March 2010: 908 died from cancer, 680 from circulatory system diseases and 967 from other diseases. We analyzed the effects of smoking, drinking, exercise, health checkup and mental health conditions on mortality separately using Cox proportional model after adjusting for sex, age and the dichotomized distance from the hypocenter. In the comparison of survivors without and with exercise, the hazard of death from cancer in the former group was 1.35-fold of that in the latter group (95% confidence interval: 1.18-1.55), the hazard of death from circulatory system in the former group was 1.68-fold of that in the latter group (1.42-1.97), and the hazard of death from other causes in the former group was 1.69-fold of that in the latter group (1.48-1.94). Similarly, in the comparison of survivors without and with health checkup, the hazard of death from cancer in the former group was 1.25-fold of that in the latter group (1.01-1.53), the hazard of death from circulatory system in the former group was 1.60-fold of that in the latter group (1.30-1.96), and the hazard of death from other causes in the former group was 1.39-fold of that in the latter group (1.16-1.66). Effects of drinking, smoking and mental health conditions showed a similar tendency, respectively. More detailed survey is needed to confirm the present results.

POSI0-06. Effect modification by smoking status on radiation effect for stomach cancer mortality among atomic bomb survivors. Ritsu Sakata, Y. Shimizu, E. J. Grant, H. Sugiyama, M. Soda, A. Suyama, K. Ozasa, Radiation Effects Research Foundation, Japan

[Background] Stomach cancer has been independently associated with both radiation as well as smoking in the Life Span Study (LSS) cohort. However, a joint analysis has not been performed. Ignoring the possibility of confounding and effect modification by smoking status could cause misleading inferences on the radiation risk on stomach cancer among A-bomb survivors.

[Objectives] In this study, we assessed the risk of stomach cancer death associated with radiation exposure while adjusting for cigarette smoking status. We also examined possible effect modification by smoking status for the radiation risk on stomach cancer mortality.

[Methods] The study involved 113,263 LSS cohort members with estimated radiation doses from the DS02 dosimetry system of whom, 55,413 replied to a question about smoking status in any of four mail surveys (1965, 1969, 1978, and 1991). Follow-up continued through December 31, 2005. Smoking status and alcohol drinking status were treated as time-dependent variables. Excess relative risk (ERR) and Excess absolute rate (EAR) estimates were obtained using Poisson regression adjusted for city, age at the time of the bombing, attained age, location at the time of the bombing, alcohol drinking status and smoking status.
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[Results] During the follow-up period 3,915 stomach cancer death cases were confirmed. ERR per 1 Gy of stomach cancer mortality was higher for females (ERR/Gy=0.51, 95% CI 0.23 – 0.85) compared to males (ERR/Gy=0.07, 95% CI -0.18 – 0.27). These ERRs did not change after adjusting for sex-specific cigarette smoking status. A sub-analysis tested whether the radiation risk estimates were modified by smoking status. ERR/Gy and EAR/1000-person-years•Gy point estimates among males were higher among non-smokers compared to those among smokers, however model fits were not significantly improved by likelihood ratio tests. Estimates were unchanged for females.

[Conclusions] Including smoking and drinking status into the background model showed no changes to the ERR of stomach cancer mortality for either male or female. However, including a term for effect modification by smoking status among male showed higher risks for non-smokers compared with current smokers. The small radiation ERR observed among men is likely influenced by the high smoking prevalence among men.


Objective: The aim of this study is to quantify the influence of radon exposure on the lung cancer mortality risk.

Introduction: In the former German Democratic Republic, the WISMUT Company conducted extensive uranium mining from 1946 and 1990. Most of the employees had been exposed to uranium ore dust and radon and its progeny. It is well established that such exposures are associated with an increased risk of lung cancer. In a recent epidemiological study, the lung-cancer mortality in a large cohort of former WISMUT miners has been analyzed by descriptive models and the influence of radon exposure on lung cancer risks has been evaluated (Walsh et al., 2010).

Materials and methods: We will present an alternative approach using a biologically-based two-stage carcinogenesis model quantifying the lung-cancer risk related to the exposure to radon. This mechanistic technique offers insight into radiobiological processes involved in carcinogenesis and allows for extrapolation to the low exposures that are important for present-day radiation protection purposes and the transfer of risk across populations.

The model is applied to a cohort of 58,987 former, male WISMUT employees with known annual exposures. The cohort comprises 2 million person years and 3,016 lung cancer deaths in the follow-up period 1946-2003. Cohort-wide information on smoking is limited and the calendar-year dependence of tobacco smoke exposure was therefore implicitly accounted for by a birth-cohort effect.

Results: The results of the largest uranium miner cohort analyzed by a mechanistic model to date and we will show preliminary results of the observed and expected lung-cancer mortality among WISMUT miners and present risk calculations associated with the exposure to radon. The results will be compared with the epidemiological risk assessment conducted by Walsh and coworkers. Finally, we will estimate the excess relative risk associated with the exposure to residential radon by extrapolation to low exposures.

POS10-08. Knowledge of and attitude to nuclear power among residents around Tianwan Nuclear power plant in Jiangsu of China, Furui Wang, N. Yu, Y. Zhang, J. Wang, X. Cao, X. Fan, X. Xu, X. Zhou, JiangSu Provincial Center for Disease Prevention and Control, China

Introduction: As a high quality clean energy, nuclear power plays an increasingly important role in the worldwide electricity production. In China, the country nuclear power generation accounts for only 1.5% in 2002 and is expected to 6% of the total generating capacity. The sitting or operating of a nuclear power plant often faces widespread public opposition. Experience of nuclear power development shows that in addition to technical and economic factors, the public acceptance and attitude to the nuclear power play an important role in the development of nuclear power, while the level of public awareness of nuclear power, which called familiarity is an important impact factor on public acceptance. Although western developed countries had conducted series of nuclear risk perception research from the 1990s, there still lacked systematic research on this area in China. The purpose of this paper is to report a study on the level of knowledge and attitude of nuclear power among Chinese residents around the Tianwan nuclear power plant within a radius of 30km in Jiangsu of China.

Materials and Methods: The study adopted a descriptive, cross-sectional design with self-administered questionnaires to assess the level of knowledge and attitude of nuclear power among Chinese residents. It was conducted through surveyed each participants who lived around the Tianwan in Lianyungang of Jiangsu, a representative city of nuclear power plant in China, in December 2010.

Results and discussion: A total of 1,616 eligible participants were recruited into our study and accepted epidemiological survey. Our investigation has shown that the level of knowledge of nuclear power was generally not high. Integrated the findings of radiation awareness and nuclear power knowledge, we can draw that the public understanding of radiation and nuclear power was obviously insufficient while this lack of understanding existed difference among different characteristics people. In summary, although nuclear energy is an economical, safe and clean energy source, the public concerns about its impact are widespread.

POS10-09. Terrain shielding effects of Nagasaki atomic bombing on cancer mortality. Kenichi Yokota, M. Mine, Y. Shihata, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Japan

On 9 August 1945, the second atomic bomb was detonated at a height of 503 m above Nagasaki city. Since Nagasaki city is hilly and there are two mountains of 366 m and 285 m in height southeast at approximately 2 km from the hypocenter, some people exposed to the atomic bomb over these and other mountains might be shielded. The people exposed to the atomic bomb in the shielded areas should have received less radiation dose than those exposed at the same distance from the hypocenter in the unshielded areas, and hence the cancer mortality will probably be lower in the former than in the latter. To examine the lastly stated hypothesis, we first classified the areas located 2-5 km from the hypocenter into 5 groups using GIS (Geographic Information System) visibility analysis, i.e., shielded and unshielded areas located at 2-3 km from the hypocenter (denoted by S2-3 and U2-3, respectively), and unshielded areas located at 3-4 km (U3-4) and 4-5 km (U4-5). We then compared the cancer mortality among atomic bomb survivors exposed in the respective areas; the comparison was made on the basis of the Cox proportional hazard model after adjusting for sex, age at the bombing, and attained age. Of 37,357 survivors who were exposed at the age of less than 30 years and were living in Nagasaki city on 1 January 1970, those exposed in areas of S2-3, S3-4 and U4-5 were 1663, 2341, 3062 and 3313, respectively. From 1 January 1970 through 31 December 2009, the number (percent) of cancer deaths observed among survivors exposed in areas of S2-3, S3-4 and U4-5 were 108 (11.9%), 202 (8.6%), 435 (8.6%) and 5062 and 3313, respectively. The present study demonstrates the terrain shielding effects and suggests that such effects are similar to those by remoteness from the hypocenter for the distance of 3 km or over.

POS11 Molecular imaging in diagnosis and therapy

POS11-01. 18F-EFS uptake in preclinical tumor models is predictive for post-radiation response. Rehan Ali, S. Apte, M. Vilalta, G. Nelson, E. Graves, Stanford University, USA

AIM: To investigate whether 18F-[2-18F-fluorodeoxyglucose (FDG)] uptake in xenograft human lung tumor models is predictive of post-radiation response.

METHODS: A549 lung carcinoma cells were injected subcutaneously in the left and right shoulders of nude mice. When tumors reached 8mm diameter, mice were injected intraperitoneally with 267 (81G) of 18F-EFS, and imaged using PET/CT after 3 hours post-injection. Tumor EFS uptake was quantified by decay-correcting data, converting units to percent injected-dose-per-gram (%ID/g), drawing...
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manual tumor boundaries, and computing mean tumor:muscle (T/M) ratios. Left-side tumors were irradiated with 20 Gy (n=6) or 7.5 Gy (n=8) the next day, using a Philips RT-250 irradiator. Right-side tumors were not treated (control). Tumors follow-up was done using calipers measurements. Differential equation based models of tumor growth and linear-quadratic (LQ) radiation damage were developed and fitted to the data.

RESULTS: Irradiated tumors were stratified on the basis of intratumoral 18F-FDG uptake, and exhibited a distinct dose dependence on tumor size which was dependent on EF5 levels. The dependence of response on initial tumor size was not significant. Non-irradiated tumors were 1.28x (+/- 0.23) their pre-treatment sizes at 1 month post-treatment. In comparison, 20 Gy-irradiated non-hypoxic (T/M < 2.5) and hypoxic (T/M > 2.5) tumors were 0.67x (+/- 0.17) and 1.09x (+/- 0.37) their pre-treatment sizes, and 7.5 Gy-irradiated non-hypoxic and hypoxic tumors were 0.95x (+/- 0.29) and 1.35x (+/- 0.08) their pre-treatment sizes respectively. A follow-up 18F-EFS PET scan taken 1 month post-treatment showed that the hypoxic regions in non-irradiated tumors became significantly brighter in the second scan (tumor %ID/g increased from 0.75 +/- 0.21 to 1.33 +/- 0.38), whereas treated tumors exhibited smaller increases in %ID/g (0.74 +/- 0.21 to 1.02 +/- 0.12). A model which incorporated an oxygen-dependence for the LQ parameters showed good agreement with the experimental growth curves.

CONCLUSIONS: 18F-EFS PET can predict radiation response in preclinical subcutaneous tumor models. Our imaging data and models allow us to infer additional biological features of, and optimize therapy for irradiated tumors.


It is given an evaluation of the genetic polymorphisms (Hp, Tf, Gc, Fb, PGD, ESd, ACP, PGM1, microsatellite loci CSFP1PO, TPOX, TH01 and F13A01, detecting genes GSTT1, GSTM1 and GSTP1), individual radiosensitivity (by the criterion of ribosome gene fragments) and DNA-damage rate ( Comet-assay) in two cohorts comprised by VNIEF personnel subjected chronically to gamma-neutron ionizing radiation and to B-iradiation of Tatum in comparison with the effects in non-irradiated cohort. The evaluation of radiation type influence on radiosensitivity parameters (nucleotide sequence contents in the extracellular DNA, nuclease activity, and DNA antibody induction rates, all being apoptosis products) showed credible differences between the 3 cohorts in respect to the majority of the analyzed radiosensitivity parameters (11 from 13 studied ones). Evaluating the type influence on DNA damage rate (using the DNA Comet Assay) revealed credible differences between all the three cohorts (P=0.0000) in regard to all the comet parameters, i.e. the DNA concentration percentage in the comet tail, comet tail momentum, and Olive momentum. Five (5) of the 14 analyzed radiosensitivity and DNA damage indicators demonstrated credible dependence on genotype; six (6) of the 18 analyzed gene loci influence credibly on the radiosensitivity and DNA damage rate. Epidemiological analysis showed: - three cohorts differ credibly in the rates of 14 disease groups from the 21 ones; - the genotype influence is credible in respect to the rates of 9 disease groups from the 21 analyzed ones; - the genotype influence is credible in respect to the nine (9) from the 18 analyzed gene loci.

The analysis conducted allowed finding the genotypes and phenotypes, which are credibly different in terms of their radiosensitivity to the natural background, gamma-neutron and tritium radiation. The analysis allowed defining the genotypes and phenotypes, which differ credibly in terms of the association with specific disease risks in the natural, gamma-neutron and tritium radiation environments (the risk genotypes and protective genotypes).

POSI1-03. Tumor Hypoxia and Reoxygenation of Human Tumors Imaged by 18F-misonidazole (F-MISO) Positron Emission Tomography (PET). Yasunasa Nishimura, I. Tachibana, T. Shibata, S. Kanamori, K. Nakamatsu, R. Koske, T. Nishikawa, K. Ishikawa, M. Tamura, M. Hosono, KinKi University Faculty of Medicine, Japan

Purpose: To visualize intratumoral hypoxic areas and its reoxygenation during fractionated radiation therapy (RT) in human tumors, PET/CT using 18F-misonidazole (F-MISO) was performed for patients with various cancers.

Methods: Ten patients including four head and neck cancers, four gastrointestinal cancers, one non-small cell lung cancer, and one uterine body cancer were included. The maximum tumor size ranged from 16 mm to 68 mm. As a protocol, F-MISO PET/CT was performed twice before RT and during fractionated RT of approximately 20 Gy/10 fractions. PET scan was obtained 3 hours after injection of 3.7MBq of F-MISO. F-MISO maximum standardized uptake values (SUVmax) of normal muscles (bilateral neck, back, and buttock muscles) was measured in ten patients. For each study, F-MISO SUVmax values of both primary tumors and metastatic lymph nodes were measured. In addition, the tumor-to-muscle SUV ratios (T/M) of F-MISO SUVmax were also calculated.

Results: In total, 18 F-MISO PET/CT studies for 10 patients were performed. Eight of the 10 patients underwent FMISO PET/CT twice, before RT and during fractionated RT. Mean ± SD of SUVmax of the normal muscles was 1.25 ± 0.17. Based on this data, accumulation of F-MISO above a value of the mean ± 2 standard deviation (SD) (≥ 1.60 SUV) was regarded as indicating a hypoxic area. Except for one patient with stage II nasopharyngeal cancer, F-MISO SUVmax values prior to RT were ≥1.60 in the remaining nine patients. In terms of reoxygenation, seven of the eight tumors, excluding one patient with postoperative recurrent uterine body cancer, showed a decrease in SUVmax and/or T/M ratio after approximately 20 Gy of fractionated RT.

Conclusions: In our F-MISO PET/CT system, accumulation of F-MISO of ≥ 1.60 SUV was regarded as hypoxic areas. Most human tumors (90%) in this small series had hypoxic areas before RT, although reoxygenation was observed for most tumors after approximately 20 Gy of fractionated RT.

POSI1-04. Molecular Imaging of Radiation-Induced Abscopal Effects. Rao Papineni, Carestream Health Inc, USA

A number of distinct radiation response biological events occur in vivo that fail to fit within the framework of the classical radiobiology. These events are recently classified under bystander effects, abscopal effects, and cohort effects. Here, we demonstrate the capabilities of non-invasive near-invasive molecular imaging in determining both radiation induced changes in the bone physiology at the irradiated region and abscopal effects of bones off-target simultaneously. Athymic nude mice were subjected to 0, 5 Gy, and 10 Gy X-ray irradiation focally over the entire right hind limb and subjected to non-invasive near-infrared fluorescence (NIRF) imaging. Molecular probes including NIR dye conjugated bisphosphonate were intravenously injected to determine the radiation-induces changes at the target and off-target sites. We propose that such molecular imaging approaches utilizing different molecular probes can be used to determine the clinically relevant abscopalian effects. With appropriate interventional measures can be designed and reduce morbidity. Also, we will discuss how such approaches can be employed in biodosimetry for radiation disasters or in radiation threat.

POSI1-05. Tumor-specific imaging with radiolabeled human anti-transferrin receptor antibody in pancreatic cancer mouse model. Aya Sugyo1, A. Tsuji1, H. Sudo1, C. Yoshida1, Y. Uki2, G. Kurosawa2, Y. Kurosawa2, T. Saga1, 1: National Institute of Radiological Sciences, Japan 2, Fujita Health University, Japan

Noninvasive imaging with the radiolabeled antibodies specific for various targets of immunotherapy would be useful for selecting appropriate patients for immunotherapy. Transferrin receptor (TIR) is a carrier protein of transferrin and necessary for the import of iron into the cell, and its expression is regulated in response to intracellular iron concentration. TIR has been reported to be overexpressed in many types of cancer including pancreatic cancer and is regarded as an important target of treatment. Recently, we isolated four human monoclonal antibody clones specific for TIR and demonstrated that one clone strongly suppressed tumor growth in the pancreatic cancer mouse model. In this study, we evaluated this monoclonal antibody clone against TIR in vitro and in vivo to develop as a new noninvasive imaging probe. We radiolabeled the antibody with 125I by the chloramine-T method, and with 68Ga or 89Zr using deferoxamine. We conducted in vitro cell binding and competitive inhibition assays using a human pancreatic carcinoma cell line highly expressed TIR and a mouse fibroblast cell line A4, which highly expressed human HER2 but not human TIR. We inoculated MIAPaCa-2 and A4
cells subcutaneously into each side of hind limb and conducted positron emission tomography (PET) imaging from one to six days postinjection of the $^{185}$Zr-labeled antibody. The cell binding assay showed that radiolabeled antibodies specifically bound to MIAPca-2 cells, but not to A4 cells. The Bmax of $^{125}$I- $^{186}$Ga- and $^{90}$Zr-labeled antibodies was 69, 86 and 64 %, respectively. The competitive inhibition assay showed that Kd of $^{125}$I- and $^{186}$Ga-labeled antibodies was 15 and 16 nM, respectively. Temporal PET imaging with the $^{185}$Zr-labeled antibody showed that the MIAPca-2 tumor was readily visualized on day 1 postinjection and became clearer thereafter, and the uptake in the MIAPca-2 tumor increased with time and reached 24.8% of injected dose per gram on day 6, whereas that in the A4 tumor was low and decreased with time (10.7% on day 1 to 8.7% on day 6). In conclusion, the radiolabeled anti-TfR antibody could be applicable for TIR-specific PET imaging and help in selecting appropriate patients for TIR-targeted treatments.

**POSI1-06. Novel EPRI imaging to visualize cycling hypoxia associated with a defect of vascular integrity in transplanted tumors**, Hironobu Yassui,1, 2; Yuki,1, 3; Matsunaga, 2; J. B. Mitchell, 2; M. C. Krishna, 1: Graduate School of Veterinary Medicine, Hokkaido University, Japan 2: Radiation Biology Branch, Center for Cancer Research, NC/NIH, USA

The fluctuation of blood flow is one of tumor phenotype, which influences oxygen concentration and subsequently invokes lack of cancer treatment efficacy. Both to improve therapeutic planning effectively against solid tumor and to make precise prognosis in cancer treatment, noninvasive dynamic imaging of spatial and temporal distributions of oxygen density is needed. Here, we present the novel EPRI imaging method to directly monitor fluctuations oxygenation i.e. cycling hypoxia in murine squamous cell carcinoma (SCCVII) and human colorectal carcinoma (HT29) tumors. A common resonator platform for both EPRI and magnetic resonance imaging (MRI) provided pO$_2$ maps with anatomical guidance and microvessel density without positional movement. Oxygen images every 3 min in pO$_2$ are dependent on tumor size and tumor type. The magnitude of fluctuations in pO$_2$ in SCCVII tumors ranges between 2- to 18-fold, whereas the fluctuations in HT29 xenografts were of lower magnitude. Alternating breathing cycles with air or carboxin (95% O$_2$ plus 5% CO$_2$) distinguished higher and lower sensitivity regions, which responded to carbogen, corresponding to cycling hypoxia and chronic hypoxia, respectively. Immunohistochemical analysis for CD31 and αSMA revealed that cycling hypoxia correlated with pericyte density rather than vascular density in the tumor. In conclusion, this EPRI technique, combined with MRI may offer a powerful clinical tool to noninvasively detect variable hypoxic status in tumors.

**POSI2 Hadrontherapy**

**POSI2-01. Acellinity of Two Quantum Annihilation Radiation of Positrons: A Model Suited for the Monte Carlo Method**, Till Böhlen, 1; A. Ferrari, 2; V. Patra, 1; CERN/KI/SL, Switzerland 2: CERN 3: University Roma, Italy

Electron-positron (e-p) pairs annihilate predominantly by emitting two $511$keV photons with opposite directions in the centre-of-mass frame ($\gamma + \gamma \rightarrow 2\gamma$). However, in the laboratory frame the two emitted annihilation photons are generally not exactly collinear. The slight angular deviation from $180^\circ$ is due to the motion of the annihilating e-p system. The excess of positrons slows down to thermal energy before they annihilate. The motion of the bound electrons is consequently dominating and can lead to deviations from collinearity up to several degrees. The two quantum positron annihilation is the underlying physical process which is made use of by positron emission tomography (PET) to obtain information about the location of positron emitters by assuming that the annihilation reaction occurs on the line of response between two detectors activated by the annihilation photons. The acollinearity of the annihilation photons in the laboratory frame is one of the factors intrinsic to the annihilation process which limits the spatial resolution theoretically achievable with the PET technique. This work proposes a model describing acollinearity of two quantum positron annihilation in a wide range of media which is suited for Monte Carlo calculations. It describes the annihilation at rest based on the independent particle approximation with shell-specific electron velocity distributions (Compton profiles) of unperturbed atoms and in the frame of a free Fermi gas for metals. Omitted e-p correlations which modify the annihilation site probability and the resulting annihilation momentum distribution are introduced additionally by the means of enhancement factors, similar to the ones discussed by Gupta and Siegel (1979) and Alatalo et al. (1995). Predicting the correct angular distribution of two photon annihilation radiation helps to simulate the spatial resolution of PET tomography more realistically. At the same time, it also describes the energy distribution of the emitted photons, the so-called Doppler broadening. Comparisons of the predictions of the model with measurements of Doppler broadening are presented for different media.


Introduction and Purpose: During therapy with carbon beams many neutrons are produced in the patients’ tissues. These neutrons have the potential to induce new secondary cancers and cause other harmful effects. Therefore, if C-ion therapy has to be conducted, all possible ways to reduce the production of secondary neutrons have to be under taken. The only possible way to reduce these neutron doses is to adjust the C-ion energy in a way that have the required penetration with minimum amount of secondary neutron production. We have termed this as the “Compromise Optimum Energy”. Materials and Methods: There is no reliable data on the production of secondary neutrons from patients under bombardment with C-ions, especially at the production site which is most relevant from the radiation- dose point of view. Measurements at some distance away from the source of neutron production are going to give erroneous results due to the absorption and scattering of the neutrons. Using the measured neutron fluence and energy distributions from different materials constituting tissue we have estimated the fluence and energies of these neutrons produced by C-ions of 100-400 MeV/u energies within patients. Results: Our results show that, for a physical treatment dose of 20 Gy in the Bragg Peak, the total fluence of neutrons produced in patients are 1.6 x 10$^{9}$/cm$^2$. 2.5 x 10$^{9}$/cm$^2$ and 4.1 x 10$^{9}$/cm$^2$ respectively at carbon energies of 400, 300, 200 and 100 MeV/u. The doses to different organs due to these neutrons have also been estimated for the organs in the immediate neighborhood of the Bragg Peak, while for organs further away suggestions are made to compute the respective doses. Our graphical data would help the users of carbon- therapy to select their own “Compromise Optimum energy” 

Conclusions: In our opinion the large number of secondary neutrons produced from patients during therapy with carbon ions, and their corresponding doses to various organs, indicate they could have real potential to cause new primary cancers and cause other harmful side-effects in patients. However, we have provided graphical data which would help to minimise this effect and still make use of any advantage the carbon ions might have in therapy.

**POSI2-03. Telomeric status and radioresistance in glioma: a predictive biomarker for steering patients to hadrontherapy**, Sylvain FERRANDON, 1; M. Priscilla, 2; S. Paul, 1; B. Michael, 1, A. Gersenive, 1, H. Ngoc-hanh, 1, R. Claire, 1, P. Delphine, 1, Laboratory of Cellular and Molecular Radiobiology 2: Laboratory of Cellular and Molecular Radiobiology, 3: Laboratory of Cellular and Molecular Radiobiology, France

Glioma is the most common adult brain tumor, accounting for 52% of primary tumors and 20% of all intracranial tumors. Given their aggressiveness and their resistance to treatment, the median survival after standard treatment is of 14 months. Conventional treatment protocols include ablative surgery (if possible) followed by radio-chemotherapy. New therapeutic strategies are needed to improve the poor prognosis and the high risk of relapse. Carbon Ion hadrontherapy is based on the use of carbon ions beam to treat solid tumors. It demonstrates an excellent ballistic (avoiding
damage to the healthy tissue surrounding the tumor) and relative biological effect (RBE) (comparatively to photon irradiation). In this way, the overall survival is significantly increased (by 9 months) when patients are treated by carbon irradiation instead of photon irradiation. It becomes necessary to identify predictive markers of radioresistance of gliomas for referring patients to this new type of radiation.

Telomeric status - Size of telomere (ST) and hTERT expression level (hEL) - modulate the sensitivity to radiotherapy in vitro and in vivo in different tumor types and are prognosis markers of glioma. We propose to investigate whether the ST and the hEL would be good predictors of radioresistance.

We modulated the telomere status in a cell line of intermediate radiosensitivity of glioblastoma (U87MG) by stably transfecting with a plasmid encoding hTERT (catalytic subunit of telomerase) or a control plasmid. We obtained in a same genetic background, two stable cell lines with increasing ST and hEL (Telo 0 Telo + +). A positive correlation appears between 1) the size of telomeres and radioresistances (<0.05) 2) hTERT level and radiosensitivity (p <0.005) when cells are irradiated with photons. These correlations disappear upon irradiation by carbon ions. We also find this correlation between telomere length and the 2Gy survival fraction (photon) on a panel of 10 glioblastoma cell lines (p = 0.049). Thus the telomere status determines response to photon irradiation but does not preclude the response to hadron by carbon ion. These results would support an in vitro evaluation of the ST and hEL on patient biopsy to guide patients to the hadron by carbon ion.

**POSTER 04**

Targeting the main causes of recurrence in Head and Neck Squamous Cell Carcinoma to overcome resistance to carbon ion irradiation. ALPHONSE Gersende1, H. Maître2, B. Anthony1, B. Gerald1, M. Mira3, B. Michael1, F. Claudia1, A. Dominique1, R. Claire1, 1: Department of Cellular and Molecular Radiobiology EMR 3738 Lyon-Sud Medical School, France; Hospices Civils de Lyon, Lyon, France , 2: Department of Cellular and Molecular Radiobiology EMR 3738 Lyon-Sud Medical School, France; Syngeie Lyon Cancer, Lyon France 3: Department of Cellular and Molecular Radiobiology EMR 3738 Lyon-Sud Medical School, France; Syngeie Lyon Cancer, Lyon Lyon France 3: Department of Cellular and Molecular Radiobiology EMR 3738 Lyon-Sud Medical School, France; Hospices Civils de Lyon, Lyon, France

It is now well established that hadrotherapy can offer some potential benefit over conventional radiotherapy. However recent clinical trials have shown that the local treatment of head and neck squamous cell carcinoma (HNSCC) is much less efficient than in other head and neck cancers (Mizoe et al., 2004) and leads to locoregional recurrence. In order to obtain more insight into this recurrence, we studied the mechanisms of cell death in a radioresistant HNSCC cell line (SQ20B). These cells underwent a transient G2/M arrest after exposure to carbon ions which is more pronounced than after photon. Although SQ20B cells showed typical signs of mitotic catastrophe, a subpopulation of cells escaped this process and re-entered the cell cycle.

Two hypotheses were proposed in order to explain this cell regrowth. Firstly, we have investigated whether or not cancer stem cells (CSC) could be involved in this recurrence. After cell sorting, it is confirmed that the SQ20B-CSC were demonstrated to be highly resistant to irradiation and displaying high Reactive Oxygen Species scavenging systems. Secondly, the study of the genomic instability in SQ20B surviving cells showed that both types of irradiation induced chromosomal rearrangements and that the nature of DNA damage (DD) greatly impacted them.

In order to radiosensitize these surviving cells, two pharmacological strategies were investigated. SQ20B-CSC cells were first treated with UCN-01, an inhibitor of the G2/M arrest which resulted in their radiosensitization through the triggering of apoptosis instead of mitotic catastrophe. Another strategy focused on the depletion of the endogenous glutathione content in SQ20B-CSC and non-CSC before irradiation which led to the activation of the intrinsic apoptotic pathway. Moreover, this treatment potentiated the effects of radiation with an increasing number of scattered DD after photon exposure without any consecutive chromosomal changes (CC). By contrast, this treatment increases the complexity of DD and minimizes CC after heavy ion radiation.

Taken together, our results demonstrated that adjuvant therapies targeting CSCs and exploiting the minimization of transmissible CC could optimize the local control of tumors after hadrotherapy.

**POSTER 05**

ANTHROPOLOGICAL DESCRIPTION OF THE RADIAL DISTRIBUTION OF DELTA-RADE DOSE AND ITS EFFECT ON THE OUTCOME OF RADIOTHERAPEUTIC PREDICTIONS IN AMORPHOUS TRACK STRUCTURE MODELS. Leszek Grzanka1, M. Korczy2, P. Olko1, M. Waligorski1, 1: Institute of Nuclear Physics PAN, Poland 2: Institute of Physics 3: The Maria-Sklodowska-Curie Centre of Oncology, Poland

**Purpose:** The Cellular Track Structure (CTS), a radiobiological model of cellular survival, developed by Katz and co-workers, is an efficient tool for studying biological effects of heavy ion beams for various endpoints, such as cellular survival. The central part of this model – the radial dose distribution function (RDD) used in the original model of Katz features special scaling which permits certain analytical expressions to be used in calculating the cell inactivation cross-section. We have investigated the possibility of extending this model by incorporating more accurate RDD formulations and studied the effects of such substitutions on model predictions.

**Materials and methods:** A suitable software library was developed in which the Katz model was implemented. The implementation includes various analytical representations of the RDD (also with options to use different delta-electron range formulations). For radiobiological endpoint calculations a set of published survival curves for normal human cell fibroblasts irradiated in vitro by various heavy ion beams was applied. Radiobiological endpoint calculations were performed with various RDD formulation and those calculated analytically without it were compared via survival calculations at different levels, for various ions over their full range of energies.

**Results:** Radial integration of the RDD should result in the appropriate value of Linear Energy Transfer for the ion considered. We demonstrate which RDD formulations give such LET normalization. Moreover, for RDD distributions which do not follow the original scaling of Katz’s model, we compared model predictions of survival curves calculated using the “direct” and “analytical” (i.e. model-scaled) versions of these amorphous track structure models. The observed differences between these versions occurred mainly for low-energy proton irradiations.

**Conclusions:** Appropriate scaling of the radial-dose distribution function is an important factor when investigating the possibility of its use in the Katz CTS model. Preferable are RDD formulations which exhibit the scaling applied in Katz’s model, which also correctly represent the measured distribution of delta-ray dose around the path of the ion and correct LET normalization.

**POSTER 06**

In vitro enhancement of TMZ-induced glioma cell killing by high-LET radiation. Ilaria Improta1, D. Bettega2, P. Calzolari2, L. Manti1, R. Marchesini2, F. Margaret Perozzoli1, E. Pignoli1, P. Scampoli1, V. Scanziani2, G. Grossi2, 1: INational Institute for Nuclear Physics, Naples Section, Italy, Italy 2: Department of Physics, University of Milan and National Institute for Nuclear Physics, Italy 3: Department of Nuclear Physics, University of Naples Federico II and National Institute for Nuclear Physics, Naples Section, Italy 4: IRCCS Istituto Tumori di Milano, Italy 1: Department of Radiation Oncology and LHEP University of Bern Inselspital CH-3010 Bern, Switzerland 6: Department of Physical Sciences, University of Naples Federico II, National Institute for Nuclear Physics, Naples Section and Centre for Radiation Protection and Health Physics, University of Naples Federico II, Italy

The glioblastoma multiforme (GBM) is the most common primary brain tumour and the most aggressive among malignant gliomas, with less than 10% of patients surviving more than two years after diagnosis. It is associated with poor prognosis, it is frequently localized deeply in the gray matter, shows a high incidence among young people, and it is characterised by intrinsic and hypoxia-induced radioresistance. Hence, GBM is a cancer eligible for hadrotherapy. Promising results on radiosensibilization gliomas have been recently obtained using the chemotherapy drug temozolomide (TMZ), a clinically well tolerated alkylating agent in combination with conventional radiotherapy. Both in vitro and preclinical studies in preclinical have shown that the TMZ, either as an additive or a synergistic activity of TMZ resulting in improved patient survival.

We exposed four human glioblastoma cell lines (LN229, T98G, U87MG and U373MG) to photons and high-LET carbon ions, in combination with, or in the absence of, TMZ aiming to investigate whether drug-induced cytotoxicity could be further enhanced by the expected gain in radiosensitivity resulting from the exposure to accelerated ion beams. In vitro cell killing is measured by clonogenic
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survival assay. Preliminary results seem to indicate that TMZ does increase ion-irradiated cell lethality. Further experiments are underway for measuring the RBE for cell inactivation in combination with TMZ as well as the mode of the observed effect (i.e. additive vs. synergistic).

POS12-07. TRACK STRUCTURE MODEL PREDICTIONS OF THE RBE- AND OER-LET DEPENDENCES IN HSF AND V79 CELL CULTURES. Marta Koczy, L. Grzanka, M. P.R. Waligorski, P. Olkon, Institute of Nuclear Physics Polish Academy of Sciences, Poland

To study track structure effects in cells irradiated by heavy ions we have performed a model analysis of an extensive data set published by Tsuoruka et al. [1] of over 40 survival curves of normal human skin fibroblasts (HSF) cells irradiated in vitro in aerobic conditions by energetic carbon, neon, silicon and iron ions. Additionally we have performed model analysis of the data set published by Furusawa et al. [2] concerning survival curves representing the survival of V79 cells irradiated in vitro by energetic helium, carbon and neon ions in both aerobic and hypoxic conditions. All considered cell cultures were irradiated in track-segment conditions. We have fitted four parameters of the cellular track structure theory (Katz model) [3] representing the survival endpoint in normal human skin fibroblasts and V79 cells irradiated in aerobic or hypoxic conditions. In particular, we modelled the dependence of Relative Biological Effectiveness (RBE) on ion type, LET and cell type. We show the compatibility of m-target formalism with the linear-quadratic approach in the calculated RBE-dose dependences. The Katz-model predictions of the Oxygen Enhancement Ratio (OER) for V79 as a function of LET for different ion species are compared with experimental data published by Furusawa et al. [2]. We discuss all results in the context of applying Katz’s track structure theory to a treatment planning system for carbon ion radiotherapy.

References

POS12-08. Gene expression changes after pulsed and continuous proton irradiation. Dörte Michalski,1 O. Zlobinskaya2, G. Dellinger2, S. Moertl1, V. Hable2, C. Siebenwirth2, O. Zlobinskaya1, P. Olko, Institute of Nuclear Physics Polish Academy of Sciences, Poland

Introduction: One possible application of laser driven accelerators may be radiotherapy of malignant tumors by high energy protons. In conventional proton irradiation the dose is delivered within milliseconds, while the laser-activated protons would be applied within nanoseconds. The actual debate is whether there is a difference in biological terms. The present experiments aimed at elucidating gene expression changes after continuous and pulsed irradiation with 20 MeV protons in vitro and in vivo using real-Time PCR.

Methods: A 3D human skin model (EpidermFTM, MatTek, USA) was used to determine changes of mRNA expression of Gadd45a and Bcl-2. A pulsed proton beam with up to 2 x 1011 protons per pulse was prepared for irradiation at the ion microprobe SNAKE at the 14 MV Munich tandem accelerator. These irradiation experiments with an average pulse dose of 80 mGy and a mean dose rate of 0.5 Gy/min. Investigated biological endpoints were the clonogenic cell survival and residual DNA-double strand breaks (DSB) 24 h post irradiation via γ-HAX-p53BP1 assay.

Conclusion: The measured dose effect curves show no difference in biological effectiveness between laser accelerated ultra short pulse and conventional continuous proton beams in clonogenic cell survival and residual DNA DSB.

The work was supported by the German Ministry of Education and Research (BMBF), grant no. 03ZIK445.

POS12-10. Evaluation of Polymer Gel dosimeter for High Energy X-rays, Electrons, and Protons. Masato Saga, K. Koshida1, K. Matsubara2, E. Yamamoto2, H. Iida3, S. Ueda2, N. Isomura3, K. Kume4 Department of Radiological Technology, Graduate School of Medicine, Kanazawa University, Japan, 1: School of Health Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University, 2: Ishikawa prefectural Central hospital, 3: Kanazawa University Hospital, 4: Department of Particle Beam Therapy, Wakasawan Energy Research Center, Japan

Introduction: In recent years, treatments that allow accurate high dosage radiation therapy to the target while preserving healthy tissues, such as intensity modulated radiation therapy (IMRT), radiosurgery

irrespectively of the irradiation mode, while the other genes were not regulated.

Conclusion: Previous studies showed slightly lower DNA damage after pulsed proton irradiation when analyzing more than one endpoint together. An explanation for this could be the anti-apoptotic function of Bcl-2 in DNA damage-induced apoptosis.


The development of proton and ion acceleration by ultra-high intensity lasers for cancer therapy promises the realisation of compact and economic particle accelerators that can be integrated in already existing clinics. However, particle acceleration by high intensity lasers leads in comparison to the conventional used acceleration technique to ultra short beam pulses, generated with low pulse frequency, that apply a very high pulse dose. Prior to a clinical application the radiobiological consequences of laser accelerated and therewith ultra short pulsed particle beams have to be investigated. For this in vitro dose effect curves have been determined, which required a high power laser system with a stable and reproducible acceleration of protons, precise beam monitoring and the technical ability to apply a prescribed dose to a cell sample and to determine the absolute dose received by the cells.

Reference irradiation were performed at the 150 TW Ti:Sapphire laser system DRACO at HZDR that delivered laser pulses with an energy of 3.5 J, a pulse duration of 30 fs and a frequency of 0.1 Hz. By focusing the laser on a 2 μm thin Ti foil, protons were accelerated from the target rear surface. The generated exponential energy spectrum was limited downwards to 6-20 MeV. An in-house developed integrated dosimetry and cell irradiation system was tested and calibrated, allowing precise dosimetry as well as the exact positioning of each cell sample.

In the present experiment radiosensitive head and neck tumour cells SKBr3 were irradiated with a pulse dose range from 0.5-4 Gy with an average pulse dose of 80 mGy and a mean dose rate of 0.5 Gy/min.

Received reference irradiation was performed with continuous, conventional accelerated 7.2 MeV proton beams at the Tandem accelerator at HZDR with a dose rate of 1.1 Gy/min. The measured dose effect curves show no difference in biological effectiveness between laser accelerated ultra short pulsed and conventional continuous proton beams in clonogenic cell survival and residual DNA DSB.
procedures, and particle radiotherapy, including those using proton and carbon beams, have been performed. The capabilities of ionization chamber dosimeters and dose verification using film are too limited to allow accurate treatment with such a complex dose distribution. Therefore, a method to measures 3D dose distribution directly is required, which has led to the development of gel dosimeters with high radiation sensitivity in an attempt to establish a 3D dose distribution measurement method. One of the detectors, pion, proton gel dosimeter, which is a chemical dosimeter in which gel increases its white turbidity and solidifies depending on the dosage as a result of radical polymerization of a monomer through atomic radiation. Ultimately, the 3D absorbed dose distribution can be estimated based on the $T_2$ value measured by MRI. In this study, dose dependence, dose rate dependence, energy dependence, $PDD$, and time-dependent changes for X-ray, electron, and proton beams were examined. Materials and methods: The polymer gel used in this experiment was BANG gel from MGS Research Inc. BANG gel uses gelatin as a gelling agent, methacrylic acid as a polymerization agent, ascorbic acid as a deoxygenating agent, and copper sulfate as a polymerization modifier. In addition, BANG gel does not use prepared gel, but is a do-it-yourself system dosimeter where the user prepares the gel by adding the deoxygenating agent and polymerization modifier to the gelling agent along with the polymerization agent. Results: The dose dependency of the polymer gel had a coefficient of determination of collinear approximation above 0.9884 throughout this experiment, and was very good in dose regions between 0.5 and 15 Gy for all proton, electron beam, and proton beam irradiation. The transverse relaxation ( $R_2$) value for 122 MU/min tended to be higher than that for 254 MU/min in this experiment. However, the differences were ≤6.7%, with the $R_2$ value for 254 MU/min being higher on some occasions. Thus, there appears to be no dose rate dependency based on the dose rate difference between 122 and 254 MU/min. Using two types of beam (X-ray and electron beams), the $R_2$ values were measured with varying energies of 4 MV, 10 MV, 4 MeV, and 9 MeV. The maximum difference in $R_2$ value depending on the energy was 10%, and in many cases was less than 5%; thus, the differences were very small. The PDD of X-rays measured using the polymer gel dosimeter matched the error within several percent with that measured using the ionization chamber dosimeter. The PDD values of proton beam obtained from the polymer gel dosimeter were lower in areas shallower than SOBP compared to those obtained with the semiconductor detector, and the value at the terminal of SOBP was slightly lower. The PDD for X-ray irradiation measured using the polymer gel dosimeter matched that measured by the ionization chamber dosimeter. The PDD values of proton beam obtained from the polymer gel dosimeter were lower in areas shallower than SOBP compared to those obtained with the semiconductor detector, and the value at the terminal of SOBP was slightly lower. The PDD for X-ray irradiation measured using the polymer gel dosimeter matched that measured by the ionization chamber dosimeter. The PDD values of proton beam obtained from the polymer gel dosimeter were lower in areas shallower than SOBP compared to those obtained with the semiconductor detector, and the value at the terminal of SOBP was slightly lower. The PDD for X-ray irradiation measured using the polymer gel dosimeter matched that measured by the ionization chamber dosimeter. The PDD values of proton beam obtained from the polymer gel dosimeter were lower in areas shallower than SOBP compared to those obtained with the semiconductor detector, and the value at the terminal of SOBP was slightly lower. The PDD for X-ray irradiation measured using the polymer gel dosimeter matched that measured by the ionization chamber dosimeter.
the measurements of DNA base damage and DNA double strand breaks (DSSB) was useful or not. In addition, the protective effects of a radical scavenger edaravone were evaluated using this system. [Materials and Methods]: Salmon sperm DNA (ssDNA) solution and MOLT4 cells were irradiated with 155 MeV PB or 200 keV x-rays. PB irradiation was performed at two different (y) points, i.e., at plateau (P) and at Bragg peak (B). After each irradiation with or without edaravone. 8-OHdG yield was measured by high performance liquid chromatography. DSSB yield was evaluated by agarose gel electrophoresis. DSSB in nuclei of MOLT4 cells were visualized and quantitatively evaluated by immunohistochemical staining of gamma-H2AX foci. [Results]: Production of 8-OHdG in ssDNA solution was significantly higher at plateau than at Bragg peak of proton beams. Agarose gel electrophoresis demonstrated that DSSB was significantly higher at Bragg peak than at plateau. Edaravone reduced the production of 8-OHdG after every irradiation. There were no remarkable differences in gamma-H2AX focus formation in MOLT4 cells at plateau and at Bragg peak. However, edaravone significantly suppressed the appearance of gamma-H2AX focus at plateau. [Conclusions]: For the standardization of proton beam radiotherapy, the fusion of proton microdosimetry and biological outcomes is mandatory. This study demonstrated that a linkage of lineal energy (y) measurement and DNA base damage and DSSB yield could be a promising candidate for such an evaluation system.

POS12-14. Pilot Study for Standardization of RBE Intercomparison of Hadron Therapy Beams in vitro. Akiko Uzawa, B. Vischioni1, S. Koike1, R. Hirayama1, Y. Matsumoto1, C. Tsuruoka1, Y. Furusawa1; 1: NIRS, Japan 2: CNAO Cancer therapy with carbon ions at the HIMAC has started in 1994, more than 5,000 lesions have been treated by 2010, and fruitful results have been demonstrated. Following those results, few heavy-ion treatment facilities are working, some are under construction and many new facilities are planning all over the world now. To start treatments with HIMAC clinical experiences such as treatment planning, the beam quality at those new facilities must be very similar to the HIMAC in physics and biology. Biological results are easy to affected by the biological conditions, techniques or so on. We tried to establish a standard biological protocol for this purpose. Both C3H/He mice head at NIRS (N) and Charles River Co. (C) were employed, and the crypt cell survival assay 3.5 days after the irradiation was performed with whole body single irradiation of carbon 290 MeV/u beam having 6 cm SOBP at HIMAC. Crypt survival data was taken from (N) and (C) mice, by both trained and fresh observers. The radio-sensitivity of the mice crypt was different by the breeders. The Dq values were similar between them (C and N), but the Do for (N) was higher than that for (C). Recognition level of the existing crypts at counting was different by the observers, but this technical difference is acceptable by simple counting. Change in recognition level with elapsed time could not found for an observer within 6 years. Use of mice supplied by worldwide breeder with good quality control may be required for intercomparison of RBE. Short training could unify technique of new observer.

POS12-15. The relative biological effectiveness (RBE) of 20 MeV protons at nanosecond pulse lengths. Olga Zlobinsky1, T. Schmid1, B. Roep1, M. Molls1, D. Michalski1, C. Greube1, V. Hable1, G. Dollinger2, G. Multhoff1; 1: Klinikum rechts der Isar, Technische Universitaet Muenchen, Germany 2: Universitaet der Bundeswehr Muenchen, Germany Purpose: One possible application of laser driven accelerators (LDA) may be radiotherapy for malignant tumours by high energy protons. Tumor irradiation by LDA is expected to differ from conventional irradiation by a dose delivery rate that is increased by ~8 orders of magnitude (10^9 Gy/sec vs. 10 Gy/sec). To prepare its clinical use, we explored the relative biological effectiveness (RBE) of protons with nanosecond pulses in comparison with continuous proton irradiation in cell experiments and tissues. Methods: HeLa cells, A5 cells and blood lymphocytes were used for cell experiments. A 3D human skin model (EpidermFTM) was obtained from MatTek, USA. A pulsed proton beam with up to 10^9 protons per pulse was prepared for irradiation experiments using the ion microprobe SNAKE at the Munich tandem accelerator. Results: Table 1 presents the summary of our data. The RBE for 20 MeV protons at both irradiation modes was calculated using the dose of the reference radiation 70 kV X-rays that produced equal responses. Conclusion: The effectiveness of the pulsed and continuous proton irradiation modes did not differ significantly from one another in single experiments. However, analyzing more than one biological endpoint in a cell, a small but significant lower RBE has been observed for the pulsed irradiation.

Results: Table 1 presents the summary of our data. The RBE for 20 MeV protons at both irradiation modes was calculated using the dose of the reference radiation 70 kV X-rays that produced equal responses. Conclusion: The effectiveness of the pulsed and continuous proton irradiation modes did not differ significantly from one another in single experiments. However, analyzing more than one biological endpoint in a cell, a small but significant lower RBE has been observed for the pulsed irradiation.

Table 1. Summary of experimental results

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Biological system</th>
<th>RBE for continuous PB vs pulsed PB</th>
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</thead>
<tbody>
<tr>
<td>PB irradiation</td>
<td>MOLT4 cells</td>
<td>1.14 ± 0.21</td>
</tr>
<tr>
<td>PB irradiation</td>
<td>C3H/He mice</td>
<td>1.36 ± 0.09</td>
</tr>
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POS13 Interdisciplinary studies

POS13-01. X-ray microbeam line for single cells irradiation. Sebastian Bożek1, J. Bielecki2, A. Wieczorek3, E. Lipiec3, J. Leki2, Z. Stachura4, W. M. Kwiatek2; 1: Institute of Nuclear Physics PAN, Jagiellonian University Medical College, Poland, 2: Institute of Nuclear Physics PAN, Poland Influence of the ionising radiation on living organisms is well known in the area of high doses [1]. In the area of low doses health consequences are still not-learned. According to the linear extrapolation model, ionising radiation could be harmful in all range of doses, and the probability of negative health effects reveal a linear dependence on the dose. Other hypothesis, known as a radiation hormesis theory [2], propagates that the radiation is unharmful below a threshold dose, or even healthy in the area of small doses. However, influence of low doses of radiation cannot be determined through a "victim" examination, because possible negative consequences (i.e. cancer) are not unique, and moreover could also appear many years after the exposition. The only possibility to assess the risk (or benefit, according to the radiation hormesis theory) is to study the radiation effects at the cellular level, analysing the biological response of cells after irradiation one by one with an exact dose. This microdosimetry research could be realised with a use of a classic wide beam, however the accurate quantitative analysis are possible only with the application of microbeam facilities. An x-ray microbeam facility has been already constructed at the Center of Nuclear Physics PAN, which is dedicated for single live cells irrations [3]. The facility is based on an open type x-ray tube with microfocusing. Radiation is emitted from a spot of about 3 micrometers in diameter into a 120 degrees cone beam. In the cells irradiation experiment a Titanium anode with 4.5 keV K-characteristic energy is applied. The cone beam is focused on the sample with the use of elliptical multilayer mirrors, which are optimized for 4.5 keV energy. Due to the Bragg reflector rule, the radiation reflected from the multilayer is monochromatic, which enable the exact dose calculations. Cells are seeded and irradiated on Petri dishes. The dishes have a 6 mm hole in the central part of the bottom, where the dish material was replaced by a 1.5 um thick Mylar foil [4]. A population of about 10^4 cells in 4 ul medium is seeded on the foil 16-18 hours before the experiments. 20 hours after irradiation, necrotic and apoptotic cells are being visualized under a fluorescent microscope. The poster presents the details of the facility construction and alignment, results of the research on different cell lines and results of the dose numerical simulations. Acknowledgements: Construction of the Krakow X-ray microprobe was supported by the Foundation for Polish Science and Technology (Grant no. 222/FNP/119/005), the European Cooperation in Science and Technology (action MIP061) and the Polish Ministry of Science and Higher Education (Grant no. DPN/N15/COST/2010). Currently, the research is supported by the National Science Centre (Grant no. NN 518 295 540, dedicated by the Polish Ministry of Science and Higher Education). All these institutions are thankfully acknowledged.
Many experimental studies suggest correlation between topological proximity of chromosomes located inside cell nucleus and frequency of interchromosomal translocations observed after ionizing radiation. Additionally, relations between genomic regions, gene activity and relaxation of local chromatin structure are elaborated. In our study we modelled the chromosomal rearrangement in human lymphocyte nuclei by performing computer MC simulations. Based on information of radial distance between the nucleus center and mimicking of local chromosome domains and chromosome territories with respect to DNA content of each chromosome are investigated.

POLYS13-02. Three-dimensional simulations of nucleus architecture. Joanna Deperas-Standylo1, M. Ciesla1, E. Gudowska-Nowak1, 1). Marian Smoluchowski Institute of Physics and Mark Kac Complex Systems Research Center, Jagiellonian University, Kraków, Poland; 2). Laboratory of Information Technologies, Joint Institute for Nuclear Research, Dubna, Russia

Vascular endothelium, a cellular layer lining the inner surface of blood vessels, is an important, metabolic and active organ that regulates cardiovascular functions. Healthy endothelium is essential for proper functioning of the cardiovascular system, while endothelial dysfunction leads to various pathologies and diseases. It was recently shown that the selected pyridinium salts exhibit strong antithrombotic activity mediated by prostacyclin derived from vascular COX-2. The mechanism of action is not fully understood yet, but it is very likely that the interactions of these biologically active cations with endothelial cells is to some extent enhanced through their binding to endogenous glycosaminoglycans (GAG) present commonly on cell surfaces.

Because of their diversity, a large part of those cellular counterparts of those endothelial glycosaminoglycans may be of biological importance. Here we present the results of the pulse radiolysis investigation. The reactivity of pyridinium cations with hydrated electrons in water solution in the presence and absence of GAG was measured. The rates of reactions were slower in the presence of glycosaminoglycans and depended on the kind of pyridinium cations.

POLYS13-03. Interactions of selected pyridinium cations with glycosaminoglycans studied by the pulse radiolysis. Malgorzata Jakubowska, A. Sikora, A. Marcinek, J. Gębicki, Technical University of Lodz, Poland

INTRODUCTION: a recombination between the nuclear body and membrane is essential for the formation of the nuclear import complex and nuclear transport. The nuclear import complex is a dimeric protein complex consisting of two karyopherins (Knap and Knap2) that are able to bind the nuclear localization signal (NLS) of the substrate protein. The karyopherins are involved in the nuclear transport of a wide variety of proteins, including transcription factors, hnRNAs, and ribosomal proteins. The karyopherins are also involved in the nuclear transport of viral proteins, such as HIV-1 and EBV. The karyopherins are also involved in the nuclear transport of mitochondrial proteins, such as the mitochondrial F1-ATPase subunit alpha and the mitochondrial DNA polymerase delta.

METHODS: We used a combination of in vitro and in vivo assays to study the nuclear import function of the karyopherins. In vitro assays included the nuclear import assay, the nuclear export assay, and the nuclear transport assay. In vivo assays included the nuclear transport assay, the nuclear export assay, and the nuclear transport assay.

RESULTS: We found that the karyopherins are involved in the nuclear transport of a wide variety of proteins, including transcription factors, hnRNAs, and ribosomal proteins. The karyopherins are also involved in the nuclear transport of viral proteins, such as HIV-1 and EBV. The karyopherins are also involved in the nuclear transport of mitochondrial proteins, such as the mitochondrial F1-ATPase subunit alpha and the mitochondrial DNA polymerase delta.

CONCLUSION: The karyopherins are involved in the nuclear transport of a wide variety of proteins, including transcription factors, hnRNAs, and ribosomal proteins. The karyopherins are also involved in the nuclear transport of viral proteins, such as HIV-1 and EBV. The karyopherins are also involved in the nuclear transport of mitochondrial proteins, such as the mitochondrial F1-ATPase subunit alpha and the mitochondrial DNA polymerase delta.
The Radiation Injury Treatment Network® (RTIN) is a cooperative effort of the National Marrow Donor Program (NMDP) and The American Society for Blood and Marrow Transplantation (ASBMT). The goals of RTIN are to educate hematologists, oncologists, and stem cell transplant practitioners about their potential involvement in the response to a radiation event and provide relevant treatment expertise in the aftermath of non-therapeutic radiation exposures. Following a radiological or nuclear incident, RTIN centers may be asked to accept patient transfers to their institutions and provide guidance to practitioners caring for victims at other centers. RTIN member centers periodically submit capabilities reports indicating immediate resource availability (24-48 hours). Results from a recent survey of 39 centers include the following data on specific preparedness. On the day of a 100% event of centers could only receive 1-10 patients within their BMT units without changes to their normal procedures. Assuming the maximum utilization of all available facilities, including transfer to affiliated centers, and major alterations of standards of care number of patients that could be received were:

<table>
<thead>
<tr>
<th>number of patients</th>
<th>% of centers</th>
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<tbody>
<tr>
<td>1-10</td>
<td>2.6</td>
</tr>
<tr>
<td>11-50</td>
<td>12.8%</td>
</tr>
<tr>
<td>51-100</td>
<td>25.6</td>
</tr>
<tr>
<td>101-499</td>
<td>41.0</td>
</tr>
<tr>
<td>&gt;500</td>
<td>17.9</td>
</tr>
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</table>

Given the estimates that an improvised nuclear device detonation would lead to casualties in the hundreds of thousands, it is clear that planning and preparation for additional surge capacity for radiological and nuclear events is necessary including a better exploration of what major alterations of standard of care could yield in increasing patient care capacity. An optimal medical response to an improvised nuclear device detonation may only be achieved by a coordinated international response effort.

**POSTER PRESENTATIONS**

Lehn*/France, B Lown*/USA, Fürst HA v Liechtenstein, C Rubbia*/Italy, Bishop D Tutt*/Afr.-Africa (* Nobel Laureate).

**POS13-06. Potential for Surge Capacity in Response to a Radiological or Nuclear Incident: Observations from the Radiation Injury Treatment Network, Joel Ross1, N. Chao1, C. Case Jr2, D. Weinstock3, J. Chute4, D. Weisfert5, R. Krawisz2, J. Wilhauk2, D. Confer1, 1: Duke University, USA 2: National Marrow Donor Program 3: Dana-Farber Cancer Institute 4: University of Minnesota Cancer Center 5: American Society for Blood and Marrow Transplantation 6: University of Kansas Hospital, USA**

**POS13-07. Fully automated interpretation of ionizing radiation-induced gH2AX foci by the pattern-recognition system AKLIDES®, Roswitha Runge1, R. Hiemann1, M. Wendisch1, U. Kasten-Pisula1, K. Storch2, C. Fritz1, G. Wunderlich1, J. Kotzerke1, 1: Carl Gustav Carus Medical School, University of Technology Dresden, Germany 2: Lausitz University of Applied Sciences 3: University Medical Center Hamburg-Eppendorf 4: OncoRay-Center for Radiation Research in Oncology, University of Technology Dresden 5: Medipan GmbH, Dahlewitz, Germany**

**Purpose:** The application of ionizing radiation requires the quantification of induced DNA damage. Assessment of phosphorylated histone H2AX (gH2AX) foci as a measure for double-strand breaks (DSBs) is a common technique. However, visual assessment of gH2AX foci is time consuming and influenced by subjective factors. Here, we compared automated and visual reading of gH2AX foci to identify the interobserver variability and the need for standardization of gH2AX foci assessment.

**Materials and methods:** DSB formation was assessed by detection of gH2AX foci after exposition of thyrocytes (PC 13) to 0-5.0 Gy 131I-Re. Immediately after irradiation cells were washed and fixed for immunostaining. We used the pattern recognition algorithms of the interpretation system AKLIDES® for automated evaluation of gH2AX foci. Manual investigation was performed by three laboratories using five observers.

**Results:** Both manual and automated quantification resulted in increasing focus numbers depending on dose. Comparison of automated reading with visual assessment for five manual observers resulted in a determination coefficient of R² = 0.889. When comparing automated reading with visual assessment results obtained by three observers from one laboratory a higher determination coefficient was found (R² = 0.931). The interobserver variability determined by interscarnite ranges and relative deviation for five manual investigators of three laboratories was 38.4 %.

**POS13-08. Preparation of Calibration Sources for the Measurement of Alpha and Beta Self-absorption Correction Factors for Water Samples, Chloé Blanchon Prigent, VALENTIN1, J. Kratky2, A. Brandl3, 1: Nuclear Engineering Seibersdorf GmbH, Austria 2: Austrian University of Technology, Vienna, Austria 3: Environmental and Radiological Health Sciences, Austria**

Measurement of the radioactivity in water samples is concerned with the total dissolved solids (TDS) in water. Dissolved solids in water samples include soluble salts that yield ions such as sodium (Na⁺), carbonate (CO₃²⁻), calcium (Ca²⁺), sulfate (SO₄²⁻) and chloride (Cl⁻). They are found by evaporating the water samples and then finding the mass of the dry residue. In the present study it is investigated if the proper salt and an appropriate preparation method to produce calibration sources for low level Alpha/Beta counting in water sample measurement. First, the individual behavior of four different kinds of salts - KCl, Na₂SO₄, CaCO₃ and CaCl₂ - was investigated by applying three preparation methods: boiling with sodium nitrate and without adding nitric acid and evaporating/drying under an IR lamp. After having obtained the proper salt and preparation method, Na₂SO₄ and CaCO₃ were chosen to further investigate the self-absorption factor in the residue. In a second step, Na₂SO₄, CaCO₃ and Na₂SO₄·CaCO₃; 1:1 in various masses ranging from 5-200 mg for Alpha- and 5-500 mg for Beta measurements were spiked with the radionuclides 131I-Am and 99Co and then dried by an IR lamp. To obtain sufficient counting statistics, the efficiencies of a low level Alpha/Beta counter (Protean MPC 9604) were determined by measuring these prepared sources for 10 000 counts each. The self-absorption factor was calculated directly from the efficiency. Visual inspection of the residues showed that both, Na₂SO₄ and the mixed salt (Na₂SO₄·CaCO₃; 1:1), presented inhomogeneous surfaces. CaCO₃ appears to be the most reliable source for both radionuclides, with a regressive coefficient for curve fits above 0.999. It can be concluded that the evaporation method is reliable, simple, and reproducible in the production of calibration sources. Due to the homogeneity of the CaCO₃ residue, it can be considered a representative salt in a self-absorption study. The Alpha/Beta efficiency-curves from this study served as self-absorption corrector factors in water sample measurements.


Introduction: Globalization needs new models for international-interdisciplinary cooperation, eg foundation of first international institute for radiation science (IIRS) via network of national selected ones.IARR/IAEA in context of integrative general & special rad. sci., eg rad. physics, chemistry, medicine, etc. are necessary. [ref.] Conception for IIRS discussion: 1: Commissions for IIRS to IARR/IAEA + organizations of rad. sci. - African, American, etc. 2: Philosophical topics to ICRR about radiation - sci theory, ethics, interdisciplinarity, sci. structure (a), intern radiation research programmes (b). 3:Conceptions for intern/European post graduate education in general-interdisciplinary cooperation, eg foundation of intern. sci. – physicists, chemists, biologists, physicians, ecologists, philosophers, etc. 4: Political-financial support of IIRS for

POS513-10. KERALA UNIQUE POSSIBILITIES FOR NOVEL CONCEPTUAL INSIGHTS INTO THE NATURE OF BRAIN DISEASES. Sergey Volovyk1, K. Loganovsky2, D. Bazyka3, R. Kefee4, J. Siedow1, 1: Department of Psychiatry & Behavioral Sciences, Duke University Medical Center, USA 2: Resarch Center for Radiation Medicine, Academy of Medical Sciences of Ukraine 3: Department of Psychiatry & Behavioral Sciences, Duke University Medical Center 4: Department of Biology, Duke University, USA

Immanent Earth (and Space) radiation have been a recurring terrestrial (and extraterrestrial) presence ever since the origin of life, throughout evolution, and part of the natural milieu of man's physiological functioning throughout its existence. Kerala (India) natural background radiation area (singular monazite sands coastal belts with non-uniform distribution of radiation exposure and dense population ~400K (with adjacent control area under normal radiation level) living there during 50 generations; the incidence of schizophrenia and suicide rate are the highest in India and worldwide, etc) ultimately may give an ample unique opportunities to conduct in vivo dose response studies on humans at all stages of life (and brain development), and to investigate gene-environment interactions, mt-DNA, Y-chromosome mutations, and V adaptational
evolutional mutations in pleiotropic genes and possible discovering unknown SNPs (with GWADS), for schizophrenia etiology, etc. Methods: Pertinent Epidemiologic, Dosimetry, Biospecimen, Molecular Biomarkers & Genomics, Specific Cognitive Batteries Element. Generalization/conceptualization of mapping between environmental (radiation) exposure (to quantify effects), molecular (free-radical/redox) biomarkers, gene expression signatures, loss of hemispheric dominance, hippocampal neurogenesis, psychotic symptoms and cognitive impairment relevant domains as pertinent plausible vestibies gives novel opportunities for theoretical and experimental insights into etiology and molecular nature of psychoses and dementia including determination of operational ability/capacity thresholds and global mental health phenomena. Cognitive impairment pertinent domains for natural radiation neurodevelopmental model of schizophrenia, especially impaired language, may be regarded as plausible vestibies of psychosis with respect to environmentally induced brain disorders/mental health phenomena. Environmental (ionizing radiation) determinant is logical, natural necessary complement to socio-economic ones for understanding the global mental health phenomena in Kerala and other HBRA (Mecape, ES, Pocos de Caldas, MG, Brazil, etc) and manmade radiation zones (Chernobyl, Fukushima, etc.).

POS513-11. CONNECTIONS OF RADIATION RESERACH TO ORIGINS OF LIFE. Zhiguen Zagorski, E. Maria Kornacka, Institute of Nuclear Chemistry and Technology, Warszawa, Poland

Radiation research has some unexpected and far reaching connections to other scientific disciplines. One of such relations is to a complex of problems concerning origins of life and early evolutionary phenomena. Studies in that field must take into consideration a higher than present intensity of ionizing radiation on early Earth (from radioactive isotopes) and on all objects in the space (from cosmic radiation), not protected, like meteorites or only slightly protected by an atmosphere, like in the case of present Mars. Our investigations are started from the analysis of radiation induced phenomena on early Earth (eg. by Z.P. Zagorski, Nukleoinika 55, 555, 2010). We have also applied present data on the intensity of ionizing radiation in outer space (eg by Benton C. Clark in Origins of Life and Evolution of Biospheres 51, 185 [2001]) to the analysis of situation of life on objects like asteroids and meteorites. Our experimental approach consists in simulation of radiation conditions in space and obtaining in hours information about thousands of years of the Earth's evolution.

In conclusion, application of radiation research to so called astrobiology helps us to elucidate the origins of life and to better understand and verify the most important hypothesis like panspermia and the mechanisms of evolution by radiation chemistry of the DNA. Our presentation draws attention to the chapter 5 by Z.P. Zagorski “Role of radiation chemistry in the origin of life, early evolution and in transportation through cosmic space” in the monograph Astrobiology: Emergence, Search and Detection of Life, by American Scientific Publishers, USA 2010, pages 97-154.

The project is supported by the Polish Ministry of Science and Higher Education No 365/N and is part of the European COST Action CM0703 (2012).

Tuesday
POS14 DNA repair

POS14-01. DNA-PK phosphorylation in XRCC4 and XLF and its role in DNA double-strand break repair through non-homologous end joining. Makesh Kumar Sharma1, M. Fukuuchi2, S. Inamichi3, Y. Maizumoto4, 1: Department of Zoology, R.L.S. Govt. (PG) College, Kaladera (Jaipur), India; 2: Research Laboratory for Nuclear Reactors, Tokyo Institute of Technology, Tokyo, Japan

In mammals, the most deleterious double strand breaks are repaired either by non-homologous end joining (NHEJ) pathway or by homologous recombination pathway. NHEJ pathway includes Ku70/86, DNA-PKcs. Artemis, XRCC4, DNA Ligase IV and Cernunnos/XLF. In the process of NHEJ, DNA-PK is considered an important or pivotal enzyme; it phosphorylates many of DNA repair proteins. Nevertheless, it has remained to be elucidated which protein, and for what reason, should be phosphorylated by DNA-PK. We have identified four phosphorylation sites in XRCC4 and two phosphorylation sites in XLF in vitro and verified in vivo phosphorylation using phosphorylation specific antibodies against these new sites. By the use of these phosphorylation specific antibodies, we have also observed that these phosphorylation sites in XRCC4 are found to be phosphorylated in living cells in response to ionizing radiation. These phosphorylation sites in the XRC4 are also biologically important in the process of DNA repair, we have mutated these phosphorylation sites into alanine and found that three of these phosphorylation sites might be important for DNA repair function, as loss of them lead to elevated radiosensitivity with deficient DNA repair capability. Altogether, these results would indicate that XRC4 phosphorylation by DNA-PK is an essential event in NHEJ repair pathway of DNA double strand break. To analyze the biological importance of XLF phosphorylation further studies are currently under progress. The results of this study will have implications on cancer radiotherapy, providing us with novel strategy to modify or predict radiosensitivity.

Supported by Grant-in-Aid for Scientific Research from JSPS (JSPS Post Doctoral Fellowship) and MEXT, Japan.

POS14-02. Investigating the interplay of TGFbeta and ATM in the DNA damage response. Jennifer Anderson1, F. Cucinotta2, F. O'Neill1, 1: University of Oxford, UK 2: NASA Lyndon B. Johnson Space Center, USA

It is believed that crosstalk occurs between the ATM and TGFbeta signal transduction pathways. Both pathways are essential for cellular and tissue control responses to ionizing radiation (IR) and aberrant modifications to these pathways are extensive in cancer. We hypothesize that the ATM and TGFbeta signaling pathways are fully induced at high doses of acute low-LET radiation, whereas only partially induced at low doses. The aim is to investigate the effect of radiation quality on modulating the cross-talk between these pathways.
and the consequences for repair of DNA double strand breaks (DSB), the formation of which activates ATM.

In rat fibroblast cells we have previously shown that addition of exogenous of TGFbeta to the low dose gamma- or alpha-radiation triggers intracellular signalling causing translocation of Smad1/2/3 from the cytoplasm to the nucleus. We now shown using immunofluorescence that in human breast epithelial cells (MCF10A) irradiated with either high-LET 28Si heavy ions (150 MeV/n) or low-LET gamma-radiation the level of TGFbeta signal increases 1/2/3 increased by 4 h after irradiation, consistent with perturbation of TGFbeta signaling in human cells. The percentage of MCF10A cells with nuclear Smad 1/2/3 is however decreased on inhibition of ATM, suggesting that cross-talk between the ATM and TGFbeta signal transduction pathways may be independent of radiation quality. Using gammaH2AX foci as a marker for DSB and RAD51 as a marker for homologous recombination, the levels of gammaH2AX and RAD51 foci numbers returned to background levels by 24 h following irradiation with gamma-rays (2 Gy) or 28Si heavy ions (1 Gy) but interestingly they both remain high even 24 h after exposure to alpha particles. These preliminary findings indicated that IR leads to perturbation of TGFbeta levels with potential crosstalk with ATM at the DNA damage level. The differences observed at the DNA damage between 28Si ions/gamma-rays and alpha-particles will be explored in relation to DNA damage complexity and radiation dose.

**POSTER PRESENTATIONS**

**POS14-03. DNA Double-Strand Breaks and Mismatch Repair Efficacy in Lymphocytes as Prognostic Markers for Melanoma Patients.** Dmitry Artamonov1, V. Tronov1, L. Gorbatcheva1, 1: Institute of Biochemical Physics, Russian Federation 2: Institute of Chemical Physics, Russia

Background: Melanoma is characterized by primary or acquired resistance to multiple cytotoxic drugs. It is the urgent aim to predict the response of patients to chemo therapy using non-invasive molecular markers.

Methods: In our work we investigated the response to standard chemotherapy of blood lymphocytes of melanoma patients. DNA single and double strand breaks were determined using comet assay; intracellular levels of marker proteins (APE1, hMSH2, HMLH1) were detected using immunocytochemistry. This parameters allows to characterize two mechanisms of DNA repair (base excision repair, BER and mismatch repair, MMR) which together with apoptosis proneness underlie response of tumor cells to chemotherapy.

Results: We found AP sites and single strand breaks to be the most numerous lesions produced by N-methylated bases in response to chemotherapy. Single strand breaks were formed as intermediates and eliminated through the course of repair of AP sites by BER mechanism. Despite of interindividual variability of BER efficiency in lymphocytes of patients there was no damage in single strand DNA (ssDNA) but low level of gamma-chemotherapy. Less abundant but highly cytotoxic DNA lesion O’-methylguanine (O’meG) induced apoptosis in stimulated lymphocytes. Cell death caused by O’meG adducts is promoted by MMR system by inducing unrepaired double strand breaks in DNA. There was a linear correlation between the level of dsDNA breaks in lymphocytes after the 1-st cycle of chemotherapy and MMR efficiency in them (R=0.89, p=0.0001). We observed a correlation between the level of dsDNA damage after the 1-st cycle of therapy and the response of patients to the full course of therapy: lymphocytes of patients exhibiting stable disease and progressive disease contained less dsDNA damage (less effective MMR) compared with lymphocytes of patients exhibiting partial remission of the disease.

Conclusion: Damage at the level of ssDNA (AP-sites and single strand breaks) and BER mechanism associated with it couldn’t be a good prognostic factor of this protocol of chemotherapy. The level of double strand breaks in DNA after the 1-st cycle of chemotherapy is predictive of clinical outcome.

**POS14-04. DNA double strand break resection occurs in the G1 cell cycle phase upon heavy ion irradiation.** Nicole Averbeck1, M. Herritz2, O. Rivas2, M. Durante2, C. Taucer-Scholz2, 1: GSI Helmholtzentrum for Schwerionenforschung, Germany 2: GSI Helmholtzzentrum für Schwerionenforschung and Department of Condensed Matter Physics, TU Darmstadt, Germany

It is well known that DNA double strand break (DSB) resection occurs as a pre-requisite of the DNA repair pathway of homologous recombination (HR) that takes place in the late S and G2 cell cycle phase. Interestingly, we found that at DSBs induced by heavy ion irradiation resection also takes place in G1. Heavy ions represent ionizing radiation that generates strictly localized DNA lesions including DSBs. Using immunofluorescence microscopy we show that with increasing linear energy transfer (LET) of the ion irradiation applied RPA foci are increasingly found not only at DSBs in G2 but also at DSBs in G1 phase cells. We further reveal that the exocynuclease CipT which promotes resection in G2 is also important for the observed resection in G1 since down regulation of CipT expression by RNA interference decreases RPA foci formation in G2 as well as G1. While in G1-phase lymphocytes gH2AX prevents DSB resection in V(D)J recombination, we did not observe such an influence of gH2AX on resection in mouse fibroblasts upon DSB induction by heavy ion irradiation. Thus, the regulatory role of gH2AX on resection might be unique to V(D)J recombination and might not apply to genomic DSBs. Taken together, since increasing LET causes increasing complexity of DSBs, most likely lesion clustering plays a critical role in the decision of DSB resection in G1. The regulation of G1 resection and the connection to repair pathways is the matter of our ongoing studies.

**POS14-05. Radiation-induced recruitment and phosphorylation of cohesin in human cells.** Christina Bauerschmidt1, M. Woodcock1, D. L. Stevens2, M. A. Hill2, K. Röthkamm2, T. Helleday1, 1: Gray Institute for Radiation Oncology and Biology, University of Oxford, UK 2: Health Protection Agency, Centre for Radiation, Chemical & Environmental Hazards, UK

The cohesion between sister chromatids depends on cohesin, a heterohexameric protein complex. SCMC1 and SMC3 form heterodimers and are joined together by Rad21 which also binds to either SA1 or SA2. We wanted to test whether cohesin is recruited to and phosphorylated at sites of IR-induced DNA damage in human cells. Therefore, HeLa cells were exposed to conventional X-rays or partially shielded ultra-soft X-rays. Immunofluorescence, Western blotting and immunoprecipitation were used to determine relocation and phosphorylation of cohesin subunits following irradiation. RNAi was used to identify factors mediating this response. Recruitment of the cohesin factor Rad21 to sites of X-ray-induced DNA damage was observed in G2-phase cells, but not in G1, and only when DNA damage was concentrated in 1 μm-wide stripes across the nucleus, generated by partially shielded ultra-soft X-rays. SCMC1 and SMC3 were phosphorylated following IR. These phosphorylations occurred at cohesin complexes located at the damaged site throughout the cell cycle and depended on ATM. FRAP experiments show that the irradiation induced phosphorylation of SCMC1 alters its mobility. Individual RNAi knockdown of H2AX, 53BP1 and MDC1 had no effect on the formation of SCMC1pS966 whereas levels of SCMC3pS1083 were reduced to about 50%. Both phosphorylation events were severely reduced when all three damage response mediators were targeted simultaneously. Human cohesin is involved in the cellular response to IR-induced DNA damage and promotes DNA double-strand break repair.

**POS14-06. Searching for rad51 homologue in radiation resistant tardigrades.** Eliana Beltran1, R. Bermúdez2, A. Wojcik3, J. Bernal1, I. Jönsson4, S. Haghdoost4, 1: Universidad Javeriana, Colombia 2: Cnvvestav 3: Jan Kochanowski University 4: Stockholm University, Sweden

Tardigrades can resist extreme conditions as desiccation and ionizing radiation in doses above 1 kGy. It has been reported that tolerance to radiation is similar in both dehydrated and hydrated tardigrades, then it was suggested that rather than preventing biochemical damage, repair mechanisms may be responsible for the observed survival. According to the current hypothesis, the tardigrades must have evolved an effective DNA repair system, but none of their proteins has been characterized. For this reason, the study of the DNA repair systems in tardigrades could aid to understand the radiation resistant mechanism, and also contribute with more information about this important system in the genome integrity maintenance.

We are interested in the characterization of the DNA repair system in tardigrades. Among the numerous proteins known to participate in the DNA repair process, RAD51 is a very important recombinate that is highly conserved. This protein is in charge of promoting strand exchange during homologous recombination, thus maintaining genomic stability.
For searching Rad51 homolog in tadgrides, different sets of primers were designed. A 200-bp PCR product was obtained, cloned and sequenced. The predicted protein sequence was analyzed by BLAST against NCBI database, resulting in 32% and 82% of identity and similarity, respectively with Rad51 proteins. This fragment was used to express a bacterial recombinant peptide that was injected into mice in order to generate a specific polyclonal antibody (ab). This ab allowed us to detect full Rad51 protein in tadgrides by western blot. Our preliminary data reveals that theRad51 levels increase after 45 min of 70 Gy radiation exposure, and they keep this tendency until 3h. As expected, Rad51 levels were upregulated in response to ionizing radiation in tadgrides, this may suggest that homologous recombination could be the immediate response to double strand breaks, at least partly.

**POSTER PRESENTATIONS**

**POS14-07. Structural and biochemical investigation of the irreversible inhibition of Lactococcus lactis Fpg by DNA base analogs.** Artur Biela1, A. Biela1, F. Coste2, J. Ciesla1, B. Tudek1, B. Castang2 1: Centre de Biophysique Moléculaire/Centre National de la Recherche Scientifique, France 2: Centre de Biophysique Moléculaire, CNRS, Orléans, France 3: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The Formamidopyrimidine-DNA glycosylase (Fpg or MutM) is a bifunctional DNA glycosylase/AP lyase base excision repair enzyme. Fpg belongs to the structural H2TH superfamily like the E. coli Nei enzyme and the human Nei-like 1 protein (hNEIL1) [1]. This enzyme displays a broad substrate specificity by excising numerous oxidized base lesions (the mutagenic 8-oxoG, the replication block FapyG, the hydantoins of purines and pyrimidines, and oxidized pyrimidines, such as dihydroxyuracile and the hydantoins of pyrimidines). As reveals by crystal structures of Fpg bound to damaged DNA, the enzyme displays different extruded binding modes of the base lesion inside its substrate binding pocket [2,3].

In previous work, we have identified DNA base analogs that reveal inhibitory properties of the E. coli Fpg protein [4]. As expected, we show that the L. lactis Fpg enzyme is also inhibited by some of these small compounds. The molecular inhibition was decoupled at the atomic level by solving the X-ray crystal structure of the L. lactis Fpg enzyme bound irreversibly to a DNA base thio-analog. As anticipated by the non-competitive behavior of this inhibition, 3D structures identify the target site of the inhibitor in the zinc finger motif of Fpg. This suicide reaction (covalent cross linking of the inhibitor to the enzyme) leads to the loss of the zinc ion abolishing thus the DNA binding properties of the enzyme. Because, most of the DNA glycosylases of the H2TH superfamily contain this DNA binding motif, our finding open the way to target this enzyme class in pharmacologic strategies.


**POS14-08. The changing dose rate: A new factor of importance in radiobiology?** Karl Brehwens, A. Bajinskis, E. Staaf, S. Haghdooost, A. Wojcik, GMT Department, Stockholm University, Sweden

Many factors are known to influence the biological outcome of an absorbed dose of ionizing radiation, such as linear energy transfer, temperature and dose rate. However, very little is known about the effects of changing dose rates, although such exposure scenarios are very common where either the source or the exposed subjects are in motion with respect to another. Consequently, the first aim of this study was to elucidate if there was any difference in biological effect between three cell samples all exposed simultaneously to the same total dose of X-rays, but where one sample was moving towards the source, one sample away from the source, and third sample was stationary in the beam. Cells were exposed either at 37°C or 0.8°C and at both irradiation temperatures the sample moving away from the source sustained the most damage as seen in the form of micronuclei induction. Irradiating at the lower temperature resulted in a similar inter-sample relationship as for the 37°C irradiation, but with the total number of micronuclei being lower. The mechanisms of this changing dose rate effect are not clear. Possibly, cells sense the DNA damage differently when exposed to increasing or decreasing dose rates.

**POS14-09. Conjugated linoleic acid sensitizes human colon cancer HT-29 cells to X-radiation by impairing DNA double strand break repair.** Kamil Brzóska, B. Sochanowicz, I. Grądzka, Institute of Nuclear Chemistry and Technology, Warsaw, Poland

Conjugated linoleic acids (CLA) are natural components of fat in milk and meat of ruminants. Individual CLA isomers may significantly differ in their biological activities. The most abundant isomer present in dietary products, 9c,11t CLA, exerts anticancer, anti-inflammatory, anti-atherosclerotic and anti-diabetic activities. In the present work, we studied the influence of 9c,11t CLA on the induction on the response of human colon cancer HT-29 cells to ionizing radiation. The cells, pre-incubated with 70 µM CLA for 22 h, were X-irradiated with a range of doses from 0-6 Gy. Cell survival and recovery ratio were assessed by clonogenicity test. Rejoining of double strand DNA breaks (DSB) was monitored with the use of pulse-field electrophoresis. Additionally, 2 hours after irradiation, expression of genes involved in DSB repair was measured by real-time PCR. The clonogenicity test showed that CLA sensitized HT-29 cells to X-rays with a dose modification factor, D$_{25}$/D$_{2}$ = 1.55; it also decreased cell recovery ratio from 3.62 to 2.64. In the initial phase of repair (up to 15 min) DSB level was substantially increased upon CLA supplementation. This initial DSB accumulation was not related to any CLA effect on the expression of the 84 genes analysed (e.g. ATM, ATR, PARP1, DNA-PKcs). Nevertheless, DNA dependent protein kinase (DNA-PK) activity in X-irradiated, CLA-supplemented cells was significantly lower than in X-irradiated and non-supplemented cells. Thus, the observed radiosensitizing effect of CLA in HT-29 cells may consist in a transcription-independent reduction of the activity of DNA-PK, the key enzyme in DSB repair in mammals.

Our results show that CLA could be considered as natural compound that may improve effectiveness of radiotherapy. Supported by The Ministry of Science and Higher Education grant no. 494/N-Niemcy/2009/0.

**POS14-10. DNA end resection by Exo1 influences the initial double-strand break repair and damage signaling decisions.** Sandeep Burna1, N. Tomunatsu1, K. Deland2, E. Bolderson3, K. Khanna4, B. Mukherjee1, 1: University of Texas Southwestern Medical Center, USA 2: University of Texas Southwestern Medical Center, USA 3: Queensland Institute of Medical Research, Australia 4: Queensland Institute of Medical Research, Australia

The mammalian DNA damage response (DDR) consists of a vast network of signal transduction cascades that amplify and relay the signal from DNA double-strand breaks (DSBs) to DNA repair, cell cycle arrest, and apoptotic machineries. Three related kinases – ATM, ATR, and DNA-PKcs – are the primary responders to DSBs. ATM and ATR enforce cell cycle checkpoints, while DNA-PKcs is primarily involved in DSB repair. According to the existing paradigm, ATM is activated directly by DSBs, while ATR is activated after resection of DSBs into ssDNA in an ATM/Mre11-dependent manner. We modify the existing paradigm by demonstrating that the activation of an ATM/Mre11-independent pathway for ATR activation and cell cycle checkpoint implementation. In addition, we show that a subset of proteins involved in chromatin remodeling can be phosphorylated in a spatio-temporally restricted manner by DNA-PKcs. The “ATM/Mre11-independent” pathway of ATR activation elucidated by us involves DSB resection by Exo1, a 5’ to 3’ exonuclease [1]. We also show that DSB resection by Exo1 plays a very important role in homologous recombination (HR) [2]. Indeed, the resection of DSBs into ssDNA is increasing assumingly a pivotal role in DDR, possibly regulating the balance between NHEJ and HR repair pathways on one hand, and between ATM- and ATR-driven cell cycle checkpoints on the other. The two-step model of resection, recently elucidated in yeast, posits that initial minimal resection by ATM/Mre11/CtIP is necessary for extensive resection by either Exo1 or BLM/Dn2. By using a combination of biochemical and live-cell imaging techniques, we show that Exo1 is an immediate responder to DSBs and can function independently of ATM/Mre11/CtIP in promoting DSB resection. Importantly, we find that Exo1 is a critical component of a recently elucidated NHEJ to HR switching mechanism involving Brcal and 53BP1. Finally, we show that a transition from ATM- to ATR-mediated cell cycle checkpoint signaling is facilitated by Exo1-dependent DNA resection. In sum, these results identify Exo1 as an independent and critical mediator of DNA repair and damage signaling decisions in response to DSBs. Interestingly, our preliminary results indicate that chromatin remodeling influences DSB resection by Exo1 and this aspect of DDR is currently under investigation.


**POSTER PRESENTATIONS**

**POS14-11.** An association between the XRCC2 and XRCC3 gene polymorphisms and outcome of non-small cell lung cancer patients after radiotherapy. Dorota Butkiewicz1, A. Drosik2, M. Krzeziak1, R. Sawinski1, M. Rusin1, A. Kosarewicz1, J. Rachan1, I. Matuszczyk2, M. Gawkowska-Suwinska1, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw Branch, Poland 1: M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch 3: Centre of Oncology, M. Sklodowska-Curie Memorial Institute, Cracow Branch, Poland

The XRCC2 and XRCC3 proteins belong to DNA double-strand break repair (DSBR) pathway. This type of lesion is induced by ionizing radiation and platinum compounds used in cancer patients treatment. Polymorphisms in genes involved in DNA repair processes are known to affect level of DNA damage, individual repair capacity and cancer risk. They may also modulate a therapy response and prognosis. The purpose of our study was to evaluate a possible impact of the two known DNA-PKcs polymorphisms – XRCC2 -234G>C and XRCC2 -4514A>G - on overall survival (OS) in radiotherapy-treated non-small cell lung cancer (NSCLC).

The study group comprised of 231 patients, mainly with squamous cell carcinoma (SCC) and in advanced stage III-IV. There were 52% of patients treated with a curative intent. Platinum-based chemotherapy was administered to 67% of the cases. DNA was obtained from frozen peripheral blood. SNPs were identified using PCR-RFLP. Kaplan-Meier survival curves were compared by log-rank test. Un- and multivariate Cox proportional regression models were also used.

The XRCC3 -4541 AG+GG combined genotype was significantly associated with better survival in the SCC subgroup, especially among those patients treated with cumulative radiation dose ≥ 60Gy or after chemotherapy. The XRCC2 -234 GC+CC combined genotype significantly correlated with improved OS among adenosccarcinomas, especially when additionally received induction chemotherapy.

Presented results show that selected DNA repair polymorphisms may serve as biomarkers of prognosis in NSCLC patients after irradiation.

**POS14-12.** Repair kinetics of DNA damage by XRCC1 in real time following irradiation of mammanial cells, Sarah Cooper, P. Reynolds, P. O’Neill, M. Lomax, Gray Institute for Radiation Oncology and Biology, UK

Irradiating radiation induces a variety of DNA damage including single strand breaks (SSBs), double strand breaks (DSBs), abasic sites and oxidised lesions. A specific feature of ionising radiation is the formation of clustered DNA damage where two or more lesions form within one to two helical turns of the DNA induced by a single radiation track. The complexity of ionising radiation induced damage increases with increasing ionisation density and it has been shown that complex DNA damage has reduced efficiency of repairability. In mammalian cells, base excision repair (BER) is the predominant pathway for the repair of clustered DNA lesions and is sub-classed as short patch and long patch. The repair of DNA damage induced in EMC11 cells (deficient in XRCC1) by both near infrared (NIR) multiphoton laser microbeam and ultrafast X-rays (USX) has been investigated in real time through recruitment and loss of a key protein in BER, X-ray cross complementing 1 (XRCC1), to sites of damage. USX irradiation is a form of low LET radiation where the damage formed is proposed to be a lot less complex than that induced by NIR multiphoton laser microbeam irradiation. We have found that following USX irradiation XRCC1-YFP is involved in a single repair component with a half life of 3.5 min, in contrast to NIR laser irradiation where XRCC1-YFP is involved in two components of repair, a fast component (half-life of 14.8 min) and a slow component (half-life of 25-45 min), which represent the repair of at least two types of damage.

We have gone on to show that in the presence of a PARP inhibitor less XRCC1 is recruited to damage sites induced by both USX and NIR irradiation. However, the kinetics of recruitment and loss of XRCC1 at these damage sites remain the same as in the absence of the PARP inhibitor. In addition, we have looked at the influence of chromatin compaction/relaxation on the recruitment of XRCC1. Preliminary results using histone deacetylase (HDAC) inhibitors suggest that when chromatin is relaxed, more XRCC1 is recruited to the damage sites following USX irradiation.

In conclusion, XRCC1 is involved in repair of more than one type of damage and the repair kinetics reflect the complexity of the damage, which is largely dependent on the type of radiation. Also, the recruitment of XRCC1 can be affected by inhibiting other proteins involved in DNA repair.

**POS14-13.** Non-linear DNA damage response to low-dose ionizing radiation suggests clustering of DNA breaks in normal human cells. Sylvain Costes1, A. Asaithambey2, T. Neumaier2, A. Polyzos3, C. Pham1, 1: Berkeley Lab, USA 2: University of Texas Southwestern Medical Center, USA 3: Institute for Radiation Protection, Helmholtz Zentrum München, Germany

The number of radiation-induced foci (RIF) measured at any time point following exposure to IR only reflects a net number of RIF: this number does not account neither for RIF that have already been resolved, nor for RIF that have not yet appeared. Novel image analysis algorithms for automatic RIF detection coupled with mathematical modeling allow us to deduce from the measured RIF kinetic the total number of RIF produced for a given dose of IR (cumulated count). Our fits from immunochemistry human specimen data suggest that the number of RIF/Gy at 1 Gy is normalized to dose is not constant, but rather is 2 to 3 fold higher at a low dose of 5 cGy compared to what is measured at a higher dose of 200 cGy of X-rays. In addition, we show that RIF are produced faster and removed more slowly with increasing doses. Similar trends are also observed when depositing very high local doses of IR in the nucleus by using high LET ions. For example, RIF induction is 25 times faster after exposure to 1 GeV/amu Fe ions. Interestingly, damages outside the ion tracks, which are estimated to be equivalent to 17 cGy of X-rays, elicit a RIF kinetic comparable to that of X-rays.

Cumulated RIF counts can only be measured by live cell microscopy. We thus report here live cell imaging of 53BP1-GFP in irradiated human fibrosarcoma cells HT1080 and non-malignant human lung. For both cell types, cumulated RIF counts are 2 to 3 times higher at low dose (40-60 RIF/Gy at 0.1 Gy) than high dose (15-20 cumulated RIF/Gy) at 1 Gy. These live data allow us to validate our mathematical model, as the cumulated counts derived from fitting net RIF kinetic are similar to the experimental values.

Assuming the number of DNA double-strand breaks (DSBs) is proportional to dose, we show with Monte Carlo simulations that these results can be interpreted by the existence of an attractive force between DSBs. Simulations assuming an interaction range between DSBs of 1 mm indicate that the probability of DSB clustering increases as a quadratic function of dose. Consequently, as dose increases, the percentage of RIF made of clustered DSBs increases, leading to faster RIF induction but slower RIF resolution.

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**POS14-14.** Processing of 8-oxo-7, 8-dihydroguanine and 2’deoxyribonolactone when present within a clustered DNA damage site, Siobhan Cunniffe1, V. Shah2, P. O’Neill2, M. Greenberg2, M. Lomax1, 1: Gray Institute for Radiation Oncology and Biology, University of Oxford, UK 2: Organic and Bioorganic Chemistry, Johns Hopkins University, USA

The formation of clustered DNA damage sites is a unique feature of ionizing radiation (IR), defined as two or more lesions formed within one or two helical turns of DNA, from a single radiation track. Due to the proximity of the lesions it has been hypothesised that they could pose a challenge to the repair machinery of the cell, in particular the base excision pathway (BER), leading to the lifetime extension of the lesions within the cluster. Two major lesions formed by IR are 8-oxo-7, 8-dihydroguanine (Go) and 2’deoxyribonolactone (dL, an oxidised abasic site). Oligonucleotides, containing dL and Go positioned one base 5' or 3' to each other in a bistranded cluster, were treated with mammalian cell extracts or purified BER enzymes to assess the efficiency of repair of the dL lesion within the clustered damage site. Limited repair of dL was seen with nuclear extract, unless the extract was supplemented with an excess of FEN1, consistent with the repair of dL following the long patch (LP) pathway of BER. When LP repair is reconstituted using purified proteins, the efficiency of base incorporation by pol β is not affected by an opposing Go during the repair of the dL lesion however its dPRase activity is reduced. Strand
displacement and ligation are retarded when Go is positioned either one base 5' or 3' to dl, compared to when dl is present as a single lesion. An E.coli reporter assay was used to determine the mutation induction of the clustered Go and dl lesions compared to isolated lesions. Mutation frequencies were found to be significantly higher when the lesions were present in a cluster after transformation into wild-type and mutant E.coli backgrounds. These results give insights into how IR damage to DNA is repaired and thus may lead to the exploitation of steps of repair pathways for cancer therapy.

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POS14-15. DNA glycosylase NEIL1 is involved in vivo repair of radiation-induced (5'R)- and (5'S)-8,5'-cyolo-2',3'-deoxyadenosines. Miral Dizdaroglu1, B. C. Nelson1, V. Vartanian2, R. Stepniewski3, J. P. Jurand3, 1: National Institute on Aging, NIH, Bethesda, USA 2: Oregon Health and Science University, USA

POS14-16. Stabilization of TP53 protein in selected DNA repair deficient cell lines by lipid peroxidation product trans-4-hydroxy-2-nonalen (HNE), Dorota Dzuban1, K. Hulka1, A. Winczura1, L. Maddukuri1, P. Kowalczyk1, 1: Institute of Biochemistry and Biophysics, PAS, Poland 2: Institute of Genetics and Biotechnology, University of Warsaw, Poland 3: Institute of Biochemistry and Biophysics, PAS, Poland 4: A. B. Hancock Jr. Memorial Laboratory for Cancer Research 5: Interdisciplinary Center for Mathematical and Computational Modelling, University of Warsaw, Poland

POS14-17. Influence of XPD (codon 312) gene polymorphic variants on the repair kinetics of DNA damages induced by ionizing radiation in HCT116 cell line. Agnieszka Gdowicz-Klosok1, W. Gdowicz-Klosok1, W. Pignoli2, J. Rzeczowska-Wolny2, 1: Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Poland 2: Silesian University of Technology, Katowice, Poland

Introduction: Various cancers are the ultimate consequence of a series of events involving single cells and leading accumulation of mutations in the genome, loss of division and growth control and, finally, to functional disorders and cancerous transformation. During the initiation of carcinogenesis genetic predispositions leading to mutations and gene polymorphisms play crucial roles. It is generally accepted that even slightest changes in the activity of proteins crucial to cell functioning may lead to oncogenesis. Particularly important seem the changes affecting proteins responsible for DNA repair. Many population-level studies have shown that there is a strong correlation between genetic polymorphisms of proteins involved in DNA repair and individual cell reaction to genotoxic factors, and which is manifested by varying levels of endogenous damage observed in donors' lymphocytes, and by different phenotypes of repair.

Materials and Methods: Analysis of the repair kinetics of DNA damage induced by ionizing radiation was performed in HCT116 cell line colorectal tumors. NEIL1 is a DNA glycosylase involved in excision repair (BER) of oxidatively induced DNA damage, exhibiting a strong preference for excision of 4,6-diamino-5-formamidopyrimidine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine with no specificity for 8-hydroxyguanine. R-CdA and S-cdA are repaired by nucleotide excision repair (NER). Since the accumulation of R-cdA and S-cdA in in vivo mice strongly points to the failure of their repair, these data suggest that NEIL1 is involved in NER of these compounds. We also showed that R-cdA and S-cdA exist in human urine, and can be identified and quantified by LC-MS/MS. These compounds had hitherto not been considered or investigated to be present in urine as possible biomarkers of DNA damage. Our data strongly suggest that R-cdA and S-cdA may be well-suited biomarkers for disease processes such as carcinogenesis or used as markers of exposure to ionizing radiation or other sources of oxidative stress, and can be accurately measured by LC-MS/MS in vivo.

Results: Analysis of the functional meaning of XPD 312 gene polymorphic variants revealed that, in the case of repair kinetics of DNA damages induced by ionizing radiation, individual cell reaction strictly depended on particular XPD genotype. In agreement with earlier reports, two different cell subphenotypes could be identified in the HCT116 cell line. The more frequent Asp312Asp genotype was linked to low efficiency of the repair systems whereas Asp312Ser and Ser312Ser showed faster and more effective DNA repair.

POS14-18. Impact of GBM-specific oncogenic events on DNA repair pathways: implications for targeted therapy. Carlos Gil del Alcazar, B. McEllin, B. Mukherjee, K. Amancherla, N. Pichamoorthy, C. Camacho, N. Tomitatsu, D. Boothman, R. Bachoo, S. Burma, UT Southwestern Medical Center, USA

Glioblastoma multiforme (GBMs) are lethal brain tumors that are refractory to radiation and chemotherapy. The only therapeutic regimen that has modestly improved survival of GBM patients is radiation in combination with the DNA alkylating agent temozolomide. Recent mapping of the GBM genome by the Cancer Genome Atlas Network revealed that these cancers commonly exhibit several signature mutations that promote gliomagenesis (EGFR amplification/activation, PTEN loss, p53 loss, Ink4a/Arf loss). How these genetic changes modulate responses to radiation and DNA alkylating agents is not well understood. Using several in vitro and in vivo approaches, we find that key GBM-specific oncogenic events influence non-homologous end joining (NHEJ) and homologous recombination (HR) DNA repair pathways in specific ways. We show that expression of constitutively-active EGFR and III promotes the repair of radiation-induced DNA double-strand breaks (DSBs) by NHEJ, resulting in increased radiosensitivity both in vitro and in orthotopic tumors. Our data indicate that this is due to hyperactivation of the DNA repair enzyme, DNA-PKcs, in an Akt-dependent manner [1]. In contrast, we find that PTEN loss results in increased DNA alkylating agents like temozolomide [2]. We show that these agents induce secondary replication-associated DSBs that are poorly repaired in PTEN-null astrocytes and trigger apoptosis. We further show that this sensitivity is due to impairment of HR, the predominant pathway for the repair of replication-associated DSBs. These newly discovered deleterious signaling and DNA repair pathway relationships suggest that there may be inherent differences in DNA repair capabilities based upon tumor genotypes. This information can help uncover new approaches to target tumors based upon their genomic map. For example, our in vitro results indicate that PTEN-null GBMs (about 36%) may be vulnerable to novel PARP inhibition. These agents are now in clinical trials and show "synthetic lethality" with HR deficient (BRCA1/2 null) tumors. We are currently using orthotopic
POSTER PRESENTATIONS

Conjugated linoleic acids (CLA) are natural components of human diet. Previously, we have shown that the most abundant isomer, 9c,11t CLA, sensitized human colon cancer HT-29 cells to X-radiation. The increase in radiosensitivity was associated with a transcription-independent decrease in DNA-dependent protein kinase (DNA-PK) activity, that resulted in a delay in DNA double-strand break (DSB) rejoining; it was, however, not followed by a significant increase of chromosomal aberration frequencies.

To further explore mechanisms of CLA action, we extended our investigations: We found that the transient increase in DSB levels during repair in CLA supplemented cultures was reflected in DSB repair foci number (histone g-H2AX), monitored immunocytochemically, and in chromatin fragmentation frequencies, measured by premature chromosome condensation. Cell cycle distribution, assessed by flow cytometry, was not affected by CLA. On the contrary, thin layer chromatography combined with gas chromatography analysis has shown that 9c,11t CLA easily incorporated into cellular lipids: triacylglycerides and phospholipids, displacing saturated fatty acids. This, in turn, considerably affected distribution of cholesterol, epidermal growth factor receptor (EGFR) and caveolin-1 in lipid raft fractions, obtained by flotation of the cell homogenates through the Opti-Prep gradient. Moreover, the X-irradiation-stimulated accumulation of EGFR in the nuclei was inhibited in CLA-supplemented cells, as estimated by enzyme-linked immunosorbent assay. As evidenced by other authors (Dittmann K. et al., J.Biol.Chem. 280 (2005):31182-31189), the EGFR import to the nuclei is indispensable to increase the DNA-PK activity in response to ionizing radiation. We hypothesized that the radiosensitizing effect of 9c,11t CLA lies in its ability to modify structure/properties of lipid rafts. The resulting decrease in EGFR migration to the nuclei and loss of its ability to modify structure/properties of lipid rafts. The resulting decrease in EGFR migration to the nuclei and loss of its ability to modify structure/properties of lipid rafts.

Acknowledgment. This work was supported by the Polish Ministry of Science and Higher Education grant, nr 494/N-PNcyi/2009/0.

POSTER PRESENTATIONS

[Results] Mean translocation frequency in mammary epithelial cells was 3.5% at 6-45 weeks after fetal exposures. This value was almost the same as that observed in their mothers (2.9%). On the other hand, clearly lower translocation frequencies (0.6-0.9%) were observed immediately after treatment was the highest with FA and the lowest with T lymphocytes of the rats irradiated as fetuses compared to that of their mothers (3.0-4.0%).

[Conclusion] The results indicated that, following fetal irradiation, mammary glands were found to record cytogenetic damage when examined as adults, and the translocation frequencies were nearly the same as that of their mothers, whereas lymphocytes did not record the chromosome damage as observed in mice. Thus, the results suggest that the lack of translocation dose response following fetal exposure is tissue-dependent.

POSTER PRESENTATIONS

When cells were irradiated with ionizing radiation, proteins are often covalently trapped on DNA, forming DNA-protein crosslinks (DPCs). DPCs are preferentially formed by irradiation under hypoxic conditions. They are also produced by exposure to aldehydes, heavy metal ions, ultraviolet light, and anticancer agents. Since immobilized proteins are much larger than conventional bulky lesions, DPCs may impair repair of DNA-PKc interaction and to inhibit DNA-PK activation in response to radiation was tested. Results from these experiments will be presented.

POSTER PRESENTATIONS

In vitro and in vivo studies of the formation and repair of DNA-protein crosslinks. Hiroshi Ide, M. I. Shoulkamy, M. Oshshima, M. Miyamoto-Matsubara, T. Nakano, Hiroshima University, Graduate School of Science, Japan

In the present study, we treated human MRC5-SV cells with aldehyde compounds, which are known to be potent DPC-inducing agents, and analyzed the formation, stability, and repair of DPCs. Cells were treated with acrolein (ACR), chloroacetaldehyde (CAA), crotonaldehyde (CRA), glutaraldehyde (GA), formaldehyde (FA), and trans-2-pentenal (PEN). According to cell survival, the cell killing efficacy of aldehydes decreased in the order of CAA > ACR > GA > CRA > PEN > FA. Cells were treated with aldehydes at concentrations that gave 10% survival (LD10), and genomic DNA was isolated. The amount of DPCs was measured by the fluorescence labeling and immunological methods. The amount of DPCs immediately after irradiation was the highest with ACR or GA, and the lowest with PEN, but the amounts of DPCs with the two aldehydes differed by only a few fold, suggesting that DPCs produced by the aldehydes inactivate cells with a comparable efficiency.

After aldehyde treatment, DPCs were gradually eliminated from genomic DNA with apparent half-lives between 5-8 h in vivo. We also measured the half-lives of DPCs in vitro using DNA that had been isolated from aldehyde-treated cells. The half-lives in vitro were

POSTER PRESENTATIONS

[Purpose] To provide biological information for radiation response and to develop biological agents for radiation therapy with temozolomide or poly(ADP-ribose) polymerase inhibitors.

Acknowledgment: This work was supported by the Polish Ministry of

POSTER PRESENTATIONS

Glioblastoma multiforme (GBM) is a primary brain tumor with poor prognosis, characterized by an exceptionally high degree of radiosensitivity. The radiosensitivity of GBMs has been attributed to both the presence of glioma tumor stem cells which resist apoptosis and the ability to efficiently repair damaged DNA by the non-homologous end joining pathway (NHEJ). A critical player in the NHEJ pathway is the DNA-dependent protein kinase (DNA-PK). Previous work in our lab has shown that knocking down the protein phosphatase 6 catalytic subunit (PP6c) or its regulatory subunit PP6R1 impairs the radiation induced DNA-PK activation and DNA repair resulting in radiosensitization of GBM tumors in vitro. We hypothesize that the DNA-PKc interaction and expression of a PP6R1 mutant which inhibits the DNA-PKc-Pp6c interaction will prevent radiation induced DNA-PK activation resulting in radiosensitization. To test this hypothesis, various PP6R1 deletion mutants were generated and tested for their binding to DNA-PKc in co-precipitation from transiently transfected cells. A PP6R1 deletion mutant that binds to DNA-PKc, but not PP6c was identified and the ability of this mutant to compete for the DNA-PKc-Pp6c interaction and to inhibit DNA-PK activation in response to radiation was tested. Results from these experiments will be presented.

POSTER PRESENTATIONS

Conjugated linoleic acids (CLA) are natural components of human diet. Previously, we have shown that the most abundant isomer, 9c,11t CLA, sensitized human colon cancer HT-29 cells to X-radiation. The increase in radiosensitivity was associated with a transcription-independent decrease in DNA-dependent protein kinase (DNA-PK) activity, that resulted in a delay in DNA double-strand break (DSB) rejoining; it was, however, not followed by a significant increase of chromosomal aberration frequencies.

To further explore mechanisms of CLA action, we extended our investigations: We found that the transient increase in DSB levels during repair in CLA supplemented cultures was reflected in DSB repair foci number (histone g-H2AX), monitored immunocytochemically, and in chromatin fragmentation frequencies, measured by premature chromosome condensation. Cell cycle distribution, assessed by flow cytometry, was not affected by CLA. On the contrary, thin layer chromatography combined with gas chromatography analysis has shown that 9c,11t CLA easily incorporated into cellular lipids: triacylglycerides and phospholipids, displacing saturated fatty acids. This, in turn, considerably affected distribution of cholesterol, epidermal growth factor receptor (EGFR) and caveolin-1 in lipid raft fractions, obtained by flotation of the cell homogenates through the Opti-Prep gradient. Moreover, the X-irradiation-stimulated accumulation of EGFR in the nuclei was inhibited in CLA-supplemented cells, as estimated by enzyme-linked immunosorbent assay. As evidenced by other authors (Dittmann K. et al., J.Biol.Chem. 280 (2005):31182-31189), the EGFR import to the nuclei is indispensable to increase the DNA-PK activity in response to ionizing radiation. We hypothesized that the radiosensitizing effect of 9c,11t CLA lies in its ability to modify structure/properties of lipid rafts. The resulting decrease in EGFR migration to the nuclei and lowered DNA-PK activity may impair pro-survival signaling in the cell.

Acknowledgment. This work was supported by the Polish Ministry of Science and Higher Education grant, nr 494/N-PNcyi/2009/0.

POSTER PRESENTATIONS

Chromosomal aberration frequency following fetal exposure to ionizing radiation differs by the tissue. Kanya Hamasaki1, M. Nakano1, K. Ohtaki2, Y. Shimada2, M. Nishimura2, M. Yoshida2, A. Nakata2, N. Nakamura2, Y. Kodama1, Radiation Effects Research Foundation, Japan 2: National Institute of Radiological Sciences, Japan 3: Hiroasaki University, Japan

In the present study, we treated human MRC5-SV cells with aldehyde compounds, which are known to be potent DPC-inducing agents, and analyzed the formation, stability, and repair of DPCs. Cells were treated with acrolein (ACR), chloroacetaldehyde (CAA), crotonaldehyde (CRA), glutaraldehyde (GA), formaldehyde (FA), and trans-2-pentenal (PEN). According to cell survival, the cell killing efficacy of aldehydes decreased in the order of CAA > ACR > GA > CRA > PEN > FA. Cells were treated with aldehydes at concentrations that gave 10% survival (LD10), and genomic DNA was isolated. The amount of DPCs was measured by the fluorescence labeling and immunological methods. The amount of DPCs immediately after irradiation was the highest with ACR or GA, and the lowest with PEN, but the amounts of DPCs with the two aldehydes differed by only a few fold, suggesting that DPCs produced by the aldehydes inactivate cells with a comparable efficiency.

After aldehyde treatment, DPCs were gradually eliminated from genomic DNA with apparent half-lives between 5-8 h in vivo. We also measured the half-lives of DPCs in vitro using DNA that had been isolated from aldehyde-treated cells. The half-lives in vitro were
Among various types of DNA damages, DNA double-strand breaks (DSBs) are considered most critical determinant of the fate of the cells or organisms exposed to radiation. DSBs are repaired mainly through two pathways; non-homologous end joining (NHEJ) and homologous recombination. XRC4C association with DNA-LigaseIV is considered one of the proteins. Our observation that NHEJ pathway in Ciona intestinalis is involved in hairpin formation, while also contributing to the evolution of organisms. All DNA repair proteins have the potential to be used as important predictive, prognostic and therapeutic biomarkers in cancer and other diseases. The measurement of the expression level of these enzymes may be an excellent tool for this purpose. Mass spectrometry is becoming the technique of choice for the identification and quantification of proteins. We applied liquid chromatography/isotope-dilution tandem mass spectrometry (LC-MS/MS) for the identification and quantification of DNA repair proteins human 8-oxoguanine-DNA glycosylase (hOGG1) and E. coli formamidopuridine DNA glycosylase (Fpg), which are involved in base-excision repair of DNA damage. We over-expressed, purified and characterized 15N-labeled analogs of these proteins to be used as internal standards. 15N-labeled whole proteins are ideal internal standards to ensure the accuracy of quantification of proteins by mass spectrometry. DNA glycosylase activities of 15N-labeled hOGG1 and 15N-labeled Fpg were determined and found to be essentially identical to those of their respective unlabeled counterparts, indicating that the 15N-labeled proteins do not perturb their catalytic sites. hOGG1, Fpg and their 15N-labeled analogs were digested with trypsin and analyzed by LC-MS/MS. A large number of tryptic peptide fragments expected from the tryptic digestion and provided statistically significant protein scores that would unequivocally identify these proteins. We also recorded the product ion spectra (MS/MS spectra) of the tryptic peptides and defined the characteristic product ions. Mixtures of the analyte proteins and their 15N-labeled analogues as internal standards were analyzed by selected-reaction monitoring (SRM) on the basis of the previously identified product ions in the MS/MS spectra. The results obtained in this work suggest that the methodology developed would be highly suitable for the positive identification and accurate quantification of DNA repair proteins in vivo as potential biomarkers for cancer and other diseases.
Secondly, we conducted nicking assays to determine whether CiNth has a DNA glycosylase activity and trapping assays to determine whether CiNth has an AP lyase activity. We used double-stranded oligonucleotides containing a thymine glycol, 8-oxoguanine or 5-formyluracil as DNA substrates. Our results revealed that CiNth is a bifunctional glycosylase/AP lyase, like human Nth1 and E. coli Nth1.

Finally, we performed a reporter assay to investigate the CiNth expression level at various developmental stages in Ciona intestinalis. We will make a spatial and temporal map of CiNth function during development.

\textbf{POSTER PRESENTATIONS}

\subsection*{POSTER 14-27. The link between MRN complex and RAD51. Akihiro Kato, K. Komatsu, Kyoto University, Japan}

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disorder characterized by growth retardation, immunodeficiency, and cancer predisposition. Cells from NBS patients are hypersensitive to ionizing radiation and display chromosomal instability. NBS patient cells also exhibit the ability to repair DNA double-strand breaks (DSBs) by homologous recombination (HR). The protein product of the gene responsible for NBS, NBS1, is a component of the MRE11/RAD50/NBS1 (MRN) complex. Because the MRN complex has nuclease activity, it has been thought that the MRN complex plays a role in the processing of DSBs, the initial step of DSB repair. This processing step is required for RAD51-staining profiles along with those of wild-type DNA to start DNA strand exchange reactions. However, molecular links between the DNA end-processing step and RAD51-mediated steps remain unclear. In the screening of NBS1-interacting proteins, we found the physical interaction between RAD51 and NBS1, suggesting that NBS1 complex has another role in HR, as well as processing the DNA ends at the initial step. Here, we show the results of domain analysis and functional analysis of this molecular interaction. Roles of the MRN complex in HR will be discussed.

\subsection*{POSTER 14-28. A comparison of the roles of XRCC4 and Artemis in the cellular response to DNA-damaging agents in human cells. Takanori Katsube 1, M. Morl 1, H. Tsuji 1, T. Shiomil 1, N. Shiiomi 2, A. Fujimori 1, M. Onoda 1, 1: National Institute of Radiological Sciences, Japan 2: National Institute of Radiological Sciences, Japan}

DNA double-strand breaks (DSBs) represent the most serious threat among the cellular effects of ionizing radiation (IR). XRCC4 and Artemis play key roles in non-homologous end-joining (NHEJ), the predominant repair pathway of DSBs, in multicellular eukaryotes. XRCC4 is an essential component of DNA ligase IV complex, which repairs broken DNA ends. On the other hand, Artemis is a nuclease required for trimming of some, but not all, types of broken DNA ends prior to rejoining by the DNA ligase IV/XRCC4 complex. To better understand the roles of these factors on risks related to radiation, we examined the roles of Artemis and XRCC4 in the cellular DNA damage response. We studied the repair of the AT-rich sequence-containing cell line HCT116, and assessed their cellular responses to various DNA-damaging agents including X-rays. As anticipated, kinetic analyses of γ-HAX foci and chromosomal aberrations after IR demonstrated a serious incompetence of DSB repair in the XRCC4-deficient cells, and relatively moderate impairment in the Artemis-deficient cells. The XRCC4-deficient cells were highly sensitive to etoposide and 5-fluoro-2'-deoxyuridine as well as IR, and showed moderate sensitivities to camptothecin, methyl methanesulfonate, cisplatin, mitomycin C, aphidicolin and hydroxyurea, compared to the parental HCT116 cells. Meanwhile, consistent with the restricted role of Artemis in NHEJ repair system, the Artemis-deficient cells were not as sensitive to most of these DNA-damaging agents as the XRCC4-deficient cells. Nevertheless, the Artemis-deficient cells were unexpectedly more sensitive to DNA cross-linking agents, such as mitomycin C and cisplatin, than the XRCC4-deficient cells. By contrast, the Artemis-deficient cells were significantly more resistant to hydroxyurea than the parental cells. These observations suggest that Artemis also functions in some DNA damage response pathways other than NHEJ in human cells.

\subsection*{POSTER 14-29. Enhanced repair fidelity under non-cycling conditions in X-ray irradiated fibroblasts accounts for increased potentially lethal damage repair but not in high-LET irradiated fibroblasts. Tetsuya Kawata 1, C. Liu 1, N. Shigematsu 1, K. George 1, H. Tsuji 1, H. Ito 1, K. Forsli 1, R. Furusawa 2, H. Ito 1, K. George 1, E. Cucinotta 1, 1: Radiology, Keio University, Japan 2: National Institute of Radiological Sciences, Japan}

Finally, we compared repair in fibroblasts exposed to X-ray and high-LET irradiated cells that were either held in non-cycling G0 phase or forced to proliferate immediately after irradiation. Fusion premature chromosome condensation (PCC) methods were combined with fluorescence in situ hybridization (FISH) to study chromosomal aberrations in interphase. The rejoining kinetics of PCC breaks was similar for each culture condition in X-ray or high-LET irradiated samples. However, under non-cycling conditions misrepair peaked at 0.55 exchanges per cell 3 hours after exposure, and under cycling conditions a peak of 1.1 exchanges per cell occurred 6 hours after exposure in X-ray irradiated cells. On the other hand, misrejoined breaks were similar for high-LET irradiated samples under either condition. Since the majority of repair in G0/G1 occurs via the non Homologous End Joining process, increased PLDR in X-ray irradiated cells may result from improved cell cycle specific rejoining fidelity of the NHEJ pathway, but not the case in high-LET irradiated cells.

\subsection*{POSTER 14-30. AT-dependent down-regulation of USP7/HAUSP by PPM1G activates p53 response to DNA damage. Svetlana Khronenkovka, I. Duanova, J. Parsons, G. Dianov, Grey Institute for Radiation Oncology and Biology, Department of Oncology, University of Oxford, UK}

The ubiquitylation enzyme USP7 plays a major role in regulating genome stability and cancer prevention by controlling the key proteins involved in the cellular DNA damage response. USP7 regulates cellular sensitivities to camptothecin, mitomycin C, aphidicolin and hydroxyurea, compared to the parenta 1, H. Tsuji 1, T. Shiomil 1, N. Shiiomi 2, A. Fujimori 1, M. Onoda 1, 1: National Institute of Radiological Sciences, Japan 2: National Institute of Radiological Sciences, Japan

\textbf{POSTER 14-31. Effect of ppri mutation on global transcriptional profiles in Deinococcus radiodurans. Dongho Kim, S. Lim, M. Joe, Korea Atomic Energy Research Institute, South Korea}

Ppr1 is known as an important regulatory protein responsible for radiation resistance in Deinococcus radiodurans. In this study, we constructed a D. radiodurans R1 ppr1 disruptant, KDH001, and monitored its global transcriptional profiles along with those of wild-type cells in response to gamma radiation in order to identify genes regulated by Ppr1. When comparing the transcriptionomes from exponentially grown wild type and KDH001 cells without irradiation, mutation in ppr1 affected the expression of 137 genes by more than two fold. Among the up-regulated genes, 118 genes including recA and ddrABCD were reduced while 19 genes including ppr1 and ach showed an increased expression. "Time-course

\textbf{POSTER 14-32. A comparison of the roles of XRCC4 and Artemis in the cellular response to DNA-damaging agents in human cells. Takanori Katsube 1, M. Morl 1, H. Tsuji 1, T. Shiomil 1, N. Shiiomi 2, A. Fujimori 1, M. Onoda 1, 1: National Institute of Radiological Sciences, Japan 2: National Institute of Radiological Sciences, Japan}

 Ultimately, we performed a reporter assay to investigate the CiNth expression level at various developmental stages in Ciona intestinalis. We will make a spatial and temporal map of CiNth function during development.

potentially lethal damage (PLD) and its repair (PLDR) was studied in confluent human fibroblasts by analyzing the kinetics of chromosome break rejoining and misrejoining in X-ray or high-LET irradiated cells that were either held in non-cycling G0 phase or forced to proliferate immediately after irradiation. Fusion premature chromosome condensation (PCC) methods were combined with fluorescence in situ hybridization (FISH) to study chromosomal aberrations in interphase. The rejoining kinetics of PCC breaks was similar for each culture condition in X-ray or high-LET irradiated samples. However, under non-cycling conditions misrepair peaked at 0.55 exchanges per cell 3 hours after exposure, and under cycling conditions a peak of 1.1 exchanges per cell occurred 6 hours after exposure in X-ray irradiated cells. On the other hand, misrejoined breaks were similar for high-LET irradiated samples under either condition. Since the majority of repair in G0/G1 occurs via the non Homologous End Joining process, increased PLDR in X-ray irradiated cells may result from improved cell cycle specific rejoining fidelity of the NHEJ pathway, but not the case in high-LET irradiated cells.

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global gene expression profiling after 30, 60, and 120 min of post-irradiation (2 kGy) showed that 266 genes were differentially expressed by more than two fold in response to the gamma radiation for at least one point in time. Gene expressions of various gene groups were affected by pprI mutation. In particular, the gene group designated as DNA metabolism showed a constant down-regulation indicating a positive regulation by PprI. Additionally, expressions of most DNA damage responsive (ddr) genes were highly affected. The down-regulation of ddABCDN0 was observed at all points in time, whereas ddhFKP showed an up-regulation for at least one point in time. According to the microarray data and qRT-PCR results, pprI mutation triggered a consistent up-regulation. Moreover, the gamma ray radiation increased its expression more than that of the wild type cells. The expression pattern of pprI indicated a down-regulation/partial autoregulation or presence of an upper regulatory protein. Many genes functioning in mobile and extrachromosomal element were up regulated at all points in time, indicating that mutation in pprI creates a stressful condition. Ten genes required for protein synthesis were up regulated after 60 min of post-irradiation, whereas three genes encoding serine protease were down-regulated, indicating the involvement of PprI in protein synthesis for recovery processes. Conclusively, these data defined PprI regulon and support the idea that PprI functions as a master regulator for radiation resistance, particularly in DNA repair.

POS14-32. Nucleolin participates in MDC1-related DNA damage response. Junya Kobayashi, H. Fujimoto, K. Komatsu, Kyoto University Radiation Biology Center, Japan

H2AX is an important factor for chromatin remodeling to facilitate accumulation of DNA damage-related proteins at DNA double-strand break (DSB) sites. In order to further understand the role of H2AX in the DNA damage response (DDR), we attempted to identify H2AX-interacting proteins by proteomics analysis. As a result, we identified nucleolin as one of candidates. Here, we show a novel role of a major nucleolar protein, nucleolin, in DDR. Nucleolin interacted with endogenous gamma-H2AX and accumulated to label micro-irradiated DSB damage sites. Nucleolin-depleted cells exhibited repression of both ATM-dependent phosphorylation following exposure to gamma-ray and subsequent cell cycle checkpoint activation. Furthermore, nucleolin-knockdown reduced HR and NHEJ activity and showed the decreased in IR-induced chromatin accumulation of HR/NHEJ factors, agreeing with the delayed kinetics of gamma-H2AX focus. Moreover, nucleolin-knockdown decreased MDC1-related events such as focus formation of 53BP1, RNF168, phosphorylated ATM, and H2A ubiquitination. Taken together, nucleolin could promote both ATM-dependent cell cycle checkpoint and DSB repair by functioning in an MDC1-related pathway.

POS14-33. FANCd1/BRCA2 plays predominant role in the repair of DNA damage induced by ACNU or TMZ. Natsumi Kondo, A. Takahashi, E. Mori, T. Noda, M. Zdziebienka, L. H. Thompson, T. Helleday, Minoru Suzuki, Y. Kinashi, S. Masunaga, K. Ono, M. Hasegawa, T. Ohnishi, Research Reactor Institutes, Kyoto University, Japan

Nimustine (ACNU) and temozolomide (TMZ) are DNA alkylating agents which are commonly used in chemotherapy for glioblastomas. ACNU is a DNA cross-linking agent and TMZ is a methylating agent. The therapeutic efficacy of these agents is limited by the development of resistance. In this work, the role of the Fanconi anemia (FA) repair pathway for DNA damage induced by ACNU or TMZ was examined. Cultured mouse embryonic fibroblasts were used: FANCa−, FANCc−, FANCa−c−, FANCd2− cells and their parental cells, and Chinese hamster ovary and lung fibroblast cells were used: FANCd1/BRCA2mt/FANCd1 and their parental cells. Cell survival was examined after a 3 h ACNU or TMZ treatment by using colony formation assays. All FA repair pathways were involved in ACNU-induced DNA damage. However, FANCd1 and FANCd1/BRCA2 played notably important roles in the repair of TMZ-induced DNA damage. The most effective molecular target correlating with cellular sensitivity to both ACNU and TMZ was FANCd1/BRCA2. In addition, it was found that FANCd1/BRCA2 small interference RNA efficiently enhanced cellular sensitivity towards ACNU and TMZ in human glioblastoma A172 cells. These findings suggest that the down-regulation of FANCd1/BRCA2 might be an effective strategy to increase cellular chemosensitization towards ACNU and TMZ.

POS14-34. Reduced level and defective protein-protein interaction of DNA-PKcs in a radiosensitive cell line LB5. Susan Loong, S. Fong Yap, C. Shiao Kee Boo, National Cancer Centre Singapore, Singapore

Purpose and objectives: To understand molecular mechanisms underlying radiation necrosis, EBV-immortalized lymphoblastoid cell lines were established from healthy and radiosensitive individuals. Amongst these, L5B was from a patient with cervical carcinoma at age 40 and a recto-vaginal fistula 2 years post-RT. We have previously shown that LB5 was radiosensitive in vitro, with reduced fast-phase DNA-DSB (double-strand break) rejoining, suggesting defective non-homologous end-joining (NHEJ) in these cells. In an in vitro assay using DNA-end joining assay, LB5 cells exhibited higher rates of end-joining of non-cohesive DNA-ends but sequencing of the end-joined products revealed that the process is error-prone with higher frequency of deletions compared to control cells.

Methods and Results: By a gel-based in vitro assay, using EcoRV digested pCDNA3 as substrate, the rate of end-joining by LB5 was greater than for control cell line SNC3. On immuno-deleting LB5 cell free extract, with DNA-PKcs antibody, of the majority of the other known NHEJ factors, the immuno-depleted extract now joined the plasmid DNA ends at a reduced rate compared to itself and to SNC3. However, when the depleted LB5 extract was mixed with SNC3 extract, the end-joining rate was higher than that of SNC3 alone. The expression of the known NHEJ factors in LB5 (by western blotting) was present in the same quantity as SNC3, apart from DNA-PKcs, which was reduced. There was no significant variation in the coding sequences of the cDNA of all the NHEJ factors in LB5 - in particular, the DNA-PKcs sequence was exactly the same as SNC3. We tested the in vitro kinase activity of LB5 using anti-phosphoserine[2056] DNA-PKcs antibody - this was reduced in LB5. To test if there were other phosphorylation defects, we used anti-phospho-threonine and anti-phospho-threonine glutamine/sine glutamine antibodies and found that a phospho-protein of about 50 kDa was not phosphorylated in LB5. Taken together we wondered if the DNA-PK function was reduced in the presence of wild type cDNA sequences because of a defect in protein-protein interactions. Using a known interacting partner as bait, we were able to pull down DNA-PKcs in SNC3 but not LB5. Conclusion: The cellular phenotype of LB5 may be related to a defect in protein-protein interaction of DNA-PKcs.


NHEJ factors rapidly recruit to the DBSs. However, it has not been clarified how pol μ recruits to the DBSs in vivo. We report here the detailed mechanism of pol μ’s recruitment to the DBSs. We first observed pol μ at DBSs in living cells after laser micro-irradiation. pol μ accumulated at DBS immediately after irradiation. By photo-bleaching and photo-conversion techniques, we found that pol μ exchanges between the DBSs and the nucleoplasm within a few seconds. Furthermore, pol μ accumulated at DBSs independently of Ku80. These observations are quite different from those of Ku80-interacting NHEJ core factors, i.e., Ku70, DNA-PKcs (DNA-dependent protein kinase), XRCC4, and XLF (XRCC4-like factor), which Ku80-dependently accumulate at DBSs. Whereas the N-terminal region of pol μ containing BRCT domain, which binds to Ku80, Ku80-dependently accumulated at DBSs, the C-terminal pol β-like region of pol μ Ku80-independently accumulated at DBSs. The pol β-like region of pol μ contains DNA-binding motif BH1 (Helix-hairpin-Helix) and β-NHEJ-interacting motif (PM)-like region. Both of them retained the ability of accumulating at DBSs. Biochemical analyses showed that γ-ray irradiation stimulates pol μ-PCNA interaction and the interaction is sensitive to ethidium bromide, suggesting that dsDNA affects on the pol μ-PCNA interaction. Our studies show that pol μ recruits to DBSs at the first step during the NHEJ process and three functional domains in pol μ individually have abilities to accumulate at DBSs after irradiation.

Radiation-induced damage to genomic DNA can lead to severe errors in transcription and replication, and if not repaired correctly, may lead to mutations, genomic instability, and cell death. Understanding the nature of radiation-induced changes to DNA in tumour cells and identification of potential targets are important in the treatment of cancer by radiation. Near real time dynamics of proteins recruited in response to DNA damage within mammalian cells has previously been investigated using molecular mechanisms such as recruitment however induce a plethora of complex DNA damage (including UV damage, base damage, SSBs and DSBs). We have developed an arrangement based on a 6 MeV electron pulse linear accelerator coupled to an inverted epifluorescence, automated, time-lapse microscope imaging system. An integrated robotic system is used to image remotely and repetitively custom-designed sample dishes between irradiation and imaging locations. This will enable the study of DNA repair kinetics in living mammalian cells under physiological conditions immediately following damage induction with (sub) microsecond electron pulses, where the ionising radiation LET is comparable to that used in the majority of radiotherapy clinics. Following the development of the linear accelerator and associated imaging devices, we have carried out preliminary investigations using 53BP1, an established biomarker of radiation-induced DNA damage. Our arrangement irradiates ~100 mm using a horizontal beam. The dose distribution across the sample is in agreement with depth-dose expectations and correlates with foci formation associated with DNA damage. The use of short, high dose rate, single pulses of radiation coupled with techniques to visualise and follow in real time recruitment of proteins to damage sites, in large numbers of mammalian cells, will contribute to a better understanding of the DNA repair mechanisms under physiological conditions. In the longer term, we envisage this arrangement to complement studies of cell responses to high LET particle beams. This work has been supported by Cancer Research UK (CS255/1A12678 and CS255/A9194) and Medical Research Council (G070300).


DNA is continuously insulted by not only endogenous metabolic products but also low levels of ionizing radiation and a wide variety of chemicals. Low levels of DNA damage does not disturb cell division cycles. Since DNA lesions usually inhibit DNA synthesis, cells have evolved mechanisms to restore stalled replication and complete duplication of damaged DNA. One of such mechanisms is translesion DNA synthesis (TLS), which can extend primer ends by specialized TLS polymerases using damaged DNA as templates. In eukaryotes, RAD6-RAD18 (E2-E3 complex) has an activity to mono-ubiquitinate PCNA. The modification of PCNA stimulates TLS through recruitment of TLS polymerases. However, this process generally has risk of mutations because of lack of fidelity. TLS is used during these DNA repair mechanisms. Under physiological conditions, the mechanism, template switching (TS), could also rescue the stalled replication in which the stalled primer end could be annealed to the newly synthesized daughter strand. This process is stimulated by polyubiquitination of PCNA with additional factors such as MMS2-UBC13 (ubiquitin E2 variant-E2 complex) and HLTF (E3). Since TS is essentially error-free because of utilizations of non-damaged templates and high-fidelity DNA polymerases, regulation of TS pathway seems to be important to prevent mutagenesis and confer damage tolerance. To analyze molecular mechanisms of its regulation, we reconstituted PCNA-ubiquitination reactions in vitro using recombinant human proteins, and show evidence of a novel molecular mechanism for PCNA-ubiquitination.


The purpose of the study was to evaluate a DNA-PK inhibitor designed to sensitize hypoxic cells to ionizing radiation. Hypoxia is a common feature of solid tumours imparting overall resistance to radiotherapy. Hypoxic cells are resistant to DNA-PK inhibition by DNA-dependent protein kinase (DNA-PK), a holoenzyme that is a central component of non-homologous end joining (NHEJ), one mechanisms responsible for repair of DNA double-strand breaks (dsb’s). We have demonstrated that a prodrably activated prodrug that releases a DNA-PK inhibitor. Cell suspensions of human non-small cell lung carcinoma (NSCLC) H460 cells and mouse liver microsomes were used to investigate cofactor requirements, metabolic kinetics and oxygen dependence on prodruk bioreduction. Using the resazurin assay the modulation of radiosensitization of HeLa cells was examined under hypoxic and aerobic conditions. Clonogenic assays with H460 cells were performed to assess cytotoxicity and preclinical pharmacokinetics were studied in C3Hu/HaN mice following single i.p. administrations. Microsomal reduction was enhanced significantly under hypoxic conditions and was inhibited by NADPH. We reduced the compound with maximal release of the DNA-PK inhibitor at <0.2% O2. Clonogenic survival confirmed the compound selectively radiosensitized hypoxic H460 cells. Murine in vivo studies revealed peak plasma concentration occurring 30 min following i.p. administration, allowing co-treatment with a radiosensitizer. Our results suggest that targeting the DNA repair capacity of hypoxic cells is a practical new anticancer drug strategy.

POS14-39. IA-6, an indazole analog of Hycanthone, a more efficient direct inhibitor of Translesion Endonuclease 1 (APE1), than Lucanthone/ Hycanthone, is also less toxic. Manta Nadu, P. Chaudhary, Z. Sanchez, Brookhaven National Laboratory, USA.

IA-6, a des-chloro indazole analog of hycanthone was first synthesized during attempts to segregate Promedonantly, IA-6 has been used as a free base and found to be fast bioavailable. Lucanthone/ Hycanthone at 20-40 µM concentration. Lucanthone and its active metabolite hycanthone are both well known thioxanthene DNA intercalators used in the 1980s as antitumor agents. Lucanthone is in Phase I clinical trial without any reports of drug-related death or lasting side effects, whereas hycanthone was pulled out of Phase II clinical trials due to severe hepatotoxicity. Our studies confirmed that lucanthone inhibitedbase excision repair (BER) enzyme apurinic endonuclease 1 (APE1) without affecting its redox activity. We further showed that this inhibition was due to direct interaction between lucanthone/ Hycanthone and the hydrophobic site of Ape1, which overlaps with active site of its endonuclease activity. Our goal is to decipher this direct mechanism of inhibition and scout for/synthesize other thioxanthene analogues for higher APE1 inhibitory activity with lower toxicity, so their potential as countermeasures for tumor therapy can be elucidated. Our results show that like lucanthone/ Hycanthone, IA-6 is a good radio sensitizer for glioma cell lines, we find that glioma cells show clavage of APE1 in the presence of increasing concentrations of lucanthone/ hycanthone and IA-6. In addition, lucanthone-treated recombinant APE1 also showed peptide cleavage and reduction in its endonuclease activity. Our data also indicate that oxidative damage may be one of the mechanisms of lucanthone/ hycanthone/ IA-6 -induced direct cleavage of APE1 as this cleavage is inhibited by TRIS, DMSO and Ascorbic acid. Finally, this research will help guide future design of clinically efficacious thioxanthene analogues, which would bind and alter the hydrophilic site of APE1 protein.

POS14-40. ChiP-Seq genome-scale analysis of gamma-H2AX induced by X-rays or heavy ions. Francesco Natale*, A. Rapp*, A. Gogol-Doering, W. Chen*, M. Durante*, G. Taucher-Scholz*, M. Cristina Cardoso1, 2; 1: FFAS - Frankfurt Institute for Advanced Scende, Germany; 2: TUD - Technische Universitaet Darmstadt, Germany 3: MDC - Max Delbrueck Center Berlin, Germany 4: GSI - Gesellschaft fur Schwerionenforschung mbH Darmstadt, Germany

After exposure to ionizing radiation (IR), the DNA damage response is activated and the DNA double strand break (DSB) marker phosphorylated histone H2A.X (gamma-H2AX) is produced. Despite gamma-H2AX being extensively studied, the distribution of such histone modification in the context of chromatin density (euchromatin/heterochromatin) remains unclear. Heavy ions are used at GSI as a tool to study DNA repair processes and chromatin structure dynamics. Using next generation sequencing technologies, we aim to provide a genome-scale sequence-based map of gamma-H2AX signature induced by different types of IR (X-rays, heavy ions). Moreover, by multi-parametric characterization (e.g. histone modifications, GWAS, single cell sequencing) we aim to investigate the effects of chromatin density on IR-induced gamma-H2AX distribution and DNA repair kinetics. gamma-H2AX was induced in unsynchronized human hepatocellular carcinoma cells (HeP2) by exposure to 10 Gy X-rays (250 kV, 16m A) and nickel ions (1 GeV/nucleon). Samples were incubated for 0.5 and 3 hours to allow the propagation of gamma-H2AX and the progress of DNA repair, respectively. Chromatin immunoprecipitation
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was performed to select gamma-H2AX-enriched chromatin fractions. gamma-H2AX-chromatin-associated DNA was then sequenced using next generation sequencing technologies (ChIP-Seq) and aligned to a reference genome. The output data was finally subjected to multi-parametric bioinformatic analysis. Results of our genome-scale analysis will be presented and their implications for DNA damage susceptibility and repair kinetics throughout the genome and correlation with chromatin structure will be discussed. Taking advantage of the high throughput next generation sequencing technology, we are able to provide a precise genome-scale sequence-based map of IR-induced gamma-H2AX. Moreover, by multi-parametric characterization of chromatin domains (e.g. various states of compaction), our current investigation provides new insight in chromatin structure dynamics and DNA repair processes.

POS14-41. Comparison of RBE Values of High-LT α-particles for the Induction of DNA-DSBs, Chromosome Aberrations and Cell Reproductive Death. Franken Nicolaas, LexOR, AMC, Netherlands

Introduction: Various types of radiation effects in mammalian cells have been studied with the aim to predict the radiosensitivity of tumours and normal tissues, e.g. DNA double strand breaks (DSB), chromosome aberrations and cell reproductive inactivation. However, variation in correlations with clinical results has reduced general application. An additional type of information is required for the increasing application of high-LT radiation in cancer therapy: the Relative Biological Effectiveness (RBE) for effects in tumours and normal tissues. Relevant information on RBE values might be derived from studies on cells in culture.

Methods: To evaluate relationships between DNA-DSB, chromosome aberrations and the clinically most relevant effect of cell reproductive death, for ionizing radiations of different LET, dose-effect relationships were determined for the induction of these effects in cultured SW-1573 cells irradiated with gamma-rays from a Cs-137 source or with α-particles from an Am-241 source. RBE values were derived for these effects. Ionizing radiation induced foci (IRIF) of DNA repair related proteins, indicative of DSB, were assessed by counting gamma-H2AX foci. Chromosome aberrations were determined by scoring fragments and translocations using premature chromosome condensation. Cell survival was measured by colony formation assay. Analysis of dose-effect relations was based on the linear-quadratic model.

Results: Our results show that, although both investigated radiation types induce similar numbers of IRIF per absorbed dose, only a small fraction of the DSB induced by the low-LT gamma-rays result in chromosome rearrangements and cell reproductive death, while this fraction is considerably enhanced for the high-LT alpha-radiation. Calculated RBE values derived for the linear components of dose-effect relationships. The DNA damage reductive death, chromosome fragments and colour junctions are 1.0 ± 0.3, 14.7± 5.1, 15.3 ± 5.9 and 13.3 ± 6.0 respectively.

Conclusions: These results indicate that RBE values for IRIF (DNA-DSB) induction provide little valid information on other biologically-relevant end points in cells exposed to high-LET radiations. Furthermore, the RBE values for the induction of the two types of chromosome aberrations are similar to those established for cell reproductive death. This suggests that assays of these aberrations might yield relevant information on the biological effectiveness in high-LET radiotherapy.

POS14-42. Inhibition of DNA-PK and its effect on fractionated irradiation of normal and cervical cancer cells. Eva Novotná2, F. Karolína1, L. Emilie, CSC1, V. Jirina, CSC1, R. Martina, PhD2, 1: University of Defence, Faculty of Military Health Science, Czech Republic 2: Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic 3: Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Objective: Radiotherapy has been one of the most effective tools used for treatment of cervical cancer. It appears that differences in radiosensitivity of tumours are often associated with the ability to repair the DSBs (double-stranded breaks). The non-homologous end joining (NHEJ) proteins DNA-PKcs and Ku 70, Ku 80 have a major role in repairing DNA lesions. The aim of this study was to analyze reparative ability of HeLa cells influenced by fractionated irradiation (IR) in the presence of specific inhibitor of DNA-PK, NU 7441. For comparison, we analyzed reparative ability of normal human dermal fibroblasts (NHDF), which are equally exposed to IR during radiotherapy. Methods: HeLa cells and NHDF were irradiated with single doses of 2 and 8 Gy, and with fractionated doses of 2 x 2, 3 x 2, 4 x 2 Gy at intervals of 24 h. Cells were divided into 2 groups: without inhibitor and treated by 1 μmol/l NU 7441. Clonogenic survival assay was carried out and expression of IRIF (ionizing radiation inducing foci) has been evaluated immunocytochemically by visual counting of γH2AX-MDC1 co-localisations. Results: Phosphorylated histone H2AX and protein MDC1 co-localize soon after IR in site of DSB. 1 h after IR the dose of 2 Gy led to production of a large number of small IRIF, which were rapidly repaired 24 h after IR. Repair of DSBs after 2nd, 3rd and 4th dose was slower and, 24 h after IR, we observed more IRIF. In case of addition of NU7441 2 h before IR, we observed increase in number of cells with larger, unpaired IRIF, 24 h after IR as 3rd and 4th dose. This kind of cells do not further divide and die later after IR. Inhibition with NU 7441 sensitized cells to IR much more than IR alone and larger IRIF were observed even after 1st dose of 2 Gy. After 4th dose most of cells died.Conclusions: We conclude that inhibition of DNA-PK with small-molecule inhibitor NU 7441 has radiosensitizing effect on HeLa cells.

POS14-43. Experimental and theoretical study of the induction and repair of DNA damage following acute and split-dose irradiations. Giacomo Maria Pirovano1, V. Giovanni Mariotti1, A. Ottino G. Schettino2, P. Tolas3, Eva Novotná1, R. Martina1, Ph. D.1, 1: Università di Pavia, Dipartimento di Fisica Nucleare e Teorica & INFN, Pavia. Italy 2: Centre for Cancer Research and Cell Biology, Queen’s University, Belfast, UK

The cellular response to radiation is known to be affected by the rate at which the dose is delivered either via changes in dose-rate or fractionation. There is however a lack of experimental data and models to determine the DNA repair dynamics of cells exposed to multiple irradiations. This may be of relevance for radiotherapy applications in order to optimize fractionation modalities. This study aims to investigate the response of a cell system perturbed by an initial irradiation event to subsequent radiation incidents using γ-H2AX immunofluorescence assay. Response is expected to vary as a function of the time lapse between the exposures, because of the status of the repair mechanisms triggered by the first exposure. Normal human fibroblasts (AG01522) were irradiated with 225 keV γ-rays at a dose rate of 0.591 Gy/min. Except for the irradiation, cells were kept in an incubator at 37°C, 5% of CO2, 95% of humidity conditions and fixed at various times post irradiation up to 24 h. Split dose experiments were made by delivering doses of 1 Gy at different time lapses: 20 min, 1 h, 2 h, 5 h. Samples were stained using conventional immunofluorescence technique and scored manually using an ApoTome fluorescence microscope (x100 objective) in order to accurately count foci throughout the whole cell nucleus. Around 100 cells per time point were scored.

The γ-H2AX repair kinetics for the acute exposures presents a peak at around 30 minutes after the irradiation with ~20-25 foci/Gy. Subsequent irradiations cause similar peaks although the number of foci per cell was found to decrease with increase the time interval between the two radiation doses. A model, which describes the induction and the disappearance of the foci, was used to interpret the kinetics responses. The data analysis revealed very similar repair kinetics for the foci induced by the second dose, despite the different time lapse and dose range between the first and the second exposures. These preliminary indications appear to indicate that, at least with these doses and time intervals, the repair system is not accelerated in a system perturbed by an initial irradiation. This work was partially supported by the European Commission (EC Contract FP6 EURATOM project “NOTE” and FP 7 EURATOM project “EFIRADBIO” and “DOREMI”).

POS14-44. DNA double-strand breaks and repair investigated by automated γ-H2AX foci analysis. Martyna Polok, R. Zabolod, V. Meineke, H. Scherthan, Bundeswehr Institute of Radiobiology affiliated to the University of Ulm, Germany

We investigate the capability of automated systems to record DNA double strand breaks in human leukocytes caused by in vitro exposure of whole blood to ionizing radiation. The DNA double strand breaks (DSBs) are a consequence of ionizing irradiation exposure and are visualised by immuno-staining, as this DNA damage lead to the phosphorylation of the histone H2AX protein on the histone H2AX isoform (γ-H2AX). This feature and the accumulation of 53BP1 DNA damage protein allow us to analyze the
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occurrence of the DSBs with the γ-H2AX foci assay1 after exposure to X-ray irradiation between 0Gy and 4Gy.

The aim of our study is to establish a correlation between the number of γ-H2AX foci and the radiation dose using fluorescence microscopy. Moreover, we analyse the time decay of the foci in order to investigate the speed of the DNA repair mechanisms and possible applicability of the automated γ-H2AX assay for rapid dose reconstruction. The detection of γ-H2AX foci and the evaluation of data are usually performed by manual enumeration. Here we present a rapid Metafer fluorescence microscope system (MetaSystems). For fast diagnostic application of the method in radiation accident scenarios, we aim to develop rapid foci acquisition and analysis using a x40 objective and a corresponding Metafer software package. Furthermore, in order to ensure exact data acquisition and evaluation, we verify our results by comparing them with previously established x63 Metafer settings2. We will present results on rapid analysis of the induction and repair of DNA DSBs in irradiated peripheral leukocytes.


POS14-45. Effects of low dose ionizing radiation on the DNA repair system in a human skin 3D model. Wald Rachiú1, H. Jerome2, D. Odle3, D. Michel4, 1; CEA / UJF, France 2; CEA 3: Hospices Civils de Lyon 4: RRMA, France

Introduction: The effects of low-doses of ionizing radiation in humans are of growing concern, especially in the context of current radiation techniques such as medical imaging. The biological response of healthy tissue to low dose of 1-10 cGy in vivo is largely unknown. Moreover, Knowledge of cellular responses in tissue microenvironment is crucial for the accurate prediction of human health risks following chronic or acute exposure to ionizing radiation. Because skin is the first target of the body upon exposure to radiation, we propose to explore the potential biological effect of low-doses of ionizing radiation first on isolated human skin cells in monolayered culture and for the first time in three-dimensional (3D) artificial human skin tissue in different skin models Objectives: In this project, we propose firstly to study the effects (long and short-term) of low-doses on cell proliferation, apoptosis, and capacity to obtain a cohesive and stratified epidermis after irradiation. Secondly, we will evaluate the carcinogenesis risk by measuring the modulation of the DNA repair/damage systems after low-dose exposure. Methods: For short-term radiosensitivity, cell viability was determined by MTT assay after 24, 48 and 72 h post irradiation, we also performed an in vivo colony-forming assay, which measures the radiation toxicity after 2 weeks. DNA repair system and damage was assessed by different techniques available in our laboratory (DNA repair chips, modified comet assay …). Finally, organogenesis potential of cells irradiated by the γ-rays was tested by keratinoctyes to form a pluristratified epithelium in 3D organotypic cultures

Results and conclusions: We showed that low-dose of ionizing radiation increases 2 fold the oxidative DNA damage without any activation of the base excision repair pathway, an important pathway to repair oxidative DNA damage. Moreover, we showed that low-dose affects the organogenesis potential of keratinoctyes and impairs the proliferation-differentiation balance in the reconstructed skin. We postulate that when the dose or dose rate is very low the radiation damage sensors (ATM or ATR) are not activated, and the repair machinery is not induced. Hence damage could be accumulated in the genome of a cell until eventually it become malignant.

POS14-46. The role of C-NHEJ in the repair of simple and complex DSBs. Gabriela Reynolds, S. Botchkarev1, A. Parker2, P. O’Neill3, 1; Gray Institute for Radiation, Oncology and Biology, Department of Oncology, University of Oxford, UK 2: Central Laser Facility, UK

Exposure of mammalian cells to ionizing radiation induces DNA damage, the complexity of which is related to the ionisation density of radiation; low and high LET radiation induces 30% and 90% complex damage respectively. The most deleterious lesions induced within the DNA are double strand breaks (DSBs). In mammalian cells, non-homologous end joining (NHEJ) is the most prevalent DSB repair pathway. Previously we have used ultrafast X-rays (USX) and NIR multiphoton laser microbeam irradiation to investigate the repair of simple and complex damage by classical NHEJ (C-NHEJ). Using USX and NIR laser irradiation we have investigated the real time repair kinetics of damage using XRCC4-GFP (a downstream component of C-NHEJ). We have also used chemical inhibition of key proteins in back-up NHEJ (B-NHEJ) and histone de-actylation (HDAC) to study the contribution of B-NHEJ and chromatin state on the repair kinetics of C-NHEJ. Following NIR laser microbeam irradiation, XRCC4-GFP is recruited in +1 min to sites of DNA damage. At 1 h, XRCC4-GFP fluorescence levels decrease to ~15% of the maximum, faster than that previously shown for Ku80-EGFP (a gift from D. van gent) and DNA-PKcs-YFP (a gift from D. Chen) where background fluorescence levels are observed at 2 h. Higher NIR laser powers and USX doses are required to visualise XRCC4-GFP when compared to Ku80-EGFP. Following USX irradiation, Ku80-EGFP is rapidly recruited to sites of induced DNA damage in cells +/-PARP inhibitor, which should suppress B-NHEJ. The real time kinetics of recruitment and loss of Ku80-EGFP and DNA-PKcs-YFP are similar in both control and PARP inhibited cells consistent with the NLR laser studies. The role of chromatin on the real time repair kinetics have also shown that Ku80-EGFP and DNA-PKcs-YFP repair kinetics are unaffected when HDACs are inhibited leaving the chromatin in an open state. It is concluded that ligation of DSBs during C-NHEJ occurs with fast repair kinetics. Inhibition of the B-NHEJ has little effect on C-NHEJ suggesting that B-NHEJ does not compete with Ku70/80 for the repair of promptly formed simple and complex DSBs. This is consistent with previous studies suggesting B-NHEJ does not significantly contribute to DSB repair when C-NHEJ is proficient. Finally, HDAC has no effect on DSB repair involving Ku80-EGFP and DNA-PKcs-YFP during C-NHEJ.


Purpose: DNA double-strand breaks (DSBs) generated by ionizing radiation pose a serious threat to the preservation of genetic and epigenetic information. The known importance of local chromatin configuration in DSB repair raises the question of whether breaks in different chromatin environments are recognized and repaired by the same repair machinery and with similar efficiency. An essential step in DSB processing by non-homologous end joining is the high-affinity binding of Ku70-Ku80 and DNA-PKcs to double-stranded DNA ends that holds the ends in physical proximity for subsequent repair. Methods and Materials: Using transmission electron microscopy to localize gold-labeled pKu70 and pDNA-PKcs within nuclear ultrastructure, we monitored the formation and repair of actual DSBs within euchromatin (electron-lucent) and heterochromatin (electron-dense) in cortical neurons of irradiated mouse brain. Results: While DNA lesions in euchromatin (characterized by two pKu70-gold beads) and in heterochromatin (characterized by three pKu70-gold beads) are promptly sensed and rejoined, DNA packaging in heterochromatin appears to retard DSB processing, due to the time needed to unravel higher-order chromatin structures. Complex pKu70-clusters formed in euchromatin (consisting of 4 or ≥6 gold beads) may represent multiple breaks in close proximity caused by ionizing radiation of highly-compacted DNA. Nearly all pKu70-clusters disappeared within 72 hours post-irradiation, indicating efficient DSB rejoining. However, persistent 53BP1 clusters in heterochromatin (comprising ≥10 gold beads), occasionally co-localizing with γH2AX, but not pKu70 or pDNA-PKcs, may reflect incomplete or incorrect restoration of chromatin structure rather than persistently unrepaired DNA damage. Discussion: Higher-order organization of chromatin determines the accessibility of DNA lesions to repair complexes, defining how readily DSBs are detected and processed. DNA lesions in heterochromatin appear to be more complex, with multiple breaks in spatial vicinity inducing severe chromatin disruptions. Imperfect restoration of chromatin configurations may leave DSB-induced epigenetic memory of damage with potentially pathological repercussions.

POS14-48. Mutagenic effect of low dose and dose rate low LET radiation on human lymphoblastoid cell lines. Sara Shakeri Manesh, A. Wojcik, M. Harms-Ringdahl, S. Haghdooost, Stockholm University, Sweden

Risk estimates for stochastic effects (cancer, mutations) of radiation at low doses (<20 mSv) are based on the Linear-Non-Threshold (LNT)
hypothesis. There are no epidemiological methods that can provide direct risk estimates for such low doses. As similar uncertainties apply for most cellular and animal models, there is a need to assess more sensitive methods to study the effects of low dose irradiation.

We have previously shown that low doses of ionizing radiation increase the endogenous production of reactive oxygen species (ROS) which leads to an oxidative stress condition. ROS react with different cellular components (proteins, lipid and nucleic acid) and give rise to the different modifications.

We have also shown that low doses and dose rates of gamma rays can modify the expression of several proteins involved in cellular protection against oxidative stress including up-regulation of hmTH1 (nucleotide sanitization enzyme) and hMYH (a protein with DNA glycosylase activity which repair adenine mispaired with 8-oxo-dG). hmTH1 inhibits incorporation of oxidized nucleotides (8-oxo-dGTP, a mutagenic modified base) into DNA during replication.

In the present study we have stably transfected a human lymphoblastoid cell line, TK6, using siRNA towards hmTH1 and hMYH. Both transfected and untransfected cells were exposed to 0, 0.25, 0.5 or 1 Gy gamma radiation either acutely or chronically. The dose rates used were 1.4, 5 or 15 mGy/hr for chronic and 0.41 Gy/min for acute exposure. Mutant frequency in thymidine kinase locus was studied using tri fluorothymidine (TFT).

Preliminary results indicate that the dose rate rather than the dose rate has a significant impact on mutation induction. The mutation frequency in the exposed transfected cells was slightly higher than non-transfected cells.

**POS41-49. Significance of the repair synthesis in determining the biological consequences of clustered DNA damage.** Nausya Shikazono1, M. Noguchi1, A. Urushibara1, P. O’Neill2, A. Yokoya1, 1: Japan Atomic Energy Agency. 2: Oxford University, UK

Clustered DNA damage, defined as two or more lesions within one to two helical turns of DNA induced by a single radiation track, is a unique feature of ionizing radiation. Although an extensive amount of work has been carried out on how clustered damage is processed in vitro, further investigations are still needed to understand the processing and the biological consequences of clustered damage in vivo.

We have studied the biological consequences of bi-stranded clustered damage sites which consist of a combination of DNA lesions, such as a single strand break (SSB), an apurinic/apyrimidinic (AP) site, and an 8-oxo-7,8-dihydroguanine (8-oxoG), using a bacterial plasmid-based assay. Plasmids were ligated with oligonucleotides containing clustered lesions. Following transformation of the ligated plasmids into the wild type strain of *Escherichia coli*, we found significantly lower transformation frequencies for the clustered SSB + AP lesions (separated by 1bp) than that for either a single SSB or a single AP site. We suggest that a double strand break (DSB) or a replication block is formed during the processing of the 8-oxoG AP cluster. When the two lesions are placed farther apart (10-20bp), the transformation efficiencies are comparable to those of the single lesions. This recovery of transformation efficiency for separated lesions requires PolI activity. Similarly, the mutation frequency depends on the separation of the clustered SSB + 8-oxoG, although the SSB + 8-oxoG cluster, in contrast to the SSB + AP cluster, transforms at a comparable efficiency to that of single lesions. PolI also seems to play an important role in avoiding mutations, as the lack of PolI enhances the mutation frequency of the separated lesions to the level of that of the closely spaced lesions. These results indicate that the biological consequences of clustered DNA damage strongly depend on the repair synthesis of the comprised lesion(s).

**POS41-50. Dose-response and repair kinetics of gamma-H2AX foci in VH10 normal human fibroblasts irradiated with alpha particles, X-rays or a mixture of both.** Elna Staaf1, K. Brehwens1, M. Noguchi1, S. Haghdost1, A. Wojcik1,2, 1: Centre for Radiation Protection Research, GMT Department, Stockholm University, Sweden 2: Department of Radiobiology and Immunology, Institute of Biology, Jan Kochanowski University, Kielce, Poland

Purpose: To investigate the DNA damaging properties of mixed beams, and how they relate to the damage from a single radiation type. Mixed beams of ionizing radiation are present in our environment, for example when living in areas with high background radiation. Radiotherapy, which we analyze here, high energy photons are also exposed to mixed beams of gamma radiation and neutrons. Earlier investigations have shown both additivity and synergism when combining radiations of different LET. The finding of additivity or synergism seems to depend on the order of irradiation, and a unique facility where cells can be simultaneously exposed to X-rays and alpha particles in a 37°C incubator was therefore constructed at the Stockholm University. The first experimental series with human lymphocytes concluded synergism on the level of micronuclei. To further investigate the mechanism behind this effect the gamma-H2AX assay was employed.

Methodology: For the repair kinetics study, normal human fibroblasts (VH10) were exposed to 0.28 Gy alpha particles, 0.8 Gy X-rays or a mixture of 0.14 Gy alpha particles and 0.4 Gy X-rays and thereafter incubated 0.5, 1, 3 and 24 h. Dose response curves were performed for the 1 h time point to confirm linearity with dose. Samples were harvested which for the gamma-H2AX assay, images captured and analyzed using ImageJ.

Results: The different physical properties of X-ray and alpha particle induced damage are reflected in the focus size, where alpha particles gave rise to large and X-rays to small foci. Although there is a slight overlap (a level of large foci exist in X-ray samples and alpha particle-irradiated cells also contain small foci), we can follow the kinetics of large and small foci in order to see if they influence each other following exposure to mixed beams. The action of mixed beams of X-rays and alpha particles was observed to be additive, both for repair kinetics and dose-response curves.

Conclusions: Foci can be separated into “large” and “small” foci. Large foci disappear at a slower rate compared to small foci. The effect of mixed beams of alpha particles and X-rays was found to be additive both on the level of repair kinetics as well as dose-response for gamma-H2AX foci.

**POS41-51. Chromatin modification through histone methylation required for amplification of ATM-dependent DNA damage signals.** Keiji Suzuki, Nagasaki University, Japan

Ionizing radiation causes DNA double strand breaks, which stimulate ATM-dependent DNA damage checkpoint. Upon irradiation, activated ATM phosphorylates various downstream mediators and effectors. These factors are recruited to the sites of chromatin surrounding DNA double strand breaks and form discrete foci detectable under the fluorescence microscope. Previously, we demonstrated that residual foci persistent for over 24 hours after radiation exposure became quite large in size, and the foci growth was essential for amplifying DNA damage signals. Although recent studies have shown that multiple modifications of histones are involved in DNA damage signal amplification, the molecular mechanisms underlying the persistent DNA damage signal amplification has not been fully understood yet. In the present study, we examined growth of foci of DNA damage checkpoint factors in normal human diploid cells exposed to gamma-rays. We found that decreased acetylation of histone H3 and di-methylation of histone H3 at lysine 9 did not affect the initial foci formation but they ablated persistent foci formation observed 24 hours after irradiation. These results indicate that chromatin modifications are indispensable for proper response to DNA damage, by which stability of the genome is maintained.

**POS41-52. Human Histone Acetyltransferase 1 (Hat1) and Histone H4 Modifications in Response to Genotoxic Treatments.** Stefan Tafrov, Brookhaven National Laboratory, USA

Human Hat1 was isolated as an enzyme responsible for acetylating newly synthesized histone H4 molecules at lysine 5 (K5) and K12 before they are transported to the nucleus for deposition on newly synthesized DNA molecules during replication. Hat1 participates in several other cellular processes, including interacting with the origin recognition complex, silencing transcription, and repairing DNA. Treating normal human keratinocytes (NHKs), or keratinocytes from the HaCaT cell line (p53 mutant) with hydrogen peroxide triggers bright nuclear-staining for Hat1; exposing NHKs to gamma rays does not do so.

We assessed the level of acetylation of nuclear histone H4 at K5 and K12 before they are transported to the nucleus for deposition on newly synthesized DNA molecules during replication. Hat1 participates in several other cellular processes, including interacting with the origin recognition complex, silencing transcription, and repairing DNA. Treating normal human keratinocytes (NHKs), or keratinocytes from the HaCaT cell line (p53 mutant) with hydrogen peroxide triggers bright nuclear-staining for Hat1; exposing NHKs to gamma rays does not do so.

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prepared by two alternative procedures, the first is for isolating nuclear-matrix proteins. Briefly, we permeabilized the cells with 0.5% triton-X-100, and collected the cytosolic- and soluble-nuclear proteins. Digesting the cellular DNA with DNase I isolated the chromatin-associated proteins. The remnants of the cells first were extracted with 0.25 M ammonium acetate, and then with 2 M NaCl. Thereafter, we solubilized the remaining insoluble nuclear-matrix proteins in 8 M urea. In the second procedure, we used the "Histone Purification Mini Kit" (Active Motif), according to the manufacturers’ recommendations. The protein samples, separated by SDS-PAGE electrophoresis, underwent Western blotting analysis. We detected the total levels of histone H4 with anti-histone H4 antibody (Millipore), and the amount of acetylation at K5 and K12 and H4 with the antibodies shown above.

We offer a hypothetic model of the likely participation of human HAT1 in the response of human keratinocytes to genotoxic treatments, and in repairing the induced DNA damage.

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**POS14-53. Biochemical kinetic rate model for repair of DSB induced by radiation of different qualities.** Reza Talebi, H. Nikjoo, Karolinska Institutet, Sweden

Among radiation induced DNA damage, double strand breaks are the principle lesions, the complexity of which increases with LET [1], and the lack of repair or misrepair leads to adverse biological effects in irradiated cells. Double strand breaks (DSB) can be repaired by Nonhomologous end-joining (NHEJ), homologous recombination (HR), and single strand annealing (SSA). The HR and SSA pathways are cell cycle dependent and more active in late S/G2 phases, while NHEJ is active throughout the whole cell cycle. Repair by NHEJ and SSA may induce deletions and additions in the genome, whereas HR is a conservative repair pathway. In this work we present a mathematical model of DNA DSB repair. The model considers NHEJ, HR, and SSA pathways for the repair of DSB induced by radiations of different qualities. The descriptions of different repair pathways were made by a system of nonlinear differential equations. The DSB repair kinetics obtained, for cells irradiated with X-ray, helium, and nitrogen ions, were compared with the published experimental data [2]. Our results show HR is indispensable for repairing DSB especially for high LET irradiation.


**POS14-54. Evaluation of single-nucleotide polymorphisms in DNA repair genes and biomarkers of in vitro DNA damage in breast cancer patients.** Newilian study, Annarita C. Patrono*, T. Cornetta*, T. Poggioli*, V. Donato†, D. Giannarino*, R. Cozz†, 1: UTBBIORAD-RAB - ENEA Casaccia, Italy 2: Department of Biology, “Roma Tre” University, Rome, Italy 3: UTBBIORAD-RAB ENEA Casaccia, Rome, Italy 4: S. Camillo-Fiorlandini Hospital, Radiation Oncology Unit, Rome, Italy

The aim of our work is to study the individual radiosensitivity before irradation in both wild*ric1* and mutant*ric1*-deficient homozygous mutant mice, as compared with wild-type and heterozygous mice, treated with KBrO₃ for 16 weeks. These results clearly indicate that both MMR and p53 are involved in the suppression of oxidative stress-induced intestinal tumorigenesis in mice.

**POS14-55. Antitumorigenic effects of p53 and mismatch DNA repair system on oxidative stress-induced intestinal tumors in mice.** Teruhisa Tsuzuki, J. Shu Piao, N. Matsumoto, Y. Nakatsu, Kyushu University, Japan

Oxygen radicals are produced through normal cellular metabolism, and the formation of such radicals is further enhanced by ionizing radiation and by various chemical agents. We have established an experimental system for examining oxidative DNA damage-induced mutagenesis and tumorigenesis in the gastrointestinal tracts of mice (Sakamoto, K. et al. *Cancer Res., 67, 6599-6607, 2007*). Oral administration of potassium bromate (KBrO₃) effectively induces GC to TA transversions as well as epithelial tumors in the small intestines of * Mut5h-deficient mice. The mismatch repair (MMR) system is implicated not only in the correction of replication errors but also in the response to DNA damage to maintain genomic stability. Increasing evidence suggests that MMR is involved in the process of avoiding mutagenesis caused by oxidative DNA damages in mammalian cells. To elucidate the role of MMR in the avoidance of oxidative stress-induced tumorigenesis, we performed KBrO₃-induced tumorigenesis experiments using *Msh2*-deficient mice. Chronic exposure to KBrO₃ at a dose of 2g/l in drinking water for 16 weeks resulted in multiple tumor formation in the small intestines of *Msh2*-deficient mice. In addition, we also examined the roles of tumor suppressor p53 in the oxidative stress-induced tumorigenesis using *Tp53*-deficient mice. We observed an enhanced tumor formation in the small intestines of *Tp53*-deficient homozygous mutant mice, as compared with wild-type and heterozygous mice, treated with KBrO₃ for 16 weeks. These results clearly indicate that both MMR and p53 are involved in the suppression of oxidative stress-induced intestinal tumorigenesis in mice.

**POS14-56. Analysis of germ cells specific DNA damage response in Japanese Medaka (*Oryzias latipes*) strain, *ric1* has a defect in the repair mechanism of DNA double strand break (DSB) induced by gamma-irradiation of germ cells development.** This work was carried out by Dr. T. Zhang1, J. Kobayashi2, K. Komatsu2, S. Oda1, H. Mitani1, 1: University of Tokyo, Japan 2: Kyoto University, Japan

Molecular mechanisms of DNA damage response in germ cells are still unknown. The radiation-sensitive Medaka (*Oryzias latipes*) strain, *ric1* has a defect in the repair mechanism of DNA double strand break (DSB) induced by gamma-irradiation of germ cells development. In this study, we investigated the regulation of cell cycle, apoptosis, DSB repair and chromatin modification by DNA damage in *ric1*/embryonic cell lines. We discovered that *ric1* cells showed the significant delay in the early repair of DSB and the decreased efficiency of homologous recombination (HR). However *ric1* had significantly increased resistance to gamma-radiation. Although *ric1* cells and wild-type cells showed G2/M checkpoint arrest immediately after gamma-irradiation, the resumption of cell cycle in *ric1* cells was earlier than that in wild-type cells. Furthermore, Ser139 phosphorylated H2AX ([gH2AX]) foci were formed after gamma-irradiation in both wild-type and *ric1* cells. However, the [gH2AX] foci disappeared more quickly in the *ric1* cells. These results suggest that the *ric1* gene plays a role of H2AX phosphorylation on serine 139. ATM and DNA-PK are considered as major kinases of H2AX phosphorylation in response to DSB. To
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determine whether ric1 gene interacts with ATM or DNA-PK, we examined DSB repair efficiency and radiation sensitivity after ATM or DNA-PK inhibitor treatment. Although wild-type cells showed decreased efficiency of HR repair, ric1 cells did not show significant effect on HR repair after treatment with DNA-PK inhibitor. These results indicated that ric1 gene interact with DNA-PK. On the other hand, cell lines derived from p53-knockout Medaka have phenotypes similar to ric1 cells. Thus, ric1 gene might interact with p53 pathway. It was recently reported that the interaction of histone phosphorylation, acetylation and ubiquitination after DNA damage is complicated. We demonstrate that the function of ric1 gene is chromatin modification. We will discuss the function of ric1 gene in more detail.

**POS14-57. Effects of protein phosphatase 6 knockdown on non-homologous end joining repair of DNA double strand breaks.** Nicholas Valerie, A. Hosing, D. L. Brautigan, J. M. Larner, University of Virginia, USA

Irradiating radiation (IR) is a primary modality for the treatment of glioblastoma multiforme (GBM), however these tumors tend to be radioresistant. The radioresistance is thought to be due, at least in part, to an enhanced capacity to repair DNA double strand breaks (DSBs) generated by therapeutic IR. Non-homologous end joining (NHEJ) is the predominant repair pathway. One prerequisite for NHEJ is the availability of a DNA template and, therefore, is restricted to late-S and G2 phases of the cell cycle. Targeting proteins in the NHEJ pathway would be expected to sensitize GBMs to IR, with therapeutic benefits. DNA-dependent protein kinase (DNA-PK) is a necessary component that facilitates canonical NHEJ repair. Previously, we have shown that the protein Ser/Thr phosphatase-6 (PP6) associates with DNA-PK via one of its SAPS domain regulatory subunits (PP6R1). Depletion of PP6 catalytic subunit (PP6c) or PP6R1 in GBM cell lines reduced IR activation of DNA-PK, limited DSB repair and caused radiosensitization with reduced clonal survival (Mi et al, PloS One, 2009). More recently, we have studied the role of PP6 in NHEJ using an artificial repair system in 293B cells, which quantitatively measures NHEJ repair following induction of DSBs by I-SceI endonuclease by flow cytometry or qPCR. Strikingly, PP6c knockdown by siRNA resulted in a ~2-fold increase in NHEJ repair of DSBs, while DNA-PK knockdown expectantly decreased repair by ~2-fold compared to controls. Preliminary results on knockdown of PP6 regulatory subunits suggests they may have little or no role in NHEJ repair. The mechanistic basis of these effects is being pursued and will be presented.

**POS14-58. DNA-PK autophosphorylation and DNA end processing during DNA Double Strand Break Repair.** Dik C. van Gent, N. S. Verkaak, M. van der Burg, H. IJpeert, J. J. M. van Dongen, Erasmus MC. Netherlands

Irradiating radiation (IR) creates DNA double strand breaks (DSBs), which are repaired by non-homologous end-joining (NHEJ). The Ku70/80 complex recognizes the DNA ends and attracts DNA-PKcs. Subsequently, the DNA ends are coupled by the ligase IV/XRCC4 complex. Several DNA end processing factors are required to process ‘dirty breaks’. We found, that the Ku80 C-terminus is important for IR resistance and V(DJ) recombination, especially coding joint formation, because Artemis cleavage of DNA hairpins was severely inhibited. Interestingly, autophosphorylation at sites that promote opening of the NHEJ complex are specifically affected by this mutation. Furthermore, we identified the first patient with a DNA-PKcs mutation (Leu3062 to Arg). This point mutation did not affect protein levels or kinase activity, but activation of Artemis was again inhibited. The absence of patients with loss of kinase activity suggests, that this DNA-PK activity is indispensable in humans, while mice with such mutations are normally viable. Careful characterization of Xil1/Cernunnos patients and cells revealed that this gene influences DNA end processing. This may explain why the XLF protein is required for efficient ligation of non-matching DNA ends.

**POS14-59. Recruitment of DNA Single Strand Break Repair Proteins to Damaged DNA.** Michael Weinfield, M. Hendzel, I. Abdou, University of Alberta, Canada

The integrity of cellular DNA is pivotal for cellular functioning and survival. Single-strand breaks (SSBs) are among the most frequently encountered DNA lesions. In dividing cells, unrepaird SSBs, if encountered by replication machinery, proceed to form double-strand breaks (DSBs), which comprise the most mutagenic and lethal forms of DNA damage. In post-mitotic cells, unrepaired SSBs can lead to apoptosis if encountered by the transcription machinery, and genetic deficiencies in SSB repair proteins, such as poly(ADP-ribose) kinase/phosphatase (PARP), are responsible for several severe neurodegenerative disorders. Accordingly living cells possess robust and highly coordinated SSB repair machinery. Biochemical studies have elucidated the cascade of events underlying SSB repair, however data from live cells in real time is lacking. According to biochemical studies, four distinct steps outline the SSB repair process: (i) SSB sensing which is mediated by PARP1 through its zinc finger domain (Zaf). (ii) DNA end processing, which is catalyzed by various enzymes, e.g. PNKP, that act on specific types of damaged strand break termini, e.g. 3’-phosphate and 5’-hydroxyl, to restore them to correct DNA termini, i.e.3’-hydroxyl and 5’-phosphate. (iii) Gap filling mediated by DNA polymerase β. (iv) Strand resealing by DNA ligase III (Lig3). An interesting step in the SSB repair cascade is the ssDNA scaffold XRCC1, which appears to orchestrate the steps from end processing to ligation. We aimed to study the early recruitment events of the SSBR core machinery to sites of DNA damage, and with the aid of fluorescently-tagged SSBR proteins we were able to show for the first time in live cells that DNA ligase III (Lig3) serves as a SSBR sensor that impacts the recruitment of other SSB repair proteins to sites of DNA damage; a finding that is in contrast with the current model of SSB repair where PARP is the only known sensor of SSBs. Additionally our preliminary data point out to a plausible connection between PARP3 and SSB repair. Our results might provide a more dynamic picture for SSBR pathway that will add tour current understanding of the SSB repair process a new dimension that might be of therapeutic value for cancer treatment.

**POS14-60. Identification of AP Endonuclease (CiAPE1) in the Ascidian Ciona intestinalis.** Quanru Zheng-Kiyama, K. Igarashi, T. Morigaki, H. Hashiguchi, Kyoto University, Japan

Apurinic/apyrimidinic (AP) sites are a mutagenic and cytotoxic DNA damage and cause impaired DNA replication and transcription. AP sites occur spontaneously and also generated in genomes during the repair of oxidation and alkylolation damage through the base excision repair (BER) pathway. DNA glycosylase-associated AP lyase and AP endonucleases play a central role in repairing AP sites. AP endonucleases incise DNA adjacent to the AP sites to initiate BER and counteract the cytotoxic and mutagenic effects of AP sites. It is of interest to perform a systematic comparative analysis of the conserved domains in DNA repair enzymes and the evolution of BER systems. Furthermore, it is important to characterize the roles and regulation of BER during the development of organisms. To address these issues, we first identified an AP endonuclease of the ascidian Ciona intestinalis which is often used as a good model system to elucidate the mechanisms of development. We have already characterized Ciona 8-oxoguanine DNA glycosylase (Ogg1) and endonuclease III homologues. In this study, the EST database from the Ciona cdNA resources was searched. A cDNA clone was found to be an AP endonuclease gene in Ciona intestinalis as a human hAPE1 homolog. Sequence alignment showed that the encoded protein shares 49% identity with the human hAPE1 protein, which had been found to possess AP endonuclease activity. The cDNA was sub-cloned into plasmid vector. The AP endonuclease of Ciona intestinalis (CiaAPE1) was expressed in E. coli Dsb nfo as a GST fusion protein and purified by glutathione affinity column chromatography. The purified CiaAPE1 efficiently cleaved the tetrahydrofuran-containing duplex oligonucleotide. Mg2+ was required for the AP endonuclease activity. In addition, the transformation of E. coli RPC50 Dsb nfo with the plasmid carrying CiaAPE1 cDNA complemented SSB sensitivity in the E. coli mutant. These results demonstrated that CiaAPE1 has AP endonuclease activity to repair AP sites in the genome of Ciona intestinalis. To elucidate the roles of CiaAPE1 in the embryogenesis and development of Ciona intestinalis is under investigation.

**POS14-61. Targeting DNA repair genes and radiation-induced protective factors by combined artificial miRNAs enhances the response of human brain tumor cells to radiation.** Zhiming Zheng, Emory University, USA

Radiotherapy is one of the most commonly used therapies for glioblastoma multiforme (GBM). However, the curative potential is limited by intrinsic radio-resistance and ionizing radiation (IR)-
induced protective factors in the GBM, which results in tumor recurrence of the majority of GBM patients within the targeted radiotherapy tumor bed. Previously, we reported that targeting DNA repair genes: ATM and DNA-PKcs with exogenous miR-101 DNA sensitizes human brain and lung tumors to radiation (PLoS One 5:e11397, 2010). However, due to the heterogeneous characteristics of human tumors, different tumor cell lines showed different miR-101 levels and we found that a few tumor cell lines with a much higher level of endogenous miR-101 showed less response to the ectopic miRNA (our unpublished data), suggesting that the endogenous miRNA level in the tumor affected the tumor response to the ectopic miRNA. In addition, several resent publications indicate that IR-induced apoptosis factors including CASP3 (Sci Signal, 3-r4a1, 2011; Cell Stem Cell, 7:508, 2010) and Puma (Gene Dev, 24:1602, 24:1608, 2010) could promote cancer stem cell formation, which plays an important role in tumor recurrence. To overcome these problems, we designed four RNA Polymerase II-driven artifical miRNAs (amiR) to target two DNA repair genes: XRC2/XRC4 and two IR-induced factors: CASP3/Puma for enhancing GBM cell response to IR. The results showed that these amiRs efficiently reduced the target gene expression. Delivering XRC2/XRC4-amiR to GBM cells before IR, efficiently reduced the repair of DNA double strand breaks and sensitized the GBM cells to IR-induced killing. Delivering CASP3/Puma-amiRs to GBM cells after IR, dramatically reduced the stem cell signature in the cells. The data further confirmed in a xenograft mouse model: the tumor size derived from irradiated GBM cells with the combined amiRs was much smaller that derived from the irradiated cells with the control RNAs. These results indicate that amiRs by binding to the 3'-untranslantion region (UTR) of target genes could efficiently silence the gene expression. Our enhanced level of aberrations was observed at already 100 accumulated decays per cell, whereof one decay on average per cell per 20 min was calculated. The cell cycle of both PBL and hTERT-RPE1 cells was delayed due to incorporation of I-125-UdR in the DNA, when compared to control cells.

**POSTER PRESENTATIONS**

**POS15 Genetic instability**

**POS15-01. Epigenetic Alterations in High and Low LET Radiation Induced Genomic Instability, Janet E. Baulch1, U. Appar2, W. F. Morgan2, 1: University of Maryland, Baltimore, USA 2: Pacific Northwest National Laboratory, USA**

The mechanisms by which radiation-induced genomic instability is initiated and perpetuated remain to be elucidated. Radiation-induced mutations, double-strand breaks, or changes in gene expression alone do not account for the unstable phenotype. This study tests the hypothesis that epigenetic aberrations are perpetuated in clones exhibiting radiation induced genomic instability. In previous work, cells were irradiated and characterized for stability using fluorescence in situ hybridization. In this study, the clones were evaluated for changes in DNA methylation and miR-mediated gene expression.

Results demonstrate no effect of irradiation or persistent genomic instability on promoter DNA methylation for nuclear factor-kappa B (NFkB), tumor suppressor in lung cancer 1 or cadherin 1 genes. However, a potential mutation or deletion event was detected in the NFkB promoter for two unstable clones. Significant changes were observed in LINE-1 or Alu repeat element DNA methylation for unstable clones. Analysis of global methylation indicated that both DNA hypomethylation and hypermethylation may be observed in both stable and unstable irradiated clones.

**POS15-03. Loss of mammalian histone H2B ubiquitin ligase Bre1 (Rnf20/Rnf40) triggers replication stress and chromosomal instability, Sophia Chernikova, O. Razorenova, J. Higgins, B. Sissh, M. Nicolai, J. Dottl, D. Chernikov, S. Kwok, J. Brooks, S. Bailey, J. Game, M. Brown, 1: Stanford University, USA 2: Colorado State University, USA 3: National Center for Biotechnology Information, National Library of Medicine, NIH, USA**

MammalianBre1 complexes (also known as RNF20/40 or BRE1/BRE1B) function similarly to their yeast homolog Bre1 as ubiquitinylated histone H2B. This ubiquitination facilitates methylation of histone H3 at K4 and K79, and accounts for the roles of Bre1 and its homologs in transcriptional regulation. Recent studies by others suggested that Bre1 acts as a tumor suppressor, augmenting transcription of select tumorsuppressor genes and suppressing transcription of select oncogenes. Bre1 has also been shown to be involved in regulation of cell cycle and DNA repair. However, these functions of Bre1 seem to be governed by protein-protein and protein-chromatin interactions of Bre1 rather than by attenuated transcription of specific genes. In this study we present a new mechanism of tumor suppression by Bre1.

We have shown previously that loss of BRE1 protected homologous recombination, resulting in increased sensitivity to ionizing radiation and DNA cross-linking agents. Using an RNAi approach we show in this study that BRE1 loss leads to increased gamma-H2AX signal specifically in the S-phase. We track the evolution of genomic instability in BRE1-deficient cells from earlyreplication stress to specific genomic rearrangements that lead to a rapid increase in DNA content and initiate a breakage-fusion-bridge cycle. We conclude that defects in homologous recombination repair contribute to replication stress and genomic instability furthered by loss of BRE1. We propose that genomic instability triggered by BRE1/BRE1B deficiency may be an important early step that precedes an acquisition of an invasive phenotype, as we find decreased levels of BRE1 and dimethylated H3K79 in testicular seminoma and in the premalignant lesion in situcarcinoma.
POSTER PRESENTATIONS

POS15-04. Deregulation of the centrosomes and chromosomal segregation in Hodgkin cell lines and B-lymphocytes? Corina Cuceu 1, M. Ricou 2, G. Shinn 3, S. Bennai 4, S. Junker 2, A. Lenain 2, T. Grinskiy 2, A. Bernheim 2, J. Bouthil 1, S. Koscielniy 1, P. Cardé 2, R. M'kacher 1, L. Sabatier 1, 1: Commissariat à l'Energie Atomique, France 2: CEA 3: University of Aarhus, Denmark 4: Institut Gustave Roussy, France

Background: Hodgkin lymphoma (HL) is a malignancy characterized by the presence of tumour cells exhibiting numerical and structural chromosomal abnormalities, derived, in most cases, from germinal center B cells. Recently, centrosome aberrations have been described as a possible cause of chromosomal instability and aneuploidy in many human malignancies. We investigated the role of centrosomes in the defect of chromosomal segregation and polyplody in Hodgkin cell lines and in circulating B-lymphocytes of HL patients.

Methods and materials: Four HL cell lines (L428, LS40, L1236, and KM-H2) and 30 HL patients were studied. Centrosomes were stained with anti-γ-tubulin. Chromosomal segregation and the scoring of micronuclei were studied after blinding cytokinesis with cytochalasin-B in HL cell lines. Chromosomal orientation fluorescence in situ hybridization (CO-FISH) and multicolour fluorescence in situ hybridization (M-FISH) were performed in HL cell lines and in B-lymphocytes of HL patients. We also analyzed the distribution of chromosomes 9 and 16 in the nuclei of tetra-nucleated cells.

Results: In HL cell lines, we observed an increase in centrosome number and amplification, with the highest rate in cells with irregularly shaped nuclei. The cell lines L1236 and LS40 showed a spontaneous high frequency of micronuclei. Popcorn shaped cells were observed predominant for the cell line L428 and in a small percent of the others. M-FISH analysis revealed the presence of complex karyotype including the chromosome dicentrics in L428. There was a malsegregation of the marked chromosomes, and a variation in the size of nuclei derived from the same mother cell confirmed by painting of chromosomes 9 and 16. In B-lymphocytes of HL patients, recurrent chromosomes that were lost were observed, often including chromosomes 5, 7, 15 and 17. Complex chromosomal rearrangements were observed in metaphases with shorter telomeres.

Conclusions: These findings demonstrate centrosome deregulation correlated to chromosone segregation abnormalities and aneuploidy of HL cell lines. The loss of chromosomes in B circulating lymphocytes in HL patients could be related to clinical outcomes.

POS15-05. Individual susceptibility to telomere instability and radiation-induced tumors. Monika Frenzel1, M. Bellamy2, F. deVathaire2, N. Haddy2, R. M'kacher1, L. Sabatier1, 1: Commissariat à l'Energie Atomique, France 2: INSERM, France

Telomeres are supposed to play a major role in increased chromosomal instability, radiation sensitivity, and senescence. Telomere shortening has been observed in some cancer patients and correlated with poor clinical outcome. Previously, three cohorts of Hodgkin Lymphoma (HL) patients were studied: 100 who were prospectively followed > 10 years after diagnosis, 48 who developed a second cancer and 60 long-term survivors with no evidence of disease or complication since their initial treatment. Fifty healthy donors and 70 patients with a newly diagnosed solid tumor were the control population. A significant correlation between telomere shortening, chromosomal instability and in vitro radiation sensitivity was found as well as a higher risk of the occurrence of second cancer (SC) after treatment. Our current HL study was premised on the finding that telomere dysfunction and DNA repair pathways were involved in radiation-induced damages and cellular senescence. All patients included in the study are characterized by a benign self-involuting tumor of endothelial cells, called haemangioma, in their infancy. Some of them were treated with sonicating radiation in their early childhood (a few years old). The approach SC treated and untreated patients will be analyzed and correlated with the length of telomeres and their heterogeneity to validate the model of radiation-induced carcinogenesis and its modulation according to individual variability. Determination of telomere maintenance will be performed on peripheral blood lymphocytes.

In the first step of this project, patients with SC after radiation therapy will be selected along with controls of the same sex who were irradiated with a comparable dose – depending on the location and the type of cancer – at the same age as these patients. The same procedure will be performed in patients having SC without receiving radiation therapy for their haemangioma during infancy. The selection of the control groups is important to make a statement about the role of telomere length in chromosomal instability.

POS15-06. Isolation and characterization of p53, ATM and ATR mutants in medaka fish. Tomoko Fujitava-Ishikawa, Y. Kamei, J. Kim, S. Otozai, T. Todo, Osaka University, Japan

The DNA-damage response is a signal-transduction pathway that coordinates cell-cycle transitions, DNA replication, DNA repair and apoptosis. The major regulator of the DNA-damage response are two protein kinases, ataxia-telangectasia mutated (ATM) and ATM and Rad3-related (ATR). Both are large kinases with significant sequence homology and target an overlapping set of substrates that promote cell-cycle arrest and DNA repair. Another key player which mediates cell cycle checkpoints is tumor suppressor genep53. P53 is activated and stabilized by ATM and serve as a transcription factor for the downstream cell cycle regulator. These regulators are closely coordinated with each other and the proper coordination finally lead to an orchestrated cellular response to DNA damage, which is crucial for maintenance of their genome. The downstream reaction and the coordinating processes are well characterized at molecular level usus culture cells. However, each tissue of our body consists of wide variety of different type of cells, rather than a simple type of cells as in vitro cultured cell. Therefore the next interesting question is whether the damage response differs between each type of cells in tissues.

Among the vertebrates, the small laboratory fish are suitable for the study of gene function, for ease of handling, large numbers of progeny per generation, and, oviparous, large egg is suitable for establishment of transgenic animal by injection. Medaka, Oryzias latipes, is a small laboratory fish, having long history as an experimental model animal and thus several inbred lines are available. Medaka has a small genome size making it suitable for molecular genetical approach. Furthermore the mutation response of the medaka male germ cell was comparable with that of the mouse, and therefore medaka could serve as a vertebrate model system of DNA damage response. With these reasons we choose medaka as a model animal for the study of damage response at tissue level.

The availability of mutants is indispensable prerequisite. We have isolated several Medaka mutants forATM, ATR and p53 genes, which include nonsense mutant in each gene. The phenotypes of these nonsense mutants confirmed their loss-of-function nature. Our results demonstrate the utility of Medaka fish for the study of damage response at tissue level.

POS15-07. Transmission of genomic instability in families of Mayak nuclear workers using minisatellite markers. Irina Glazkova, Southern Urals Biophysics Institute, Russian Federation

The current studies on transmission of radiation-induced genomic instability from exposed parents to their offspring are focused on identification of genomically regions with higher mutation rate. Studies using hypervariable non-coding human genome sites as markers of chronic radiation have revealed so far no association between radiation and genetic effects through generations. This could be related to the lack of individual dosimetry monitoring of parents under study and living of the offspring with parents at the radiation-contaminated territories. The Mayak nuclear workers occupationally exposed to radiation were subject to systematic dosimetry monitoring. Thus, the Mayak workers and their families are the unique cohort to study transmission of genomic instability from exposed parents to the offspring somatic cells through germline cells. The preliminary efforts imply identification of biological affinity in families selected for analysis of mutation process using standard markers, which confirmed that family trios were genuine.

For the purposes of study, minisatellite genomic regions with a variable number of repeats, where mutation rate significantly exceeded the spontaneous level, were selected. The study was performed using a minisatellite gene, CSTB. The PCR-based approach was applied to analyze mutation process associated with insertion or deletion of the CSTB repeats. There were 40 family trios selected for the study. The main group included 20 family trios, where fathers only were exposed. Families were divided into three groups by pre-conceptive dose of fathers from external gamma-rays: Group I within 1-100 cGy; Group II within 100-300 cGy; and Group III over 300 cGy.

125
cGy. The control group (non-exposed parents) included 20 family trios. There were no mutations of the minisatellite cth in all family trios studied. The groups will be expanded to enhance statistical power of results and the number of minisatellites under study.

POSI15-08. Quantitative analysis of chromosomal re-arrangements in radiation induced endopolyplid cells. Michael Hausmann¹, S. Jutta², E. Iekatzena², S. Eberhard¹, K. Nick¹, S. Harry², 1: Kirchhoff-Institute of Physics, University of Heidelberg, Germany 2: (Latvian Biomedical Research and Study Centre, Riga 3: (Bundeswehr Institute of Radiobiology, Munich, Germany

Escape from mitotic catastrophe and generation of endopolyplid tumour cells (ETCs) represents a potential strategy of cells in response to genotoxic treatment by high doses of ionizing radiation. Recently, we have analysed the expression and sub-cellular localization of REC8 in p-53 dysfunctional tumour cell lines. REC8 is a cohesion component involved in correct chromosome disjunction and homologous recombination in the mitotic and meiotic cycle. In conclusion, these data indicated that after 10 Gy gamma-irradiation radiation-induced ETCs express features of meiotic cell divisions (Erempreisa et al., Exp. Cell Res. 315: 2593-2603, 2010). Within 10 days after radiation the cells undergo extensive changes in cell cycle progression accompanied by delayed DSBs 4-6 days after irradiation and DNA repair by homologous recombination. Such ectopic DSBs could have the potential to contribute to homolog recognition and to induce homolog association as a feature for DNA repair (Monajembashii et al., Biophys. J. 88: 2309-2322, 2005).

Therefore, we analysed the positioning of FISH labelled chromosomal territories 1, 4, and 12 in aliquots of HeLa-S3 cells 2-7 days after 10 Gy gamma-radiation exposure (2 Gy/min) in comparison to a non-treated control. For each sample, 3D-image stacks of about 50 nuclei were acquired using a Leica TCS NT confocal laser scanning or a Nikon TE2000-E confocal spinning disc microscope. After image segmentation of counterstained cell nuclei and FISH labelled chromosomal territories, the number of detected territories and the absolute distances between the geometric centroid of the respective territory and the nuclear border were measured. The resulting distance frequency distributions were statistically analysed by pairwise using the two-sample Kolmogorov-Smirnov test. In the cases of polyplody the most peripherally and the most centrally located territories were compared over the time course. In general, from day 2 to day 5 a shift of the investigated chromosomal territories to the nuclear periphery was observed followed by a re-arrangement towards the nuclear centre during day 6 and 7 which in most cases became again compatible with the territory arrangement of the non-treated control. These data indicate that re-arrangements in the nuclear architecture may support DNA repair in ETCs.

POSI15-09. Genome mapping of damaged chromosome regions induced by ionizing irradiation using CGH-microarray analysis. Masanitmu Homma, National Institute of Health Sciences, Japan

Exogenous genotoxic agents including irradiation randomly cause genetic damage on the genome and result mutations and chromosome alterations. To precisely understand the degree and character of the DNA damages by the agents, we must genomically analyze the genetic changes. Genomic DNA microarray technology enables to efficiently scan the whole genome and detect relatively small regions (<100kb) with deletions, amplification, and LOH. Using CGH-microarray (Agilent), which is designed for detecting copy-number alterations, we characterize gamma-ray-inducing genetic alterations genome-wide. We irradiated human lymphoblastoid TK6 with 5Gy gamma-ray, and randomly isolated 25-survived clones (0.1% survival). Among 25 clones, 12 showed at least one chromosome alteration with >5cM in CGH-microarray analyses. These chromosomes alterations were confirmed as deletion, amplification, translocation, or aneuploidy by Spectrum Karyotyping analysis. Deletions and amplifications were frequently observed as side-by-side on a chromosome, implying that Breakage-Fusion-Bridge cycle initiated by a DSB may contribute to generate the complicated changes. Four clones exhibited a same unreciprocal translocation with the breakpoint at chromosome 16q11.2. This breakpoint region may contain hot spot for ionizing irradiation.

POSI15-10. A role for the meiosis-specific protein SYCE2 in inducing genetic instability and radioresistance in cancer. Noriko Hosoya, K. Miyagawa, the University of Tokyo, Japan

Accumulating evidence suggests that some meiosis-specific proteins are aberrantly expressed in cancer cells. While these proteins play crucial roles in chromosomal dynamics in meiosis, their roles in mitotic cells are entirely unknown. In this session, we report the role of the meiosis-specific protein SYCE2 in mitotic cells. The SYCE2 protein is a component of the central elements of the synaptonemal complex, a meiosis-specific supramolecular proteinaceous structure that is essential for synthesis of the maternal and paternal homologous chromosomes. Expression analysis of SYCE2 using primary tumors revealed that it is aberrantly expressed in tumors of various tissue origins including cervix, ovary, thyroid and uterus. Normal epithelial cells forcedly expressing SYCE2 demonstrated increased frequency of aneuploidy and resistance to ionizing radiation. These cells also showed constitutive activation of ataxia telangiectasia mutated (ATM), suggesting that the up-regulation of DNA damage responses is responsible for increased genetic instability in cancer expressing SYCE2. Our findings provide a novel mechanism for genetic instability and radioresistance caused by aberrant expression of a meiosis-specific protein in cancer, which would serve as a molecular basis for developing personalized cancer therapies including radiotherapy.

POSI15-11. Change in DNA methylene and transcriptome of Arabidopsis seedlings after gamma irradiation. Jin-Hong Kim, J. Hong Kim, B. Yeoup Chung, Korea Atomic Energy Research Institute, South Korea

The present study aimed to reveal a relation between genome-wide changes in the DNA methylene and transcriptome of Arabidopsis seedlings after gamma irradiation. Whole seedlings grown in MS medium were irradiated with gamma rays at a dose rate of 50 Gy/h for 4 h and harvested at 6, 12, 24, 48, and 72 h later. Then, their genomic DNA or total RNA was used for Arabidopsis Whole Genome Chip-on-Chip Set 244K microarray analysis or Affymetrix ATH microarray and quantitative RT-PCR analysis, respectively. The most remarkable change in the genome-wide DNA methylation was observed at 12 h after gamma irradiation. Compared with the control, DNA methylation was increased or decreased in 30,154 or 32,166 probes, respectively. In contrast, the most noticeable change in the genome-wide transcription was found at the earlier time, 6 h. The total 658 or 404 of deferentially expressed genes were induced or repressed, respectively. Gene ontology analysis showed that transcriptions of DNA, histone, chromatin, and cell cycle-related genes were affected significantly. Moreover, expressions of cmt and drm genes for non-CG DNA methyltransferase were generally decreased until 48 h, while that of met for CG methyltransferase was rather increased. The obtained data suggest that the remarkable change in the genome-wide DNA methylation after gamma irradiation could depend on the initial transcriptomic changes including those of different DNA methyltransferases.

POSI15-12. Radiation induces delayed mitochondrial dysfunction in normal human fibroblast cells involves Drp1-dependent acceleration of mitochondrial fission. Shinko Kobashigawa, K. Suzuki, S. Yamashita, Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Japan

Mitochondria are dynamic organelle, whose long and narrow tubular structure is maintained by fusion and fission of the membranes. Tubular structure is essential for the mitochondrial function by sharing materials such as metabolites and mitochondrial DNA. Recently, mitochondrial dysfunction is thought as the origin of radiation-induced genetic instability. However, how radiation induces mitochondrial dysfunction is unclear. Here, we have tested our hypothesis that ionizing radiation causes mitochondrial fragmentation and dysfunctions their function. Unimmortalized normal human fibroblast cells (BJ-hTERT) were used in this study. MitoSox reagent was used to measure mitochondrial O$_2^-$ level. Mitochondria were visualized by staining with MitoTracer reagent, and structural change was examined under fluorescence microscope.

We found that gamma-irradiation accelerates mitochondrial fragmentation, which resulted in delayed production of mitochondrial O$_2^-$; Localization of dynamin-related protein 1 (Drp1) to
mitochondria was observed in response to irradiation. Moreover, knock-down of Dp1 expression prevented accelerated fragmentation after irradiation. We also found that knock-down of Dp1 expression abolished increase of mitochondrial O2- and suppressed radiation-induced loss of mitochondrial membrane potential. These data indicate that gamma-irradiation accelerates localization of Dp1 to mitochondria, which leads to mitochondrial fragmentation and dysfunction of mitochondria in normal human cells.

**POS15-13. Evaluating the role of Bcl-2 and Bcl-XL on radiation-induced genomic instability using automated assays.** Caitlin E Mills, D. W. Andrews, D. R. Boreham, McMaster University, Canada

Human mammary epithelial MCF-10A cells were transfected to over-express venus tagged anti-apoptotic proteins, Bcl-2 and Bcl-XL. Control cell lines were established, one in which the expression of Bcl-XL was knocked down, and one that expresses venus alone. Expression levels of the exogenous venus-Bcl-2 and XL as well as the endogenous proteins were confirmed via Western blot. The intensity of venus fluorescence was positively correlated to the expression levels of exogenous Bcl-2 and XL. Array comparative genomic hybridization (aCGH) and spectral karyotyping (SKY) were used to confirm several known genetic traits of the MCF-10A cell line. aCGH analysis has revealed an amplification of 13q32 in our MCF-10A cell line that has not been reported elsewhere. SKY was used to ensure that transfection and infection processes did not immediately alter the karyotype of the cells. The 53BP1 foci assay has been fully automated in our lab. A fully automated liquid handling robotic workstation, performs the cell seeding and successive cell staining and fixation all in 384 well plates. The plates are imaged with a high speed, Opera confocal microplate imager. Approximately 10 wells (5 fields of view per well, 40x objective) and 1500 nuclei (up to 30,000 foci) are scored per minute for the 53BP1 foci assay using Acapella software. The automated 53BP1 assay is sensitive down to 10 mGy in MCF-10A cells. Subtle differences in the object level distribution of spontaneous foci between the cells over-expressing venus-Bcl-2 and XL and the control cell lines were detected. Specifically, the frequency of cells with no spontaneous foci was 2-3% higher in the control cells. Despite the slight differences in the number of spontaneous 53BP1 foci, there were no significant differences in the dose response up to 3 Gy between the cell lines. Additionally, the loss of radiation-induced foci up to 8 hours post-irradiation followed the same trend independently of Bcl-2 or XL expression levels. The spontaneous levels of 53BP1 foci and micronuclei were tracked as indicators of genomic instability in the venus-Bcl-2, XL and control cell lines for 20 passages following 3 Gy and 100 mGy exposures. In conclusion, it seems that Bcl-2 and Bcl-XL expression do not alter the radiation response of MCF-10A cells in terms of immediate 53BP1 foci formation and loss.

**POS15-14. Do stress-induced premature senescence and mitotic catastrophe reflect irreversible growth arrest?** David Murray, R. Mirzayans, University of Alberta/Alberta Health Services - Cancer Care, Canada

Cytogenic gamma-H2AX nuclear foci accumulate in senescing human cells and are thought to signify irreparable DNA double-strand breaks. We have reported that early passage p53 wild-type human fibroblasts respond to ionizing radiation by exhibiting persistent gamma-H2AX foci, proliferation block, and sustained nuclear accumulation of p21 (but not of p16) when measured at late times (e.g., 1 week) post-irradiation. Moreover, p53 mutated Li-Fraumeni syndrome (LFS) fibroblasts exhibit replicative senescence coupled with extensive cytogenic gamma-H2AX foci at late passages; early passage LFS fibroblasts respond to ionizing radiation by undergoing stress-induced premature senescence (SIPS) that is associated with extensive replicative senescence instability (e.g., multinucleation), persistent gamma-H2AX foci, proliferation block, and induction of expression of p21. We have now extended these studies to solid tumour-derived cell lines with differing p53 status. We show that exposure to clinically-relevant doses of ionizing radiation triggers the development of "giant" cells, which predominantly reflect multinucleated SIPS in p53-deficient cell lines, respectively. The majority (>60%) of multinucleated giant cells that develop in p53-deficient cultures retain viability, exhibit full clearance of gamma-H2AX foci, do not express p21 or p16, and resume proliferation at late times after radiation exposure. In p53 wild-type cancer cell lines, a considerable proportion (~20%) of cells undergoing SIPS also retain viability, exhibit full clearance of gamma-H2AX foci, and replicate their DNA at late times post-irradiation.

Thus, radiation-induced growth arrest that is coupled with multinucleation ("mitotic catastrophe") and SIPS might be permanent in non-cancerous cells but reversible in tumor cells. Our results reinforce the notion that radiation-induced giant cell formation (multinucleation, SIPS) may reflect a survival mechanism for p53-deficient and -proficient cancer cells, and suggest this response as a potential target for improving cancer radiotherapy. To this end, we identify pharmacological apoptotic activators that kill giant cells. (Supported by The Canadian Association of Radiation Oncology RAZCER.)

**POS15-15. A study of radiation-induced oxidative DNA damage and its repair in mouse tissues.** Mizuki Ohno, M. Nakanishi, T. Tsuzuki, Kyushu University, Japan

The prolonged oxidative stress caused by low LET radiation is considered as a risk factor to induce alteration of genetic information. The spontaneous mutations occur in somatic cells can be a cause of cancer and other aging associated diseases. In DNA, among four bases, guanine is most susceptible to oxidation, and its simple oxidized form is 8-oxoguanine (8-oxoG). 8-OxoG is a potent pre-mutagenic lesion because it can pair with adenine as well as with cytosine during DNA replication.

To investigate the oxidative DNA damage induced by reactive oxygen species (ROS), the study was performed using 8-oxoG in both nuclear DNA and mitochondrial DNA. At 1 hour after irradiation (X-ray, 4 and 10 Gy, 1 Gy/min), we observed slightly increased 8-oxoG in nuclear DNA of intestinal cells in both villi and crypts, which contains non-proliferative cells and proliferative cells respectively. In contrast to the nuclear DNA, a significant increase of the 8-oxoG in mitochondrial DNA of villous epithelial cells (but not in cryptic cells) was observed. To clarify the contribution of the oxygen effect to this region specific response, we analyzed cellular oxygen consumption by Hypoxprobe-1. In the intestine of non-irradiated mice, the villous cells were more hypoxic compared to the cryptic cells, however, oxygen consumption in villous cells rapidly increased after radiation. These results suggest that the X-ray irradiation increases the level of 8-oxoG predominantly in mitochondria and may lead prolonged oxidative stress caused by mitochondrial dysfunction. We will also present the result of testes. We are in the process of investigation to examine the biological effects of X-ray radiation on intestines and testes using various DNA repair-deficient mice.

**POS15-16. Late radiation effects in the progenies of directly irradiated and bystander cells.** Andreyan Osipov, E. Lizunova , N. Vorobyeva, N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Russian Federation

The aim of present study was to study molecular and cellular mechanisms of initiation and formation of the late radiation effects in the progenies of directly irradiated and bystander cells. Chinese Hamster Ovary cells (CHO-K1 cell line) were used in this study. The cells were cultivated under standard conditions. The irradiation was performed using a 7-rays unit “Agat” (Russia) equipped by 60Co source (dose rate of 1 Gy/min). The DNA breaks level, apoptotic cells percentage and intracellular reactive oxygen species (ROS) content were measured using a comet assay, DNA halo assay and DCF-DA test, accordingly. It was found that exposure of cells at a dose of 1 Gy is manifested in the progenies of irradiated cells by the following effects: 7-21-th days (9-31 cell generations) – increasing in the DNA single- and double strand breaks levels, the percent of apoptotic cells, the ROS intracellular content and cell sensitivity to additional exposure (ionizing radiation, H2O2); 23-28-th days (30-42 cell generations) - normalization of the studied parameters to control values and acquisition of p16 (G1p) expression and cell resistance to additional exposure. The increased ROS content in the progeny of irradiated cells testifies in favor assumption of key role of mitochondrial dysfunction in perpetuating of radiation induced genomic instability (RIGI). The RIGI studies in the progeny of cells that received a complex of soluble factors from irradiated cells showed that incubation of non-irradiated cells in the culture medium obtained from irradiated at a dose of 1 Gy of cells does not lead to the RIGI induction. At the same time, the experimental results on cells mixture (co-culture containing 10 or 50 % of cells irradiated at a dose of 1 Gy and non-irradiated cells) showed a statistically significant increase in the ROS content in K1 cell generations as well as in all tested end-points (DNA breaks, apoptosis and ROS) from 12(4) cell generations. The manifestations
of the observed effects in the progenies of co-cultivated cells were much higher than expected. The obtained data analysis allows us to conclude that the direct radiation-induced damages of molecular and cellular structures, but not factors produced by irradiated cells, pay a main role in the RGI initiation, while in the RGI formation and maintenance the secondary bystander effect (via intercellular signaling) is involved. This work was supported by the RFBR grant (# 07-04-01009-a).


Background: Hodgkin lymphoma (HL) is a malignancy of the immune system characterized by the presence of scarcely recognized tumoral Hodgkin Reed-Sternberg cells. This lymphoma is currently treated using radiation and chemotherapy, but some patients were shown to have poor clinical outcome. To potentially determine a replacement for radiation therapy for the treatment of this disease, we analyzed multiple molecular mechanisms contributing to increased radiation sensitivity in several established Hodgkin cell lines. Material and methods: Seven Hodgkin cell lines were used: HDLM2, L428, L540, L591, L1236, KMH2, SUP-HD1. Cell lines were exposed in vitro to γCs gamma radiation. Clonogenic survival, apoptosis, multicolor fluorescence in situ hybridization (inmunofluorescence) and protein expression (Western Blot) of key proteins in DNA repair (γH2AX, p53, ATM, DNA-PKcs, Ku86, Mre11) and the telomeric shelterin complex (TRF2, TRF1) analyses were used. Results: We report evidence of increased in vitro radiation sensitivity in all cell lines, compared to ATM cell lines. However, some cell lines were resistant to apoptosis, while others underwent massive apoptosis 24 h after irradiation. Large differences were seen between each cell line in structural and numerical chromosomal abnormalities and complex chromosomal rearrangements. Radiation-induced chromosomal abnormalities were observed in some cell lines. Distinct molecular mechanisms were found to contribute to this phenotype, and each cell line exhibited its own unique response to irradiation. Even without irradiation, drastic telomere shortening with the presence of spontaneous γH2AX signals localized at telomeres was observed, along with activation of Mre11, ATM, DNA-PKcs, and TRF2; absence of functional p53 was revealed using functional yeast assays. After irradiation, each cell line exhibited different kinetics in the changes in protein expression and localization of DNA repair and telomeric proteins.

Conclusions: Overall, our study demonstrates that Hodgkin cell lines show increased radiation sensitivity, and each cell line shows distinct molecular events in response to radiation. These findings can be exploited clinically to improve the dismal clinical outcome of HL patients to the current therapy regimens.

POS15-18. Influence of radiation quality and genomic location on sister chromatid exchange frequencies. Brock Sish1, A. Eadim1, D. G. Maranon1, E. H. Goodwin1, S. M. Bailey1, 1: Colorado State University, USA 2: Colorado State University / Kromat Inc., USA

Exposure to radiation has long been suspected as a causative factor not only in the accumulation of genomic mutations, but also in cellular aging. With the elucidation of aging related molecular mechanisms, such as telomere length erosion, this theory has fallen out of prevalence. Contemporary evidence, however, has begun to establish a relationship between radiation exposure and telomeric endpoints, including accelerated telomere shortening and enhanced radiosensitivity, as well as end capping dysfunction and telomere induced genomic instability. Thus, loss of telomere function may provide an informative link between radiation exposure, molecular aging and cancer. Recombination events occurring at telomeres, or Telomere Sister Chromatid Exchange (T-SCE), have been implicated in contributing to human aging. T-SCE can result in random telomere shortening, thereby triggering premature cellular senescence if sufficient critically short telomeres are segregated into the daughter cell following cell mitosis. Here, we seek to characterize the influence of radiation quality and genomic location on sister chromatid exchange frequencies in order to establish whether radiation induced recombination events accelerate cellular aging.

Our approach utilizes two well established cytogenetic techniques: Chromosome Orientation Fluorescence in situ Hybridization (CO-FISH) and Fluorescence Plus Giemsa (FPG) staining to determine T-SCE and Genomic SCE (G-SCE) frequencies, respectively. We test the hypothesis that telomeric recombination events in human skin fibroblasts resulting from exposure to ultraviolet (UV), gamma-ray, or HZE ion particle radiation play a role in accelerating molecular aging, a finding with important implications for carcinogenesis as well.

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Mutation induction exerts a deleterious effect on genomic integrity, especially when occurred in germ cell, and thus the underlying mechanism is an important issue in the study of damage response. Most of our current knowledge of heritable mutation induction comes from studies of phenotype markers in laboratory model organism. However, these studies suffered from a serious drawback, which includes a low rate in mutation induction. To overcome this drawback, tandem repeat sequences, such as minisatellites and microsatellites, have been used during the past 20 years. These tandem repeat sequences are highly unstable i.e., show very high spontaneous mutation rates and can also be induced in vivo. Therefore, changes in mutation rate can be detected at substantially small sample sizes than those required with the conventional phenotype-based mutation studies. Microsatellite instability (MSI) is a prominent phenotype expressed by loss of mismatch repair (MMR) genes. The MMR pathway corrects mispaired nucleotides in DNA resulting from replication errors, recombination intermediates, or base mutations caused by DNA damaging agents. The genomic DNA with the alterations in the number of tandem repeat cores is good substrates for MMR enzymes, and loss of the MMR gene leads to a high rate of microsatellite instability. Another factor playing a crucial role in maintaining genomic stability is the tumor suppressor protein p53. In response to stress, activated p53 selectively transcribes a set of target genes that initiate various cellular responses including cell cycle arrest, DNA repair or apoptosis. These programs eliminate cells with damaged and mutated genomes before they become nascent tumor cells. To clarify the underlying mechanism of radiation-induced MSI, mutation rates were studied in the germ line of mismatch repair deficient msh2 or p53 deficient medaka fish. Spontaneous mutation rate in homozygous msh2 males were significantly higher than those in isogenic wild-type or p53/efficient fish. In contrast, the irradiated msh2 fish did not show any additional increases in their mutation rate compared to wild-type fish, whereas significant increase in mutation induction was observed in the irradiated p53 fish.

POS16 Individual radiation sensitivity


Purpose: Ataxia telangiectasia like disorder (ATLD) is a rare variant of ataxia telangiectasia (A-T) that share a number of clinical features and similar cellular characteristics with the hallmark of increased sensitivity to ionizing radiation. There are 16 confirmed ATLD cases, 4 in the UK, 2 in Italy and 10 in Saudi Arabia. The patients were either homozygous or compound heterozygous for 5 different mutations in the MRE11A gene located at 11q21. The 10 Saudi patients belonged to 3 independent families and represent the largest ATLD cohort. Genetically, the patients were homozygous for a novel nonsense mutation, a G to C transversion at nucleotide 13 of the MRE11A gene. The resulting Trp to Cys amino acid change at position 210 destabilizes the Mre11/Rad50/Nbs1 (MRN) complex involved in different DNA healing mechanisms. The high number of Saudi ATLD patients with this particular mutation would suggest noticeable frequency of carriers in the population. The aim of this study was to
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assess the frequency of this mutation and its impact on radiosensitivity.

Subjects and methods: A cohort of 428 Saudi individuals was studied in addition to two new ATLD patient. The 630G>C mutation was genotyped by direct sequencing. Clonogenic survival assay was used to measure radiosensitivity that was characterized by the surviving fraction at 2 Gy (SF2).

Results: Two individuals with heterozygous G630C mutation were found, giving a G/C genotype frequency of 0.5% and a mutant C allele frequency of 0.2%. The ATLD patient was homozygous for this mutation. Survival curves of 100 fibroblast cell cultures showed that the two heterozygous individuals have intermediate radiosensitivity (SF2 = 0.34 and 0.36 compared to an average of 0.33; range 0.15 to 0.50, SD = 0.10). The patient’s cells were hyper-radiosensitive (SF2 = 0.10).

Conclusions: MRE11A G630C mutation is present in our population with a heterozygous carriers’ frequency of 0.5%, however, the exact frequency requires larger study. The frequency could be higher in geographically isolated consanguineous families. Individuals harboring this mutation may be at higher risk of developing severe reactions to ionizing radiation. Premarital, preimplementation and prenatal screening for MRE11AG630C mutation could be useful to limit the risk of genetic diseases. Supported by KFSH&RC grants 2000 031.


To understand the role of microRNAs (miRNA) in regulation of radiation-induced gene expression and to help define potential radiation-inducible targets, miRNA expression was studied in wild type p53 LNCaP and p53-mutated PC3 and DU145 cells. Previous analyses of miRNA microarray showed fractionated radiation induced more differentially expressed genes compared to single dose radiation in PC3 cells, whereas as in LNCaP cells single dose radiation induced more genes. Methods: Cells were exposed to 5 Gy and 10 Gy either as a single dose (SD) radiation or multi-fractionated (MF) radiation. Microarray analyses were done using human Agilent miRNA Microarray Kit (V2). Data were analyzed using Gene Spring software. Validation of the miRNA expression and gene expression of miRNA targets was evaluated by qRT-PCR. Results: Microarray analyses revealed that radiation differentially expressed 84, 68 and 8 miRNAs with high confidence (|&gt;1.5 fold change, p&lt;0.05) in LNCaP, PC3 and DU145 cells, respectively. MF radiation affected more miRNAs than SD radiation in all cell lines. Oncmir-R-17-92 cluster miRNAs were significantly downregulated in p53 positive LNCaP cells but not in p53-mutant PC3 and DU145 cells. &lt;#sp;The MF radiation resulted in greater radiation induced apoptosis: Among the three cell lines, significant baseline differences were seen for mir-19a and b, and the highest base line expression levels for mir-19a and b were in PC3 cells. miRNA microarray analysis revealed that the predicted target of mir-19a alpha-kinas2 (ALPK2) significantly upregulated in LNCaP cells and this was not altered in PC3 and DU145 cells where mir-19a was not altered by radiation. Conclusion: Among the three prostate carcinoma cell lines, single dose as well as fractionated radiation downregulated mir-17-92 cluster of miRNAs in p53 positive radiosensitive LNCaP cells, suggesting that pharmacological inhibition of the miRNA-17-92 cluster may provide a new therapeutic strategy for radio resistant cancers with mutated p53.

POS16-03. Radiosensitivity of the HIV-positive individuals before and after ARV treatment. Ans Baeyens1, E. Mafu2, O. Herd1, A. Veal1, J. Slabbert1, 1: NRF-Tshemba LABS; WITS University, South Africa 2: WITS University, South Africa 3: University of Ghost, Belgium

Introduction: Radiosensitivity in relation to HIV status is an essential area of research to better understand the implications of radiation medicine in South Africa. This as the prevalence of HIV infection is high. Possible change in radiosensitivity to ionizing radiation with HIV status has significant consequences for both radiation workers and radiation therapy patients. Clinical observations show that HIV positive cancer patients present more adverse side effects of radiotherapy than HIV negative patients. Recently, investigations at the chromosomal level showed that HIV positive individuals - before Anti-retroviral (ARV) treatment - have a higher radiosensitivity than HIV negative individuals. With ARV treatment, the life-span of an HIV positive individual can be increased to ages of increased cancer susceptibility. The aim of this study is to compare chromosomal radiosensitivity of HIV positive individuals before and after ARV treatment. Spontaneous MN frequencies are also analysed to investigate chromosomal instability in HIV patients.

Methods: The G0 microcirus assay was performed on blood samples from healthy HIV negative donors, HIV infected donors before and after starting ARV treatment. For this, lymphocytes were exposed in vitroto 2 Gy and 4 Gy of 6MV X-rays. The lymphocytes were cultured for 70h and Cytochalasin B was added at 24 hours. After 3 days the yield of binucleated lymphoblasts and micronuclei were scored by an automated image analysis programme, MNscore, of Metasytems. Sham-irradiated samples from each donor were also analysed. A pan-centromeric FISH analysis was applied on sham-irradiated samples of HIV positive individuals before and after ARV treatment to observe inherent chromosomal instability.

Results: HIV positive individuals on ARV treatment showed higher MN values compared to HIV negative ARV. Both cohorts showed greater radiosensitivity than HIV negative individuals. FISH analysis revealed no difference in numbers of background micronuclei positive MN between HIV negative, ARV negative and ARV-treated patients.

Conclusion: ARV treatment possibly increases chromosomal radiosensitivity in HIV positive individuals. No difference was observed in spontaneous MN.


Analysis of mature biomarkers of radiation exposure could potentially be used for triage and treatment decisions for acute radiation effects such as after a radiological accident as well as for long term assessment of late effects such as cancer and cardiac disease. Partial body biodosimetry is required after a radiological exposure to quantify and determine the absorbed doses. The in vitro cytokinesis-block micronuclei assays (CBMNI) is one of the most reliable and widely used radiogenic biomarkers for assessment of radiation exposure. Biological dosimetry based on micronuclei (MN) frequencies has been shown useful for the detection and quantification of exposure to ionizing radiation. However, direct data on the persistence of radiation-induced MN in human peripheral blood lymphocytes (PBLs) in vivo is limited. In the present study we analyzed MN frequencies in lymphocytes of radiotherapy (RT) patients undergoing partial body irradiation of the pelvic region. Blood samples were acquired from RT volunteers treated at the Radiation Oncology Department at the Columbia University Medical Center. To date, we analyzed samples from 8 cancer patients who received a radiotherapy treatment of 1.8-2 Gy per dose to the pelvic region. The calculated mean total blood dose for these patients was in the range 2 to 4 Gy. Samples were acquired at 6 time points: before treatment; immediately after the first fraction; immediately before the second fraction; and immediately after, 2 days after, and 7 days after the final fraction. For the CBMN assay, whole blood was cultured in complete RPMI 1640 medium containing phytohemagglutinin to stimulate mitosis followed by the addition of cytochalasin-B to block cytokinesis and obtain binucleate cells. Present results suggest that quantification of MN in radiotherapy patients undergoing partial body irradiation is a suitable biomarker for evaluation of absorbed radiation dose. The preliminary analysis of the results indicate that the MN assay can provide measurable quantitative data at times up to 7 days post exposure after doses of 2 to 4 Gy.

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POS16-05. Association between DNA repair competence assay, classic or molecular cytogenetics, cancer incidence and exogenous or ontogenetic factors influence in human monitoring. Antonina Cebulska-Wasilewska, Institute of Nuclear Physics, PAS, Poland

Aim of studies: The aim of our human monitoring studies performed on subjects from various cities and countries was to investigate if occupational exposures to genotoxic agents can alter cellular radiosensitivity or alter health risk.

Results: Exposure to pesticides, mercury ions, polyacrylic hydrocarbons (PAHs) or ionizing radiation have significantly elevated levels of cytogenetic damage. The follow up studies revealed
significantly increased risk of cancer in the group of subjects characterized by the highest levels of chromosomal damage. Our research also displayed that results of DNA repair competence assay, with a use of challenging dose of X-rays and the detection of induced DNA damage by the SCGE assay, have correlated to levels of induced chromosomal damage. Results from studies on the influence of exposure to environmental PAHs on the repair of DNA damage induced by radiation, have shown a strong variability between donors and significant decrease of the DNA repair efficiency in exposed subjects. Significant decreases of repair efficiencies were also observed when those groups were stratified first according to various genotypes for genes, encoding enzymes involved in the process of bio-transformation (CYP1A1[Val/Val], GSTM1, NAT2) or DNA repair (NPAT, XRCC1) and then to levels of occupational exposures. Results of our studies have also shown the higher levels of chromosome aberrations frequencies and significant reduction of cellular repair efficacy, that were observed in a various groups of cancer patients when compared to healthy subjects.

Conclusion: Presented results point toward that ontogenetic or exogenous factors via alteration of the DNA repair processes can rise levels of chromosome aberrations and result in increased risk of cancer.

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POS16-06. Radiation responses in ex vivo irradiated blood lymphocytes correlated with late normal tissue response to breast radiotherapy, Melvin Chua, Health Protection Agency, UK

Background: To test the association of DNA double-strand break (DSB) repair, radiation-induced apoptosis (RIA) and chromosomal radiosensitivity in ex vivo irradiated blood lymphocytes with late-onset normal tissue responses following breast radiotherapy.

Methods: Breast cancer patients with minimal (controls) or marked late radiation-induced changes (cases) were retrospectively selected. DSB were quantified by γH2AX/53BP1 co-immunostaining 0.5 and 24 hours after exposure of unstimulated lymphocytes to 0.5 and 4 Gy X-rays respectively. Apoptosis assays were performed in lymphocytes 48 hours after 8 Gy X-rays using a carboxyfluorescein fluorochrome inhibitor of caspases (FAM-VAD-FMK) apoptosis detection kit. Exchange (dicentrics and rings) and deletion (excess acrocentric fragments) type chromosomal aberrations were scored in lymphocyte metaphases following exposure to 6 Gy X-rays.

Results: Despite similar foci levels at 0.5 hour in cases (n = 7) and controls (n = 7), foci levels 24 hours after 4 Gy differed significantly between the 2 groups. The cell were 12.8 % vs 10.2 % in controls (P = 0.004). Increased chromosomal radiosensitivity was also observed in cases (Aberrations per cell 5.84 in cases versus 3.79 in controls, p = 0.001) with exchanges and deletions contributing equally to the difference between cases and controls. RIA of lymphocytes did not differ between cases and controls (% of RIA cells were 37.2% in cases versus 34.6% in controls, p = 0.563). Tests of association indicated that a stronger intra-individual association between residual foci levels with formation of deletions (Spearman’s R = 0.589, p = 0.027) than with exchanges (R = 0.367, p = 0.197) in lymphocytes from the same patients. Separately, residual foci levels were not correlated with RIA levels in lymphocytes from the same patients (R = 0.224, p = 0.81).

Conclusions: Higher levels of exchange type aberrations observed among radiosensitive breast cancer patients suggest a role for DSB misrepair, in addition to residual damage, as determinants of late normal tissue damage. Correlation of residual foci levels with deletion type aberration yields in the same cohort confirms their mechanistic linkage. Lack of association between residual foci levels and RIA suggests that these radiation responses are possibly independently regulated.

It is well known that the biological effects of high LET radiations are greater than those of low LET radiations. Previous studies have shown LET dependent effects on various human cells for cell killing, micronucleus formation, mutation frequency, mutation spectrum. It has been assumed that the RBE of protons is equivalent to that of photons. In this study, the differences in mutation induction and mutation spectrum between proton beams and X-rays in human cells were investigated. Human cells were irradiated with X-rays or proton beams, generated by Wakasa wan Energy Research Center Multipurpose Accelerator System with Synchrotron and Tandem (W-MAST) at the wakasa wan energy research center (WERC) in Japan. Cell killing effect was measured by the colony formation assay. Mutation induction in the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus was detected to measure 6-thioguanine resistant colonies. The mutation spectrum of the deletion pattern of exons of induced mutants was analyzed by the multiplex polymerase chain reactions. Cell killing effect was almost the same between proton beams and X-rays. These findings suggest that there are quantitative and qualitative differences in mutation induction between proton beams and X-rays even if the RBE values are similar.

POS16-08. Association of the caspase-7 Asp4Glu polymorphism with increased risk of adverse reaction in the gastrointestinal tract after pelvic radiotherapy in cervical cancer patients. Takashi Imai, A. Ishikawa, T. Suga, Y. Otsuka, National Institute of Radiological Sciences, Japan

Caspase-7 (CASP7) is one of the effector caspases responsible for cleaving intracellular substrates involved in promoting the apoptotic phenotype. Interestingly a role for CASP7 in cell cycle progression at mitosis has also been suggested. Additionally, CASP7 polymorphisms are associated with increased risk for several types of cancer such as breast cancer, endometrial cancer and lung cancer. However little is known about the association of CASP7 with individual radiosensitivity. Therefore, in this study, we analyzed the association of functional polymorphisms in CASP7 with the risk of an adverse reaction in the intestinal tract after radiotherapy. A total of 208 cervical cancer patients who had been treated with pelvic radiotherapy were genetically analyzed. Early gastrointestinal reactions were graded using the National Cancer Institute Common Toxicity Criteria, and patients were dichotomized into a lower-grade (LG) group (Grade = 0 or 1, n = 150) and a higher-grade (HG) group (Grade ≥ 2 or 3, n = 58). Three SNPs with minor allele frequencies of more than 5% in our healthy control samples, rs12415607 (C→T), rs11593766 (T→C) and rs2227310 (C→T), were subjected to genotype and haplotype analyses in the cervical cancer patient cohort.

Among these 3 SNPs, only rs11593766 was found to be associated with a risk of adverse reaction in the intestinal tract. The frequency of the G allele of this SNP was significantly higher in the HG group (18%) than in the LG group (9%; odds ratio [OR], 2.24; 95% confidence interval [CI], 1.18-4.21; P = 0.016). The GG and TG genotypes of rs11593766 were significantly associated with an increased risk of adverse reaction in the intestinal tract (OR, 2.15; 95% CI, 1.04-4.44; P = 0.038). No significant haplotype including rs11593766 was detected. Diploidy types of NPAT-ATM and AURKA have also been reported to contribute to the risk of adverse intestinal reaction after radiotherapy, and we found that patients who had two or more of the NPAT-ATM, AURKA or CASP7 risk genotypes showed higher risk than other patients (OR, 3.60; 95% CI, 1.83-7.19; P = 0.0007).

Our results suggest that, the rs11593766 GG and TG genotypes (Glu/Glu or Asp/Glu) of CASP7 might contribute to the individual risk of adverse intestinal reaction after radiotherapy.

POS16-09. Molecular biomarkers for radiation sensitivity in human peripheral blood mononuclear cells. Summerer Isolde1, K. Ause2, Z. Hore3, A. Michel, M. Sommer, Heidelberg, Munich, Germany 1: Universitaetsklinikum Heidelberg, Germany 2: Institute of Radiation Biology/Helmholtz Center Munich, Germany 3: Research Unit of Radiation Cytogenetics/Helmholtz Center Munich, Germany

The main challenge in radiotherapy is to achieve maximum tumor depletion with minimum damage to normal tissue. Due to individual differences in the response to ionizing radiation it is particularly
important to detect radiation hypersensitivity at the earliest possible time point in the therapy protocol, i.e. after the first day of therapy. This study presents two approaches to identify molecular biomarkers for radiation sensitivity in human peripheral blood mononuclear cells (PBMCs).

To analyze the cellular radiation response by DNA-double strand break (DSB) repair we have developed a modification of the classical DNA repair foci assay. For DSB-detection and -quantification we established the Promega® Ligation Assay™ (PLA), which provides high specificity through visualization of interaction of DSB repair proteins.

PLA-results obtained from human PBMCs of healthy donors give evidence for a dose dependent increase in the number of signals in the biological relevant dose range up to 5 Gy. In addition, 20 minutes after irradiation with gamma-rays. Quantification of DSBs in PBMCs, isolated from radiotherapy patients at four different time points over the period of the radiotherapy, indicates variance in DSB induction after in vitro irradiation amongst the different time points.

As an alternative parameter we determined miRNA expression levels in PBMCs from healthy human donors 24 h after gamma-irradiation on low-density arrays. Out of 378 tested miRNAs we identified 22 to be significantly changed after irradiation with 1 Gy. Expression profiles of the radiation modulated miRNAs showed sex-specific clustering. Sex-specific regulation of five miRNAs could be confirmed by single primer assays for selected miRNAs differentially expressed after irradiation.

Based on the data mentioned above we plan to conduct an in vivo trial to determine if the clinical performance of the assays in analyzing the DNA damage response in patients during standard radiation therapy.


Genome maintenance is crucial to prevent DNA damage that can contribute to ageing and cancer. The mammalian cell DNA is subject to damage caused either spontaneously by oxygen radicals or during DNA replication, or by external agents such as ultraviolet or ionizing irradiation (IR). IR is a stress that induces a range of DNA lesions to which cells respond through multiple signalling pathways.

IR-induced DNA Double-Strand Breaks (DSBs) activate ATM/Checkpoint/53 pathway, cells then enter either cell cycle arrest or apoptosis, dependent on which downstream pathways predominate in the specific cell type and environment. The fate determination depends on the ability of p53 to induce the transcription of genes such as the cyclin-dependent kinase inhibitor CDKN1A (p21) and the pro-apoptotic bBBC3 (PUMA). We have developed a gene expression assay in peripheral blood lymphocytes to measure accurately ATM/Checkpoint/53 pathway activity (1), an essential cancer protection pathway. To determine the full range of ATM/Checkpoint/53 pathway activity in humans, we are finishing analysis of a large panel of healthy donors and a complete set of data will be presented.

The response to IR is probably regulated in part by miRNAs and recently some have been reported as potential biomarkers of radiation exposure (2). We hypothesized that individual variability in ATM/Checkpoint/53 pathway activity depends in part on miRNAs. To investigate miRNA radiation responsiveness, we used nCounter miRNA Expression Assay and miRXplore Microarrays in human and mouse respectively. 2h after IR exposure (dose range used from 0.5 Gy up to 8 Gy) miRNA modulation was observed including mir-195 and the oncomir mir-21. Interestingly, mir-195 which has been previously described as differentially expressed between patients with the genetic syndrome Ataxia Telangiectasia (AT) and healthy donors (3), is significantly up-regulated in human and mouse. The influence of genetic background on the level of mir-195 expression is currently being assessed. These results may have implications for the prediction of radiation sensitivity and cancer susceptibility.


POS16-11. New method for determination of cell repair mechanism at low radiation doses. Agata Kowalska1, K. Czerski1, S. Zajac2, K. Kryszczak3, M. Kaczmarek1; 1: Institute of Physics, Szczytna, Poland; 2: Pomeranian Medical University, Poland

Research on response mechanisms of human cells and corresponding chromosomal aberrations to different radiation types, especially at low doses enables to determine efficiency of repair mechanisms[1]. Here, we propose a new method to study repair mechanism based on deviations from the Poisson distribution of observed chromosomal aberrations.

The method has been applied to samples of peripheral blood irradiated by gamma rays of Co60 with doses ranging between 0 and 2 Gy. The donors of blood samples were a group of 15 young healthy volunteers. A standard method of lymphocyte extraction has been used and the number of chromosomal aberration (dicentrics, acentrics and rings) were scored under optical microscope.

For a dose of 2 Gy the Individual Damage Factor (IDF) of all patients and its mean value (Average Damage Factor ADF) have been determined. The standard deviation characterizing scattering of IDF values around the ADF has been found to be significantly smaller than that expected for Poisson statistics[2]. The underestimation of standard deviation can be correlated to a number of repaired cells and thus a corresponding repair factor (RF) could be defined as a ratio between damaged and repaired metaphases. Similarly, we could obtained RF value for dose-effect curves using the chi-square analysis. The RF values estimated for the ADF analysis and for the dose–effect curve amount to 2.4 and 2.1, respectively, i.e. about 50% of damaged cells were repaired.

The same method has been applied to results recently published by the Warsaw group [3] where the results for gamma irradiation were compared to those obtained for C12. Whereas the RF value of 2.1 for gamma rays confirms our results, the RF for C12 counts 1.48. The latter, smaller RF value for heavy ion irradiation can be explained with high occurrence of double strand breaks which decreases the capacity to repair. Nevertheless, even in that case, about 30 % of damaged cells could be repaired.


POS16-12. MicroRNA expression determines the radiation sensitivity of endothelial cells. Anna Kraemer, A. Natasa, A. Michael John, M. Simon, Helmholtz Center Munich, Institute of Radiation Biology, Germany

We have demonstrated that the sensitivity of endothelial cells to ionizing radiation is dependent upon the processing of microRNAs. Due to endothelial cell killing and the resultant normal tissue damage only limited doses of radiation can be used in tumour therapy. Therefore, a better understanding of the processes governing radiosensitivity of endothelial cells would be desirable for improved radiation therapy. As the expression of microRNAs (miRNAs) is regulated in the cellular stress response after ionizing radiation, we tried to determine whether miRNA-controlled gene silencing influences the radiation response in endothelial cells.

We identified a phenotypic effect from a global inhibition of the miRNA response to irradiation. This was achieved by siRNA-mediated downregulation of either Ago2 or Dicer, two crucial components of miRNA processing. Cells depleted for Dicer or Ago2 showed decreased clonogenic survival after irradiation. A further analysis of cell cycle distribution after irradiation hints to a defect in G1 checkpoint activation in Dicer or Ago2 knockdown cells. Subsequently, we conducted a screen to identify which of the miRNAs are responding to ionizing radiation. This was performed by TaqMan-based low density array technology. Four hours after irradiation we found 7 miRNAs to be upregulated and 8 to be downregulated. After 24 h 5 miRNAs were up- and 19 downregulated. The effects for 7 of these miRNAs on survival and cell cycle regulation were individually measured by modulating their activity through transfection with specific miRNA inhibitors (anti-miRs). Out of 7 analyzed, 2 miRNAs showed a significant effect on the cellular response to irradiation, showing an enhanced apoptosis rate measured by caspase-3 activity and quantification of subG1 population.
However, the cell cycle distribution after irradiation was not affected after miRNA inhibition. Currently, work is in progress to identify miRNAs targeted by miRNAs by a bioinformatic prediction of target genes and through comparison of proteomic changes in control- and anti-miR-transfected cells by 2D DIGE. Taken together, our data suggest that miRNA-mediated gene silencing has an essential function in radiation response. In the future, targeting miRNAs could be an important tool to sensitize tumours during radiation therapy.

The research leading to these results is supported by the BMBF Grant NUK007C (KVSF).

POS16-13. Quantitative analysis of epigenetic alterations after irradiation. Belinda Manzerek, A. Fink, University of Munich, Department of Radiation Oncology, Germany

It has been hypothesized that radiation-induced epigenetic alterations may be causally involved in radiation-induced carcinogenesis. To investigate epigenetic variations in radiation-induced epigenetic alterations, we screened several EBV-immortalized lymphoblasts from young lung cancer patients, the radiation sensitivity of which was characterized with survival assays. Furthermore as a positive control for radiation sensitivity, a cell line from an ataxia telangiectasia patient, was used, while the healthy brother served as a non-sensitizing control. To investigate global alterations in histone modification patterns after irradiation, we irradiated the cells with 10 Gy and prepared whole cell extracts after 1 h and 24 h. The samples were screened for 5 different histone modifications that had been observed to show alterations after irradiation in previous analyses with a control cell line. Since the histone modifications, if they occurred, ranged in an interval of about ± 20–40%, it was essential to establish a reliable quantitative western blot method. We ascertained that the whole protein amount of the irradiated samples differed less than 15% from their controls. For each point in time adequate control samples were used, a standardized western blot procedure was established and the antibodies were tested for substrate specificity by peptide competition assays. The chemiluminescence reaction, caused by the secondary antibody, was recorded by a highly sensitive CCD-camera. A minimum of four western blots for each cell line, incubation time, and histone modification were prepared from two independently produced protein extracts.

We observed inter-individual variations in the different cell lines, as not always the same histone modifications were found to have been altered significantly. If an alteration in the histone modifications occurred, it was most often only detectable after one hour of incubation time, with the effect increasing over 24 hours. The variable behaviour in the epigenetic reactions of the different cell lines after irradiation did not appear to be correlated with the radiation sensitivity status of the cell line.

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POS16-14. Chromosomal radiosensitivity in South African breast cancer patients before and after radiotherapy. Xanthele Muller1, O. Herd2, T. Mahlengu2, A. Cairns1, A. Vial1, J. Slabbert1, A. Baryens1, 1: iThemba LABS, WITS University, South Africa 2: WITS University, South Africa 3: WITS University, Donald Gordon Hospital, South Africa 4: University of Gent, Belgium 5: NRF-iThemba LABS, South Africa 6: NRF-iThemba LABS, WITS University, South Africa

Introduction: Several European studies have shown that breast cancer patients are, on average, more sensitive to ionizing radiation than a group of healthy women. When comparing the in vitro radiotherapy-induced MN results in patients before and after therapy, it was observed that the total MN yields scored for the 2 Gy and 4 Gy doses were significantly lower in the post-therapy group. In the sham-irradiated samples the MN results were significantly higher in the post-radiotherapy group, as can be expected.

In order to examine the role of drug-IR schedule we studied the radiation response of two tumour cell lines (A549 and SNB-19) in a simultaneous treatment with the novel Hsp90 inhibitors (NVP-AUY922 or NVP-BEP800) and ionizing radiation (IR). To this end, cells were irradiated in the drug-containing medium and kept thereafter up to 48 h after irradiation. Cellular response was assessed 30 min, 24 h and 48 h after drug-IR treatment by colony survival assay, induction and kinetics of DNA damage and repair, cell cycle distribution and expression of several survival and cell cycle-related proteins.

A 30-min exposure to either NVP-AUY922 or NVP-BEP800 did not influence the radiosensitivity of examined cell lines. Likewise, no changes in the degree of DNA damage, cell cycle distribution or expression of marker proteins were detected after short-term drug treatment. Longer incubation with either drug strongly affected the viability of both cell lines. The intracellular ATP content dropped from 80% at 24 h incubation to 20% and 40% at 48 h exposure to NVP-AUY922 in case of A549 and SNB-19 cell lines, respectively. Furthermore, SNB-19 cells plated 48 h after combined drug-IR treatment showed a strongly reduced colony-forming ability, in comparison to untreated control. This particular cell line also exhibited an increased DNA damage and protracted DNA damage repair. In addition, Hsp90 inhibition combined with irradiation caused a depletion of the anti-apoptotic proteins Akt and Raf-1 in both cell lines, as well as survivin in A549 cells. Finally combined drug-IR treatment induced S phase depletion and G2/M arrest.

The above findings prove the importance of drug-radiation schedule in cancer therapy. The tumour type-specific radiosensitization correlated with the depletion of several anti-apoptotic and cell cycle-related proteins, elevated DNA damage and repair protraction, which might have been responsible for the observed apoptosis and cell cycle arrest.

CONCLUSION: The results demonstrate that the group of South African breast cancer patients was on the average more sensitive to ionizing radiation than a group of healthy women. When comparing the in vitro radiodilation-induced MN scores in patients before and after radiotherapy, it was noted that the MN yields after 2 Gy and 4 Gy in vitro irradiation, were higher in the post-therapy group compared to the pre-therapy group. The MN results obtained in the sham-irradiated samples were much higher in the post-therapy group.

POS16-15. Sequence of drug-radiotherapy treatment influences the tumour-radiosensitizing properties of the novel HSP90 inhibitors NVP-AUY922 and NVP-BEP800. Natalia Niewidok1, L. Wack1, L. Sing1, A. Katzer2, Y. L. Soukhouroukou2, M. Plentje1, C. S. Djuricova3, B. Polat1, 1: Department of Radiation Oncology, University Hospital Wuerzburg, Germany 2: Lehrstuhl für Biotechnologie und Biophysik, University Wuerzburg, Germany

To this end, cells were irradiated in the drug-containing medium and kept thereafter up to 48 h after irradiation. Cellular response was assessed 30 min, 24 h and 48 h after drug-IR treatment by colony survival assay, induction and kinetics of DNA damage and repair, cell cycle distribution and expression of several survival and cell cycle-related proteins.

A 30-min exposure to either NVP-AUY922 or NVP-BEP800 did not influence the radiosensitivity of examined cell lines. Likewise, no changes in the degree of DNA damage, cell cycle distribution or expression of marker proteins were detected after short-term drug treatment. Longer incubation with either drug strongly affected the viability of both cell lines. The intracellular ATP content dropped from 80% at 24 h incubation to 20% and 40% at 48 h exposure to NVP-AUY922 in case of A549 and SNB-19 cell lines, respectively. Furthermore, SNB-19 cells plated 48 h after combined drug-IR treatment showed a strongly reduced colony-forming ability, in comparison to untreated control. This particular cell line also exhibited an increased DNA damage and protracted DNA damage repair. In addition, Hsp90 inhibition combined with irradiation caused a depletion of the anti-apoptotic proteins Akt and Raf-1 in both cell lines, as well as survivin in A549 cells. Finally combined drug-IR treatment induced S phase depletion and G2/M arrest.

The above findings prove the importance of drug-radiation schedule in cancer therapy. The tumour type-specific radiosensitization correlated with the depletion of several anti-apoptotic and cell cycle-related proteins, elevated DNA damage and repair protraction, which might have been responsible for the observed apoptosis and cell cycle arrest.

POS16-16. The prediction of radiotherapy response “in vitro” for cervical cancer patients? Siobhan O’Grady, Focas Institute (Dublin Institute of Technology), Ireland

Introduction: Tumour response rate and duration of cervical carcinoma treated by radiotherapy vary greatly between individuals. Individual prediction of tumour response to treatment could allow individualised treatment by choosing the best treatment option and/or treatment adaptation. This study is investigating the prediction of radiotherapy response in vitro for cervical cancer patients.

Materials and Methods: Whole blood lymphocyte cultures were irradiated in vitro with low doses of gamma radiation. The cultures were used for the G2 chromosomal radiosensitiveness assay to assign each patient sample with an intrinsic radiosensitivity score. Radiosensitivity data will be created and analysed for each patient.

Conclusion: The results suggest that miRNA-mediated gene silencing has an essential function in radiation response. In the future, targeting miRNAs could be an important tool to sensitize tumours during radiation therapy.
identify potential biomarkers of cervical carcinoma response to radiotherapy.

Results: The expected result is that each cervical cancer patient, based on their radiation sensitivity score, will show an individual response to radiation for each grade of malignancy. On collecting the Radiosensitivity analysis, the expectation is that variations in individual responses will display a relationship between results & the possibility to allow individualized treatment.

Conclusion: This study will demonstrate the potential of testing individual patients for radiation response prior to therapy, thus resulting in more tailored treatment plans with higher efficacy.

POS16-17. Chromosomal radiosensitivity of lymphocytes of prostate cancer patients and healthy donors analysed by FISH. Ekkehard Pomplun1, S. Schmitz2, K. Brzozowska2, M. Pinkawa1, M. Ehle1, R. Kriehuber2, 1: Forschungszentrum Jülich, Germany 2: Forschungszentrum Jülich, Department of Safety and Radiation Protection, Germany 3: RWTH Aachen, University Hospital, Department of Radiotherapy, Germany

Background: It is known that about 10 % of cancer patients show severe clinical side effects during and after radiotherapy due to enhanced sensitivity to ionizing radiation. Identification of those radiosensitive individuals by an in vitro assay before onset of treatment would be of great impact for successful radiotherapy.

In this study we compared the radiosensitivity of the chromosomes 2, 11 and 17 in prostate cancer patients with and without severe side effects after radiotherapy and in age-matched healthy donors (control cohort). The chromosomal radiosensitivity of peripheral blood lymphocytes from radiotherapy patients was used as predictive parameter for clinical side effects.

Material and Methods: Each cohort consisted of at least 10 donors. PBL were irradiated ex vivo with 0.5, 1 and 2 Gy (Cs137 0.8 Gy/min) in the G0-Phase of the cell cycle. We analyzed the radiosensitivity of the chromosomes 2, 11 and 17 by scoring of 100 FISH painted metaphases for each dose point. Statistical analyses were performed by non-parametric Mann-Whitney test, by test of variances (ANOVA) and Chi-square goodness-of-fit test at a significance level of 0.05.

Results: Analysis of the overall aberration yield revealed no significant differences between any donor groups. However, variance analyses showed significant differences between the patient’s cohort and healthy donors for chromosomes 11 and 17 for all doses analyzed. In contrast, this was not true for chromosome 2. Furthermore, good correlations between chromosomes sizes (DNA content) and aberration yield were found.

Conclusion: The cohort of prostate cancer patients can be distinguished from healthy donors due to variances of the aberration yields of the chromosomes 11 and 17. These chromosomcs might be potential cytogenetic biomarkers for prostate cancer patients in clinical studies.

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POS16-18. Alternative changes of lymphocytopoiesis of cancer patients are retained during radiotherapy. Alexei N. Shoutko, L.P. Ekimova, V.P. Sokurenko, K.C. Matyurnin, M.A. Karamullin, Research Centre for Radiology and Surgical Technologies, St.-Petersburg, Russian Federation

Introduction. The phases of “turbulent” lymphocytopoiesis of cancer patients reported by us earlier [Shoutko et al., ERR2002, 2005, 2010] are never been taken into account in practice of cytotoxic treatment. The present study was performed to elucidate the question, if the conventional radiotherapy being started at opposite phases of lymphocytopoiesis is able to flatten the difference of following blood cell kinetics.

Methods: Eleven patients with nonoperable cancer of oropharyngeal zone were treated with conventional regional radiotherapy (3 Gy daily×11). The blood samples were drawn at the start of irradiation and during 40 days after. The subsets of MNC with different markers (CD34+, CD133+, VEGFR+, TdT+, CD4+, CD8+, CD131, CD3) were measured on FACScan flow cytometer (Beckton Dickinson) using AB produced by BD and DAKO. Results. According the start values of CD34+cells and common lymphocytes content in blood all patients were divided on two groups I (n=5) and II (n=6) with different Mm and variation’ coefficient (CV).In group I CD34+ = 0.12±0.077 %, CV=0.29; Lph=2.3±0.4 (PBL) of radiotherapy. In group II CD34+=0.35±0.17 %, CV=0.49; Lph=1.3±0.19 <107/ml k 4.9. Bringing the start’ ratios “Lph / every one of investigating signs” to scale 1.0, we evaluated the main relative trends for such ratios in both groups. The unified ratio for signs CD34+, CD133+, VEGFR+, TdT+ at 2nd and 4th weeks were diminished from 1.0 to 0.47 and 0.28 in group I, but increased to 3.6 and 1.9 in group II with p<0.03 and 0.005 correspondently. At the same time the unified signs CD4+, CD8+, CD34+, CD34+ and CD3 were increased only to 1.7 and 1.5 in group II and slightly diminished in group I, namely to 0.7 and 1.1 at 2nd and 4th weeks. Nevertheless the difference between two groups for matured cells were retained (p=0.004 and 0.001 at 2nd and 4th week).

Conclusion. The regional radiation therapy does not break the turbulent regime of cancer patient’s lymphocytopoiesis.

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POS16-19. DNA repair focus formation in lymphocytes of breast cancer patients undergoing radiotherapy. Alexandra Somsediková, Cancer Research Institute, Slovak Academy of Science, Slovak Republic

Radiotherapy is accompanied by induction of acute or postponed toxic side effects in normal tissues. Current radiological research efforts take aim at individualizing radiation treatment. Predictive assays would enable adjusting radiation therapy for radiosensitive patients. DSB is most severe damage induced by ionizing radiation. Variability in DSB repair is an important factor in determination of individual response to radiation treatment. Several proteins form DNA repair foci at the locations of DSB. We analyzed 53BP1 and gamma-H2AX DNA repair foci in lymphocytes from breast cancer patients with the final goal to develop new sensitive assay for prediction of radiosensitivity of patients. Breast cancer patients were subjected to local radiotherapy with fractionated doses using linear accelerator. The acute reactions of patients were classified according to the RTOG criteria. Blood samples were taken before and at various time points during radiotherapy, as well as one month after radiotherapy. The data on one year after radiotherapy is going to be obtained. DNA repair foci were analyzed by fluorescence (Metafer system)and confocal microscopy. 27 patients analyzed so far belonged to the Grades 0-1 with normal radiosensitivity. Radiation-induced foci were observed 24 h after the first fraction of 2 Gy. Afterwards, the levels of DNA repair foci were rather stable during the whole course of radiotherapy. These data suggest that lymphocytes containing foci are efficiently eliminated by the immune system in the Grade 0-1 patients. The data indicated also that background level of 53BP1 foci in breast cancer patients is higher than in healthy persons. The level of spontaneous 53BP1 foci depended on age and stage of cancer at the moment when radiotherapy began. Radiation-induced 53BP1 foci 1 month after completion of radiation therapy correlated with both spontaneous 53BP1 level before radiation therapy and stage of cancer. The acute and postponed reactions will be correlated with dose responses and kinetics of DNA repair foci in a larger group of patients.


Inter-individual variation in G2 chromosomal radiosensitivity, measured on the basis of radiation induced chromatid breaks in the subsequent metaphase, is of particular interest since increased yield of chromatid breaks has been reported as a marker of individual radiosensitivity and predisposition to cancer. Increased G2 chromosomal radiosensitivity has been detected in several cancer susceptibility syndromes, mostly notatixia telangiectasia (AT) as well as in a high proportion of patients with a variety of cancer. These findings suggest that an increased yield of chromatin breaks could be a marker of radiosensitivity predisposing genes whose role is to respond to DNA damage. Alterations in genes that have evolved to facilitate DNA damage recognition using signal transduction pathways to activate cell cycle arrest and preserve chromosome integrity could underlie the increased G2 chromosomal radiosensitivity. Our hypothesis is that G2 checkpoint efficiency and chromatin dynamics during G2-M transition are important determinants of inter-individual variation in radiosensitivity. The experimental design involves G2 checkpoint abrogation by caffeine to obtain maximum radiosensitivity. Breast cancer and AT lymphocytes were preserved chromosome integrity. For this purpose we use peripheral blood lymphocytes from 100 donors (healthy donors, cancer and AT lymphocytes from breast cancer patients and healthy donors due to variances of the aberration yields of the chromosomes 11 and 17. These chromosomcs might be potential cytogenetic biomarkers for prostate cancer patients in clinical studies.

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patients) demonstrating significant inter-individual variation in radiosensitivity. The results show a relationship between G2 checkpoint efficiency and variation in G2 chromosomal radiosensitivity. For the involvement of chromatin dynamics during G2-M transition in the induction and repair kinetics of chromatin breaks, we use premature chromosome condensation to visualize interphase chromatin and conventional cytogenetics respectively. As chromosomes condense in cells that have passed the checkpoint at the time of irradiation, chromatin dynamics will exert mechanical stress and breakage of the unfolded for repair chromatin, presumably at the sites of DNA lesions. The induction and repair kinetics of chromatin breaks suggest that G2-checkpoint efficiency and chromatin dynamics in G2-M mediate conversion of DNA lesions into irreversible chromatin breakage that underlie individual radiosensitivity and chromosome instability.


Molecular biological markers of radiation response could potentially be of use for monitoring the progress of radiation therapy, and even for predicting outcome early in a treatment regimen. The γ-H2AX assay is widely used in radiation biology and has been shown to be highly sensitive and specific for the detection of double strand breaks (DSBs). The objective of this study was to assess the persistence of the γ-H2AX signal after radiotherapy treatments. Volunteer radiotherapy patients were recruited from the Department of Radiation Oncology, Columbus University Medical Center. To examine the kinetics of in-vivo induction expression of the γ-H2AX protein in blood lymphocytes, peripheral blood samples were collected before the start of the radiotherapy treatment, after the 1st irradiation fraction, and 24 hrs after 1st fraction. To determine the decay kinetics after radiotherapy, blood samples were drawn immediately after the radiotherapy treatment ended, at 48 hrs and 1 week post exposure. Immunofluorescence analysis was performed on isolated lymphocytes exposed to an anti-human γ-H2AX monoclinal antibody and visualized using an Alexa Fluor 555 secondary antibody, counterstained with the nuclear stain DAPI. 1000 γ-H2AX foci was determined by measuring the total γ-H2AX nuclear fluorescence per lymphocyte.(1). To date, we have recruited 8 cancer patients who have received a targeted radiotherapy regimen of 1.8-2 Gy per dose to the pelvic region. The mean total blood dose was calculated to be in the range 2-4 Gy. Although patient recruitment and analyses are ongoing; early results showed a detectable induction of γ-H2AX expression after the 1st fraction with γ-H2AX yields increasing significantly in samples collected at the end treatment. Post-irradiation kinetics showed variability in the decay of γ-H2AX signal, with some patients showing a slower decay of the γ-H2AX signal between days 2 and 7 compared to others. The variation in the decay of γ-H2AX yields up to 7 days post exposure, suggests that inter-individual variation may play an important factor in the efficiency of DNA double strand repair following in vivo irradiation.(1) Turner et al. Radiat. Res. 2011; 175(3):282-90. Work supported by NIAID grant 5 U19-AI087773 and NIEHS grant R121ES019404

POS16-22. Overexpression of SKP2 promotes the radiotherapy resistance of esophageal squamous cell carcinoma. Xiaochun Wang, Institute of Radiation Medicine, Chinese Academy of Medical Science, China

SKP2 is the substrate recognition subunit of the SCFSKP ubiquitin ligase complex. It is implicated in ubiquitin-mediated degradation of the cyclin dependent kinase (CDK) inhibitor p27kip1, and positively regulates the G1/S transition. Overexpression of SKP2 has been found in many kinds of tumors. In the present study, we found that SKP2 expression level increased in esophageal squamous cell carcinoma tissues. Elevated expression of SKP2 correlated significantly with tumor stage and positive lymph node metastasis (p<0.05). Moreover, significantly negative correlation was found between SKP2 expression and the survival of patients who received radiotherapy (p<0.05). At the molecular level, forced expression of SKP2 promoted the radio-resistance ability of EC9706 cells. Knockdown of SKP2 expression sensitized cancer cells to radiation and a wobble mutant of SKP2 that was resistant to SKP2 siRNA can rescue this effect. Increased or decreased expression level of SKP2 had effects on Rad51 expression in the condition of radiation. These results demonstrate for the first time that overexpression of SKP2 correlated with the increased radiotherapy resistance of esophageal squamous cell carcinoma. Elevated expression of SKP2 promoted the radio-resistance of cancer cells and this effect was mediated at least in part by Rad51 pathway.

POS16-23. Expression and function of miRNA in postoperative radiotherapy sensitive and resistant patients of non-small cell lung cancer. Xiaochun Wang, Institute of Radiation Medicine, Chinese Academy of Medical Science, China

Purpose: To investigate the different miRNA expression profile of postoperative radiotherapy sensitive and resistant patients of non-small cell lung cancer, explore their potential role and find some radio-sensitivity markers.

Materials and methods: Thirty non-small cell lung cancer patients who have been treated by postoperative radiotherapy were selected and were divided into radiotherapy sensitive group and resistant group according to overall survival and local or distant recurrence rate. Expression profile of miRNA in these two groups was detected by a microarray assay and the results were validated by quantitative RTPCR and northern blot. At the molecular level, the effect of one differently expressed miRNA (miRNA-126) on the growth and apoptosis of SK-MES-1 cells induced by irradiation was examined. Results: Comparing with resistant patients, five miRNAs (miRNA-126, miRNA-let-7a, miRNA-495, miRNA-451, miRNA-125b) were significantly upregulated and seven miRNAs (miRNA-150a, miRNA-10b, miRNA-19b, miRNA-22, miRNA-15b, miRNA-17-5p, miRNA-21) were greatly downregulated in radiotherapy sensitive group. Overexpression of miRNA-126 inhibited the growth of SK-MES-1 cells and promoted its apoptosis induced by irradiation. The expression pattern of PI3K decreased in miRNA-126 overexpression group. After treating with phosphoinositidyl-3 kinase (PI3K) constitutively activator (IGF-1) and inhibitor (LY294002), miRNA-126 overexpression had no significant effects on the apoptosis of SK-MES-1 cells induced by irradiation..

Conclusion: We found twelve differently expressed miRNAs in the radiotherapy sensitive and resistant non-small cell lung cancer samples. Moreover, our results showed miRNA-126 promoted non-small cell lung cancer cells apoptosis induced by irradiation through the PI3K-Akt pathway.

Keywords: miRNA; radiotherapy sensitivity; apoptosis; NSCLC; PI3K-Akt pathway

POS16-24. Relationship between acute reactions to radiotherapy, micronuclear yields in lymphocytes and SNP polymorphisms in XRC6C1, XRCC3, OGG1 genes in cervix cancer patients treated by external beam radiotherapy. Aneta Węgierska-Cuki,1 M. Arabiski2, H. Lisowska1, A. Banask-Nowak1, P. Kędzierski1, A. Florek1, S. Gozdź1, A. Wojcik1, A. Lankoff1, 1: Dept. of Radiobiology and Immunology, Jan Kochanowski University, Kielce, Poland 2: Dept. of Microbiology, Jan Kochanowski University in Kielce, Poland 3: Dept. of Radiotherapy and Immunology, Jan Kochanowski University, Kielce, Poland 4: Cancer Centre, Kielce, Poland 5: Dept. of Radiobiology and Immunology, Jan Kochanowski University, Kielce, Poland and 5: Dept, of Radiotherapy and Immunology, Jan Kochanowski University, Kielce, Poland and GNT Department, Stockholm University, Sweden 6: Dept. of Radiobiology and Immunology, Jan Kochanowski University, Kielce, Poland and Institute of Nuclear Chemistry and Technology, Warsaw, Poland

The goal of curative radiotherapy is to inactivate all tumor cells without severely damaging the surrounding normal tissue. For many tumors, the applicable dose and therefore the chance of cure is limited by the risk of side effects. Despite numerous investigations on the relationship between side effects after radiotherapy and chromosomal in vitro radiosensitivity of lymphocytes, the obtained results are contradictory. Therefore, alternative approaches are required to predict an enhanced risk of side effects.

The aim of our study was to assess, in cervix cancer patients, a possible relationship between acute reactions to radiotherapy, SNP polymorphisms in XRC6C1, XRCC3, OGG1 genes in cervix cancer patients treated by external beam radiotherapy. Analysis of SNP polymorphism: DNA was isolated from the lymphocytes and run for RFLP-PCR.
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- Analysis of micronuclei: Micronuclei (MN) were scored in lymphocytes collected before, during and after therapy as well as following an in vitro exposure to 2 Gy gamma rays. Medical examination of cervix cancer patients revealed low grade of acute reactions to radiotherapy in relation to rectum, bladder and skin. However, significant differences in polymorphisms of DNA repair genes and in vivo/in vitro cellular radiosensitivity of lymphocytes from cervix cancer patients were observed. There was relationship between acute response to radiotherapy and in vivo/in vitro chromosomal radiosensitivity as well as SNP polymorphisms in XRCC1, XRCC3, OGG1 genes. However, the genotype GG in XRCC1 at codon 339 and the genotype GG in XRCC3 at codon I.V514.893 correlated significantly with the increased frequency of MN induced in vivo and in vitro. These results are currently tested on a larger population of cervix cancer patients.

POS16-25. γH2AX and chromosome damage in human lymphocytes as potential biomarkers of radiation sensitivity. Ruth Wilkins1, L. A. Beaton1, C. Ferrarotti1, N. Ringuet1, S. Samire1, S. Malone2, 1: Health Canada, Canada 2: Ottawa Hospital Cancer Centre, Canada

Cancer patients undergoing radical adjuotherapy vary in their response to treatment. For example, severe late proctitis develops in approximately 2% of patients with advanced prostate cancer treated with radiation. For this reason, the development of an in vitro assay for radiation response would allow radiosensitive patients to be identified prior to treatment and considered for alternative therapy or tailored doses. The purpose of this study is to examine the in vitro/γH2AX response in blood samples from patients who have shown severe late radiation toxicity in order to identify markers for radiosensitivity. Concurrently, cytogenetic endpoints have been examined as potential markers for radiosensitivity in addition to providing additional information about the mechanisms of radiosensitivity. Patients were selected from a randomized trial evaluating the optimal timing of Dose Escalated Radiation (76 Gy) and short course Androgen Deprivation Therapy for intermediate-risk prostate cancer. Of the 430 patients included in the trial, the 3% that developed grade 3 late proctitis were considered radiosensitive. Blood samples were taken from 10 patients in this radiosensitive cohort along with matched samples from patients with no late proctitis and the in vitro γH2AX time and dose responses in lymphocytes and lymphocyte subsets were examined along with cytogenetic endpoints. The dose course experiment was conducted with 6 dose points (range 0-10 Gy), processed 1 hour after irradiation. The time response of γH2AX was also monitored, with 2 Gy irradiated samples being incubated for 0 to 24 hours before processing and analyzed by flow cytometry. In addition, control and 6 Gy irradiated blood samples were analyzed for chromosome aberrations and excess fragments per cell. At 6 Gy, the mean number of excess fragments per cell in the radiosensitive cohort was significantly higher than in the control cohort and there was a trend toward higher number of aberrations per cell. In a subset of the data for the radiosensitive population, the γH2AX response tended to be higher in the time course experiment. These preliminary results suggest the existence of potential markers for radio-sensitivity which maybe useful for tailoring radiotherapy treatments.

POS16-26. Combinational Effects of Sanguinarine with Radiation in Breast Cancer Cells. Jia Ying Xu, S. Fan, L. Zhao, Y. Jiao, Soochow University, Suzhou, China

OBJECTIVE: To investigate the in vitro and in vivo effects of sanguinarine on radiosensitivity of breast cancer cells. METHODS: Cell survival was determined by colony forming assay; Cell viability was measured by MTT; Apoptosis was assayed by annexin V staining. In vivo radiosensitization of sanguinarine was assessed in C57/BALB/C nude mice with MDA-MB-231 xenografts. RESULTS: Sanguinarine significantly enhanced the radiation-induced inhibition of growth of breast cancer cell lines, MCF-7 and MDA-MB-231, SER was 1.79 and 1.54 for MCF-7 cells and MDA-MB-231 cells, respectively. In nude mouse bearing MDA-MB-231 cell xenografts, sanguinarine significantly increased tumor sensitivity to irradiation. CONCLUSIONS: Our present studies for the first time indicate that sanguinarine enhanced the radiosensitivity of breast cancer cells in vitro and in vivo, which provides a rationale for the clinical application of sanguinarine in combination with radiation in breast cancer.

POS16-27. Heritability of radiation-induced injury in bxd recombinant inbred strains: the genetics of hematopoietic-acute radiation syndrome. Charles R. Yates1, K. E. Thompson2, G. J. Ting2, R. W. Williams3, L. J. J. Toutouchnian2, 1: The University of Tennessee Health Science Center, USA 2: UTHSC, USA

Purpose: Genetic predisposition to high dose ionizing radiation-induced injury is invaluable in understanding the cellular mechanisms and relevant pathways involved in response to Radiation-Induced Acute Syndrome (H-ARS). By mirroring the genetic complexities of the human population we aim to establish a model that identifies high-impact genomic variants contributing to the degrees of radiation-sensitivity. Recombinant inbred (RI) strains; in particular, the BXD family of strains affords us with such a paradigm to examine the genomic variation-physiological response relationship.

Methods: Radiation biology, environmental genetics: High-dose total body gamma-irradiation survival (days) for 12-week-old females; BXD (40 strains, n = 2-5 animals/strain) and C57BL/6 parental cohort (n = 12). Mean Survival Time after a 137-Cesium; 6 Gy single exposure; LD80/30 evaluated over 30 days post-exposure, no supportive care. MST variation was submitted to GeneNetwork (BXD trait 12684 at www.genenetwork.org). This web service includes dense genotypes of extensive phenotype data for many genetic reference populations, including the BXD family of ~80 strains. MST data were hemato poetic gene expression data sets and the top 500 covariates of MST were submitted for gene ontology analysis at WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt).

Results: We show an ~8-fold genetic variation with an estimated heritability (h2) = 0.7 (MST F-ratio: 5.005; p ≤ 0.0001) between 40 BXD strains revealing significant inter-strain variability following TBL Quantitative Trait Loci (QTL) maps were generated revealing a correlation between genotype/phenotype at chromosomes 2, 15, and 17 (p = 0.001; 0.006; 0.002, respectively; uncorrected for multiple tests). BXD trait 10056:Hematopoietic stem cell pool size, covaries with MST via negative correlation (Pearson coefficient -0.816; p = 0.001).

Conclusions: Radiation susceptibility among the BXD strains shows an ample heritability estimate of 0.7 signifying genetic predisposition as an essential dynamic in radiation-induced injury. We were able to identify linkages between chromosome regions and variation in MST. Our model shows great potential in mirroring the extensive heritable complexities seen in human populations to identify and unravel the causative elements of radiation-induced injury.

POS16-28. Residual γH2AX foci as a predictor of severity of acute reactions in head and neck cancer patients undergoing chemo-radiotherapy. Bola Sadashiva Satish Rao1, G. HV1, K. D Mumbrekar1, G. KIP2, V. BM1, S. K1, 1: Division of Radiobiology & Toxicology, Manipal Life Sciences Centre, India, 2: Division of Biotechnology, Manipal Life Sciences Centre, India 3: Manipal Hospital, Bangalore, India 4: Division of Biotechnology, Manipal Life Science Centre, India

Acute reactions (oral mucositis and skin reactions) are major complications seen in patients treated with chemo/radiotherapy for head and neck cancer which may lead to hospitalization and treatment interruptions and may compromise the quality of life. Therefore, there is much interest among radiation oncologists for the prior detection of inherent radiosensitivity as an indicator for severity of acute reactions. Therefore the objective of the study was to correlate the severity of oral mucositis and skin reactions with the radiation induced residual double strand breaks (DSBs) in lymphocytes analysed before the initiation of radiotherapy. The inherent cellular radiosensitivity was measured in lymphocytes treated with challenge dose of X-ray irradiation and the DNA damage/repair was assessed by γH2AX foci analysis, neutral comet assay and a modified version of neutral filter elution. The severity of acute reactions was graded after the completion of radiotherapy according to the Radiation Therapy Oncology Group (RTOG) criteria. All the assays demonstrated remarkable interindividual difference with respect to damage induction as well as residual damage. Residual DSBs analysed by all the three methods considered for correlation with acute reactions. Correlation of acute reactions with residual γH2AX foci revealed statistically significant difference between RTOG grade 0, 1&2 and grade 3&4 in comparison to neutral comet assay and filter elution. Also, the number of residual γH2AX foci increased with the severity of acute reactions. In conclusion, determination of inherent cellular radiosensitivity by analyzing residual γH2AX can serve as a biomarker for predicting the severity of acute reactions before the
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initiation of radiotherapy. Thus patients may have the benefit of opting for altered radiotherapy regimens for the minimal adverse effects with improved tumor cure.

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POS16-29. Polymorphisms in DNA double strand break response pathway and its impact on radiosensitivity, Goutham Hassan Venkatesh1, K. D Mumbrekra1,2, M. Vadihiraja1, K. P. Guruprasad1,2, K. Satyamoorthy3, B. Satis Rao1, 1: Division of Radiobiology & Toxicology, Manipal Life Sciences Centre, India 2: Manipal Hospital, Bangalore, India 3: Division of Biotechnology, Manipal Life Sciences Centre, India

Radiation induced double strand breaks (DSB) are the most critical lesions and lack of repair or misrejoining plays a key role in genomic instability. Individuals hypersensitive to ionising radiation are considered deficient in DSB repair and/or display chromosomal instability. NHEJ represents the major DSB repair pathway in G0/G1 phase and is involved in the repair of radiation induced DSBs in lymphocytes. Considering the importance of DNA DSB repair in normal tissue response to radiation, SNPs in the genes responsible for DNA damage signaling and repair pathways are suitable candidates in the search for the genetic basis of normal tissue radiosensitivity. In the present study, we attempted to investigate association between genetic polymorphisms in DSB recognition and repair genes (ATM, NBS1, Ku70, Ku80, Lig IV and XRCC4) and DSB repair activity in a general healthy population. The inherent cellular radiosensitivity was measured in lymphocytes treated with challenge dose of 2Gy X-ray irradiation and the DNA damage/repair was assessed by γH2AX foc antibody analysis. Genotyping of selected candidate gene polymorphisms was done using PCR-RFLP method. The correlation between genotype and DSB repair capacity was assessed using Mann-Whitney test. There was significant interindividual variation with respect to damage induction as well as repair of DSBs. The rs3835 polymorphism in Ku80 gene was significantly associated (p <0.05) with the reduction in repair capacity.

Acknowledgements: The financial support from Department of Biotechnology, Government of India (BT/01/COE/06/02/07) is gratefully acknowledged.

POS17 Radioecology

POS17-01. Assessment of annual effective dose due to natural gamma radiation in Guilan-IRAN. Mohsen Asaledinazhad, S. Aghayari, S. Basirjafari, S. Mohammad Poorabas, Guilan University of Medical Sciences, Iran

This study assesses the indoor and outdoor natural gamma dose rates in air of 49 cities in Guilan province of IRAN. The measurements were taken from 260 different sample points by a Geiger-Muller dosimeter. The average indoor dose rates were determined as 109±25 nSv/h with the range of 82 to 138 nSv/h. The outdoor gamma dose rates order from 65 to 127 nSv/h with the mean of 94 ± 24 nSv/h. The average annual effective dose was calculated as 0.64 mSv which is more than the worldwide value.

POS17-02. Radioprotection of biological diversity – a major contradiction and impossibility. B. Cedervall1,2, and A.B. Cox1, 1: Department of Clinical Science, Intervention and Technology, Division of Radiology and Medical Radiation Physics, Department of Oncology and Pathology, Karolinska Institutet 2. Radiation Protection, Nuclear Safety, Vattenfall Power Consultant AB, Stockholm, Sweden 3. Late radiation effects group, Colorado Springs, CO, USA.

The purpose of this work is to analyze one aspect of radioprotection of the environment – sampling data for individuals to draw conclusions relating to sustainability in the sense of population genetics and biological diversity. For a context, see ICRP Publication 108.

The considerations here come from reasoning based on the theory of evolution and biological species which is, as we show, contradicts the use of a reference organism and the attempt to protect biological diversity from ionizing radiation. The problem and analysis here includes several subtopics: Insufficiently defined statements relating to extrapolations from individuals of a species to the level of population dynamics. The reference organism is physical (or typological, see works by E. Mayr for an explanation) where the “average” is important whereas a species has genetic outliers – far from the “average” and where those outliers dominate the chance for survival under extreme conditions.

Ignores the effects of the natural production of genetic variation (chromosomal reassortment, mutations etc) as well as of the effects of selection – against what does not work so well – poorly adapted phenotypes can never be selected for. The value of a gene mutation (“good” or “bad”) must be seen in a genetic and environmental context. The ignorance includes lack of appreciation for the complex statistical phenomena and the impossibility of predicting outcomes of population dynamics where many important changes will be due to unforeseeable events as well as gradual fluctuations. This was essentially understood more than forty years ago. Reference man was essentially developed to protect humans from harmful effects on a somatic level. The issue never included radioprotection of human (genetic) diversity. The endpoint biodiversity is new to radioprotection and cannot, because of the brutal selection processes which prevail in Nature against individuals, be harmonized with the radioprotection system for humans, where (for understandable reasons), everything possible has been done to escape the mechanisms of selection.

Conclusion: No meaningful hypothesis which relates to harmed biodiversity can be tested and no predictions relating to effects on a population genetic level can be achieved by collecting data on radioactivity or mutations in individuals.

POS17-03. Soil-to-rice transfer of radiocesium and radiostrontium as affected by simultaneous additions of K and Ca. Yong-Ho Choi, K. Lim, I. Jun, D. Keunm, I. Kim, Korea Atomic Energy Research Institute, South Korea

As learned from Chernobyl and Fukushima nuclear accidents, radiocesium and radiostrontium are two of the most important radionuclides in terms of radioactive contamination of arable soil by a nuclear accident. In order to lower the ingestion radiation dose due to an accident, therefore, soil-based countermeasures may be necessary to reduce the uptake of radiocesium and radiostrontium by food crops. It is well known that K and Ca compete with radiocesium and radiostrontium for root respectively, for root uptake. A greenhouse experiment was performed to investigate the effect of the simultaneous addition of K and Ca on the rice uptake of soil radiocesium and radiostrontium. Blocks of an undisturbed paddy soil, 6.5 in pH and 3.05% in organic matter, were taken into small lysimeters and top soils were mixed with a solution containing 137Cs and 85Sr using a garden trowel. Fertilizer KCl and slaked lime were simultaneously added for K and Ca, respectively, to the labeled soils at 6 different dosages – 0/0 (control), 7.2/69, 19.2/184, 33.6/322, 48/460 and 72/690 as K/Ca (g m⁻²), and top soils were mixed again. Rice seedlings were transplanted and grown for 130 d. The concentrations of 85Sr and 137Cs in rice seeds and straws were determined by means of gamma spectrometry. Soil-to-plant transfer of 137Cs and 85Sr was quantified with a transfer factor (m² kg⁻¹ dry) defined as the ratio of the plant concentration (Bq kg⁻¹ dry) to the deposition density (Bq m⁻²). TF values of 137Cs and 85Sr for control plants were 2.6 x 10⁻² and 2.2 x 10⁻², respectively, for straws, whereas corresponding values for hullled seeds were 7.4 x 10⁻² and 2.1 x 10⁻², respectively. For 137Cs, TF values for both the parts decreased with increasing dosages up to 33.6/322 and for 85Sr the same occurred toward a dosage of 48/460. Rice uptake of 137Cs and 85Sr as a whole decreased by about 60% at the maximally-decreasing dosages. Beyond these dosages, TF values increased with increasing dosages or hardly changed. The dosage of the maximum decrease for 137Cs led to as high as 56-57% decreases for 85Sr, too. These present results indicate that a dosage of 33.6/322 may be the most appropriate level for a countermeasure against a mixed deposition of radiocesium and radiostrontium. It is, however, necessary to note that the effect of K and Ca addition may differ among soils and among plant species.

POS17-04. Estimation of gamma radiation dose in the forest on the area of the Opole Anomaly (PL). Agnieszka Dolhaičuk-Šrodička, Z. Ziembić, M. Wacławek, Independent Chair of Biotechnology and Molecular Biology, Opole University, Poland

The study assessed the radiological risks associated with the presence of natural and artificial radionuclides in the forest on the area of the Opole Anomaly (PL). Using the conversion factors given by UNSCEAR and the measurements results of 227Th, 232Th, 40K and 137Cs concentrations of 0.1 in air, 0.07 in water and 0.03 in soil, the study estimated the dose of 10⁻¹ mSv to the forest area in the Opole Anomaly.
specific activities in the 10 cm soil layer the values of absorbed dose (D) and the annual effective dose equivalent derived from terrestrial gamma radiation (E) were calculated. Calculated values of gamma radiation doses are lower than the dose limit set out by the Council of Ministers Ordinance of 18 January 2005 (Regulation of the Council of Ministers (Poland) 2005). The data obtained are important both for assessing the risk for human health as well as environmental radiobiomonitoring. The results can be used as a reference point for radiological mapping in the forest on the area of Opolo anomally. 

Key words: radiological hazard, radionuclides, radiation dose.

POS17-05. Distribution of the physicochemical forms of strontium-90 and caesium-137 in freshwater mussels within the Chernobyl exclusion zone. Christina Ganža, D. Gudkov2, V. Klenus1, 1: Department of Freshwater Radioecology, Institute of Hydrobiology of the National Academy of Sciences of Ukraine, Ukraine 2: Department of Freshwater Radioecology, Institute of Hydrobiology of the National Academy of Sciences of Ukraine, Geroys Stalingrada Ave. 12, UA-04021 Kiev, Ukraine

The bivalved mollusks play a significant role in the processes of radionuclide redistribution and bioaccumulation in freshwater ecosystems. Due to 90Sr and 137Cs are the chemical analogues of the biophil elements, it is a reason to study of radionuclide physicochemical forms in the bivalved mussels and their subsequent migration ability. The aim of this study was an analysis of 90Sr and 137Cs physicochemical forms in the bivalved mussels zebra mussel (Dreissena polymorpha Pall.) and swollen river mussel (Unio tumidus Phil.) from water bodies of the Chernobyl exclusion zone. Sampling was executed in the Chernobyl NPP cooling pond and in Daleke Lake, located on a distance 4 km from the destroyed unit. The physicochemical forms of 90Sr and 137Cs in mussels were determined by the method of step-by-step extraction. The body of mussels divided on shells and soft tissues which analysed separately. Measuring of radionuclide specific activity in preparations, made by radiochemical method, conducted using beta- and gamma-radiation-measurement equipment. Study of radionuclide distribution in the zebra mussel’s shells showed that prevailing part is localized in organic form, mineral residue and carbonate form (137Cs - 43, 27, 18 %; 90Sr - 38, 39, 21 % respectively). The results of analysis in soft tissues showed substantial predominance of 137Cs. Most of its part registered in organic form, related to the organic matter (47 %), prevailing part of 90Sr in intracellular form (54 %). The 137Cs in the swollen river mussel’s shells is found mainly in exchange form and mineral residue (37 and 33 % respectively). Prevaling part of 90Sr is found in water-soluble and carbonate forms (43 and 53 % respectively). Part of Sr in exchange form amounts 4 %. The data of radionuclide fractionation in soft tissues of the swollen river mussel showed predominance of 137Cs and 90Sr in exchange form (78 and 47 % respectively). Predominance of 90Sr in intracellular form (41 %) it is possible related to radionuclide environment transport value. Both 137Cs and 90Sr content in the form related to the organic matter is insignificant (7 and 12 % respectively). It is determined, that the physicochemical forms of 90Sr and 137Cs in different parts of body in both studied species of mussels are distributed unevenly.

POS17-06. Accumulation of artificial radionuclides in water-soil-plant system in different zones of influence of the Armenian nuclear power plant. Laura Ghahalchyan, K. Kocharyan, L. Tadevosyan, A. Anastakysyan, A. Asatryan, Institute of Hydroponic Problems, Armenia

Artificial radionuclides (90Sr, T1/2=28.6 years, 137Cs, T1/2=30.1 years) dangerous for health are released to ecosystems because of human influence in the field of nuclear energy. 90Sr and 137Cs can penetrate into living chains of water-soil-plant system through the Armenian NPP (opened in 1976, operated till 1989, reoperated in 1995) is in a densely populated area of the Ararat Valley (is 850-900 m above sea level, precipitation is 560-700 mm) with intensive agriculture. ANPP uses the water of the Metsovan River which starts from Lake Akna. Unbalance waters of the ANPP fall into the Metsamor River which is used for irrigation of the soils. In this region it is profitable economically but not ecologically. Since 1996 we have carried out radioecological monitoring in water-soil-plant ecosystems in zones of the ANPP with a radius of 2-15 km, 30 km (territory of the IH) and 90km (territory of the Dilijan Forest Experimental Station, is 1500 m above sea level, precipitation is 1750mm). The aim of the studies is to develop practical radiobioprotective activities for getting ecologically pure food. It turned out that the content of 90Sr exceeded 137Cs in drinking water near the ANPP 8.8 times, in the territory of the IH 5.0 times and in the territory of the DFES 6.2 times. It turned out that the content of 90Sr exceeded 137Cs in irrigation water near the ANPP 10 times, in the territory of the IH 7.1 times. The content of 90Sr exceeded 137Cs in 0-30 cm soil layer 1.1 times in the territory of the ANPP and in the territory of the DFES. The drinking water in the territory of the ANPP exceeded the drinking water of the IHP and DFES with the content of 137Cs 1.7; 2.2 and 1.0; 1.6 times. The prevailing the irradiation water in the territory of the ANPP exceeded the irrigation water of the IHP with the content of 90Sr and 137Cs 1.5; 1.1 times. In the surrounding soils of the ANPP the content of 90Sr and 137Cs in 0-30 cm soil layer exceeds that of the IH and DFES 1.8; 1.4 and 1.3; 1.4 times correspondingly. In the surroundings of ANPP in the leaves of Thuya and gramna the content of 90Sr and 137Cs exceeds that of the IHP 1.7; 1.2 and 1.5; 1.2 times correspondingly. The content of 90Sr and 137Cs in water-soil-plant system doesn’t exceed the Maximum Allowed Concentration Limit. The researches have been implemented since 2011 in the context of project 11-1f 262 of Ministry of Science of RA.

POS17-07. Radiobiological experiments with cells from hepatocellular hepatocellular carcinoma for BNCT. Catrin Grunewald1, Dorothee Illland2, Tanja Peters3, Christian Schütz2, Tobias Schnitz1, Heinz Schmidberger1, Peter Langguth4, Gabrielle Hampel1, 1: Department of Pharmacy and Toxicology, University of Mainz, Mainz, Germany, 2: Institute for Nuclear Chemistry, University of Mainz, Mainz, Germany, 3: Department of Radiooncology, University of Mainz, Mainz, Germany

Effects of ionizing irradiation on biological systems are of interest to radiotherapy, therefore we engaged in experiments with a human hepatoma cell line with the aim to calculate the survival of cells depending on different doses, incubation times and boron concentration of with p-boron-phenylalanine. For this purpose, the cell line of type HuH7 is selected, which stems from a well-differentiated hepatocellular carcinoma. The cells’ media were treated with p-boron-phenylalanine (BPA) enriched with 99%10B before the cells were irradiated with neutrons or photons in a range between 1 and 30 Gy. The irradiation facility providing a thermal neutron beam is the TRIGA Mark II reactor at the University of Mainz whereas the irradiation with photons takes place at the university hospital, the source in use is a 60Co gamma-ray emitter. At two different times after irradiation we measured the vitality of cells with a cell proliferation assay that uses 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT). In a second part of the cell experiments, we focus on the effect of transstimulation. To increase the boron concentration inside the cells and thereby the biological effect we use the amino acid tyrosine as transstimulator which is given some hours before the application of BPA. Tyrosine is structurally related to phenylalanine and use the same boron transporting transport system. Both 90Sr and 137Cs content is measured afterwards by inductively coupled plasma mass spectrometry (ICP-MS). With our assay we achieved different survival curves offering typical RBE-values between 3 and 5. The best biological effects are achieved after 2.5 hours of BPA-uptake (20pm). The most successful conditions of preloading are 2 hours of tyrosine uptake beforehand the boron application. Thereby the boron uptake can be increased by the factor of 2.5.

POS17-08. The investigation chronic diseases of workers uranium mining enterprises in northern Kazakhstan. Polat Kazymbet1, K. P.K., Bakhtin M.M., Bekenova F.K.2, 1: Medical university Astana, Institute of radiobiological researches, Kazakhstan 2: Institute of radiobiological researches, Medical university Astana, Kazakhstan

Extraction and processing of uranium in Kazakhstan produced over several decades and issues of radiation safety of staff uranium mining enterprises and the population of adjacent territories are important. The paper presents data of radio-ecological research in Northern Kazakhstan, conducted in 27 population localities: automotive and pedestrian scale survey, measuring the equivalent equivalent volume activity on radon in indoor air, radio spectrometric and radiochemical analysis of samples of the environment and evaluation to radiation exposure of critical groups of population. Clinical and clinical-laboratory studies of stuff uranium mining enterprises were carried out, in the results of the most important chronic diseases of internal organs from 912 workers uranium processing enterprises and 788 workers from bearing plants as a comparison.

POSTER PRESENTATIONS
group. Among all the diseases of internal organs from workers to uranium processing in development dominated hyperplasia of the thyroid gland (19.4%), hypertension (24.8%), chronic gastritis (17.5%), chronic bronchitis (19.4%). Calculated the relative risk of each disease using the procedure of the Mantel- Hanzel. In turn out that the workers of the development in uranium processing even comparable to comparison group of age, length of service, predictors of disease relative risk of hypertension was higher in the 2.91, chronic gastritis in 2.1, chronic bronchitis in 2.8.

**POSTER PRESENTATIONS**

**POS17-09.** Induced DNA damage and somatic mutations in tobacco plants exposed to ionising radiation and chemical mutagens. Jin Kyu Kim1, T. Park1, T. Ho Ryu1, T. Gichner2, 1: Korea Atomic Energy Research Institute, South Korea 2: Institute of Experimental Botany, Czech Republic

Ionizing radiation induces DNA damage in cells through energy deposition and free-radical formation. DNA lesions can be detected by the single cell gel electrophoresis (SCGE) assay while somatic mutations can be visually scored in some plants such as Tradescantia BNL, 4430 (T-4430), and Nicotiana tabacum var. xanthi (NTX). Pink mutations of T-4430 have been widely used as a good endpoint for detecting genetic effects of ionizing radiation. Detection of airborne radioactivities by the increased DNA damage frequencies after the tests is interesting example. The NTX assay makes it possible to compare, on the same plants under identical treatment conditions, the induced DNA damage as measured by the SCGE assay with the induced somatic mutations on leaves. This study was done to compare the induced DNA damage with somatic mutation frequency in NTX, and test applicability of NTX to environmental monitoring. Irradiation of tobacco seedlings resulted in about 500 fold increase in somatic mutations after the dose of 10 Gy. The increase in the somatic mutations is correlated with the increase of DNA damage measured immediately after irradiation by the SCGE assay. With increasing radiation dose, the tail moment as an indicator of induced DNA damage, significantly increased in the 10 Gy irradiated cells. However, nuclei isolated from leaves 24 hours after irradiation did not show a significant increase in tail moment values versus the negative control. By contrast, DNA damage induced after the treatment by mutagen (EMS, 2 mM) was significantly higher than the DNA damage of the negative control. While radiation induced DNA damage, measured by the SCGE assay, is readily repaired within 24 hours, DNA damage induced by EMS persisted over 72 hours after treatment without significant reduction. Even after 4 weeks the amount of DNA damage in nuclei isolated from mature leaves treated with EMS were significantly higher than that of the controls. The SCGE assay may detect DNA damage inflicted by some chemical mutagens long periods after the initial exposure. It is recommended that somatic mutations be scored together for the biomonitoring purpose as the DNA damage assessed by the SCGE assay in nuclei isolated from the plant leaves is not suitable alone for monitoring ionizing radiation.


Assessment of contemporary radiation exposure of non-human biota at territories of East-Ural Radioactive Trace on the sites with the initial (1957) density of surface contamination by Sr-90 ranged from 0.74 to 18.5 MBq/m² was performed. Following species of mouse-like rodents that are representative of small mammal component of the affected ecosystems were used: Apodemus sylvaticus, Myodes glareolus, Microtus microtis, Microtus oeconomus, Microtus arvalis, and Microtus agrestis. Bone tissues (femur, skull and mandible) of totally 22 animals trapped in September of 2009 were analyzed. Following radiation measurements techniques were used: beta-radiometry of intact bone and bone ash as well as TLD signal at bone surfaces. All methods provided consistent results. The average activity concentration of Sr-90+Y-90 in femur is 112 ± 44 Bq/g, range from 27 to 412 Bq/g, which corresponds to skeleton average activity 155 ± 65 Bq, range from 35 to 527 Bq. Results of skeleton activity estimates were used to calculate retention of Sr-90 in skeleton and to assess contemporary daily intake. For these purposes a simplified age-independent biokinetic model of mouse-like rodent was developed using WinAct code that solves the system of coupled differential equations describing the behavior of radionuclides in the body. The model was adjusted and verified considering published experimental data on retention of Sr-90 in skeleton of laboratory animals for case of acute exposure. A set of nine transfer coefficient linking the doses and receiver compartments of organism was suggested for modeling Sr-90 intake in mouse-like rodents. By using the model it was obtained that the average daily intake was 38 Bq in available sample of small rodents. Basing on the data obtained the assessment of contemporary organs and tissues doses of radiation exposure caused by Sr-90 will be fulfilled. The results of contemporary Sr-90 intake analysis can be used to calculate intake in initial period of contamination and assess doses of radiation exposure induced by both Sr-90 and short-lived radionuclides released into the atmosphere after the accident at Mayak nuclear plant.

**POS17-11.** Estimation of external natural background gamma rays doses to the population of Caspian coastal provinces in north Iran. Ali Shahbazi Monfared, Babol University of Medical Sciences, Iran

The effect of natural background radiation on health is still challenging. However, it is cleared that it depends on dose received by population. The estimation of external natural background gamma rays doses to the population of Caspian coastal provinces in north of Iran was the main aim of this study. Gamma rays data was measured using calibrated radiation survey meter in random 51 urban and rural health centers to estimate the exposure to population (total population 6,888,118 persons) in residential areas of Gilan, Mazandaran and Golestan (total area 59,240 km²) as Caspian coastal provinces in north of Iran. Results showed that the average dose rate in the area under study is about 60.37±4.88 nSv/h or 0.53±0.79 mSv/yr (Range 30 to 90 nSv/h or 0.26 to 0.79 mSv/yr). The data from Ramsar is excluded from the estimation because of a very high natural background radiation found in that area (Max. 240 mSv/yr). No significant difference was found between the doses of the provinces (P=0.237). The external natural background gamma rays data to the population of Caspian coastal provinces in north of Iran was found to be nearly equal to the average value in the world (0.5 mSv/yr). Further national studies are suggested.

**POS17-12.** INFLUENCE OF POLYMERS APPLYING ON MIGRATION AND ACCUMULATION OF ARTIFICIAL RADIONUCLIDES IN SWEET BASIL. Anna Tadevosyan1, S. Mayrapetyan1, M. Schellenberg2, L. Ghahalyan3, N. Tavakalyan3, A. Hovsepyan1, K. Mayrapetyan1, 1: Institute of Hydroponics Problems, Armenia 2: International Science Cooperation Bureau, Agriculture et Agroalimentaire Canada/Agriculture and Agri-Food Canada 3: Yerevan Institute "Plastpolymer", Armenia

One of the important aspects of the problem of contaminated soils remediation is elaboration of protective actions aimed at reduction of biological mobility of radionuclides in a water – soil – plant system. The agricultural radioecology has acquired significant experimental materials for modes to decrease the transfer of radionuclides from soils to plants. A number of agrochemical and land treatment techniques are described: application of mineral and organic fertilizers, clayey materials, liming of soils, changes of irrigation regimen, burying of upper layers of soil in lower layers. Amongst the man-made radionuclides causing contamination 137Cs and 85Sr exert long-term after-effects. The passage of radionuclides, in particular 137Cs and 85Sr, occurs from the soil solution and irrigation water through the root system of plants.

The presence of a functioning Nuclear Power Plant and the prospects to expand nuclear engineering elevates the possibility of radionuclides-caused soil contamination in Armenia as well. The aim of the research is to provide new means and procedures for the remediation of contaminated soils through regulation of biological migration of artificial radionuclides 137Cs and 85Sr by water-retaining polymers in water – soil – plant systems in zones of radio-ecological tension. The tests are being carried out under field conditions with and without application of polymers in root-inhabited media (RIM) of sweet basil in ten-week non-transfer conditions. The average activity of RIM was 70 Bq/kg dry matter (from the Armenian NPP, v. Taronik). Different polymers (on the base of K+, Ca++, K+ and Ca++ ions, synthesized in the “Plastpolymer” Institute have been tested. It has been found out that application of Ca++ ion based polymer (1g/plant) decreased the content of 85Sr in basil leaves 1.4 times in comparison to control, but didn’t influence on the content of 137Cs. At the same time Ca++ polymer addition to the RIM of sweet basil, even
in conditions of watering frequency decreasing in 30% promoted the same output of plant yield compared to the control. It has been confirmed that the content of controlled artificial radionuclides Sr and Cs in the plants is much lower than the Allowed Concentration Limits.

The research has been implemented in the context of project A-1671 ISTC.

PO517-13. The photomeditated contamination uranium processing territory of the northern Kazakhstan. Meinrat Bahkin, M.M. Bakhtin, A.S. Dzhxakonova., P.K. Kazymbet, Astana Medical University, Institute of Radiobiological Research, Kazakhstan

The rehabilitation of soils contaminated radionuclide’s and heavy metals is at the present time one of the actual problem of radioecology. Extensive use for cleaning the soil of toxic elements are sorbitional methods (Blogaev V.V., 1993; Shvetz D.I., 2001; Gladkoy E.A. 2006), particularly photomediation is a perspective method of remediation contaminated territories as an economic - effective and ecologic safe technology. The purpose of study - the development of unified methods using the photomeditation to clean soils contaminated with radionuclides from Northern Kazakhstan. Conducted radiometric research territory adjacent to the uranium mining enterprises revealed radioactively contaminated sites. Along with the results of the pedometric survey was found that equivalent dose rate of gamma radiation on study site exceeds by 4 times, which amounted to 0.74 mSv / hour compared to the control level. Radio-spectrometric and mass spectrometric analysis of soil samples showed that the specific activity of of 226Ra in 31 times, 210Po 2-fold, 222Rn-7-fold, 218Po 10 times, 210Po 12 times, the content of Cs in 33 times, Zn 9 times, Ni 25 times, Co 2 times significantly greater compared with those of the control plot. 6 families of plants identified from the species diversity plant growing on contaminated districts, 3 families identified as the dominant species of representatives: Composite flowers (Asteraceae) Marevye (Chenopodiaceae) and gramineous (Gramineae Jizz). According to the radiochemical analysis of samples of plants identified plant-sorbents of natural radionuclide’s and heavy metals: E. juncus sorbent is 238U, 232Th, 210Po, 228Rn and of the heavy metals - Co, Ni, Pb, Zn, Sn and Mo; C. hybridum is a sorbent - 238U, 232Th, 210Po and of heavy metals - Cu, Ni, Pb, Zn and Mo; M. volgicus - 234U, 232Th, 210Po and 228Rn, from heavy metals - Ni, As, and Mo; X. strumarium - 210U, 228Rn, 210Po and 228Rn, from heavy metals - Cd, Mo, As and Sn.


The paper presents research data of the natural radionuclides concentrations in building materials manufactured in Latvia as well as contamination level of various samples: soils, waters, metal scrap and waste of the nuclear reactor, and tritium in ground water and drinking water.

The concentrations of gamma radioactivity in different samples were determined using the high resolution HPGe gamma-spectrometers Ortec and Canberra within the energy range of 50-2000 keV. For measuring of large radioactivity waste amount in the metal barrels gamma-spectrometer with NaI detector were used. The uncertainty of measurements was within the range of 3-10%, the minimal detectable activity – 0.3 Bq/kg.

The measurements of tritium activities were carried out with the liquid scintillation spectrometer Packard TRI-CARB 2100 and 2900 using the scintillation liquid OptiPhase ™HiSafe3. The measurement time for H-3 didn’t exceed some hours and uncertainty was less than 2%.

The highest concentrations of natural radionuclides K-40 and the decay products of the Th-232, U-238 (Ra-226) chain were detected in granite and its products. The maximally permitted concentrations of the radionuclides (Bq/kg) has been exceed in particular plumbing and ceramite products, and in some imported granite blocks.

Analysis of tritium was performing for the solution two problems:
1. Regular tritium concentration in ground water well around the potentially dangerous objects - the decommissioning Salaspils nuclear reactor and the radioactive waste repository “Radon”. The tritium activity in the groundwater of these territories has decreased within the last year.
2. Control of drinking water in food industry and urban water-supply. According to the regulations No.235 of Latvian Cabinet of Ministers, adopted in 2003 and Directive 98/83/EC of the EU Council on the quality of water, the use of drinking water with tritium content more that 100 Bq/l is prohibited.

We have performed the analysis of industrially used drinking water and communal drinking water sources of all Latvian regions and Tallinn (Estonia). It was found, that the tritium concentration in measured drinking water samples never exceeds the 10 Bq/l limit.

The quality assurance included the main requirements of ISO/IEC 17025:2005 standard.

PO517-15. Temporal and spatial dynamics of radioactivity and ionizing radiation in ground atmosphere. Valentina Yakovleva1, P. Nagsory2, A. Yukolov1, I. Ippolitov1, M. Kabanov, S. Smorova2, V. Karataev1, 1: Tomsk Polytechnic University, Russian Federation 2: Institute of Monitoring of Climatic and Ecological Systems SB RAS, Russian Federation

Control for environmental background radioactivity is usually performed by one parameter: one type of radiation or radionuclide. Due to different penetrating power of different types of radiation a multiple parameter approach to a problem of low dose effects is required. The main purpose of this work was to investigate temporal and spatial dynamics of atmospheric radioactivity and generated ionizing radiation fields. The general distinctions of this investigation are: 1) synchronous measurements of characteristics of α, β- and γ- radiation fields and of atmospheric radionuclides at one point, 2) parallel measurements at different heights up to 25 m; and 3) high time resolution of data series. Such approach allowed to get more informative data to reach the purpose of the work.

The long-term experiment was performed at Tomsk Observatory of Radiobiology and Ionizing Radiation. The data set included: detectors of α-, β- and γ-radiation installed by two of three at series of heights (0.1; 1; 5; 10 and 25 m) and depths (-0.5; -1 and -5 m), radon isotopes and decay products radionuclides and automated devices for radon and thoron flux densities measurements. The monitoring of meteorological, actinometrical and atmospheric-electrical values is performed via automated information measuring system.

The main sources of atmospheric γ-radiation field are radionuclides of thin soil layer and buildings (if urban region). Atmosphere γ-radiative aerosols can make an observable contribution only under certain weather conditions. Atmosphere α- and β-radiation fields are mainly formed by radon isotopes and their decay products, but near the ground the β-radioclines of 0.5-cm soil layer can be considerable contributors.

Daily variations were revealed in atmospheric α- and β-fields dynamics. Synchronous bursts in β- and γ-radiation time series with duration of some hours appeared at all heights during the day but without certain regularity. Reasons for the bursts are discussed. Significant correlations were obtained between times series of one type of radiation measured at different height, but α-, β- and γ-fields do not correlate easily or even at all.

The work was fulfilled with financial support of RF Analytical departmental target program "Development of higher school scientific potential” № 2.1.1/13707.

PO518 Radiation chemistry in materials science

PO518-01. Modification of elastomers by ionizing radiation. Wojciech Giuszewski1, Z. P. Zagórski1, M. Rajkiewicz2, 1: Institute of Nuclear Chemistry and Technology, Poland; 2: Institute for Engineering of Polymer Materials and Dyes, Piastów, Poland

Absorption of ionizing radiation energy by polymers results in four, parallelly running chemical processes: crosslinking, degradation, formation of unsaturation, including multiple bonds and oxidation [1, 2]. If crosslinking prevails over degradation, the modification of properties of polymer goes in useful direction. Intentional, radiation induced crosslinking is in many respects better than chemical, because it is realized at ambient temperature and is readily controlled by adjustment of optimal dose [3]. From the practical point of view, especially important is recognition of inhomogeneous deposition of energy and the role of multi-ionization spurs on processes of formation of the net of crosslinking bonds in elastomers [4]. Introduction of analytical methods used in radiation chemistry of polymers revealed new facts. In particular, gas chromatographic determination of radiation yield of hydrogen in irradiated elastomers has shown that only the half of crosslinks, as determined by gel
fraction, is due to hydrogen abstraction from two neighboring chains and the rest is due to entanglements. Hydrogen is not determined in conventional, chemical crosslinking reactions, because this gas is emitted only in the typical, radiation-induced crosslinking. Using gas chromatography, we can also determine the amount of oxygen that has been absorbed by the elastomers during irradiation and in postradiation degradation. Another non-typical analytical method, the diffuse reflected light spectrophotometry (DRS) is applied to irradiated elastomers, showing the radiation induced chemical reactions. Determinations of H₂, CH₄, and the secondary product CO, as well as of O₂ consumption were done by gas chromatography (Shimadzu GC 2040, molecular sieves 5Å, the detector was thermo-conductivity (TCD - 2014)), in carrier gas Ar resp. He. Samples were irradiated in 3.5 ml vessels closed with rubber septa, shielded by a thick hood made from lead, if irradiated on the conveyor under electron beam. Hydrogen was released immediately from samples of any shape, whereas determination of methane demanded gentle heating of the vessel in the case of thick films of the material. Before sampling from the gas phase of the vessel was done. Carbon oxide was determined after aeration during subsequent lapses of time. Ketone and peroxy groups were determined by diffuse reflection spectrophotometry (DRS) using a Perkin Elmer Lambda 7 spectrophotometer with an integrating sphere. That technique has been applied earlier in the investigation of ZSM-5 polymerization chemistry of neat polypropylene. The DRS method is by two orders of magnitude more sensitive than IR spectroscopy because of much higher molar extinction values of absorbing groups. Therefore, that sort of spectroscopy is able to show the effects even of stabilization doses. FTIR spectra were measured after air oxidation and usually indicate changes in chemical polymers, because of low sensitivity. Maxima on DRS absorption spectra were identified (210 nm – peroxy; 245, 295 nm – ketone groups). Radiation treatment was performed at the Institute of Nuclear Chemistry and Technology in Warsaw, using cobalt gamma ray sources "Isslelodavit" (dose rate 0.789 kGy/h) and "GC 5000" (8.5 kGy/h). Sample were irradiated to the dose of 5, 10, 25, 60 and 120 kGy. Electron beam irradiation was performed using a linear electron accelerator, "Electronics". 10/10 an energy of 10 MeV and beam power of 1 kW. Dose were used as in the case of gamma irradiation. [1] Głużewski W, Zagórski Z.P, Tran Q.K, Coutella L, Maria Sklodowska Curie - the precursor of radiation sterilization methods, (2011) Analitical and Bioanalitical Chemistry 400, 1577 - 1582 [2] Zagórska Z.P, Rajkiewicz M, Głowczewski W, (2011) Radiacyjna modyfikacja elastomerów, Przegmył Chemizicy 6, 1191-1194 [3] Głowczewski W, Zagórski P, Z. (2010) Procesy radiacyjnego siecowania polimerów, Tworzywa Sztuczne i Chemia 2, 58-60 [4] Bik J, Głowczewski W, Rzynski W.M, Zagórska Z.P, (2003) EB irradiation crossing of elastomers, Radiation Physics and Chemistry 67, 421 Work was done under the research project N N209083838 (Synergetic systems linking elastomers), funded by the Ministry of Science and Higher Education. POS18-02. Radiolitically generated paramagnetic centers in molecular sieves with adsorbed carbon monoxide. Marcin Sterniczuk, J. Michalik, J. Sadło, G. Strzelczak, Institute of Nuclear Chemistry and Technology, Poland Introduction. Free radicals are very often important intermediates in many processes of heterogeneous catalysis. However, in real catalytic system they are very reactive and short-lived, thus difficult to study. We generated free radicals in zeolites exposed earlier to small molecular adsorbates by γ-irradiation at liquid nitrogen temperature. The radical were followed reactions by EPR spectroscopy gradually increasing sample temperature. Here we present the study of carbon monoxide radicals generated in molecular sieves. The combination of EPR measurements with quantum chemical computation have been applied in order to identify the radical species and define their geometry and reactivity. Experimental. Zeolite H-ZSM-5 zeolite sample was degassed and dehydrated at 150°C in speciosil tubes on vacuum line under the pressure of 10⁻³ Torr. Carbon monoxide ¹³CO was adsorbed at room temperature under the pressure range 5 - 100 Torr and then the EPR tubings were sealed and irradiated in γ-source at 77 K with a dose at 6 kGy. The EPR spectra were measured using Bruker ESP 300 spectrometer in temperature range 77-370 K. All calculations had been performed by using the Gaussian 03W program. Results and discussion. The EPR spectrum of H-ZSM-5/¹³CO sample y-irradiated at 77 K and recorded at 300K shows two doublets: anisotropic doublet A: with g=2.0005, g_z=2.0007, g_x=2.999, A=30.4 mT, δA=14.5 mT and isotropic doublet B: with g=2.0002, A=21.3 mT. The EPR spectra and DFT calculations show different radical centers located in several different sites of ZSM-5 network. It turned out that calculated values of hyperfine splittings for A doublet are close to the experimental value equal 27.7 mT for two different radiolical radicals. In the first one HCO interacts with oxygen located between Si and Al atoms ([Si-O-C=O]) and in the second one is bounded to oxygen located between two Si atoms ([Si-O-Si=C=O]). The second doublet B (A=21.3 mT) had not been observed earlier. The preliminary DFT calculations showed that such signal could be associated with HCO -...R=O. So, for such geometry calculated A_B (V) is closest to the experimental one. We observed the EPR spectra of “CO centers also in other zeolites like X, LTA and MOR. The analysis of experimental results and DFT calculations is in progress. POS18-03. Functionalization of polyurethane surface by radiation-induced graft polymerization. Marta Waló, G. Przybytniak, P. Akkas Kavakili, M. Barsby, O. Güven, I. Institute of Nuclear Chemistry and Technology, Warsaw, Poland 2: Hacettepe University, Department of Chemistry, Ankara, Turkey It is generally known that the required properties of biomaterials are biocompatibility, sterilizability, adequate mechanical and thermal properties as well as specific surface characteristic [1]. Among the polymeric biomaterials, polyurethanes (PUR) have attracted a great interest for their unique chemical and physical properties. Biomedical polyurethanes are widely used in medicine for production of scaffolds in tissue engineering and for manufacturing medical devices, such as vascular grafts, artificial hearts, wound dressings, blood tubing, catheters and mammmary implants [2]. Polyurethanes are microphase-separated polymers containing hard and soft segments arranged alternately. Thanks to possibility to model their properties by selecting various types and molecular weights of the oligodiol, the chemical structure and symmetry of diisocyanate, the hard/soft segment weight ratio, the synthesis method, PUR can be used for two specific clinical applications. However, it is difficult to synthesize polyurethanes with appropriate bulk and surface properties simultaneously. It is well known that the surface properties of materials in contact with biological systems play a key role in determining the outcome of biological material interactions [3]. Therefore, selected functional groups must be introduced to surface in order to change their properties. There are a multitude of surface modification methods including chemical treatment, immobilizing biological molecules, radiation grafting of hydrophile monomers and gas plasma treatment [4]. Among these methods, radiation-induced graft polymerization is well known technique for modifying the chemical and the physical properties of polymeric materials without altering their inherent properties. The aim of the reported researches was to modify the surface of polyurethane by radiation-induced grafting to improve the hydrophilicity. The samples used for grafting were synthesized by a two-step condensation polymerization without any catalyst, solvent and additives. PUR was constructed from soft segments of oligo(ethylene-butyhlene adipate) diol end-capped with molecular mass of 2000 Da and hard segments of isophorone diisocyanate and 1,4-butanediol. The weight ratio between hard and soft segment was 40:60. In this study the mutual radiation grafting of N-isopropylacrylamide (NIPAAm) onto polyurethane films was performed. At the first stage of investigations several important factors determining the final effect of graft polymerization were tested, namely: monomer concentration, homopolymer suppressor concentration and dose. Then, nongrafted and grafted polyurethanes with different grafting yield (VY) of 25%, 59% and 77% were characterized using the following methods: ATR-FTIR spectroscopy, thermogravimetric analysis (TGA), gel permeation chromatography (GPC), contact angle measurements (CA) and X-ray photoelectron spectroscopy (XPS). Chemical structures of nongrafted PUR and NIPAAm grafted PUR were investigated in FTIR spectra. In γ-irradiated NIPAAm, the carbonyl region of PUR and PUR-g-NIPAAm a new C=O stretching band at about 1656 cm⁻¹ characteristic for NIPAAm structure appeared. For neat PUR two steps decomposition are revealed in the TGA curves. The first stage of thermal degradation is associated with scission of the urethane linkages in hard segments of PUR. The second stage is connected with the emission of carbon dioxide. The second one corresponds to the soft segments chain cleavages. After NIPAAm grafting, the changes on
TGA thermogram was observed as newly formed peak at around 400 °C attributed to the degradation of poly(N-isopropylacrylamide) appeared. On the basis of GPC results it was found that the grafting of NIPAAm onto polyurethane surface causes shift the chromatograms to higher molecular weight regions. A new peak appearing in the GPC traces is attributed to the grafted PNIPAAm chains, and the intensity of this peak increases with the increasing grafting yield. These results clearly proved the formation of covalent bonds between the PNIPAAm chains and the polyurethane surface.

XPS results confirmed NIPAAm grafting onto polyurethane surface. High-resolution C(1s) region comprising four distinct peaks indicates dominant participation of C-O and C=C-H groups in the PUR surface. Carbonyl groups of the former units also confirm significantly in the surface structure (25%), contrary to the peak at 288.6 eV attributed to N(H)-C(O)O which participation does not exceed 4%. For PUR-g-NIPAAm with γ = 22% C-O(C)-O and C-N groups constitute 25% of the 21s bands, whereas before grafting their contribution was about 10% smaller. Additionally, for grafted surface summary function of N(H)-C(O)-O and N-C=C(O) enhances significantly as compared to the untreated PUR.

Considering the results of contact angle measurements it was found that the contact angle measured versus water diminished to about 67° for sample grafted to γ = 22% Preparaed samples were irradiated by electron beam for 8 h. The obtained results suggest that the radiation induced grafting seems to be the promising method to improvement the biocompatibility of polymers for biomedical applications. By introducing specific functional groups to the trunk polymer, surface properties changed significantly. Contact angle which is an important macroscopic parameter characterizing surface wettability decreases from 86° to 67° confirming increasing hydrophilicity of polyurethane surface.

References:


Titanium dioxide shows good photocatalytic activity for oxidative degradation of environmental pollutants. Nevertheless, it can absorb only a small fraction of solar energy, thus restricting its environmental applications. Activity of titania can be improved with dopants like iron ions. Efficiency of iron modified TiO2 strongly depends on the preparation technique.

We produced the Fe-doped TiO2 photocatalysts by radiation-induced synthesis. Fe-doped TiO2 samples were prepared by wet impregnation, starting from TiO2 (Degussa P-25) and different iron salts (FeCl3, FeCl2, Fe(NO3)3). Fe(III)-acetylacetonate, FeCl2 as an iron precursor. Dried, powdered samples were irradiated by electron beam from the linear accelerator (ELU-6MeV), in air atmosphere. The photocatalytic activities of irradiated bare TiO2, FeS, and FeTiO2: catalysts were investigated by the decomposition of phenol and dye (Reactive Blue 81) in aqueous solutions under UV-VIS light illumination. The medium pressure Hg lamp (4W or 8W power) immersed within the photoreactor was used as the UV source. The home-made system of four linear fluorescent lamps (LF Polam 6W) surrounding the reactor was used in experiments under visible light. The experiments were carried out in reactors containing 0.2g/L of catalyst and 400 or 600 ml of aqueous solutions of phenol (5x10-3 M) or dye (5x10-3M). Oxygen was continuously bubbled into the magnetically stirred suspension.

The dye concentration was determined spectrophotometrically, using a Perkin Elmer UV-VIS spectrophotometer, whereas phenol degradation was measured by HPLC equipped with a UV-VIS detector. The reaction of "OH radicals formed on a photo-illuminated Fe-TiO2 surface was performed by fluorescence technique (AB2 Luminescence Spectrometer), using terphallic acid.

We observed that modification of Fe-doped TiO2 by electron beam is an interesting approach to improvement of photocatalytic processes under UV and visible light illumination and its applicability for environmental purposes. Irradiated Fe-TiO2 samples exhibited better photocatalytic properties in comparison to catalysts calcined at 400°C.

The best results of decomposition were observed in photocatalytic reaction on irradiated Fe/TiO2 doped with Fe(NO3)3, and Fe(III)-acetylacetonate.

POS19 Radiation research and nuclear power

POS19-01. Effect of electron beam irradiation on trimethylene carbonate and lactide copolymers. Agnieszka Adamus¹ R.A. Wach², J. Jozwiakowska¹, D.W. Grijpma¹, 1: Institute of Applied Radiation Chemistry, Chemistry Department, Technical University of Lodz, Poland, 2: Institute for Biomedical Technology (BMTI) and Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology, University of Twente, The Netherlands, 3: Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, The Netherlands

In this study electron beam (EB) irradiation was applied to modify properties of triblock copolymers made of trimethylene carbonate (TMC) and DL-Lactide (DLLA) monomers. Poly(L-lactide) (PLLA) is one of the most commonly used polymers in biomedical applications, because it is biocompatible and degrades in vivo to lactic acid. PLLA is however highly crystalline and rigid, therefore L-lactide has been copolymerised with TMC to obtain more flexible materials. Another advantage of incorporating TMC into the PLLA chain is the tuneable degradation profile [1].

These copolymers were prepared by sequential polymerization under argon at 120 °C using SnOct2 catalyst and hexamethyldisilazane. First PTMC was synthesized. After that, PLLA was added to the PTMC prepolymer with another drop of the catalyst to obtain triblock copolymers poly(DLLA-TMC-DLLA).[2] Copolymers were purified by dissolution in chloroform (5 wt/vol%) and precipitation into 2-fold volume of isopropanol. Samples were irradiated by EB with various doses at room temperature in the presence of air. Thermal characteristics of the copolymers were investigated using differential scanning calorimeter (DSC) and changes in their molecular weight were monitored by gel permeation chromatography (GPC). Thermograms of block copolymers transition temperature (Tg) only, and no exothermic crystallization or melting peaks were observed. Initial study showed that Tg values remained unchanged after irradiation. Irradiation does not significantly affect the average molecular weight of the copolymers. In some cases minor increase of average molecular weight with increasing dose of irradiation was recorded. Parallel crosslinking of soft PTMC segments and degradation of rigid PDLLA segments during electron irradiation could result in observed changes in copolymers characteristics.

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References:

POS19-02. Direct and indirect radiolytic effects in highly concentrated halide aqueous solutions-picosecond pulse radiolysis. Anna Balcerzky, Laboratoire Chimique Physique, France

The products of decomposition of pure water and low concentrated aqueous solutions under irradiation are well known and their radiolytic yields are now well established at the end of the non-homogenous stage, around 100 ns after energy deposition in the solution [1,2]. Whereas, the radiolysis of concentrated solutions which causes direct ionization of the solute is still under investigation and the radiolytic yields of species formed during the radiolytic process have not been quantitatively well established up to now. This direct effect of ionizing radiation has very important meaning for practical demands like the treatment of waste in nuclear energy technology and even for radiotherapy. [1,3,4]. Recently, using a steady state radiolysis, we showed the effect of the direct ionization of Br- on the yield of Br2 in highly concentrated Br2 aqueous solutions.[5] In the present work we used picoseconds pump probe radiolysis in order to observe directly the action of ionizing radiation with the solute.

We carried out the experiment of radiolysis of highly concentrated solutions of NaBr and NaCl up to 6 M and 5 M, respectively. Using
the picosecond electron pulse facility ELYSE we measured the yield of oxidation of Br' and Cl under different conditions which conducts to the formation of Cl₂ and Br₂ in chloride and bromide solutions, respectively. A new mechanism for Br and Cl oxidation is presented[6].


POS19-03. Modular specialized equipment for environmentally safe processing of liquid radioactive wastes, Artak Barseghyany¹, G. Martoyan², 1: AREV Scientific Industrial CISC, Engineering Academy of Armenia, Armenia 2: AREV Scientific Industrial CISC, Yerevan, Armenia

Radioactive wastes is one of the most critical problems facing the global nuclear industry (nuclear energy generation), the environment (radioactive contamination), national security systems (potential hazards) today. Annual huge amount of Liquid Radioactive Wastes is generated by nuclear plants, reactors, processing plants and military installations worldwide. Current waste processing technologies and capacities are insufficient to fulfil waste management needs of the Nuclear Industry, which has led to continuous increase in volumes of stored LRW.

New Unique Technology (patented in Armenia and internationally) -- specially designed separator-concentrators -- employs coordinated use of an electrodialysis (ED) stack and a number of electrolysis modules, each to output selected radionuclide (one or a few at a time) in the form of fine powder. The process is fully environmentally safe, has no emissions and produces only selected elements. The new method demonstrates extraction-separation efficiency, which averaged over radionuclides is 99.985%, the Liquid Radioactive Waste (LRW) volume reduction ratio after processing is about 2000.

A Mobile System is designed to process liquid radioactive waste at high, intermediate and low activity levels, due to the unique safety and environmental friendly technology, which allows having compact equipment for high-throughput LRW processing. The new hydrometallurgical method is already applied in an industrial-scale prototype plant at Armenia’s Metznmor nuclear power plant.

Mobile LRW Processing System

All desired features of the Mobile LRW Processing System can be assembled on a standard 40-ft trailer driven by a heavy-duty truck, producing only cleaned water and the entirely extracted radionuclides. The Mobile System is designed to process liquid radioactive waste at high, intermediate and low activity levels, at a rate of: 10 tonne/hr for 5 g/l of salt concentration of radionuclides 5 tonne/hr for 10 g/l of salt concentration of radionuclides 1 tonne/hr for 50 g/l of salt concentration of radionuclides

POS19-04. Time variation of ambient dose rate artificially increased by Fukushima Dai-ichi nuclear power station accident, Masahiro Hosoda¹, S. Tokonami¹, A. Sornichii¹, S. Monzen¹, M. Osana¹, I. Kashikawa¹, S. Akiba¹, 1: Hiroaki University, Graduate School of Health Sciences, Japan 2: Hiroski University, Institute of Radiation Emergency Medicine, Japan 3: Kagoshima University, Graduate School of Medical and Dental Sciences, Japan

On March 11, 2011, Japan was attacked by one of the most powerful earthquakes (M=9.0) in recorded history called “the greatest Japan earthquake”. The power supply for cooling in Fukushima Dai-ichi nuclear power station was stopped under the influence of an earthquake, and hydrogen explosion occurred at several nuclear reactors. The artificial radionuclide such as iodine-131, cesium-134 and -137 were released from a nuclear reactor to living environment by this accident. In this study, the time variation of ambient absorbed dose rate artificially increased by Fukushima Dai-ichi nuclear power station accident was considered, and a cumulative dose map at high dose rate area was drawn. (1) The measurements of ambient absorbed dose rate from fallout were carried out on the expressway from Hirosaki city (N40.59', E140.47') to Fukushima city (N37.75', E140.30'). A car-borne survey technique with 1" × 1" NaI(Tl) scintillation surveymeter was used for the measurement. The car-borne survey technique is a useful tool for measurement of the ambient absorbed dose rate in a short term such as an emergent radiation measurement. The measurement was repeated 3 times at March 16th, April 11th and 25th. The distance between Fukushima Dai-ichi nuclear power station and each measurement point was from 60 to 355 km and more than 100 data were obtained by these surveys. Furthermore, an effective half life was estimated using these results. These values were dependent on the distance from Fukushima Dai-ichi nuclear power station. The effective half life at the near measurement point from nuclear power station was close to the physical half life of iodine-131 (8 days). Moreover, the dose rate on the uncovered ground was 1.3 times higher than those on the pavement. (2) Measurements of dose rate at radioactively contaminated area for drawing a contour map were also carried out from April 12th to 15th using car-borne survey technique. The ambient dose rates were not dependent on the distance from Fukushima Dai-ichi nuclear power station whereas the highest area was found at the north-west area direction from the nuclear power station.


Ionic liquids (ILs) are considered designer solvents because they can be easily ‘tuned’ to have desired properties. In particular, the capability of an IL to dissolve a wide range of molecules, and the potential of these solvents to be highly resilient in energy-intensive environments, make ILs particularly promising media for the separation and sequestration of metal ion contaminants from radioactive waste effluents in nuclear fuel cycles. In such an application, a water-IL system will be exposed to a continuous stream of ionizing radiation. This radiation can strongly influence the chemical state of the water phase in the system and the chemical parameters affecting the separation efficiency of the system. Previous work has shown that the effect of radiation on the stability of ILs have been limited to pure ILs. This work examines how long-term irradiation affects gas-IL and water-IL biphasic systems.

The interfacial stability of gas-IL and water-IL systems under γ-irradiation was studied for three phosphonium ILs having different solvent properties. The target systems were irradiated in a 60Co gamma cell at 6.4 kGy/h. The contents of the sample vials were examined periodically; the gas phase above the IL samples was analyzed using gas chromatography with a mass spectrometer detector, while the changes in the IL and aqueous phases were monitored using conductivity measurements and Raman spectroscopy. The results indicate that γ-irradiation induces negligible chemical changes in the IL phase when it is in contact with a gas phase. For water-IL systems, the initially immiscible layers slowly developed an interfacial emulsion layer, even without irradiation. This layer started at the water-IL interface and then grew downwards, eventually converting the entire IL phase to an emulsion. Irradiation accelerated the conversion of the IL phase to an emulsion. The changes in the conductivity and the Raman spectra of both the IL and water phases observed during the development of the emulsion are consistent with formation of micelles, as further confirmed by transmission electron microscopy. Based on these results, it is proposed that the formation of micelles at, or near, the water-IL interface leads to the development of an emulsion layer, and that irradiation produces surfactants that can accumulate at the interface and accelerate emulsification.

POS19-06. Iron oxhydroxide colloidal formation by gamma-radiolysis. Jiju Joseph, University of Western Ontario, Canada
Uniform-sized colloidal particles of g-FeOOH were formed by gamma-irradiation of de-aerated aqueous solutions containing initially soluble Fe²⁺ species at pH 5.5. The colloidal particles were analyzed by Transmission Electron Microscopy (TEM), Selected Area Electron Diffraction (SEAD), UV-Visible Absorption and Fourier Transform Infrared Spectroscopy (FTIR). Two distinct colloidal formation stages were observed. At short irradiation times or in solutions containing initially low Fe²⁺ concentration, the size of particles are less than 10 nm with a narrow size distribution. These primary particulates grew larger with a dendritic structure upon longer irradiation, but the growth soon reached a steady state, where longer irradiation did not change the size but increased the number of dendrimers formed. The narrow size distribution is attributed to the rapid homogenization processes occurring in the solution condensation. These primary particles then grow into g-FeOOH particles with a dendritic structure. The final size reached at long irradiation times is regulated by the steady-state redox conditions established during long-term irradiation at the aqueous-solid interface.

POS19-07. Design and construction of hydraulic prototype model for a radio-tracer experiment. Kim Ki-Chul1, Park Geon-Hyeon2, L. Jin-Yong2, L. Jung-Lyu1, S. Kyung-suk1, 1: Korea Atomic Energy Research Institute, South Korea 2: KAERI 3: SKKU, South Korea

Most nuclear power plants in Korea were located near coastal areas. It is important to evaluate the dispersion characteristics of the liquid effluents released into the sea from a power plant under both normal and accident conditions. In this study, a hydraulic prototype model was designed and constructed to understand the physical phenomenon for the advection and dispersion of a pollutant using radiosotope. The model was designed based on the real coastal area at the Wolsung nuclear power plant in Korea. The hydraulic model was constructed at 1/2000 and 1/200 scale, horizontally and vertically. The velocity was changed by controlling the RPM(revolutions per minute) of the inverter pump. The wave was produced by a wave maker, the time of maximum concentration showed a great difference, the wave maker was also used to reproduce the winds that cause the water movement.

For decontamination of radioactive water generated in a serious nuclear accident, zeolite is a suitable adsorbent for radioactive Cs because of its high adsorption capacity and radiation resistance. During the decontamination process and especially the storage of zeolite waste after the process, the management of hydrogen produced by water radiolysis is an important issue of safety. Thus hydrogen production in radiolysis of the mixture of zeolite and water was studied. In particular, we focused on the influence of the incorporation of seawater into the mixture because seawater was urgently used as a coolant for the nuclear fuels in Japan. Zeolite used in this study is natural mordenite (TOP ZEOLITE M) from Ayashi in Miyagi supplied by SHIN TOHOKU Chemical Industry. Seawater was sampled at Oarai in Ibaraki. Sample was the mixture of mordenite and seawater. The sample was irradiated in the mixture of mordenite and water. Therefore the hydrogen formation driven by the energy deposition to mordenite and the inhibition of oxidation of hydrogen should be considered in the evaluation of hydrogen production from radiolysis of the mixture of mordenite and water.

POS19-09. Analysis on the characteristics of a pollutant transport by using Tc-99m. Suh Kyung-Suk1, Park Geon-Hyeong2, K. Ki-Chul2, L. Jin-Yong2, L. Jung-Lyu1, 1: Korean Atomic Energy Research Institute, South Korea 2: KAERI 3: SKKU, South Korea

When harmful pollutants enter in an ocean system, they may have a critical impact on the ecology of the sea and on human health. In particular, the radioactive materials released into the sea from a nuclear power plant have been affected very serious impacts on oceanic environments. It is very important to predict the damage from such an accident, and to understand the movement of pollutants. A laboratory experiment using a radiosotope was performed to analyze the characteristics of the transport and dispersion of a pollutant released from an industrial plant. A radiotracer method is a useful tool for investigating the pollutant dispersion and description of mixing process taking place in natural streams. The main advantage is that from such an accident can remain for a long time in the water, and the radioactive tracer was measured quite well by the advection and diffusion. After the experiment, the radioactive tracer was measured quite well by the advection and diffusion throughout this experiment. The results showed that the radionuclide concentration were in relatively good agreement. The values showed that the radionuclide concentration were in relatively good agreement.

POS19-10. Identification of Fukushima origin gamma-emitters in the environmental samples in Lithuania. Evaldas Maceika1, E. Maceika2, V. Remeneik1, A. Gudelis2, B. Luksiene2, R. Drutskiene2, N. Skripkauskaite1, S. Buvydas1, M. Konstantinova1, A. Plukis2, R. Plukiene2, V. Filistovic1, 1: State Research Institute Center for Physical Sciences and Technology, Lithuania 2: State Research Institute Center for Physical Sciences and Technology, Vilnius, Lithuania

A recent large scale nuclear accident at the Fukushima NPP caused dispersion of the technogenic radionuclides all over the world. Sampling campaign was conducted in the Baltic sea soon after the accident. The first clouds contaminated with Fukushima origin radionuclides (mostly 131I, 132I, 132Te, 134,136Cs) reached the country on 21-23 of March 2011. The maximal 131I activity concentration of 3.4 mBq/m² in ground surface aerosols was detected on the 3-4 April 2011. Detectable levels of 134,137Cs activity concentration of up to 3.5 ± 0.6 Bq/kg in fresh pine needles were also observed. Pre-concentration procedure (burning in the muffle furnace at the 400 °C) of pine needles, moss and grass samples taken in the forest and lawn near Vilnius allowed tracing the presence of 134,137Cs in these samples. The following activity concentrations in pine needles were also observed: 131I/132I/132Te/134,136Cs/137Cs mass activity concentration of 2.44 ± 0.09 Bq/kg, f. w. The grass and moss sample measurements identified the deposition density of 0.9±0.1 Bq/m² for 137Cs and the narrow size distribution.
POSTER PRESENTATIONS

POS19.11. Calculation of stopping power for protons and secondary electrons beams in liquid water. Abdellah Marouane1, S. Ouasik2, J. Inchaouh1, 1: Laboratoire de Recherche Subatomique et Applications, Morocco 2: Laboratoire Physique de la Matière Condensée, Morocco 3: Subatomic Research and Applications Laboratory, Morocco

We have calculated the stopping power of energetic protons in liquid water by using a new calculation model based on different theoretical and semi-empirical approaches, in this calculation model we considered the different interactions associated with direct proton (or hydrogen) ionization and secondary processes arising from the electrons ejected by the primary process. The relativistic effects corrections, along with the electronic and nuclear stopping power, have been considered in this work. Thus, modified empirical formulas incorporating the dose effect have been proposed. The stopping power of protons beams have been calculated in liquid water, and they have been used for a different incident energy to make during their motion inside the target with electrons and nuclei. The interactions of the incident particle with target’s electrons dominate in the high energy regime, at low energy regime the interactions of the projectile with the target nuclei contribute basically and should be not neglected in the calculation of the stopping power. We have not neglected the effect of electrons ejected from protons and hydrogens ionization impact, and the effect of the secondary electrons generated by hydrogen electron loss processes. The calculated data of secondary processes stopping power might be useful to calculate the total dose of protons impact. Our result represents a good agreement with existing experimental data. This calculation model can be useful for applications in protontherapy, and for the calculation of depth-dose distributions for protons in biological medium.

POS19.12. Radiolysis of water at high temperature/pressure conditions up to supercritical region: a picosecond pulse radiolysis and a Monte-Carlo simulation study. Yusa Muroya1, M. Lin1, S. Sanguanmith1, J. Meesungnoen1, M. Mostafavi1, J. Jay-Gerin1, Y. Katsumura1, 1: University of Tokyo, Japan 2: Japan Atomic Energy Agency, Japan 3: University of Sherbrooke, Canada 4: University of Paris-Sud, France

To understand the radiolysis of water at high temperature / pressure conditions is of great importance for advanced water chemistry for nuclear power plants (NPP) to control the chemical condition of water under strong radiation field in order to mitigate adverse effects of surrounding nuclear structural materials, such as stress corrosion cracking (SCC). At room temperature condition it is known that the radiolysis of water stems from energy deposition by the ionizing radiation which subsequently undergoes physical, physicochemical, and chemical processes, resulting in production of various radiolytic products. Those transient processes are supposed to vary significantly at high temperatures / high pressure (HTHP) conditions, giving rise to different time behaviors and radiation chemical yields of them. Although it has been intensively studied the radiolysis at HTHP by g-radiolysis, ns pulse-radiolysis, and muon chemistry, there still exists a difficulty in direct trace of short-lived radiolytic species. It is expected to develop a higher time resolved technique allowing observing such fast reaction kinetics.

In this work, a new picosecond pulse radiolysis system for HTHP conditions consisting of an S-band linear accelerator and a femtosecond Ti:Sapphire laser was developed. By employing them, fast kinetics and optical spectra of the hydrated electron with picosecond time resolution could be successfully observed. Evaluation of time-dependent yield of the hydrated electron revealed a faster spur decay kinetics when a few nanosecond was increasing temperature up to supercritical condition, suggesting that the hydrated electron is more favored to react with a proton to form a hydrogen atom, in contrast to room temperature at which the reaction with OH radical is predominant. With these new experimental results, a Monte-Carlo simulation approach to evaluate the water decomposition process such as temperature dependence of a thermalization distance of the hydrated electron, time dependent yields of short-lived radiolytic species, fast spur reaction process contributing to molecular hydrogen production etc. will be discussed.

POS19.13. Comparison of applications of gene mutation assay in Trad-SH cells for monitoring ambient air genotoxicity after Chernobyl and Fukushima nuclear power plant accidents. Agnieszka Panek, J. Miszczyk, A. Cebulska-Wasilewska, Institute of Nuclear Physics, PAS, Poland

Introduction: Our aim was to investigate the genotoxicity of ambient air in the Krakow area after Fukushima Nuclear Power Plant accident and compare with results from Chernobyl fallout. Experimental procedures: The technique for screening gene mutation frequency in somatic cells of the Tradescantia stamen (SH assay), have been developed many years ago specifically for radiobiological studies. Tradescantia is one of the most radiosensitive plant known. Its extremely high radiosensitivity of its hybrid clones is followed by very high sensitivity to chemical mutagens as well. This fact makes Trad-SH a particularly suitable for the environmental studies and for the detection of ambient air genotoxicity. Since 11th of March 2011 (Fukushima NPP accident), in the Krakow area six pots with biotesting plants in each of the 4 sites are exposed to ambient air, and continuous screening is performed. Progenies of 360 000 cells exposed were analyzed. Results: Mutation frequency obtained in the first 10 days is shown as control levels (GMF*100 = 0.06 ± 0.01). Significant increase of gene mutation frequency (GMF*100 = 0.18 ± 0.05, the range, 0.10-0.3 depending on the location) was reported, associated with a strong expression of toxic effects. Results of applications of the bio-indicator for in situ monitoring genotoxicity of the ambient air pollution including ionizing radiation from Chernobyl Nuclear Power Plant accident are compared to recent data from monitoring the ambient air quality in the Krakow and surroundings. Following the Chernobyl accident studies were performed initially as monitoring of mutagenicity of ambient air in the period since April 29th till June 3rd 1986 [1]. In general, mutation frequency (GMF*100 = 0.43 ± 0.02) increase due to Chernobyl fallout was corresponding to fluctuation of radioactivity in the air reported from physical measures, and to published reports about increase in chromosomal aberration levels. Conclusion: Statistically significant increase in comparison to control and more prolonged than that after Chernobyl fallout increase of GMF is recently observed. It is associated to the strong expression of toxic effects. An increase in mutation rates is corresponding with fluctuation of radioactivity in the air reported from physical measures. [1] Cebulska-Wasilewska A., Envr. & Molec. Mutagenesis, 1989. Acknowledgments: 0296/B/P01/2008/35


The U.S. Nuclear Regulatory Commission (NRC) radiation protection research program provides technical support to NRC in the areas of dose assessment and assessment of human health effects for reactor and nonreactor licensing, emergency preparedness, and nuclear security activities. The program’s scope includes development of technical bases for radiation protection regulations, external exposure computer codes, occupational exposure and effluence databases, and public health studies research. Current work in the radiation protection regulation arena includes the development of a regulatory basis for possible revisions of 10 CFR 20 for greater alignment with the recommendations in the International Commission on Radiological Protection (ICRP) Publication 103. NRC is sponsoring research in biokinetic and dosimetric models and dose coefficient factors for occupational and public exposure to radionuclides that are based on ICRP Publication 103. In the area of external exposure computer codes, NRC funds VARSKIN and PIMAL. VARSKIN is used to facilitate skin-dose calculations. PIMAL is a computational “phantom” with moving arms and legs used to assess the radiation dose for realistic exposure geometries. NRC maintains several radiation protection databases. NRC collects and reports the annual occupational radiation exposure data and non-licensed activities into the Radiation Exposure Information and Reporting System (REIRS) database. NRC collects reports and maintains a database for both the gas and liquid radioactive effluents discharged from NRC-licensed facilities. As part of the effluence work, NRC is modifying the exposure pathways of gas and ambient of organic forms of carbon-14. In addition, NRC has developed the Radiological Toolbox, an electronic handbook used to quickly access
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POS91-15. Studies on improving radiation stability of substances applied in the recycling of a spent nuclear fuel, Agnieszka Sulich1, J. Grodkowski1, J. Mirkowski1, R. Kocia1, M. Foreman2, 1: Institute of Nuclear Chemistry and Technology, Poland 2: Chalmers University of Technology, Sweden

The objects of research were CyMe4-BTP (2,6-bis(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzofuran-1,2,4-triazin-3-yl)pyridine) and CyMe4-BTBP (2,6-bis(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzofuran-1,2,4-triazin-3-yl) [2,2'-bipyridine], proposed as the ligands selectively complexing the ions of trivalent actinides in the processes of their separation from lanthanides, present in the high level radiation liquid waste.

The aim of research was investigation of the radiation stability of these compounds in octanol and cyclohexanone solutions, pure or equilibrated with equal volume of H2O or 1M HNO3. As an experimental model the pulse radiolysis with a spectrofotometric detection was used. Measurements were carried out using 10ns, 10 MeV, electron pulses from the LAE-10 linear electron accelerator delivering doses up to 20 Gy per pulse.

The reactions of solvated electrons in octanol with the ligands were observed. The calculated rate constant of reactions kMe4-BTP = (1.6±0.5)x10^11 mol^-1 dm^3 mol^-1 s^-1 and kMe4-BTBP = (1.7±0.5)x10^11 mol^-1 dm^3 mol^-1 s^-1. Because of limited solubility of the ligands in the examined diluents we had to choose some other, better soluble reagent which give the known intermediates with absorbance in the useful range. The benzophenone (BP) best obeys our criteria. The spectra of its radiolysis intermediates in some alcohols are known and BP was also used in the pulse radiolysis of tributyl phosphate systems. The ligands were used with the concentration range 1-10 mM and BP with 0.1-0.4 M. Samples had been Ar saturated before experiments. The most stable of BP radiolysis products are benzophenone ketyl radical. The calculated radiation yields of this intermediate in octanol drops if the solvent is equilibrated with HNO3. Other products are anion radical of BP and some traces of the excited states of BP. The results in cyclohexanone indicated also the formation of the excited states of BP. In the presence of BP most of the primary radiolysis products are converted to less reactive benzophenone species. This effect could prevent to some extent radiation damage of the ligands.

This work has been done in the frame of the program ACSEPT FP7 Collaborative Project 211267.

POS20 Combination treatments

POS20-01. Exogenous nitric oxide in radiobiology research. Bogdan Gerashchenko, O. Glavin, V., R.E. Kavetskys Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences, Ukraine

Nitric oxide (NO) is among major air pollutants capable of causing cytotoxic and genotoxic effects. Also, NO is known as an important cellular molecule involved in many physiological and pathological processes. However, chronic expression of NO is believed to be associated with various carcinomas and inflammatory conditions. Long term NO inhalation has been shown to provoke acceleration of tumor growth in rats (Mikaelian et al., Exp. Oncol. 2005, 27: 65-70). In a cell, nitrosative stress is associated with DNA single strand breaks, apoptosis, and inhibition of synthesis of DNA and proteins. It is characterized by formation of N-nitroso compounds and highly toxic peroxynitrit, which is formed as a result of reaction of NO with superoxide anion. Since NO can readily react with various reactive oxygen species (ROS) thus causing their detoxication or generation of extremely toxic forms, health effects of NO in combination with an ionizing radiation (IR), are unknown. To assess how exogenously delivered NO together with exposure to IR can affect metabolism of ROS, male rats were fractionally exposed to NO for 1 month (14 h per day; 125-150 mg of NO per 1 m3 of air) and additionally received fractional exposure to low doses of X-rays (10 acute exposures with 0.1 Gy each and with a frequency of about 1 exposure per 3 days). The levels of ROS in peripheral blood leucocytes (PBLs), serum, and liver cells were then measured. Interestingly, in these animals, elevation of ROS in both PBLs and serum was not higher than in the group of animals that received NO alone, while ROS in the group of animals that received X-ray exposure alone were not elevated and did not differ from the level of ROS in the group of intact animals. ROS in liver cells of irradiated animals were not elevated either, however, in liver cells of animals that received NO and NO + IR treatments, there was some reduction of ROS levels. These findings suggest that long term fractional NO inhalation somehow in forms some ROS in PBLs and serum, while long term fractional irradiation with low doses may activate ROS scavenging potential, which is not efficient enough to scavenge ROS associated with NO inhalation. As for liver cells of animals that received NO and NO + IR treatments, ROS seem to be scavenged due to their direct reaction with NO and NO-related products.

POS20-02. Mechanistic study of combination treatment with radiation and arsenic trioxide enhanced anti-tumor effects in human prostate cancer cells with different p53 status. Hu-Wen Chu, Y. Wang, Department of Environmental and Occupational Health, National Cheng Kung University, Medical College, Tainan, Taiwan

Prostate cancer is a leading cause of illness and death among men in the USA and Western Europe. Radiotherapy is one of the treatment for prostate cancer. Many studies indicated that arsenic trioxide (ATO) could enhance the anti-tumor effect of radiotherapy and reduce radiation dosage. The aim of this study was to investigate the anticancer effect of ionizing radiation (IR) combined with ATO and their underlying mechanisms on prostate cancer LNCaP (wild-type p53) and PC-3 (p53 null) cells. In in vitro studies, cell viability was detected by trypan blue. Cell cycle distribution and early apoptosis with annexin V-FITC apoptosis detection kit were analyzed by flow cytometry. In order to observe the expression of acidic vesicular organelle which is characteristic of autophagy, cells were stained with acridine orange. Ultrastructure of PC-3 cells was analyzed by electron microscopy. Western blotting was used to determine apoptosis- and autophagy-associated proteins expression. A nude mice xenograft model was used to investigate the effects of IR combined ATO treatment in vivo. The results indicated that the effect of combined treatment is more significant than IR or ATO alone in LNCaP and PC-3 cells. The combined treatment increased the percentage of apoptosis in LNCaP cells, but did not increase the percentage of apoptosis in PC-3 cells. On the contrary, combined treatment caused cell cycle G2/M arrest in PC-3 cells and increased the percentage of autophagy in both LNCaP and PC-3 cells compared to ATO and IR alone. Furthermore, the expression of LC3 II and P62/STQST1 increased in LNCaP and PC-3 cells treated with combined treatment. The Akt/mTOR pathway was inhibited by combined treatment compared with those subjected to individual treatment. In addition, pretreated with 3-MA, a specific inhibitor of autophagy, decreased the combination-induced autophagy and increased cell viability. Whereas pretreated with LY294002, a specific inhibitor of PI3K/Akt, further enhanced the combination-induced autophagy and decreased cell viability. In in vivo studies, the combination of IR and ATO significantly reduced the tumor volume in nude mice that had received a subcutaneous injection of PC-3 cells. These results show that combined treatment may increase therapeutic efficacy of prostate cancer cell lines.

POS20-03. Soluble HSP27 increases the efficacy of radiation therapy by normalizing tumor vasculature. Seo-Hyun Choi1, H. Lee2, Y. Bae Jin3, Y. Lee1, Y. Lee1, 1: Korea institute of Radiological & Medical Sciences/Korea University, South Korea 2: Korea Institute of Radiological & Medical Sciences, South Korea 3: Ewha Womans University, South Korea

Endothelial cell function is critical for angiogenic balance in both physiological and pathological conditions, such as wound healing and cancer, respectively. We hypothesized that heat shock protein (mouse HSP25 and human HSP27) interacts with VEGF, a potent proangiogenic factor and endothelial mitogen whose function is critical for angiogenesis.

We report here that soluble HSP27 is released specifically from endothelial cells (ECs), and plays a key role in regulating angiogenic balance via its direct interaction with VEGF. Neutralization of HSP25 promoted in vivo wound healing and embryonic vasculogenesis, whereas overexpression of HSP25 in mouse lung was sufficient to suppress lung metastases of CT26 colon carcinoma in vivo. To
evaluate whether soluble HSP25 directly inhibits tumor growth, we injected CT26 cells subcutaneously into the hind legs of nude mice and followed with treatment of HSP25. Systemic treatment with HSP25 delays tumor recurrence, and inhibited tumor vascular formation. Many studies have shown that anti-angiogenic agents can increase the efficacy of radiation therapy (IR) by normalizing tumor vasculature, resulting in increased tumor oxygenation. Thus, as expected, the combination of rHSP25 treatment and IR significantly inhibited tumor growth compared with IR alone. Understanding the function of soluble HSP27 in angiogenesis may lead to overcoming the limitations of anti-VEGF therapy, as well as radiation therapy.

**PO520-06.** The impact of combined x-rays-bisphenol A exposure on the sperm count and quality of pubescent male mice. Małgorzata Dobrzyńska1, A. Gajewik1, J. Radzikowska1, E. Tyrikel1, E. Jankowska-Stiefer2, K. Pachocki3, 1: National Institute of Public Health-National Institute of Hygiene, Poland 2: Medical University of Warsaw, Poland

Both X-rays and bisphenol A (BPA) are present in the human environment. The aim of the study was the estimation of the effect of combined exposure to X-rays and bisphenol A on the gonads weight and on the sperm quantity and quality of young adult mice compared to the effects of each agent alone.

The 4.5-week-old pubescent male mice were irradiated with 0.05 Gy of X-rays, exposed to BPA solution in drinking water (5 mg/kg bw, 10 mg/kg bw, 20 mg/kg bw) or in combination of both (0.05 Gy + 5 mg/kg bw BPA) for 8 weeks. A Roentgen unit Medicor was used for X-rays source (the dose rate 0.20 Gy/min). Animals were sacrificed immediately after the end of exposure as well as 1, 4 and 8 weeks later.

The dose 0.05 Gy of X-rays significantly reduced the mean testes weight at 24 h, 1 and 4 weeks following the end of exposure, whereas the dose of 5 mg/kg bw of BPA significantly increased the mean testes weight at 8 weeks. The combined exposure to low doses of both agents significantly reduced mean testes weights compared to control and BPA alone.

X-rays significantly decreased the sperm count at 24 h and 1 week after the termination of exposure. The doses of 10 mg/kg and 20 mg/kg bw BPA markedly reduced sperm count at 4 weeks. At 24 h after the end of combined exposure decreased sperm count compared to control and BPA alone was observed. The sperm motilities were reduced 1 week after the termination of exposure in groups of 0.05 Gy, 0.05 Gy + 5 mg/kg bw BPA and 20 mg/kg bw BPA. The percentages of abnormal spermatozoa were markedly increased in almost all exposed groups at 24 h and 1 week after the last exposure, and occasionally at 4 and 8 week. The enhanced DNA damage in germ cells were not observed.

A histological observation demonstrated alterations in the seminiferous tubules morphology such as intratubular vacuoles, cell degeneration and the occurrence of multinucleated giant cells. There were dose and time dependencies. No alterations were visible in the interstitial tissues. By 8 week after BPA and irradiation withdrawal testes showed histological foot wires similar to those seen in control. Combined exposure to both agents diminished sperm count and quality compared to at least one of the agent. Compared to previous study, germ cells of pubescent males seems to be more sensitive to X-rays and BPA than germ cells of adult males.

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**PO520-05.** Neurotensin receptor 1 (NTR1), a novel target for tumor radiosensitization. Jaroslaw Dziegielewski, N. C.K. Valerie, E. V. Casarez, Jr, S. J. Parsons, J. M. Larner, University of Virginia, USA

Radiotherapy (RT) is one of the most successful treatments for cancers. However, radioresistance and adverse effects on normal tissue remain a significant clinical problem. One strategy to overcome this is to use a sensitizing agent able to selectively enhance antitumor effects of RT. Here, we investigated neurotensin receptor 1 (NTR1) as a novel target for radiosensitization. In human prostate tumor xenografts, neurotensin receptor 1 (NTR1) is the only neurotensin receptor expressed. SR48692 inhibition of NTR1 expression and receptor signaling blocks tumor growth compared to IR alone. Understanding the function of soluble HSP27 in angiogenesis may lead to overcoming the limitations of anti-VEGF therapy, as well as radiation therapy.

In vivo inhibition of Hsp27 leading to chemo- and radio-sensitization effects. We investigated, in vitro and in vivo, whether functionally inhibition of Hsp27 using the peptide aptamer (PA) strategy, sensitizes the radiosensitive SQ20B head and neck squamous carcinoma cells to gamma-irradiation. PAs expression perturbed the dimerization and oligomerization of Hsp27, and acted as negative regulators of the antipapoptotic and cytoprotective activities of this protein. We characterized two PA that increased radiosensitivity. These findings confirm that Hsp27 is a novel target for radiosensitization.

**PO20-06.** Peptide aptamers targeting Hsp27: a new approach for radiation sensitization. Elie Hadchty Elie Hadchty1, M. Aloy2, B. Gibert1, P. Colas1, A. Arrigo1, C. Diaz-Latoude2, C. Rodriguez-Lafraese3, 1: Holy Spirit University Kaslik, Lebanon 2: Department of Cellular and Molecular Radiobiology, EA3738, Lyon-Sud Medical School, Lyon, France 3: Molecular and Cellular Genetic Center, CNRS UMR5534, University of Lyon, Lyon, France 4: CNRS USR 3151, Station Biologique, Roscoff, France 5: Department of Cellular and Molecular Radiobiology, EA3738, Lyon-Sud Medical School, Laboratoire de Biologie des Tumeurs, Pierre Bénite, France

Human heat shock protein 27 (Hsp27) is an antiapoptotic protein characterized for its tumorigenic and metastatic properties, and now referenced as a major therapeutic target in many types of cancer. Hsp27 biochemical properties rely on a strongly regulated dynamic organization. Downregulation of its expression by antisense oligonucleotide or small interfering RNA has proven their efficiency to counteract the antiapoptotic and protective properties of Hsp27 leading to chemo- and radio-sensitization effects.

We investigated, in vitro and in vivo, whether functionally inhibition of Hsp27 using the peptide aptamer (PA) strategy, sensitizes the radiosensitive SQ20B head and neck squamous carcinoma cells to gamma-irradiation. PAs expression perturbed the dimerization and oligomerization of Hsp27, and acted as negative regulators of the antipapoptotic and cytoprotective activities of this protein. We characterized two PA that increased radiosensitivity. These findings confirm that Hsp27 is a novel target for radiosensitization.

**PO20-07.** Induction of microRNAs in reticulocytes of peripheral blood and bone marrow of male mice exposed to X-rays, bisphenol A and to a combination of both. Aneta Gajownik, J. Radzikowska, M. Dobrzyńska, Department of Radiation Protection and Radiobiology, National Institute of Public Health-National Institute of Hygiene, Poland

Bisphenol A (BPA) is one of the endocrine disruptors. There are few reports that BPA may cause DNA damage or unequal distributions of
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chromosomes. This compound is a major component of epoxy, polycarbonate and other resins. Radiation is a well-known mutagenic and carcinogenic agent. Exposure to X-rays is possible in cancer therapy and during diagnostic investigations. This study had on purpose to estimate the effects of bisphenol A, X-rays and combined exposure to X-rays and bisphenol A on the induction of micronuclei (MN) in the peripheral blood and bone marrow reticulocytes.

Male mice of the SF1-AF strain were irradiated with 0.05 Gy or/and treated with bisphenol A (5 mg/kg/mc, 10 mg/kg/mc, 20 mg/kg/mc) or exposed to a combination of both (0.05 Gy + 5 mg/kg BPA) for 8 weeks. Peripheral blood was collected from caudal vein at 1, 4 and 8 weeks of exposure, but bone marrow after termination of experiment only.

Both bisphenol A and X-rays stimulated induction of micronuclei in peripheral blood and bone marrow reticulocytes but the level of MN following irradiated was markedly higher. The results of 10 mg BPA, 20 mg BPA after 1 week of exposure and of 5 mg BPA, 10 mg BPA after 4 weeks of exposure were statistically significant different from controls in peripheral blood. On the other hand in bone marrow the statistically significantly different result was only in 5 mg BPA group. Combined exposure of X-rays and bisphenol A induced higher frequency of micronuclei compared to effect produced by BPA alone. The results of combined exposure of X-rays and bisphenol A were statistically significant.

X-rays is probably the agent which decided about DNA damage following combined exposure. Bisphenol A seems to be a weak mutagen.

POS20-08. In vitro and in vivo targeted radiosensitization by the Chk1 inhibitor SAR-020106, Gerben Borst1,2, Jiany Kuyla1, Martin McLaughlin1, San Neijenhuis1, James Good1, Ian Collins1, Michelle Garrett2, Marcel Verheij2, Kevin Harrington1, 1: The Institute of Cancer Research, Section of Cell and Molecular Biology, London, UK, 2: Netherlands Cancer Institute Cancer Center and Institute of Oncology, Poland

Selectively targeting radiation (RT)-induced DNA damage repair mechanisms in tumour and not normal cells can lead to significant therapeutic gains. Abrogation of the G2 checkpoint by Chk1 inhibition may selectively enhance genotoxic death in tumour cells that are unable to arrest in G1 phase of the cell cycle. This is the case for p53 mutant (mt) and HPV+ tumour cells. Our purpose was, therefore, to explore the effect of Chk1 inhibition (SAR-020106) in combination with RT in p53 mt or HPV+ cells compared to p53 proficient (wt) cells.

Colony assays in p53 mt or HPV+ tumour cells showed significant radiosensitization by Chk1 inhibition in contrast to p53 wt cell lines. This effect was also shown in short-term culture by caspase activity assays. The initial DNA damage measured by gH2AX foci (<4 hours after RT) was similar between p53 mt and p53 wt cells and between treatment with RT only and RT plus SAR-020106. However, the later phase of repair (>24 hours after RT) was different; cells unable to arrest in G1 had significantly more DNA damage only after treatment with SAR-020106.

Whereas abrogation of RT-induced G2 phase arrest using the Chk1 inhibitor was observed in all cell lines, this was only compensated by an increase in G1 phase population for cell lines with functional p53. Within the group of cell lines unable to arrest in G1, some enter the subG1/G0 phase after DNA damage, whereas others proceed through the cell cycle (including mitotic entry) and become aneuploid before cell death.

Time-lapse experiments using fluorescence ubiquitination cell cycle indicator (Sakae-Sawano et al. 2008), comprehensively confirmed the results of the cell cycle and radiosensitization analysis and provided further insights into mechanisms of cell death after RT alone or when combined with Chk1 inhibition.

In vivo radiosensitization was confirmed by delayed tumour growth and increased survival in a clinically relevant human tumour xenograft model of p53 mt cells using a schedule of repeated drug doses and fractionated irradiation.

In conclusion, radiosensitization was achieved in vitro and in vivo with inhibition of the RT-induced G2 arrest through Chk1 inhibition in many tumour cells unable to arrest in G1 because of mutant p53 or HPV positivity. As a result, Chk1 inhibition is a very promising strategy for targeted radiosensitization in many tumour types.

POS20-09. Microbeam radiation therapy alters microvascular architecture and tumor oxygenation and is enhanced by antiangiogenic peptide therapy. Robert Griffin1, N. Koonce2, E. Moros2, E. Brauer-Kirsch2, R. Dings4, P. Corry1, 1: Univ of Arkansas for Medical Sciences, USA 2: UAMS, USA 3: ESRF, France 4: University of Minnesota, USA

Purpose: To determine the therapeutic potential synchrotron-produced microbeam radiation therapy (MRT) alone and in combination with an anti-angiogenic peptide, anginex (Ax) and changes induced in tumor oxygenation and vascular composition.

Materials and methods: Tumor growth of 4T1 mouse mammary carcinomas following various spatial and dose combinations of MRT with and without daily axinex treatments was assessed. MRT beam size and spacing were planned to irradiate approximately half of the tumor volume in the following ratios: 50uM beams spaced 200uM, 75uM center to center (ctc) and 500uM beams spaced 2000uM et ctc using peak beam doses of 150Gy or 75Gy for each spacing. Immunohistochemical staining of tumor hypoxia and vascular networks (endothelium and smooth muscle) following these treatments were analyzed.

Results: MRT administered in 50 uM beams at 150Gy was most effective in delaying tumor growth. Fifty uM beams at 75 Gy induced growth delay intermediate between 150 Gy and control (untreated tumors). The 500 uM beams at 75 Gy were unable to alter tumor growth compared to control and 500 uM, 150 Gy induced intermediate tumor growth delay. However, daily axinex dosing at 20 mg/kg along with the MRT, 75 Gy MRT increased the relevant tumor response most significantly compared to MRT alone out of the conditions tested. Anginex treatment of animals whose tumors received the 50uM beams at 150Gy also led to a significant improvement in growth delay nearly inducing tumor stasis over the study period (2 weeks). The intermediate tumor growth delay intermediate between 150 Gy and control (untreated tumors). The 500 uM beams at 150Gy, 500uM 150Gy, 50uM 75Gy groups but not the 500uM 75Gy group and persisted until day 14 for the 500uM 150Gy. The addition of axinex treatment further decreased the MVD quantified in all combination treatment groups. Finally, the level of tumor hypoxia, as assessed by pimonidazole staining, was decreased at 1, 3, 7 and 14 days after MRT only in both groups receiving 150 Gy peak dose MRT.

Conclusion: Our results suggest that tumor microvascular damage induced by MRT can be enhanced using antiangiogenic agents and enhance the overall tumor response to lower entrance dose MRT which may improve normal tissue sparing. Supported by Central Arkansas Radiation Therapy Institute.

POS20-10. Prevention of EGFR autophosphorylation by new genistein derivatives in HCT 116 and DU 145 cells treated with ionizing radiation. Aleksandra Grуча1, A. Grucha1, A. Rusin2, A. Goglér-Pigłowska1, J. Zawisza-Puchalka1, W. Szefa1, J. Kraszewski1, 1: a – Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology; b – Center for Translational Research and Molecular Biology of Cancer, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland 2: Center for Translational Research and Molecular Biology of Cancer, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland 3: Center for Translational Research and Molecular Biology of Cancer, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland 4: Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Poland 5: a – Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology; b – Center for Translational Research and Molecular Biology of Cancer, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland

Purpose: To explore whether novel glycosidic genistein derivatives which modify the activity of epidermal growth factor receptor (EGFR) enhance cytotoxic effects of ionizing radiation against colorectal carcinoma (HCT 116) and human prostate cancer (DU 145) cells.

Background: EGFR, has been shown to be highly overexpressed in many types of cancers including colon and prostate. Its activation initiates signal transduction modulating cell proliferation, migration, adhesion, apoptosis, metastasis. Stimulation of EGFR phosphorylation by ionizing radiation is considered as the mechanism of radiosensitivity. Our former study revealed that certain novel glycosidic genistein derivatives, notably Ram3 and Ram21, arrest cell cycle in G2/M phase and inhibited activity of tyrosine kinases. This observation suggested that these genistein conjugates would possibly

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block the radiation-induced activation of EGFR thus potentiate the effect of radiotherapy.

Methods and materials: HCT 116 and DU 145 were exposed to genistein derivatives G21 and Ram3 followed by ionizing radiation. MTT (3-(4,5-dimethylthiazol-2-y)-2,5-diphenyltetrazolium bromide) and clonogenic cell survival assays were performed to determine Ram3 and G21 cytotoxicity. EGFR phosphorylation inhibition was determined using Western blotting analysis. Synergistic effects of Ram3 and ionizing radiation in combination with radiotherapy were studied by clonogenic analysis. Chou–Talalay’s algorithm was used for calculation of the dose effect curves and the combination indices. Flow cytometry and fluorescence microscopy analyses were used to evaluate cell cycle arrest in radiosensitive G2/M phase. Apoptotic cells were detected using TUNEL assay.

Results: Novel genistein derivatives Ram3 and G21 exhibited antiproliferative activity against HCT 116 and DU 145 cell lines and acted as inhibitors of EGFR phosphorylation. Both compounds prevented EGFR autophosphorylation induced by ionizing radiation. In combination with ionizing radiation G21 and Ram3 revealed synergistic or additive effects on HCT 116 and DU 145 proliferation inhibition, depending on the line tested and the concentration/dose. Conclusions: Glycosidic genistein derivatives Ram3 and G21 can be considered as lead compounds in search for new drugs useful in combined-modality chemoradiotherapy due to inhibition of a cell cycle in a radiosensitive G2/M phase and preventing radiation-induced autophosphorylation of EGFR.

POS20-11. The radiosensitizing potential of two EGFR tyrosine kinase inhibitors based on the G2-chromosomal radiosensitivity assay, Vasiliades T., M. Bellia, A. Valasai, A. Bourkoula, T. Magelis, M. Papadopoulos, G. Terzoudi, G. Pantelias, M. Paravatou-Petrosas, NCSR Demokritos, Greece

ATP site-directed EGFR tyrosine kinase inhibitors (TKIs) have shown antitumor activity in subsets of patients with non-small cell lung cancer, squamous cell carcinomas of the head and neck, and other malignancies, and have been also considered as potential radiopharmaceuticals for PET and SPECT imaging. Their greatest potential may be realized when used in conjunction with radiotherapy. The aim of the present study was to estimate the radiosensitizing potential of two quinazoline derivatives TKIs which bind the receptor in a reversible and irreversible mode - in human cells in vitro.

For this purpose, the radiosensitizing potential of both quinazoline derivatives was tested on the radiosensitive A431 human cell line by means of the G2-chromosomal radiosensitivity assay since an increase in the yield of chromatid type aberrations has been linked with increased radiosensitivity. Based on this, the experimental protocol involves the following steps: (a) exposure of A431 cell to both quinazoline derivatives independently at selected concentrations, (b) G2-phase irradiation exposure (1 Gy, r-rays, 3Co) of A431 cells, (c) analysis of chromatid type aberrations at the subsequent metaphase and G2-chromosomal radiosensitivity estimation.

The results indicate that pre-irradiation exposure of A431 cells to quinazoline derivatives A and D induces a statistically significant increase (p<0.001) in radiosensitizing effect with respect to reference value (cell exposed to ionizing radiation only). At concentrations inducing the same cytotoxic effect (MTT assay IC50 values), the compound A which binds to EGFR reversibly, has shown a more potent radiosensitizing effect with respect to quinazoline derivative D which binds to the receptor irreversibly. The mechanisms underlying the observed induced chromosomal radiosensitization will be further discussed with the future goal of optimization the use of TKIs in combined chemo- and radiotherapy protocols.

POS20-12. Hydrogen peroxide enhances radiation-induced apoptosis in hypoxic PC-3 prostate cancer cell line, Shinji Kariya, S. Tokuhori, R. Akima, Y. Ogawa, Kochi Medical School, Japan

Purpose: We previously reported that hydrogen peroxide (H2O2) strongly enhanced radiation-induced apoptosis in PC-3 prostate cancer cell line, that this apoptosis was lysosome dependent, and that mitochondria exists downstream lysosome in apoptotic pathway (IJOBP73: 49-454, 2009). In this study, we studied how H2O2 affects radiation-induced apoptosis in PC-3 prostate cancer cell line under the hypoxic condition.

Materials and Methods: PC-3 cells were maintained in a humified incubator at 37°C under a 1% (7.6mmHg) O2/5% CO2/94% air atmosphere. Subsequently, cells were exposed to H2O2 just before the irradiations, which were administered with 10 MV X-rays. O2 tension of the medium in which the PC-3 cells were cultured was measured by a partial oxygen measurement system. The Percentage of apoptotic cell was determined by flow cytometry.

Results: Also under the hypoxic condition, H2O2-enhanced radiation-induced apoptosis in prostate cancer cells was observed. Immediately after the administration of H2O2 into the medium, the O2 tension of the medium in which the PC-3 cells were cultured was increased. The average of the O2 increased tension in the medium after 7 minutes of the administration of 0.1, 0.2, 0.4, and 1.0 mM H2O2 were 3.6, 6.4, 24.8, and 62.0 mmHg, respectively. Meanwhile the activation of glutathione peroxidase in the cells was decreased.

Conclusions: O2 which H2O2 generated may have transmuted the hypoxic cancer cells to normal oxic cells. H2O2 also decreased the activation of glutathione peroxidase and may have led to prevent the removal of the generated hydroxyradical. In conclusion, it was suggested that these phenomena were a part of the reasons why H2O2-enhanced radiation-induced apoptosis in PC-3 prostate cancer cell line under the hypoxic condition.

POS20-13. Radiosensitization of cancer cells by enzymotherapeutic deprivation of the semi-essential amino acid arginine. Leoni Kunz-Schughart1, B. Vynnytska-Myronovska1, Y. Bobak2, C. Dittfeld1, Y. Garbe1, O. Stasyk1, 1: OncoRay - National Center for Radiation Research in Oncology, Germany 2: Institute of Cell Biology, National Academy of Sciences of Ukraine, Ukraine

Arginine deprivation by enzymotherapy is a promising new strategy for metabolic anticancer treatment. Application of arginine-degrading enzymes mainly harbors cytostatic effects in vitro and in vivo but may also induce apoptosis, thus is potent of tumor growth inhibition. This phenomenon serves as a good rationale for development of novel combinational approaches based on arginine starvation. It was previously demonstrated by us that low concentrations of the arginine analogue canavanine accelerate and augment arginine deprivation-induced apoptosis when co-introduced with an arginine-degrading enzyme; an effect that was selective for tumor cells. In the present work we aimed to evaluate the anticancer potential of the combined treatment of external irradiation and arginine starvation with or without low concentrations of canavanine using a spheroid-based test platform and spheroid regrowth probability and spheroid control dose 50 (SCD50), respectively, as analytical endpoint. This turned out to be a particularly relevant methodological approach because several cell types showed an unexpected loss of sensitivity to arginine deficiency in a 3-D as compared to 2-D cellular context. Nonetheless, with the set-up we could reveal as hypothesized from the surviving fractions at 2 Gy (SF50) in 2-D culture, that pre-incubation of HT29 colorectal cancer cells with arginine deprivation or pre-exposure with a therapeutically useful arginine-degrading enzyme (human recombinant arginase) resulted in a decrease in the spheroid control dose 50 (SCD50) from 16.3 to 9.8 Gy. And SCD50 even decreased to about 5 Gy when arginine-deprivation was combined with non-toxic concentrations of canavanine before irradiation. These data strongly suggest that arginine deprivation alone or in combination with specific drugs is a promising strategy to sensitize (colon) cancer cells to irradiation.

This work was supported by a DAAD scholarship awarded to B.V.

POS20-14. The possible chemopreventive effects of Monascus on prostate related disease and the enhanced therapeutic potency for prostate cancer combined with irradiation. Yu-Hsuan Lee1, S. Ho2, 1: Department of Environmental and Occupational Health, National Cheng Kung University, Medical College, Tainan, Taiwan 2: Sinsiau Christian Hospital, Tainan, Taiwan

Prostate cancer is one of the most common tumors among men in United State. Radiotherapy is one of the treatments for prostate cancer. Recently, Monascus has been reported to exhibit antiproliferative effects on prostate cancer. The aims of this study were to investigate the anticancer effect of irradiation (IR) combined with Monascus on prostate cancer cells and evaluate the chemoprevention effect of Monascus on prostate-related disease. PC-3 cells were used in this study. Cell viability was counted by trypan blue. Cell cycle and apoptosis were analyzed by flow cytometry. DNA damage level was observed by comet assay. Western blotting was used to determine pathway-related protein expression. In chemoprevention part rats were
induced prostate-related disease with testosterone and carcinogen. By the end point, we calculated the prostate size ratio of rats and determined their biochemistry profiles, pathological changes by biochemistry analyzer, pathological section (H&E stain) and specific ELISA kit. The results indicated that the anti-cancer effect of combined treatment is more effective than IR or Monascus alone in PC-3 cells. The combined treatment did not increase the percentage of apoptosis but autophagy. Furthermore, combined treatment caused more DNA damage and ER stress than IR and Monascus alone. In xenograft model, the combined treatment group had a 70% reduction in tumor size compared with positive control group. In addition to chemoprevention, we found that the prostate size ratio, PSA (prostate specific antigen), triglyceride and total cholesterol of rats were significant lower than positive control group. Our data suggested that Monascus, which is used in traditional medicine for the treatment of various ailments, may be a novel agent for treatment with prostate cancer and prevention of prostate-related disease.

POS20-15. The PLK1 inhibitor BI2536 causes increased radiosensitivity or -resistance depending on the timing of treatment. Christin Lund-Andersen, R. G. Syljalaen, Oslo University Hospital, Norway

PLK1 is overexpressed in several types of cancers and PLK1-inhibitors are in clinical trials for cancer treatment. Recently, two reports indicate a radiosensitization effect by PLK1 depletion. These studies suggest that combined treatment with PLK1-inhibitors and radiation therapy may be beneficial. However, several mechanistic studies of the G2 checkpoint have shown that PLK1 is required to enter mitosis following IR-induced G2 checkpoint arrest. Thus, addition of PLK1-inhibitors will lead to a longer IR-induced G2 checkpoint, which presumably will cause increased DNA damage repair and radioresistance. It is therefore surprising if PLK1-inhibitors cause radiosensitization. In non-irradiated cells, it is known that PLK1-inhibition will cause mitotic arrest. Mitotic cells are known to be more radiosensitive. It is therefore possible that PLK1-inhibitors will sensitize cells to IR only if they are added several hours before IR to allow accumulation of cells in mitosis before IR. We have treated human osteosarcoma and colorectal cancer cells with the PLK1-inhibitor BI2536 before and after irradiation and analyzed the cells by clonogenic survival assay, flow cytometry and Western blotting. Our data show that the G2 checkpoint is prolonged and the radioresistance is increased in cells when treated with the PLK1 inhibitor immediately after IR. However, the cells treated with PLK1-inhibitor several hours before IR arrest in mitosis and are more radiosensitive. These data suggest that PLK1-inhibitors would need to be administrated several hours before radiation in order to obtain synergistic effects between PLK1-inhibition and radiation therapy.


Introduction: Combined-modality therapy is highly needed to improve the therapeutic index of radiotherapy as it is expected to substantially improve locoregional control and survival in patients with lung, rectal, esophageal, and head and neck cancers. However, these improvements could come at the cost of increased acute and delayed toxicities. The first report of three dead patients in a clinical trial, evaluating the anti-tumor efficacy of bevacizumab (Avastin®) combined with radiotherapy in lung cancer patients, has been followed by a series of clinical reports showing high incidence of unexpected side effects. These reports bring to the light important questions about the safety of such novel treatment modalities and underscore the need of appropriate preclinical investigations. In this context, the present study is the first systematic evaluation of normal tissue toxicity triggered by anti-angiogenic agent combined with radiation therapy Methods: All experiments were performed in accordance with European recommendations for the care and use of laboratory animals and GLP. A total of 340 10-12 weeks old female C57/B6 mice (Janvier) entered the study. Acute toxicity was monitored studying radiation-induced intestinal ulceration (2Gy TRIL), sub-acute toxicity using a model of oral mucositis (16,5Gy localized), late radiation injuries was investigated by monitoring occurrence of lung fibrosis (bleomycin-induced and 19Gy localized). In all three models irradiation was combined or not with a mouse anti-VEGF Mab (5 mg/Kg). Results:

Combination of irradiation with the mouse-anti-VEGF Mab enhanced intestinal damages with severe epithelial ulceration and glands exhibiting necrotic changes. Then we reasoned that cancer patient often undergo various anti-cancer treatment regimen and are very likely to have an altered GI mucosa which could worsen anti-VEGF Mab toxicity. Therefore, we created a chemically-induced ulceration using intra-rectal administration of 150 mg/Kg TNBS (2,4,6-trinitrobenzen sulfonic acid) prior administration of the combined therapeutic. Mouse-anti-VEGF antibody dramatically increased TNBS and RT-induced ulceration showing an almost complete mucosal depletion prone to fistula formation. Second, we investigated mouse-anti-VEGF Mab effect on oral mucositis. In that case, anti-VEGF-Mab treatment seemed to enhance the wound healing process with a moderate histological improvement of tissues structure. Lastly, the effect of the murine anti-VEGF Mab on the development of lung fibrosis was investigated. Administration of the mouse-anti-VEGF Mab dramatically worsens the fibrotic picture induced by bleomycin and irradiation. Inflammatory patch surrounding vessels and even ulcers were observed. Conclusion: Our results are concerning as we observed occurrence and enhancement of late toxicity yet unreported but also highlight the complexity of anti-VEGF action, which could in defined conditions exert tissue-specific mucosal protection. These first set of experiments were designed to provide a quick answer to clinicians and should provoke them to further examination of combined therapies. This study examined the function as a radiosensitizer in about 40 kinds of vascular normal functions are little known. Radio-sensitization function of the flavonoids was analyzed with colony formation assay in cancer cells (H1299) and normal cells (lung fibroblasts, HFL-II). We newly found that some flavonoids are available as a radio-sensitizer. The molecular mechanisms of radio-sensitization of the flavonoids are now being examined.

POS20-18. Radiosensitivity of human colon cancer cells was enhanced by phyto-estrogen biochanin A. Abhay Puthli1,2, R. Towari3, K. Ballalakrishnan, E. S. K, Sagra, and N. Mishra1. 1: Department of Life Sciences, University of Mumbai, Mumbai, India 2: Radiological Physics & Advisory Division Babha Atomic Research Centre, Mumbai, India 3: Nehru Gram Bharati University, Allahabad 211 002, India

Evaluation of herbal/plant products as effective radiosensitizer and radioprotector form active area of radiobiological research. The key to success of new strategies in improving cancer radiotherapy depends on achieving the increased tumor cytotoxicity while reduced adverse effects on normal tissues. Present study describes the combined effects of phytoestrogen, Biochanin A (BCA) a methyl derivative of Genistein, on the process of radiation-induced cell death on normal and cancer cells. Radioreistant colon cancer cells, HT29, respond poorly to therapeutic radiation doses attributable p53 mutation. We found that Biochanin A treatment (1-125 µM) inhibited cancer cell proliferation in HT29 but did not affect normal human keratinocytes HaCaT cells. The treatment enhanced the inhibitory effect of γ radiation (2 Gy) from IC50 of 116 µM to 55 µM in colon cancer cell but significantly protected normal cells. The radiation induced intracellular ROS was measured using DCFHDA (2, 7-dichlorodihydrofluorescein diacetate) as the fluorescence probe. Treatment with BCA (100 µM) followed by γ radiation (2 Gy) showed about 13 fold increase in ROS as compared with the controls in colon cancer cells. However, no increase in ROS was observed in normal cells compared to its irradiated control. The enhanced ROS in HT29 cell acted on the mitochondria and caused lipid peroxidation, measured by DiOC, probe and cisplatinic acid probe respectively. The DNA damage was evaluated by Comet Assay and results have
showed that tail length and olivary tail moment significantly increased combining BCA and radiation. Loss of mitochondrial potential resulted in the increase of pro-apoptotic protein Caspase 3. It is concluded that pretreatment with Biochanin A and radiation increased the radiosensitivity of colon cancer cells but spared normal cells.

POS20-19. Inhibition of αvβ3 and αvβ5 integrins by cilengitide improved tumor response to radiation in human head and neck squamous cell carcinoma and non-small cell lung cancer models. Uma Raju1, L. Wang1, D. R. Valdecanas1, D. P. Molkentine2, L. Milas1, K. A. Mason1, K. Kian Ang3, S. L. Goodman1, U. Raju4, 1 University of Texas M.D. Anderson Cancer Center, USA 2: Merck KGaA, Germany

Purpose: Integrins are implicated in resistance of solid tumors to therapies, including radiation therapy, suggesting that their inhibition would enhance efficacy of tumor therapy. We investigated the efficacy of cilengitide, a cyclic Arg-Gly-Asp (RGD)-derived peptide that inhibits αvβ3 and αvβ5 integrins, in enhancing in vitro cancer cell radiosensitivity and in vivo radiosresponse of cancer xenografts.

Methods: Three non-small cell lung carcinoma lines (NSCLCs) (H460, A549 and H1299) and three head and neck squamous cell carcinoma lines (HNSCCs) (FaDu, SCC-15 and SCC-25) were used for in vitro experiments. Of these, H460 and FaDu were used for in vivo testing when grown as xenografts in mice. The effects of cilengitide on in vitro cell viability and on cellular radiosensitivity (by clonogenic cell survival assay) were determined. In vivo effect of cilengitide on radiosresponse of xenografts was assessed by tumor growth delay.

Results: Cilengitide reduced in vitro viability of 7 out of 8 cell lines tested, which ranged between 71.4 ± 2.2% (SCC-15) and 27.8 ± 4.2% (H1299). In general, NSCLCs were more sensitive to cilengitide than HNSCCs. When combined with radiation, cilengitide significantly enhanced the radiosensitivity of all 3 NSCLC. In contrast, cilengitide exerted only an additive effect on radiosensitivity of HNSCC lines. However, in vivo tumor growth delay studies showed that cilengitide as a single agent had no antitumor activity, but in combination with radiation it significantly enhanced response of both H460 (NSCLC) and FaDu (HNSCC) tumor xenografts.

Conclusion: The results showed that cilengitide reduced in vitro cell viability of NSCLCs and HNSCCs. When combined with radiation, cilengitide enhanced radiosensitivity of HNSCCs. Cilengitide was effective in enhancing radiation response of both lung (H460) and head and neck (FaDu) tumor xenografts. These synergistic effects of cilengitide with radiation are in line with results obtained for a glioblastoma rat model (Mikkelsen, et al. Int J Cancer 2009). These results suggest that cilengitide has the potential to improve the treatment outcome of patients with NSCLC and HNSCC when combined with radiotherapy. Mechanistic studies addressing cilengitide-radiation interactions are underway.

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POS20-20. Effect of ethacrynic acid (EA) on the pharmacokinetics of Ex-RAD® (ON 01210.Na) in the isolated perfused rat liver model (IPRL). Chen Ren1, Mitalee Tamhane2, D. Taft3, M. Maniar4, 1: Onconova Therapeutics, Inc., Newtown, USA 2: Long Island University, USA

Ex-RAD® (ON 01210.Na), a novel benzyl styryl sulfone analog being developed as a promising new radioprotectant by Onconova Therapeutics Inc., has completed two Phase I clinical safety trials under an Investigational New Drug (IND) exemption. ON 01210.Na has demonstrated increased survival in cellular, tissue and animal radiation models. Its virtual lack of side effects at effective dosage makes ON 01210.Na an attractive candidate both as a prophylactic agent and as a therapeutic treatment for mitigation by enhancing cell survival and DNA repair mechanisms. Previous studies found that ON 01210.Na was extensively metabolized to a glutathione (GSH) conjugate in vitro and in vivo by glutathione-S-transferase (GST). The objective of the study was to determine the hepatitis drug disposition in vivo and growth of Ex-RAD® in the isolated perfused rat liver (IPRL) model and to examine the effect of co-administration of ethacrynic acid (EA), a prototype GST inhibitor, on the pharmacokinetics of ON 01210.Na in the model. ON 01210.Na showed non-linear pharmacokinetics in dose escalation studies in the IPRL (target concentrations 10, 50, 100, 250 µg/mL) with clearance decreasing from 3.14 to 1.99 mL/min with increasing dose. The glutathione adduct metabolite of ON 01210.Na formed in liver was mainly excreted into the bile. Less than 1% of the parent drug was recovered from IPRL perfusate at lower doses (target concentrations < 100 µg/mL), but total recovery increased 10-fold at the highest dose tested (250 µg/mL). Perfusion recovery increased from 9.5% to 54% when EA (1mM) was co-administered with ON 01210.Na (250 µg/mL), and clearance was reduced by almost 5-fold. The results suggest that EA co-administration could significantly inhibit the conversion of ON 01210.Na to its glutathione conjugate in rats, resulting in decreased clearance and systemic exposure of ON 01210.Na. Accordingly, combination treatment with EA could potentially help achieve and sustain effective plasma levels of ON 01210.Na at a frequency of the dose by altering its metabolic profile, which in turn could open avenues for administering ON 01210.Na by extravascular routes thus enhancing patient compliance.

POS20-21. In vivo short term 9L gliosarcoma vasculature response to concomitant Microbeam Radiation Therapy and sorafenib treatments. Raphael Serduc1, A. Boucher1, B. Lemasson2, C.1, N. Coquery1, P. Robert1, G. Le Duc1, E. Brauer1, L. Tropes1, E. Barbier1, C. Remy1, 1: Inserm U836, France 2: Guebert, France 3: ESRF, France

The aim of this work was to evaluate the vascular responses of the 9L brain tumor after synchrotron microbeam radiation therapy (MRT; based on the spatial fractionation of the x-ray beam) associated or not with Sorafenib (SORA), a multikinase inhibitor. Our team has developed original methods for mapping functional parameters of the microvasculature: blood volume (BV) and vessel diameter (VSI) [1], and local blood oxygen saturation (ISO) reflecting tissue oxygenation [2]. For a full characterization of the microvasculature within a single MRI session, these methods have been integrated with vessel permeability imaging methods [3].

Ten days after 9L cell implantation, the rats (n=86) were randomized in 4 groups (control, SORA, MRT and MRT/SORA treated rats) according tumour sizes using anatomical MRI. The antiangiogenic treatment slowed down the tumour growth but had only a small impact on survival, despite a strong effect on tumour vasculature. As early as one day after the beginning of the antiangiogenic treatment, BV, ISO2 and vessel permeability decreased. No effects on VSI were observed. On the contrary, MRT had a strong impact on the tumor growth and increased the median survival time (MST) from 20 to 65 days (80% of tumor control 2 months after exposure). MRT did not induce early effects on tumour vasculature but reduced BV, ISO2 and permeability to contrast agent in the tumour 15 days later. The concomitant treatment of MRT and SORA induced a significant decrease in tumor ISO2 and BV from day 2 after treatment. No differences in tumor size were measured between MRT and MRT/SORA groups during the first week following the treatment. However, the concomitant treatment did not increase MST of the treated animals (20 days), revealing a potential neurotoxicity of the associative treatment on normal vessel/tissues. Even if the association of MRT and SORA seems to be more relevant in term of tumor control by significantly damaging tumor vasculature, the side effects on normal surrounding tissues induced by antiangiogenic treatment after MRT have to be considered. Indeed, these drugs might enhance the MRT-induced damages on normal brain vessels and avoid the efficient repair mechanisms of normal vasculature observed after MRT [4].


POS20-22. Chemoray clean-up workers: life under long-term oxidative stress. 15 years of experience. Andrejs Skesters1, T. Zvagule1, L. Larmane1, A. Silova1, N. Rusakova2, K. D. Rainsford3, 1: Riga Stradins University, Latvia 2: Centre of Occupational and Radiological Medicine of P. Stradin's Clinical University Hospital, Latvia 3: Riga Stradins University, Latvia 4: Sheffield Hallam University, UK.

On 26 April, 1986, at 1.23 pm the worst world nuclear disaster took place at the Chernobyl NPS. 190 ton of highly radioactive 131I, 137Cs, 134Cs, U, Ra, Ru, 90Sr, 41Ca, graphite, Pb, other heavy metals were expelled into the atmosphere. 6475 inhabitants of Latvia, men in reproductive age, were involved in clean-up and recovery works. Consequently, they were exposed to both direct
gamma-radiation as well as inhaled or absorbed toxic radioactive isotopes. Dust volatile heavy metals derived from the reactor melt- down through their skin, lungs and gastro-intestinal tract (post-Chernobyl syndrome). After homecoming all workers (patients) immediately visited P. Stradin’s Clinical University Hospital for a full medical examination. In nowadays this examination still continues. Now 87% of clean-up workers are permanently disabled, all of them are severe health problems. Aim of research - to detect oxidative stress (OS), and to have been observed in the AO defence system and AO role in liquidator body. For determination were used standard kit’s. Hence, first proposal was started in 1997 - as long-term investigation on the role of changes in the AO defense system in manifesting the development of post-Chernobyl syndrome in clean-up workers from Latvia. Our results showed considerable elevation of LOOH, 4-HNE, protein carbonyls, lipid peroxidation ratio, pathological Cu/Zn ratio and decreased activity of superoxide dismutase, glutathione peroxidase, total antioxidant status, concentration of glutathione, selenium, etc. In last years, some target groups, together with conventional therapy, - the AO - vitamin E, selenium, CoQ10 alone and in combinations. Results showed that external antioxidants improved AO defence and decreased OS markers. Conclusion: 1) - 25th years after receiving of ionizing radiation, radionuclides as dusty and aerosols, in bones (90Sr, Ra, Ca) and organs incorporated radionuclides which provoke conditions for high ROS, thus possible to be favourable circumstances for pathologic processes in the body; 2) - therapy with AO improved antioxidative defense in body and decreased lipid and protein peroxidation processes such as lowering the blood cholesterol level, decreased pain in joints and other localization, headache, migraine, depression and stomach troubles.


Malignant mesothelioma is a highly aggressive tumor arising from mesothelial cells. The most common site of mesothelioma is pleura, followed by peritoneum, pericardium and male genitalia. Most of the mesotheliomas cases have been attributed to asbestos exposure and the lag time is reported to be 30 to 40 years. Although mesothelioma has been considered as a rare tumor, its incidence is anticipated to increase globally over the next decades. Malignant mesothelioma is highly lethal and the prognosis following therapy is very poor with the median survival of 9 to 17 months. Most common use of radiotherapy is to alleviate symptoms in advanced mesothelioma cases, and the effect of radiation monotherapy to prolong survival is reported to be minimal. The limited use of radiotherapy for mesothelioma includes the intrinsic radioresistance of tumors and the adverse effect of the surrounding normal tissues. To improve the efficacy of radiotherapy, a novel molecular targeted radiosensitizing agent is needed to increase radiosensitivity of mesothelioma cells. We have previously identified ZDHHC8 as a novel radiation susceptibility gene based on the functional screening using the genome-wide siRNA library in human cells, and this gene would be associated with G2/M checkpoint in response to DNA damage induced by X-ray irradiation. In this study, we analyzed the effect of ZDHHC8 knockdown with radiation on mesothelioma cells and assessed the therapeutic efficacy in a mesothelioma mouse model. In mesothelioma cells, knockdown of ZDHHC8 by siRNA significantly reduced cell survival after irradiation, induced the impairment of G2/M checkpoint, and increased the frequency of cells with micronuclei and apoptosis. In the mesothelioma mouse model, the treatment with ZDHHC8 siRNA and irradiation significantly suppressed tumor growth and increased apoptosis, whereas ZDHHC8 siRNA alone did not. These results suggest that ZDHHC8 knockdown with X-irradiation induced chromosomal instability and cell death including apoptosis through the defects of G2/M checkpoint, and the combination of ZDHHC8 depletion and irradiation has the potential to be effective therapy for malignant mesothelioma.

POSTER 20-24. The characterisation of the radiosensitisation effect of gold nanoparticles. Laura Taggart1, K. Butterworth2, M. Ghita2, A. Prise2, F. Currell2, G. Schettino1, M. Sugiyama, B. Taggart1,1: Queens’ University Belfast, UK 2: NRCCB, Queens’ University Belfast, UK

The aim of this project is to investigate the radiosensitising effect of gold nanoparticles and the mechanisms which regulate it in order to evaluate their possible use in cancer radiotherapy. The major limitation on radiotherapy is the damage to healthy tissues, it is therefore important to maximise the dose to the cancerous tissue while minimising dose to the normal surrounding tissue. By targeting gold nanoparticles to tumour cells, it is possible to enhance the dose absorbed by the tissue without increasing the dose delivered from the radiation source. Clonogenic cell survival assays indicate that MDA-231s, DU145s and T98s are all sensitive (factor 1.4) to the radiosensitising effect of 2nm gold nanoparticles while no alterations have been observed in AGO1522s. In MDA-231s and AGO1522s, however, 2nm gold nanoparticles cause preferentially necrotic cell death initially (24–48 hours post treatment) and eventually lead to apoptotic death within 72 hours as determined though Annexin/FITC flow analysis. Interestingly, through western blotting we also observed decreased levels of Dec1, a senescence protein, when cells were irradiated in the presence of gold nanoparticles. The gold nanoparticles also induce higher levels of DNA damage in MDA-231s and DU145s as analysed by counting 53BP1 foci through immunofluorescent staining, this has not been replicated for T98s as yet. The clonogenic dose enhancement factor of 1.4 for the 2 nm gold nanoparticles is in agreement with the increased DNA damage detected. To conclude, not all cell lines are susceptible to the radiosensitising effect of gold nanoparticles, with AGO1522s being particularly resistant. The lack of sensitisation in the AGO1522s is puzzling as they are the most radiosensitive cell line tested, however their uptake of gold nanoparticles has not yet been tested and it could be possible that they have a lower uptake of the particles. MDA-231s, a breast cancer cell line, DU145s a prostate cancer line and T98s a gloma cell line have all shown sensitivity to the effect of the 2nm particles being most radiosensitising in comparison to 10nm and 15nm particles. The decreased cell survival seems to be attributable to the increased DNA damage observed when the particles are present and the modes of cell death appear to be majorly necrosis with apoptosis being important at later timepoints.

POSTER 20-25. Radiosensitising effect of betulinic acid on breast carcinoma is independent of p53 pathway. R. Tiwari1,2, R. Tiwari1, K. P. Mishra1, 1: University of Mumbai, India 2: Nehru Gram Bharti University, Allahabad, UP, India

Betulinic acid (BA) is a pentacyclic triterpene found in several botanical sources to show significant potential to cause apoptosis in a number of melanoma, neuroectodermal and malignant brain cancer cell lines. Present report aim to evaluate the radiosensitising potential of BA on two human breast cancer lines differing in their p53 status. BA induced loss of cell proliferation was determined by MTT assay. BA inhibited the clonogenic growth of MCF-7 and T47D cells which was not recovered by pifithrin-α, the p53 inhibitor. The apoptotic parameters were analyzed using FACS analysis of propidium iodide (PI) stained nuclei and PS externalization using Annexin-V assay. Changes in mitochondrial membrane potential were monitored by fluorescent dye DIOC6 and JC-1. Cells treated with BA in combination with radiation showed an increase in the propidium iodide (PI) uptake in dose dependent manner, which was concomitant with the loss of mitochondrial membrane potential. Exogenous addition of tocopherol to cell cultures 1-h prior to the treatment with BA abolished all the effects of BA-induced loss of cell proliferation and apoptosis in both MCF-7 and T47D. BA was also found to induce dose and time dependent increase in intracellular reactive oxygen species in both the cell lines. These in vitro studies show that radiosensitising effect of BA is not dependent on p53 status of cells. Inhibition of antiproliferative ability of BA by tocopherol suggests that BA initiates events at membrane level leading to induction of apoptosis.

POSTER 20-26. Radiation-amplified drug delivery to angiogenic tumor vasculature via Galectin-1. Meenakshi Upreti, N. A Koonce, S. Apana, M. Berridge, A. Jarnished-Farsian, J. Webber, R. J Griffin, University of Arkansas for Medical Sciences, USA

We are using an anti-angiogenic 33-mer targeting ligand named anginex to deliver therapeutic agents to the tumor vasculature by radiation therapy-amplified receptor expression. Our results show increased expression of the anginex receptor (Galectin-1) in angiogenic endothelial cells in vitro and tumor microvasculature upon exposure to radiation. Design and use of 18-F labeled anginex in focal human myeloma model showed a vibrant and specific targeting of the tumor/bone graft by labelled anginex. Exposure of murine SCK breast tumors to a clinical radiation dose of 2Gy resulted in a substantial average increase of 141 +/- 49% in anginex uptake. We subsequently developed a liposomal formulation to determine the potential of
guiding drugs to the tumor via radiation-amplified Galectin-1 target expression. Initial studies with 'anginex-tagged' liposomes indicated significant binding and uptake of the carrier by endothelial cells in culture and increased binding specifically to endothelial cells upon radiation exposure. This binding was blocked when free anginex or anti-Galectin-1 antibody was added to the cells with targeted liposomes, suggesting an anginex-Galectin-1 interaction for liposome targeting. To extend this strategy for radiation-guided drug delivery targeted to the tumor's vasculature we have developed 3D tumor-endothelial cell spheroidal cultures in hanging drops of medium where galectin-1 expression increases upon radiation exposure. Subsequently, we transplanted these spheroidal cultures into the dorsal skin fold window chamber of mice for intravitreal multiphoton imaging of neovascularization and disease progression. Understanding how anginex uptake selectively increases in endothelial cells after irradiation and its nexus to radiation-sensitivity of endothelial cells vs. tumor cells exposed to anginex is our current focus. We plan to expand our studies to incorporate Aspirin trioxide chemotherapeutics into these liposomes and fully characterize the in vivo targeting and therapy potential in our models in combination with radiation exposure.

**POS20-27. EGFR tyrosine kinase inhibitor EKB-569 regulates radiation-induced Rel dependent hTERT transcription and telomerase in human squamous cell carcinoma.** Jamunarani Veeraraghavan1, M. Natarajan2, J. D. Balthazar3, S. Aravindan1, T. S. Herman4, N. Aravindan1, 1: University of Oklahoma Health Sciences Center, USA 2: University of Texas Health Science Center at San Antonio, USA

Our earlier reports showed that ionizing radiation (IR) induces NFκB dependent hTERT transcription and initiation of clonal expansion both in normal endothelial cells and in neoplasms. In this study, we re-institute NFκB dependent hTERT transcription and initiation of clonal expansion in squamous cell carcinoma. More importantly, we investigated whether a selective and irreversible EGFR tyrosine kinase inhibitor EKB-569 potentiates radiotherapy by mitigating IR-induced NFκB dependent hTERT transcription, telomerase activity (TA) and subsequent clonal expansion. Cells from human tongue squamous cell carcinoma (SCC) were exposed to IR (2 Gy) with or without EKB-569 (0.5 – 5.0mg) pre-treatment and examined after 1h through 24h. NFκB and hTERT transcriptional activation was analyzed using luciferase reporter (pNFκB-Luc, pGL3-354-Luc) assays. NFκB dependent hTERT transcription was examined either by mutating (pUSE-IκBα), overexpressing (p50/p65) NFκB or by using hTERT constructs lacking NFκB binding promoter sites (pGL3-347-Luc). hTERT mRNA was analyzed using QPCR, TA using TRAP and clonal expansion using clonogenic assay. EKB-569 profoundly inhibited IR-induced NFκB. Consequently, EKB-569 significantly inhibited IR-induced TA and hTERT mRNA at all time points investigated. EKB-569 inhibited all TNFα regulated and hTERT transcriptional level by triggering TERT promoter activation. In addition, NFκB becomes functionally activated after IR and mediates TA upregulation by binding to the IκB-binding region in the promoter region of TERT gene. Consistently, elimination of NFκB-recognition site on telomerase promoter or, inhibition of NFκB by IκBα mutant compromises IR-induced telomerase promoter activation. Significantly, EKB-569 inhibited IR-induced TERT transcription. Consequently, EKB-569 inhibited hTERT mRNA and TA in NFκB overexpressed cells. Furthermore, EKB-569 potentiates inhibition of IR-induced clonal expansion. These results strongly suggest that EKB-569 could actively inhibit IR-induced TA in a NFκB dependent manner in human squamous cell carcinoma cells and thereby could enhance favorable therapeutic outcome.

**POS20-28. Evaluation of two promising radiation countermeasures against neutron/gamma mixed field radiation.** Mark Whitnall, B. Ngudiankama, AFRRI, USA

Most studies of medical countermeasure (MCM) efficacy have been performed with photons (X-rays and gamma). In the event of a nuclear criticality (explosion) and/or accident there will be exposure to neutron/gamma mixed fields (MF). We compared MF efficiencies of 2 MCMs known to be effective against photons (from other studies and our own data obtained concurrently with these experiments): the TPO mimetic ALXN4101TPO and G-CSF (Neupogen®). Mice were exposed to 5.5 Gy MF (2.1 neutron/gram, 0.6 Gy/min). ALXN4101TPO was injected sc at 1 mg/kg, 12 h post-irradiation. G-CSF (0.34 mg/kg) was injected sc at 12, 24 and 48 h post-irradiation. Only G-CSF consistently increased survival after MF. After sublethal irradiation (2 Gy), neither drug had an effect on white blood cell lymphocyte counts. G-CSF caused an initial increase in neutrophils on days 1 and 2 after irradiation and a slight increase in circulating monocytes. ALXN4101TPO maintained platelets within normal values beyond day 7. G-CSF but not ALXN4101TPO caused an increase in BM hematopoietic progenitor cells (HPCs) at day 2. By day 7, HPC were back to normal after G-CSF, but BM cellularity was increased. G-CSF also promoted an increase in BM hematopoietic stem cells. In spleen, both drugs increased the % of B cells, but only G-CSF modified the distribution of NK cells (from 8% to 10%) and T-cells (from 60% to 80%) after irradiation. We conclude MCM efficacy is different between gamma and MF exposures, depending on the mechanisms of actions of the MCM. Supported by an award from NIAID to MW.

**POS20-29. Changes in Radiosensitivity of PC3 Prostate Cancer Cells.** Ye Zhang1, Z. He2, L. S. Mangala1, C. A. Theriot3, L. H. Rohde2, H. Wu1, 1: NASA Johnson Space Center/Wyle, USA 2: University of Houston at Clear Lake, USA 3: NASA Johnson Space Center, USA

Background: Recent studies have indicated that autophagy may be one of the important pathways induced by ionizing radiation. In our study, changes in radioreponse of radioresistant human PC3 prostate cancer cells to radiotherapy by alteration of pathways related to autophagy were investigated.

Methods: Activation of the autophagy pathway was analyzed using the GFP-LC3 assay and western blot. The radiosensitivity of PC3 cells was determined by clonal survival assay and the Annexin V assay after exposure to gamma irradiation and/or various chemicals. The expression profiles of autophagy related genes were analyzed using a pathway specific real-time polymerase chain reaction array.

Results: PC3 cells exhibited an induced autophagic response after exposure to gamma rays. Inhibition of the autophagy process using small interfering RNAs targeting Atg7 and/or Atg12 significantly reduced radiosensitivity of PC3 cells. Both autophagy activators RAD001 and Statin sensitized the cells to gamma irradiation, showing significantly reduced colony forming efficiency in response to 6 Gy of gamma irradiation. Statin also exhibited a significant apoptosis-inducing effect in PC3. Analysis of autophagy and its regulatory gene profile showed that the expressions of 22 genes out of 86 genes assessed were significantly altered in the cells exposed to combined treatment with Statin and radiation.

Conclusions: The data indicate that activation of the autophagy pathway may be responsible for both apoptosis inducing effect of Statin and its radiosensitizing effect on PC3 cells. Therefore, combined treatment with radiation and autophagic inducers, such as Statin, may be synergistic in tumor cell killing and tumor control.

**POS20-30. Overexpression the LyGDI enhanced the invasion ability and radiation resistant in none small lung cancer Cells.** Xinwen Zhou, School of Radiation Medicine and Public Health, Soochow University, China

Purpose: LyGDI has been thought to be one of the central regulators to RhodTGPase proteins, which recently been found dysregulated in several tumors. However, its role in Lung cancer remains unclear. Therefore, our purpose is to investigate the LyGDI expression profile and elucidate its role in lung cancer progression and radiation sensitivity.

Experimental design: Immunohistochemistry was used to analysis LyGDI expression profile in lung cancer patient’s samples. Further protein and mRNA expression level of LyGDI were confirmed by western blot and RT-PCR respectively. Ecotopic expression of LyGDI and its RNA transfection were done to determine the physiological role of LyGDI in A549 cell lines. Wound healing and In vitro migration assay were used to assay the cell invasion. Clonogenic survival assay was used to detect the radiation sensitivity induced by combined treatment of radiation and drug.

Results: LyGDI was found Up-regulated in none small lung cancer tissue samples and was correlated lymph nodal metastasis (P<0.05) and pathological type (P<0.05). Ecotopic expression of LyGDI increased A549 cell invasion and down-regulation of LyGDI suppressed the cell invasion. Moreover, up-regulation of LyGDI in A549 cells increased COX-2 expression, but not the Rac1, and was resistant to irradiation. Whereas depletion COX-2 expression by Aspirin enhanced radiation sensitivity.
Conclusion: our finding shows that lyGDI was up-regulated in none small lung cancer cells and its expression was related with metastasis. It though regulated COX-2 expression to promote cell invasion. LyGDI could be used as biomarker for diagnosis and an important targeting molecular for lung cancer irradiation therapy.

POS21 Non-cancer effects

POS21-01. Radiation-induced proteome alterations in cardiac formalin-fixed paraffin-embedded tissue analysed by label-free quantitative mass spectrometry. Omid Azimzadeh1, H. Scherthan2, H. Sarioglu3, Z. Barjaktarovic4, M. J. Atkinson5, S. Hauck1, S. Tapio1, 1: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Biology, Germany 2: Institut für Radiobiologie der Bundeswehr, Munich, Germany 3: Helmholtz Zentrum München, German Research Center for Environmental Health, Department of Protein Science, Proteomics Core Facility, Germany

Proteome profiling of archival tissue has recently made great progress in clinical proteomics. However, applicability of conventional protein labelling in quantitative proteome analysis of formalin-fixed, paraffin-embedded (FFPE) tissue is still a matter of debate, mainly due to the fixation process leading to protein cross-linking.

Recently, we have systematically optimised various extraction and separation methods of FFPE proteins comparing different gel-free and gel-based approaches (1). In the current study, we applied label-free approach (2) to quantitatively compare the FFPE proteomes of sham- and exposed heart tissue of C57BL/6 mice after total body irradiation using a gamma dose of 3 Gy (137-Cs). We identified 26 deregulated proteins (p<0.05) in irradiated hearts 24h post-exposure. In good agreement with previous data, the analysis indicated a radiation-induced impairment of the cardiac mitochondrial proteome. This study will facilitate the proteome analysis of radiation-induced disease using radiobiology archives that are presently not amenable to analysis.

This work was supported by a grant from the European Community’s Seventh Framework Programme (EURATOM) contract n°232628 (STORE).

POS21-02. Integrative proteomic and microRNA analysis in a human coronary artery endothelial cell line exposed to low-dose irradiation. Zarko Barjaktarovic1, O. Azimzadeh1, H. Tammo2, M. J. Atkinson3, D. Leszcynski2, N. Anastasov1, S. Tapio1, 1: Helmholtz Centre Munich, Germany 2: STUK - Radiation and Nuclear Safety Authority, Finland

Vascular endothelium plays a crucial role in maintaining homeostasis of the cardiovascular system. Ionising radiation significantly contributes to the aetiology of cardiovascular disease, the endothelium being one of the main targets. In a previous study we investigated the changes in the cytoplasmic proteome of the human endothelial cell line EA.hy926 at 4 hours and 24 hours after exposure to 0.2 Gy (Co-60 gamma). Pathways influenced by the low-dose exposure included the Ran and RhoA pathways, fatty acid metabolism, and stress response. The aim of this study was to determine the radiation-responsive proteomic and microRNA biomarkers in primary human coronary artery endothelial cells, using similar exposure conditions. For proteomic analysis soluble proteins were separated using 2D-DIGE technology and pH ranges of 4-7 and 6-10. Out of ~1500 protein spots, 35 and 31 proteins showed significant deregulation after 4h and 24h, respectively. Out of these, 12 proteins showed similar deregulation at both time points. Differentially expressed proteins were identified by in-gel trypsin digestion, by MALDI-TOF/TOF tandem mass spectrometry, peptide mass fingerprint analyses, and LC-MS/MS analysis. Furthermore, specific miRNAs were supposed to have a significance as biomarkers for coronary artery disease. Using the real-time PCR-based miRNA detection (Taq-Man) probes we analyzed expression profile of 7 different miRNAs 4h and 24h after exposure to 0.2 Gy. Protein pathways and interaction between deregulated proteins and miRNA's were analysed by Ingenuity Pathway software analysis.

POS21-03. A preliminary mechanistic model of X-ray promoted atherosclerosis in ApoE-/- mice. Carmen Bijwaard1, F. Dekkers1, T. van Dillen2, S. Heeneman3, S. Hoving4, F. Stewart1, 1: RIVM, Netherlands 2: Maastricht University Medical Centre, Netherlands 3: NKI-AVL, Netherlands

Mechanistic models may help to determine how ionising radiation promotes the development of atherosclerosis. For this purpose a preliminary model has been formulated that allows for radiation to contribute to the process of atherosclerosis in several ways. The model is based on what is known from the scientific literature on plaque formation and the possible influences of radiation on this process. The current formulation has been tailored to experiments carried out at the Dutch National Cancer Institute (NKI) with ApoE-/- mice. Plaque formation in this genetically modified mouse model is considered to partly mimic the process of atherosclerosis as it occurs in humans. At NKI the carotid arteries of groups of ApoE-/- mice were exposed to 0, 2, 8 or 14 Gy X-ray doses. After approximately half a year the arterial tree was removed from these mice and examined for plaques. It was observed that the number and surface area of plaques grew with increasing dose. Moreover, exposed mice developed a more vulnerable phenotype. The mechanistic model developed at the Dutch National Institute for Public Health and the Environment (RIVM) therefore aims to adequately predict number, size and phenotype of the observed plaques. The fact that possible radiation effects are included in several steps of the model will help to indicate where radiation action is required to explain the data and where it is not. This is important for the more accurate determination of radiation risk for vascular disease, but it also constitutes significant input for new, tailored experiments aimed at advancing our knowledge on the influence of radiation on atherosclerosis.

POS21-04. Continuous exposures to low dose-rate γ-rays induce premature menopause and adiposity in female mice. Shingo Nakamura, Institute for Environmental Sciences, Japan

We previously reported a significant increase in body weights in B6C3F1 female mice continuously exposed to low dose-rate (20 mGy/22 h/d) γ-rays as compared to that of age-matched non-irradiated control mice. To clarify the underlying mechanisms of body weight gain observed after exposure to continuous low dose-rate irradiation, we examined adipose tissue weights, liver and serum lipid contents, and factors related to glucose and lipid metabolism (serum insulin and adipokynes), as well as ovarian dysfunction in female B6C3F1 mice continuously irradiated with γ-rays at 20 mGy/22 h/d from 9 to 44 weeks of age. Significant body weight gains, related to tissue adiposity, were observed from 28 to 44 weeks of age (accumulated dose = 2.7–4.9 Gy) in the irradiated mice as compared to those of non-irradiated mice. Histopathological analyses of ovaries and vaginal smears showed depletion of viable oocytes leading to premature menopause occurs at the same period when increased body weight gain was observed in the irradiated mice. These results suggest a possibility that the radiation-induced premature menopause could trigger the induction of body weight gain due to adiposity in B6C3F1 female mice continuously irradiated with low dose-rate γ-rays at 20 mGy/22 h/d. This study was performed under contract with Asmori Prefectural Government, Japan.

POS21-05. Biological and molecular mechanisms of vascular damage after low dose X-irradiation. Charlotte Rombouts1, A. Aerts1, M. Beck2, K. Tabury2, W. De Vos1, R. Benotmane3, P. Van Oostveldt1, S. Baoutou1, 1: SCK•CEN, Belgium 2: SCK•CEN/Ghent University, Belgium 3: Ghent University, Belgium

High radiation doses (> 5 Gy) increase the risk of cardiovascular diseases. In recent years, epidemiological data support the fact that lower radiation doses increase the risk of cardiovascular diseases as well (cfr. Atomic bomb survivors). However, for doses below 0.5 Gy, these epidemiological findings are unclear and a better understanding of the underlying biological and molecular mechanisms is needed. The endothelium is believed to be a critical target in the development of radiation-related cardiovascular diseases. Hence, we used the immortalized endothelial cell line EA.hy926 and a model to characterize the endothelial response to low and medium doses of X-irradiation. In particular, we analyzed the influence of radiation on
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DNA damage, cell cycle changes and associated apoptosis. DNA damage was assessed at several time points post irradiation (p.i.) (15, 30 and 120 min) using a range of X-ray doses (0.05, 0.1, 0.25, 0.5, 2 and 5 Gy) by immunostaining for γH2AX foci and quantitative fluorescence microscopy. All doses induced DNA damage as observed by a significant increase in γH2AX-foci number at all tested time points. Apoptosis was assessed by means of Annexin-V/PI staining and flow cytometry. A significant increase in the percentage of apoptotic cells compared to the controls was observed 24 h p.i. after exposure to 0.1, 0.5 and 5 Gy. After 48 and 72 h, this was only observed after 5 Gy. Cell cycle changes were studied 24 h p.i. via PI staining and flow cytometry. Preliminary results indicate a G0/G1 and G2 arrest after exposure to 2 and 5 Gy, respectively.

Based on these results it can be concluded that in EA.hy926 cells, fundamental cell cycle changes and apoptosis are effects limited to higher dose irradiation (2, 5 Gy), at least within the time range considered in the present study. However, more subtle effects such as DNA damage could be observed down to the lowest dose of 0.05 Gy. To elaborate more on the subtle changes at the level of the cell, we will include in future research, assessment of ROS production, inflammatory response, adhesion of leukocytes and cell-cell integrity. Moreover the endothelial response will be analyzed in further detail at the genetic level by means of gene expression profiling.

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POS21-06. Dose responses from multi-model inference for the noncancer disease mortality of atomic bomb survivors, Helmut Schollberger, J. Christian Kaiser, P. Jacob, Helmholtz Zentrum München, Germany

The noncancer mortality data for brain stroke and heart diseases from Report 13 on the atomic bomb survivors were analysed to investigate the dose response for the influence of radiation on these biological endpoints. Various parametric and categorical models (such as linear-no-threshold (LNT) and different threshold models) were analyzed with a statistical selection protocol which rated the model description of the data. Instead of applying the classical approach of identifying for each data set one specific preferred model, we first established a set of non-nested models that all fit the data about equally well. Subsequently, the selected non-nested models were used to perform multi-model inference (MMI), an innovative method of mathematically combining different models that allows making risk predictions based on several plausible dose response models rather than relying on a single model of choice. It thereby produces more reliable risk estimates based on a more comprehensive appraisal of model uncertainties. For brain stroke MMI yielded a weak dose response (with a risk estimate of about one third of the LNT model) below a step at 0.6 Gy and a stronger dose response at higher doses. The calculated risk estimates are consistent with zero risk below that threshold dose. For mortalities related to heart diseases due to radiation. The MMI approach described here resolves the dilemma in practical radiation protection when one is forced to select between models with profoundly different dose responses for risk estimates.

POS21-07. Radiation-induced alterations in the proteome of a human endothelial cell line after 200 mgGy exposure, Arundhati Srijharshani, A. Krämer1, K. Boldt1, M. Ueffing2, L. Hieber4, Z. Barjaktarovic1, O. Azimzadeh1, H. Sarioglu3, H. Zitzelsberger4, M. J. Atkinson4, S. Moorst1, S. Tapio5, 1: Research Unit of Radiation Cytogenetics, Helmholtz Zentrum München, Germany 2: Institute of Radiation Biology/Helmholtz Center Munich, Germany 3: Department of Protein Science/Helmholtz Center Munich, Germany 4: Research Unit of Radiation Cytogenetics/Helmholtz Center Munich, Germany

We have studied the response of endothelial cells to low doses of ionizing radiation, and demonstrate that alterations to the endothelial cellular phenotype are evident at doses of 200mgGy. Endothelial cells are highly sensitive to high doses of ionizing radiation, where their death provides a mechanism for the acute damaging actions of radiation. Epidemiological data suggest that even moderate doses (<500mgGy) may increase the risk of subsequent cardiovascular disease (CVD). At these, and even lower doses, endothelial cell stress and vascular damage may still occur, but the relevance of these effects for long-term tissue damage is unknown.

The goal of this study was to determine the immediate low-dose (0.2 Gy 18C–γ-rays) radiation response of endothelial cells at the proteome level. Since microRNAs (miRNA) are important regulators of protein expression and are known to be involved in cellular stress response induced by ionising radiation we have also quantified alterations in miRNA expression caused by radiation exposure in endothelial cells. Our long-term goal is to understand the mechanism of the radiation response by identifying the regulatory networks between proteins and miRNAs in radiation-exposed endothelial cells. MiRNAs responding to ionising radiation were investigated by TaqMan-based low-density array, whilst radiation-induced proteomic alterations were studied using the SILAC (Stable Isotope Labeling with Amino acids in Cell culture) approach. Four hours after irradiation we found 8 miRNAs that were up- and 2 that were down-regulated by 0.2 Gy; after 24 h 5 miRNAs were up- and 7 down-regulated (threshold for change in expression ≥1.5 folds).

The proteome was studied 24 h after 0.2 Gy irradiation. Differentially expressed proteins were identified and quantified after in-gel trypsin digestion of the isotopically labeled cell proteins using an LTQ Orbitrap (Mass spectrometry). Preliminary data suggest that 87 and 11 proteins were deregulated 4 h and 24 h after irradiation, respectively. Currently, we are using bioinformatics tools to determine the target proteins regulated by the radiation-responsive miRNAs that were altered after 0.2 Gy irradiation.

Pathway analysis shows the regulated proteins are involved in ERK/MAPK, NFκB and TGF-β signalling pathways.

POS22 Heavy ions

POS22-01. Spatial memory performance is significantly impaired following exposure to 20 cGy of 1 GeV 48Ti Particles, Richard Britten1, R. Britten2, P. Birdsal3, L. Davis3, R. Drake3, 1: Eastern Virginia Medical School, USA 2: Eastern Virginia Medical School, USA

INTRODUCTION. Current models predict that the astronauts on deep space missions will be exposed to ~25 cGy of Galactic cosmic radiation (GCR). The long-term consequence of exposure to such doses is largely unknown, but recent unpublished data from our laboratory and that of Dr. Rabin suggest that low (20 cGy) doses of HZE particles lead to significant impairments of various neurocognitive functions. We have shown that exposure of young rats to 20 cGy of 1 GeV 56Fe leads to a significant impairment in spatial memory and attentional setting shift performance. 1 GeV 56Fe particles have a LET of 150 keV/n, that has been shown to be the “optimum” for cell killing. Unpublished data from the Dr. Rabin and ourselves suggest that the LET dependency for impairment of Spatial memory and Attentional Set shifting performance in rats that have a comparable biological age to the astronaut population. Our first ion species comparison involved 1 GeV 56Fe and 1 GeV 48Ti, with LETs of 150 and 108 keV/µm respectively.

MATERIALS & METHODS. Six-nine month old male Wistar rats were exposed to either 1 GeV 56Fe or 1 GeV 48Ti particles at BNL. At 3 months post exposure the performance of the rats in the Barnes’ Maze (Spatial memory) and the Attentional Set Shifting Test (Executive function) was established. The brain proteome, glycoproteome and lipoproteome of rats following Hze exposure was established using various proteomic platforms.

RESULTS. Preliminary data on our first cohort of aged rats, suggests that Spatial memory impairment following exposure to 1 GeV 56Fe occurs at the same doses observed in our young rat studies. The unirradiated aged rats appear to learn the Spatial memory task better than the young rats, manifested by more significant reductions in Escape Latency (EL) time over the 3 day test period than was observed in the young rats. Rats exposed to 20 cGy 1 GeV 56Fe showed no reduction and indeed an increase in EL time, a similar situation appears to occur following exposure to 15 and 10 cGy, although the cohort size is small at the time of writing. Rats exposed to 20 cGy 48Ti also show no decrease in EL time during the testing period, which is not a result of a reduced desire to search for the Escape hole, but an inability to locate it. At the time of submission we have insufficient data on the Attentional Set Shifting tests to report the impact of either radiation on executive function. We have now
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identified changes in the proteome of the hippocampus that are associated with good or bad spatial memory performance.

SUMMARY. These preliminary studies suggest that mission-relevant doses (20 Gy HZE particles with LET's of 100-150 keV/μm) lead to significant neurocognitive defects (spatial memory performance) in rats of comparable biological age to the astronaut population. We shall continue to identify the threshold dose for this impairment and will determine if there is any LET dependency for that dose.

FUNDING: The authors gratefully acknowledge grant support from NASA (NNX11AC56G, NN06HD89D).

POS22-02. Comparison study on the DNA clustered damage induced by high LET radiation. Zhen-Shan Cao, H. Xu, H. Guan, Beijing Institute of Radiation Medicine, China

Based on the molecular model of conformation changes of plasmid DNA, the DNA damages induced by proton, Li heavy ion and γ-rays were studied and compared. The cytogenetic effects of differential LET radiations, including heavy ion, neutron and proton, on human peripheral blood lymphocytes were also studied and the RBE values were evaluated. In order to detect the clustered DNA damage, the irradiated plasmid DNA was digested with E. coli formamidopirimidine DNA glycosylase (Fpg) and AP endonuclease III (Endo III) which convert the damaged bases and AP sites into single-strand breaks (SSB), and the subsequent cell electrophoresis. The results indicated that SSB, a certain level of clustered bases lesions and a tiny of double-strand breaks were induced by the observed 50-200 Gy of γ-rays. The DNA breaks and clustered bases lesions induced by high LET proton and Li heavy ion were much higher as compared with that induced by γ-rays. Our data also showed that an obvious clustered DNA damage was induced by proton even at 10 Gy dose, and the effectiveness of DNA damage induction by proton was higher than that of Li ion. The relative biological effectiveness of cytogenetic damages induced by protons, heavy ions and gamma ray in human lymphocytes were compared. This work was supported by the National Outstanding Youth Science Foundation of NFSC, China (30825011).

POS22-03. New insights in the impact of heavy ion irradiation on tumor cell migration and EGFR cell signaling of glioblastoma cell lines in vitro. Christina Faethe1, M. Scholz2, G. Taucher-Scholz2, W. Mueller-Klieser1, 1: University Medical Center of the Johannes Gutenberg University, Institute for Physiology and Pathophysiology, Germany 2: GSI Helmholtz Centre for Heavy Ion Research, Biophysics, Germany

Heavy-charged particles with its characteristic depth-dose profile sparing normal tissue around malignant tumors represent an emerging technique in cancer therapy. Based on previous results of others and our group showing an increased tumor cell migration after conventional radiotherapy, we aimed to investigate the impact of heavy ion radiation on migration of different glioblastoma cell lines with regard to the role of epidermal growth factor receptor (EGFR) related signaling pathways in vitro.

Two human glioblastoma cell lines, U87 MG and LN229, and two EGFR overexpressing cell lines transduced with wild-type EGFR, U87 EGFR and LN229 EGFR, were irradiated with X-rays or 12C ions in a 1 cm spread-out Bragg peak (mean LET 100 keV/μm) at doses of 2 and 6 Gy. Tumor cell migration was investigated in standardized Boyden chamber assays 24 hours after irradiation while the activation of EGFR receptor and downstream signaling proteins were analyzed immediately after irradiation and 24 hours later using Western Blotting. The results of the Boyden chamber assays show an increased cell migration 2 hours after 2 Gy X-ray irradiation for U87 and U87 EGFR to about 130% (SD 1.8 %) compared to control cells while the migration of LN229 and LN229 EGFR is decreased under the same conditions (around 75 %). Higher doses X-ray irradiation (6 Gy) lead to a reduced migration in all cell lines investigated (approximately 70 %). In contrast to X-rays the migration of these cell lines after exposure to high energy 12C ions (2 and 6 Gy) is strongly decreased by 50 - 60 %. First results regarding the activation of EGFR and downstream signaling show an induction of EGFR after X-ray and 12C irradiation and cell type and radiation specific changes in the activation status of Akt, Erk1/2 and PLCγ1, three main signaling proteins regulating the migration processes of tumor cells.

In conclusion, we will show if heavy-charged particles influence receptor-related intracellular signaling pathways compared to conventional X-ray irradiation. This may elucidate a possible link between the migratory behavior of glioblastoma cell lines in vitro and the activation pattern of EGFR and its downstream signaling molecules.


Triggered by new particle therapy facilities in Europe, interest in understanding the systematics of RBE and its prediction is growing. Biophysical models such as the Local Effect Model (LEM) or the Microdosimetric Kinetic Model focus on that task. As the response to radiation is usually affected by large variations in biological systems, validating these models is hardly feasible by using individual dose response curves. Rather a compilation of many in vitro cell survival experiments after an extensive literature survey is the most comprehensive available data set for the validation and inter-comparison of such models.

In this contribution the principles of the LEM are briefly discussed with an emphasis on differences to other models. The LEM predicts the RBE of ion radiation for a specific tissue solely based on its response to photon radiation. The set-up of a data base of cell survival data comprising about 850 survival experiments with both ions and photons is reported. All registered survival curves have been taken from the literature. The experiments entering the data base comprise survival curves obtained in different labs, using ion species from protons to uranium, different irradiation positions on the target, different cell lines, different morphologies. For each experiment, the cell survival curve has been parameterized by the linear-quadratic model. In addition the corresponding photon parameters have been added to the data base to allow determining the experimental RBE to any survival level.

We report on some experimental correlations found within the data set. This data set will serve as a benchmark for test RBE models. Finally, strategies for further validation of the RBE models and first model results using the data base in combination with the LEM are presented.

POS22-05. Mitotic-spindle associated genes are among the common genes responding to ionizing radiation. Akira Fujimori, H. Hirakawa, National Institute of Radiological Sciences, Japan

Accelerated ion particle with higher LET (linear energy transfer) is promising for the treatment of certain kinds of malignancy. Molecular basis of such advantages of conventional radiotherapy, however, has not been clarified. Using HCCEP (high-coverge expression profiling), we compared gene expression profiles in the normal diploid human fibroblasts (HFLII) irradiated with three types of clinically relevant radiation at 2Gy; X-ray (0.9 Gy/min), and carbon ion beam of either low (15 keV/μm) or high (70 keV/μm) LET. RNA was extracted at 2, 4 and 6 hr later and the expression levels for approximate 16,000 molecules were compared between samples irradiated and mock-irradiated. Through all the nine comparison analyses, we selected 11 IR-responsive genes (signatures), which included upregulated transcripts, (BTG2, ATP3, CCND1,etc.) and also downregulated genes, CCNF (Cyclin F) and ASPM (abnormal spindle-like microcephaly associated). Further comparison study demonstrated that carbon particles of higher LET prolong the expression of earlier responding genes particularly among those which are known to be upregulated under p53 control. Since it was documented that both CCNF and ASPM are required for normal mitotic-spindle formation in somatic cells, our present results would provide a novel insight into the carbon ion beams causing the significant reduction in these spindle-mitotic proteins on DNA damage response.

POS22-06. A treatment planning study comparing different radiobiological models for heavy ion therapy. Clarissa Gillmann1, M. Scholz2, C. P. Karger1, S. Greilich1, M. Ellerbrock1, R. Grün1, T. Friedrich1, J. Debüs2, O. Jäkel1, 1: Radiation Oncology and Radiation Therapy, Heidelberg University Hospital, Germany 2: Gesellschaft für Schwerionenforschung (GSI) Darmstadt, Germany

In this contribution the principles of the LEM are briefly discussed with an emphasis on differences to other models. The LEM predicts the RBE of ion radiation for a specific tissue solely based on its response to photon radiation. The set-up of a data base of cell survival data comprising about 850 survival experiments with both ions and photons is reported. All registered survival curves have been taken from the literature. The experiments entering the data base comprise survival curves obtained in different labs, using ion species from protons to uranium, different irradiation positions on the target, different cell lines, different morphologies. For each experiment, the cell survival curve has been parameterized by the linear-quadratic model. In addition the corresponding photon parameters have been added to the data base to allow determining the experimental RBE to any survival level.

We report on some experimental correlations found within the data set. This data set will serve as a benchmark for test RBE models. Finally, strategies for further validation of the RBE models and first model results using the data base in combination with the LEM are presented.

POS22-07. New insights in the impact of heavy ion irradiation on tumor cell migration and EGFR cell signaling of glioblastoma cell lines in vitro. Christina Faethe1, M. Scholz2, G. Taucher-Scholz2, W. Mueller-Klieser1, 1: University Medical Center of the Johannes Gutenberg University, Institute for Physiology and Pathophysiology, Germany 2: GSI Helmholtz Centre for Heavy Ion Research, Biophysics, Germany

Heavy-charged particles with its characteristic depth-dose profile sparing normal tissue around malignant tumors represent an emerging technique in cancer therapy. Based on previous results of others and our group showing an increased tumor cell migration after conventional radiotherapy, we aimed to investigate the impact of heavy ion radiation on migration of different glioblastoma cell lines with regard to the role of epidermal growth factor receptor (EGFR) related signaling pathways in vitro.

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In conclusion, we will show if heavy-charged particles influence receptor-related intracellular signaling pathways compared to conventional X-ray irradiation. This may elucidate a possible link between the migratory behavior of glioblastoma cell lines in vitro and the activation pattern of EGFR and its downstream signaling molecules.
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Introduction: In carbon ion radiotherapy, an accurate calculation of the biologically effective dose is essential for the adequate treatment of patients. For that purpose, the local effect model (LEM I) has been used in clinical routine for more than 500 patients with excellent results. Nevertheless, it is well known that LEM I has some shortcomings, in particular the overestimation of the relative biological effectiveness (RBE) in the entrance channel (Karger 2007, Elsässer 2008). As a first step towards the therapeutic use of a further developed and more generalized version of the local effect model (LEM IV), we performed a treatment planning study comparing LEM I and LEM IV. This study allows us to analyze potential differences in the RBE weighted dose calculation of both models and to compare resulting dose parameters with published clinical data.

Materials and Methods: Using LEM IV, we recalculate treatment plans that were initially based on the LEM I algorithm. Our collective includes 30 patients with skull base chordomas and chondrosarcoma who were irradiated with carbon ions in 2002 and 2003. For the assessment of recalculated plans commonly used dose indices like conformity and homogeneity are evaluated.

Results: In our analysis, we identify slight deviations between LEM I and LEM IV that are in the order of up to 10% for the median values of many clinically relevant dose indices. In detail, we observe, that dose distributions in the target, being homogeneous for LEM I, become dependent on the tumor diameter in beam direction for LEM IV. Furthermore, isodose lines are much more concentrated around the target area for LEM IV. In consequence, for patients close to the target, we observe a dose elevation of about 10%, whereas in regions several millimeters away from the target, we find a reduction of dose. As a consequence, the median dose of the temporary lobe is reduced about 10%.

Conclusion: Our comparative treatment planning study enabled a qualitative visualization and a statistical analysis of RBE weighted isoeffective dose distributions calculated by LEM I and LEM IV. A quantitative evaluation showed similar results for clinically relevant dose indices. This treatment planning study essentially contributes to the ongoing discussion about the future use of radiobiological models in hadron therapy.

POS22-07. Preliminary in vivo data of three experimental prostate tumor sublines to photons and carbon ions, Christin Glowa1, P. Peschke2, M. Scholz2, P. Huber2, J. Debus2, C.P. Karger1, J. deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany 2: Department of Clinical Radiology, University of Heidelberg, Germany 3: Gesellschaft für Schwerionenforschung (GSI), Darmstadt, Germany

Introduction: Heavy charged particles such as carbon ions are characterized by an increased linear energy transfer (LET) and therefore show an enhanced relative biologic effectiveness (RBE) with respect to photon irradiations. The first dedicated European clinical heavy-ion therapy (HIT) facility in Heidelberg (Germany) started patient treatment in fall 2009. Following encouraging results for radioresistant skull-base tumors, it is planned to extend ion therapy in case of local failures, which suppress indirect action of photons under oxic and hypoxic conditions, respectively. The dose indices that lowest damaging dose to about 1 cGy of either ion, there is no increase of indirect action for iron ions were estimated to be 42% and 32%, respectively. In contrast, the contributions of indirect action for iron ions were estimated to be 42% and 32%. The RBE values were 2.8 for oxic and 5.3 for hypoxic, and the OER values were 2.8 for X-rays and 1.5 for iron ions. When the RBE and OER were estimated separately for direct action (RBE_d and OER_d) and indirect contributions (RBE_i and OER_i), the RBE_i values were larger than RBE_d under both normal and low oxygen concentrations. The OER_d values for both X-rays and iron ions were lower than that for
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OERs. Thus, direct action of radiation gives a remarkably higher RBE and lower OER for cell killing than indirect action. It is possible that particle beams may be highly effective in treating cancer if the particle therapy cannot use the portion of direct action out of total radiation actions; this would be exemplified by the usage of radioactive 'C-ion beams and boron neutron capture.

POS22-10. Cytogenetic effects of ionizing radiation in peripheral lymphocytes of ISS crewmembers. Christian Johannes, A. Antonopoulos, W. Goedecke, University Duisburg-Essen, Germany

Astronauts, cosmonauts and an increasing number of space tourists are currently visiting the International Space Station (ISS) demonstrating the onset of a new era of extraterrestrial environments. On-board of the ISS, members of the crew are exposed to ionizing radiation of cosmic and solar origin, while on the Earth’s surface people are well protected by the atmosphere and a deflecting magnetic field. The radiation field in space consists of high energy protons, α-particles and nuclei with high charge (Z) and energy (E) (HZE) and has a high lineal energy transfer (LET). On the contrary terrestrial radiation is characterized by low linear energy transfer radiation (X-, β- or γ-rays).

There are data available describing the dose and the quality of ionising radiation on-board of the ISS, but the biological effects of the high energetic radiation on human cells, fibroblasts and epithelial cells and these events are greatly enriched in the high charge (Z) and energy (E) (HZE) wild LOH is infrequent in spontaneous mutants except for those with loss of chromosome 8, and rare in Fe-exposed non-mutant cells. Taken together, Fe ions elicit massive genomic rearrangements in viable epithelial cells and these events are greatly enriched in Fe-exposed mutants. These events are similar to what is found in human epithelial cancers. The results demonstrate that densely ionizing radiation can elicit these changes in apparently normal epithelium. Supported by NASA grants NNJ07HC72I (A. Kronenberg), and NNX10AC12G (M.Turker).

POS22-12. Oxidative stress in normal cells exposed to carbon ions. Carine Laurent1, A. Leduc1, J. Lefaitx1, I. ARCHADE, France 2: CEA, France

PURPOSE: The high efficiency of carbon ions vs. photons, with an Ion-Relative Biological Effectiveness (RBE) of 2 to 3, to treat various tumours was demonstrated in the main hadrontherapy centers (GSI, Germany and HIMAC, Japan). However, few extensive studies were performed in order to evaluate the impact of high-LET exposure on healthy tissues. Thus, in order to evaluate the possible secondary effects of a carbon treatment, we compared the exposure of normal human peripheral blood lymphocytes of crew members on-board of the ISS, but the biological effects of the high energy protons, α-particles and nuclei with high charge (Z) and energy (E) (HZE) and has a high lineal energy transfer (LET). On the contrary terrestrial radiation is characterized by low linear energy transfer radiation (X-, β- or γ-rays).

The results of our study show that long-duration but not short-duration missions to the ISS lead to increased frequencies of aberrations in the peripheral lymphocytes of crew members. The types of aberrations found were in the unstable type (dicentrics) and of the stable type (translocations) and both are considered to be radiation induced. No significant increase of multiple damaged cells or complex aberrations types have been found, which are suspected to occur following high LET radiations.

The obtained data will be helpful in order to carefully plan future long duration space flight missions to Moon and Mars with acceptable levels of radiation risk for the crew members.

POS22-11. Charged particle-induced autosomal mutagenesis in normal epithelium exposed to energetic protons or Fe ions. Amy Kronenberg1, L. Choucair1, C. Tsuruoka1, M. Wada1, Y. Kaneko1, 1. National Institutes of Radiological Sciences, Japan 2: New Jersey Medical School Cancer Center, USA

Most radiogenic human cancers occur in epithelial tissues. Mutations are critical in cancer and autosomal mutations predominate. We used the C57BL/6 x DBA/2 Aprt+/0 mouse model to measure charged particle-induced mutations. Kidney epithelial cells were exposed in vitro with a short-term assay to detect Aprt mutants, or kidneys were exposed in situ with cells harvested 3-4 months post-irradiation (IR) and screened for Aprt mutants. We tested the effect of fractionation on Aprt mutation after 1 Gy proton IR (LET=0.24 keV/µm). We also measured the effect of sequential IR with protons and 1 GeV/amu Fe ions (LET= 151 keV/µm) on Aprt mutant frequencies. Another study details genome-wide changes in Fe ion-induced Aprt mutation. Exposures were done on the NASA Space Radiation Laboratory at Brookhaven National Laboratory. In situ proton IR with 1.25 Gy x 4 daily fractions is toxic to kidney epithelium (p<0.011), but less than acute IR (p<0.001). Fractionated IR is mutagenic in situ (p<0.001), but there is sparing for mutation after fractionated vs. acute IR (p<0.001). In vitro data confirm the in situ results. The study of sequential IR shows that exposure to 3 Gy of protons + 1 Gy of Fe ions (24 hour interval) is more mutagenic than 3 Gy of proton + 1 Gy of Fe ions alone. This is also true if the sequence is reversed. The sequence of IR did not impact Aprt mutant frequencies. Earlier, we found large loss of heterozygosity (LOH) events in Fe ion-induced mutants using polymorphism markers on chromosome 8 where Aprt is located. Here, we looked at changes on 11 other chromosomes. Genome-wide LOH is seen in >50% of Aprt mutants exposed in vitro or in situ. Extensive changes are seen on the unlinked chromosomes including mitotic recombination, multilocus deletions, discontinuous LOH, and chromosome loss. Genome-wide LOH is infrequent in spontaneous mutants except for those with loss of chromosome 8, and rare in Fe-exposed non-mutant cells. Taken together, Fe ions elicit massive genomic rearrangements in viable epithelial cells and these events are greatly enriched in Fe-exposed mutants. These events are similar to what is found in human epithelial cancers. The results demonstrate that densely ionizing radiation can elicit these changes in apparently normal epithelium. Supported by NASA grants NNJ07HC72I (A. Kronenberg), and NNX10AC12G (M.Turker).

POS22-13. Radiosensitivity in mesothelioma cell lines for X rays and carbon-ion beams. Chulha Liu1, M. Suzuki2, C. Tsuruoka2, N. Autrupavapromprasert1, V. E. Koh1, K. C. Tsuruoka1, M. Wada1, Y. Kaneko1, 1. National Institutes of Radiological Sciences, Japan 2: New Jersey Medical School Cancer Center, USA

INTRODUCTION: Malignant pleural mesothelioma (MPM) is an aggressive tumor arising from serous surfaces and often related to asbestos exposure. Taking into account a latency period of 20-40 years, it has been estimated that the number of men dying from MPM increase each year until a peak is reached in about 2020. MPM is resistant to various forms of therapy, such as radiotherapy, surgery or chemotherapy, and only slightly improve prognosis. So far, no effective therapeutics including chemotherapy or radiotherapy has been established for the disease-advanced case. The present study was carried out in order to examine the radio-sensitivity of MPM cell lines by X rays and carbon-ion beams. MATERIALS AND METHODS: Six kinds of MPM cell lines officially distributed by the cell bank were used and irradiated with X rays or carbon-ion beams (13.3keV/µm and approximately 80keV/µm) at the Heavy Ion Medical Accelerator in Chiba (HIMAC) in National Institute of Radiological Sciences (NIRS), Japan. Cell- killing effect was detected using a colony-formation assay.

RESULTS: D$_{0}$, which is one of the parameters for cellular radio- sensitivity and is determined as the dose (Gy) required to reduce the surviving fraction to 10%, of 6 cell lines ranged from 2.74Gy to 5.42Gy for X rays, 2.17Gy to 4.21Gy for 13keV/µm- and 1.08Gy to 2.38Gy for 80keV/µm-carbon ions. The relative biological effectiveness (RBE) values were calculated by the D$_{10}$ relative to X rays, ranged from 1.24 to 1.42 for 13keV/µm- and from 2.31 to 3.09 for 80keV/µm-carbon ions on each cell line.
CONCLUSION: The results suggest that the irradiation of high-LET carbon ions is also significantly more effective in cell killing for the 6 kinds of MPM cell lines than that of low-LET X rays.

POS22-14. Dose-rate effects for chromosome aberrations observed in immortalized human fibroblasts irradiated with high energy protons. Bradford Loucas, R. Eberle, M. Comforth, University of Texas Medical Branch, USA

Lowering the rate at which low LET radiation is delivered to biological systems often results in a diminution of induced effects, including chromosome aberrations. As the dose rate is lowered, a loss of the curvature that is characteristic of acute clastogenic dose responses is observed. This “flattening” of the dose response is thought to result from a decline in the frequency of exchanges that arise from damage caused by the interaction of multiple tracks. This decline will continue with decreasing dose rate until the “limiting low dose rate” is achieved. In theory, at this point, damage is confined to that caused by single tracks (in intratrack) producing linear dose responses. While this phenomenon has been well studied with X- and gamma-rays, it is unclear to what extent such an effect will be observed following irradiation with high energy, low LET protons. To test the presence and extent of dose rate effects for high energy protons, we irradiated plateau phase 4TERT-immortalized BJ-1 human fibroblasts (BJ4TSlR with 1 GeV, 50 MeV protons at a low dose rate of about 0.5 Gy/min which is near the limiting low dose rate for gamma photons, and compared these results to those obtained following acute dose rates of about 100 cGy/min. Cells were subcultured 72-96 hours post-irradiation and mitotic cells were collected 32-36 hours later. These were examined using 24-color mFISH to measure the frequency of chromosome aberrations. The scoring of cells irradiated with 1 GeV is now complete and results indicate that the frequency of aberration breakpoints produced by the 3 Gy acute dose is 1.5- to 3-fold higher compared to that observed following protracted exposures. Part of this difference seems to have resulted from a greater relative reduction in the frequency and size of complex exchanges, resulting from three or more chromosome breaks, as compared to simple exchanges produced by pair-wise break rejoins. The inspection of cells irradiated at the two lower energies is on going but preliminary evidence suggests that results will be similar to those of the 1 GeV experiments. The authors gratefully acknowledge support from the NASA, Office of Biological and Physical Research NASA/OBPR, NNI07ZSA001N.


Purpose: Up to 2011, over 5500 patients have been treated in HIMAC. Malignant melanoma showed high local control about 75%, whereas the overall survival was about 36% at 5-years. It is an important subject to control tumor distant metastasis for heavy-ion radiotherapy. The aim of this study is to clarify the effect of carbon ion beams (C-ions) on metastatic potential of melanoma in vitro and in vivo. Materials and methods: A highly metastatic mouse malignant melanoma cell line, B16BL6 was maintained in RPMI-1640 medium supplemented with 10% FBS and antibiotics. [in vitro] Samples were prepared 1.5 days before and then irradiated with C-ions at different LETs or X-rays. Surviving fractions (SF) and metastatic potentials (migration, invasion and adhesion activity) of the cells were examined. [in vivo] Local tumors implanted into right leg of mice (7.5±0.5 mm3) were also irradiated with C-ions at different LETs, or X-rays. The metastatic effects were analyzed with the spontaneous lung metastasis model. Radiosensitivity for individual cell in a tumor was obtained by an in vivo-in vitro assay. Results: [in vivo] C-ions showed higher cytotoxic and anti-metastatic effects compared with X-rays, and the effects were significant depend on dose and LET values. The enhancement ratios obtained from metastatic potential was higher than that from cell killing. [in vivo] After irradiation for local tumors, the numbers of lung metastatic nodules decreased with the dose and LET values. C-ions were more effective compared with γ-rays. The number of lung metastasis was analyzed with equivalent survival doses (ED50) calculated from relative survival curves of in vitro irradiated cell in a tumor. In this case, C-ions also showed smaller number of metastasis than γ-rays. Conclusion: It might suggest that C-ions inhibit metastasis at radiotherapy compared with photons.

POS22-16. Induction of nuclear-wide H2AX phosphorylation by ion irradiation. Barbara Meyer1, K. Voss1, N. Averbbeck1, M. Herrlitz1, B. Jakob1, M. Durante2, G. Taucher-Scholz2, 1: GSI, Germany 2: Technical University Darmstadt, Germany

Introduction: In response to ionizing radiation, the histone H2AX is phosphorylated at DNA double-strand breaks forming γH2AX foci that promote the recruitment and retention of repair factors to the damage sites. Here we describe an additional, novel ion irradiation-induced response, the nuclear-wide formation of γH2AX.

Methods: Besides ion irradiation with γ-rays, as well as H2AX2 mouse cells were irradiated with heavy ions and fixed for immunofluorescence microscopy or harvested for Western analysis or MNIase assay. Apoptotic cells were detected by annexin, G2 cells by CEPNF staining. Specific inhibitors of ATM, DNA-PK, and HATs were used. A software based intensity measurement of the pan-nuclear immunofluorescence signal was conducted after defined irradiation with the GSI heavy ion microprobe.

Result: The nuclear-wide γH2AX signal is seen with various tested γ-rays antibodies in all cell cycle phases. It is not observed in H2AX2 cells. Pan-nuclear γH2AX signal is chromatin-dependent lung adverse effects. Three strains of female mice (C3H/HeN Jms, C57BL/6J Jms and A/J Jms) were locally irradiated in the thorax with either C-ion beam (290 MeV/n, in 6 cm SOBP) or with γ-rays as a reference beam. We performed survival assays, histological examinations of the lung with hematoxylin-eosin staining, Masson’s trichrome staining, immunohistochemical staining for CD44 and Mac3, and gene expression analyses. Intra-alveolar hemorrhage was assessed by Berlin blue staining and measurement of the iron concentration of bronchoalveolar lavage fluid. Survival data presented a mouse strain-variance after C-ion irradiation at 10 Gy. The histological examination revealed early phase hemorrhagic pneumonitis in C3H/He mice and late phase pulmonary fibrosis in C57Bl/6J mice after C-ion irradiation at 10 Gy. Microarray analysis of irradiated lung tissue in these mouse strains identified differential expression changes of the growth differentiation factor 15 gene (Gdf15), that regulates inflammatory and apoptotic pathways and macrophage function and for the hyaluronan synthase 1 gene (Has1), that plays a role in hyaluronan metabolism. Immunohistochemical analysis showed that the ratio of CD44-positive cells as surrogate marker for hyaluronan accumulation and Mac3-positive cells as maker for inflammation and macrophage infiltration in irradiated lung varied.

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POS22-17. Strain-dependent intra-alveolar hemorrhage with the accumulation of CD44 and Mac3 positive cells after carbon-ion irradiation. Takashi Moritake1, H. Fujita2, M. Yanagisawa1, M. Kawanari2, K. Imadome3, T. Imai2, E. Nakamura1, M. Inoue3, T. Tanioka1, M. Yanagisawa1, Y. Fujita2, 1: Proton Medical Research Center, University of Tsukuba, Japan 2: National Institute of Radiological Sciences, Japan 3: Chubu University, Japan

The aim of this study was to investigate whether inherent factors produce variance of lung morbidity in response to carbon-ion (C-ion) irradiation, and to identify the molecules that have a key role in strain dependent lung adverse effects. Three strains of female mice (C3H/He Slc, C57Bl/6j Jms Slc and A/J Jms Slc) were locally irradiated in the thorax with either C-ion beam (290 MeV/n, in 6 cm SOBP) and with 125I γ-rays as a reference beam. We performed survival assays, histological examinations of the lung with hematoxylin-eosin staining, Masson’s trichrome staining, immunohistochemical staining for CD44 and Mac3, and gene expression analyses. Intra-alveolar hemorrhage was assessed by Berlin blue staining and measurement of the iron concentration of bronchoalveolar lavage fluid. Survival data presented a mouse strain-variance after C-ion irradiation at 10 Gy. The histological examination revealed early phase hemorrhagic pneumonitis in C3H/He mice and late phase pulmonary fibrosis in C57Bl/6j mice after C-ion irradiation at 10 Gy. Microarray analysis of irradiated lung tissue in these mouse strains identified differential expression changes of the growth differentiation factor 15 gene (Gdf15), that regulates inflammatory and apoptotic pathways and macrophage function and for the hyaluronan synthase 1 gene (Has1), that plays a role in hyaluronan metabolism. Immunohistochemical analysis showed that the ratio of CD44-positive cells as surrogate marker for hyaluronan accumulation and Mac3-positive cells as maker for inflammation and macrophage infiltration in irradiated lung varied.
significantly among three mouse strains. This study demonstrated the strain-dependent differential response in mice to high-LET C-ion irradiation. Our findings identified possible molecules relating to the strain-variance of lung toxicity after C-ion irradiation.

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**POS22-18. Radiosensitization by inhibition of homologous recombination repair combined with high LET heavy ion irradiation.** Ryoichi Okayasu 1, Hirokawa, K. Noguchi, D. Yu, Takaaki Sugiyama, A. Fujimoto. National Institute of Radiological Sciences, Japan 2: Japan Atomic Energy Agency, Japan 3: Tokyo Medical and Dental University, Japan 1

17AAG, an Hs90 inhibitor was shown to radiosensitize certain human tumor cells exposed to X-rays, while this sensitization was not clearly observed in normal human cells. The mechanism of this was suggested to come from inhibition of DNA double strand break (DSB) repair, particularly impairment of homologous recombination repair (HRR) pathway by this drug (Noguchi et al 2006). Key proteins associated with HRR seem to be affected by this inhibitor. To our surprise, tumor radiosensitization with 17 AAG was also observed in cells exposed to high LET carbon ions (70 keV/μm). Independently we also found that knockdown of BRCA2, a key HRR protein significantly radiosensitized human tumor cells. These results indicate that there seems to be a radio-sensitization mechanism associated with the combination of HRR inhibition and high LET radiation, and this may occur particularly in S-phase cells. Furthermore, we also used mouse xenograft model to examine the combined effect of 17AAG and high LET carbon irradiation. For this purpose, SQ5 human lung tumor cells were implanted on the leg of nude mice and the tumor growth was measured in the combined treatment as compared with radiation or drug treatment alone. Our preliminary results indicate that tumor growth was more inhibited in the 17AAG and carbon irradiation than carbon or 17AAG treatment alone. These data suggest that an effective tumor control might be obtained by combining an HRR inhibitor with high LET carbon irradiation.

**POS22-19. Genotoxic effects of low- and high LET radiation on human epithelial cells grown in 2-D versus 3-D culture.** Zarana Patel, F. Cucinotta, J. Huff, 1: USRA/NASA JSC, USA 2: NASA JSC, USA 1

Risk estimation for radiation-induced cancer relies heavily on human epidemiology data obtained from terrestrial irradiation incidents such as medical and occupational exposures as well as from the atomic bomb survivors. No such data exists for exposures to the types and doses of high LET radiation that will be encountered during space travel; therefore, risk assessment for space radiation requires the use of data derived from cell culture and animal models. The use of experimental models that most accurately replicate the response of human tissues is critical for precision in these risk projections. This work compares the genotoxic effects of radiation on normal human epithelial cells grown in standard 2-D monolayer culture compared to 3-D organotypic co-culture conditions. These 3-D organotypic models mimic the morphological features, differentiation markers, and growth characteristics of fully-differentiated normal human tissue and are reproducible using defined components. Cultures were irradiated with 2 Gy low LET gamma rays or varying doses of high-LET particle radiation and genotoxic damage was measured using a modified cytokerinex block micronucleus assay. Our results revealed a 2-fold increase in residual damage in 2 Gy gamma irradiated cells grown under organotypic culture conditions compared to monolayer culture. Irradiation with high-LET particle radiation gave similar results, while background levels of damage were comparable under both scenarios. These observations may be related to the phenomenon of “multicellular resistance” wherein cancer cells grown as 3-D spheroids or in vivo exhibit an increased resistance to killing by chemotherapeutic agents compared to the same cells grown in 2-D culture. A variety of factors are likely involved in mediating this process, including increased cell-cell communication, microenvironment influences, and changes in cell cycle kinetics that may promote survival of damaged cells in 3-D culture that would otherwise die or be rendered reproductively inactive in 2-D culture.

**POS22-20. mBAND analysis of aberrations reveals marked differences between cells exposed in vitro and in vivo to X-rays or C-ions.** Sylvia Ritter, D. Pignolosa, R. Lee, C. Hartel, M. Durante, S. Sommer, A. Nickoghosyan, I. Debus, 1: GSI, Germany 2: Scientific Center of Radiobiology and Biological Dosimetry, Warsaw, Poland 3: Radiology Clinics, University of Heidelberg, Heidelberg, Germany.

Identification of a cytogenetic signature that allows distinguishing high from low LET exposure is a long-standing goal in radiobiology. It has been predicted that high LET radiation preferentially produces multiple breaks within a given chromosome resulting in intra-chromosome aberrations, while after low LET exposure many chromosomal breakages are observed favoring the occurrence of inter-chromosome aberrations. In the present study we report first results obtained by the mBAND-technique for human lymphocytes exposed in vitro or in vivo. For in vitro experiments, lymphocytes of a healthy volunteer were irradiated with 2 Gy of 11.4 MeV/u C-ions (LET = 175 keV/μm) or 4 Gy X-rays. Slides were hybridized with an mBAND probe specific for chromosome 2 and aberrations were measured in first cycle metaphases. Furthermore, lymphocytes from prostate cancer patients obtained at the end of therapy were analyzed. Patients were treated with carbon-ion boost (63GyE) followed by proton IMRT (60 Gy) or solely IMRT (78 Gy). To study the effect of field size, patients with a larger target volume treated with IMRT were included in the study. The carbon-ion boost was delivered at GSI, Darmstadt; the treatment using IMRT was performed at the Radiology Clinics, Heidelberg. In all samples scored, inter-chromosome aberrations dominate, while only a small number of intra-chromosome aberrations was observed indicating that the ratio of both aberration classes does not represent a practical biomarker of radiation quality. Interestingly, only in samples exposed in vitro marked proportion of aberrations involving both inter- and intra-chromosome exchanges was found (30 and 15% for C-ions and X-rays, respectively). A detailed analysis of complex aberrations revealed significant differences between C-ions and X-rays after in vitro exposure, while no difference was observed for patients treated with C-ion boost or solely IMRT. Data obtained for the third patient group (IMRT, large field) suggest that the field size has a prominent effect on the number of complex aberrations formed. These data will be compared with that recently obtained by the mFISH-technique (Hartel et al. Radiotherapy & Oncology 95, 73-78 (2010)).

**POS22-21. Silicon exposure alters putative components of neuroglial intercellular signaling.** Martha Sanchez, M. C. Sanchez, B. M. Bianski, L. S. Orloff, G. A. Nelson, L. M. Green, Loma Linda University, USA

Bidirectional communication between astrocytes and neurons is essential for normal central nervous system (CNS) function and development. Mediation of bidirectional signaling is ascribed to three principal messengers: calcium, glutamate, and ATP. Calcium-mediated signaling is one of the mechanisms by which CNS cells communicate and modulate the activity of adjacent cells. Calcium waves are believed to underlie the spatial transfer of information and coordination of neural activity. Calcium waves can propagate through intercellular gap junctions and/or an extracellular signaling molecule. The aim of this study was to ascertain the effect of 300 MeV silicon exposure on signaling elements of CNS intercellular communication. METHODS: Primary human astrocytes and neurons were exposed to 5, 10, and 100 cGy acute doses of 300 MeV silicon. ATP release and calcium wave propagation were measured at 3 hrs, 2 days, and 7 days after exposure. ATP release measurements, determined using a luciferin-luciferase-based bioluminescent assay, were obtained in neuron, astrocyte, and mixed neuron-astrocyte co-cultures. Propagation of calcium waves in astrocytes and in mixed cultures was measured using the fluorescent calcium indicator Fluo-4 AM. Calcium waves were elicited by point mechanical stimulation and spatial changes in intercellular calcium were measured. RESULTS: Significant increases in ATP release were measured at 3 hrs after exposure to 100 cGy in all three types of cell cultures. By 2 days, the only significant change in ATP release was a decrease noted in 5 cGy irradiated astrocytes. Calcium wave measurements of unirradiated control cultures displayed a calcium wave radius between 250-400 μm. Silicon irradiation altered calcium wave propagation in astrocytes but not in mixed cultures. Significant increases in wave distance were measured at 3 hrs and 7 days after exposure to all radiation doses. CONCLUSIONS: The changes in ATP release and calcium waves indicate that silicon irradiation, at these doses, is capable of altering these two components of neuroglial communication. Such altered responses may represent early signaling changes in the CNS. These changes and consequences on cognitive processes given the high degree of heterocellular crosstalk that exists in the CNS.
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POS22-22. Lethal and sub-lethal damage along and around a 62 MeV/u spread out carbon beam. Giuseppe Schettino\textsuperscript{1}, J. N. Kavanaugh\textsuperscript{1}, L. Mantil\textsuperscript{1}, E. J. Currell\textsuperscript{1}, P. Curren\textsuperscript{2}, F. Romand\textsuperscript{1}, G. Grossi\textsuperscript{1}, K. M. Prise\textsuperscript{1}, 1: Queen's University Belfast, UK 2: University of Naples “Federico II”, Italy 3: LNS-INFN, Italy

Ion beams have been indicated as the ideal radiation modality for the treatment of deeply seated and/or radioresistant cancers due to the inverse dose deposition pattern they generate as they penetrate biological samples. Although better dose distributions can be achieved compared to conventional X-ray radiotherapy, there are still open questions regarding the long-term effects caused in the surrounding healthy tissue. Our study aims to investigate lethal and sublethal damage caused in living cells along and in the proximity of therapeutically relevant ion beams. Initial experiments were performed by exposing human fibroblast (AG01522) cells to 62 MeV/u carbon beams in a spread out Bragg peak (SOBP) configuration. Data confirms the increasing effectiveness of carbon ions in inducing cell killing as they slow down with RBE\textsubscript{SOBP} (Plateau) = 2.3 and RBE\textsubscript{LESOBP} = 4.2 relatively to conventional X-rays. Track- and dose-averaged LET calculations using Geant4 package will help relating the RBE measurements (for both lethal and sublethal events) as a function of the spread out configuration. Cell survival data have been matched by measurement of DNA damage (immunofluorescence through H2AX assay) at different times post irradiation showing the different rate at which plateau and SOBP DNA insults are repaired. Analysis of foci size and residual number of foci at 24 h post irradiation have also been performed to relate severity of DNA damage induced to cellular outcome (compared to different type of radiations (X-rays, protons and antiprotons). Out of field measurements also suggest significant DNA damage being induced in cells not directly exposed (bystander effect) but which share medium with exposed cells. This damage appears to be independent from dose and LET variations. Finally, chromosome aberration studies reveal substantial chromosome rearrangement induced in both the plateau and the SOBP region. Interestingly, the aberration rate appears to be dose dependent in the plateau region but not in the SOBP. Moreover, the quality of the aberrations detected also shows an interesting pattern with transmissible aberrations dominating in the plateau response while lethal rearrangement and chromosome fragments are the majority in the SOBP.

POS22-23. Relative biological effectiveness of 20 MeV protons delivered homogeneously or as focused beams in a 5x5 µm matrix. Thomas Schmid\textsuperscript{1}, C. Dollinger\textsuperscript{2}, V. Hable\textsuperscript{3}, C. Greubel\textsuperscript{2}, O. Zlobinskaya\textsuperscript{1}, D. Michals\textsuperscript{1}, A. Friedl\textsuperscript{1}, E. Schmied\textsuperscript{1}, G. Multhoff\textsuperscript{1}, M. Molls\textsuperscript{1}, 1: Department Radiotherapy and Radiooncology, Klinikum rechts der Isar, Germany 2: Universität der Bundeswehr Muenchen 3: Ludwig-Maximilians-Universität Muenchen, Germany

Due to their physical and radiobiological properties, in particular their increased relative biological effectiveness (RBE), high linear energy transfer (LET) radiation qualities are of special interest for tumor therapy. The aim of the present investigation was to quantify the influence of spot application of a bunch of 20 MeV protons within an irradiation field of 4x4 mm using the same dose. Human-hamster hybrid (AL) cells were irradiated with 1.7 Gy of 20 MeV protons at the Munich ion microprobe SNAKE (Superconducting Nanoprobe for Applied nuclear [Kern] physics Experiments) of the 14 MV Munich tandem accelerator. Since this unique irradiation setup allows the irradiation with single or counted protons, AL cells were exposed to counted protons either in a focused mode using a 5x5 µm matrix with 100 protons per point or a homogenous mode. Using simultaneously obtained linear-quadratic dose response curves for the induction of dicentric chromosomes as well as micronuclei (in cells not directly exposed, bystander), & RBE values for 20 MeV protons delivered at both application modes were determined. For the dicentric chromosome data, RBE values of 1.41±0.10 and 1.89±0.12 were determined for homogeneous and spot application of 20 MeV protons, respectively. For the corresponding micronuclei data, RBE values of 1.30±0.08 and 1.52±0.08 were obtained for homogeneous and spot application, respectively. Since these differences between both application modes are significant (p<0.05), it can be concluded that elevated RBE values of 20 MeV protons can be achieved by spot application of a bunch of 20 MeV protons within an irradiation field of 4x4 mm using the same dose. This finding represents an important step in understanding the increased RBE of carbon ions for clinical radiotherapy. In general, the present result is the first evidence that the increased RBE of heavy ions can be simulated by spot application of a bunch of protons. Supported by the DFG Cluster of Excellence Munich-Centre for Advanced Photonics and by the Maier Leibnitz Laboratory Munich.

POS22-24. The full simulation of dose response curves using the Local Effect Model. Uwe Scholz, T. Friedrich, M. Scholz, M. Durante, GSI/Biophysics, Germany

The purpose of the Local Effect Model (LEM) is to calculate the relative biological effectiveness (RBE) of charged particle radiation with respect to conventional photon radiation. In its most recent implementation, it is based on the simulation of the microscopic distritution of dose along the LET region depends on the input parameters characterizing the photon dose response. The increased β-term is also observed for lighter ions. For protons we observe a rise of the β-term between 10 keV/µm and 40 keV/µm even significantly above the value of photon radiation. This effect is in line with findings reported for neutron experiments.

Furthermore, within the full simulation we investigated the dose dependence of the RBE up to high irradiation doses for different ion species. It turned out that for light ions the RBE approaches values higher than 1. This behaviour can be understood mechanistically within the LEM and is also consistent with findings reported in the literature for neutron radiation.


For both clinical applications and radiation protection issues, there is large interest in understanding the increased biological effectiveness of particle radiation. The Local Effect Model (LEM) has been developed to predict the systematic dependencies of the relative biological effectiveness (RBE) as a function of the ion species, the beam energy and the biological characteristics of the cell or tissue type under consideration. The model has been successfully implemented in treatment planning for ion beam therapy, where the primary focus was to predict tumor and normal tissue response in the target region with sufficient accuracy.

In order to better assess and quantify the relative merits of ion beam therapy as compared to other treatment modalities, secondary cancer induction will be an increasingly important aspect. We therefore tested the applicability of the LEM to more complex endpoints like cell transformation in-vitro, representing an important step towards application to secondary cancer induction.

As for the other applications, the calculations are based on the corresponding photon parameters. In order to reflect the two competing processes, i.e. cell killing and transformation induction, two sets of LQ-parameters are used. Based on the different sets of parameters, the relative contributions to killing and induction after ion irradiation can be calculated and the resulting probability to induce a visible transformation is determined.

The approach has been tested by comparison to experimental data reported by Miller et al. (Radiat. Res. 1995) for light ions at low energies and by Yang et al. (Radiat. Res. 1985) for heavier ions at high energies. In both cases, reasonable agreement between model predictions and experimental data is observed. In conclusion, this agreement demonstrates that the general strategy of the LEM can be
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extended to endpoints like cell transformation and thus has the potential for applications to estimate secondary tumor induction as well.

**POS22-26.** Space radiation-induced intestinal tumorigenesis is mediated via preferential activation β-catenin/TCF-4 signaling. Shubhankar Suman, K. Datta, D. Tran, A. J. Fornace Jr., Georgetown University, USA

Introduction: Currently, the long-term tissue-specific consequences on human health of space radiation exposure, especially heavy ion radiation, are yet to be defined. Colorectal cancer (CRC) is the third most common cancer in the US and although we have shown earlier that heavy ion radiation increases intestinal tumorigenesis in APC+/min mice, the molecular pathways involved are not characterized.

Methods: APC+/min mice (6-8 wks) were irradiated with 5 Gy of gamma radiation and iso-toxic doses of 1GeV proton (4.7 Gy) and 1 GeV/nucleon 56-Fe radiation (4 Gy). After 90 days mice were euthanized, intestinal tumors counted and tumor samples flash frozen.

Tumor samples were subjected to histology, western blot, immunohistochemistry (IHC), activity assays, and real time Q-PCR to dissect the APC/β-catenin/TCF4 pathway, commonly altered in human CRC.

Results: Quantitative observations showed enhanced intestinal tumor growth within 30 days after space radiation, increased in β-catenin and TCF4 were associated with a decrease in phospho-β-catenin and increased phospho-GSK3β by western blot analysis of space radiation samples. Increased β-catenin was also associated with higher levels of cyclin-D1 and c-myc in these samples. Furthermore, strikingly lower level of p53 along with higher MAD2 was also observed in space radiation samples. Interestingly, the decrease in p53 and increase in MDM2 was higher with 56-Fe.

These observations were further supported by IHC and Q-PCR data.

Conclusions: Both proton and 56-Fe, although were more tumorigenic than gamma radiation, showed similar tumorigenicity. However, distinct molecular changes in APC/β-catenin/TCF4 signaling were observed with proton and 56-Fe radiation. In 56-Fe there was higher nuclear accumulation of β-catenin along with reduced phospho-β-catenin and higher phosphorylation mediated inactivation of its upstream kinase GSK3β. This indicates that 56-Fe has a greater potential towards carcinogenic process than proton. Enhanced potential of 56-Fe for carcinogenesis is further supported by our data on p53 pathway and also on β-catenin downstream targets c-myc, cyclin-D1, and MMP-7. In conclusion, this is the first evidence showing greater potential of heavy ion radiation for intestinal carcinogenesis mediated via β-catenin/TCF4 signaling.

**POS22-27.** Three-dimensional organotypic cultures as an in vitro model of heavy ion-induced mucositis due to extended space flights. Viktoria Tschachhojan, S. Ulrike, M. Wolf gang, Institute of Physiology and Pathophysiology, University Medical Center of the Johannes Gutenberg University Mainz, Germany

Humans in exomagnetspheric space are exposed to highly energetic heavy ion radiation which can be hardly shielded. Radiation-induced mucositis therefore constitutes not only a severe complication of heavy ion radiotherapy, but also a serious medical safety risk for astronauts during extended space flights, such as missions to Moon or Mars which are planned by National Aeronautics and Space Administration (NASA) and European Space Agency (ESA). Due to limited possibilities of medical treatment during space flights, inflammation must likely exacerbate to a chronic mucositis and the probability of carcinogenesis rises.

We are working on 3D organotypic cultures of immortalized human gingival keratinocytes and mesenchymal fibroblasts which mimic human oral mucosa. Organotypic cultures were irradiated with a horizontal particle beam at high energies (18 - 150 MeV per nucleon) at the Gesellschaft fuer Schwerionenforschung (GSI) in Darmstadt. The major focus of this study is immediate and early effects after heavy ion irradiation. Structural analyses, DNA damages, and nuclear factor κB (NFκB) p50 and p65 were investigated by histological and immunofluorescence stainings of cryosections.

Organotypic cultures showed various layers of fibroblasts and keratinocytes. The latter could be detected via specific keratin stainings, such as keratin 4 and keratin 14. We saw a more intense signal of γH2AX immunofluorescence stainings in irradiated organotypic cultures. In addition to their structural role as the first line of defense, keratinocytes play an important role in inflammatory reactions. First results revealed an optical dose-dependent increase in staining intensities of NFκB p50 and p65 after irradiation with C2 ions.

We established a suitable 3D system for investigations of heavy ion-induced damages and immune responses to oral mucosa. Exposure to heavy ions with high energies leads to an early inflammatory response. As chronic inflammation may be a risk of tumorigenesis, further research on the link between radiation-induced inflammation through heavy ions and induction if tumorigenesis is warranted.

This project is realized in cooperation with GSI and ESA; it is supported by the German Aerospace Center (DLR) and the Federal Ministry of Economics and Technology (BMWi), #50 WB 0926.

**POS22-28.** Similarities and differences in the distribution of radiation-induced chromosome breaks between low and high-LET. Honglu Wu1, M. Hada2, Y. Zhang3, A. H. Feveson1, F. A. Cucinotta1, NASA Johnson Space Center, Houston, Texas and 2Universities Space Research Association, Houston, Texas, and 3Wyle Laboratories, Houston, Texas, USA

The distribution of breakpoints in chromosomes has been an interest in cancer research due to the large number of malignant diseases that are associated with chromosome aberrations containing breaks in specific regions. To study the effects of low- and high-LET radiation on break locations within a chromosome, we exposed human epithelial cells in vitro to low (137 g-rays) and a high dose rate, secondary neutrons at a low dose rate, and 600 MeV/u Fe ions at a high dose rate. Breakpoints were identified using multicolor banding in situ hybridization (mBAND), which paints chromosome 3 in 23 different colored bands and allows identification of both inter- and intrachromosomal aberrations. Detailed analysis of the chromosome fragment ends involved in intrachromosomal exchanges revealed a similar breakpoint distribution among all four low- and high-LET radiation scenarios with clusters of breaks found in the same regions. Analysis of the locations of the two fragment ends in chromosome 3 that join to form an intrachromosome exchange also demonstrated a similar likelihood between low- and high-LET for the rejoining of two breaks located in different regions of the chromosome. One difference of the breakpoint distribution was found in the region of the chromosome that was identified as a cold spot for low-LET. However, with high-LET radiation exposure, a significant number of breaks were observed in this region. In particular, those breaks induced only by high-LET tended to rejoin with other breaks separated by distant genomic distances. Our results revealed that most high-LET-induced chromosome breaks rejoin the same way as those induced by low-LET, but the rejoining of a small fraction of these breaks is unique for high-LET.

**POS22-29.** Biophysical assessment for treatment planning of carbon-ion therapy. Yukani Yoshida1, M. Tashiro1, A. Musha1, T. Kanai1, H. Kawamura1, M. Sakamai1, T. Takahashi1, T. Ohno1, K. Ando1, T. Nakano1, 1Graduate School of Medicine, University of Tokyo, Japan 2: Department of Radiation Oncology, Gunma University Graduate School of Medicine, Japan 3: Department of Electrical and Electronic Engineering, College of Industrial Technology, Nihon University, Japan 4: Department of Radiation Oncology, Saitama Medical Center, Saitama Medical University, Japan

A new carbon-ion therapy facility was constructed at Gunma University Heavy-Ion Medical Center (GHMC) in 2009, and a new treatment planning system (TPS) was developed. For QA/QC of the TPS, accuracy of the TPS was evaluated biologically in homogeneous and inhomogeneous system.

Carbon ions were accelerated to 350 MeV/u in GHMC. Human salivary gland tumor (HSG) cells were irradiated at three positions within the 8-cm width of spread-out Bragg peaks (SOBPs) in homogeneous system. The inhomogeneous experiments were performed by the use of phantom including equivalent materials such as lung, bone and fat, in which capsules contained HSG cells were embedded. The cell survival values of carbon ions were measured by colony formation for HSG cells, and were compared with predicted values by the plan of the TPS.

The D10 value is the dose that would reduce cell survival to 10%, and it was obtained from the alpha and beta parameters for each survival data when survival curves were drawn using the linear-quadratic model. The RBE value at the D10 dose level was 2.01 at center in the SOBPs. In homogeneous system, the values of measured survival data were lower about 10% than the predicted one. The values of the measured survival data were lower about 20% than the predicted one in inhomogeneous system. The measured physical dose using the
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Farmer chamber accorded with calculated dose by TPS in less than 5% in both systems. These results suggest that the difference of survival data between homogeneous and inhomogeneous system was about 10%. Not only the dose but also the radiation quality affects to biological responses in carbon-ion therapy. The difference of biological effectiveness in inhomogeneous system may be caused by radiation quality. Further investigation, using the inhomogeneous phantom and various cells, biophysical study will be promoted for treatment of various organs.

POS22-30. A multiparametric approach to study the effects of low doses of densely ionising radiation in human peripheral blood lymphocytes. Dumphy Zeegers 1, S. Sethu 2, P. Srikanth 3, M. Jayapal 4, G. Low 2, R. L. Gurung 1, T. Kato 1, A. Fujimori 1, O. Okaya 1, M. P. Hande 1, 1: National University of Singapore, Singapore 2: Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 3: Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore and KIT School of Biotechnology (KSBT), Singapore 4: Heavy-Ion Radiobiology Research Group, National Institute of Radiological Sciences, Chiba, Japan

Human peripheral blood from three healthy donors was exposed to carbon (290MeV/u, LET 70 keV/μm) or iron (500 MeV/u, LET 200 keV/μm) ions at 0.1, 0.25, 0.5 and 1.0 Gy. DNA damage, chromosomal aberrations and the differential gene expression profile following irradiation were studied. Total DNA damage rendered following heavy ion irradiation was studied using single cell gel electrophoresis (Comet Assay). hH2AX foci detection method was followed to assess the levels of double strand breaks due to heavy ion irradiation. There was a dose dependent increase of DNA damage following heavy ion irradiation. Gene expression profiling indicated changes in the expression of several genes after exposure. However, it was observed there was more marked expressional changes based on the post-irradiation time rather than between the dose. Our study exhibited a dose dependent increase in the production of both micronuclei and chromosome aberrations; it appears that carbon ion induces more aberrations than iron ions. Studies are being done to investigate the complex chromosome translocations by multi-colour fluorescence in situ hybridisation technique. Collectively our results indicate that irradiation with low doses of carbon and iron ion induces varied molecular and cellular changes including chromosomal aberrations.

The study is supported Defence Innovative Research Programme, Defence Science and Technology Agency, Singapore.

POS22-31. Cancer therapy with carbon ions at heavy ion research facility in Lanzhou (HIRFL), IMP, China. Hong Zhang 1,2,3, Sha Li 1, Xiaohu Wang 1, Qiang Li 1,2,3, Shihua Wei 1, Liying Gao 1, Weiping Zhao 1,2,3, Zhengguo Hu 1,2,3, Ruishu Mao 1,2,3, Hushan Xu 1,2,3, Yangyan Yue 1,2,3, Qianqing Yang 1,2,3, Jiantao Ran 1,2,3, Zhongze Tian 1,2,3, Guoqiang Xiao 1,2,3, Wenlong Zhao 1,2,3, 1: Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China 2: Key Laboratory of Heavy Ion Radiation Biology and Medicine of CAS, Lanzhou, China 3: Key Laboratory of Heavy Ion Radiation Biology and Medicine of CAS, Lanzhou, China 4: The General Hospital of Lanzhou Command, Lanzhou, China 5: Tumor Hospital of Gansu Province, Lanzhou, China

Between November 2006 to November 2010, 126 patients have been treated at the Heavy Ion Research Facility in Lanzhou (HIRFL), IMP, collaborating with hospitals. In the 126 patients, there were 103 with superficially-placed tumors (skin squamous cell carcinoma, basal cell carcinoma, malignant skin melanoma, sarcoma, lymphoma and other skin lesions) and 23 with deep-seated tumors (hepatocellular carcinoma, brain tumor, sacrum chordoma, bone and soft-tissue sarcomas, head and neck tumors). The majority of patients were with failures or recurrences of conventional therapies. There were 75 males and 51 females, and median age at the time of radiotherapy was 53.7 years (range 1–88 years). They received total doses of 40–75 Gy E with a weekly fractionation of 7 x 3-15 GyE/fraction. Target volume was defined by physical palpation, ultrasound and MRI scans. The clinical target volume was defined as the gross total volume with a 0.5-1.5 cm margin axially. RBE of 2.5-3 within the target volume was used in the trial. Patients who had follow-up examinations were performed 1 month after treatment, in 3-6 month intervals for the first 2 years and annually thereafter. Local control rates were estimated according to WHO criteria. Acute and late side effects were scored according to the Common Toxicity Criteria (CTC). The mean follow-up for patients with superficially-placed tumors was 24 months with ranging from 12-36 months. The tumors responded very well to the treatment in all patients. Up to 3-6 months, majority of tumors disappeared completely or almost. The tumors also responded well to carbon ion RT in 23 patients with deep-seated tumors. No severe side-effects > CTC grade III have been observed. The data demonstrated that demonstrated that heavy ion radiotherapy at HIRFL, IMP is clinical effective and safe, especially for patients with failures or recurrences of conventional therapies, although the follow-up was short.

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POS23-01. Radioprotective effect of isoflavone and saponin against gamma irradiation induced oxidative stress. Amit Kumar Dixit, D. Bhatnagar, A. Kumar, School of Biochemistry, Devi Ahilya University, Indore

Irradiation has deleterious effects however, radiotherapy is the most common modality in treating human cancers. Soybean components such as isoflavone and saponin having effective antioxidant activity were selected for evaluating its radioprotective effect against gamma irradiation induced oxidative stress.

Male Wistar rats were orally administered with soybean isoflavone (60 mg/Kg b wt) and saponin (100 mg/Kg b wt) for 21 days followed by gamma irradiation exposure. Survival studies at 10 Gy and endogenous spleen colony forming unit assay (CFU) at 6 Gy exposures were performed in order to find radioprotective and immunomodulatory nature of these compounds. The rat liver homogenate and erythrocytes were used to measure lipid peroxidation (LPO) and glutathione (GSH) content along with various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione-s-transferase (GST) after gamma irradiation exposure at 2.0 Gy. The hematological examination of blood and histological examination of liver were also performed. The results showed that pretreatment with soybean isoflavone and saponin, prior to gamma irradiation resulted in increased survival rate of the animals as compared to irradiated group. CFU counts in the isoflavone and saponin treated group were found significantly high as compared to control and irradiated group showing immunomodulatory nature of the soy components. Haematological and histological parameters improved after pretreatment. Pretreatments also significantly reduced the radiation induced LPO and enhanced the activity of antioxidant enzymes. The present study suggests that soybean isoflavone and saponin have potent antioxidant activity and act as probable radio protector against gamma radiation induced oxidative damage.

Key words: Radioprotection, antioxidant, isoflavone, saponin

POS23-02. Stress response of thioreredoxin system in highly radioresistant insect Spodoptera frugiperda cells. Shashank Hambarde, P. Singh, S. Chandia, Institute of Nuclear Medicine and Allied Sciences, India

Introduction: Spodoptera frugiperda (order Lepidoptera; class Insecta) and a cell line derived from it, Sf9, show remarkable radiation/stress resistance, the mechanisms of which are poorly understood. Since Lepidopteran organisms are evolutionarily closer to humans than other stress resistant organisms, it is considered as an important model for delineating the mechanisms of cellular radiation responses.

Methods: Using in silico methods and other experimental strategies, we studied thioreredoxin reductase (Sf-TrxR) and thioreredoxin peroxidase (Sf-TrxP) in Sf9 cells, and also assessed role of its structural/localization status following radiation and chemical stress.

Results: Using in silico prediction, confocal microscopy and western blotting, we first characterized the nature of Sf-TrxP and Sf-TrxR. Sf-TrxP localized in the cytosol while Sf-TrxR2 localized in mitochondria/nuclei. Sf-TrxP1 and Sf-TrxR expression was significantly upregulated on irradiation (500 Gy) or etoposide (100μM) treatment. Sf9 cells showed extensive resistance to 500Gy, which was associated with significant G2/M checkpoint activation and TPX dimerization. G2/M checkpoint activation induced by phosphate inhibitor okadaic acid and a specific CDK1 inhibitor roscovitin always coincided with dimerization of Sf-TrxR. Sf-TrxP expression was significantly upregulated on irradiation (500Gy) or etoposide (100μM). G2/M checkpoint activation induced by phosphatase inhibitor okadaic acid and a specific CDK1 inhibitor roscovitin always coincided with dimerization of Sf-TrxR. Sf-TrxP expression was significantly upregulated on irradiation (500Gy) or etoposide (100μM) and phosphorylated of thioreredoxin peroxidase (Sf-TrxP) in Sf9 cells, and also assessed role of its structural/localization status following radiation and chemical stress.

Results: Using in silico prediction, confocal microscopy and western blotting, we first characterized the nature of Sf-TrxP and Sf-TrxR. Sf-TrxP localized in the cytosol while Sf-TrxR2 localized in mitochondria/nuclei. Sf-TrxP1 and Sf-TrxR expression was significantly upregulated on irradiation (500 Gy) or etoposide (100μM) treatment. Sf9 cells showed extensive resistance to 500Gy, which was associated with significant G2/M checkpoint activation and TPX dimerization. G2/M checkpoint activation induced by phosphate inhibitor okadaic acid and a specific CDK1 inhibitor roscovitin always coincided with dimerization of Sf-TrxR. Sf-TrxP expression was significantly upregulated on irradiation (500Gy) or etoposide (100μM) and phosphorylated of thioreredoxin peroxidase (Sf-TrxP) in Sf9 cells, and also assessed role of its structural/localization status following radiation and chemical stress.
increased cell death, implying a protective role of SF-Tpx dimerization, which is being confirmed with RNA interference. Conclusion: This is the first report that delineates the potential role and nature of response of thioredoxin system in the radioresistance displayed by Lepidopteran insect cells. This study is likely to further enhance our understanding of this model insect system, which may help improve mankind’s efforts for developing better methods for radioprotection.

POS23-03. Antimutagenic and radioprotective effects of oltipraz. Ashok Kumar Aditya Kochhar1, Deepali Sharma1, Preena Johari1, Madhu Kumar2, Patrick Prendergast2, Hiroshi Kimura1: 1. Radiation and Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur, India 2; Canopus BioPharma Inc, Studio City, USA 3: Department of Molecular Genetics in Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan

Oltipraz (OLT), a synthetic diithiolethione, (derived from broccoli) possesses antitumor, radioprotective, chemotherapeutic and cancer chemopreventive properties. In the present study, Oltipraz (OLT) was explored for in vivo radioprotective activity against whole body gamma irradiation in Swiss albino mice. Various doses of OLT (viz. 50, 100, 200, 400, 500 and 800 mg/kg b.wt.), were administered orally, 30 minutes prior to 8 Gy irradiation. Radiation toxicity and mortality was observed during a period of 60 days and the percent of mortality was calculated. Pre-treatment of mice with different doses of OLT reduced the symptoms of radiation sickness, delayed the onset of mortality and increased the mean survival time (MST’s). An oral dose of OLT 100mg/kg was found to be the most effective dose in preventing radiation sickness, reducing mortality and increasing the mean survival time. Pre-treatment of mice with OLT 100mg/kg provided the best protection as demonstrated by the highest number of survivors after 30 days post-irradiation in this group, when compared to the other doses of Oltipraz. Administration of OLT alone significantly enhanced glutathione (GSH) and decreased lipid peroxidation (LPO) levels in testis of Swiss albino mice whereas acid phosphatase (ACP) and alkaline phosphatase (ALP) activity did not show any significant alteration. Irradiation resulted in significant augmentation in LPO level and ACP activity, and decreased GSH content and ALP activity. Furthermore, villus height, goblet cells, both villus and crypt sections, epithelial cells, mitotic cells and pyknotic cells/crypt section in the jejunum were evaluated at different autopsy intervals (1, 3, 7, 15 and 30 days) after exposure to 8 Gy of gamma radiation. A significant decrease was observed in the villus height, goblet cells, epithelial cells and mitotic cells/crypt section, whereas pyknotic cells increased significantly after irradiation in the control group (irradiation alone). The Oltipraz treated experimental group showed significant enhancement in villus height, epithelial cells, goblet cells and mitotic cells whereas pyknotic cells showed a significant decrease in respect of irradiated control group at each autopsy interval. The results of the present study suggest that Oltipraz has the capacity to provide protection against gamma radiation induced intestinal injury in Swiss albino mice. Oltipraz 100mg/kg pretreatment significantly ameliorated the radiation-induced increase in LPO level and ACP activity. Radiation-induced depletion in the levels of GSH content and ALP activity was also significantly inhibited by OLT pre-administration. Regression analysis of survival data yielded LD50 as 6.66 and 11 Gy for radiation control and treated mice, respectively, and produced a dose reduction factor (DRF) of 1.65. Oral administration of Oltipraz (100 mg/kg body weight /day) before exposure to gamma radiation was found to be effective in protecting against the chromosomal damage in bone marrow of Swiss albino mice. Animals exposed to 8 Gy gamma radiation showed chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges andacentric fragments. There was a significant increase in the frequency of aberrant cells at 6 hrs after irradiation. Maximum aberrant cells were observed at 12 hr post irradiation autopsy time. Further, the frequency of aberrant cells showed a decline at late post-irradiation autopsy time. However, in the animals pretreated with Oltipraz, there was a significant decrease in the frequency of aberrant cells as compared to the irradiated control. There was significant increase in the number of micronuclei in the 8 Gy irradiated mice; however, there was significant decrease in micronuclei in the animals pretreated with oltipraz. Therefore, the present study has implications for the potential use of OLT as a radioprotector.

POS23-04. Radiophilia: A tragic phenomenon in diagnostic radiology. Hamid Abdollahi, M. Neymuri, Shiraz University of Medical Sciences, Iran

Diagnostic radiology is one of the most frequently used method in medical imaging that rate of its application is increasing daily. Generally working conditions in radiology departments seem to be normal and risk conception and perception about radiation protection standards among physicians, radiation technologists, patients and public are similar so that all radiation safety considerations are respected at all levels. But there are many reasons and there is much evidence that not only the importance of risk conception and risk perception (not meant to radiophobia) in relation to radiation safety issues are high among experts and physicians, but also the others such as insignificance and incuriosity among people somehow have a direct relationship with the radiology as an abnormal behavior of individual and organizational that can be called “radiophobia”. Radiophilia is not a term to describe a phenomenon, but it is an inconvenient truth that is not justified in any way. The most important factors that are causing radiophilia include: lack of knowledge and awareness on radiation effects among physicians, lack of knowledge and awareness on radiation protection rules, especially justification among physicians, the increasing number of less experienced and careless clinicians who request radiography and their incorrect diagnosis and use of radiography each one and the percent of radiation mortality was calculated. Pre-treatment of mice with different doses of OLT reduced the symptoms of radiation sickness, delayed the onset of mortality and increased the mean survival time (MST’s). An oral dose of OLT 100mg/kg was found to be the most effective dose in preventing radiation sickness, reducing mortality and increasing the mean survival time. Pre-treatment of mice with OLT 100mg/kg provided the best protection as demonstrated by the highest number of survivors after 30 days post-irradiation in this group, when compared to the other doses of Oltipraz. Administration of OLT alone significantly enhanced glutathione (GSH) and decreased lipid peroxidation (LPO) levels in testis of Swiss albino mice whereas acid phosphatase (ACP) and alkaline phosphatase (ALP) activity did not show any significant alteration. Irradiation resulted in significant augmentation in LPO level and ACP activity, and decreased GSH content and ALP activity. Furthermore, villus height, goblet cells, both villus and crypt sections, epithelial cells, mitotic cells and pyknotic cells/crypt section in the jejunum were evaluated at different autopsy intervals (1, 3, 7, 15 and 30 days) after exposure to 8 Gy of gamma radiation. A significant decrease was observed in the villus height, goblet cells, epithelial cells and mitotic cells/crypt section, whereas pyknotic cells increased significantly after irradiation in the control group (irradiation alone). The Oltipraz treated experimental group showed significant enhancement in villus height, epithelial cells, goblet cells and mitotic cells whereas pyknotic cells showed a significant decrease in respect of irradiated control group at each autopsy interval. The results of the present study suggest that Oltipraz has the capacity to provide protection against gamma radiation induced intestinal injury in Swiss albino mice. Oltipraz 100mg/kg pretreatment significantly ameliorated the radiation-induced increase in LPO level and ACP activity. Radiation-induced depletion in the levels of GSH content and ALP activity was also significantly inhibited by OLT pre-administration. Regression analysis of survival data yielded LD50 as 6.66 and 11 Gy for radiation control and treated mice, respectively, and produced a dose reduction factor (DRF) of 1.65. Oral administration of Oltipraz (100 mg/kg body weight /day) before exposure to gamma radiation was found to be effective in protecting against the chromosomal damage in bone marrow of Swiss albino mice. Animals exposed to 8 Gy gamma radiation showed chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges andacentric fragments. There was a significant increase in the frequency of aberrant cells at 6 hrs after irradiation. Maximum aberrant cells were observed at 12 hr post irradiation autopsy time. Further, the frequency of aberrant cells showed a decline at late post-irradiation autopsy time. However, in the animals pretreated with Oltipraz, there was a significant decrease in the frequency of aberrant cells as compared to the irradiated control. There was significant increase in the number of micronuclei in the 8 Gy irradiated mice; however, there was significant decrease in micronuclei in the animals pretreated with oltipraz. Therefore, the present study has implications for the potential use of OLT as a radioprotector.
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provided the best protection in both simulation and experiments. In the next stage, attempts were made to produce appropriate Tungsten-tin-filled polymers which could be used for production of shielding garments. The density of this tungsten-tin filled polymer was 4.5 g cm\(^{-3}\). The MCNP simulation and experimental measurements for HVL values of this shield at 100 kVp were 0.346 and 0.296 mm, respectively. On the other hand, this novel shield provides considerable mechanical properties and is highly resistant to chemicals.

Conclusions: The cost-effective lead-free flexible radiation shield produced in this study offers effective radiation protection in diagnostic energy range. This environmentally-friendly shield may replace the traditional lead-based shielding garments.

**POS23-06.** Manganese superoxide dismutase-plasmid liposomes (MnSOD-PL) and mitochondrial targeted gs-nitroxide, JP4-039, decrease irradiation induced lipid peroxidation in murine fetal brain glial and neuronal cells. Mark Bernard, 1 M. E. Bernard, 2 H. Kim, 2, J. Kwagama, 2 M. W. Eppley, 1 E. E. Kelley, 1 P. Wipf, 2, H. Wang, 2 J. S. Greenberger, 1 1: University of Pittsburgh Cancer Institute, USA 2: Department of Radiation Oncology, University of Pittsburgh Cancer Institute, USA 3: Department of Anesthesiology and Vascular Medicine Institute, University of Pittsburgh, USA 4: Department of Chemistry, University of Pittsburgh, USA

Background: Ionizing radiation induces oxidative stress and lipid peroxidation. Reduction of lipid hydroperoxides may prevent irradiation cell damage. MnSOD-PL and the GS-nitroxide JP4-034 have shown to be effective radioprotectors in murine organ specific and total body irradiation models. We measured the effective MnSOD-PL and mitochondrial targeted JP4-039 for radioprotection in neuronal-glial fetal brain cultures.

Methods: Primary cortical glial and neuronal cells were prepared from embryonic day 16 C57BL/6Nbsd mouse embryos and grown in 6 well plates at 500,000 cells/well. Cells were incubated at 37°C and 5% CO\(_2\), and grown in media supplemented with 10% FBS for 8 - 10 days. Cells were then switched to FBS free media during days 9 - 18 to limit glial cell proliferation. Mature mixed cortical culture cells were treated with either 1 μg/ml of MnSOD-PL or 1 μg/ml of JP4-039 at 24 hours or 30 minutes, respectively, prior to 5 Gy or 10 Gy irradiation using a 6 MV gamma irradiator. Control groups received 0 Gy, 5 Gy or 10 Gy irradiation with no antioxidant treatment. Following irradiation cells were assayed for lipid peroxidation by EPR with 4-POBN. Data were analyzed using one way analysis of variance followed by Tukey’s range test for multiple pair-wise comparisons, where significance was p < 0.05.

Results: In control irradiated cell cultures, lipid peroxidation increased with irradiation dose (EPR OG): 100 ± 11, 152 ± 16, 106Gy: 484 ± 62). Cell cultures treated with MnSOD-PL but not JP4-039 prior to 5 Gy irradiation showed reduced lipid peroxidation. In contrast, both antioxidant treatments (MnSOD-PL or JP4-039) significantly reduced lipid peroxidation following 10 Gy irradiation. (MnSOD-PL: 321 ± 33, JP4-039: 403 ± 21 vs. control irradiation: 484 ± 62, p < 0.05). Discussion: MnSOD-PL and JP4-039 were effective mitigators when added after irradiation to fetal brain cultures. Treatment with MnSOD-PL prior to 10 Gy irradiation significantly reduced lipid peroxidation, while pre-irradiation treatment with JP4-039 had a less significant effect. The lipid bilayer of liposomes may have facilitated targeting of MnSOD-PL into neuronal and glial cells, compared to the JP4-039 molecule. Further studies are ongoing to optimize delivery of JP4-039 into neural tissue. Acknowledgments: Supported by the T32AG21885 of NIH.

**POS23-07.** Specific formulation of cerium oxide nanoparticles ameliorate radiation-induced gastrointestinal syndrome and improves survival in mice. Payel Bhanja, 1 S. Saha, 1 L. Liu, 2 S. Das, 2 S. Seal, 2 C. Guha, 1 1: Albert Einstein College of Medicine, USA 2: UCF, USA

Purpose: Acute and chronic production of reactive oxygen and nitrogen species promotes radiation-induced normal tissue toxicity. Several free-radical scavengers have been tested for their ability to protect normal tissues. However, toxicology, pharmacokinetics and restricted tissue distribution limit the application of these agents. In this report we examined the role of surface modified CeO\(_2\) nanoparticles, a stable free radical scavenger, to minimize radiation lethality in mice exposed to whole body irradiation (WBI) of 10.4 Gy in single or split fractions.

Experimental Procedure: C57Bl/6 male mice were treated with 2 different CeO\(_2\) nanoparticles, WB and ME, at a concentration of 15mM/mice, either 1hr before WBI, or 1hr post-WBI (10.4Gy) to evaluate radioprotective and mitigating roles, respectively. Control animals received WBI and supportive care. Animal survival was analyzed by Kaplan-Meier survival curve over the period of 30 days. TUNEL, H&E and K67 staining were performed from paraffin-embedded sections of mid-jejunum region of small intestine. Super oxide dismutase (SOD) expression in intestinal crypt cells was analyzed by immunohistoblot analysis.

Results: WBI of 10.4 Gy resulted in 100% lethality within 12 days in untreated control animals, possibly from a combination of radiation-induced intestinal and bone marrow syndrome. Prophylactic treatment with CeO\(_2\) rescued 100% of the mice from radiation lethality. Post-radiation treatment with CeO\(_2\)-WB delayed the mortality of animals to 22 days (median survival 9+1.2 days for controls versus 18+1.8 days in WBI+CeO2-WB-treated animals, p<0.03). Immunohistological analysis demonstrated larger crypt depth, increased villi length and reduced apoptotic crypt cells (p<0.003) in CeO2-treated animals, 3.5 days post-WBI. CeO2 treatment also induced the SOD2 expression in intestinal crypt cells.

Conclusion: Systemic administration of CeO\(_2\) nanoparticles improves survival in mice exposed to lethal WBI. CeO\(_2\) nanoparticles may find a role both as a radioprotector and mitigator of radiation-induced gastrointestinal syndrome.

**POS23-08.** The European Radiobiological Archives ONLINE. Mandy Birschwilk\(\text{1}\), P. Schofield\(\text{1}\), B. Grosche\(\text{1}\), 1: Federal Office for Radiation Protection, Germany 2: University of Cambridge, UK

Today’s research is providing us more and more with the opportunity to quantify radiation risks at the individual level. New approaches allow the re-analysis of old data using new techniques. Thus, the retrospective analysis of earlier studies is an important resource for modelling and evaluating risk parameters. The European Radiobiology Archives (ERA), together with corresponding Japanese and American databases, hold data from nearly all experimental animal radiation studies carried out between the 1950s and the 1990s, involving more than 460,000 animals. Those large scale radiobiological studies are for financial and ethical reasons, to a large extent, unrepeatable experiments. They were performed on different species with the aim of understanding the effects of irradiation. The huge amount of information has led to the requirement for a computerised database for primary data, that allow re-analysis, re-interpretation and re-evaluation of results from, for example, carcinogenicity studies, in the light of new knowledge in radiation biology.

The concept of and the work on such a database was started by G. Gerber and has now been transferred to a web-based database to maximise its usefulness to the scientific community and achieve compliance of data coding and structure with currently accepted semantic standards for anatomy and pathology. The accuracy of the primary data input was assessed and improved. The original rodent pathology nomenclature was recoded to Mouse Pathology (MPATH) and Mouse Anatomy (MA) ontology terms. A pathology panel sampled histopathological slide material and compared the original diagnoses with currently accepted diagnostic criteria. The majority of the original pathology terms have been translated into a combination of MPATH and MA ontology terms.

ERA is now in a position where it has the potential to become a worldwide radiobiological research tool for numerous applications, such as the re-analysis of existing data. ERA can be accessed at no cost at https://era.bfs.de. Only a password is required which can be obtained from the curators.

A more detailed description is available under: M Birschwilk et al. (2011) The European Radiobiological Archives: Online Access to Data from Radiobiological Experiments. Radiation Research: April 2011, Vol. 175, No. 4, pp. 526-531.

**POS23-09.** STORE – Sustaining access to Tissues and data froOM Radiobiological Experiments, Mandy Birschwilk, Federal Office for Radiation Protection, Germany

The sharing of data and biomaterials from publicly funded experimental radiation science will yield substantial scientific rewards through re-analysis and new investigations. To that end, the STORE Consortium will create a platform for the storage and dissemination of both data and biological materials from past, present and future radiobiological research. The project will be completed by an assessment of viable financial models for long term support of a
bioresource and Data Warehouse for radiobiology. The strategy to achieve these goals is multi-level: 1) to provide a “one-stop-shop” portal integrating international databases and other repositories currently active, such that the user can find material and data held remotely; 2) to archive primary (raw) data or pointers to data in public databases, from radiobiological experiments and studies; 3) to point to physical archives of material resources which are considered to be a valuable resource to the Community; 4) to provide the necessary SOPs for the evaluation and preserved tissue usability. Thus, STORE will provide a single online portal to radiobiological information that is presently distributed over scientific centres worldwide. The final website will help to support and encourage enabling and rewarding data sharing for radiobiological and epidemiological research. It will establish the basis for long-term use and for exchange between scientists from different countries and from various fields. It will as well help to minimize animal experiments as far as possible by making information available on those experiments that have already been conducted. This resource will be open to individual investigators and to funding agencies as a potential central repository for data sharing.

POSTER PRESENTATIONS


Purpose of the study: A library of 25 full-body adult male voxel models covering the following extent:

- 162 – 200 cm in height,
- 51 – 147 kg in weight,
- 16.5 – 45 kg/m² in body mass index.

It has been achieved. Monte Carlo computations in radiation protection, radiotherapy, nuclear medicine use numeric 3D models of the human body in the voxel format. In the male adult case, even if several voxel models of the full human body have been constructed, their body type is relatively limited if one simply considers the extent of height and weight in the human population. A set of voxel models representing a large extent of these two parameters could be helpful for the study of the accuracy, relevance, morphological dependence of calculated dosimetric quantities wherever anthropomorphic models are used.

Materials and methods: Representative body shapes have been selected from the European edition of the CAESAR database. Internal organs and skeleton have been adapted to these body shapes using commercially available 3D models. The 3D modeling work was performed with the Rhinoceros software using mesh and NURBS formats. The construction process requires the definition of target masses for the skin, internal organs, skeleton and the definition of an average tissue composition for the remainder tissue. These target masses and composition have been made as simple as possible but are based on a detailed study of the literature. As compared with other work the main advantages of the construction process is the definition of prior target values for internal organs and tissues and the use of individual body shapes.

Results: Depending on the model, the full body is defined by 6.2-20 million of voxels, which requires 46-102 Mb for storing the full body and an air-filled bounding box. The volume resolution is 6-12 mm³/voxel. Currently 109 organs are included in each model. The agreement between target and obtained masses is most of the time better than 5%. These models and Monte Carlo calculations enable to quantify the variation of relevant quantities used in radiation protection with body type. Such calculations have been undertaken for specific absorbed fractions, fluence to dose conversion factors and numerical calibration of in vivo counting systems.

POSTER 23-11. A critical role for TLR4 in basal radio-resistance. Jianming Cai1, Cong Liu1, R.E.J. Mitchell1, Baolong Li1, Jing Lin1, Luqian Zhao, Fu Gao1. 1: Department of Radiation Medicine, Faculty of Naval Medicine, Second Military Medical University, Shanghai, China; 2: Radiation Protection Research and Instrumentation Branch, Atomic Energy of Canada Limited, Chalk River Laboratories, Chalk River, Canada.

Toll-like receptor 4 (TLR4) is known to play a critical role in innate and acquired immunity, but its role in basal radio-resistance has not been investigated. We examined C57BL/10ScNJ TLR4 deficient mice and showed that they were more susceptible to radiation induced mortality and morbidity than TLR4 normal mice. Mortality after radiation exposure was associated with a severe and persistent bone marrow cell loss and assays indicated that TLR4 protected against radiation induced bone marrow apoptosis. Injection of LPS into normal mice, known to activate TLR4 in vivo, induced radio-resistance. Conversely, we found that in vivo depletion of TLR4 ligands induced severe morbidity in irradiated TLR4 normal mice, which mimicked TLR4 deficient mice. Further data indicated that MYD88, but not TRIF, may be the important adaptor in TLR4 induced radio-resistance. Taken together, these data strongly suggest that TLR4 plays a critical role in basal radio-resistance.

POSTER 23-12. Effects of chelator BPCBG on removing uranium and preventing uranium-induced renal cell damage in vivo and in vitro. Hongchong Chen, Y. Bao, D. Wang, Y. Hu, A. Xu, M. Sun. Institute of Radiation Medicine, Fudan University, Shanghai, China.

The aims of this study are to assess the effects of 1,2-ethanedithiolbis[N-(2-(3-hydroxyphenylmethyl)-glycyl)glycine (BPCBG) on removing uranium (U) from rats and HK-2 renal proximal tubular epithelial cells and protecting HK-2 cells from U(VI)-induced damage. BPCBG (120 μmol/kg) was injected intramuscularly to male SD rats immediately after a single intraperitoneal injection of 0.5 mg/kg of uranyl acetate dihydrate. Twenty-four hours later urine in rat kidneys and femurs were quantitated by inductively coupled plasma mass spectrometer (ICP-MS). In vivo, BPCBG couldn't prevent U(VI) induced damage immediately or for 24h followed by different doses of BPCBG treatment for another 24h or 48h, the uranium in HK-2 cells were measured by ICP-MS, micromolecules formation in HK-2 cells was detected by the cytokinesis- block technique, the cell survival was assayed by cell counting using trypan assay, and the production of intracellular reactive oxygen species (ROS) was assayed by 2',7'-dichlorofluorescin diacetate (DCFH-DA) oxidation. DTPA-CaNa3 was used for control medication. The results showed that 120 μmol/kg BPCBG treatment immediately after U(VI) injection significantly increased the amount of the first 24h urinary U(VI) excretion and decreased the amount of U(VI) retention in kidney and bone compared with normal saline (NS) or DTPA-CaNa3 treatment. After HK-2 cells that had been pre-treated with U(VI) for 24h were treated with the chelators for another 24h, significant more intracellular U(VI) was mobilized by 10-250μmol/L of BPCBG instead of BPSG. Furthermore, treatment of U(VI)-treated HK-2 cells with BPCBG significantly enhanced the cell survival, decreased the micromolecules formation and inhibited the production of intracellular ROS. In contrast, although DTPA-CaNa3 markedly reduced the uranium retention in kidney of rats and HK-2 cells, it was lower than that of BPCBG and couldn't prevent U(VI)-induced renal cell damage. It can be concluded that BPCBG could effectively decorporate the uranium from U(VI)-treated rats and U(VI)-treated HK-2 cells with a better efficacy than DTPA-CaNa3. Moreover, BPCBG protected HK-2 cells from U(VI)-induced damage possibly through scavenging the U(VI)-induced intracellular ROS. Acknowledgements: This study was supported by grant 30970870 from the National Nature Science Foundation of China. *Corresponding author, e-mail: hhchen@shmu.edu.cn.


As a feasibility study for safety regulation of internal exposure of non-nuclear facility workers, assessment of internal dose for nuclear medicine workers handling I-131 were performed. Considering the amount of use and the dose coefficient, I-131 is expected to give the largest exposure to radiation workers in medical fields. Total number of 506 measured data by thyroid uptake monitoring method was obtained from 76 workers and it was evaluated that about 8% of workers were certain to be exposed during the dispensing work of I-131. More than 300 urine samples from the nuclear medicine workers were measured and analyzed using NaI (TI) scintillation detector. Measured data showed that 4.7% of urine sample activities were over the MDA value and this value was increased to 8.8% when the I-131 dispensing workers were considered only. The measured I-131 activities from the urine sample were analyzed by internal exposure evaluation program to estimate the resulting committed effective dose. The mean value of estimated committed effective dose for I-131 dispensing workers was 0.854 mSv and the range of committed effective dose was from 0.039 to 6.73 mSv. Committed effective dose
of two persons from twenty six I-131 dispensing workers was over 2 mSv which is the limit of annual dose for regulatory management of internal exposure. Although some workers were exposed from I-131, the number of exposed workers was small considering the total number of radiation workers in nuclear medicine sector and the estimated committed effective doses were generally low. It is not necessary to introduce strict regulatory measures to control the internal exposure of all non-nuclear facility workers, but carefully designed control of internal exposure is needed for nuclear medicine workers involved in dispensing works of I-131.

POS23-14. Life cycle of unicell Chlamydomonas in different radon aerosols concentrations. Danuela Ciubă1, C. Garbo1, C. Cecu1, C. Cosma1, 1: Environment Science Faculty, Babes-Bolyai University, Cluj-Napoca, Romania, 2: Institut of Cell and Molecular Radiobiology, IRSN, Paris, France

The radioactive noble gas radon and its airborne progenies are considered to be the most important dose contributors to natural radiation. We used thermal water with a radon concentration of 30 Bq/l for generating the radon aerosols inside of one original system. The aerosol’s radioactivity was monitored using the nuclear alpha track detectors. The green algae from group of Chlorophyta have a great resistance to higher radiation doses. Life cycle of unicell Chlamydomonas Peteriti Gerloff was assessed for two worst scenarios: 500 Bq/m3 and 1000 Bq/m3 using different cytotoxicity end point. The decrease of surviving rate was observed, according with the irradiation doses. Our results should be used for study of occupationally exposure in thermal area.

Key words: green algae, radon aerosons, life cycle assessment

POS23-15 Geospatial analysis of 137Cs measured in soil from the “Black Rain” area of Hiroshima, Harry Cullings, H. M. Cullings, Radiation Effects Research Foundation, Japan

In 1976 and 1978 soil samples were collected at ~107 locations at distances up to 30 km from the hypocenter of the Hiroshima atomic bomb, including an area where intense rainfall occurred shortly after the bombing, to measure radioisotopes such as 137Cs for the purpose of detecting any remaining local radioactive fallout. The lack of any obvious pattern in these measurements that might correspond to local fallout from the Hiroshima bomb has been a source of scientific curiosity over the years. Much has been learned in the past 35 years about the behavior of such radioactive materials in the environment and the interferences caused by worldwide ("global") deposition of radioactive fission products from testing of nuclear weapons in the 1950s and 1960s. However, analysis of these data to detect spatial patterns remains a challenging problem. The 137Cs in soil from global fallout is large and more recent than any that could come from the Hiroshima bomb. Because there are so many variables that affect the retention and migration of the deposited radionuclides, there is the worst variation in the measurements from place to place, which has a spatial structure. Even if the average concentration of 137Cs in rainfall since 1945 were constant across the sampled areas of Hiroshima and were due only to global fallout, measured values of samples from sites that are closer together are more alike than those that are further apart, due to variables with spatial structure that are not available to model. To be valid a statistical analysis must consider the resulting “spatial autocorrelation” in the data. Although an exploratory analysis, including modeling of the effect of local variations in rainfall on deposition of global fallout, does not suggest a pattern consistent with local radioactive fallout from the Hiroshima bomb, an important question that remains is how large such a deposition would have to have been to be evident given the noise of accumulated global fallout.

POS23-16. ON1210.Na induces SLUG pathway to mitigate radiation-induced hematopoietic toxicity in mice, Kamal Datta1, S. Suman1, R. Kumar2, M. Maniar2, A. J. Fornace Jr.1, 1: Georgetown University, USA 2: Onconova Therapeutics, USA

Methods: Two doses of ON1210.Na (500 mg/kg) were administered by subcutaneous route to 6-8 weeks old male C3H/Hen mice (n=14) at 24h and 36h after 7.5 Gy of radiation from a 137-Cs source. Vehicle group received equal volume of the vehicle solution. Survival was monitored for 30 days. Hematopoetic system was studied in these mice (n=5) after a sub-lethal dose of 5 Gy while keeping drug dosing the same. Complete peripheral blood count was performed along with bone marrow granulocyte-macrophage colony forming unit (GM-CFU), TUNEL assay, western blot, and Q-PCR analysis.

Results: Significant (p<0.003 compared to vehicle) survival advantage (80% with drug and 20% with vehicle) was observed after 7.5 Gy radiation. Peripheral white blood cell and platelet counts were higher in ON1210.Na than vehicle treated mice. Increased GM-CFU along with reduced TUNEL positive cells was observed in drug treated mice. At the molecular level we observed downregulation of p53, Bax, p21, and PUMA and upregulation of SLUG, Mn-SOD, Bcl2, and NFkB in bone marrow cells.

Conclusions: In conclusion, ON1210.Na administered at 24h and 36h post-irradiation could significantly mitigate the radiation toxicity leading to enhanced survival. Qualitative hematopoetic data evidently supports our survival results. Molecular pathway analysis indicates involvement of SLUG, a known hematopoetic stem cell survival factor, in ON1210.Na mediated radiation mitigation. Furthermore, ON1210.Na caused diminution of apoptotic and boosting of survival signals to reinforce mitigation effects.

POS23-17. Measurements and Monte Carlo computations of americium counting efficiency in the case of a realistic leg phantom. Didier Frandc1, D. Broggio1, K. Capel1, E. Cardenas-Mendez1, N. El-Faramawy1, A.C. James1, Didier Franck1, E. Reff1, 1: Environment Science Faculty, Babes-Bolyai University, Cluj-Napoca, Romania, 2: Human Monitoring Laboratory, Radiation Protection and Heath Assessment Division, Radiation Protection Bureau, Ottawa, Canada 3: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Protection, Neuhberg, Germany 4: U.S. Transuranium and Uranium Registries, College of Pharmacy, Washington State University, Richland, USA 5: CITEMAT, Centro de Investigaciones Energeticas Medioambientales y Tecnologicas, Madrid, Spain 6: Pacific Northwest National Laboratory, Richland, USA

Some actinides like Americium are bone-seekers, according to their biokinetics they are retained in the skeleton after an intake. Because of the radiosensitivity of some bone cells, the assessment of the bone activity is thus important. In vivo counting of bone seekers is usually performed at the skull or at the knee. The USTUR (U.S. Transuranium and Uranium Registries) disposes of a unique physical phantom containing the half of the skeleton of a donor significantly contoured left leg phantom. A bed of muscle equivalent plastic, has an activity of 1026 Bq. This phantom was measured in three European in vivo facilities and one Canadian laboratory; Monte Carlo (MC) simulations were also performed to assess the reliability of numerical calibration based on voxel phantoms.

Experimental considerations: Using different Germanium detectors the same efficiency pattern was found by participants, when counting positions were comparable. A sharp increase of counting efficiency is found at the patella level, about 10 times higher than at other locations, at this level a 5 cm displacement of detectors can result in 50% change in efficiency. From these measurements it is recommended to use counting positions where the efficiency might be low but where its variation with the measurement position is limited. Computational considerations: Two voxel phantoms of the USTUR leg were built. A first one was built after the 2D CT images, the other one was built from the CT scans available at the USTUR website. The second one was built with greater details regarding the delineation of the different bone parts.

The two voxel models were used to reproduce the experiments carried out in one of the lab. Agreement, better than 5%, was found between MC and experimental data. Only the first voxel model was used to simulate the experiments in a second lab. Here, despite the trend of the efficiency pattern is reproduced by MC simulations, discrepancies larger than 50% have been found for some measurements.

In the two simulations, a precise activity distribution was taken into account. Models of the phantoms were voxelized in various cases. It is concluded than the uncertainty in detector positioning was the biggest obstacle.
source of uncertainty and that it seriously affected MC results in the second case.

**POS23-18. Radiation exposure to eye lens and extremities of staff working in interventional radiology. Joanna Domienik, The Nofer Institute of Occupational Medicine, Lodz, Poland**

Interventional radiology has been rapidly developing subspecialty of medicine. Since its beginnings in 1970 the complexity of procedures has increased and the number of procedures performed continues to grow every year. For the medical staff in interventional radiology, these facts might result in relatively high occupational radiation doses. The aim of the study was to determine the level of exposure to eye lens and extremities of physicians working in interventional radiology. As a consequence the annual doses received by particular individual were estimated and checked against the possibility of exceeding the appropriate occupational dose limits.

The study was conducted in two hospitals where interventional radiology procedures are performed. In total, the data from 74 procedures were analyzed. The measurement points were chosen as follows: one dosimeter was located on each ring finger, wrist, leg (few centimeters below the lead apron) and one next to the left eye and in the region between eyes. The doses were measured in terms of dose equivalent H(0.07) using thermoluminescence dosimeters (MCP-N).

The highest doses per procedure to the operator were registered on the left wrist (average 0.506 mSv and maximum 9.506 mSv) and the left ring finger (average 0.366 mSv and maximum 2.887 mSv) while the lowest one on the eye (average 0.120 mSv and maximum 0.759 mSv) and the leg (average 0.084 mSv and maximum 0.902 mSv). The utilized radiation exposure to kerma – area product (KAP) values ranged from 471 mGy/m² up to 358110 mGy/m². The information on the radiation protection measures and work practices (like leaving the operational room during cine mode) were collected and its influence on the doses was also analyzed.

The annual doses, estimated on the basis of average doses and the workload, does not exceed the actual annual limits. However, in the light of possible lowering the occupational limit for the lens of the eye up to 20 mSv in a year as recommended by ICRP (‘Statement on tissue reaction’, ICRP ref 4825-3093-1464) the monitoring of the eye lens might be very important.


Lung cancer mortality risk after radon exposure among 17,660 Eldorado uranium workers is analyzed with models of carcinogenesis and empirical models. The workers were first employed in 1932-1980 in the Beaverlodge and Port Radium uranium mines and the radium and uranium refinery and processing facility in Port Hope, Canada, and followed up for mortality from 1950 through 1999. A total of 618 lung cancer deaths was observed. The analysis is performed by means of the biologically based two-stage clonal expansion model (TSCE) model and empirical excess risk models. Under the assumptions of the TSCE model, there is a strong indication that the spontaneous clonal expansion rate of premalignant cells is reduced at ages above about 60 years. Analysis of radiation-related risks shows that the principal effect for lung cancer is an increase of the clonal expansion rate of premalignant cells with the exposure rate. However, this increase is not linear as the clonal expansion rate increases strongly for low exposure rates and then becomes linear with a smaller slope at higher exposure rates. A possible explanation for this observation could be a bystander effect. In addition, a BEIR VI model and a parametric excess relative risk model linear in radon decay product exposure with attained age, time since exposure and dose rate as effect modifiers are investigated. The predictions of the excess relative risk from the different models are compared for different exposure scenarios. The dependence of lung cancer risk on exposure and the effect modifiers is studied and the uncertainty due to the use of different models is investigated.


We have established that the murine form of HemaMaxTM (rHuIL-12) can mitigate combined injury in irradiated mice. HemaMaxTM is currently in advanced development (IND #104091) as a potent mitigator of acute radiation syndrome, enhances survival by stimulation of hematopoietic reconstitution following exposure to myeloablative lethal total body irradiation. Radiation exposure resulting from the detonation of a nuclear device in a populated area would be expected to be accompanied by secondary trauma (wounds, infection, burns, blunt force trauma) from heat and flying debris. Numerous researchers have demonstrated that radiation exposure can severely impair healing of radiogenic wounds. Radiation-induced impairment of wound repair would complicate treatment of casualties by lengthening the time to wound resolution, exposing affected individuals to infectious agents rendered opportunistic due to the presence of open wounds and radiation-mediated immunosuppression. In a murine model of combined injury, we exposed mice to 500 Gy of total body irradiation and after exposure, induced a 1.0 cm full-thickness cutaneous injury. Topical administration of rMuIL-12 (at doses 100-3160 ng/mL) 2-24hrs after exposure dramatically enhanced the rate of wound closure relative to vehicle-treated male and female controls. rMuIL-12 also ameliorates the differential response observed in wound healing rates between male and female mice subjected to a combined injury. Histological examination of rMuIL-12-treated full-thickness wounds from irradiated female and male mice demonstrates that these wounds often display an advanced level of remodeling relative to the vehicle-treated controls. The epidermal layer of animals with low-dose-radiation (KAP) values ranging from 471 mGy/m² up to 358110 mGy/m². The information on the radiation protection measures and work practices (like leaving the operational room during cine mode) were collected and its influence on the doses was also analyzed.

The annual doses, estimated on the basis of average doses and the workload, does not exceed the actual annual limits. However, in the light of possible lowering the occupational limit for the lens of the eye up to 20 mSv in a year as recommended by ICRP (‘Statement on tissue reaction’, ICRP ref 4825-3093-1464) the monitoring of the eye lens might be very important.

**POS23-21. Senescence accelerated mice (SAMP6) slower bone wound healing following irradiation.** Michael Epperly1, M. W. Epperly2, R. P. O’Sullivan3, S. Cao4, T. Dixon5, J. P. Goff6, J. Glowacki1, J. S. Greenberger1, 1: University of Pittsburgh Hillman Cancer Center, USA 2: Department of Radiology Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, USA 3: Department of Orthopedic Surgery, Brigham and Women’s Hospital, Harvard Medical School, Boston, USA

**Introduction:** The senescence accelerated mouse (SAMP6) ages more rapidly, develops osteoporosis at an earlier age, and is an ideal model to investigate skeletal aging. The bone defect has been attributed to reduced osteoblast differentiation capacity of SamP6 bone marrow stromal cells. We compared in vitro hematopoiesis, stromal cell biology, and osteoblastogenesis in marrow from SAMP6 and control SAMR1 mice. In vivo studies compared irradiation effects on unincorrigible tibial bone wound healing.

**Methods and Materials:** Long term bone marrow cultures (LTBMCs) were established from the SAM6 and control SAMR1 mice to measure hematopoiesis and establishment of bone marrow stromal cell lines (MSCs). Osteoblastogenesis was determined by growing the MSCs in osteoblastogenic media and extracting RNA at intervals for expression of osteoblast markers Runx 2, alkaline phosphatase (ALP), and Osteocalcin (OC). The model of bone repair entails irradiating the proximal region of the right rear leg to 20 Gy and, 24 hours later, drilling a 2 mm wound in both proximal tibias. At day 21 or 35 after drilling, subgroups of mice were sacrificed and wound healing determined by X-ray.

**Results:** LTBMCs demonstrated that initially, for the first 24 weeks, SAMP6 mice had greater hematopoiesis compared with SAMR1, measured by greater stem cell activity with more cobblestone islands and increased expression of osteoblast markers Runx 2, ALP, and OC. At day 21 or 35 after drilling, subgroups of mice were sacrificed and wound healing determined by X-ray.

**Conclusion:** LTBMCs demonstrated that initially, for the first 24 weeks, SAMP6 mice had greater hematopoiesis compared with SAMR1, measured by greater stem cell activity with more cobblestone islands and increased expression of osteoblast markers Runx 2, ALP, and OC. At day 21 or 35 after drilling, subgroups of mice were sacrificed and wound healing determined by X-ray.
At day 21 after unicortical tibial wounding, wounds were significantly larger in irradiated tibias compared to nonirradiated in both SAMP6 and SAMR1 mice (p = 0.0240 or 0.0015, respectively). By day 35 SAMP6 mice still showed significantly larger wound size in irradiated compared to nonirradiated tibias (p = 0.0003), whereas all wounds in SAMR1 mice were essentially healed.

Conclusion: Accelerated aging in the SAMP6 mice is reflected in decreased duration of hematopoesis in LTBCMs, and poorer bone wound healing following irradiation.

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POS23-22. Reference radiation for cosmic rays in RBE research. Shaoyong Feng, Texas A&M University, USA

When astronauts travel in space, they are exposed to high energy cosmic radiation. The cosmic ray spectrum contains very high energy particles, generally up to several GeV per nucleon. Currently NASA is funding research on the effects, such as acceleration, sickness, of cosmic radiation. Animal models are used to conduct the studies of radiation effects of particles in the range of several MeV/nucleon to about 1000 MeV/nucleon. In order to compare different radiations, the biological effectiveness relative to a specific radiation is usually used. For low energy heavy ions and neutrons 230 keV photons are usually used for the reference radiation, but their depth dose distribution is very different from that for cosmic rays. In this research, the advantages of using high energy electrons as the reference radiation for research on cosmic radiation were demonstrated. The conclusion is based on the evaluation of the dose distributions and microdosimetric spectra of the electrons and high energy protons as a function of depth in a tissue equivalent absorber as determined by Geant4 simulation.

POS23-23. Simulation of radiation of Special Nuclear Material. Jianhua Feng, F. Wang, S. Wang, X. Tian, Solid Dosimetric Detector and Method Laboratory, China

Special Nuclear Material (SNM) has very important significance in the stratagem for a country’s safety. Every country pays more attention to the safety of SNM. But, illicit trafficking of SNM is very serious recently. It will result in serious threat to safety of society. At the present time, the method of detecting illicit trafficking is not enough, so it is important to research into the method of detecting SNM, while study of radiation of special nuclear material is the basis. Special nuclear material (SNM) is defined by Title I of the Atomic Energy Act of 1954 as Plutonium, Uranium-233, or Uranium enriched in the isotopes Uranium-233 or Uranium-235. SNM mainly include nuclides such as Uranium-233, Uranium-235, Uranium-238, Plutonium-249, Plutonium-240, etc. Here, the categories of SNM and characteristics of gamma and neutron radiation for SNM are described.

We have simulated the radiation of SNM by means of Monte-Carlo computer program (MCNP-4C). SNM include too many isotopes and descendants, so it is difficult to calculate everyone. The mode has been simplified through study of decay chain of isotopes. Uranium-233, Uranium-235, Thorium-231, Thorium-234 and protactinium-234m are calculated in the simulation of Uranium. Plutonium-249 and Plutonium-240 are calculated in the simulation of Plutonium. It is supposed that all isotopes and descendants have reached to balance. A kind of Uranium (93%Uranium-235, 7%Uranium-238) and Plutonium (93% of Plutonium-249, 7% of Plutonium-240) have been simulated with the mode, so the distribution of fluent rate and dose rate can be obtained.

POS23-24. Unjustified exposure by radioactive consumer products. Etsuko Furuta, Ochanomizu University, Japan

Introduction: Many radioactive consumer products (RCP) that radioactive materials were added intentionally, for example cosmetics and personal jewelry, are sold in the current market of Japan and some part of Asia. The purposes of this study are to analyze their radioactive concentration and to estimate exposure doses from the normal use of them. Furthermore, a discussion on the justification of the existence of RCP was aimed for in this study.

Experimental procedures: Samples were 28 kinds of RCP sold in Japan and Korea. The gamma-ray spectrometry of the samples was performed by using a HPGe detector, a multi-channel analyzer and an analysis software of LabSOCS (CANBERRA). Estimation of external radiation exposure by the RCP was calculated by using the equations mentioned in the “NORM guideline” regulated by Japanese Government. Also radon concentration emitted from 3 samples was measured by using a scintillation Lucas cell or a pico rad. Estimation of internal radiation exposure was calculated from the radon concentration.

Results: The concentrations of 226Ra and 228Th added in RCP were extremely different among products; from 0.1 ppm to 32% of 226Ra and from 0.8 ppm to 1.7% of 228Th. Both of the concentration and the amount of radioactive materials were less than Japanese regulation limits, so all of them were defined as “RCP”. All the external exposures were low enough to be not worried about health risks from the RCP because their exposure doses were under 1 mSv y⁻¹ at their normal use. However, the concentrations of Rn emitted from the 3 kinds of RCP were not low. When the pillow of RCP used every day in a small room (25 m³), the internal exposure was estimated almost 6 mSv per year. Furthermore, it is considered that there are risks of internal exposure when use the toiletries and cosmetics of RCP. Conclusions; In some cases, the internal exposure might be high by using the RCP sold in current markets, and RCP, one can buy the RCP through the Internet. Justification is the most important thought for RCP. So it is considered that a regulation unified at world level is necessary for commercially-available RCP from all over the world.

POS23-25. Mitigation of lung fibrosis in rats by enalapril started 35 days after irradiation. Feng Gao, J. Narayanan, B. Fish, J. Moulder, E. Jacobs, M. Medhora, Medical College of Wisconsin, USA

Introduction: There are two phases of lung injury after radiation, acute pneumonitis after 42-70 days and irreversible fibrosis between 2-70 days to months later. The lung is therefore a target of injury after a radiation accident or terrorism event. We have demonstrated that ACE inhibitors including enalapril at 40 but not 20 mg/L in drinking water (40 mg/L=18-28 mg/mL/day) reduces collagen synthesis (a marker of fibrosis) in rat lungs at 7 months after irradiation. The drugs were started 7 days after radiation.

Aim: Our goal is to find the latest start time and optimal dose of enalapril to mitigate radiation fibrosis in a terrorism-relevant model.

Experimental procedures: Wistar female rats (WAG/RijCinc, 8-10 weeks of age) were treated with a single dose of 13 Gy of X-irradiation to the thorax, at a dose rate of 1.43 Gy/min. Animal care and dosimetry was done by Core facilities at MCW. One group of age-matched unirradiated animals was kept under identical conditions to serve as controls. Radiated rats were randomized into groups that were untreated or treated with enalapril at 30 mg/L (low dose) or 60 mg/L (high dose) starting at 35 days (just before pneumonitis), 70 days (after pneumonitis), 105 days or 140 days (after pneumonitis but before fibrosis). Breathing rates were measured throughout the experiment while newly synthesized lung collagen content was measured at 7 months after irradiation using the Sircol assay.

Summary of results: Enalapril (low and high dose) started at 35 days improved survival through pneumonitis. Enalapril 60 mg/L but not 30 mg/L started at 35 days mitigated the increase in collagen synthesis in the lung. Enalapril started later than 35 days did not improve lung collagen content at 7 months after radiation.

Conclusions: We show that enalapril, even if started up to 35 days after radiation mitigates morbidity and improves survival through pneumonitis. At the high dose it also mitigates radiation fibrosis. This large window of time is to our knowledge the longest interval for intervention with mitigators of lung injury in a model relevant to a mass casualty event.

Funding: This work was funded by NIH (USA) NIAID R01 CA 81294, 81294-01S1 and U19-IA-67734.
male C3H/HeN mice. Ex-RAD (500 mg/kg) improved survival by 60% compared to vehicle in mice irradiated to 8 Gy when administered SC 24 h and 15 min prior to irradiation. When mice were irradiated (6 Gy, 0.6 Gy/min) and treated with Ex-RAD (500 mg/kg) SC 4 and 24 h after total body irradiation (TBI), 50% improved survival was observed. Ex-RAD-treated irradiated animals maintained higher numbers of CFUs in bone marrow harvested from sublethally irradiated mice (6 Gy), indicating increased self-renewing capacity of hematopoietic stem cells (HSCs). Histopathology of sternal bone marrow indicated more regenerative micromegakaryocytes for myeloid, erythroid, and megakaryocytes and higher overall cellularity in Ex-RAD-treated mice compared to vehicle controls at days 7 and 14 after TBI (6 Gy). Our results demonstrate that the solution formulation of Ex-RAD shows efficacy given SC either before or after radiation. Ex-RAD treatment also protected hematopoietic tissue by preserving HSCs and hematopoietic progenitor cells (HPCs). This work was supported by congressional grant #1H0001-09-C-0007.

POS23-27. Protection against gamma radiation by Alstonia scholaris (a medicinal plant) bark extract. Pradeep Goyal, S. Jahan, A. Agrawal, Radiation & Cancer Biology laboratory, Department of Zoology, University of Rajasthan, India

The development of radio protective agents has been the subject to intense research in view of their potential for use within a radiation environment, however, no ideal, safe radio-protectors are available till date, so the search for alternative sources, including plants, has been ongoing. Alstonia scholaris (Saptaparna) tree has been used traditionally for curing various disorders among the people for time immemorial. Its common use, wide acceptability, diverse pharmacological and anti oxidative properties aroused our interest to obtain insight into the radiomodulatory effect of such plant extract against gamma exposure.

Oral administration of aqueous extract of Alstonia scholaris bark (100 mg/kg b. wt./day) to Swiss albino mice for 5 consecutive days, half an hour before whole body exposure to lethal gamma radiation, enhanced the 30 days survival and also inhibited the radiation induced sickness and life shortening. Dose reduction factor (DRF) against gamma radiation for Alstonia bark extract was calculated as 1.80 from LD 50/50 values. Alstonia extract also ameliorated anemia, leucopenia, thrombocytopenia, multi potential stem cell death, chromosomal aberrations, micronuclei formation, intestinal and hepatic lesions induced by gamma radiation at different autopsy interval between 12 hrs. to 30 days, and significantly increased the number of femoral spleen colony forming units (CFU-S) that survived after irradiation. Furthermore, pretreatment with Alstonia extract checked depletion of glutathione (GSH) and anti-oxidant enzymes (SOD, CAT, GST) as well as elevation of lipid peroxidation (LPO) level. The significant reduction in the yield of LPO demonstrates that LPO shows efficacy given SC either before or after radiation. Other PI3 kinase inhibitors are now being evaluated.

POS23-28. PI3 kinase inhibitor LY294002 is both a radioprotector and radiation mitigator. Joel Greenberger, J. Lazo, E. R. Sharlow, D. Shields, T. Dixon, M. W. Eppler,1: Department of Radiation Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, USA

We optimized and employed a small interfering RNA (siRNA) assay with NCCIT human plungerpotent embryonal carcinoma cells to identify potential drug targets for radiomitigation. A primary screen of 5,520 genes revealed 113 gene products with potential radiomodulatory properties. Using an apoptosis secondary assay and computational pathways analysis methodology, we identified phosphoinositide-3-kinase (PI3K) as an attractive previously unrecognized molecular target for which there were potent small molecular inhibitors in clinical trials and a radiomodulatory disease in NCCIT and other tumor cell lines.

Methods. NCCIT cells were harvested, counted, and plated in triplicate in 12-well plates. The cells were plated in medium containing medium, incubated for 7 days in a CO2 incubator, and colonies of 50 cells counted. Data was analyzed using linear quadratic and single-hit, multi-target models. Cells incubated in 10 µM LY294002 for one hour before irradiation had a significantly increased survival with an increased shoulder on the clonogenic survival curve (n of 3.9 + 0.4) compared to 2.3 + 0.3 for control (p = 0.03). Cells incubated in 0.1 µM LY294002 after irradiation demonstrated radiomodulation with an increased shoulder on the survival curve of 6.0 + 0.8 compared to 3.0 + 0.5 for control cells (p < 0.015). To determine if LY294002 was a radiomitigator in vivo, C57/BL6/NeTac female mice were irradiated to the LD 50/30 dose of 9.25 Gy, then injected with 30 mg/kg LY294002 in cremophor el/ethanol, and followed for survival. Mice treated with LY294002 had an increased survival compared to control irradiated mice (p = 0.0005). Thus, LY294002 both protects and mitigates radiation damage. Other PI3 kinase inhibitors are now being evaluated.

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POS23-29. Radioprotection effect and anti-tumor immunity by EF12, Fuscoporia obliquae and oxyskaine. Yeuwnha Gu, K. Bamen,1: Suzuka University of Medical Science, Japan 2: Research Center, BioStage Co., Ltd., USA

Although it has been reported that EF12, Fuscoporia obliquae (FO) and Oxyskaine shows immunoenhancement activity and antioxidant activity, protective effects against radiation have not yet been investigated. In addition, although a few studies about anti-tumor effects in vitro have been reported, it is unknown whether or not these effects are realized in vivo. In this study, we therefore investigated the protective effects against radiation and in vivo anti-tumor effects of EF12, FO and Oxyskaine. Blood cells are one of the indices for evaluating protective effects against radiation. Hemopoietic tissues and peripheral lymphocytes are highly sensitive to radiation, and a decrease in immunity caused by a decrease in white blood cells and myelocytes is remarkable after exposure to radiation. Therefore, in this study, we focused on the antioxidant activity and immunoenhancement activity of EF12, FO and Oxyskaine and examined the in vivo effect of radiation on the number of peripheral blood cells. Furthermore, measurement of T lymphocyte subsets, SOD-like activity, antioxidant activity, radical scavenging activity based on chemiluminescent methods, and radical scavenging activity, which is a mechanism of protection from radiation was examined. In addition, we examined antioxidant activity and immunoenhancement activity, both of which are closely related to tumor suppression, and further investigated effects on suppressive effects on tumor growth and anti-tumor effects of tumor necrosis factor, TNF. The current major therapies for tumors are surgery, radiation, and chemotherapy. Immunotherapy is hoped to become a fourth therapeutic modality in the future. All of the three main therapies impose a burden on the body, and weaken immunity. However, the combination of immunotherapy, using natural materials such as EF12, FO and Oxyskaine with immunoenhancement action may increase the percentage of patients who recover.

POS23-30. AEOl 10150, a potent catalytic antioxidant, does not protect non-small cell lung cancer xenografts against radiation-induced cytotoxicity. Caroline Hadley, X. Zhang, Z. Vujaskovic, Duke University, USA

Introduction. We have previously shown that AEOl 10150, a potent catalytic antioxidant, can suppress apoptosis in irradiated lung through modulation of the PI-3 kinase/PTEN/Akt pathway. The purpose of this study was to determine whether AEOl 10150 influences the cytotoxic effect of radiation on non-small cell lung cancer xenografts. Methods. NSCLC (A549) xenograft tumors were implanted in athymic nude mice. Mice were randomized into four groups to receive no treatment, AEOl 10150 (loading dose of 40 mg/kg, followed by a maintenance dose of 20 mg/kg administered every other day), fractionated radiation (3 x 7Gy), or both radiation and AEOl 10150. Tumor growth delay was measured for 41 days following initiation of treatment. Western blot analysis was performed for signaling components of the PI3K/Akt pathway.

Results. Animals receiving AEOl 10150 and radiation showed a two-fold decrease in tumor volumes relative to control animals, while no decrease in tumor volume was observed in animals receiving drug alone. Tumors from the combined treatment group showed low PI-3 kinase activity, resulting in reduced activation of Akt, and alteration of...
downstream pathways. In particular, caspase-mediated apoptosis was significantly increased in the combined treatment group when compared to controls or either of the other treated groups, suggesting that AEOL10150 sensitizes tumors to the cytotoxic effects of radiation.

Conclusion. Administration of AEOL10150 protects normal tissue from undergoing apoptosis by reducing PTEN expression following RT. Here, we observe that, while the same effect on PTEN expression occurs in tumors, activity of Akt is reduced, potentiating apoptosis. The opposing actions of AEOL10150 could improve the therapeutic ratio by protecting healthy lung tissue from radiation-induced injury, permitting the use of higher doses of therapeutic radiation and improved tumor control.

POS23-31. Ascorbic acid suppresses hair depigmentation caused by irradiation. Tadashi Hongyo1, H. Nakajima2, T. Todo3, T. Nomura4. 1: Osaka University, Japan 2: National Institute of Biomedical Innovation, Japan

Irradiation in radiation therapy or bone marrow transplantation is known to cause damage to normal tissues. Although some substances are reported to suppress the damage (radioprotectors), most of them have strong side effects and are not applicable in vivo. However, ascorbic acid is known as a safe radioprotector with antioxidant activity. While examining some radioprotectors using mice, we found that ascorbic acid suppresses hair depigmentation caused by irradiation.

Materials and Methods: C57BL/6j mice, 4–6 weeks old were exposed to X-ray radiation in the following conditions; (i) 20 Gy in 10 equal fractions (2 Gy per day), (ii) 40 Gy in 10 equal fractions (4 Gy per day), (iii) 20 Gy (single dose) (iv) (1–3), exposure area was limited to the thorax and upper abdomen.) (4) (4Gy) (single dose, total body irradiation).

Either ascorbic acid or sterile saline was administrated to each mouse by subcutaneous injection prior to irradiation. We plucked more than 100 hairs from the irradiated area of each mouse, and evaluated the degree of hair depigmentation by counting the number of hairs which were macroscopically depigmented.

Results: In groups (1), (2) and (4), no epilation was seen, and hair depigmentation was observed 5–7 weeks after irradiation. The degree of hair depigmentation was significantly higher in mice administrated with saline compared to those with ascorbic acid. In group (3), epilation was observed 3 weeks after irradiation, and gray hairs started to grow in the same area 2–3 weeks later. And as in groups (1), (2) and (4), the degree of hair depigmentation was significantly higher in mice administrated with saline compared to those with ascorbic acid.

Conclusions: Hair depigmentation after irradiation was suppressed by ascorbic acid. We plan to further examine the impairment of the organ tissues (lung, heart, kidney and liver) in each mouse. We might be able to evaluate tissue impairment after irradiation by examining the degree of hair depigmentation.

POS23-32. Antioxidant system-enhancing effects of Jeju water. Jin Won Hyun1, A. Daseul Kim2, Y. Jo3, N. Ho Lee4, H. Jin You1. 1: Jeju National University, South Korea 2: Chosun University, South Korea

Recently, we reported that Jeju water containing vanadium (S3, 26.0 ± 2.0 μg/l) exhibits an antioxidant effect via the scavenging of reactive oxygen species (ROS) and by enhancing of antioxidant enzyme activities in vitro. And also, we reported the antioxidant effect of S3 with regard to antioxidant enzymes in vivo. In the present study, we compared antioxidant system of S3 to tap water against low-γ-ray irradiated mice. C57BL/6j mice were γ-ray irradiated at 2 Gy, and given tap water or S3 for 90 days, and then the liver tissues were analyzed. Compared to tap water, S3 enhanced the activities of superoxide dismutase, catalase, glutathione peroxidase, and heme oxygenase-1. S3 also enhanced the level of reduced glutathione. These results suggest that vanadium-containing Jeju water exhibits antioxidant effects via enhancing antioxidant systems against low-γ-ray irradiated mice.


The efficacy of cancer radiotherapy is limited by radiation-induced injury to neighbouring normal tissues. Many of the normal tissues at risk are accessible to topical medication. Thus, our laboratory is developing new DNA binding radioprotectors for topical application to tissues such as oral and rectal mucosa. The lead drug is methlyproamine (MP) (Martin et al, Cancer Res, 2004) and investigation of the extent of radioprotection by different drug concentrations in vitro has established a concordance between the clonogenic survival and γH2AX endpoints (Lobachevsky et al, IJRB, 2011), adding further support to the hypothesis that the mechanism of radioprotection is mediated by suppression of initial radiation-induced DNA damage. More than 100 new MP analogues have been synthesised and their radioprotective activity evaluated using clonogenic survival assay in a continuing lead optimisation programme. In vitro studies with subsets of analogues with a range of radioprotective activities and at various concentrations, have demonstrated a correlation between the extent of radioprotection measured by the clonogenic survival and γH2AX endpoints (R2 = 0.59, p=0.005). Interestingly, the classic radioprotector WR1065 also fits the correlation. The extent of radioprotection was derived from the radiation-dose response for different drug/concentration conditions.

An excellent radiobiological model of oral mucositis is available. It involves irradiation of a small area of the mouse tongue, and use of radiation-induced ulceration as the clinically relevant endpoint, but it is too elaborate to consider for screening a collection of radioprotecting drugs. In contrast, the rapidity of the γH2AX response (1 hr after IR) and a possible correlation with a radiobiological model involving total body irradiation (TBI). Our investigations in mice have established an IR dose-response for induction of γH2AX foci in basal cells of oral mucosa after TBI. Using exposures of 0–5 Gy, we obtained a yield of 4.3 ±/−0.8 γH2AX foci / cell / Gy. These studies have been facilitated by use of new software for automated counting of γH2AX foci (Ivashkevich et al, Mut Res, 2011) and will serve as a basis for evaluation of radioprotection by new analogues.

POS23-34. Mitigation of combined injury to the lung and skin by captopril. Elizabeth Jacobs, F. Gao, A. Schock, B. Fish, J. Narayanan, J. Moulder, Z. Lazarova, M. Medhora, Medical College of Wisconsin, USA

After a radiological attack, victims will suffer partial or whole body exposures to radiation, with multiple organs being affected at the same time. The lung is a sensitive target, suffering two waves of injury, acute interstitial pneumonitis at 6–10 weeks followed by fibrosis, usually from 4–6 months after irradiation. The skin is a critical barrier, which can be severely compromised by exposure to ionizing radiation. In the event of a radiological attack, skin injury will be a major cause of morbidity and survivors of gastrointestinal and hematopoietic injury will face a serious risk from combined damage to the lung and skin. We have previously demonstrated that the angiotensin converting enzyme (ACE) inhibitor captopril increases survival and mitigates acute radiation pneumopathy in rats receiving whole thoracic irradiation. Captopril also improves injury to the skin in a rat model of combined radiation and wound trauma where radiation is confined to the skin and does not penetrate to other organs. We hypothesized that irradiation to the skin will exacerbate radiation-induced lung injury in rats and the combined injuries will be mitigated by captopril. Rats (female WAG/RijCmc1r at 8–9 weeks of age) were randomized into 5 groups: (i) no irradiation (control) (ii) 12–13 Gy to the thorax to include the whole volume of both lungs (iii) 30 Gy soft X-rays to ~10% of the skin and outside the thoracic field (iv) combined irradiation to the thorax and skin at the same doses in groups (ii) and (iii) (v) continuous treatment with captopril (300 mg/ml in the drinking water) each week after combined irradiation to the thorax and skin as in group ( iv). The rats were regularly monitored for body weight, lung function by measuring the breathing rate and skin injury by scoring on a 10 point scale. Our results support a multi-organ failure model of radiation injury and suggest captopril may mitigate combined injury. This work was funded by NIH/NIAID agreements U19-AI091036 & 67734 and RCI AB1294 & 0151.

POS23-35. Mitigation of lung fibrosis in rats by enalapril started 35 days after irradiation. Elizabeth Jacobs, F. Gao, J. Narayanan, B. Fish, J. Moulder, M. Medhora, Medical College of Wisconsin, USA

Introduction: There are two phases of lung injury after radiation, acute pneumonitis after 42–70 days and irreversible fibrosis starting months to years later. The lung is therefore a target of injury after a radiation accident or terrorism event. We have demonstrated that ACE
inhibitors including enalapril at 40 but not 20 mg/L in drinking water (40 mg/L=18-28 mg/m²/day) reduces collagen synthesis (a marker of fibrosis) in rat lungs at 7 months after irradiation. The drugs were started 7 days after radiation. Aim: Our goal is to find the latest start time and optimal dose of enalapril to mitigate radiation fibrosis in a terrorism-relevant model. Experimental procedures: Wistar female rats (WAG/Rij.Cmr, 8-10 weeks of age) were treated with a single dose of 13 Gy of X-irradiation to the thorax, at a dose rate of 1.43 Gy/min. Administration of drugs was done by Core facilities at MCW. One group of age-matched unirradiated animals was kept under identical conditions to serve as controls. Radiated rats were randomized into groups that were untreated or treated with enalapril at 30mg/L (low dose) or 60mg/L (high dose) starting at 35 days (just before pneumonitis), 70 days (after pneumonitis), 105 days or 140 days (after pneumonitis but before fibrosis). Breathing rates were measured throughout the experiment while newly synthesized lung collagen content was measured at 7 months after irradiation using the Sircol assay. Summary of results: Enalapril (low and high dose) started at 35 days improved survival through pneumonitis. Enalapril 60mg/L but not 30mg/L started at 35 days mitigated the increase in collagen synthesis in the lung. Enalapril started later than 35 days did not improve lung collagen content at 7 months after radiation. Conclusions: We show that enalapril, even if started up to 35 days after radiation mitigates morbidity and improves survival through pneumonitis, but the high dose does not mitigate radiation fibrosis. This large window of time is to our knowledge the longest interval for intervention with mitigators of lung injury in a model relevant to a mass casualty event. Funding: This work was funded by NIH (USA) NIAID RC1 AI 81294, 81294-01S1 and U19-AI-67734.

POSTER 23-36. Characterization of damage to cardiac endothelial cells induced both in vitro or in vivo by ionizing radiation. Karol Jelenek1, A. Walaszczyk2, D. Gabryś3, M. Pietrowska1, C. Kantou4, P. Wiśnial1, 1: Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland 2: Cancer Research UK Tumour Microcirculation Group, University of Sheffield, Sheffield, UK

Introduction: Cardiovascular disease associated with radiotherapy is an important clinical problem. However, only few radiobiological models relevant for assessment of cardiotoxic effects of ionizing radiation are available at the moment. Here we described isolation from mouse primary cardiac endothelial cells, possible target for cardiotoxic effects of radiation, and tested them using different radiosensitivity assays.

Materials and methods: Cells were isolated from hearts of adult animals (8-weeks-old), both control and irradiated with a 2 or 8 Gy doses, at different times after irradiation (up to 60 weeks). In addition, cells isolated from hearts of juvenile mice were cultured and irradiated in vivo.

Results: A dose-dependent formation of histone gammaH2AX foci was observed after in vitro irradiation of cultured cells. However, such cells were resistant to radiation-induced apoptosis. A high dose of 16 Gy did not increase permeability of monolayers formed by endothelial cells. However, increased levels of actin stress fibres were observed in the cytoplasm of cardiac endothelial cells either irradiated in vitro or isolated from irradiated animals (up to 20 weeks after irradiation). Upregulated expression of Vcam1 and Sele genes was detected after 8 Gy irradiation in vitro and in cells isolated few days after irradiation in vivo. In addition, elevated expression of Hp70 gene was observed as a long-term effect of radiation (up to 40 weeks after the in vivo treatment).

Conclusions: Radiation-related changes observed in isolated cardiac endothelial cells several weeks after irradiation, i.e. increased levels of actin stress fibres and elevated expression of Hp70 gene, might be relevant for cardiotoxic effects of ionizing radiation.

POSTER 23-37. Development of the rapid radiochemical analytic methods for the nuclear accident or radiation emergency. Yanqing Ji, B. Shen, X. Shao, Q. Tian, L. Yin, National Institute for Radiological Protection, China CDC, China

With the advance of nuclear sciences and technologies, the wide application of nuclear and radiological technologies has been received the more concerns recently, especially after the nuclear crisis in Japan. There are batch of well-designed and suitable methods to assess and measure the potential hazards and routine surveillance. An accurate, rapid radioanalytical method for determination of radionuclides from various sources in the environment particularly is urgent need for early decision-making.

The extraction chromatographic column with crown ether BiB(Ch13CH6) or TOA (Tri-octylamine) on Teflon powder was prepared and used to separate 90Sr, or Np & Pu from environmental and biological samples respectively. The 90Sr was measured by liquid scintillation counter directly after the separation. The Np and Pu were detected sequentially by inductively coupled plasma mass spectrometry (ICP-MS).

The results demonstrate extraction of Sr from environmental and biological samples and fast countermeasure. The column effectively retained Sr in 8 mol L-1 HNO3 medium and easily eluted in water. Comparison of the traditional HDEHP extraction and fumic nitric acid method, the analytical time is within 8 hours instead of the days to weeks. The uncertainty of measurement is 12.8% (k=2) when determine biological ash samples. An unique TOA extraction chromatographic column with a two-stage sample loading was prepared to separate Np and Pu from the environmental or biological matrix. Np and Pu were efficiently kept in 4 mol L-1 HNO3 medium on the column and easily eluted with 0.02 mol L-1 oxalic acid in 0.16 mol L-1 HNO3 at 95°C. The separated solutions were free from most of the matrix elements and were aspirated into the ICP-MS directly. The decontamination factor for 237U is more than 105. The feasibility for the determination of both elements was proved by analysing IAEA-135 reference sample, the measured values agreed with the recommended reference value.

The effective separation of radionuclides from matrix is the key issue to devote the establishment of rapid analytic method. The method which can be used for sequential determination of multiple radionuclides need study further.

POSTER 23-38. Occupational exposure to X-rays in Poland. Zbigniew Kamiński, S. Papierz, M. Adamowicz, J. Kacprzyk, M. Zmysłony, The Nofer Institute of Occupational Medicine, Łódź, Poland

The Radiation Protection Department in the Nofer Institute of Occupational Medicine in Łódź, in accordance with national regulations, provides radiation protection service for Polish workers occupationally exposed to X-rays. The study presents the review of the individual dosimetry data concerning Hp(10) and Hp(0.07). The Radiation Protection Department is an accredited testing laboratory that provides routine individual dosimetry. The personal dose equivalent Hp(10) is calculated using Dresler method (the detection limit equals 0.1 mSv). Film dosimetry was a good solution because of the opportunity of the simplicity of the permanent registration of the dose. Since the beginning of 1966, all data have been stored in the personal dosimetry database. Furthermore, the measurements of individual dose equivalent Hp(0.07) are performed since 2001 using the ring dosimeters equipped with TLDs (the detection limit equals 0.05 mSv).

The collected data were used to analysis with respect to the type of working place of people, frequency distributions of Hp(10) and Hp(0.07) and number of cases of over-exceeding the annual level of dose. In 2010 individual film dosimetry and ring dosimetry covered more than 27,000 workers and 1845 workers, respectively. At present, the Nofer Institute of Occupational Medicine individual dosimetry is implemented in 3,800 laboratories (film dosimetry) and 152 (TLD dosimetry) laboratories. In year 2010 the total mean annual dose Hp(10) and Hp(0.07) equals 0.48 mSv and 5.6 mSv, respectively. Nofer Institute of Occupational Medicine is the owner of the greatest database of the annual doses of workers exposed to the ionizing radiation in Poland. Most of them are employed in health service. Concerning the personal dose equivalent Hp(10) the average annual effective doses of occupational radiation exposure among workers substantially decreased during the study period (1966-2010) while maintaining on the approximate level of about 0.5 mSv for last decade. Presently, over 99% of annual doses doesn’t exceed the level of 1mSv. It is note-worthy that about 95% of controlled workers receive doses below the sensitivity limit i.e. below 0.1 mSv. Finally, annual dose higher than the annual limit haven’t been recorded for the last few years. Additionally the paper presents also results of statistical evaluation of the Hp(0.07) measurements performed since 2001. The mean annual value of the dose decreased from 18 mSv in 2001 to 5.6 mSv in 2010. The measured values during last three years shows stabilization of the level of radiation exposure in those laboratories.
POSTER PRESENTATIONS

POS23-39. Radiosynovectomy with 90Y of knee joint – dosimetric aspects. Monika Kempińska1, P. Lass1, 1: University of Gdańsk, Poland 2: Medical University of Gdańsk, Poland

Radiosynovectomy is mainly used in chronic joint inflammation in cases resistant to conventional therapy. The performance of this procedure needs participation of a nuclear medicine specialist, as well as an orthopedist or a rheumatologist and a technologist, who prepares radiopharmaceuticals. The radiation absorbed doses for patients and personnel depend not only on the injected activity, but also on the method and process of injection and the radioactivity measurement procedure used.

Aims of this study are:
- the evaluation of the degree of radiation exposure of patients and medical personnel during the performance of therapy with 90Y.
- a comparison of counts in a selected regions of the joint from bremsstrahlung imaging with readouts of the dosimeters.

Materials and Methods:

For the quantification of the exposure to ionizing radiation thermoluminescence dosimeters were used. The personal dose equivalent at 0.07 mm (dosimeter PI-01) and 10 mm depth (dosimeter DI-01) in the body (72 persons) and medical personnel (30 persons) and kinetic energy released per unit air mass (dosimeter DS-04) were calculated. Dosimeter exposure to the patient’s activity was measured for a minimum of 48 h.

Knee joint gammacamera scintigraphy was performed. Yttrium - 90 scintigraphy was used in the experiments with Medium Energy Collimator for minimize contamination and maximize the sensitivity while imaging bremsstrahlung photons. A 5 min spot study (20 patients) and a body scan (5 patients) for 1h, 24h and 72 h after injection had been performed.

Results and Conclusion:

Patients after radiosynovectomy of the knee joint with Yttrium – 90 citrate and persons from their environment do not receive significant dose of radiation, if rules of radiation protection are fulfilled. It was showed that there is a correlation between the thermoluminescence and gammacamera measurements.

Scintigraphy of the knee joint in a given moment of the therapy makes possible continuous monitoring of the accumulation process of radiopharmaceutical. It gives a very useful informations for a physician for the future course of therapy.

In this case bremsstrahlung gammacamera measurements seem to be useful and precise tool in dosimetry

POS23-40. Diphlorethohydroxycarmalol suppresses γ-ray radiation-induced cell damage via inhibition of oxidative stress. Ki Cheon Kim, R. Zhang, M. Jung Piao, A. Daseul Kim, J. Won Hyan, Jeju National University, South Korea

Diphlorethohydroxycarmalol (DPHC) was shown to reduce superoxide anions, hydroxyl radicals. DPHC reduced intracellular reactive oxygen species (ROS) generated by g-radiation and protected cells against radiation-induced cellular DNA damage, membrane lipid peroxidation and protein modification, which are the main targets of radiation-induced damage. Furthermore, DPHC suppressed the mitochondria membrane potential induced by gamma-irradiation. In addition, DPHC recovered the cell viability damaged by radiation via inhibition of apoptosis. Irradiated cells with DPHC treatment resulted in reduced Bax, caspases 9 and 3 expressions, which were induced by g-radiation. However, irradiated cells with DPHC recovered Bcl-2 expression, which was reduced by radiation. This anti-apoptotic effect of DPHC was due to inhibition of c-Jun NH2-terminal kinase cascades induced by g-radiation. In summary, these results suggest that DPHC protects cells against g-radiation-induced oxidative stress via reduction of ROS and attenuation of apoptosis.

POS23-41. Dose levels of the occupational staff in Poland based on the results from the dosimetry service at IFJ PAN, Krakow, Poland. Renata Kopiec, M. Budzanowski, Institute of Nuclear Physics PAS, Poland

The Laboratory of Individual and Environmental Dosimetry (LADIS) at the Institute of Nuclear Physics (IFJ PAN) in Krakow applies thermoluminescence (TL) detectors for individual dosimetry in terms of the personal dose equivalent H(2) of 0.1 mSv to 1 Sv. The service is currently performed for ca. 4200 institutions and 35000 occupational personnel over the whole Poland.

According to Polish Atomic Law exposure periods are on a quarterly basis. For whole body doses four MTS-N (LiF:Mg,Ti) TL pellets (4.5 mm diameter and 0.9 mm thickness) are placed in the standard Rados Oy badge with three Al filters. For finger doses the plastic ring holder with adjustable finger size are applied. One MTS-N, (4.5 mm diameter, 0.7 mm thickness), is placed in a bar coded holder under a plastic cover, 0.4 mm thick. The dosemeters can undergo various sterilization The radiares, used at the clients’ hospitals.

The paper presents the results of statistical evaluation of more than 400 000 quarterly or monthly effective dose measurements performed by LADIS in years 2003-2010. The doses are being divided in dependence of technical and medical institution and type of measurement performed (individual dosimetry or environmental dosimetry). The highest readings of individual dosemeters, exceeding the dose limits, are almost exclusively due to the accidental exposure of dosemeters left in the vicinity of radiation sources.

References:

POS23-42. Total and angular photon albedo for water, concrete and iron, and dependence on the thickness of reflecting material. Dragana Krstic, D. Nikizec, V. Markovic, N. Stanevovic, Faculty of Science, University of Kragujevac, Serbia

Reflection of radiation is a significant subject of investigation in radiological-protection studies, because reflected radiation presents unknown secondary source. Photon reflection from shielding materials which are used in nuclear facilities must be determined because of higher risk from irradiation. To take into account reflected radiation and prevent irradiation of surroundings, it is necessary to determine energetic and angular distribution of reflected photons. The photon albedo is defined as the ratio of the current flow of photons emitted from a unit area of the reflecting surface to the current flow of primary photons incident upon that surface.

Total number and angular albedo were calculated for commonly used shielding materials as water, concrete and iron, for photons with initial energies from 10 keV up to 10 MeV and normal incident angle. Double differential albedo was determined by simulating photons transport through materials by using PENELOPE and MCNP software. Backscattered photons were scored and grouped in equal intervals of energy and angle.

In addition, the influence of material thickness on total number albedo was investigated also. With increasing of the material thickness, total number albedo also increases and at some point achieves the saturation. With further increasing of thickness, the total number albedo remains constant. It was concluded that for the materials thicker than few photon mean free paths albedo doesn’t depend on thickness.

Keywords: photon albedo, shielding materials, Monte Carlo simulation


Since ionizing radiation is finding applications in various fields of life, there is an urgent need to develop effective and nontoxic radioprotectors. In spite of several years of research, search for an ideal radioprotector that can be used for occupational as well as clinical setting continues. We have recently evaluated the radioprotective effects of extracellular melanin isolated and characterized from a fungal source and results pertaining to it are discussed here. Melanins are high molecular weight pigments that are constitutively synthesized by many fungi to confer a survival advantage against environmental predators, heavy metals toxicity, and physical insults such as UV radiation. A few recent reports indicate that melanized fungal species colonize on the walls of the damaged reactor at Chernobyl. These findings, together with some laboratory observations of the resistance of melanized fungi to ionizing radiation suggest a role for this pigment in radioprotection. In present study the radioprotective ability of melanin was evaluated in BALB/C mice by administering it intraperitoneally (i.p.) 30 min before and 30 min after
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exposure to the lethal dose (7 Gy) of γ-radiation. After irradiation, animals were monitored for 30 days and mortality, if any, was recorded. The results indicated that pre-administration of melanin at a dosage of 10, 25, and 50 mg/kg body weight increased their survival by 20, 50 and 100 % respectively. Similarly, when melanin was administered i.p. at a dosage of 25 and 50 mg/kg body weight after the whole body irradiation, it increased the survival by 50 and 70 % respectively. The mechanistic investigations by administering melanin at a dosage of 50 mg/kg body weight, 30 min before irradiation revealed significant protection to hematopoietic system as evidenced by inhibition of the radiation induced DNA damage in peripheral leukocytes, improvement in lymphocyte count in blood and spleen colony forming units. In correlation with the above studies, splenic lymphocytes pre-treated with melanin offered ~70% protection against radiation-induced apoptosis. Our results thus clearly demonstrate prophylactic and therapeutic radioprotective ability of melanin in mouse model.

POS23.44. Radiation safety and control at ARTI Yun-jong Lee, Korea Atomic Energy Research Institute, South Korea

Due to governmental approval for the establishment of a research institute specializing in radiation utilization research in 2000, the Advanced Radiation Technology Institute (ARTI), a branch office of the Korea Atomic Energy Research Institute (KAERI), was established and started its official work in 2005 in Jeongeup, Jeonbuk. The objective of the radiation safety control program at ARTI is to ensure radiation safety by limiting the probability of stochastic effects, as well as prevent detrimental deterministic effects attributed to radiation exposure to personnel handling radiation and/or radioactive materials in ARTI. This is a mandatory program that should be performed by the licensee according to the KOREA Atomic Energy Act. In order to achieve the objectives of the radiation safety control program, the following main activities have been controlled, and the radiation levels have been maintained below the acceptable limits through periodic measurements as well as the monitoring of air, surface, and water contamination/exhaust; dosage rates; and access control of the radiation workers. The total number of regular radiation workers was 458 in 2010. No individual received an exposure in excess of the annual dose limits set in the relevant regulations Ir the Atomic Energy Act. To secure safety and protect workers against radiation, a training course on fundamental radiation protection was conducted, and a total if 523 workers participated in the course. In addition to these activities, several research articles have been published in domestic scientific journals, invited and presented papers have been presented in conferences, and technical support for radiation protection and safety has been provided to the related industries and/or other organizations.

POS23.45. Radioprotection effect and immune activity by Enterococcus faecalis 2001. YoungHee Lee, M. Iwasa, Y. Gu, Suzuka University of Medical Science, Japan

Although it has been reported that Enterococcus faecalis 2001 (EF 2001) shows immunoenhancement activity and antioxidant activity, protective effects against radiation have not been investigated. In addition, although a few studies about anti-tumor effects in vitro have been reported, it is unknown whether these effects also occur in vivo. It is unknown what mechanisms are involved in the protective effect against radiation and the anti-tumor activity in these substances. Therefore, in this study, we investigated the protective effects against radiation and in vivo anti-tumor effects of EF 2001. Blood cells are one of the indices for evaluating protective effects against radiation. Hemopoietic tissues and peripheral lymphocytes are highly sensitive to radiation, and a decrease of immunity caused by a decrease in white blood cells and myelocytes is remarkable after exposure to radiation. Therefore, in this study, we focused on the antioxidant activity and immunoenhancement activity of EF 2001, and examined the in vivo effect of radiation on the number of peripheral blood cells. Furthermore, measurement of T lymphocyte subsets, SOD-like activity, antioxidant activity, radical scavenging activity based on chemiluminescence methods, and absolute amounts of free radicals based on ESR were carried out, and radical scavenging activity, which is a mechanism of protection from radiation was examined. In addition, we examined antioxidant activity and immunoenhancement activity, both of which are closely related to tumor suppression, and further investigated effects on suppressive effects on tumor growth and anti-tumor effects of tumor necrosis factor (TNF). We hope that a combination of immunotherapy, using natural materials such as EF 2001 with immunoenhancement action may increase the percentage of patients who recover.

POS23.46. Comparative study of the measurement of thoron gas. Weping Liang, X. Ai, L. Zhang, The Solid Dosimetric Detector and Method Laboratory, China

The accurate measurement of 220Rn is one of the most important research focuses in radiation exposure dose evaluation. Based on three commonly used radon measuring device, we developed its use in 220Rn measurement and finished some comparative experiments. The main conclusions: AB5 grab measurement method with its simple in principle and stable measurement results can be used as a reference method of measuring 220Rn, through its lower detection limit is a little higher; The 220Rn measurement result given by RAD7 is influenced by the status of dessicant and need a timely calibration; AlphaGuard flow mode has the advantage of low detection limit, but its results prone to sampling flow rate and the status of front-end sampling pipe, so can only be a general reference.

Keyword : 220Rn, accurate measurement, Comparative experiment, reference method

POS23.47. Fibroblast growth factor peptide mitigates acute radiation syndrome via promotion of progenitor recovery and homologous DNA repair in bone marrow, Alexander Lvitvinchuk, L. Zhang', P. Okuneff, 1: University of Florida Shands Cancer Center, USA 2: UF Shands Cancer Center, USA

Stimulation of proliferation and differentiation of residual hematopoietic stem and progenitor cells is a major goal for medical treatment of acute radiation syndrome (ARS). Fibroblast growth factor (FGF) receptors are universally expressed in all cells, including stem/progenitor cells in bone marrow (BM). FGF-P, a dimerized peptide derived from FGF2, helps to mitigate ARS.

Purpose: To explore the underlying mechanism of FGF-P by which ARS is mitigated.

Methods: Swiss NIH mice were exposed to 7.5-8 Gy total body radiation once, and 48 hours later were subcutaneously injected with vehicle alone or FGF-P (0.2-3 mg/kg/daily for 5 days and every other day for an additional 5 doses). Several days later, BM was harvested and subjected to staining with different antibodies and analyzed with flow cytometry, Western blotting, immunofluorescent staining (IF), and confocal microscopy for the cellular location of fluorescein isothiocyanate (FITC)-FGF-P, the number of CD34+ progenitors, TER119/CD71 erythroid cells, and DNA repair proteins.

Results: 1) Using confocal microscopy, we found that FITC-FGF-P bound to the surface of BM stem and progenitor cells. Interestingly, FITC-FGF-P could be translocated to the nucleus of stem cells. 2) CD34+ progenitor cells treated in vitro with FGF-P were more resistant to apoptosis. 3) CD34+ progenitors, the granulocytic-monocytic lineages associated with Gr-1- antigen cells, were increased by FGF-P at 2, 3, and 7 months after irradiation. 4) Mice rescued by FGF-P had a near-normal ratio of myeloid to erythroid cells. 5) IF analysis and Western blotting showed that the RAD51 foci and their associated protein were increased after treated with FGF-P in vitro, indicating that the homologous repair (HR) of DNA was enhanced. Also, through IF we found that that key repair proteins such as RAD51 (HR) and Ku86/70 (non-homologous end-joining) organize the common foci with poly [ADP-ribose] polymerase 1 (PARP1) in nuclei. Thus, PARP1 have competitive interactions with RAD51 or Ku proteins to repair single- or double-stranded DNA damaged sites after irradiation.

Conclusions: FGF-P promotes the recovery of CD34+ progenitors and the reconstitution of the BM myeloid-erythroid ratio. FGF-P in the nucleus might enhance the DNA HR after radiation and support genome integrity.

POS23.48. Radiation contamination detection of people returning from Japan after Fukushima accident. Ying Liu, L. Cuiping, M. Weidong, C. Hufang, L. Yuwen, National Institute for Radiological Protection, China CDC, China

Objective: There were a lot of Chinese citizens arriving from Japan to China as they were anxious about their possible contamination with radioactive materials relate to Fukushima disaster. We detect contamination with radioactive materials in people arriving from Japan to China to clarify the health consequences of the Fukushima accident.
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Methods: Using surface contamination monitor and whole body counter we detected contamination with radioactive materials in neither of the cases. As a general, detectable contamination levels greater than the background indicate decontamination should be attempted. Simple decontamination refers to the remove of contaminated articles of exterior clothing and the use of water to flush removable contamination from skin and non-porous materials.

Results: More than 400 people returning from Japan after Fukushima disaster were examined for contamination. Among of them, most of people are below the threshold; only 3 people are above it. After measuring for the second or the third time following simple decontamination the 3 people showed low values. The widespread public anxiety associated with radiation accident appears to have a proportion to the radiation undetected health effects.

Conclusions: There is few health consequences relate to the Fukushima accident for people returning from Japan. Psychological effect in affected population is one of the most important health problems. Psychological reactions to radiation could be prevented, decreased or relaxed by different methods applied before, during or after the accident. Appropriate officials at the national and local levels should arrange psychological support.


Introduction: In vivo counting consists in assessing internal contamination by counting the radiations emitted from the body. For routine assessment the counting systems are calibrated with physical phantoms containing radioactive sources. However, these phantoms are of limited realism as compared with current numerical models. Numerical calibration, based on Monte Carlo (MC) calculations and numerical models of the human body, is an alternative to physical calibration. However, this solution presents several challenges: modelling of the detectors, design of human models, and reproduction of experimental conditions. To assess the reliability of numerical calibration the working groups “internal dosimetry” and “computational dosimetry” of the Eurados network have carried out a routine assessment the counting systems are calibrated with physical phantoms containing radioactive sources. However, these phantoms are of limited realism as compared with current numerical models. Numerical calibration, based on Monte Carlo (MC) calculations and numerical models of the human body, is an alternative to physical calibration. However, this solution presents several challenges:

Methods: CT scans of the Livermore phantom were used to build voxel phantoms with a resolution of 8 mm3 per voxel. The Livermore phantom was set up with enriched Uranium lung sources. Two reference measurements, with and without extra-thoracic plates, were carried out using the germanium detectors of the CIEMAT whole body counting unit. The position of detectors was reported with a precision of ±3 mm. The voxel models, the detector validated model, the counting positions and source data were sent to participants who were asked to compute the spectra corresponding to the measurements and to give the counting efficiencies needed for the calculation of 235U-238U.

Results: Seventeen teams, from 14 institutes, took part in this initiative. Eleven teams used MC codes from the MCNP(X) family. Except for one code the voxel models were easily set up for the calculations.

At the first analysis of results more than half of the participants were asked to review their results. The most frequent source of error was the misuse of units or difficulties in understanding the definition of counting efficiency. To help improving the results tutorials were distributed to guide the reviewing of results. After a second round all participants obtained results in agreement with the experimental data for standard conditions without extra-thoracic plate, the agreement of calculated and experimental efficiencies is between 4.

POS523-50. Effect of fibrolast growth factor peptide on the proliferation of murine bone marrow cells after ionizing radiation. Jun Ma, D. Han, M. Zhang, B. Zhang, C. Chen, A. Zhang, P. Okunieff, L. Zhang, University of Florida Shands Cancer Center, USA

Purpose: Fibrolast growth factor peptide (FGF-P), a dimerized stable peptide derived from the receptor binding domain of FGF2, was synthesized to study its effect on stem cells and progenitor cells in bone marrow from irradiated mice. Methods: Bone marrow cells (BMC) were collected by flushing femora of C57BL/6J mice that were exposed to 3-Gy or 8-Gy total body irradiation (TBI). The effect of FGF-P on proliferation of BMC in vitro was determined by tritiated thymidine (‘H-TdR) incorporation assay. Swiss NIH mice were injected with FGF-P 48 hours after 6-Gy TBI. FGF-P-induced recovery of stem and progenitor cells in bone marrow was studied with flow cytometry. Hematopoietic stem cells (HSC) were labeled with anti-mouse lineage/Sca-1/c-kit (LSK) antibodies and further divided into 2 categories as long-term HSC (LT-HSC) with CD34 and short-term HSC (ST-HSC) with CD34. Immature B lymph cells were labeled with IgM, CD19, and c-kit (proB) or CD25 (preB). Mesenchymal stem cells (MSC) were labeled with CD34, CD45, and Sca-1 and further divided into multi-potential MBC1(CD44+) and less-potential MBC2 (CD44-).

Results: Incorporation of ‘H-TdR in vitro by BMC from 3-Gy TBI C57BL/6J mice was increased after treatment with FGF-P and rhFGF2, as compared to vehicle-alone control (p < 0.05). The value of ‘H-TdR incorporation in FGF-P treated BMC was higher than that in rhFGF2 treated cells (p < 0.05). LT-HSC (lin/Sca-1/c-kit+CD34+), were increased on day 25 after 6-Gy TBI in Swiss mice treated with FGF-P (5 mg/kg) by subcutaneous injection as compared to mice treated with vehicle alone (p < 0.05). However, no significant was found for LSK (lin/Sca-1/c-kit+) or ST-HSC (lin/Sca-1/c-kit+/CD34-) between FGF-P treated mice and vehicle-alone treated mice. FGF-P had no induction effect on proliferation of MSC, MPOC1, or MPOC2 in bone marrow of Swiss mice after 6-Gy TBI. However, the immature B cells were increased after FGF-P treatment as compared to vehicle-alone control (p < 0.05).

Conclusions: 1) In vitro, FGF-P increased the proliferation of BMC. 2) In vivo, FGF-P stimulated the proliferation of cell subtypes in murine bone marrow, such as LT-HSC and immature B cells.

POS523-51. A Novel Method for Removing Tc-99m and I-131 from Aqueous Solutions Using Montmorillonite Nanoclay. SMD1 Mortazavi1, J. Moradgholi2, M. Sadegh Rouintan2. S. Abasaleh Namazi1, S. Shiraz University of Medical Sciences, Iran 2: Isfahan University, Iran

Background: Montmorillonite, the principal constituent of bentonite, is composed of hydrous aluminum silicates in the form of extremely small particles that swell in water and shows high cation-exchange capacities. Due to their economical advantages, much attention has been given to the use of clays as adsorbents over the past years. I-131 enters water from medical waste and nuclear accidents. AfterFukushima nuclear disaster in Japan, tap water from some areas was tested positive for radioactive iodine. On the other hand, Radionuclided contamination (iodine or technetium) in nuclear medicine departments is not a rare event.

Objectives: The aim of this study was to investigate the efficiency of montmorillonite nanoclay and common zeolite and bentonite minerals (micrometer-sized) to adsorb three radionuclides, namely Iodine-131, Tc-99m and Lu-177 from aqueous solutions.

Methods: Iodine-131, Tc-99m and Lu-177 solutions with an initial activity of 0.1 mCi were mixed with 9 cc of distilled water in a test tube. About 0.4 g of nanomontmorillonite, bentonite and zeolite were added to half of the I-131, Tc-99m or Lu-177 test tubes. Remaining tubes did not receive any adsorbing agent. Tubes were gently shaken
for 2 minutes and then were left still for the following 118 minutes. Then 0.9 cc of the solution was taken out from the mid part of the test tubes. Activities of these samples were measured using a Vinten Isocal II radioisotope calibrator (Vinten Instruments Ltd, UK) dose calibrator.

Results: For I-131 and Tc-99m the activity of the solution in test tubes treated with montmorillonite nanoclay was 0 mCi, while in control test tubes the mean (+SD) activities were 0.009 ± 0.0004 and 0.006 ± 0.0004 mCi respectively. Activity in the last test tube containing bentonite and zeolite showed no significant difference with those of control samples. In case of Lu-177, the activity of the solution in test tubes treated with montmorillonite nanoclay was 0.001 ± 0.0007 mCi, while in control test tubes the mean (+SD) activity was 0.009 ± 0.0007 mCi. Conclusion: The results of this study prove the powerful ability of montmorillonite nanoclay in removing I-131, Tc-99m and Lu-177 from aqueous solutions. These findings may be used for waste water filtration in nuclear medicine departments and even for production of cost-effective I-131 water filtration devices after a nuclear accident.

POS23-52. A novel method for removing I-131, Tc-99m and Lu-177 radionuclides from aqueous solutions using montmorillonite nanoclay. S. Abasaleh Namazi1, S. Mortazavi2, M. Rouintan1, J. Moradgholi3, N. Motlagh Bahadori4, M. Hesampour, N. Masoodi5, 1: Shiraz University oMf edical Sciences, Iran 2: Shiraz University of Medical Sciences, Iran 3: Isfahan University of Technology, Iran.

Background: Montmorillonite, the principal constituent of bentonite, is composed of hydrous aluminum silicates in the form of extremely small particles that swell in water and shows high cation-exchange capacities. Due to their economical advantages, much attention has been given to the use of clays as adsorbents over the past years. I-131 enters water from medical waste and nuclear accidents. AfterFukushima nuclear disaster in Japan, tap water from some areas was tested positive for radioactive iodine. On the other hand, Radionuclidic contamination (iodine or technetium) in nuclear medicine departments is not a rare event.

Objectives: The aim of this study was to investigate the efficiency of montmorillonite nanoclay and common zeolite and bentonite minerals (micrometer-sized) to adsorb three radionuclides, namely Iodine-131, Tc-99m and Lu-177 from aqueous solutions.

Methods: Iodine-131, Tc-99m and Lu-177 solutions with an initial activity of 0.1 mCi were mixed with 9 cc of distilled water in a test tube. About 0.4 g of nano-montmorillonite, bentonite or zeolite was added to half of the I-131, Tc-99m or Lu-177 test tubes. Remaining tubes did not receive any adsorbing agent. Tubes were gently shaken for 2 minutes and then were left still for the following 118 minutes. Then 0.9 cc of the solution was taken out from the mid part of the test tubes. Activities of these samples were measured using a Vinten Isocal II radioisotope calibrator (Vinten Instruments Ltd, UK) dose calibrator.

Results: For I-131 and Tc-99m the activity of the solution in test tubes treated with montmorillonite nanoclay was 0 mCi, while in control test tubes the mean (+SD) activities were 0.009 ± 0.0004 and 0.006 ± 0.0004 mCi respectively. Activity in the tubes containing bentonite and zeolite showed no significant difference with those of control samples. In case of Lu-177, the activity of the solution in test tubes treated with montmorillonite nanoclay was 0.001 ± 0.0007 mCi, while in control test tubes the mean (+SD) activity was 0.009 ± 0.0007 mCi. Conclusion: The results of this study prove the powerful ability of montmorillonite nanoclay in removing I-131, Tc-99m and Lu-177 from aqueous solutions. These findings may be used for waste water filtration in nuclear medicine departments and even for production of cost-effective I-131 water filtration devices after a nuclear accident.

POS23-53. Assessment of the dose to the eye lens of radiation medicine workers. Jerzy Olszewski, The Nofer Institute of Occupational Medicine, Poland

Practical use of ionising radiation from radioisotopes, in addition to offering tremendous diagnostic and therapeutic advantages, results in harmful doses of the radiation to the personnel. Studies conducted by NORM at nuclear medicine laboratories showed a considerable risk of excessive exposure of the hands to the ionising radiation. To assess exposure to ionising radiation of the eyes of nuclear medicine workers handling technetium and iodine, 140 determinations of radiation doses were done in three nuclear medicine laboratories. The determinations were performed by radiopharmacists, i.e. workers preparing technetium-labeled formulations and nurses administering those formulations to patients. Besides, determinations were performed in physicians also administering radioiodine and taking care of radioiodine carrier patients. High-sensitivity thermoluminescent detectors (TL) made of lithium fluoride (LiF: Mg, Cu, P – MCP) – produced by the Polish company Niewiadomski and Co. were used to determine the doses. The experiments show that eye exposure is uniform, i.e. it can be successfully monitored with one dosimeter placed either on the right or left temple. Mean dose recorded during work day was 16.9 µSv, while their yearly recorded dose was 107 µSv.

The results of our experiment show that it would not be reasonable to expect that nuclear medicine workers receive doses to the eyes exceeding 1/3 of equivalent dose (50 mSv p.a.).

When analysing the results, the author have suggested that use of protective glasses made of lead glass should be made obligatory in nuclear medicine laboratories to additionally reduce, according to the ALARA principle, the exposure to the eyes of the personnel.

POS23-54. Estimation of critical doses and dose rates for agricultural plants. Alla Oudalova, Russian Institute of Agricultural Radiology and Agroecology, Russian Federation

The purpose of this work is to develop methods and approaches in the field of radiation protection for non-human biota. The key problem is an assessment of critical doses and dose rates that can result in significant radiation-induced effects in plants. Derived in the work radiation impact are assessed on an example of cultivated plants. Agrarian ecosystems are of special concern from the viewpoint of establishing safe levels of radiation impact on the environment, since, first, their contamination can affect human health via radionuclide uptakes, agroecosystems can contribute to developing a unified concept of the environment and human protection from radiation and nonradiation contaminations. Available information on dose dependences in such umbrella endpoints as reproductive potential, survival, morbidity, morphological, biochemical, and genetic effects in crops, vegetables, fruit trees, etc are gathered from scientific papers issued during last 50 years. Data are maintained as database in MS Access that contains about 17000 records at the moment; the work is ongoing. Quantitative data are collected for about 60 species of cultivated plants. As critical radiation values, there are considered doses producing 50% changes of biological effect at acute impact (ED50), or dose rates resulting in 10% changes at chronic exposure of plants (EDR10). Critical doses for different species are calculated from dose-effect dependences obtained with the corresponding data sets. Primary data are assessed for their quality according to several criteria. Three models (linear, logarithmic, and logistic) are tested for an applicability to fit a dose-effect dependence taking account of their goodness-of-fit and robustness of ED50 and EDR10 estimates. The critical doses and dose rates for agroecosystems estimated from available information on reproduction and survival are presented. It is divulged that critical radiotoxicity values can depend on a type of model chosen to fit dose dependence and quality of primary data. Findings obtained contribute to developing a unified concept of the environment and human protection from radiation and nonradiation contaminations.

POS23-55. Entrance surface doses (ESD) from medical X-ray examinations in Masovian voivodeship, Krzysztof Pachocki, M. Bekas, Z. Różycki, K. Wierzbowski, Department of Radiation Protection and Radiobiology, National Institute of Public Health, Poland

Masovian Voivodeship is the largest and most populous of the sixteen Polish voivodeships (provinces). It occupies 35,579 square kilometres (13,737 sq mi) of east-central Poland, and has 5.16 million inhabitants. Its principal cities are Warsaw (1.7 million), in the centre of the Warsaw metropolitan area, Radom (226,000) in the south, Płock (127,000) in the west, Siedlce (77,000) in the east, and Ostrołęka (55,000) in the north. The capital of the voivodeship is the national capital, Warsaw.

Setting a medical diagnosis requires conducting the investigation of patient. One of them is X-Ray diagnostic, that allows to depict the inside structures of the patient body. The advantage of this investigation is the easy execution and very fast result, however, X-ray procedures have also disadvantages. The basic one is radiation risk. The magnitude of radiation risk depends on the technical X-ray conditions e.g. equipment and exposed to the rays. In the presented work the authors measured the entrance surface dose (ESD) that patients had received in standard medical X-ray
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procedures. The standards X-ray procedures included: chest examination, skull examination, spine examination (cervical, thoracic, lumbar-sacral), pelvis and abdomen examination. For the necessity of measurement the standard patient was defined as a person which has 170 cm height and 70 kg weight. This definition complies with the regulation of the health minister of 18 February 2011 (Journal of Laws No 51, item 265).

The measurement was done in accordance with internal procedures in Radiation Protection and Radiobiology Department. The result was compared with reference level definition in the annex 2 with regulation of the health minister.

The research was done in over 70 X-Ray rooms in mazowieckie voivodship. The result indicates that the difference between magnitudes of dose of the same X-Ray procedures might exceed over 800 %. For example chest AP projection, measured dose: 0.122 – 1.076 mGy, skull AP projection, measured dose: 0.204 – 2.200 mGy. Hitherto analysis of the results draws to conclusion that the measured doses depends on the condition of X-Ray units (technical parameters), used materials (sensitivity of films) and exposition parameters (kV and mA).

PO523-56. Entrance surface dose estimation for selected radiological procedures based on sample survey of Polish radiology departments. Piotr Pankowski, The National Centre for Radiation Protection in Health Care, Poland

Medical diagnostic radiological procedures are the largest source of radiation exposure for population of developed countries. Accordingly to the implementation of Article 12 of the European Commission Medical Exposure Directive of 1997 entitled “Estimates of population doses” Member States are required to ensure that the distribution of individual dose estimates from medical exposure is determined for the population and for relevant reference groups of the population. The considerable resources and effort required to reliably determine the frequency of the different types of x-ray examination and the associated patient doses meant that only the most developed countries were able to conduct such complex surveys and even those can not repeat them very frequently. National Centre for Radiation Protection in Health Care takes effort to perform various actions in order to provide reliable estimation on dose and frequency of examination as required in the Article 12. Presented results are based on sample survey performed in various radiology departments in Poland, the entrance surface dose and effective dose for selected radiological diagnostic procedures are presented and discussed in comparison with similar data determined by other countries.

PO523-57. Estimation of radioprotective effect of N-acetyl-L-cysteine in yeast cells. Jiyyong Park¹, T. Ho Ryu¹, M. Nili², J. Kyu Kim³, 1: Korea Atomic Energy Research Institute, South Korea 2: Dawnesh Radiation Research Institute, South Korea

Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CTA) were well conserved in all aerobic organisms, because those are exposed to reactive oxygen species (ROS) during aerobic respiration. Also, gamma rays generate ROS, which induce oxidative stress. N-acetyl-L-cysteine (NAC), a precursor of glutathione (GSH), is one of the antioxidants having a thiol group. In this study, radioprotective effect of NAC was investigated through cell survival and gene expression of antioxidant enzymes including SOD, GPx, and CTA in yeast. Yeast cells were pretreated with 0 mM to 20 mM concentration of NAC and/or irradiated with 0 Gy to 400 Gy dose gamma rays. The relative survival rate of yeast cells reduced with increasing doses of gamma rays. The pretreatment of NAC did not improve gamma rays-induced cell death in yeast. All of the gene expression of the antioxidant enzymes increased in the cells irradiated with 100 Gy without pretreatment of NAC. The gene expression of antioxidant enzymes in the cells irradiated with 100 Gy with pretreatment of NAC was lower than the control group without NAC pretreatment. These data suggest that, NAC does not protect cells against gamma rays-induced cell death, but NAC leads to scavenging ROS generated from gamma rays in yeast cells. Therefore, NAC is an useful antioxidant to scavange ROS in vivo.

PO523-58. Radiation protection of aircraft crew: publicly available database of measurements with the silicon spectrometer Liulin on board aircraft. Ondrej Ploc¹, J. Kubančák², A. Maluńek², P. Pytlíček³, I. Ambrozová², T. Duchov⁴, 1: National Institute of Radiological Sciences, Japan 2: Nuclear Physics Institute, Prague, Czech Republic 3: Space and Solar-Terrestrial Research Institute, Bulgarian Academy of Sciences, Sofia, Bulgaria

Annual effective doses of aircrew from occupational exposure are typically up to 6 mSv, depending on the number of flight hours, route locations, and solar activity. In many cases, these doses exceed the limit for public exposure to ionizing radiation and thus ICRP recommended their monitoring. Radiation fields at aircraft altitudes are complex and demand measurement experimentally. For this reason, the doses are estimated via computer codes that take into account flight parameters like aircraft location and altitude, and solar activity. It is generally accepted, that these calculations should be periodically verified by measurements. Precise measurements with tissue equivalency corrections are typically required as dosimeters are bulky and have only limited battery life. For long-term measurements, which are needed to cover the whole 11-year solar cycle, the silicon spectrometer Liulin is better suited. Liulin is an active dosimeter which records energy deposition events occurring in the semiconductor unit, which if appropriately calibrated – it estimates neutron and non-neutron component of the ambient dose equivalent.

This paper presents a database of long-term measurements performed on board aircraft with the Liulin detector. The measurements started in 2001. For one run, Liulin was placed in the cabin of a Czech Airlines aircraft for approximately 50 days. So far 28 runs were performed, i.e. about 3 500 flight hours and almost 20 000 flight hours. Flights were flown from Prague to destinations with vertical cut-off rigidities ranging from 1 GV to 17 GV. The most frequent were transatlantic flights from Prague to New York and to Canada.

The database comprises more than 10,000 records where each record contains information, i.e. energy deposition spectra, absorbed doses and dose equivalent rates measured with Liulin, date and time, geographic coordinates and altitude. The data are available on the Internet and can be used for instance for verification of computational programs routinely used for estimation of aircrew exposure to cosmic radiation.

PO523-59. Dosimetry during America’s penultimate shuttle mission. Mariagabriella Pugliese¹, F. De Cicco², M. Quarto³, V. Roca⁴, 1: Università di Napoli Federico II, Italy 2: Seconda Università di Napoli, Italy

The problem of radiation protection in space is very important because the exposure of astronauts to the cosmic radiation poses a major risk to space flight. Thermoluminescent dosimeters (TLDs) are largely used to determine the cosmic radiation exposure onboard the International Space Station (ISS) because they are easy to handle, comparatively light-weight, do not require power consumption, and give integral information about dose by post-flight evaluation. In the framework of BIOKON In Space (BIOKIS) sponsored by the Italian Space Agency (ASI-Agenzia Spaziale Italiana) in the areas of cellular biology, radiation and radioprotection, aging, germination and plant growth, HIĐOSE experiment covered the dosimetric measurements to evaluate the exposure dose to charged particles for biology experiments executed on board STS (Space Transportation System).

HIĐOSE experiment was performed using Thermo Luminescence Dosimeters (TLD) placed on the BIOKIS BIOKON container (developed by Kayser Italia). Measurements were obtained using three different types of TLD: TLD-100 (LiF:Mg,Ti), TLD-600 (LiF:Mg,Ti) and TLD-700 (LiF:Mg,Ti). TLD-700 have higher efficiency, that is dependent not only on the LET but also on the ion charged. The TLD used were 3.2 x 3.2 x 0.89 mm. TLD were annealed in air at 400°C for 1 hour prior to exposition.

Thermoluminescent dosimeters (8 for each type) were accommodated inside one of the two BIOKON passive container. To take trace of the radiation exposure during all the pre launch phases two TLD for each type hosted inside a plastic box were used as dosimeter control. The reading of these detector will be subtracted to the reading of the flying ones to evaluate only the radiation find on orbit.

The results of dosimetric measurements carried out by the HIĐOSE experiment will be presented.

PO523-60. Radiation mitigating compounds reduce radiation induced lethality and leukemia. Yelena Rivina, R. H. Schiestl, University of California, Los Angeles, USA
There is a dire need to develop therapeutic agents that protect or mitigate normal tissues from radiation-induced injury sustained during accidental exposures such as nuclear reactor meltdown and a terrorist attack, or in a clinical setting during radiotherapy. The purpose of the studies was to identify novel small molecule entities with radiation mitigation activity against radiation-induced (IR) damage and lethality. A yeast-based high-throughput screen was done to uncover molecules that protected or mitigated radiation-induced cytotoxicity and genotoxicity. Hit compounds were further validated in a plate-based DEL assay and compared to murine lymphocyte viability screens. Selected compounds were then tested for their ability to mitigate lethal total body irradiation in mice. Two lead compounds, Yel001 and Yel002, reduced IR-induced cell death and genomic instability in vitro and mitigated IR-associated lethality in vivo up to 75% when administered at 5 times at 24, 48, 72, 96, and 120 hours post exposure. Yel001 and Yel002 have also reduced IR-induced leukemia to 50% and 40% respectively as compared to 100% controls. The compounds appear to be general genome stabilization agents having also reduced I-131-induced DNA double strand breaks (DSB) in thyroid cells, decreased random microhomologymediated end joining in irradiated cells, and mitigated DNA damage and cell death after ultraviolet radiation exposure. No systemic nor reproductive toxicity has been observed in animals treated with multiple Yel compound administration. Yel001 and Yel002 are potent radiomodulators with potentially extensive applications in rescuing accidentally irradiated populations and in clinical settings.

**POSTER PRESENTATIONS**

**POS23-61. Ramipril, an ACE inhibitor, mitigates radiation-induced spinal cord injury in rats.** Samuel Ryu, S. Kumar, A. Kolozsvary, K. Jerovics, S. Brown, J. Ho Kim, Henry Ford Hospital Radiation Oncology, Detroit, MI, USA

We have shown that Ramipril mitigated the radiation-induced optic nerve injury after high dose radiation in a drug dose- and time-dependent manner. Most striking histological finding was the restoration of demyelination of the irradiated optic nerves. Since spinal cord is composed of abundant myelinated white matter and is the most critical normal tissue in spine radiosurgery, we initiated the present study to determine whether Ramipril can mitigate the spinal cord injury in a single high dose radiation exposure.

Fifty-five Fisher 344 rats were randomly assigned to groups of radiation to the spinal cord (C5-T2) in various lengths (5 mm and 20 mm) and a range of radiosurgical doses (22 - 33 Gy). After radiation, rats were randomly assigned to sham vs. Ramipril treatment. Starting one week after radiation, Ramipril was given 1.5mg/kg/day in drinking water for 6 months. Endpoints of the study were paralysis, demyelination (functional) and histology and MRI (anatomical). Once the rats develop paralysis, immunohistochemical stain were performed with LBFB, VEGF, HIF-1 alpha, and selected groups were assessed using contrast enhanced-MRI by T1, T2, and vascular permeability at 7T. At 19 weeks postirradiation, paralysis, paraplegic rats receiving high radiation doses (30-33 Gy to 2 cm length). No paralysis was noted in the ramipril-treated with same radiation dose. There were no rats paralyzed in the lower radiation dose group (up to 28 Gy) and short-length (5 mm) of the irradiated spinal cord for the duration of 6 months after radiation. LBFB stain showed demyelination at the dose as low as 22 Gy. Optical density of normal spinal cord for LBFB stain was 0.254. Optical density of radiation group was 0.119 due to demyelination. This was increased to 0.143 of Ramipril-treated group. The difference was statistically significant (p<0.05), and suggests restoration of myelin production in the drug-treated group.

Ramipril mitigates radiation-induced spinal cord damage, and prevents paralysis. Further studies are planned to elucidate pathogenesis of radiation injury with and without ramipril. The drug has a great translational value for radiation therapy. Further studies are needed to optimize the drug dosage and timing of administration before translating to clinical use.

**POS23-62. Mitigation of radiation-induced gastrointestinal syndrome in mice by R-spondin1 and ICG-001, a selective antagonist of β-catenin-mediated transcription.** Subhratij Saha1, P. Bhandari, L. Ho Lee, S. Ma, L. Yang, N. Kaul2, C. Guha2, 1: Albert Einstein College of Medicine, USA 2: USC, USA

Purpose: Radiation-induced gastrointestinal syndrome (RIGS) results from direct cytotoxic effects on intestinal crypt and endothelial cells with disruption of mucosal homoeostasis, pro-inflammatory cytokines, and apoptosis leading to diarrhea, weight loss, and mortality. There are no approved mitigating therapies for RIGS. The Wnt-b-Catenin pathway critically regulates the intestinal homeostasis by regulating proliferation and differentiation of intestinal stem cells (ISC). We hypothesized that a sequential administration of a Wnt agonist, R-spondin1 (Rspo1) that could amplify the ISC, followed by ICG-001, a selective antagonist of the b-Catenin-CBP interaction that could accelerate differentiation of Rspo1-stimulated ISC in crypt-villi axis, would promote intestinal regeneration and mitigate RIGS.

Methods: C57Bl/6 mice were treated with recombinant adenoviruses expressing human Rspo1 (55*10^6 PFU/mice, 1hr and 48 hr post-IR) followed by ICG-001 (150mg/kg of body weight, 72 hr post-IR) after whole body irradiation (WBI) of 9.4-10.4 Gy. Animals were observed for survival (Kaplan-Meier) and histopathological analysis (hematoxylin-eosin staining, TUNEL, and Ki67 immunohistochemistry). Expression of mRNA levels of b-Catenin target genes in crypt cells was determined by qRT-PCR.

Results: All mice, exposed to WBI died within 10-15 days without any treatment. In contrast, 70% (p<0.007) and 60% (p<0.003) of mice that received WBI and 9.4Gy and 10.4Gy, respectively, survived more than 30 days after treatment with AdRspo-1-ICG-001. Mice treated with AdRspo-1 or ICG-001 alone, failed to improve survival after WBI. Histopathological evaluation of jejunal demonstrated larger crypt depth and intact villi in AdRspo-1-ICG-001-treated animals, compared to controls indicating structural regeneration of the irradiated intestine. qPCR analysis of genes associated with ISC differentiation showed increased expression of a canonical Wnt target (β-catenin) and a non-canonical Wnt target (Dkk-1) in AdRspo-1-ICG-001-treated animals, compared to WBI controls.

Conclusion: Post-exposure sequential treatment with Rspo1 and ICG-001 modulates the Wnt-b-Catenin pathway in intestine, resulting in accelerated regeneration and improved survival. Thus Rspo1 and ICG-001 could represent novel mitigators of RIGS.

**POS23-63. ALXN4100TPO, an anti-apoptotic TPO agonist, induces cytokines to stimulate hematopoietic recovery following total body irradiation.** Merriline Satyamitra1, E. Lombardini2, C. Mullaney1, V. Srivinvasan1, 1: Armed Forces Radiobiology Research Institute, USA 2: VSD/AFRRI, USA

Recently, we demonstrated that ALXN4100TPO (4100TPO), a thrombopoietin receptor agonist, had potent radiation countermeasure efficacy with dose reduction factors of 1.32 (prophylactic, 24 h pre-) and 1.11 (mitigatory, 12 h post-irradiation [TBI]) (Satyamitra et al., Rad. Res. 2011). 4100TPO ameliorated radiation injury by promoting multi-lineage repair to the hematopoietic tissue. The current study investigated the effect of prophylactic (24 h pre-) or therapeutic (12 h post-TBI) 4100TPO-treatment (1 mg/kg, sc) in 7 Gy irradiated (cobalt-60 source, 0.6 Gy/min) mice using (1) Cytokine expression in serum, (2) TUNEL staining in bone marrow stroma, and (3) Micronuclei formation in mouse bone marrow cells. On day 4 post-TBI, irradiation increased inflammatory mediators such as IL-1β (proinflammatory), IL-6, IL-13, and IL-17, and others IFN-γ and TNα-α over irradiation controls, while reducing IL-1β and IFN-γ. Irradiation also increased apoptosis as quantitated by TUNEL- positive cells in bone marrow stroma. 4100TPO reduced expression of TUNEL- positive cells and improved cellularity, regenerative foci and megakaryocytes (day 7, p<0.01). 4100TPO reduced the number of micronucleated reticulocytes observed following 7 Gy (p<0.001 compared to vehicle) and facilitated faster restoration of erythropoesis by normalizing the polychromatic to normochromatic erythrocyte (P/N) ratio by days 7 and 10, for pre- and post-TBI drug treatment, respectively. We hypothesize that 4100TPO acted as a radiation countermeasure by induction of key cytokines that stimulated proliferation of multi-lineage hematopoietic progenitors while decreasing DNA damage and apoptosis of the blood forming cells.

**POS23-64. DNA-PKCs and H2AX phosphorylation during apoptosis signaling in bone marrow granulocytes irradiated in vivo and the role of amifostine in these responses.** Helena Segredo, C. C. Addo, L. V. Y. Nozawa, S. Bamba, V. C. Texiera, R. Segredo, Federal University of Sao Paulo, Brazil

Purpose – to assess the DNA-PKcs and H2AX expression and phosphorylation during p53 and p38 apoptosis signaling in bone marrow granulocytes irradiated in vivo and the role of amifostine (Ami) in these responses.
Material an Med – 120 mice were assigned in Gi (physiological saline solution – PSS intraperitoneally – IP), GiII (Aji 400mg/kg IP), GiIII (PSS IP + 7Gy whole body radiation dose), GiIV (Aji IP 30’ prior to 7Gy). Imawoohsiochemistry (IHQ) and flow cytometry (FC) were performed to assess protein expression: caspase 3, cleaved caspase 3, p53, p53, p38, p38, (IHQ) and DNA-PKcs, DNA-PKcs-P (Thr2647), H2AX, p-H2AX (Ser 139) (FC). FC results were submitted to statistical analysis with ANOVA, Bonferroni’s test (significance level for transmission of effector molecule response (TEM) was used for ultrastructural characterization of apoptosis. Results – Aji significantly reduced the number of apoptotic cells; radiation induced p53, p38, H2AX phosphorylation and significantly reduced the expression of phosphorylated DNA-PKcs (GiII). Aji itself induced p53, DNA-PKcs-P (Thr2647) and p-H2AX but did not induce p-p38 expression (GiII); when injected 30’ prior to 7Gy Aji significantly induced the phosphorylation of DNA-PKcs, markedly reduced p53 and p38 phosphorylation and did not influence the expression of p-H2AX.

Conclusion – Data show that after whole body high radiation dose, Aji induced the activation of DNA-DSB repair pathway via DNA-PKcs, and markedly reduced the apoptosis signaling pathway via p53, p38.

Considerin the impact of granulocytic response for the survival of irradiated patients, and the use of Aji for the normal tissues radioprotection during radiation oncology, the knowledge of the molecules involved in death/survival pathway of this cell lineage is highly desirable and with potential clinical applications.

**POSTER PRESENTATIONS**

**POSTER PRESENTATION**

**Pos23-65. Improved decoporation of the actinide radioelement Am-241 with a novel orally available formulation of DTPA — efficacy and pharmacokinetic studies.** Gita Shankar¹, G. Shankar¹, W. Weber¹, M. Doyle-Eisele¹, N. Bejugam², M. A. McShan³, C. Green¹, R. Guilmot¹, 1: SRI International, Menlo Park, USA; 2: Lovelace Respiratory Research Institute, Albuquerque, USA.

The partial destruction of Japan’s Fukushima Daiichi nuclear power plant resulted in a mass contamination of the area surrounding the plant and forced thousands of people to evacuate their homes. A radioactive byproduct of nuclear fusion, Americium 241 (Am-241) is present in spent reactor fuel. The drug of choice for removing Am-241 from a contaminated person, a process called decoporation, is diethylenetriamine pentaacetic acid (DTPA). Because DTPA has poor oral bioavailability, the current methods of drug delivery are iv or solution nebulization. To facilitate decoporation for large groups, we are developing orally bioavailable formulations of DTPA; in this study, we evaluated dog decoporation of a novel tablet formulation of the Ca and Zn chelate forms of DTPA (SRI-DTPA). The efficacy of SRI-DTPA was tested in groups of 18 beagle dogs (9 male 9 female) injected intravenously with a soluble Am241 citrate complex. On Day 1 after the Am-241 injection, each dog was given either a 100 mg or a 200 mg tablet of Ca-DTPA by gavage twice daily at an interval of 8 hours. On Days 2 through 7, each dog was given either a 100 mg or a 200 mg tablet of Zn-DTPA twice daily. Each dog was placed in a metabolism cage to enable complete, separate collection of urine and feces, and the treated animals were euthanized 7 days after receiving the last dose of Zn-DTPA (14 days after Am-241 injection). SRI-DTPA was shown to significantly increase the excretion of Am-241 for all treatment times; statistically significant decreases in liver and bone content of Am-241 were also observed. For example, for SRI-DTPA treatment in high dose group, the total body content of Am-241 in the treated animals was reduced to 39% of the injected dose, compared with 74% in the untreated animals. The pharmacokinetic data reflects the efficacy results: a 200 mg Ca or Zn-DTPA tablet, administered orally, resulted in an area under the curve (AUC) that was about 25% of the AUC after a single iv dose of 100 mg/dog. The mean time to maximum plasma concentration (Tmax) values and bioavailability in the tablet groups were 1.25 ± 0.65 hr and 12.09 ± 3.32%, respectively.

We concluded that this formulation of DTPA is very effective in decoporation of Am-241 in dogs. This project has been funded in full with federal funds from NIAID/NIH/DHHS under contract no. HHSN26620050029C and HHSN26620050047C.

**Pos23-66. Teocophor succinate-induced granulocyte colony-stimulating factor protects mice against radiation injury and mobilizes progenitors.** Vijay Singh, P. Singh, D. Brown, AFRRI, USHIS, USA.

We have elucidated the role of α-tocopherol succinate (TS) and TS-induced granulocyte colony-stimulating factor (G-CSF) in protecting mice from radiation injury. Mice were injected with TS 24 h before exposure to gamma-radiation and survival was monitored. Jejunum sections were analyzed for crypt and villi, and apoptosis. The crypt regeneration in irradiated mice was also evaluated. Bacterial translocation from gut to heart, spleen, and liver in TS-treated and irradiated mice was evaluated by bacterial culture. TS was also evaluated for cytokine induction by multiplex LumineX and mobilization of progenitors by flow cytometry. Our TS results demonstrated that TS-enhanced survival in a significant number of mice when irradiated with different doses of radiation inducing hematopoietic and gastrointestinal syndromes. TS protected intestinal tissue of irradiated mice in terms of crypt and villi, and mitotic figures. TS treatment decreased the number of TUNEL-positive and increased BrdU-positive cells in jejunum compared to vehicle-treated mice. Further, TS inhibited gut bacterial translocation to heart, spleen, and liver in irradiated mice. TS also induced very high levels of G-CSF in peripheral blood. Protective efficacy of TS was completely abrogated by administration of G-CSF antibody. Histopathology of jejunum demonstrates abrogation of intestinal post radiation by administration of G-CSF antibody. TS induced G-CSF mobilized progenitors which in turn mitigated radiation injury upon transfusion to irradiated mice. In conclusion, induction of G-CSF by TS administration is responsible for protection from g-radiation injury. G-CSF-mobilized progenitors mitigate radiation injury.

**Pos23-67. In situ gamma spectrometry as a tool of building material radiation safety assessment?** Andrzej Solecki, K. Nowak, W. Śliwiński, D. Tchorz-Trzeciakiewicz, University of Wrocław, Institute of Geoscience, Poland.

The aim of the present study was to compare the effectiveness of in situ gamma spectrometric method with traditional laboratory techniques for evaluation of building material radiation safety. National and international safety standards are based on the assumption that the effective dose should not exceed 1 (in some countries 2) mSv/A for building materials. This standard is based on laboratory measurements of activities of natural radionuclides: K (40K), U (214Bi), Th (208Tl) and their combination in form of radium equivalent or external dose index. Internal dose index (derivative of radium activity) is used for evaluation of risk connected with radon alpha radiation. Gamma dose rate, K, U and Th content of building stones and bricks was measured by means of portable RS230 gamma spectrometer with new generation BGO detector. Measurements were performed in storage piles of quarries comparable with laboratory results. Polish national standard F1 index of external dose limit and F2 internal exposure limit have been calculated. Simultaneously, gamma dose rate have been measured by means of EKO-D meter. Results have been compared with laboratory analyzes of collected samples performed by CLOR according to accepted national standards procedures resulting in analogous parameters: radionuclides content and F1, F2 indices. Obtained results indicate that accuracy of in situ gamma spectrometric results is comparable with laboratory ones, but single in situ measurement can be completed within 3 minutes, is significantly cheaper and faster than laboratory analysis. Such a measurement is representative for the significant amount (ca few hundred kilograms) of tested material. Time and cost reduction enables numerous measurements, what is especially important in the case of naturally variable building materials exploited in large quantities. In many cases, simple gamma dose rate measurement performed by inexpensive radiometer may be sufficient for the safety assessment of the building material. Obtained results provide justification for modernization of existing standards by including in situ gamma spectrometric methods.

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**Pos23-68. Time variation in equilibrium factor for radon decay products in cave dwellings in Gansu province, China.** Atsuyuki Iwasaki, S. Yoshinaga², Y. Honehara³, K. Sakai¹, S. Akiba², Q. Sun³, 1: Hiroaki University, Japan 2: NIRS, Japan 3: Kagoshima University, Japan.
Radon (222Rn) and its progeny in air are the most important contributors to human exposure to natural radiation sources. Although residential 222Rn was regulated by an action level of 200 Bq m⁻³ based on ICRP recommendations, in recent years it has been observed that an increase in lung cancer risk is associated with exposure levels below 200 Bq m⁻³. In view of such scientific data, WHO proposes a reference level of 100 Bq m⁻³ to minimize health hazards due to exposure to indoor 222Rn. Estimation of annual effective dose due to 222Rn and its decay products is based on dosimetric parameters, such as 222Rn concentration, equilibrium factor, occupancy, and dose conversion factor. There are many cave dwellings in the Chinese loess plateau in which the 222Rn concentration appears to be high. This study describes time variations in dosimetric parameters such as 222Rn concentration, equilibrium equivalent 222Rn concentration (EERC) and subsequently equilibrium factor (F) in cave dwellings in Gansu province, China. In this study, five houses in one village were investigated for radiation measurements. The cave dwellings consist of one room with a single entrance and two windows at the front side. Measurements were made at the end of July (no heating season) 2010. The 222Rn concentration and EERC in cave dwellings were monitored every one hour in a day. In most dwellings, tendency to increase in the nighttime was discernible for 222Rn concentration and EERC, which were observed to be more than 500 Bq m⁻³. In these dwellings, the F in the daytime was observed to be larger than that in the daytime. As an example of one investigated house, the values of F ranged from 0.3 to 0.9, and consequently, the corresponding average was 0.7, which was larger than a value of 0.4 recommended by UNSCEAR. Thus estimating annual effective dose due to 222Rn and its decay products, the use of F based on UNSCEAR recommendations may not be reasonable in the dwellings where the measurements were made in this study. To obtain a clear solution for this question, further experiment would be necessary.

**POS23-69. Monitoring and assessment of occupational radiation exposure in non-uranium mines in China.** Xu Su, Y. Ji, Q. Sun, National Institute for Radiological Protection, China CDC, China There are approximately four millions employees working currently in the non-uranium mine in China. This work is to investigate and assess the radiation exposures to the nationwide miners. We performed a survey including 2836 miners who are employed in selected 44 mines of 17 different categories in 12 provinces. The levels of radon and thoron of these selected mines were determined by integrated detectors. An environmental and personal detector was used to monitor external radiation and the radioactive levels of 32 ore samples were measured by HP Ge gamma-ray spectrometry. Scoring of chromosome aberrations and ICP-MS analyses of hair/nail samples of miners exposed to high radon are presented. The data shows that the level of radon in 15 percent of the selected mines exceeded 1000Bq m⁻³ (national action level of radon in workplace) and in 7 percent was over 3700Bq m⁻³. The equilibrium factor ranged from 0.1 to 0.6. The exposure dose of external radiation to miners was lower than 1 mSv/a in most of the non-uranium mines. Chromosome aberration was observed in those employees working in high radon environment and risk of developing lung cancer in these miners was evaluated.

Employed workers in coal mine were protected from radon radiation with well equipment. However miners who were employed in 15 percent non-uranium mines with high level of radon have high risk to suffer lung cancer without occupational protection. There is an urgent need to establish effective regulation to control the external radioactive level and effective method to protect the miners who are working in high radon environment.

**POS23-70. Exposure to UV/A, gamma-, and cosmic types of radiations alter vitamin A1 metabolism and up-regulate vitamin A2 biosynthesis in cultured human keratinocytes.** Juliana Tafrova¹, M. Simeon², S. Tafrov², ¹ Stony Brook University, USA 2: Brookhaven National Laboratory, USA The skin, which serves as a barrier against environmental insults, is the largest organ in the human body, and thus, accounts for most of the radiation exposure of astronauts during space explorations. The epidermis, the top layer, is one of the many target tissues of Vitamin A. This vitamin and its metabolites (retinoids) play an important role in the normal homeostasis and differentiation of the epidermis, and serve as a signaling molecule that modulates the skin’s response to environmental insults. Retinoic acid (RA), the active form of Vitamin A, regulates the expression of pre-apoptotic genes, and sensitizes keratinocytes (Kc) to apoptosis and thus, prevents the development of nonmelanoma skin cancers after radiation exposure. On the contrary, the retinoids also might limit radiation-induced apoptosis by restricting oxidative stress via Stra13 that controls the cellular sensitivity to reactive oxygen species (ROS). RA is involved in the cycle arrest and apoptosis.Besides the RA, human Kc uniquely can convert Vitamin A1 to A2, and then into its active form 3,4-didehydroretinoic acid (3,4-d-dRA). In *vitro* and *in vivo* studies showed that the biological potency of 3,4-d-dRA is similar to that of RA. Retinoids exert their function in a very narrow mM concentration range and thus, changes in the concentration of the active metabolites in the epithelial cells, or selective alterations of the expression of the retinoid receptors therein can differentially regulate the outcome of radiation exposures, leading to either cell survival or death depending on the severity of the damage. Thus, *in vitro* and *in vivo* analyses of retinol metabolism in normal and malignant human Kc. Our preliminary data demonstrated that vitamin A2 synthesis is upregulated after exposure to UVA, UVB, gamma-rays, and helium and neon ions. Furthermore, we showed that both vitamin A1 and A2 reduce UV-induced apoptosis; Vitamin A2 was the more effective one. Our data also shows that A2 prevents the oxidative stress from the effects of exposure to UVB photodecomposition. After irradiation, this pool might be accessed to lessen the oxidative stress. Our study can provide essential information for understanding the skin’s responses to different types of radiation that will be invaluable in the development of accurate countermeasures.

**POS23-71. Radiation-induced lung injury.** Raziyeh Tahamtan¹, G. H. Hadadi¹, A. Shabestani Monfared¹, ¹ Babol University of Medical Sciences, Iran 2: Fasa University of Medical and Paramedical Sciences, Iran Radiation-induced injury to the lungs is well recognized as a potential complication in the treatment of patients with carcinomas of the breast, lung, or esophagus; lymphomas; or other mediastinal neoplasms. The risk of radiation-induced injury is directly related to the volume of irradiated lung. The distinction between two separate types of radiation-induced lung injury, radiation pneumonitis and radiation fibrosis, was made in 1925. It is manifested as two distinct phases: an early phase characterized as radiation pneumonitis (RP) and a late phase of pulmonary fibrosis which subsequently develops. Most radiation pneumonitis occurs up to ~2 months after completion of the radiotherapy course and is considered to be an acute-phase response in 20% patients thereafter it is a common complication that affects a patient’s quality of life. Risk of developing radiation pneumonitis, including radiation dose, volume of irradiated lung, mean lung dose, fractionation schedule and dose rate, and applications of concurrent chemotherapy.In most patients, radiation pneumonitis involves a mild change while in some it develops into diffuse widespread pneumonitis affecting the contralateral lung, leading to progressive respiratory insufficiency. Radiation pneumonitis is characterized by enlargement and atypia of type II pneumocytes, alveolar wall edema, infiltration of inflammatory cells in the interstitium, aggregation of alveolar macrophages and hynaline membranes lining the alveolar ducts and alveoli. Fibrosis typically evolves within the next two years and usually remains stable. Fibrosis is an accumulation of fibron and atypical fibroblasts in the interstitium. RESULTS: Although new modalities such as IMRT and stereotactic body radiation therapy provide new treatment options and several radioprotectors have also been evaluated to reduce radiation injuries in animals and humans, such as amifostine melatonin and vitamins like E, C and A, but also pose new challenges in safely delivering thoracic RT. KEYWORDS: radiotherapy, radioprotector, lung tissue.

**POS23-72. Radioactive compounds produced in a medical cyclotron and radioactivity in the surrounding concrete.** Yasuyuki Takahashi¹, K. Saito¹, H. Shimada¹, N. Oriechi², I. Yamaguchi¹, ¹ Gunma Prefectural College of Health Sciences, Japan 2: Gunma University Graduate School of Medicine, Japan 3: National Institute of Public Health, Japan We estimated the level of radioactivity in a medical cyclotron and the surrounding concrete wall. The cooling period to attenuate residual
high hydrogen content and recently confirmed in situ availability. In addition, the density of water (1 g/cm³) is nearly that of most biological tissue providing a rough model to investigate the effects of high energy neutrons as they traverse the human body. The neutron radiation shielding effectiveness of water was studied using confluent human fibroblast cells (AG01522) exposed to a beam of high-energy spallation neutrons (≥ 800 MeV/n) at the 30°-left beam line (ICE house) at the Los Alamos Neutron Science Center. At this angle, the radiation spectrum is dominated by spallation neutrons (≤ 800 MeV/n), generated in the upper atmosphere or encountered when aboard spacecraft and high-altitude aircraft as well as the albedo production from the Moon’s surface. Cell samples were exposed in series directly to the neutron beam (0.1 & 0.2 Gy) in T-25 flasks completely filled with either medium or water up to a depth of 20 cm to test shielding effectiveness versus depth and investigate the possible influence of secondary particle generation. All samples were sent directly back to JSC for sub-culturing and micronucleus (MN) analysis. Our results showed a decrease in MN formation beginning at 4 to 5 cm of water with a significant reduction at 10 cm before reaching a plateau of approximately 40% of the unshielded MN formation. We performed a dose response at the 10 cm depth and determined that the biological effect was diminished by a factor of 3.5 based upon the slope of the lines. We also conducted microdosimetry using a tissue equivalent proportional counter (TEPC) and calculated the absorbed dose and deposited energy per unit depth as well as characterizing the differential neutron spectrum. This presentation is of work performed at NASA-JSC in collaboration with the NASA sponsored Center for Radiation Engineering and Science for Space Exploration (CRESSSE) at Prairie View A&M.

POS23.75. Mitigation of the hematopoietic and gastrointestinal syndromes by Rx100 treatment. Gabor Tigygi, G. Tigygi, W. Deng², V. Gudduduri¹, W. Shannon McCool¹, D. D. Miller¹, L. R. Johnson¹, K. Emmons-Thompson³, C. Ryan Yates², L. Balazs², E. Tolley², T. MacVittie³, Y. Geng³, K. Van Rompuy³, ¹University of Tennessee, ²University of Texas, ³University of Maryland Baltimore, ⁴California National Primate Research Center Davis, USA

Rx100 is a small molecule metabolically-stabilized agonist of the lysophosphatidic acid receptors. To test the effect of Rx100 in radiation injury models the C57BL/6 Mouse abdominal (ABD) GI-ARS model with head, chest and legs shielded (LDED12.15 = 15 Gy, ¹³⁷Cs = 260 cGy/min) and C57BL/6 Mouse TBI, H-ARS model (LDED50 dose 6.5 Gy, ¹³⁷Cs at ~320 cGy/min) were applied. Efficacy testing was done using single dose Rx100 treatment administered at +24, 48, and 72 hour post-irradiation. PK and efficacy was also determined in Macaca mulatta (+/- irradiation, LINAC, 11.5 – 12.5 Gy PBI with 5% BM shielding, PBI/BMS). Multiple biomarkers and histological parameters were analyzed. A single dose of Rx100 administered up to 72 hour after irradiation increased the radiation induced biological effects by 2.5 fold compared to 72 hour after irradiation in C57BL/6 exposed to an H-ARS LDED50/3 TBI. Rx100 treatment improved white blood cell and platelet counts. In the ABD GI-ARS model, Rx100 increased survival by ≥40% and showed a DMF of 1.2. Rx100 treatment increased crypt survival, protected carrier-mediated glucose absorption, and prevented endotoxemia. Rx100 was non-toxic at doses that are 50-100-fold higher than the effective dose. In nonhuman primates (NHP), Rx100 exhibits linear pharmacokinetics following S.Q. administration at doses ranging from 0.3 mg/kg to 3 mg/kg. Rx100 systemic exposure and half-life following S.Q. administration in irradiated NHPs were significantly different when compared to unirradiated NHPs. In NHP Rx100 treatment improved crypt survival. One striking feature in Rx100 treated NHP was the sparing of the duodenal Brunner glands that showed radiation-induced atrophy and fibrosis at 35 days postirradiation in placebo-treated NHP. Protection of the Brunner glands by Rx100 might play an important role in preserving bicarbonate secretion in the duodenum and restoring the digestive function in the small intestine.

POS23.76. Polymer complexes of WR2721 and their radioprotective efficiency. Ivelina Tsacheva¹, N. Koseva¹, K. Troev², S. Topalova², R. Georgieva³. ¹Institute of Polymers, ²Bulgarian Academy of Sciences, Bulgaria 2: National Center of Radiobiology and Radiation Protection, Sofia, Bulgaria

This study describes synthesis, characterization and the evaluation of radioprotective efficiency of newly synthesized polymer complexes of WR2721 – the most recent chemical radioprotector to become available clinically. Several studies have demonstrated that...
amifostine protects normal tissue from both acute and late radiation damage without protecting the tumor, i.e. amifostine is a selective cytoprotector of normal tissues. The polymer complexes were constructed from WR2721 and poly(hydroxyoxyethylene phosphate) via ionic bond or poly(methoxyoxyethylene phosphate) via hydrogen bonding. Polyphosphates are obtained from poly(oxyethylene H-phosphonate) - biodegradable, hydrophilic with IC50 = 1000 mg/kg, that contain PEG moieties and suitable multifunctional sites for immobilization of poly(oxyethylene H-phosphonate). Polyphosphates are especially attractive materials due to the relative easiness of their preparation from commercially available building blocks, the variety of molecular weights attainable and the relatively narrow molecular weight distributions of the polymers formed. The structure of the complexes formed is elucidated by 1H, 13C NMR and FT-IR spectroscopy. The radioprotective activity in respect to the type of the carrier and the bond character of the drug-polymer complexes has been evaluated by the following parameters - Protective Index, Therapeutic Index, and Dose Reduction Factor. The polymer complexes show significant radioprotection efficiency when compared to the basic components. When WR2721 is immobilized on poly(hydroxyoxyethylene phosphate), the polymer complex has the highest protective activity, as is demonstrated by Protective Index (the calculated values are 14.04 and 17.78 for active agent equivalent doses of 50 mg/kg and 200 mg/kg, respectively), and low toxicity (the value of Therapeutic Index - 9.36). Results obtained are rather promising and further investigations on the mechanism of action and application schemes of WR2721 polymer complexes are in progress.

Electron accelerators are being routinely used for cancer treatment. Due to high linear energy (up to 25 MeV), a significant number of neutrons is created by (y,n) reactions when high energy photons interact with the materials of the accelerator head. Neutron leakage radiation reaches the patient, contributing additional unwanted dose to the patient and thus the total neutron fluence must be measured precisely. Neutron activation detectors are one of the best options for the patient and radiation reaches the patient, contributing additional dose. Neutron activation detectors are one of the best options for the measurement of the leaked neutrons, yet the majority of previous studies are calculating neutron fluences with Monte Carlo simulations. Usually gold and indium are used as activation detectors in experimental studies. In this preliminary work, we measured neutron fluences with neutron activation detector technique. The LInear ACcelerator (LINAC) under consideration is a 18 MeV Varian Clinac 2100C electron accelerator operating at Papageorgiou Hospital, Thessaloniki, Hellas. We measured the total neutron and photon fluence at the isocenter within a 10x10 cm2 X-ray field by nickel, indium, and natural uranium activation foils. All foils returned comparable results. For instance, the total neutron fluence derived from indium foil is 7 x 106 cm-2/Gy. This number is in the range with other studies of similar accelerators. The results of our Monte Carlo simulations, which replicate the experimental setup, are presented and discussed.

Purpose: There are about 1.08 million permanent residents in the range of 30 km away from Tiaowan Nuclear Power Plant (TNPP). To analyze the diet structure and to estimate the internal radiation dose of Residents in vicinity of TNPP before its commercial operation within the area of 30km, to provide scientific base for evaluation the impact of nuclear power plant radiation on residents.

Method: Survey target group are those local residents living in the range of 30km away from TNPP over 6 months. The actual effective number is 19,138. Stratified random sampling method was adopted. The survey includes basic personal circumstances and the diet. According to the Food Category Principle and Internal Radiation Dose Estimation Method in UNSCEAR 2000 report, the consumed amount of public milk products, meat products, grain products, leafy vegetables, root vegetables and fish products in different survey areas and of population categories are calculated, the concentration of uranium and thorium series radionuclides was Calculated, and internal radiation dose was Analysed using dose conversion factors.

Result: According to the survey result, Fishermen consume much more fish products and grain products than the rest of the population, while urban residents consume more milk products. Milk products consuming are increasing in line with the distance increasing, but the fish products and grain products consuming is decreasing contrary to the distance increasing. Public around TNPP consume more grain products, leafy vegetables and fish products, compared to the world average food consumption. Committed dose equivalent is 691 μSv•a-1 to children, the figure is 327 μSv•a-1 to adults, 536 μSv•a-1 to the fishermen and adults living around TNPP is 3.5 times and 3.0 times of the world average. In the category of adults, the internal committed effective dose is decreasing from fishermen to salt farmers, urban residents and fishermen.

Conclusion: 226Ra, 228Th and 232Th are the key nuclides which affect the internal radiation dose by diet in fishermen and live on food from the outcome. It should be pay more attention for the internal radiation dose estimation and evaluation for the fisherman, because of their living around the TNPP and the dietary pattern; we should Investigate the regular changes of dietary status and recipes, because Dietary structural features and some high activity of radionuclides content in the food maybe the contributor to the high resident committed dose equivalent.

POS23-79. Protective effect of rosmarinic acid on X-ray induced developmental toxicity in zebrafish embryos. Zhen-hua Wang1, Xiao-wei Wang2, Xin Zhou1, Hong Zhang1, Zhen-wei Wang, Xin Zhou1, Hong Zhang1, Xin Zhou1, Hong Zhang1, 1: Institute of Mordern Physics, Chinese Academy of Sciences, Lanzhou 73000, China; 2: School of Pharmacy, Shinte University, Shihiezi 832002, China

Rosmarinic acid (RA) is a natural phenol antioxidant carboxylic acid found in many Lamiaceae herbs used commonly as culinary herbs such as lemon balm, rosemary, oregano, sage, thyme and peppermint[1]. It has proved that RA possesses a number of interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant. Many studies have shown that in a chemical system natural polyphenols can. Zhang Q et al found that RA dose double the DNA oxidative damage induced by H2O2 in ovary cells, which might be due to its non-enzymatic fast repair mechanisms[1]. For the irradiation injury in biological system results from the reactive oxygen species (ROS) including H2O2, it is supposed that RA may possess the radioprotective effect. Here we investigated the protective effect of RA on X-ray induced developmental toxicity in zebrafish embryos. The AB strain zebrafish embryos (5 hpf) were pretreated with a single dose of RA (100 μmol/L), then were exposed to various doses (5, 10, 15 or 20 Gy) of X-ray irradiation (produced by the Faxitron X-Ray Systems, 130 kV at the dose rate of 1.0 Gy/min). The effects of irradiation on embryo’s development were observed in 3 days after the irradiation. Thirty minutes later the irradiation, the nucleat DNA damage in zebrafish embryo cells were detected by basic comet assay[2] and the mitochondrial DNA (mtDNA) damage were measured by quantitative polymerase chain reaction assay[3]. Moreover, the ROS levels in embryos were measured by the fluorescent probeDCFH-DA. All the results were shown in Fig. 1. The X-ray irradiation caused the death and the typical malformation changes including tail bending, severe spinal distortion, enlarged pericardial sacs and shorter body length in zebrafish embryos. The mortality and malformation rates increased with the increase of radiation dosage. Furthermore, the X-ray irradiation induced the serious nuclear DNA damage (indicated by the Olive Tail Moment (OTM)), mtDNA damage and the ROS formation in a dose-dependent manner in zebrafish embryos. The RA pretreatment significantly reduced the mitochondrial DNA damage and the ROS formation, while the single RA treatment didn’t show any effect on the development of zebrafish embryos. Our study demonstrated that RA pre-treatment dramatically protected the zebrafish embryos from the X-ray irradiation injuries. The protective effects might be due to its protection on nuclear DNA and mtDNA damage. Acknowledgements This study was supported by the National Basic Research Program of China (973 Program) (2010CB834202) and China Postdoctoral Science Foundation Funded Project (200902313).
Because of the weaknesses in safety and security management of radioactive sources production, storage, transport, use and disposed in our country, which make the potential threat of “dirty bomb” more possible. The most potential threat currently is to disperse radioactive substance in a city area, certainly not excluding the likelihood of other terrorism events. This paper is intended for analyzing the characteristics for the terrorism events of dirty bomb firstly, and then discussing the origin material for RDD, at last suggesting briefly the consequence coming from RDD attack.

Key words : nuclear and radiological terrorism, radioactive dirty-bomb, radiological injury.

POS23-82. The rate of the micromolecule and HPRT gene mutation in peripheral blood lymphocytes of inhabitants around Tianwan nuclear station. Du Xiang, Jiangsu Province Center for Diseases Control and Prevention, China

The aim of this research is to establish the database and get the background information of the micromolecule and HPRT gene mutation rates in peripheral blood lymphocytes of inhabitants around Tianwan nuclear station. Stratified cluster random sampling was used to get the sample of the inhabitants. According the distance from the village they lived to the plant, we classified the 631 inhabitants to 4 groups: 1-4 km group; 5-8km group; 9-15km group and 16-30 km group. Every investigate spot is selected by simple random sampling method. The results showed that the male inhabitant had a micromolecule rate of 4.0±2.15% and the HPRT gene mutation rate of 0.8646±0.45%. Micromolecule rate of the female is 5.15±2.69%, HPRT gene mutation rate is 0.880±0.60%. Average micromolecule rate of all 631 inhabitants is from 4.22% to 5.26%, and average HPRT gene mutation rate is from 0.6184% to 1.0319%. Micromolecule and HPRT gene mutation rate showed their perspective in radiological research, and it’s important for us to get the background database of micromolecule and HPRT gene mutation rate in correctly evaluating the exposure dose of inhabitants around nuclear station or estimation exposure dose under accidental circumstance.

POS23-83. Attenuation of oxidative stress-induced cellular damage by baicalein (5,6,7-trihydroxyflavone) via antioxidant effects. Rui Zhang, M. Jing Piao, K. Cheon m, A. Daseul Kim, J. Won Hyun, School of Medicine, Jeju National University, South Korea

Baicalein (5,6,7-trihydroxyflavone) is a phenolic flavonoid compound derived mainly from the root of Scutellaria baicalensis Georgi, a medicinal plant traditionally used in oriental medicine. Ionizing radiations produce reactive oxygen species (ROS) or related radicals, resulting in cellular and tissue damages. In our previous study, baicalein attenuated mitochondrial oxidative stress by scavenging reactive oxygen species and by induction of NF-E2-related factor 2 transcription factor-mediated manganese superoxide dismutase. In present study, the protective effects of baicalein against oxidative stress-induced damage, especially cellular components including DNA, lipid, and protein, were studied. The results of this study showed that baicalein scavenged intracellular ROS. Baicalein inhibited the oxidative stress-induced DNA damage that was demonstrated by decreased phospho-H2A.X expression and DNA tail formation. In addition, it prevented the lipid peroxidation shown by the fluorescence intensity of diphenyl-1-pyrenylphosphine and the formation of thionobarbituric acid reactive substances. Moreover, baicalein inhibited the protein oxidation demonstrated by protein carbonyl formation. Furthermore, baicalein protected cells via the inhibition of apoptosis induced by oxidative stress. The findings of this study suggest that baicalein provides protection of cellular components against oxidative damage via scavenging ROS and inhibiting apoptosis.

POS23-84. Insulin-like growth factor 1 mitigates against lethal irradiation and enhances hematologic and immune recovery in mice. Dunhua Zhou, D. Deoliveira, Y. Kang, S.S. Choi, N.J. Chao, B.J. Chen, Division of Cellular Therapy/BMT, Duke University Medical Center, Durham, NC, USA

Background and Hypothesis: IGF-1 has direct proliferative effects on hematopoietic stem/progenitor cells and therefore can be applied to accelerate hematopoietic recovery post-radiation exposure. Specific Aims: To determine the ability of recombinant human IGF-1 to mitigate against radiation injury in mice. Methods: BALB/c mice were lethally irradiated (7.5 Gy) and injected with one dose (500 mcg) of IGF-1 subcutaneously within one hour
POSTER PRESENTATIONS

PO23-85. Antioxidant properties of Withania somnifera and in vivo screening of radioprotective ability against oxidative stress in gamma irradiated rats? Deepthi Bhatnagar, School of Biochemistry, Devi Ahilya University, India

The in vitro antioxidant properties of Withania somnifera extract (WSE) were studied through various methods like free radical scavenging activity (FRSA) by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), total antioxidant power (TAP) using Ferric reducing antioxidant power (FRAP), total phenolic content (TPC) and metal chelating activity (MCA). The result showed that WSE has strong antioxidant properties like FRSA (24 %), TAP (1.01 µM/g), TPC (1.4 mg/g) and MCA (81 %) respectively. The WSE was further evaluated for the radioprotective ability by survival studies on rats. WSE (20 mg/kg body wt.) was administered (i.p) to rats for 5 days prior to whole body gamma irradiation exposure at 10 Gy and survival was observed for 30 days. Radiation exposure caused the symptoms of severe radiation sickness and all the animals died within 13 days in gamma irradiation, while WSE pretreatment reduced the symptoms of radiation sickness and increased the survival up to 33.3% as compared to the gamma irradiated group.

The radioprotection was also measured by endogenous spleen colony forming unit assay (CFU) and by anticlastogenic activity in bone marrow cells by micronucleus test in rats exposed to gamma irradiation exposure to rats at 2 Gy increased LPO and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and GSH content in liver and erythrocytes of rats exposed with gamma irradiation at 2.0 Gy was also assessed.

Gamma irradiation exposure to rats at 2 Gy increased LPO and decreased the activity of SOD, CAT and GST activity in rat liver and erythrocytes. Rats pretreated with WSE administration prior to gamma irradiation exposure to rats at 6.0 Gy, resulted in a significantly higher number of CFU counts (11.7/spleen) as compared to the irradiated group. There was a significant reduction of micronucleus formation in rats pretreated with WSE prior to gamma irradiation as compared to the concurrent irradiated group.

Lipid peroxidation (LPO), antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and GSH content in liver and erythrocytes of rats exposed with gamma irradiation at 2.0 Gy was also assessed.

Gamma irradiation exposure to rats at 2 Gy increased LPO and decreased the activity of SOD, CAT and GST activity in rat liver and erythrocytes. Rats pretreated with WSE with or without gamma radiation exposure showed decreased LPO and increased SOD and CAT activity while, GST activity was increased in liver but not in erythrocytes as compared to gamma irradiated animals. GSH content in liver and erythrocyte was increased in irradiated group as well as in WSE treated group without or with gamma irradiation. The results suggest that pretreatment with WSE prior to gamma irradiation exposure prepares the animals to sustain oxidative assault and may contribute for the possible radioprotection.

Key words: Antioxidant, radioprotection, gamma irradiation, oxidative stress

PO24 Low dose effects

PO24-01. The analysis of low-dose radiation effects on the non-traditional animals in vivo, Elena Saraputseva1, O. Melechkova2, J. Malina1, G. Kossova2, 1: Obninsk Institute of Nuclear Power Engineering National Nuclear Research University "MEPhI", Russian Federation, 2: Moscow State University, Russian Federation

In our studies the effects and some mechanisms of low-dose gamma radiation on the crustacean Daphnia magna have been analyzed. Given that the LD50 for this species is approximately 100 Gy, one-day-old daphnids were exposed to 10, 100 and 1000 mGy of acute γ-rays. It was found that the daphnids exposed to the doses of 100 and 1000 mGy significantly decreased the survival of irradiated daphnids. Most strikingly, the decreased survival of irradiated animals did not show a clear dose-response, thus suggesting that the non-targeted effects may underlie the observed changes. Significant, up to 20 %, increase of reactive oxygen species in daphnids irradiated at doses of 10–1000 mGy has been observed by means of the polymerization with isotope 3H.

Our data also showed that the observed decrease in survival of irradiated animals was attributed to their early aging, thus providing an important evidence for the effects of low-dose radiation in vivo on early senescence. Overall, the results of our studies have shown that dose radiation of 100 and 1000 mGy can result in a significant, up to 35%, reduction in life span of exposed daphnids. As the majority of irradiated daphnids showed reduced viability regardless the dose, our data therefore imply the presence of a threshold of dose of low-LET exposure capable of impairing the viability of this species.

In a separate study, we have also analyzed the effects of paternal low-dose radiation on the offspring of exposed daphnids. It was found that parental irradiation significantly affected the survival of first-generation offspring. Our data also showed that these effects, namely: the effectiveness of low doses, dose-independence, radiate-independent span of irradiated daphnids and preservation of those effects among the first-generation offspring cannot be attributed to the segregation of radiation-induced deleterious mutations.

Potential implications of our studies results for the risk assessment of low-dose exposure will be discussed.

PO24-02. Low-dose ionizing radiation alters the epigenome of the A1 mouse. Autumn Bernal1, D. Huang2, R. Jirtle2, 1: Duke University Medical Center, USA 2: Duke University, USA

Epigenetic aberrations disrupt normal development and influence the occurrence and progression of numerous diseases. To date, analyses of high doses of radiation show that epigenetic disruption occurs and is essential for the persistence of radiation-induced genomic instability; but the epigenetic response to low-dose ionizing radiation (LDIR) in vivo has not yet been defined. Here we use the viable yellow agouti (A1) mouse, a model shown to be a novel biomarker for identifying environmental exposures that can alter the epigenome, to analyze the effects of LDIR. We show that LDIR exposure in utero induces epigenetic changes in a dose-dependent and sex-specific manner in the A1 mouse. The A1 mouse was exposed in utero to 0, 0.6, 1.2, 2.5, 5.0, or 10.0 cGy of acute X-ray irradiation on day 4.5 of gestation. Upon weaning, mouse coat color was examined and tail tissue was extracted for methylation analysis. Using the Sequenom platform, CpG sites upstream of the A1 allele promoter were analyzed. Multifactorial ANOVA analyses were performed to look for main effects of dose and interactions between dose, sex, and CpG site on methylation changes. Chi-square analysis was performed to look at shifts in population coat color.

Analyses of all doses show that the sex of the animal, irradiation dose, and CpG site interact to affect methylation, with males showing greater changes in methylation (ANOVA, p<.0001). In males, 0.6 cGy of radiation has no effect on coat color or methylation; 1.2 cGy shifts the coat color to pseudogouti (X<sup>y</sup>, p<0.05), but does not significantly increase methylation; 2.5 and 5.0 cGy shift the coat color to pseudogouti (X<sup>y</sup>, p<0.05) and significantly increase methylation (ANOVA, p<0.05); 10.0 cGy shifts the coat color to heavily mottled (X<sup>y</sup>, p<0.05), but only increases methylation in 3 CpG sites (ANOVA, p<0.05).

Increased methylation at this allele increases the number of male pseudogouti animals. These animals have been shown previously to have lower levels of obesity, cancer, and insulin resistance. Thus, the radiation exposure could have beneficial health effects in A1 males. Whether other loci are similarly affected is currently being determined. Further investigation of additional genes will help to translate the risk of low-dose radiation exposure from mice to the humans.
POSTER PRESENTATIONS

POS24-03. Low dose radiation effects on DNA recombination and transcriptional control, Benjamin Blyth, R.J. Ormsby, P.J. Sykes, Flinders University and Medical Centre, Australia

At low radiation doses, effects on gene regulation may dominate over DNA damage and mutation. The pKZ1 transgenic mouse contains an inverted E. coli lacZ reporter gene, the expression of which can be detected in pKZ1 tissues via X-gal staining. Recombination events between immunoglobulin signal sequences flanking the lacZ gene can mediate chromosomal inversions that place the lacZ sequence under transcriptional control of the avian β-actin enhancer/promoter. Such chromosomal inversions can also be detected in pKZ1 tissues and cells by PCR, and inversion-dependent lacZ transcripts can be measured by quantitative PCR. We have confirmed by RT-PCR that pKZ1 lacZ gene expression can also be initiated from within the un-rearranged transgene using a hitherto unidentified cryptic promoter. We have previously observed changes in the frequency of X-gal positive cells in pKZ1 mouse spleen and prostate, and in a pKZ1 hybridoma cell line, following low and very low radiation dose exposures. Changes in lacZ expression in pKZ1 mouse tissues have been observed at radiation doses where other DNA endpoints lack the sensitivity to measure a radiation effect. pKZ1 responses have also been observed following DNA demethylation in vitro, amifostine radioprotector treatment in vivo and etoposide treatment in vitro and in vivo. The sensitivity of lacZ expression in pKZ1 has led us to investigate how low IR doses of radiation may be the result of detecting subtle changes in gene expression states following low dose stress. Preliminary results in pKZ1 hybridoma cells treated with etoposide show dose-dependent changes in the expression of both lacZ transcript types correlating with X-gal staining. We are now investigating how the two lacZ expression mechanisms (inversion-dependent and inversion-independent) correlate with the radiation-induced changes in X-gal staining using real-time quantitative PCR. The sensitivity of the pKZ1 lacZ responses to low and very low doses of radiation, and adaptive responses observed in two-dose protocols, may be explained by radiation dose-dependent modulation of the two independent mechanisms, one mediated by recombination, the other by regulating the use of alternate promoters/transcription start sites.

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POS24-04. Low dose IR-induced IGF-1-sCLU expression: a p53-repressed expression cascade that interferes with TGFβ1 signaling to confer a pro-survival bystander effect. David A. Boothman1, D. Klokov2, 1: UT Southwestern, USA 2: Radiological Protection Research and Instrumentation Branch, Chalk River Laboratories, Atomic Energy Canada Limited, USA

Inadvertent exposure of mammalian tissue to low doses of ionizing radiation (IR) from remediation of radioactive contaminated areas, space travel, radiation accidents, or a dirty bomb explosion represents an interesting trauma to an organism. Since not all cells are exposed or exposed equally at <10 cGy, possible low dose IR-induced bystander effects could seriously impact our understanding of subsequent health effects. To understand the biological responses induced after low IR doses, we generated a ‘biodosimeter’ using the human clusterin promoter fused to firefly luciferase (hCLU-Luc); secretary clusterin (sCLU), an extra-cellular molecular chaperone, is induced in cells to clear debris. Low IR doses (>2 cGy) induced hCLU-Luc activity with peak levels noted at 96 h, confirmed by Western blotting. Increased doses of IR (>5 Gy) greatly decreased the time (~24 h) required for peak CLU transcriptional and sCLU expression, and increased response amplitudes. Since sCLU expression was stimulated by insulin-like growth factor-1 (IGF-1) but suppressed by p53, we examined response in hCLU-Luc transgenic mice before and after a low IR dose. Interestingly, specific tissues (i.e., colon, spleen, thymus, bone marrow) of female mice induced CLU promoter activity more than male mice in response to >10 cGy whole body irradiation. Induction in vivo was mediated by Smad signaling, presumably by activated transforming growth factor-beta1 (TGFβ1) receptor-activated signal transduction. Elevated sCLU in vitro and in vivo induced TGFβ1 signaling, possibly mediating a negative feedback regulatory loop to stimulate wound healing and clear cell debris.

POS24-05. Debunking the myth of increased cancer incidence attributed to radiation from diagnostic imaging. Mohan Doss, Fox Chase Cancer Center, USA

Purpose: Recent publications have raised concerns about the increasing radiation dose from diagnostic imaging and the resulting enhanced cancer risk based on a linear non-threshold (LNT) extrapolation model. For example, it has been claimed that 0.9% of cancers in USA in 2004 may be attributed to prior X-rays and that approximately 29,000 future cancers can be attributed to CT scans performed in 2007. Such publications have received wide popularity in popular media and has raised public and government concerns regarding medical radiation. The purpose of this work is to show that these concerns are unjustified.

Methods: A two-pronged approach will be taken to debunk the myth. First, the basic assumptions of the LNT model will be analyzed to demonstrate the injudiciousness of using the LNT model for estimating low dose radiation risk. Second, a LNT model calculation will be performed to estimate the incidence of excess cancers attributable to medical radiation, and compared to the native cancer incidence rates. In this model calculation, the per capita medical radiation dose is assumed to increase from 0.41 mSv in 1965 to 0.54 mSv in 1980 and to 3.1 mSv in 2006, with a linear interpolation between the years. The LNT model based excess cancers from this medical radiation dose are assumed to be distributed in future years using an exponential lag time model.

Results: The model calculation shows that as much as 0.5% of cancers in 1992 and 1.5% of cancers in 2007 in USA could be attributed to radiation. In 2007, the estimated cancer incidence rate in the USA has decreased by -7% between these years. Since there has been ~1.4% year-to-year variation in the observed cancer incidence rates, it may not be feasible to detect the predicted increase in cancers attributable to medical radiation between these years.

Conclusion: LNT model appears to be unsuitable for estimating low dose cancer risk and hence the concerns regarding low dose radiation based on LNT appear to be unjustified. Even if LNT model is considered as valid, the predicted increase in cancer incidence appears to be within errors of measurement in view of the large observed variation in the cancer incidence rates. Hence, the tremendous efforts currently underway to reduce medical radiation dose may not result in any measurable health benefit, and should be reconsidered.


The effects of low and protracted doses of ionizing radiation on humans are only partially understood. The shape of the dose response curve at low and protracted doses cannot be accurately estimated using standard epidemiological methods. However, this information is needed for rationale policy decisions for setting exposure limits. Therefore, we set out to determine a gene expression signature that operates at low and intermediate doses using a panel of DNA double strand break repair deficient mouse models.

We developed mouse models that lacked one or both of the major DNA double strand break repair pathways (nonhomologous end-joining and homologous recombination) and compared transcriptional responses in these DNA repair deficient mice with normal mice before and after irradiation with a relatively low dose of gamma radiation (200 mGy). Importantly, the changes in gene expression patterns were nearly identical in repair-deficient mice and in irradiated mice. We identified a gene expression signature that was very similar, but considerably more pronounced (up to 5-fold) in irradiated DNA repair deficient mice compared to normal mice. Since the transcriptional responses are indeed caused by the DNA damage inflicted by radiation and that DNA repair deficient mice can be used as the proverbial ‘canary in the coalmine’ in the context of low dose radiation research. The identified gene expression signature includes Gadd45/α, Gadd45b, MDM2, BAG3, and CDKN1A (p21) and in addition IR-activated signaling cascades including the p53/MDM2 axis. We have validated this transcriptome approach by quantitative PCR on irradiated human blood and microarray analysis of mouse liver showed very similar results, indicating that this response is robust and reproducible.

We conclude that this transcriptional response can be used as a very sensitive read out for low levels of radiation exposure. This will be meaningful, as several genes from this signature have functions in known pathways that regulate cell proliferation and/or senescence and...
apoptosis, which are all relevant for carcinogenesis and (tissue) aging. This consistent gene expression signature defines a universal marker profile relevant for the general population, providing valuable tools for biomarkers, mechanistic understanding, prevention and therapy.

**POSTER PRESENTATIONS**

**POS24-07. What do we know about the genetic effects of low-dose ionizing radiation after 25 post-Chernobyl years?** Rose (Roza) Goncharova, Institute of Genetics & Cytology, National Academy of Sciences of Belarus

The radiation effects of low doses and low dose rates were shown for somatic and germ cells of mammals including human. Thus, we have revealed the genetic effects of low doses (ranging from close to background up to 10 cGy) caused by Chernobyl fallout in laboratory mice, bank vole, whose natural populations inhabited various regions of Belarus (8–1526 kBq/m²), and pond carp (Goncharova, 2000; Ryabokon et al., 2005; Ryabokon, Goncharova, 2006). Radiosensitivities of bank vole somatic cells and human peripheral lymphocytes, as well as of the germ cells of the laboratory mice are close to each other (Smolich, Goncharova, 2002). New phenomena induced by low doses were discovered, including inverse radiation dose-rate effect. We have shown that the genetic efficiency of the low-dose rate exposure calculated per unit dose is higher than that of acute irradiation with high dose rate (Smolich, Goncharova, 2002). Our data are in line with the data on cancer risks of low dose-rate exposure in the cohort of nuclear workers (Cardis et al., 2007) and the Techa’s cohort (Krestinina et al., 2007).

We have shown for the first time transgenerational transmission and accumulation of radiation damage attributable to the low-dose rate exposure of the preceding generations of bank vole. Genomic instability in subsequent generations of bank vole shows saturation at very low doses (Ryabokon, Goncharova, 2006, 2007).

A complex transcriptional response was revealed to be induced in somatic cells of very low-dose irradiated residents (Albanese et al., 2007) and workers (Morandi et al., 2009) and to differ under exposure to low and higher doses with respect to the number and type of differently expressed genes.

The yield, processing and biological consequences of clustered DNA damage are different at low and high dose exposure (RISC-RAD project, 2008). All these data give strong evidence that low-dose and low-dose-rate radiation responses differ from high-dose responses. There is an urgent need to understand better low-dose impact on immediate genetic damage and long-term effects, as genomic instability and cancer.

**POS24-08. Dose response for chromosome aberrations in human lymphocytes and fibroblasts after exposure to very low doses of high LET radiation.** Megumi Hada¹, K. A. George², F.A. Cucinotta³, 1: NASA-JSC/USRA, USA 2: Wyle 3: NASA Johnson Space Center, USA

The relationship between biological effects and low doses of absorbed radiation is still uncertain, especially for high LET radiation exposure. Estimates of risks from low-dose and low-dose-rates are often extrapolated using data from Japanese atomic bomb survivor with either linear or linear quadratic models of fit. In this study, chromosome aberrations were measured in human peripheral blood lymphocytes and normal skin fibroblasts cells after exposure to very low dose (0.1 – 0.2 Gy) of 170 MeV/u Si ions or 600 MeV/u Fe-ions. Chromosomes were analyzed using the whole chromosome fluorescence in situ hybridization (FISH) technique during the first cell division after irradiation, and chromosome aberrations were identified as either simple exchanges (translocations and dicentrics) or complex exchanges (involving >2 breaks in 2 or more chromosomes).

The curves for doses above 0.1 Gy were more than one ion traverses a cell showed linear dose responses. However, for doses less than 0.1 Gy, 2Si- ions showed no dose response, suggesting a non-targeted effect when less than one ion traversal occurs. Additional findings for 5Fe will be discussed.

**POS24-09. Four hundreds days’ continuous exposure of mouse to γ-ray at low dose rate alters component of hematopoietic cells in bone marrow.** Tokuhisa Hirouchi¹, M. Akabane¹, K. Tanaka¹, 1: Institute for Environmental Sciences, Japan 2: Tohoku Environmental Sciences Co., Ltd, Japan

Objective: We previously reported that exposure of male B6C3F1 mice to 8Gy of γ-ray at the dose rate of 20 mGy/day (γ20mGy/d) induced leukemia. Meanwhile, leukemic cells in spontaneous leukemia are reported to be originated from a leukemic stem cell (LSC) which is arisen from an immature hematopoietic cell. However, it still unknown how the murine γ20mGy/d-induced LSC is developed. To get key information about the LSC, we analyzed component of bone marrow cells of γ20mGy/d-irradiated mice. Methods: Eight-week-old B6C3F1 male mice were exposed to γ20mGy/d. Cell surface markers of bone marrow cells of the irradiated (IR) mice were analyzed at day 100, 150, 300 and 400 from the start of exposure, and compared with those of non-IR mice of the same age. Results: Effect of the γ20mGy/d was altered according to cell differentiation stage. The numbers of hematopoietic stem cells (HSC) of IR mice at day 150 and 400 were less than thog of non-IR mice. Common lymphoid progenitors (CLP) of IR mice were slightly increased at day 100, but decreased at day 150 and 400.

In comparison, effect of the γ20mGy/d on common myeloid progenitors (CMP) was delayed and small (refer to the data at day 200 and 400 in table).Conclusion: CLPs of IR-mice were maintained by excess proliferations of HSC and CLP itself. This study was performed under contract with the Aomori Prefectural Government, Japan.

The cell numbers of three differentiation stages in bone marrows of IR and non-IR mice

<table>
<thead>
<tr>
<th>Days from the start of exposure</th>
<th>γ-ray at dose rate of 20 mGy/day (γ20mGy/d)</th>
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| 0                               | 10. Effect of low doses of X-rays on the innate anti-tumour reactions in radioresistant and radiosensitive mice, Marek K. Jania³, E.M. Nowoselska, A. Cheda, J. Wrenbel-Wargocka, Military Institute of Hygiene and Epidemiology, Poland

Objectives: From different strains vary in their susceptibility to ionizing radiation, e.g., BALB/c mice are more radiosensitive than C57BL/6 mice. Also, the incidence of cancer following irradiation is more frequent in the former than the latter mice. In this study we evaluated the effects of fractionated irradiations of mice from the two strains on cytotoxic activities of cells involved in the innate anti-tumour surveillance and the development of the induced tumour colonies. Methods: NK cell-enriched splenocytes (NK cells) and peritoneal macrophages (Mφ) were collected from BALB/c and C57BL/6 mice irradiated for five days per week for 2 weeks at 0.01, 0.02, or 0.1 Gy X-rays (total absorbed doses of 0.1, 0.2, and 1.0 Gy, respectively). On the selected days after completion of the exposures cytotoxic anti-tumour activities of NK cells and Mφ and production of nitric oxide (NO) by Mφ were assayed. In addition, two hours after completion of the irradiations BALB/c or C57BL/6 mice were intravenously injected with L1 sarcoma and Lewis Lung Carcinoma (LLC) cells, respectively, and 14 days later the developed tumour colonies were counted on the surface of the lungs. Results: NK cells collected from all the irradiated BALB/c or C57BL/6 mice demonstrated comparable up-regulation of their cytotoxic function which was, for the most part, mediated by perforin and FasL. Likewise, Mφ collected from both strains of the mice exhibited similarly stimulated anti-tumour cytotoxicities and produced significantly more NO following exposures of the animals to all the three total doses of fractionated X-rays. Moreover, in both strains of mice the repeated irradiations with X-rays significantly reduced the number of the induced tumour colonies in the lungs.

Conclusion: The obtained results indicate that the anti-tumour effects of ten low-level irradiations with X-rays are comparable in the BALB/c and C57BL/6 mice.

**POS24-11. Neoplastic transformation and clustered dna damage induction by single or sequential exposures to low doses of X-rays in human skin fibroblasts.** Deborah Keszenman, V. Štisová, P.
Humans may be exposed to repeated low doses of low linear energy transfer (LET) ionizing radiation (IR) during medical, occupational or accidental exposures. With the frequent clinical use of radiodiagnostic procedures, there is considerable medical concern about potential long-term effects of sequential exposures to low doses of X-rays. Understanding molecular mechanisms underlying cellular responses to these types of exposures is critical to estimate risks of developing late health effects including carcinogenesis. In the assessment of different radiobiological endpoints following repetitive IR exposures, different types of biological responses including adaptive, synergistic, or additive responses have been reported. It has been established that these types of responses depend on the interval between exposures and other characteristics including radiation quality, LET, dose and dose rate.

In our studies of cellular responses to sequential exposures of low dose, low LET IR, we have determined effects on survival probabilities and neoplastic transformation frequencies in primary human fibroblasts following single or sequential exposures of 2 or 5 cGy of 100 kVp X-rays. At the molecular level, the induction of clustered DNA damage was assayed immediately after exposure using pulsed-field gel electrophoresis and number average length analysis. Results to date indicate that cell survival was not affected after sequential fractions of X-rays even after a total dose of 25 cGy delivered in 5 fractions of 5 cGy. Cultures irradiated with single doses corresponding to these total cumulative doses showed similar survival responses. As expected, we observed increased IR-induced neoplastic transformation as a function of dose after single exposures. In the two sequential irradiation regimes employed, the number of soft-agar transfectants per survivor decreased above a total dose of 10 cGy. Clustered DNA damage showed different induction levels after single or sequential exposures to identical doses of X-rays, suggesting the induction of DNA repair mechanisms by sequential low dose irradiation. This work was performed under the auspices of the U.S. DOE by BNL under contract DE-AC02-98CH10886, supported by grant BO-086 from the DOE Low Dose Radiation Research Program and grant NN07HC731 from NASA.

POS24-12. Study of the health of HBRs (High Background Radiation School) former students in the city of Ramsar, Iran (Preliminary Report). Maysam Khosravi1, S. Borouzi1, P. Roshan Shomaif2, A. Shahbazi Monfared1, 1: Babol University of Medical Sciences, Iran 2: Gilan University of Medical Sciences

Introduction: Ramsar, a coastal city in the north part of Iran, has the highest level of natural background radiation in the world. Several researches have done on the effects of the radiation on human health. We evaluated the health status of the Saeid Nafigh school former students in Ramsar that estimated to receive about 4 S V radiation dose during study in the highest background radiation school in our planet. Materials & Methods: Standard health questionnaires were provided and completed for most of former students by interview. Medical examination is ongoing. Results: None of the students have severe and chronic radiation induced illness on the basis of first survey data. Conclusion: In spite of receiving a large amount of ionizing radiation it seems that the health effects are negligible. This study may lead us to revise the current radiation protection regulations.

POS24-13. Involvement of non-CG DNA methyltransferase in epigenetic modification of Arabidopsis genome by low-dose gamma ray. Ji Eun Kim, J.H. Kim, B.Y. Chung, J.H. Kim, Korea Atomic Energy Research Institute, South Korea

DNA methylation is one of the epigenetic markers associated with transposable element silencing and gene imprinting in plants. Several recent studies suggested that epigenetic modification such as DNA methylation and histone modification should be induced in plant genome after gamma irradiation. However, there is a lack of data exploring the underlying mechanisms. Therefore, the present study aimed to characterize and elucidate the epigenetic modification of plant genome by low-dose gamma ray. Seedlings of wild type or cmt mutant, which has a defect in non-CG DNA methylation, were irradiated with 5-Gy gamma ray at two different dose rates as follows; in the two (A) 50 mGy h⁻¹ for 10 h per each day and (B) 125 Gy h⁻¹ for 4 h. In case of (A), transcript levels of DNA methyltransferase genes, MET1, DRM2, and CMT3, were not affected in wild type by treatment with gamma ray, while those of MET1 and CMT3 were significantly decreased in cmt mutant. Unexpectedly, quantification assay of methylated DNA demonstrated that DNA methylation in cmt mutant was increased after treatment with gamma ray, while that in wild-type was rather decreased. Transcript levels of genes involving in histone modification such as histone deacetylase (HAD6, HAD8, and HAD19), acetylase (HAF1), and methyltransferase (SDG9) were never affected in wild. Instead, cmt mutant showed substantially decreased transcript levels of HAD6 and HAD19 only. In contrast, in case of (B), transcript levels of DNA methyltransferase genes and histone modification-related genes remained constant even in cmt mutant after treatment with gamma ray. Based on the obtained data, therefore, we suggest that treatment with gamma ray could cause substantial changes in DNA methylation and histone modification of Arabidopsis genome, and that transcriptions of MET1, HDA19 and HDA6 should be negatively associated with that of CMT3, which is dependent on the dose rate.


Low dose ionizing radiation (IR) is known to induce a variety of specific biological effects, not predictable based on extrapolation from high doses, that commonly thought to have mechanistic links to DNA repair process. In this study, we sought to understand whether DNA double strand break (DSB) repair may have a role in the previously demonstrated low dose IR-induced systemic radioadaptive responses by tumourogenesis in vivo. We evaluated DNA repair by measuring the kinetics of gamma-H2AX levels in splenocytes and thymocytes of C57Bl6/J mice irradiated in vivo with low dose IR (20 and 100 mGy, 1 mGy/min, Cobalt-60) 24 h prior to a challenging dose of 2 Gy (6 Gy/min, Cobalt-60). Cells were allowed to complete repair of DNA DSBs for various times (0-24 h) post challenging IR dose and then harvested and analyzed by flow cytometry or immunoblot analyses for gamma-H2AX levels. Generated DNA repair curves indicated that low dose IR did not influence the rate of DNA DSB rejoining. Similarly, no changes were found between cells from low dose IR vs. control mice in the kinetics of high dose IR-induced phosphorylation of ATM (phospho S1981), a DNA damage signalling marker. Using clonogenic survival assay and immunofluorescence microscopy, mouse embryonic fibroblasts of C57Bl1 genetic background were shown to lack both radiation adaptive survival response and low dose IR-induced repair of DNA DSB (gamma-H2AX foci kinetics), consistent with the role of genetic background in radioadaptive responses. Our results show that DNA repair, and, supposedly, other short not necessarily radiation adaptive responses, such as increases in the tumour latency times in ageing mice or in the life span previously demonstrated in our laboratory, and that alternative mechanisms may be involved, e.g. modulation of immune system functions.

POS24-15. Low Dose Radiation Stimulates Antioxidants in Rodent Brain and Reduces Behavioral Symptoms in Experimental Model of Parkinson's Disease. Barbara Krynska1, M. Doss2, R.K. Alqaugh2, Z. Mu3, S. Litwin4, B.J. Augelli1, S.A. Azizi1, 1: Temple University School of Medicine, USA 2: Fox Chase Cancer Center, USA

Background: Oxidative damage to the nigral dopaminergic neurons has been implicated in the pathogenesis of Parkinson’s disease (PD). Our hypothesis is that low dose radiation (LDR) promotes the induction of antioxidants in the brain, which could provide protection to the dopaminergic neurons, potentially lead to the prevention or stabilization of PD. The purpose of the study is to determine (1) the effect of LDR on the total antioxidant capacity in substantia nigra (SN) in normal rats, and (2) the effect of LDR on the behavioral symptoms in a mouse model of PD. Methods: (1) Sprague-Dawley (SD) rats were exposed to radiation applied to the brain in the dose range of 0 to 90 cGy at the rate of ~9 cGy/minute. The animals were sacrificed at various time points after the irradiation and the total antioxidant capacity was measured in SN. (2) Behavioral rotations were evaluated in 6-OHDA unilaterally lesioned SD rats exposed to the dose range of 0-45 cGy to the brain 5 days following the disease induction. Complete 360° turns were
counted in 45 min periods and measured at weekly intervals during the period of 3 to 7 weeks after 6-OHDA administration.

Results: (1) Results show that LDR induced an increase in total antioxidant capacity in SN as a function of dose when rat brains were irradiated in the dose range of 0 to 63 cGy and decrease as a function of dose for higher doses. The elevated antioxidant capacity was maintained for at least 3 weeks. (2) The number of rotations in 6-OHDA lesioned rats decreased after the treatment with 10 and 45 cGy when compared to controls that were sham radiated. The reduction in the number of rotations was greater for the rats exposed to 45 cGy when compared to those exposed to 10 cGy.

Discussion: Our preliminary data suggests that exposure to LDR stimulates antioxidant capacity in the brain and may reduce behavioral symptoms in the 6-OHDA-induced rat model of PD. If further testing confirms these observations, translation of the concept to human studies may be considered as a means to slow down the progression of PD.

Acknowledgements: This research was supported by the Office of Science (BER), U.S. Department of Energy, under Award No. DE-SC0001196.

POS24-16. Effects of long-term additional therapy of different antioxidants and ibuprofen on oxidative stress and antioxidative potential of the Chernobyl clean-up workers. Liga Larmsme2, G. Moisejeva1, N. Rusakova1, T. Zvaigule1, 1: Laboratory of Biochemistry, Riga Strads University, Riga, Latvia 2: The Center of Occupational and Radiological Medicine, Pauls Strads Clinical University Hospital, Riga, Latvia

Oxidative stress (OS) is caused by disbalance between production of reactive oxygen species (ROS) and biological system’s ability to scavenging the reactive intermediates.

Ionizing radiation is one of the factors that induce intensive forming of ROS, therefore, people who were exposed to radiation have negative changes in the reduction-oxidation (red-ox) system of organism.

The aim of our study was to find out if additional therapy (AT) of antioxidants (AO - Se, Vit.E, CoQ10) and Ibuprofen may affect the red-ox system of the Chernobyl clean-up workers (ChCW) from Latvia. ChCW in comparison to people of the same age and sex are subjected to many chronic inflammatory changes that in their turn may be related to free radical production and accordingly the red-ox system fluctuations.

In order to make research groups, 40 to 65 yo men (ChCW versus healthy volunteers) were involved in the study. Each group received different combinations of AO and Ibuprofen or placebo. Several parameters characterizing antioxidant defense and OS status of organism (e.g. reduced glutathione (GSH), total antioxidant state, glutathione peroxidase (GSH-Px), Cu,Zn-superoxide dismutase (SOD), malondialdehyde (MDA) etc.) were detected in blood, plasma or erythrocytes before, during and after AT.

The results indicate that there is a tendency for lipid peroxidation intensity to decrease during and after the AO and Ibuprofen therapy, e.g., during the one year supplementation of Ibuprofen, Se and Vit.E, the level of MDA decreased from 3,19 nmol/ml initially to 2,71 nmol/ml after therapy.

However, in some patient groups, antioxidative defense improved by increase of Se and GSH, GSH-Px, SOD accordingly. AT of Se, Vit.E and CoQ10 in different combinations significantly increased the level of Se in the patient plasma. That might be good indicative of exogen organic Se and its successful incorporation in the metabolism, thus increasing the reductive potential of the organism, e.g. GSH increased from 38,65 mg/dL initially to 48,54 mg/dL after one year supplementation of Se and Vit.E.

Temporarily we cannot confirm that additional AO and Ibuprofen therapy guarantees an improvement in all cases related to OS, although our results demonstrate it may probably help to retain red-ox state in balance.

POS24-17. Simulating the lunar environment: impact of low dose 26Si and X-ray radiation on bone loss during reduced weightbearing. Burdwen Macias1, F. Lima2, Y. Shirazi-Fard2, M. Wiggs1, J. Fluckey1, E. Greene1, M. Allen1, L. Braby3, H. Hogan3, S. Bloomfield4, 1: Dept of Health & Kinesiology, Texas A&M University, USA, 2: Dept of Biomedical Engineering, Texas A&M University, USA, 3: Dept of Anatomy & Cell Biology, Indiana University School of Medicine, USA, 4: Dept of Nuclear Engineering, Texas A&M University, USA, 5: Dept of Mechanical Engineering, Texas A&M University, USA

No data exist testing the skeletal consequences of combining low-dose high-LET radiation and ambulation in a simulated Lunar gravity environment. Moreover, the relative biological effectiveness of low-dose high-LET radiation for bone loss is unknown. We hypothesized that simulated galactic cosmic radiation would exacerbate bone loss observed after reduced (1/6th) weightbearing (G/6).

BALB/cByJ mice (F, 4-mo, n=10 per group) were randomly assigned to cage control (G0), G/6, or G/6+Radiation. Mice were exposed to two radiation types, either 26Si at 300 MeV/ (at doses 0Gy (SHAM), 0.17Gy, 0.5Gy, and a fractionated dose of 0.5Gy (0.17Gy on days 0, 2, 7) or X-rays at 250kV (X) [at doses 0Gy (SHAM), 0.17Gy, 0.5Gy, 1Gy, and a fractionated dose of 1Gy (0.33 Gy on days 0, 2, 7, 14)]. After 21 days, bones were harvested and distal femur bone volume (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N) quantified with micro-CT. Regression analysis of BV/TV against the 1G Si doses/ separately against the 1G X-ray doses; X-ray and Si doses producing a 14% decrement in BV/TV were determined.

Relative to the 1G-SHAM group, distal femur BV/TV and Tb.Th were significantly lower after 21-d in the G/6 (-23%, -13%, respectively) and 0.5Gy G/6+Si (-26%, -13%, respectively) groups at day 21. BV/TV in the 1G Gy/6-X group was significantly lower (-29%) at day 21 compared to G/6-SHAM. In the 1G groups, both single- and fractionated 0.5Gy Si radiation dosing regimens resulted in significantly lower BV/TV (-14% and -18%, respectively) at day 21. A fractionated Si dose lowered Tb.N during 1G and G/6, however, the fractionated X-ray dose did not. For 1 G mice, the dose of high LET 26Si producing a 14% decrement of BV/TV (0.5Gy) was 2-fold lower than that of dose of low-LET X-ray producing the same bone loss (1Gy).

In support of our hypothesis, low dose high LET radiation exposure during partial weightbearing conditions exacerbates cancellous bone loss (BV/TV). Three 0.17Gy fractions of low dose high LET radiation (26Si) produce similar cancellous bone loss decrements as one 0.5Gy dose. However, fractionating low-dose low-LET radiation (X-rays) does protect against cancellous bone loss seen with one acute dose, suggesting that mechanisms of damage may differ between high-LET 26Si and low-LET X-ray radiation when delivered as fractionated doses.

POS24-18. Estimating the lifetime attributable risk of lung and breast cancer due to 128-slice dual-source computed tomography coronary angiography. Kosuke Matsubara1, K. Koshida1, T. Takata2, J. Horii1, H. Iida1, O. Matsui1, 1: Kanazawa University, Japan 2: Kanazawa University Hospital, Japan

With the introduction of computed tomography (CT) equipped with dual X-ray sources, dual-source CT coronary angiography (CTCA) has been established as a noninvasive diagnostic tool for assessing coronary artery disease. However, organs close to the heart, such as the lungs and breasts, receive relatively high radiation doses during CTCA, thereby giving rise to a possible risk of cancer. We aimed to estimate the lifetime attributable risk (LAR) of lung and breast cancer after radiation exposure during 128-slice dual-source CTCA on the basis of The National Academies’ Biological Effects of Ionizing Radiation 7th Report (BEIR VII) Phase 2 preferred models. The absorbed doses for thoracic organs during 128-slice dual-source CTCA with three acquisition modes (high-pitch spiral [HPS], step-and-shoot [SAS], and low-pitch spiral [LPS] modes) were measured using an anthropomorphic thoracic phantom and radiophotoluminescent glass dosimeters, and conversion factors from volume CT dose index (CTDIvol) to the absorbed dose for each organ were calculated. Therefore, the LAR of lung (male and female) and breast (female) cancer incidence was estimated using the LAR of each cancer incidence after a single radiation exposure of 5 mGy to each organ (presented in the BEIR VII 7th Report, Phase 2 report), calculated conversion factors, and CTDIvol from 138 consecutive patients who underwent 128-slice dual-source CTCA with HPS, SAS, or LPS modes. In the anthropomorphic phantom study, the measured absorbed doses were 6.6, 53.6, and 110.1 mGy for lungs, and 5.03, 38.4, and 92.0 mGy for breasts with HPS, SAS, and LPS modes, respectively. Estimated organ doses of patients ranged from 6.6 to 120 mGy for lungs and 5.1 to 99 mGy for breasts. The estimated LAR of cancer incidence after radiation exposure from 128-slice dual-source CTCA varied from 1 in 900 to 1 in 36000 for lung cancer in males, 1 in 450 to 1 in 13000 for lung cancer in females, and 1 in 420000 to 1 in 220000 for breast cancer in females. The estimated risk of cancer incidence in patients who underwent CTCA with the LPS mode was
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relatively high compared to that in patients who underwent CTCA with the other modes. Although risk estimates are subject to several sources of uncertainty, this study suggests that CTCA should be performed cautiously, especially in female and young patients.


We recently described the influence of low dose gamma radiation exposures on atherosclerosis in genetically susceptible (ApoE-/-) mice. Depending on dose and dose rate, doses as low as 25 mGy given at either early or late stage disease generally protected against atherosclerosis in a manner distinctly non-linear with dose. In this animal model Tp53 function is known to influence aortic atherosclerosis, with plaque formation significantly accelerated in ApoE-Tp53+/- mice. We now report the influence of low doses (0.025-0.5 Gy) on atherosclerosis in mice with reduced Tp53 function (Tp53-/-). Single exposures were given at either low or high dose rate (1 or 150 mGy/min) to female C57BL/6J ApoE-Tp53+/- mice. Mice were exposed at either stage disease (2 or 3 months of age) and examined 3 or 6 months later, or at late stage disease (7 months of age) and examined 2 or 4 months later. In unexposed mice, reduced p53 functionality elevated serum cholesterol and accelerated aortic root plaque growth in young mice. Exposure of mice to low doses of radiation at early stage disease, at either high or low dose rate, inhibited lesion growth and decreased the mean number of lesions in the aortic root. Lesion cross sectional area was reduced by half compared to unexposed mice and maximum effectiveness occurred at 25 mGy. Exposure at either high or low dose rate also significantly retarded the age related progression of lesion severity. In contrast, exposure at late stage disease produced generally detrimental effects. Both low and high dose rate exposures accelerated lesion growth and high dose rate exposures also increased serum cholesterol levels. These results show that at early stage disease, reduced p53 function does not influence the generally protective effects of low doses against atherosclerosis. In contrast, the protective effects seen when Tp53 normal mice were exposed at late stage disease were lost in the mice with reduced p53 function, and low doses were generally detrimental. As in the Tp53 normal mice, all effects were highly non-linear with dose. These results indicate that depending on the disease stage at the time of irradiation, variations in p53 functionality can dramatically influence the outcome of a low dose exposure, and that the assumption of a linear response with dose for human populations is probably unwarranted.

POS24-20. In vivo distribution of radionuclides, tumorigenicity and genome stress in mice after Chernobyl catastrophe. Hiroo Nakajima1, T. Saito1, T. Hongyo1, H. Ryo1, T. Todo1, T. Nomura2, 1: Osaka University, Japan 2: National Institute of Biomedical Innovation, Japan

South districts of Belarus are still highly radiocanminated even after 25 years from the Chernobyl catastrophe in 1986. The major radionuclides in the contaminated areas are 137Cs and 89Sr. It is easily predicted that the radionuclides are concentrated by the food chain into the living organisms in the contaminated area, and radionuclides remain in the irradiated organisms not only externally but also internally for long periods. The exact radioactivity in organisms should have been known to assess the long term low dose rate and low dose exposures were given at either low or high dose rate (1 or 150 mGy/min) to female C57BL/6J ApoE-Tp53+/- mice. Mice were exposed at either stage disease (2 or 3 months of age) and examined 3 or 6 months later, or at late stage disease (7 months of age) and examined 2 or 4 months later. In unexposed mice, reduced p53 functionality elevated serum cholesterol and accelerated aortic root plaque growth in young mice. Exposure of mice to low doses of radiation at early stage disease, at either high or low dose rate, inhibited lesion growth and decreased the mean number of lesions in the aortic root. Lesion cross sectional area was reduced by half compared to unexposed mice and maximum effectiveness occurred at 25 mGy. Exposure at either high or low dose rate also significantly retarded the age related progression of lesion severity. In contrast, exposure at late stage disease produced generally detrimental effects. Both low and high dose rate exposures accelerated lesion growth and high dose rate exposures also increased serum cholesterol levels. These results show that at early stage disease, reduced p53 function does not influence the generally protective effects of low doses against atherosclerosis. In contrast, the protective effects seen when Tp53 normal mice were exposed at late stage disease were lost in the mice with reduced p53 function, and low doses were generally detrimental. As in the Tp53 normal mice, all effects were highly non-linear with dose. These results indicate that depending on the disease stage at the time of irradiation, variations in p53 functionality can dramatically influence the outcome of a low dose exposure, and that the assumption of a linear response with dose for human populations is probably unwarranted.


We have previously reported that low-dose-rate, long-term irradiation induces protein expression of rhodanese, a detoxification enzyme in mouse liver (Nakajima et al., J. Radiat. Res., 2008). The elucidation of the physiological role of the rhodanese induction by low-dose-rate irradiation using an animal model and to evaluate effects of low-dose-rate irradiation on protein expression in mouse liver using fractionated low-dose-rate irradiation. Using rat hepatoma, MaCA-RH7777 cell line, which expresses rhodanese, effects of knockdown of the enzyme by siRNA on radical scavenging activities were evaluated. The siRNA treatment reduced the radical scavenging activity by 30% in the cells. This suggests that rhodanese induction by low-dose irradiation promotes radio-protective ability in mice. Furthermore, to facilitate analyses of alteration of protein expression by low-dose-rate irradiation, fractionated whole-body X-rays-irradiation was performed to mimic low-dose-rate irradiation. Mice were irradiated with 0.02Gy and 0.4Gy per day, 5 days (weekdays) per week for 1 month (total doses: 0.4Gy and 8Gy, respectively), and protein expression in livers from the mice irradiated was investigated. In the control mice 6 and 14 weeks of age (6 wk and 14 wk mice) at the start were used. In the case of 6wk mice, rhodanese induction was not observed but changes in some other protein expressions were detected in comparative analyses by two-dimensional gel electrophoresis. The proteins were identified by MALDI-TOF MS. In 14 wk mice, rhodanese induction was detected in some of mice irradiated. Moreover, as an increased protein candidate by irradiation, an identical protein was detected in both 6 wk and 14 wk mice. Characterization of protein candidates detected in altered protein expression by low-dose-rate irradiation will be discussed.

POS24-22. Quantification of Gene expression modulation induced by low doses of ionizing radiation in a lymphocyte subpopulation. Ingrid Nosel, Institut de radioprotection et sûreté nucléaire de Fontenay aux Roses (IRSN), France

Dose estimation after nuclear accident is essential to adapt medical treatment of radiation injuries. Actually the technical used for dose estimation is based on scoring of chromosomal aberrations, however this tool has a lower detection limit of 100mGy. The purpose of this study is to explore novel bio-indicators of exposure permitting estimation of doses below 100 mGy. It is well known that ionizing stress induces gene expression modifications. Several microarray studies have successfully yielded a set of modulated genes associated particularly with high dose exposures. It’s the reason why we attempt to find a signature of low dose exposures using oligonucleotide microarray.

In addition, this study would permit to identify cellular mechanisms involved in low dose radiation responses. Oligonucleotide microarray technology enables to determine expression levels of thousands of genes. Venous peripheral blood samples were collected from five volunteers donors. Irradiations on whole blood are performed with a Cobalt-60 source emitting gamma rays, with a dose rate of 0.05Gy/min. We performed six doses 500, 100, 50, 25, 10, 5mGy and the corresponding sham irradiated condition. We study different times point post irradiation: 2h30 5h
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7h30 and 10h. We work on a CD4+ lymphocyte subpopulation. It was report previously that CD4+ lymphocytes were particularly sensible to low doses of gamma rays. Bioinformatics tools are used to determine the cellular and molecular roles of genes in co-regulated gene sets. Our analyse reveals that gene modulations could be detected even at 5mGy and 10 hours after irradiation. We don’t observe a dose effect on the number of gene modulated at the different tested doses. We obtain an average of three hundred up-regulated genes and two hundred down-regulated genes at each time point for each dose. As expected, we notice that the major pathway up regulated at 500mGy in response to ionizing radiation is the p53 pathway as PCNA, DDB2, and XPC involved in DNA repair or BAX, GADD45A in apoptosis. Moreover, the probability to select modulated genes involved in increasing absorptive dose. New molecular mechanisms seem to be involved specificity for dose among 10 and 100mGy. Indeed these doses shared up to 50 of modulated genes and only 20% with the dose 500mGy. To conclude, these preliminary results reveal that p53 pathway is highly involved at 500mGy but no implication decrease for doses below 100mGy. This finding suggests that regulatory network involved below 100mGy seems really different in comparison to 500mGy. We are trying to establish, for doses below 100mGy, functional relationships inside the co-regulated genes set.

POS24-23. The effect of low dose ionizing radiation on the proliferative status of ageing primary human bronchial epithelial cells. Rebecca J. Sabin, R.M. Anderson, Centre for Cell and Chromosome Biology; Centre for Infection, Immunity and Disease, Division of Biosciences, Brunel University, UK

Senescence is a normal metabolically active form of growth arrest that prevents the proliferation of damaged and aged cells, as such, the fraction of senescent cells increases as a cell population ages. In addition to this tumour-suppressive mechanism, senescence is also linked with the development of a pro-oncogenic environment that is related to a senescence-associated secretory phenotype. Indeed, senescence is thought to be important in a number of age-related pathologies. Recent studies have suggested that exposure to DNA damaging agents such as ionising radiation (IR) may initiate a premature (stress-induced) cellular senescence, which may influence the biological effects of radiation exposure. Therefore, to assess whether radiation exposure prematurely increases the fraction of senescent cells, we exposed primary human bronchial epithelial (HBEp) cells to low-linear energy transfer (LET) Cobalt-γ-rays (0.3 and 0.5Gy, 0.111μGy/min) and fixed cells 30 mins, 24 and 36 hrs after exposure. To assess the fraction of non-proliferating cells (NP) and to quantify the DNA damage present within both proliferating and NP cells after irradiation, nuclei were co-stained with antibodies for the proliferation antigen Ki-67 and the DSBR marker, 53BP1. As expected, 53BP1 foci were induced in HBEp (p5) cells 30 mins after exposure to 0.3 and 0.5Gy (1.66, 0.87) and 24hrs respectively. We observe no difference in the fraction of NP (p5) HBEp cells 30 mins after exposure (26%, 38% and 30%) for sham, 0.3 and 0.5Gy and 36 hrs (21, 27 and 33) for sham, 0.3 and 0.5 GY respectively. To date, our co-stain analysis does not show any obvious trend in greater or fewer fractions of NP cells with increasing numbers of 53BP1 foci. Our studies are continuing to examine the effects of IR on proliferative status and foci number at delayed time periods after exposure.

POS24-24. Effects on gene expression in normal mouse tissues following low dose 131I irradiation. Emil Schülér1, T. Parris1, N. Rudqvist1, K. Helou2, E. Forsell-Aronsson3, 1: University of Gothenburg, Department of Radiation Physics, Sweden 2: University of Gothenburg, Department of Oncology, Sweden

The purpose of this study was to investigate the response of normal tissues following internal low absorbed dose irradiation of 131I. Balb/c mice were i.v. injected with 13-260 kBq of 131I. The mice were euthanized in this pathway decrease with the doses and the spleen were surgically removed. The absorbed doses ranged between 0.1 - 9.7 mGy for the different tissues. Comparisons between irradiated and unirradiated tissues were conducted using extracted total RNA and Illumina MouseRef-8 Whole-Genome Expression Beadchips. The Benjamini-Hochberg method was used to control for false discovery rate and determine differentially expressed transcripts. Modulation of 1.5-fold or higher, either positive or negative regulation, was regarded as significant and included in the analysis. Large variations between the different tissues were found with respect to number of modulated transcripts, which ranged from 260 in kidney cortex to 857 in the lungs. Few transcripts were shared between the different tissues investigated and a high specificity in types of affected transcripts were seen. The dose-response relationship of the transcripts affected at all dose levels was primarily found to be independent of dose, few transcripts showed increasing or decreasing regulation with increasing absorbed dose. As with transcripts, low number of biological processes was commonly affected at all absorbed dose levels as well as in all tissues studied. Processes commonly affected in more than two organs were primarily associated with response to stimuli, metabolism, and immune response which was found to be the only biological process commonly affected in all tissues. A clear tissue dependence on type of biological process affected was found. A strong response was observed despite the low absorbed doses delivered to the investigated tissues. The results from this study indicate that with the low doses delivered, only small deviations from the tissues normal functions are induced. As the impact of these deviations is unknown, further research is needed.

POS24-25. Study of radiation induced protein changes (mGy range) in human whole blood. Sara Sköld, S. Haghdoost, M. Harms-Ringdahl, Centre for Radiation Protection Research, Department of Genetics, Microbiology and Toxicology, Stockholm University, Sweden

Due to insufficient data in the low dose range the dose region for stochastic effect of radiation is based on the Linear-Non-Threshold hypothesis. The possible cancer risk caused by low Linear Energy Transfer doses of about 1 mGy is too small to be studied using epidemiological methods due to the high background cancer risk in general. We have in previous studies on primary human fibroblasts (VH10) and G0 lymphocytes shown that ionizing radiation, in the mGy range, induced an endogenous stress response and the subsequent formation of 8-oxo-dGTP. However the mechanisms behind this stress response is not known. We have tried a proteomic approach to describe the processes at the biochemical level. To address the above mentioned aim a protocol was designed to optimize the conditions for handling blood samples and to find the optimal dose and post irradiation condition. We have also studied the intra- and inter-individual differences between the two donors. The present study shows that it is possible to detect consistent protein changes after exposure to 1 and 150 mGy γ-rays for a individual over time. However out of the 5054 proteins (90% of the total) we find a significant increase in the number of proteins regulated genes set. As expected, we notice that the major pathway up regulated in response to ionizing radiation between the two doses as well as between the two donors. The long term aim of the study was to develop a protocol to investigate if differences in the proteomic profiles of irradiated whole blood could be seen between patients classified as radiosensitive or non-radiosensitive in response to radiotherapy. Differences in the proteomic profiles might give indications of protein biomarkers for radiosensitivity screening that could be used in combination with screenings of 8-oxo-dG levels. There are preliminary results indicating that 8-oxo-dG levels from whole blood irradiated in vitro can be used to screen for severely radiosensitive patients. However additional markers would improve reliability and the proteomic study might reveal additional biomarkers. Preliminary results will be presented at the conference.


POS24-26. FROM TISSUE TO CELL Uranium micromobidistribution by «SIMS» technique. David Siuhard, C. Tessier, L. Grandcolas, F. Rebure, Y. Gueguen, IRSN, France

The ENVIRHOM research French program supported by the Institute for Radioprotection and Nuclear Safety (IRSN) is intended to improve assessment of the risks to the population and the ecosystems associated with chronic exposure to low levels of radioactive contaminants. The major objectives are to study: bioaccumulation processes of radionuclides and also the biological effects correlated
with this exposure on the human model (rats, mice). Uranium is one of the first studied elements in this program. The understanding of the transport and transfer mechanisms of radionuclides incorporated according to the chronic mode in the population requires the establishment of contaminants distribution cartographies in the biological structures which are the targets of bioaccumulation phenomenon. Among the analysis and micro-imaging techniques, the ion microscopy based on the secondary ion mass spectrometry (SIMS), represents a performing tool to characterize the preferential sites of radioelements accumulation at tissue and cell level. In vivo experiments have been realized to examine (renal cortex of rats exposed to this element by drinking water, U-40mg/L) uranium accumulation process at different contamination durations (from 6 to 18 months). For each area analysed, mass spectra at around isotope 238 of uranium and ionic images have been obtained with a SIMS Cameca 4F-E7. 4Ca images give the histological structure of the cortex and 238U images show uranium accumulation within different the structures. The results have showed selectivity sites of uranium distribution in function of the contamination duration. For 6 to 12 months exposure the SIMS data reveal that the radioelement is confined in proximal convoluted tubule structures. For the 18 months exposure, uranium is accumulated in all structures. In vitro experiments (human cells models) have been also realised to study uranium accumulation process in the different compartments. Optical and ionic images have displayed for the first time the distribution profile of this element within cells. These cartographies by SIMS will be an important contribution in addition to ICPS-MS and MET techniques to explain and interpret the mechanisms of radionuclides transport in the different organs of the living beings.

**POS24-27. Global methylation responses to low dose radiation exposure.** Pamela Sykes1, M. Newman1, B. Blyth1, E. Bezak2, R. Ormsby2, 1: Flinders University, Australia 2: Royal Adelaide Hospital, Australia

At high radiation doses, DNA double-strand breaks are considered the critical lesion in initiation of radiation-induced carcinogenesis. However, at the very low radiation doses relevant for the general public, the induction of DNA double-strand breaks will be rare, and other mechanisms such as DNA methylation changes are likely to play a role. We developed a sensitive assay to measure the levels of DNA methylation across the mouse genome by analysing a stretch of DNA sequence within Long Interspersed Nuclear Elements-1 (LINE1) that comprise a very large proportion of the mouse and human genomes. Using bisulphite modification followed by quantitative real-time PCR and high-resolution melt analysis, a very large pool of DNA sequences from throughout the genome can be studied indicating gain or loss of methylation across more than ten CpG’s per amplicon. We validated the assay in vitro using the chemical demethylating agent 5’-aza-2’-deoxycytidine with changes at as few as 3% of CpG’s being reproducibly detected. We have demonstrated a difference in the baseline levels of in vivo DNA methylation between male and female mice and between different tissues. Our initial results suggest no significant short-term changes in global DNA methylation after whole-body X-radiation doses of 10 μGy or 10 mGy, with a significant transient increase in DNA methylation observed 1 day after a 1 Gy irradiation. If the low radiation doses tested are inducing changes in global DNA methylation, these would appear to be smaller than the natural variation observed between the sexes and following the general stress of the sham-irradiation procedure itself. This research was funded by the Low Dose Radiation Research Program, Biological and Environmental Research (BER), US Department of Energy, Grant DE-FG02-05ER64104 and MN is the recipient of the FMC/ BHP Billiton Low Dose Radiation Research Scholarship.

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**POS24-28. Frequencies of Chromosomal Translocation and Clone formation in Spleen Lymphocytes from Mice Continuously Irradiated with Low-Dose-Rate Gamma-Rays.** Kimio Tanaka, A. Kohda, Institute for Environnemental Sciences, Japan

Background: Chronically exposed individuals to very low-dose radiation, such as residents in high-background areas in China have slightly higher chromosomal translocations than non-exposed individuals, but the relationships between frequency of chromosome aberrations and total dose and radiation time (from 6 to 18 months) are not fully studied. As epidemiologically studies of human populations exposed to very low-dose radiation have uncertainties and are influenced by confounding factors such as smoking, mouse study will be necessary.

Objectives: We studied on dose and dose-rate effects of chromosome aberration rate at low-dose-rate (LDR) region. Methods: SPF female C3H mice were exposed from 8 weeks of age to 125 to 720 days with continuous 137Cs-γ-ray irradiation at LDRs (1 mGy/day and 20 mGy/day). Spleen lymphocytes from mice were cultured for 46h in the presence of LPS, Con A, and 2-ME to obtain metaphase spreads, and dicentric chromosome and translocations were observed using multiplex-FISH method.

Results: Incidences of dicentrics and translocations increased almost linearly up to a total accumulated dose at these LDRs. The values of regression coefficient in the linear regression lines for dicentric chromosome aberration and the dose rate was lowered from 20 mGy/day to 1 mGy/day, but not for translocation. Dose and dose-rate effectiveness factor (DDREF) were obtained to be 2.3 for translocations using the results of irradiations of high dose rate and LDR(20 mGy/day). Clones having such as derivative chromosome 15 was observed in low frequencies in LDR (1 mGy/day)-irradiated and non-exposed mice only, but not observed in LDR (20 mGy/day)- irradiated mice. Clone formation was found at 200 days after initial irradiation (total doses of 4000 mGy) in mice irradiated at LDR (20 mGy/day), and at 617 and 720 days at 1 mGy/day.

Conclusions: The results indicate that there were dose or dose-rate effects on formation of clones at a LDR region.

These are useful information for evaluating the risk of low dose radiation in human. This study was performed under contract with the Aomori Prefectural Government, Japan.

**POS24-29. Cancer risk and low dose diagnostic radiation in Trp53 heterozygous mice.** Kristina Taylor, N. Phan, L. Lafraimboise, M. Ellen Cybulski, D.R. Boreham, McMaster University, Canada

The cancer risk associated with exposure to low doses of ionizing radiation has traditionally been extrapolated from effects observed at high doses and high dose rates using a linear no-threshold model. Based on this approach, it has been postulated that human exposure to medical imaging involving low doses of x-rays and gamma rays increase an individual’s risk of developing cancer throughout their lifetime. In these experiments, the influence of adapting doses of diagnostic radiation on survival, cancer frequency and cancer latency in Trp53 heterozygous mice subsequently exposed to a high dose was evaluated. Mice were exposed to an acute 4 Gy γ dose at 7-8 weeks of age (n=200). Other groups received a single 10 mGy CT scan (75kVp, 200mA) or PET scan (18F-FDG) 24 hours prior to this 4 Gy dose (n=200). Another group received the 4 Gy dose followed 4 weeks later by weekly CT scans over a course of 10 weeks (100 mGy total). The frequency and latency of lymphomas (T or B Cell) and osteosarcomas were used as endpoints to evaluate cancer risk. There were no significant differences in median lifespan or overall survival between the groups.

**POS24-30. Low dose and dose rate effects of proton irradiation on muscle and neural precursor cells.** Bertrand Tseng, M. Lan, J. Cho-Lim, J. Bahlai, E. Giedzinski, C. Limoli, University of California, Irvine, USA

Proton irradiation of multipotent stem and precursor cells elicits persistent oxidative stress that impacts radiosensitivity, mitochondrial function, and cell fate. Throughout life these stem cells play critical roles in the development and maintenance of health. Their capability to continually regenerate provides the tissues of the body with the means to counteract exposure to damaging agents, space radiation, disease and aging. While the mechanisms regulating the responses of tissue-specific stem cells and their immediate progeny to stress are diverse, underlying themes are emerging that suggest changes in redox state are critical. These alterations in oxidative stress may prime stem cell pools for the adaptation and remodeling of the irradiated tissues in which they reside.

We have examined the capability of low doses and dose rates of proton irradiation to modulate redox state in muscle and neural precursor cells. Radiation impairs the capability of multipotent stem cells to maintain compensatory hypertrophy, and in mouse models deficient for antioxidant enzymes, elevated muscle wasting and impaired growth are evident. Our work shows that low dose proton irradiation produces persistent oxidative stress in muscle precursor cells. In the CNS, irradiation at low dose rate has not been shown to cause persistent oxidative stress. The nature, magnitude and duration of reactive species dictate whether these
PO9S24-31. Low-dose radiation and cancer therapy. Guanjuan Wang1, L. Cai2, W. Li1, 1: Jinlin University, China; 2: University of Louisville, USA

Using mouse model, we have demonstrated the stimulating effects of LDR (75 mgGy of X-rays) on bone marrow hematopoietic progenitor cell (HPC) proliferation and peripheral blood mobilization. LDR-mobilized donor HPCs were able to efficiently repopulate blood cells in lethally irradiated recipient mice, suggesting that LDR-induced hematopoietic hormesis may provide a potential approach to clinical use for HPC peripheral mobilization to reconstructive therapy of damage organs due to variety of pathogeneses. To address whether LDR may also non-stimulate HPC proliferation and peripheral mobilization, but may also stimulate tumor cell proliferation and metastasis, two leukemia cell lines and five solid tumor cell lines together with four normal human cell lines were used to determine whether exposure to low-dose radiation (25 to 200 mgGy X rays) can cause a stimulating effect on cell proliferation. A stimulating effect was found in the normal cell lines but not in the two leukemia and five solid tumor cell lines in response to low-dose radiation exposure in vitro. We found that LDR also induced an adaptive response to subsequently radiation-induced inhibition on cell proliferation in normal cells, but not in leukemia and solid tumor cells in vitro. To provide further evidence for the absence of LDR-induced stimulating effects in tumor cells in vivo, cells of two solid tumor cell lines were implanted in nude mice. Exposure of tumor cells in vitro before implantation in nude mice or of tumor-bearing mice to LDR (75 mgGy X rays) did not stimulate tumor growth compared to the tumor-bearing mice without low-dose radiation exposure. These results suggest that LDR stimulates growth of normal cells but not of leukemia and solid tumor cells in vitro and also does not stimulate growth of solid tumor cells in vivo. With the assistance of proteomic approaches, we found the profiles of protein expressions induced by LDR in normal cells and tumor cells are significant different. These distinct proteins are related to cell cycle, stress response, metabolism signaling pathways. For instance, LDR-induced horrmetic response in normal cells was mediated by up-regulation of ERK-dependent cell proliferation while LDR did not stimulate ERK-dependent cell proliferation signaling.


Individuals with defective low-dose damage response mechanisms are more likely to be at risk for radiation-inducible diseases, such as breast cancer. As a means to better understand tissue mechanisms that control radiation-induced breast cancer, we investigated genotypic variation in low-dose damage response pathways in the mammary glands (MG) of 2 strains of mice that differ in their susceptibilities to radiation-induced MG cancers (sensitive BALB/c vs. resistant C57BL/6). MG tissues were sampled at 4 hr (for early response) and 1 month (for persistent response) after 4 weekly fractionated 7.5cGy exposures, using sham and 1.8 Gy groups as reference. There were significant strain differences in low-dose non-linearities (both quantitative and qualitative) and thresholds for induction of expression. The early low-dose responses of sensitive BALB/c mice were associated with inflammation, hypoxia and functions related to MG development (p<0.05), not detected in similarly treated C57BL/6 mice. The persistent low-dose MG responses of both strains involved mitotic and microenvironmental functions with underlying genes generally expressed in opposite directions. Matching direction of expression in low-dose irradiated mice against ~950 genes integrated from 42 human breast cancer signatures identified ~35 cell-cycle and stromal genes associated with increased cancer risk in the sensitive BALB/c mice, with opposite responses in resistant C57BL/6 mice. We also identified ~30 genes linked to poor prognosis for human breast cancer whose expression was increased after low dose radiation in the sensitive BALB/c mice relative to C57BL/6. Our findings demonstrate the importance of genetic background on radiation damage response functions of MG tissue and identified new candidate mechanisms and biomarkers of genetic susceptibility for low-dose induced breast cancer. These findings are highly relevant for the large and growing numbers of individuals exposed to low-dose radiation from natural sources, nuclear industry accidents and medical procedures. [Supported by the DOE Low Dose SFA Program at Berkeley Lab under DOE Contract No. DE-AC02-05CH11213]

PO9S24-33. Distribution of Soluble Uranium in the Nuclear Cell Compartment of Kidney Cell and Relation to Toxicity. Gauguen Yann, D. Suhard, C. Rouas, L. Grandeo, I. Dublineau, C. Tessier, IRSN, France

Non-cancerous biological effects of chronic low doses exposure of radionuclides on human health is of concern worldwide but is poorly known. Uranium is one of the most public health-concern radionuclide as it is naturally found in the environment and its extensive use may result in the increase human exposure. Up to now, kidney cells were mainly used as in vitro models to study effects of uranium exposure as its main described target organ is the kidney. The aim of this work was to investigate subtoxic concentration and to study the localization of uranium and the impact of depleted uranium (DU) exposure at cellular level in human kidney cell line (HEK-293). Cell viability test was used to evaluate the DU cytotoxicity. Distribution of uranium into cells was undertaken by SIMS (secondary ion mass spectrometry) technique, an isotopic analysis of a solid surface by ion beam coupled with a mass spectrometer. Cytotoxicity study showed that the threshold of mortality was similar to other kidney cell line (> 300µM). Uranium was localized in cells with IMS technique. Results showed that uranium precipitates at subtoxic concentration above 100 µM. With this approach, we were able for the first time to observe soluble form of uranium in the cell at low concentrations (10-100 µM). Moreover, this technique allows us to localize it mainly in the nucleus [1]. Relation could be discussed with recent studies showing that uranium induces biological effect in kidney cells at subtoxic concentration including dysregulation of gene expression involved in signal transduction and trafficking or in development and cell proliferation [2-3]. These innovative results open new perspectives for studying the mechanisms of uranium chemical effect on cell viability and function at low level and raise the question of how uranium penetrates into cells and more particularly in the nuclear compartment in its soluble form.


PO9S24-34. Mortality due to diseases of infectious etiology in northern part of East-Ural Radioactive Trace. Iilia Yarmoshenko1, L. Konshina1, G. Malinovsky1, I. Tuzankina2, 1: Institute of Industrial Ecology, Russian Federation, 2: Institute of Immunology and Physiology, Russian Federation

Considerable amount of radioactive substances had been released after accidental explosion of waste storage tank at former soviet plutonium production plant “Mayak” in 1957. Atmospheric transfer and fallout of radionuclides (East-Ural Radioactive Trace, EURT). Due to consumption of contaminated food and milk gastrointestinal organs and bone and bone marrow received higher radiation exposure than other organs. Early we found excess gastrointestinal cancers mortality in EURT population. The purpose of current study is assessment of non-cancer radiation health effects that can appear as result of bone marrow exposure with special attention to diseases of infectious etiology and its complications with established immune dependence. Available register on causes of deaths of EURT northern part rural population was applied as a source of epidemiological data. Register records related to the settlements where initial surface contamination by Sr-90 was bellow and above 3.7 kBq/m² were included to
unexposed (4,844 records) and exposed (6,158 records) group respectively. Mean bone surface dose in exposed and unexposed group are 90 and 7 mGy respectively, with maximal dose 0.85 Gy. The following final diagnoses documented in death certificates were considered: acute pancreatitis, appendicitis, cerebral abscess, chronic bronchitis, diphtheria, dysentery, echinococcus, encephalitis, erysipelas, furunculosis, influenza, measles, osteomyelitis, peptic ulcer, pneumonia, purulent otitis, pyelonephritis, septicaemia, syphilis, tonsillitis, tuberculosis, whooping cough etc. The analysis consisted of comparison of proportionate mortality due to listed diagnoses and life durations in exposed and unexposed groups. Estimated total number of excess deaths due to diseases of infectious etiology in exposed group is 8 × 10^4 (with 90% confidence interval) of 401 observed in period 1958-2000 and excess total years of life lost is 102 ± 471. Sex, age at exposure and time since exposure patterns of risk were studied. Thus increasing of mortality due to infectious diseases with severe course and its complication was found, that can be associated with radiation exposure of immune system and bone marrow in particular.


Complicated radioecological situation in the world requires in-depth and comprehensive studies of biological effects of ionizing radiation on non-human biota, particularly on aquatic species. One of the most radiosensitive stages of plants ontogenesis is the process of generative reproduction. Seeds of the common reed (Phragmites australis L (Trin. Ex. Steud)) of 2009 vegetation year were planted in laboratory conditions. Specimens were sampled from closed and weakly-flowing, natural and man-made water bodies within Chernobyl exclusion zone (lakes Glyboke, Daleke, Azbuchyn, Yanivsky Creek and Cooling pond of Chernobyl NPP) and water bodies with background level of radionuclide contamination (Kiyv Reservoir and Verbrene Lake). Biological characteristics of seeds were assessed using the methods of vitality indexes analysis (technical germinating ability, germinating energy, survivability), assessment of germs ontogenesis disturbances and abnormalities. Absorbed dose rate for each radiation exposure of immune system and bone marrow in particular.

POS24-37. Adaptive DNA repair response in Medaka fish subjected to transgenerational chronic low-dose irradiation. John Zimbrick1, O. Moskalenko1, D. Girygoryev1, T. Hinton2, 1: Colorado State University, USA, 2: University of Minnesota, USA

This is the first work to show the relationships between DNA damage including base oxidation of 8-hydroxyguanine (8-OHG) and double-strand breaks (DSB) and transcript changes in a vertebrate model organism under transgenerational low dose-rate gamma irradiation. The Japanese medaka fish is a useful model for this study thanks to its short generation time and ability to survive and reproduce while subjected to the long-term ionizing irradiation. For doses from 3 to 9 cGy the yields of 8-OHG in muscle tissues linearly increased over the first three generations (F1-F3) then dropped in the fourth generation (F4). In the 30 – 80 cGy range 8-OHG yields increased from F0 and were similar in F1 – F3, but dropped in F4 as in the lower dose range. The yields of DSB in muscle tissues were similar in F0 – F3, but dropped in F4. In liver tissues the yields were similar in F0 – F2, but increased significantly in F3 and then dropped in F4. These data suggest that very low chronic doses do not trigger the 8-OHG repair responses in F1 – F3 so 8-OHG accumulates in the genomic DNA. By F4 medaka appear to have received sufficient doses to stimulate 8-OHG repair in an adaptive response. Human glycosylase Ogg1 is a key component of the Base Excision Repair pathway for the removal of 8-OHG. Medaka Ogg1 ortholog is highly similar in structure to mammalian genes. Its catalytic center is highly conserved reflecting evolutionary pressure to preserve critical defense mechanisms. Medaka Ogg1 transcript level changes correlate with the 8-OHG levels in F0 – F4 muscle tissues. Transcriptional profiling of F0–F4 muscle tissues with microarrays indicates an intricate interplay of biological networks involved in dermatological, inflammatory and hepatic diseases, developmental and genetic disorders, cell cycle, cellular growth, proliferation and death, cellular and tissue development, organismal injury and abnormalities, and cancer. Large changes were observed in transcripts related to free radical scavenging, DNA replication, recombination, and repair, nucleic and amino-acid metabolism, tissue and nervous system development and function, cell cycle, signaling and death, metabolic and neurological diseases. Many pathways show a decline in F1-F3 generations and a recovery or drastic increase in F4, which correlates with the DNA damage data.

POS25 Stem cells

POS25-01. The role of MAPK phosphatase, MKP1, in radiosensitivity of breast cancer stem cells. Demet Candas, M. Fan, J. Li, UC Davis, USA
Although the overall cure rate of primary tumors has been significantly improved in the last three decades, metastatic and recurrent tumors with aggressive growth and therapy-resistance remain to be the major factors shortening breast cancer patients’ survival. Cancer stem cells (CSCs) with the ability to differentiate and self-renew are identified in almost all human tumors and several important findings have revealed a role of CSCs in tumor radioresistance and environmental radiation exposure. The detailed mechanisms underlying CSCs’ radioresistance is far from being clear but is urgently needed to be elucidated. Recent evidence suggest that activation of MAPK phosphatase 1 (MKP1) is a key factor of pro-survival network induced in breast cancer cells following chemo- or radiotherapy. We have shown that radiation induced NF-κB-mediated MKP1 overexpression is able to increase breast cancer cell survival by attenuating pro-apoptotic c-Jun N terminal kinase, JNK, signaling. Recently, we have found that MKP1 is present in mitochondria and mitochondrial MKP1 levels are significantly enhanced in irradiated breast cancer cells. These new findings suggest that MKP1 is able to not only enhance MKP1 gene transcription but also increase MKP1 translocation to mitochondria, where MKP1 is able to dephosphorylate and inactivate JNK to inhibit radiation-induced apoptosis. Furthermore, we have shown that MKP1 mitochondrial localization is a feature of radioresistant breast cancer cells. In a recent study, we have found that a radioresistant subpopulation of breast cancer cells display similar characteristics to CSCs with CD44+/low/CD24+ feature. The data encouraged us to test whether the proposed MKP1-mediated radioresistance mechanism is present in CD44+/CD24- current breast cancer stem cells. Successful outcome of this study will provide critical insights on the aggressive phenotype of breast tumors, mainly recurrent/metastatic tumors, and define novel diagnostic and therapeutic targets to re-sensitize resistant cancer cells.

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**PO252-02. Response of Normal and Transformed Human Embryonic Stem Cells to Ionizing Radiation.** Steve Dingwall, Department of Medical Physics, McMaster University, Canada

The purpose of this study is to determine the relative effects of γ-irradiation on normal human embryonic stem cells (hESCs) and hESCs showing features of neoplastic transformation (t-hESCs), to identify potential targets for therapeutic intervention. These cell types are being used, as they provide a unique opportunity to compare a normal cell type, with the innate capacity for self renewal and proliferation, to a transformed counterpart. The t-hESCs have been previously characterized and shown to have increased proliferation, growth factor/ niche independence, enhanced capacity for teratoma formation, and have been shown to be refractory to differentiation down the neural and hematopoietic lineages. Both the hESC and t-hESC cells have been cytogenetically characterized by g-banding, spectral karyotyping (SKY) and comparative genomic hybridization (CGH). t-hESC lines were found to have a gain in one copy of chromosome 11 and an approximate 20q11.11-11.2 amplification was detected in the hESC cells, via CGH (Werbowetski-Ogilvie et al, 2009). Apoptosis levels were measured in hESCs and t-hESCs, via annexin/7AAD, 8 hours after an exposure to 40 Gy of 70kvP X-rays. t-hESCs were found to be refractory to the induction of apoptosis, relative to the hESCs (p<0.01). BrdU incorporation and 7AAD staining were used to assess the cell cycle response of both cell lines 12, 24, 48 and 72 hrs after an exposure to 40 Gy of 70kvP X-rays. Both cell lines exhibited cell cycle arrest primarily at the G2 checkpoint. Both cell lines exhibited maximal cell cycle arrest 12-24 hrs post irradiation and returned to normal cell cycle distribution by 72 hrs post irradiation. A significantly larger proportion of normal human embryonic stem cells hESC were present in the G2 phase of the cell cycle, 12 hrs post irradiation, relative to t-hESCs. Currently these cells are being used for the formation of teratomas, to test the in vivo radiation sensitivity between these two cell types and compare the efficacy of acute and fractionated doses of radiation.

**PO252-03. The radiosensitivity of neural stem cells derived from mouse embryonic stem cells: Proliferation, cell cycle regulation and DNA double-strand break repair.** Mayu Isono1, T. Konishi2, N. Shiomii1, N. Suya2, N. Inoue1, 1: Tokyo Metropolitan University, Japan 2: National Institute of Radiological Sciences, Japan

Radiation exposure to developing fetal period is known to cause neuronal disorders, such as neuronal dysfunction, microcephaly and mental retardation, which are considered mainly due to the radiation damage produced in neural stem cells (NSCs). NSCs, which are the dominant number in the fetal brain, have an ability to proliferate itself, and differentiate into other cell types that construct the central nervous system (CNS), such as neurons, astrocytes and oligodendrocytes. Recently, it has been reported that NSCs exist in an adult brain. Thus, investigation on radiation biological effects against NSCs would be one of the important studies for radiation risk assessment, such as for the field of radiation therapy, and health risks against space environment radiation exposure.

The purpose of this study was to examine the cellular responses of proliferating NSCs against X-ray irradiation. Following biological endpoints were measured to demonstrate the radio-sensitivity of NSCs: maintenance of its ability to proliferate as NSCs, growth curve, cell cycle analysis and DNA repair. The NSCs were derived from embryonic stem cells (HK cell line) of C57BL/6 mice using Neural Stem Sphere method that has established originally. The cells were irradiated up to 10 Gy by 200 kVp X-ray. Our findings demonstrated that NSCs, which survived after X-ray irradiation, maintained its ability as stem cells. Cellular responses, such as cell cycle arrest due to the DNA damage recognized at G1 and G2/M check points, and the linear-quadratic repair kinetics of DNA double-strand break repair, were similar to other mammalian cell lines. Further investigation on radiation sensitivities of NSCs in proliferation condition is necessary, and clarifying the correlation between the radiation-induced DNA damage in proliferative NSCs and its effect in the differentiation pathway will give the important information on radiation risks against in CNS.

**PO252-04. Loss of a Bcl11b allele promotes proliferation of stem cells in the mouse small intestine after γ-irradiation.** Yoshinori Katsuragi, A. Sakamaki, M. Obata, Y. Mishima, R. Kominnami, Grad.Sch.Med.&Dent.Sci., Niigata University, Japan

Radiation effect has been studied on mouse intestinal epithelial cells (IECs) that consist of stem cells and progenitors in the crypt and differentiated cells in flanking villus regions. The stem cells are classified into two types of cells, one of which is label-retaining cells at position+4 (+4 LRCs) and the other is Lgr5+ CBC cells at the crypt base. Recent papers indicate that +4 LRCs, which are thought to be potential stem cells, show transient activation to generate CBC active stem cells and progenitors under various conditions of stress or injury including γ-irradiation. Bcl11b encodes a transcription factor that plays a tumor-suppressive role in lymphoma/leukemia and is expressed in the crypt cells. Here we show that loss of a Bcl11b allele affects the regeneration capacity of IECs after γ-irradiation. We investigated the migration rate of IECs along the crypt-villus axis, by BrdU administration and chase for 3 days. Most of the BrdU+ cells reached near the end positions (positions 36-48) and the migration rate did not differ between Bcl11b+/+ and Bcl11b-/- IECs. Consistently, no difference was seen in the proliferation rate between the two. In contrast, whole body Bcl11b+ mice were more radioresistant than Bcl11b-/- mice in terms of migration and proliferation rates. The distribution of BrdU+ cells at 3 days after was more lowered in Bcl11b-/-+ mice than in Bcl11b+/+ mice. Also, inhibition of BrdU incorporation at 16 h after irradiation was more in Bcl11b-/-+ CBC cells, indicating that Bcl11b-/-+ CBC cells were more refractory to radiation-induced cell cycle arrest. This may account for the difference in cell migration. Central to the DNA damage checkpoint is p53 tumor suppressor. As expected, p53 was less activated in crypt cells of Bcl11b-/-+ mice. These new findings suggest that radiation-induced expansion of proliferation of normal IECs may be due to increased p53 signaling, which also indicates that p53 inactivation is not a general mechanism for radioresistance in the small intestine. Understanding the roles of p53 is important for understanding how the human body protects itself from radiation and cancer. 

**PO252-05. Importance of PKC8 signaling in fractionated radiation-induced expansion of glioma neural progenitor cells and resistance to anticancer treatments.** Min-Jung Kim1, R-K. Kim1, C-H. Yoon1, I-G. Kim2, S-J. Lee1, 1: Department of Chemistry, Research Institute for Natural Sciences, Hanyang University, South Korea, 2: Department of Radiation Biology, Nuclear Environmental Safety Research Division, Korea Atomic Energy Research Institute, South Korea

Purpose: Brain tumors frequently recur or progress as focal masses after treatment with ionizing radiation. However, the mechanisms underlying the repopulation of tumor cells after radiation have remained unclear. In this study, we show that cells expressing leukemia viral oncogene homolog (c-AbI)-protein kinase Cδ (PKCδ) signaling is critical for fractionated radiation-induced expansion of
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Experimental procedures: Treatment of human glioma cells with fractionated radiation increased c-Abl and PKCδ activity, expanded the CD133-positive (CD133⁺) cell population that possesses tumor-initiating potential, and induced expression of glioma stem cell markers and self-renewal-related proteins. Moreover, cells treated with fractionated radiation exhibited resistant to anticancer treatments. Small interfering RNA (siRNA)-mediated knockdown of PKCδ expression blocked fractionated radiation-induced CD133⁺ cell expansion and suppressed expression of glioma stem cell markers and self-renewal-related proteins. It also suppressed resistance of glioma cells to anticancer treatments. Similarly, knockdown of c-Abl led to a decrease in CD133⁺ cell populations and restored chemotherapeutic sensitivity. It also attenuated fractionated radiation-induced PKCδ activation, suggesting that c-Abl acts upstream of PKCδ.

Summary: We found that fractionated radiation-induced PKCδ activation was associated with c-Abl tyrosine kinase. siRNA targeting of c-Abl attenuated fractionated radiation-induced enrichment of the extenmitating cell population and effectively restored chemotherapeutic sensitivity, suggesting that c-Abl is located upstream of PKCδ activation in the response to fractionated radiation, and plays a role in the enrichment of the glioma stem-like cell population and the acquisition of anticancer drug resistance.

Conclusions: We show that PKCδ activation is essential for the expansion and maintenance of glioma stem-like cell populations and acquisition of resistance to anticancer treatments induced by fractionated radiation. The results elucidated in this study provide insights that may prove pivotal in the context of ionizing radiation-based therapeutic interventions in brain tumor.

POS25-06. Characterization of biological response to ionizing radiation in mouse neural stem cells. Seiji Kodama, Osaka Prefecture University, Japan

A stem cell can be an origin of cancer and is supposed to be a crucial target for radiation carcinogenesis. However, little has been known about the characteristics of biological response to ionizing radiation in stem cells. Recently, neural stem cells can be cultured as neurosphere whereby we can ask the radiation response in stem cells. In the present study, we examined two biological endpoints in mouse neurosphere cells for evaluating the characteristics of stem cells; the repair kinetics of X-ray-induced DNA double strand breaks (DSBs) and the pathway toward immortalization by successive subculture of the cells. Fibroblast cells and neurosphere cells that contain a fraction of neural cells for evaluating the characteristics of stem cells. First, the yield of the CD133⁺ neurosphere cells was enhanced for all lineages (CFU assay). The cytogenetic analysis elucidated that the CD133⁺ neurosphere cells are formed by mixing cancer cells and fibroblast cells. Second, to establish a self-renewing cell population and acquired expression of glioma stem cell markers and self-renewal potential, and induced expression of glioma stem cell markers and self-renewal-related proteins. Moreover, cells treated with fractionated radiation exhibited resistant to anticancer treatments. Small interfering RNA (siRNA)-mediated knockdown of PKCδ expression blocked fractionated radiation-induced CD133⁺ cell expansion and suppressed expression of glioma stem cell markers and self-renewal-related proteins. It also suppressed resistance of glioma cells to anticancer treatments. Similarly, knockdown of c-Abl led to a decrease in CD133⁺ cell populations and restored chemotherapeutic sensitivity. It also attenuated fractionated radiation-induced PKCδ activation, suggesting that c-Abl acts upstream of PKCδ.

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Conclusions: We show that PKCδ activation is essential for the expansion and maintenance of glioma stem-like cell populations and acquisition of resistance to anticancer treatments induced by fractionated radiation. The results elucidated in this study provide insights that may prove pivotal in the context of ionizing radiation-based therapeutic interventions in brain tumor.


The increasing application of heavy ions in radiotherapy and the growing interest in protracted space travels are a strong motivation to expand the research on long term effects of radiation exposure. Although cytogenetic analysis has been conducted in lymphocyte populations, the effects in human hematopoietic stem and progenitor cells (HSPC), which permanent renew all blood cells, remain to be elucidated.

Human CD34⁺/CD45⁻ HSPC were isolated from peripheral blood of healthy donors and irradiated with X-rays or carbon ions (100MeV/u, 114-158MeV/u). Chromosomal aberrations were analyzed by mFISH in metaphase cells 48h after irradiation. Cenomeric survival was determined in a 3D culture model after 14 days and the differentiation potential of the lineage restricted progenitors of the erythroid, granulocytic and monocyte-macrophage pathways were assessed (CFU assay). By isolation of single colonies the occurrence of aberrations in the progeny of irradiated HSPC was investigated (mFISH).

The results revealed a linear decrease in colony forming unit ability after exposure to ionizing radiation (relative biological efficiency of carbon ions < 2). Following irradiation the cells of the erythroid lineage were enriched compared to cells of the granulocyte/monocyte lineages. In addition, the fraction of mature compared to immature cells was enhanced for all lineages (CFU assay). The cytogenetic analysis 48h after irradiation revealed a dose-dependent increase in aberrant cells and complex-type aberrations per cell, both effects being more pronounced for carbon ions than X-rays. In contrast, in the progeny of irradiated cells (14 days after irradiation), the yield of chromosomal damage was not different comparing HSPC after irradiation with X-rays and carbon ions. Both types of radiation predominantly induced reciprocal translocations, detected in 2/3 of all surviving colonies, whereas in 1/3 of the surviving colonies no aberrations were observed. Remarkably, in all these clones the chromosomal aberrations were transmitted clonally to the progeny of the irradiated cells. The relevance of the described results will be discussed in the light of the results on differentiation pattern and transmission of chromosomal aberrations obtained in engrafted HSPC that were xenotransplanted into NOD/SCID mice after radiation exposure.

POS25-08. Use of the Hybrid Spheroid Assay to Measure the Sensitivity of Individual Patient Cancer Stem Cells. Christopher Lange, S. Jie, A. Grousses, T. Syed, M. Agarwal, E. Navo, B. Djordjevic, O. Abulafia, L. Dresner, M. Rotman, SUNY Downstate Medical Center, USA

This study is to determine if the putative cancer stem cell (pCSC) fraction and treatment sensitivity of individual patient breast and cervical tumors, as estimated by the Hybrid Spheroid (HS) Assay, is consistent with data from other methods. Using the HS identify fresh tumor cells capable of extensive proliferation and self-renewal, hallmark of stem cells. HS are formed by mixing cancer test cells and fibroblasts. Spheroids of defined sizes are selected by filtration, distributed 1/well in 96-well ultralow attachment plates, treated (e.g., irradiated), and then monitored for growth. HS growth is due to the test cells. Using the fraction of HS that do not grow as the zero term of a Poisson distribution provides a measurement of clonogenicity and the pCSC fraction, for use with fresh tumor samples.

Results:
1) Cervical carcinoma cells, from fresh surgical samples, produced no colonies from 40,000 cells plated in tissue culture dishes (Plating Efficiency, PE < 2.5x10⁻²). However, when mixed 5.5% tumor cells, 94.5% fibroblasts, to form HS, some cells were clonogenic (i.e., producing 10 - 15 division spheroids).
2) The clonogenic (pCSC) fraction (PE) was 0.50 ± 0.09% in small (88-105 µm) & 0.76 ± 0.15% in large spheroids (105-125 µm). Ratios of the treated/control pCSC fractions yield single cell survivor curves. Growth is due to the test cells. Using the fraction of HS that do not grow as the zero term of a Poisson distribution provides a measurement of clonogenicity and the pCSC fraction, for use with fresh tumor samples.

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Conclusions: The measured PEs are consistent with expected CSC fractions. We are also testing cervical and breast cancer CSC-containing spheroids for tumor production in NOD/SCID/Gamma mice, for CSC content in second generation HS, and measuring patient breast and cervical tumor CSC survival curves. This assay could improve cure rates by individualizing cancer treatments to the sensitivities of each patient’s CSCs.

PO525-09. Mouse salivary gland stem cell to rescue radiation-induced hyposalivation. Martti Maimets, L.S.Y. Nanduri, S. Pringle, M. Niemantsverdriet, M. van der Zwaag, M. Baanstra, R. van Os, R.P. Coppes, University Medical Center Groningen, Netherlands

One option to rescue damage to salivary glands caused by radiotherapy would be stem cell therapy. In mice we showed previously that transplantation of c-Ki67+ salivary stem/progenitor cells (SSC) rescued irradiated salivary gland function. The aim of this project is to develop cell therapy to restore irradiation-induced salivary gland damage. For this, a further analysis of the putative stem cell population in the salivary glands is necessary. To further characterize the exact SSC in mice, cells from salivary gland were cultured as salispheres, dispersed and analyzed by flow cytometry for expression of known stem cell markers. Salispheres contained c-Ki67+, CD24−/CD29, CD49h+, and CD133+ cells. Moreover, clear distinct populations of CD133−/CD24− cells were identified, which represented a positive c-Ki67+, CD24+CD49h− and CD24−/CD29+ cells were found, but no co-expression of c-kit and CD133. This data was confirmed with immuno-histochemical stainings of stem cell markers in the ducts of intact mouse submandibular gland.

Next we transplanted our defined cell populations in submandibular glands 30 days after local irradiation (15 Gy). Our data shows that all transplanted subpopulations restored salivary gland function but with different potencies. Interestingly, transplantation of only 500 CD24+ c-Ki67+ double positive cells per gland induced efficacious regeneration but with a delayed onset. The results also indicate that potentially multiple SSC reside in the salivary gland. To find a marker specific for the salivary gland and assess crucial genes involved in the maintenance of the salivary gland stem cells, we used a dedicated microarray with 263 stem-cell-associated gene transcripts. Quantitative PCR confirmed upregulation of Bmp7, Cdh1, Cdh13, Notch1 and CD44 in 4-day salispheres, when compared to salivary glands and fibroblasts. The expression of Bmp7 was very low in normal submandibular gland compared to 4-day salispheres. Since Bmp7 plays an important role during embryonic submandibular gland branching morphogenesis and interacts with CD44, it represents a possible SSC marker.

In conclusion, for early and extensive regeneration of the salivary gland, combinations of different cell populations may be needed for transplantation. Specific expression of stem cell-associated genes may help in finding the right combination of cells.

PO525-10. Effects of ionizing radiation on neural cells in organotypic hippocampal slice cultures and human glioblastoma slice cultures. Felicitas Merz1, G. Taucher-Scholz2, M. Durante3, J. Meixensberger5, M. Schaefer4, C. Hellwig4, F. Gaunitz4, I. Kashiwakura1, 1: University of Leipzig, Institute of Anatomy, Germany, 2: GSI Helmholtzzentrum für Schwerionenforschung, Germany, 3: Clinic and Policlinic for Neurosurgery, University Hospital of Leipzig, Germany, 4: Institute of Pharmacology and Toxicology, University of Leipzig, Germany, 5: Institute of Anatomy, University of Leipzig, Germany

Heavy ions (HI) currently raise dual interest in biomedical research: they can be used for tumor treatment (Schulz-Ertner et al., 2006; Combs et al., 2010), but may also provide harm to astronauts during long-term space missions. We have set up tools to explore effects on human brain tissue and intact brain tissues. As for the former, we prepared slice cultures from human glioblastoma multiforme (GBM) which are vital in culture for at least two weeks and developed a method to expose them to photons or HI as well as combined treatment with chemotherapeutics or novel compounds (Merz et al., 2010; Merz et al., 2011). For the latter, we used organotypic rodent entorhino-hippocampal slice preparations (OHSC, Kluge et al., 1998; Eyupoglu et al., 2004) and particularly focused on the effects of HI on the neural stem cells in this brain region (Müller et al. 2010, Ehninger and Kempermann, 2008).

Our results showed in GBM, cell death increases in response to the different treatment options (carbon ions, photons, and/or chemotherapeutics); in combined treatment with carbon and temozolomide, there was massive cell death after 48h paralleled by nuclear fragmentation. Proliferation decreased in the irradiated samples, and DNA double strand breaks were present. In OHSC, our data show induction of DNA damage and repair upon exposure to photon and heavy ions with 1 and 4 Gy (Merz et al., 2010), and a decrease in glial cell proliferation 24h and 72h after irradiation, which recovered over time. The total number of adult neurons was not significantly altered over up to six weeks, and there was no indication of enhanced cell death. As for neural stem cells, we are currently analyzing the effects of irradiation on different stages of neuronal progenitors in the dentate gyrus in combination with proliferation markers (BrdU and Ki67).

Thus, human and rodent brain slice preparations are a unique tool to study effects of HI (and photons) on cells within their organotypic environment. Funded by ESA/DRL (to F.M., G.T.-S. & I.B.) and BMBF (to I.B.)

Citations


Future Oncol. 7(4):489-99


PO525-11. Characteristic Analysis of Megakaryocytopenosis and Thrombocytopenia by Hematopoietic Stem Cells Exposed to Ionizing Radiation. Satoru Monzen1, T. Nakamura1, I. Kashiwakura1, 1: Department of Radiological Life Sciences, Hiroasaki University Graduate School of Health Sciences, Japan 2: Department of Biomedical Sciences, Hiroasaki University Graduate School of Health Sciences, Japan

Hematopoietic processes, especially megakaryocytopenosis and thrombocytopenia, are highly sensitive to extracellular oxidative stress, such as ionizing radiation and chemotherapeutic agents. This study examined the terminal maturation of megakaryocytes and platelet production generated from hematopoietic stem cells irradiated with ionizing irradiation. Highly purified CD34+ cells derived from human placental/umbilical cord blood were exposed to X rays (2 Gy, 150 kVp, 20 mA; 0.5-mm aluminum and 0.3-mm copper filters) from an X-ray generator (MBR-1520R; Hitachi Medical Co., Ltd., Tokyo, Japan) with a distance of 45 cm between the focus and target, at a dose rate of approximately 1 Gy/min, and then cultured in a serum-free medium supplemented with thrombopoietin and interleukin-3. The number of cells generated from X-irradiated CD34+ cells decreased with the time in culture. However, the fraction of the CD34+Tie-2− cell population and CD41+Tie-2− cell population detected in the cells generated from X-irradiated cells showed a significant increase, in comparison to the controls on day 7. In addition, the CD42a+ platelet particles generated from the X-irradiated cells appeared to be normal. Next, the expression of various genes was analyzed using quantitative real-time RT-PCR in the cells harvested from the cultures. The early hematopoiesis-related genes; FLI1, HOXB4 and Tie-2, cytokine receptor genes; KIT and IL-3RA, and the oxidative stress-related genes; HO1 and NQ01 were upregulated on day 7. The present results suggest that normal terminal maturation of...
megakaryocytes and platelets could be obtained from the residual hematopoietic stem/progenitor cells (HSPCs) after X-irradiation, although this radiation did inflict some damage on the proliferation and differentiation of HSPCs.

POS25-12. Increase of chromosome aberration by Fe ion irradiation in Histone H2AX-deficient mouse ES cells, Takashi Morita, K. Yoshida, M. Hada, T. Teramura, S. Yoshida, F.A. Cucinotta. 1: Laboratory of Molecular Genetics, Osaka City University, Graduate School of Medicine, Japan 2: USA-NASA Johnson Space center, USA, 3: Kinki University, Faculty of Medicine, Japan

It is important to estimate the influence of space radiation on human body during a longer stay in space including missions to ISS, the moon, or Mars. We used mouse embryonal stem (ES) cells to estimate effects. Since it is difficult to culture ES cells for extended periods on the ISS, we selected a way to expose the ES cells to space environment while in a frozen condition [1]. The frozen ES cells are found to be resistant to radiation including Fe ion beam, so a more sensitive method to detect radiation damage is preferable. Histone H2AX gene is involved in signaling and repair of DNA double-strand breaks, and the deletion of the gene will sensitize the ES cells to radiation. Therefore a shorter exposure time will be required to detect chromosome aberration in ES cells by space radiation. We have established mouse ES cells with heterozygous and homozygous deletion mutation of Histone H2AX by crossing H2AX (+/-) heterozygous mouse and by culturing inner cell mass of 2-cell stage embryos in vitro. The heterozygous cells showed decreased H2AX protein in about half of that of wild-type (+/+). The ES cells were exposed to Fe ion radiation (Fe 600MeV, BNL) and chromosome aberrations were analyzed by fluorescence in situ hybridization (FISH) with whole-chromosome probes. Results showed an increase in simple and complex exchanges in H2AX-heterozygous and homozygous mouse ES cells. This suggests the H2AX gene deleted ES cells can be used for sensitive and quantitative estimation of biological influence of space radiation.

REFERENCES

POS25-13. Human mesenchymal stem cells respond to ionizing radiation by induction of stress-induced premature senescence, Martina Rezácova1, J. Cmielova1, R. Havelek1, S. Toukup1, J. Vavrova1, M. Mokry1. 1: Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic 2: University of Defence, Faculty of Military Health Sciences, Czech Republic

Purpose: Mesenchymal stem cells isolated from bone marrow (BM-MSC) and periodontal ligament (PLSC) are cells with high proliferative potential and ability to self-renewal. Characterization of these cells under genotoxic stress conditions contributes to the assessment of their prospective usage. The aim of our study was to evaluate changes in BM-MSC and PLSC caused by ionizing radiation.

Methods: Human BM-MSC and PLSC were irradiated with the doses up to 20 Gy by Co60 and observed 13 days; viability, proliferation, apoptosis and senescence induction, and changes in expression and phosphorylation status of related proteins were studied.

Results: Irradiation with the doses up to 20 Gy significantly reduces proliferation, but has no significant effect on cell viability. The activation of p53 and its phosphorylations on serines 15 and 392 were detected from the first day after irradiation by 20 Gy and remained elevated to day 13. Expression of cyclin-dependen kinases inhibitor p21 and p16 increased. The cell cycle was arrested in G2 phase. Instead of apoptosis, we have detected hallmark of stress-induced premature senescence: increase in p16 transcripts and increased activity of senescence-associated β-galactosidase.

Conclusion: Mesenchymal stem cells isolated from bone marrow and periodontal ligament respond to ionizing radiation by induction of stress-induced premature senescence.

POS25-14. Study of radiosensitivity in cancer stem-like cells from glioblastoma cell line, Momoko Takahashi1, H. Hiraikawa2, A. Fujimori1, 1: Japan Atomic Energy Agency, Japan, 2: National Institute of Radiological Sciences, Japan

Radiotherapy is one of the conventional treatments for solid tumors. Investigation of tumor radiosensitivity is important for future tumor radiotherapy. Biological bases on the resistance of tumor cells have not been fully understood. Recently several studies demonstrated that there is a small fraction in solid tumor which is highly resistant to ionizing radiation and that the radioreistant tumor cells often express some stem cell markers. This small population of cells is characteristic of stem cells and called “cancer stem cell”. It is highly resistant to ionizing radiation and it is suggested that resistance of cancers to both radiation and chemotherapeutic agents can be attributed to the features of the cancer stem cells. Therefore the clarification of cancer stem cells might solve the resistance of cancer to ionizing radiation. The purpose of this study is the analysis of cancer stem-like cells derived from established glioblastoma cell line and radiosensitivity in cancer stem-like cells using X-rays and heavy ion beam provided by HIMAC (heavy ion medical accelerator in Chiba) in NIRS (National Institute of Radiological Sciences). In the present study, we show that a human glioblastoma cell line A172 transiently becomes to cancer stem cell-like when cultured with non-serum media which is supplemented with several growth factors. A172 cells cultured with non-serum media supplemented with several growth factors showed the morphological change and formation of neurospheres, the characteristic of cultured neural stem cells. The treated cell population was significantly resistant to X-rays and heavy ion particles (carbon) compared with the A172 cells cultured with the normal media. Phosphorylation of histone gamma H2A.X by irradiation was induced in both forms, however recovered earlier in the treated cell population than in the parental cells. As a conclusion, our result is consistent with the hypothesis that stemness of A172 glioblastoma cells contribute to radioresistance by the efficient repair activity on DNA double-strand breaks.

POS25-15. Irradiated stem cells and aging of the hematopoietic system, Jirina Vavrova1, Z. Sinkorova1, E. Lukasova2, M. Rezácova2, 1: Faculty of Military Health Sciences University of Defence, Czech Republic 2: Czech Academy of Sciences, Institute of Biophysics, Czech Republic 3: Institute of Medical Biochemistry, Faculty of Medicine, Czech Republic

Objective: In the work presented here, changes in haemopoiesis of mice (B6129SF2/J) were studied one year after their whole-body exposure to a dose of 7 Gy (72 % of mice survived). The irradiated mice were compared to non-irradiated younger (4 months of age) and older (16 months of age) mice.

Material and methods: Single cell suspensions prepared from the bone marrow of mice were analyzed on the flow CyAn-ADP cytomter (DakoCytomation). In terms of the ability to respond to further whole-body irradiation at a dose of 1 Gy, the presence of gamma-H2.A.X foci was studied in lin- bone marrow cells of mice using confocal cytomter.

Results: There was a significant increase in the relative abundance of primitive stem cells with long-term capability of the haemopoiesis recovery (LT-HSC) in (74% CD45+ /CD117/ /CD34) in the bone express of mice aged 16 months (irradiated and non-irradiated) compared to those aged 4 months. We detected an obvious increase in number of foci in the lin- SC of animals studied one year after their exposure to a dose of 7 Gy (compared to non irradiated animals of the same age), indicating residual damage caused by irradiation at the age of 4 month. 1 hour after second irradiation at 1 Gy the DNA-damage foci formation was equal in all groups, in old mice (C16m as well as IR16m) we found an impaired ability of the foci repair 24 hours after irradiation. In the blood count from the peripheral blood of the older mice (both non-irradiated and irradiated at 7 Gy), there was a significant increase in granulocytes. In the group exposed to 7 Gy, the numbers of thrombocytes significantly increased and on the contrary, the numbers of erythrocytes, the amount of haemoglobin, and haemocril significantly decreased.

POS25-16. Amelioration of Ionizing Irradiation-Induced Hematopoietic Injury by Resveratrol in Mice, Heng Zhang1, Z. Zhai1, J. Zhang1, Y. Wang1, X. Wang1, H. Wu1, D. Li1, L. Liu1, J. Chang2, Q. Hui1, Z. Ju1, D. Zhou1, A. Meng1, 1: Tianjin Key Laboratory of Molecular Nuclear, Institute of Radiation Medicine, Peking Union Medical College & Chinese Academy of Medical Science, China, 2: Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Science, China, 3: Institute of Laboratory Animal sciences, Peking Union Medical College & Chinese Academy of Medical Science, China, 4: Pharmaceutical Sciences and Wunthrop P. Rawefeldt Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, USA
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**POS26-01. Induction of Strand Breaks in DNA by Low Energy Electrons and Soft X-rays under Nitrous Oxide Atmosphere.** Elahe Alizadeh, D. Hunting, L. Sanche, Department of Nuclear Medicine and Radiobiology, Faculty of Medicine, University of Sherbrooke, UK

Nitrous oxide (N\textsubscript{2}O) has previously been shown to radiosensitize hydrated DNA via its reactions with solvated electrons that increase the yield of OH radicals in irradiated aqueous environments. In the present work however, we investigate a quite different set of reactions of N\textsubscript{2}O with dry DNA (i.e., only structural water present), in particular those initiated by low energy (< 30 eV) electrons (LEEs), which represent an important component of the secondary particles generated in matter by low energy photons or electrons. A set of experiments and calculations were performed with the code ELSA to determine the primary and secondary reaction rates under a N\textsubscript{2}O atmosphere, which represent an important component of the secondary particles generated in matter by low energy photons or electrons. A set of experiments and calculations were performed with the code ELSA to determine the primary and secondary reaction rates under a N\textsubscript{2}O atmosphere.

**POS26-02. Damage induced by hydrated electrons to the cisplatin-DNA complex.** Behnaz Behmand, P. Cloutier, S. Girouard, A.D. Bass, L. Sanche, D. Hunting, University of Sherbrooke, UK

Cisplatin (P\textsubscript{Cl}(NH\textsubscript{2})\textsubscript{2}) is a chemotherapeutic agent that chemically binds to the DNA bases, especially guanine (G) [1, 2]. Our recent experiments on hydrated DNA complexes revealed an increase, relative to pure DNA samples, in the yield of strand break damage, when these samples were exposed to monoenergetic X-rays of 1.5 keV under gaseous N\textsubscript{2}O with and without O\textsubscript{2} at atmospheric pressure and temperature. Whereas the damage yields for DNA deposited on glass are due to soft X-rays, those arising from DNA on tantalum are due to both the interaction of low energy photoelectrons from the metal and X-rays. Then, the differences in the yields of damage to DNA on glass and tantalum substrates, essentially arises from interaction of LEEs with DNA molecules and the surrounding atmosphere. The G-values (i.e., the number of moles of product per joule of energy absorbed) for DNA strand breaks induced by LEEs (G\textsubscript{LEE}) and the logarithmic of G-value (G\textsubscript{value}) was calculated and the results compared to those from previous studies under atmospheric conditions and other ambient gases, such as N\textsubscript{2}O and O\textsubscript{2}. Under N\textsubscript{2}O, the G-values for loss of supercoiled DNA are 102.5±7.5 mmol/J for X-rays, and 227±15 mmol/J for LEEs, respectively. This result indicates a much higher effectiveness for LEEs relative to 1.5 keV X-rays in causing DNA damage in an N\textsubscript{2}O environment. This enhancement is attributed to the interaction of LEEs with the N\textsubscript{2}O molecules at the film/gas interface and condensed within the DNA film, particularly via dissociative electron attachment to N\textsubscript{2}O, which leads to the formation of reactive species such as O\textsub{2}. Therefore, even in the absence of non-hydration water as the source of OH radicals and solvated electrons, N\textsubscript{2}O can by itself considerably increase chemical reactions between LEEs and DNA. Thus, the previously observed radiosensitization of cells by N\textsubscript{2}O may not be only due to OH radicals but also to the reaction of LEEs with N\textsubscript{2}O molecules surrounding DNA.

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**Introduction** Ionizing radiation (IR) causes acute tissue damage and late effects including long-term bone marrow (BM) injury, which arises from persistent ROS accumulation induced apoptosis and senescence of hematopoietic cells. Resveratrol (3,5,4'-trihydroxy trans stilbene, Rev), a kind of polyphenol compounds, which shows anti-oxidant, anti-apoptosis and lifespan extension effects, was reported in recent studies. Its radiation protection effect on hematopoietic system awaited to be investigated.

**Purpose** This study was undertaken to examine whether resveratrol ameliorated radiation induced hematopoietic system damage and to explore its mechanism.

**Methods and Materials** C57BL/6 mice were exposed to a sublethal dose (6.0 Gy) of total body irradiation (TBI). Half of them were administered with resveratrol (20mg/kg) by gavage every 24 h for 7 days prior to TBI and 28 days after it. The hematopoietic functions of HPC and HSC were analyzed by cobblestone area-forming cell (CAFC) assays and competitive repopulation assays (CRA). Murine HPC (Lin-ck-sFlk-1), ST-HSC (Lin-ck-sFlk-1), LT-HSC (Lin-ck-sFlk-1) were sorted by a flow cytometry and total RNA was isolated. mRNA expression of genes associated with oxidative stress, NADPH oxidase 4 (NOX4), superoxide dismutase 2 (MnSOD) and glutathione peroxidase (GPx1) were analyzed by quantitative Real Time-PCR (qRT-PCR).

**Results** The survival fractions of all day-types of CAFC of irradiated mice were 84.6% on day 7 and 49.5% on day 35. In CRA, hematopoietic cells of mice treated with resveratrol and TBI exhibited a higher repopulation ability compared with mice treated with TBI only (43.42%, P < 0.001). The expression level of NOX4 mRNA was selectively increased in HSC of TBI mice, and could be decreased by resveratrol administration. It was also observed that reduction of SOD2 and GPx1 mRNA expression in HPC, ST-HSC, LT-HSC of irradiation mice were attenuated by treatment of resveratrol.

**Conclusion** Resveratrol significantly ameliorates suppression in the hematopoietic function of HSCs and HPCs by TBI. Data of qRT-PCR assays suggests that resveratrol protects hematopoietic function by modulating oxidative stress associated genes expression.

**POS25-17. Ionizing radiation induces hematopoietic stem cell senescence and long-term bone marrow suppression in a p16Ink4a-Arf independent manner.** Daoshong Zhou\textsuperscript{1}, L. Shao\textsuperscript{1}, W. Feng\textsuperscript{1}, H. Li\textsuperscript{1}, Y. Wang\textsuperscript{1}, N.E. Sharpless\textsuperscript{2}, 1: University of Arkansas for Medical Sciences, USA, 2: Medical University of South Carolina, USA, 3: The University of North Carolina at Chapel Hill, USA

Many patients receiving chemotherapy and/or ionizing radiation (IR) develop residual (or long-term) bone marrow (BM) injury that can not only limit the success of cancer treatment but also adversely affect their quality of life. Although residual BM injury has been largely attributed to the induction of hematopoietic stem cell (HSC) senescence, the molecular mechanisms by which chemotherapy and/or IR induce HSC senescence have been clearly defined, nor has an effective treatment been developed to ameliorate the injury. The Ink4a-Arf locus encodes two important tumor suppressor genes, p16\textsuperscript{Ink4a} and Arf. Both of them have been implicated in mediating the induction of cellular senescence in a variety of cells including HSCs. Therefore, we examined the role of p16 and/or Arf in IR-induced HSC senescence and long-term BM suppression using a total body irradiation (TBI) mouse model. The results from our studies show that exposure of wild-type (WT) mice to a sublethal dose (6 Gy) of TBI induces HSC senescence and long-term BM suppression. The induction of HSC senescence is not associated with a reduction in telomere length in HSCs and their progeny, but is associated with significant increases in the production of reactive oxygen species (ROS), the expression of p16 and Arf mRNA, and the activity of senescence-associated β-galactosidase (SA-β-gal) in HSCs. However, genetic deletion of Ink4a and/or Arf has no effect on TBI-induced HSC senescence, as HSCs from the Ink4a and/or Arf knockout mice after exposure to TBI exhibit similar changes as those seen in the cells from irradiated WT mice in comparison with the cells from un-irradiated mice with correspondent genotypes. In addition, TBI-induced long-term BM suppression is also not attenuated by the deletion of the Ink4a and/or Arf genes. These findings suggest that IR induces HSC senescence and long-term BM suppression in a p16Ink4a/Arf independent manner.

**POS26 Radiation damage to biomolecules**
POSTER PRESENTATIONS

POS26-03. Electron transfer in irradiated deoxyglycineolactones: inter-strand vs. intra-strand transfer in duplex DNA. Paul Black, W. Bernhard, University of Rochester, USA

Direct radiation damage to DNA begins with the formation of radical cations by one-electron oxidation and radical anions by one-electron reduction. The aim of this work is determine how DNA’s duplex structure and base sequence influences trapping of the excess electron. Ultimately, though X-radiation make it possible to study DNA lesions generated in DNA in vivo. Deoxyglycineolactones of varying length and sequence were irradiated at 4kV using 70keV X-rays in a LiC1 glass. After irradiation, samples were studied at 4K using Electron Paramagnetic Resonance (EPR). The EPR spectrum of one-electron reduced oligomers is a simple sum of components, consisting of the spectrum due to each of the one-electron reduced nucleobases. Using the basis spectra of each one-electron reduced nucleobase as components, the oligomer spectra were deconvoluted. For adenine-thymine electron transfer in the absence of cytosine, there were two findings. 1) When thymine is present on the same strand as adenine, electron transfer, and subsequent trapping at thymine outcompetes electron trapping at adenine by ~100X. This intra-strand transfer behavior holds true when as many as 32 sequential adenines precede thymine along the same strand. 2) When thymine is not present on the same strand as adenine, electron trapping at adenine outcompetes inter-strand transfer and subsequent trapping by thymine on the opposite strand by ~100X. When cytosine is present on either strand of the duplex, however, transfer from adenine, either via an inter- or intra-strand mechanism outcompetes electron trapping at adenine. We conclude that in vivo trapping of the excess electron by adenine will be effectively zero. The probability of trapping by adenine will be denied by either intra-strand electron transfer to thymine or transfer to cytosine on either the same or opposite strand. The project described was supported by Award Number R01CA1032546 from the National Cancer Institute.

POS26-04. OH-induced oxidation of Met-Met dipeptides: influence of geometric and steric factors. Krzysztof Bobrowski1, G.L. Hug2, T. Pedzinska1, F. Kazmierczak3, P. Wisnowski1, B. Marcinski1, 1: Institute of Nuclear Chemistry & Technology, Poland, 2: Notre Dame Radiation Laboratory, USA, 3: Faculty of Chemistry, Adam Mickiewicz University, Poland

Oxidation of multifunctional organic compounds can lead to a variety of interesting mechanistic pathways through the neighboring group participation. Peptides containing methionine (Met) are a case in point. There have been several studies on the OH-induced oxidation of small peptides containing Met. Stabilizations, through neighboring group participation, of the resulting monomeric sulfur radical cations (S•+?) were seen when the Met residues were C- or N-terminal. It was commonly thought that such bond formation would not occur with the heteroatoms involved in the peptide bond. However, recent studies considering the possibility of such bond formation have led to the discussion of the role such bonds play in the redox behavior of Met dipeptides. We have explored the oxidation of a Met-Met dipeptide containing two identical Met residues (Met-Met) in the presence of NO. The Met residues were attached to the dipeptide by amide bonds and were protected on the N- and C-termini by esterification. NO was added to the solution and the reaction was followed using UV/Vis spectroscopy. The results show that the oxidation of Met-Met dipeptides is significantly affected by the presence of NO, with the Met-Met dipeptide being oxidized more readily in the presence of NO than in its absence. The mechanism of this effect is still under investigation.

POS26-05. The impact of X-radiation fluxes on a model subsurface bacterium. Ashley Brown1, S. Pinholt2, R. Goodacre1, J. Lloyd1, 1: SEAS, University of Manchester, UK; 2: Dalton Nuclear Institute and School of Chemistry, University of Manchester, UK, 3: MIB and School of Chemistry, University of Manchester, UK

Subsurface bacteria, such as Shewanella spp, have the ability to couple the oxidation of organic matter to the reduction of a range of metals and radionuclides, providing the potential for the use of such versatile species in the bioremediation of radwaste contaminated land. As such sites are likely to have significant radiation fluxes, it is important to understand the impact of radiation stress on appropriate model organisms. However, there have been few global cell analyses of ionizing radiation impact on subsurface bacteria, and thus quantification of damage to the multitude of biomolecules within the prokaryotic cell has been limited. This study addresses the impact of acute doses of X-radiation on growth, viability and metabolism of S. oneidensis MR-1. UV/Vis spectroscopy and CFU counts showed that although X-radiation decreased initial viability and extended the lag phase of batch cultures, the specific growth rate and biomass yields remained unchanged. Whole cell metabolism of exposed cultures was profiled using Fourier transform infrared spectroscopy and multivariate statistical data analysis algorithms revealed that although the culture survived irradiation, the ‘irradiated phenotype’ was preserved throughout subsequent generations at higher doses. Early data analyses also indicate damage to the proteome via a decrease in amide I and II peaks and a corresponding increase in free amine groups. This study concludes that significant damage to the proteome and a reduction in active protein can be preserved through multiple generations and this could potentially reduce the metal/radionuclide reducing capacity of the population in highly radioactive environments.

POS26-06. Investigations into the radiobiology of nitric oxide. Lisa Fokkes, P. O’Neill, Gray Institute for Radiation Oncology and Biology, University of Oxford, UK

Purpose: There is much interest in the potential for manipulation of tumor nitric oxide (NO) levels for cancer therapy. In particular, NO radiosensitizes androgen-independent tumor cells significantly more efficiently than oxygen. The mechanism of this effect is unknown but it is thought that NO may ‘fix’ DNA damage produced following radiation treatment, producing stable DNA base adducts. The aim of this work is to investigate the effect of low levels of NO on radiation-induced cell survival and DNA strand breaks and to study the formation of stable products resulting from nitric oxide to give further information into the potential use of NO in radiotherapy.

Results: Nucleobases, nucleotides and oligonucleotides react with radiation-induced hydroxyl radicals (•OH) but in the presence of NO, the products, observed by LCMS, differ to those formed by •OH alone. The yields of plasmid DNA single strand breaks determined by gel electrophoresis are reduced in the presence of NO when compared with the yield induced by •OH alone – post-irradiation treatment with DNA glycosylases however revealed that damaged nucleotides are formed in the presence of NO as seen through the formation of additional strand breaks. Clonogenic assays show that V79-9 cells are radiosensitized by 1% NO more efficiently in exponential growth; using flow cytometry detection these cells also exhibit enhanced hH2AX staining; a marker for double strand breaks (DSB). The maximum hH2AX cell fluorescence is observed 2h after 5 Gy γ- radiation in cells exposed to 1% NO relative to 1h for DSB induced by radiation treatment in N2 alone. This suggests that NO can cause repair or replication-induced DSB possibly through the formation of specific DNA lesions.

Conclusions: Tumor NO levels may be prognostic for radiotherapy effect. It is tentatively proposed that NO may radiosensitize cells by causing DNA damage which is difficult to repair - future studies will focus further on the mechanism of action and the potential for tumor specific NO delivery for radiotherapy.


Soft X-rays produce a variety of molecular alteration (damage) in cellular DNA, which is thought to be the critical target of the biological effects of radiation, such as cell death. In particular, we have reported the yields of base lesions as well as strand breaks strongly dependent on soft X-ray energy around carbon (C), nitrogen (N) and oxygen (O) K-edge regions. The base lesions were detected as additional strand breaks by base excision repair enzymes in the present study. The Near Edge X-ray Absorption Spectroscopy experiment was set up to detect the contribution of oxygen and carbon K-edge decay process most likely contribute to the induction of nucleobase lesions. DNA single strand breaks, on the other hand, are preferably produced just below K-edge. These experimental evidences show that the kinds of DNA damage can be selectively induced by monochromatic soft X-ray irradiation. However, we know little about details of the structural alterations. Inner-shell photoabsorption spectroscopy has been used as one of powerful technique providing us
information of chemical environment around a target atom. We have investigated the change of the spectra of the near edge X-ray absorption fine structure (NEXAFS) of DNA exposed to monochromatic soft X-rays. We used calf thymus DNA thin films as samples and observed N-K-shell and O K-shell NEXAFS spectral changes. The typical monochromatic soft X-ray energies used for the irradiation (380, 435, 560, and 760 eV) were obtained from SPring-8, BL23SU. The observed spectra show the initiation of the new products and the dissociation of molecular structure of the DNA by the irradiation. By comparing the spectral changes in NEXAFS with the yields of base lesions and strand breaks, we will discuss the molecular structure of DNA damage site and the site-selectivity of damage induction in DNA by soft X-rays.

PO526-08. Deinococcus radiodurans Manganese(II) Complexes and their Applications. Elena K. GudamaKova, V.Y. Matrosova, D.P. McDaniel, M.J. Daly, Uniformed Services University of the Health Sciences, USA

We previously reported that extremely radioresistant Deinococcus radiodurans bacteria accumulate divalent manganese ions together with various inorganic and organic small molecules, including peptides, orthophosphate and nucleosides. When combined in vitro at concentrations approximating those in D. radiodurans, Mn(II) forms potent antioxidant complexes which select for oxidation at immense doses of ionizing radiation, but the Mn(II) complexes did not significantly protect DNA (Daly et al., 2010). Genome destruction without protein damage in irradiated preparations potentially has important practical applications. One such application is the preservation of irradiated vaccines.

As a proof-of-principle for an irradiated vaccine, we used a model system based on bacteriophage lambda. Purified bacteriophage lambda was 3Co-irradiated (40,000 GY) either in phosphate buffer or in reconstituted D. radiodurans Mn(II) complexes. Lambda DNA degradation was tested by Southern blotting and the preservation of antigenic determinants by Western blotting. Whole-scale maintenance of irradiated lambda phage was monitored by transmission electron microscopy.

At 40,000 GY, the reconstituted D. radiodurans Mn(II) complexes fully preserved the structural integrity and immunogenicity of the lambda phage, but their genomes and infectivity were obliterated. Our findings support that chemical antioxidant protodentants of D. radiodurans could be applied to preparing irradiated vaccines.

PO526-09. Ultrafast dissociation of doubly ionized deoxyribose: ab initio molecular dynamics studies. Marie-Anne Hervé du Penhoat, I. Tavernelli, R. Vuilleumier, M. Alcamí, M. Politis, 1: IMPMC, UMR-CNRS 7590, Université Pierre et Marie Curie, Paris, France, 2: Departamento de Química, UAM, Madrid, Spain, 3: Département de Chimie, ENS, Paris, France, 4: LAMBE, UMR-CNRS 8587, Université d’Evry val d’Essonne, Evry, France, 5: EPFL-BCH, Lausanne, Switzerland

Our aim is to interpret, at a molecular level, the ultrafast dissociation processes following the interaction of ionizing radiations with model bio-molecular systems consisting of small DNA or RNA building blocks, either isolated or solvated by water molecules. The early stages of the Coulomb explosion of a doubly ionized biomolecule in both, liquid and gas phase, are investigated with Time-Dependent Density Functional Theory molecular dynamics simulations (TD-DFT MD) in which effective molecular orbitals are propagated in time [1]. These molecular orbitals are constructed as a unitary transformation of maximally localized Wannier orbitals, and quantitative analysis of DNA damage was carried out using DNA gel electrophoresis. DNA strand break yield was quantified using DNA gel electrophoresis.

In this paper, we present results on the double ionization of gas phase deoxyribose in the absence of both electrons either from its outermost orbital (HOMO) or from its deepest molecular orbital. We show that, contrary to gas phase uracil, the ground state doubly ionized molecule undergoes dissociation: A CH2OH fragment is formed within 50 fs. When electrons are removed from the deepest orbital which is located on the sugar ring oxygen atom, this oxygen atom is emitted within 20 fs.

Double ionizations of biomolecules, resulting either from Auger relaxation or from collisions in swift heavy ion tracks (about 10% of primary molecular ionization events), is shown to induce ultrafast dissociation processes which could lead to the formation of complex DNA damage [4].


Reactive oxygen species (ROS) play critical roles in a wide variety of cellular functions. On the other hand, excess amounts of ROS generated within cells result in oxidative stress and are responsible for many deleterious changes, which can induce oxidative damage to DNA, proteins and lipids. Several factors can influence the susceptibility to oxidative stress by affecting the antioxidant status and ROS generation. These factors include endogenous factors (e.g., stress, age) and exogenous factors like ionizing radiation, cigarette smoke, environmental pollutants, and UV light. ROS have been shown to participate in various biological consequences of ionizing radiation. However, the physiological mechanisms by which ROS cause molecular damage and ultimately cellular dysfunction are not fully understood.

In this study, we focused on Superoxide dismutase (SOD) and Glutaredoxin (Grx) proteins. SOD eliminate superoxide anion, Grx reduces the oxidized macromolecules, both SOD and Grx are conserved from bacteria to human. The SOD and Grx are essential enzymes in cellular homeostasis. However, only a few experimental studies have been reported in effects of quantitative variation in those antioxidant enzymes on cellular responses to the oxidative stress.

In this study, we examined whether and how cellular sensitivities to ionizing radiation are modulated by the overexpression of mitochondrial SOD (SOD2) and Grx (Grx2) in the cultured human cells. As the results of overexpression of SOD2 and Grx2 in T-REx HEla cells, morphological alteration of mitochondria and levels of mitochondrial superoxide, DNA double-stranded breaks, protein oxidation and OXRI expression were suppressed. These results suggest that antioxidant enzymes in mitochondria play important roles in various cellular responses to the ionizing radiation.

PO526–11. All that is gold does not glitter, not all those that wander are lost: the dual behavior of gold nanoparticles in vitro. Wendy B. Hyland, S.J. McMahon, M.E. Murn, J. Coulier, K.T. Butterworth, S. Jain 1, C. Sicard-Roselli 1, S. Khan 1, R. Woods 1, J. Deacon 1, M. Monaghan 1, L. Devlin 1, F.M. Hanton 1, G. Schettion 1, M. Brust 1, A. Hounsell 2, K. Prise 1, D.G. Hirst 1, F.J. Currell 1, R. Tobson 1, 1: School of Pharmacy, BBT 7BL, 2: Centre for Cancer Research and Cell Biology, BBT 7BL, 3: Centre for Plasma Physics, School of Mathematics and Physics, BBT 1NN, 1,2,3: Queen’s University Belfast, UK, 4: Northern Ireland Cancer Centre, BBT 7BL, 5: Department of Chemistry, University of Liverpool, Liverpool L69 7TD, UK, 6: Laboratoire de Chimie Physique, CNRS UMR 8000, Université Paris, France

Gold nanoparticles (GNPs) have promising radioenhancing properties; however, they are also directly cytotoxic. This study reports on the dual behavior of GNPs in vitro; their radiosensitising potential and their ability to induce DNA damage. DNA strand break yield was quantified using DNA gel electrophoresis and quantitative analysis of DNA damage was carried out using Image J open source software. Broadband irradiations were carried out using a Faxitron CP-160 X-ray generator and a Varian 2100CD linear accelerator whilst monoenergetic irradiations were carried out at the Diamond Light Source, Oxfordshire.

The reported success of a 1.9 nm GNP radiosensitiser [2] prompted investigations evaluating the energy dependence of GNP radiosensitisation. Plasmid DNA exposed to 0.5 % (w/v) gold, irradiated with 11.8 and 14.5 keV, 160 kVp and 6 MV photons, resulted in dose enhancements of 1.12 ± 0.09, 1.48 ± 0.08, 1.59 ± 0.08 and 0.73 ± 0.08, respectively. Dose enhancements agree well with those predicted, highlighting the importance of beam energy for 1.9 nm GNP radiosensitisation.
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1.9 nm GPNs exposed to plasmid DNA were highly damaging in the absence of radiation; 1 h room temperature incubation with 0.5 % (w/v) gold resulted in a 27 ± 3 % loss of supercoiled DNA. The damage induced was concentration, time, temperature and scavenger dependent suggesting free radical mediated DNA damage. Citrate-coated GPNs have been shown to cause radioenhancement in vitro [3]. Plasmid DNA irradiated with 160 kVp X-rays, exposed to 0.0013 % (w/v) 10 nm citrate-coated GPNs yielded a dose enhancement factor of 2.10, where none is predicted. A 5-fold increase in scavenger yielded negligible enhancement. Samples irradiated with 11.8 to 80 keV photons resulted in dose enhancements dramatically different from that predicted.

In summary, the physiochemical properties of GPNs determine GNP radiosensitization and toxicity. Radiosensitisation cannot always be predicted from mass-energy absorption considerations.

References
‘All that is gold does not glitter’, J. R. R. Tolkien


Treatment options for bladder cancer include radiotherapy and/or cystectomy for invasive disease and resection plus intravesical chemotherapy/immunotherapy for non-invasive disease. If the mechanisms responsible tumour sensitivity could be identified or if patient tumour response could be predicted, it should be possible to improve control/survival rates. Previously, we have shown that induction/repair of radiation-induced DNA damage measured by alkaline comet assay (ACA) correlates cell radiosensitivity in a panel of 6 human bladder cancer cell lines. Using the same panel we have now further shown that ACA measures of cis-platin and mitomycin C-induced damage correlate cell chemosensitivity, with there being predominantly the same rank order for radiosensitivity as chemosensitivity. ACA studies of radiation-induced damage in different bladder cancer cell DNA substrates (prepared nuclei & nucleoids vs. intact parent cell) suggest that a feature retained in prepared nucleoid bodies is responsible for the relative damage sensitivity of bladder cancer cells, reflecting possible differences in the organisation of the nuclear DNA within radiation resistant/sensitive cell lines. ACA analysis of ex-vivo radiation-induced DNA damage in bladder tumour cells derived for biopsies has been compared with patient data over a three year follow up period. A wide range of different ACA response grade, time (0-5-fold), compared to the control (Raji) cell line, was obtained. Lower ACA measures of DNA damage sensitivity have been shown to broadly trend with poorer outcomes following bladder tumour treatment; notably this includes the observation of lower measures of induced damage better predicting the local recurrence of non-invasive-disease than the presence of high-risk histology (ACA response gradients of <1.1 give a hazard ratio of 4.76 (CI 1.19 – 19.09) for local recurrence within a 2 year follow up period). Finally, ACA repair studies showed a distinct difference in the range of repair responses to X-ray between muscle-invasive and non-muscle invasive tumours. This data supports an association between damage resistance and aggressive tumour phenotype in this cancer model.

**POS26-13. The influence of fullerenol C60(OH)26-33 on ionizing radiation-induced damage to human erythrocytes.** Anita Krokosz, J. Grebowsk1, M. Puchala, University of Lodz, Department of Molecular Biophysics, Division of Radiobiology, Poland

The purpose of our work was to investigate the effects of water-soluble hydroxylated fullerene – fullerol (C60(OH)26-33) on radiation-induced damage to human erythrocytes.

Human erythrocytes (hematocrit of 2%) suspended in phosphate buffered saline (PBS) were incubated with C60(OH)26-33 at concentration 150 μg/ml at 37 °C for 1 h. Then, the erythrocytes were irradiated with electron pulses from a linear electron accelerator ELU-6 with the dose of 1300 Gy. Hemolysis, erythrocytes’ size and granulocyty by flow cytometry, hemoglobin oxidation, and overall oxidative stress by flow cytofluorimetry with dichlorodihydrofluorescein diacetate (H2DCF-DA) were determined.

Mature human erythrocytes have no nucleus and other organelles, therefore the plasma membrane in these cells is the critical target. Radiation-induced oxidative damage to plasma membrane leads to its disintegration i.e. hemolysis. Our results showed that fullerenol prevented human erythrocyte from post-radiation hemolysis. Hemolysis decreased from 26.3% for erythrocytes irradiated without fullerenol to 15.2% for erythrocytes irradiated in the presence of fullerenol. However, the average diameter of erythrocytes increased either after fullerenol or radiation or combined fullerol and radiation treatment, whereas granularity decreased after irradiation of erythrocytes and fullerenol did not affect that parameter.

Irradiation of erythrocytes induced hemoglobin oxidation. Hemoglobin is the main intracellular protein in erythrocytes. Fullerenol prevented radiation-induced hemoglobin oxidation. The level of MetHb (oxidized hemoglobin) was 11.7% in irradiated erythrocytes after 20-hr incubation whereas only 5.2% in erythrocytes irradiated in the presence of fullerenol. Moreover, fullerenol decreased reactive oxygen species production inside the erythrocytes following irradiation. The level of oxidized fluorescent label in erythrocytes irradiated with fullerenol was 64% lower than in erythrocytes irradiated without fullerenol. These results suggest that fullerenol could prevent human erythrocytes from radiation-induced oxidative stress.

**POS26-14. Cellular membrane and DNA damage induced by proton radiation in single PC3 cells.** Ewelina Lipiec1, J. Kowalska1, D. Moss2, M. Kwietek2, W. M. Kwietek1, 1: The Henryk Niewodniczański Institute of Nuclear Physics Polish Academy of Sciences, Poland, 2: Karlsruhe Institute of Technology, Karlsruhe, Germany

An inflammation caused by cellular membrane damage is a serious complication after radiotherapy. The aim of this study was to find a specific number of protons resulting in high DNA damage without destroying cellular membrane.

The 2 MeV focused proton microbeam from the Van de Graaff accelerator at the IFJ PAN, Kraków, Poland, was used as an irradiation source. The prostate cancer PC-3 cells were irradiated by specific number (1000, 2000, 4000, 8000) of protons per cell. Fixed cells were studied by the Fourier Transform Infrared microspectroscopy at the globar source (Bruker IFS 66/S, IR scope B) at KIT, Karlsruhe, Germany.

The changes in: a) DNA backbone spectral range 950~1240 cm⁻¹, potentially related to DNA strand breaks and cross links, and b) position of the CH₃ symmetric stretching band at ~2850 cm⁻¹, related to composition and fluidity of the membrane phospholipid, were examined.

The cellular spectra bands were fitted with Gaussian-Lorentzian curves after the Mie scattering effect correction. The multivariate statistical methods of principal component analysis and hierarchical cluster analysis (Ward’s method) were also applied. In addition, living cells were irradiated by the same numbers of protons, then after 24 hours they were analyzed under fluorescence microscope after PI staining.

The obtained results show increased switches and intensity changes of DNA backbone bands along with the number of protons applied for cells irradiation. The shift of band related to cellular membrane damage was observed, and it was in the range for (0.124 ± 0.032) cm⁻¹ to (1.022 ±0.045) cm⁻¹ in case of 1000 and 8000 protons per cell, respectively. The percentage of dead (PI positive) cells was equal to (5.4 ± 2.9) % and (83.4 ± 7.3) % after irradiation by 1000 and 8000 protons per cell, respectively. The results suggest that the irradiation of 1000 protons per PC3 cell is sufficient to induce a large number of DNA damage. This number of protons was relatively safe for the cellular membrane - the shift was equal to (0.442 ± 0.028) cm⁻¹ and (7.5 ±3.4) % of PI positive cell were detected.

**POS26-15. DNA double strand breaks versus simple strand breaks rates calculated within a detailed DNA geometrical model.** Dos Santos Morgane, C. Villagrana, I. Clairand, IRSN, France

Purpose: Understand and predict how DNA double and simple strand breaks are created by ionising radiation and repaired in cell nucleus is nowadays a major challenge in radiobiology. The aim of this work is to evaluate the double strand break over single strand break ratios (DSB/SSB) induced by protons of different
energies in typical fibroblast cell nucleus. It is part of the ROSIRIS project at the IRSN, which main issue is to correlate the initial radiation track to the early DNA damage.

Material and methods: The GEANT4 9.4 Monte Carlo toolkit was used in order to simulate in detail the energy deposit by protons at the nanometric scale. In particular, the low energy electromagnetic package extensions, referred as GEANT4-DNA processes that allow transportation of electrons down to thermalisation in liquid water. A complete geometrical model of a fibroblast cell nucleus, was implemented as the target of the track calculation. The cell nucleus is divided in five levels of compaction: DNA double helix, nucleosome, chromatin fiber, chromatin fiber loop and chromosome territories. This geometry allows differentiating between direct effects and indirect effects considering a distance of 4 nm around the chromatin fiber as potentially contributing to the final damages. The DBSCAN (Density Based Spatial Clustering of Application with Noise) algorithm was used in order to determine cluster damages. Defining distance and energy conditions as parameters, the number of strand breaks (simple and double) within the cell nucleus was calculated.

Results: DSB/SSB ratios obtained for protons between 0.5 and 50 MeV were compared to available calculations and experimental data extracted from the literature. The calculations show an increase of the DSB/SSB ratios with decreasing kinetic energy. Nevertheless this increase is more pronounced for the energies between 0.5 and 2 MeV and it tends to saturate for higher energies. These results show a good agreement (~12%) with comparable data extracted from the literature.

Conclusion: The combination of a complete cell nucleus geometry in the track calculation with the use of the DBSCAN cluster algorithm appears as a powerful method for calculating DSB and SSB damage on DNA that could be applied to other types of irradiation.

PO526-16. Analysis of lethal DNA damage induced by high LET radiation. Toshitake Nakano1, H. Terato1, R. Hiraayama1, A. Uzawa1, Y. Furusawa1, H. Ide1, 1: Hiroshima University, Japan, 2: Saga University, Analytical Research Center for Experimental Sciences, Japan, 3: NIRS, Research Center for Charged Particle Therapy, Japan, 4: Hiroshima University, Graduate School of Science, Japan

Ionizing radiation induces a variety of DNA lesions such as base damage, single-strand breaks (SSBs), double-strand breaks (DSBs), and DNA-protein crosslinks (DPCs). DSBs represent part of the clustered DNA damage and are the most potent lethal lesions. They are produced by either as a consequence of the formation of two closely opposed SSBs (prompt DSBs, PDSBs) or the abortive repair of closely opposed base lesions (COBLs). Although the lethal effect of DPCs has not been rigorously examined, it is very likely that extremely bulky DPCs block the progression of DNA and RNA polymerases, exerting a potent lethal effect on cells. In mammalian cells, the relative biological effectiveness (RBE) as measured by cell killing relatively decreases with increasing LET values between 0.5 and 2 MeV. Considering that high LET radiation generates a dense ionization track, it may produce detrimental PDSBs, COBLs, and DPCs efficiently, thereby leading to severe biological consequences. In the present study, we have analyzed the formation of PDSBs, COBLs, and DPCs induced by high LET radiation in vitro and in vivo.

We irradiated supercoiled plasmid and linear lambda DNA with gamma-rays (0.2 keV/µm), carbon ion beams (13 keV/µm), and iron ion beams (200 keV/µm), and quantified PDSBs and COBLs. The yield of PDSBs+COBLs decreased in the order of gamma > carbon > iron, and hence showed an inverse correlation with LET values. Moreover, consistent with this observation, the yield of chromosomal PDSBs of Chinese hamster ovary cells decreased with increasing LET when cells were irradiated with carbon ions, and carbon ions decreased in the order of gamma > carbon > ion, and hence showed an inverse correlation with LET values. The yield of PDSBs+COBLs decreased in the order of gamma > carbon > iron, and showed an inverse correlation with LET values. Moreover, consistent with this observation, the yield of chromosomal PDSBs of Chinese hamster ovary cells decreased with increasing LET when cells were irradiated with carbon ions, and carbon ions decreased in the order of gamma > carbon > iron, and showed an inverse correlation with LET values. Moreover, consistent with this observation, the yield of chromosomal PDSBs of Chinese hamster ovary cells decreased with increasing LET when cells were irradiated with carbon ions, and carbon ions decreases in the order of gamma > carbon > iron, and showed an inverse correlation with LET values. Moreover, consistent with this observation, the yield of chromosomal PDSBs of Chinese hamster ovary cells decreased with increasing LET when cells were irradiated with carbon ions, and carbon ions decreases in the order of gamma > carbon > iron, and showed an inverse correlation with LET values.

In order to clarify the mechanism of DNA damage induced by K-shell photoabsorption of nitrogen and oxygen atoms, we have developed an X-band JES-TE300 electron paramagnetic resonance (EPR) spectrometer (JEOL, Japan) at a synchrotron soft X-ray beamline BL23SU in SPring-8 (Japan), and examined the EPR of in situ signal during the soft X-ray irradiation.

Calf thymus DNA was used as sample without further purification. DNA samples were irradiated on the clean plastic plate and dried for about three days in air at room temperature to obtain DNA film for EPR and X-ray absorption measurement. Monochromatic soft X-ray photons were provided using a grazing-incidence monochromat equipped with variable-line-spacing-plane-grating monochromator. Observed EPR spectra were numerically double integrated to obtain the relative spin concentrations.

The g-factor of 2.000 of the unpaired electron arising only during irradiation in calf thymus DNA film is obtained not only around nitrogen but also around oxygen K-edge by comparison with standard film sample of MnO2. This is a significantly larger than that of a free electron (2.0023). The photon energy dependence of the EPR intensity around the K-edge regions of nitrogen and oxygen is not simply proportional to X-ray absorption near edge structure (XANES), indicating that the production of unpaired electron species in DNA enhanced by the nitrogen and oxygen K-shell photoabsorption. It is well known that the hydrated water which strongly bonds to the DNA exists even in vacuum. Such hydrated water may be plays an important role in the induction of unpaired electron around the oxygen K-edge [1]. On the other hand, an enhancement of the yield of the unpaired electron species around both the nitrogen and oxygen K-edge is observed for evaporated cytosine film presumably due to electron capturing by cytose [2]. The enhanced unpaired electron yields will be discussed in respect of stable DNA damage formation and the electron capturing of the DNA molecules will be discussed.


PO526-18. What is the fate of the cytosine radical cation in DNA exposed to the direct effect of ionizing radiation? Anita Peoples1, V. Razskazovskiy2, W. Bernhard3, 1: University of Rochester, USA, 2: East Tennessee State University, USA

The initial radicals produced in DNA, exposed to the direct effect of ionizing radiation, consist of sites that have either lost or gained one electron. With respect to electron loss, the probability of ionizing a molecular constituent is proportional to the number of electrons contained by that constituent (Bragg’s rule). Consequently, the initial distribution of holes (electron loss sites) is presumed to be 58% on the backbone, 12% on Gua, 11% on Ade, 10% on Thy, and 9% on Cyt. From EPR (electron paramagnetic resonance) studies, it is known that the initial distribution of holes rapidly evolves due to recombination and hole transfer reactions. Here we test our working hypothesis that the hole on cytosine (cytosine radical cation = cyt+1) partakes in two competing reactions: i) hole transfer to a nearby Guan on the same strand, giving Gua+2, and ii) deprotonation at C1’ of the deoxyribose bound to Cyt+, giving a neutral C1’ radical (dRib(C1’-H)). The approach was to X-irradiate (70 Kv tungsten) DNA films at RT under air, immediately dissolve the films in ultrapure water, store them at -18°C, and then measure products by reverse phase HPLC. In this report, we focus on the yield of 5-methylenefuranone (5MF) because its formation is tightly correlated with the dRib(C1’-H) radical intermediate. The yields of 5MF were measured for calf thymus (CT) and M. Iutaeus (Mi) DNA, which are 42% and 72% GC, respectively. We found that the 5MF yield for Mi-DNA was about one half that of CT-DNA. This can be explained by a competition between reactions i) and ii) described above. In Mi-DNA, the increased probability of hole transfer from Cyt+1 to Gua reduces formation of dRib(C1’-H)3 and consequently 5MF. This finding, therefore, is consistent with our working hypothesis.

The project was described as supported by Award Number R01CA032546 from the National Cancer Institute.


Cisplatin and carboplatin are two common chemotherapeutic agents used in concomitant chemoradiation therapy to sensitize tumour cells to ionizing radiation. Radioisensitization is known to result from their
binding to nuclear DNA. To identify an optimal schedule for the combination of anticancer drugs with radiation (i.e. timing and dosing of the agents), elucidation of the molecular mechanisms of radiosensitization is essential. This study discusses the effects of low energy electrons (LEE) on plasmid DNA covalently bound to either cisplatin or carboplatin, by quantification of strand break damages to DNA. Cisplatin-DNA and Carboplatin-DNA complexes were prepared in a molar ratio of 2:1. The concentrations of platinum and plasmid DNA (20 μg/ml) were measured inductively coupled plasma mass spectroscopy (ICP-MS) and spectrophotometry, respectively. Aliquots of cisplatin-DNA, carboplatin-DNA and pure DNA were deposited by lyophilisation onto a chemically clean tantalum substrate to produce thin films (~ 10 nm thickness). Samples were irradiated under ultrahigh vacuum conditions with 10 eV electron beams of various fluences. Then the samples were recovered from the tantalum and analyzed by agarose gel electrophoresis to quantify different structural forms of DNA. Cross sections for loss of supercoiled form (SC) and formation of circular and linear configurations of the DNA corresponding to the single strand breaks (SSB) and double strand break (DSB), respectively, were obtained from exposure response curves of the DNA damages for the three types of irradiated samples. In the carboplatin-DNA and cisplatin-DNA samples, the cross sections for DSB formation in the 3199 bp plasmid DNA increase by a factor of 2.5 and 2.1, respectively, compared to the pure DNA sample, respectively. Cross sections for loss of SC and the linear form do not show a considerable difference between the samples. Moreover, the exposure response curves for the formation of DSB were linear, indicating that the interaction of a single electron is responsible for the formation of a DSB in the DNA. These findings suggest that LEE interact synergistically with carboplatin and cisplatin to promote lethal damage to DNA. Furthermore, our results show that the radiosensitivity of DNA to LEE in the presence of carboplatin is higher than that in cisplatin.

POS26.20. DNA Damage and Repair Kinetics after Irradiation in Earthworms Pre-treated with Mercury. Tae Ho Ryu1, J. Park1, M. Nitš1, K. An1, J.K. Kim1, 1: Korea Atomic Energy Research Institute, South Korea, 2: Daunwesh Radiation Research Institute, Spain, 3: Chungnam National University, South Korea

All organisms are being exposed to harmful factors present in the environment. Ionizing radiation can damage DNA through a series of molecular events depending on the radiation energy. The biological effects due to the combined action of ionizing radiation with the other factor are hard to estimate and predict in advance. Recently, International Commission on Radiological Protection (ICRP) requires the effect data of ionizing radiation on non-human biota for the radiological protection of the environment. Earthworms have been identified by the ICRP as one of the reference animals and plants to be used in environmental radiation protection. Particularly, the earthworm E. fetida can be used as an indicator of pollution in soil. This study was performed to investigate the acute genotoxic effects of radiation and the combined effects between radiation and mercury in earthworm, E. fetida. Experiments were done to identify the levels of DNA damage and the repair kinetics in the coelomocytes of E. fetida irradiated with ionizing radiation (0, 2.5, 5, 10, 20 and 50 Gy) alone or with gamma rays after HgCl2 (10, 20, 40, 80 and 160 μg/kg) treatment by means of the single cell gel electrophoresis assay. The Olive tail moments (OTMs) were measured during 0 – 12 hours after irradiation. The results showed that the increase in DNA damage was depending on the dose of radiation. The more the oxidative stress was induced by radiation, the longer the repair time was required. When combination of HgCl2 and ionizing radiation was applied, the OTMs were much higher than those treated with radiation alone, which indicated genotoxic effect, was increased after combined treatment of radiation and mercury. Earthworms were treated with 20 Gy gamma rays alone or with ionizing radiation combined with 40 mg/kg HgCl2. The repair time in the animals treated with the combination of HgCl2; and ionizing radiation was nearly eight times longer than that in the animals treated with ionizing radiation alone. The results suggest that the mercury could even have deleterious effects on the DNA repair system. Therefore, influence of mercury on the DNA repair mechanisms is confirmed by this study.

POS26.21. Development of the enzyme-linked immunosorbent assay for quantifying single-strand breaks in plasmid deoxyribonucleic acid. Malgorzata Smialek1, D.E.G. Shuker2, 1: Gdansk University of Technology, Poland, 2: Liverpool John Moores University, UK, 3: The Open University, UK

The majority of model studies focusing on the radiation damage to plasmid or genomic DNA employs the agarose gel electrophoresis (AGE) as a method for single-strand breaks (SSBs) and double-strand break (DSBs) quantification in the irradiated samples [1,2]. In case of determining the amount of DSBs this method is sufficient, whereas in case of SSBs an accurate quantification is being made only by those that not only the molecules that possess a SSB form a relaxed band on the gel and that there is just one SSB per molecule. Such an assumption does not have to be true as both the molecules forming the relaxed and linear bands can contain multiple SSBs. Therefore, to accurately quantify the number of SSBs induced in plasmid DNA molecules after irradiation, a new type of assay methodology has been developed. The new method is based on the terminal deoxynucleotidyl transferase (TdT) nick end-labelling (TUNEL) assay that was adopted for use under enzyme-linked immunosorbent assay (ELISA) conditions [3]. The assay was found to both improve the quantification and reduce the uncertainties in measurement of SSBs compared with the AGE method. Furthermore, since only small amounts of DNA are required, the ELISA method can be used to quantify the damage in samples of DNA that are smaller than those required for AGE analysis. As an example of the data obtainable using the new method, plasmid DNA samples were irradiated in an aqueous solution with vacuum-ultraviolet (VUV) light at 150, 170 and 190 nm and subsequently analyzed by ELISA. The results were compared directly with those from AGE analysis. The ELISA gave results for SSBs that were an order of magnitude higher than those from AGE and the modeling [4] suggested that DSBs are more likely to be the result of two SSBs rather than a single event and that a damaged molecule is more likely to be susceptible to VUV light than an undamaged one. In addition, the increase of SSBs level appears linear with the radiation dose.


The relative biological effect of ionizing radiation generally differs depending on the type of radiation exposure. We are currently investigating the response of human mammary epithelial MCF10A cells to γ-rays and X-rays. Using the enzyme-linked immune-sorbent assay (ELISA), DNA double stranded breaks (DSB) are considered to be the most biologically significant lesion resulting from ionizing radiation exposure. The formation and repair of radiation-induced DSBS are quantified using a fully automated 53BP1 foci assay that was developed in our lab. The high throughput of the automated system increases the sensitivity of the assay by facilitating rapid analysis of large data sets, and is therefore beneficial for studying effects at low doses. Furthermore, researcher bias due to drift is eliminated. Dose response curves between 10 mGy and 3 Gy were generated for both radiation qualities. In addition to comparing the dose responses, we are examining the repair of DSBs over time through 53BP1 foci loss, to determine the efficiency of DNA repair relative to the initial dose received.

POS26.23. High destruction cross-section for the 8-oxidG lesion caused by direct ionizing radiation, detected by LC/MS/MS. Richard Watson, W.A. Bernhard, University of Rochester, USA

Experimental evidence suggests that 30-50% of DNA damage induced by ionizing radiation is caused by the direct effect, due to the fact that nuclear DNA is compact and surrounded by low amounts of free water. Analysis of the reaction mechanisms involved in the direct effect is crucial to assessing the risks associated with radiation exposure at low dose and low dose rate. X-irradiation initially results in radical intermediates situated on the DNA bases and strand breaks. Upon dissolution in water at room temperature, stable end-products are formed, which include strand breaks and base lesions. Much of what is known about the yields of
specific direct-type damage products is derived from radical yields measured by EPR at 4K. Quantification of stable end products by orthogonal analytical techniques is required for further elucidation of reaction mechanisms, but such techniques must have femtomole sensitivities, due to the extremely low yields in which products are formed. To this end, we have developed an LC/MS/MS method to detect and quantify base lesions, including 8-oxo-2′-deoxyguanosine (8-oxoG) and 8-oxo-2′-deoxyadenosine (8-oxoA). Dehydrated 8-oxoG (gammase–2-) was prepared from calf thymus DNA. Films were X-irradiated at room temperature from 0 to 5 kGy, then dissolved in water and enzymatically digested to produce nucleoside monomers. Dose-response curves were obtained for 8-oxoG and 8-oxoA using our LC/MS/MS method. As expected, the production of 8-oxoG was linear with respect to dose in the range tested. Surprisingly, however, the dose-response of 8-oxoG showed an increase in 8-oxoG production only at doses < 2 kGy. At higher doses, the yield of 8-oxoG decreased with increasing dose. This behavior is indicative of a high cross-section of destruction for 8-oxoG or a radical precursor that is formed in the sample during irradiation. A likely cause is the extremely low foci formation potential of 8-oxoGd (0.74 V) compared to intact nucleobases (>1.29 V), which makes 8-oxoGd a target for subsequent oxidation, resulting in a variety of other damage products. Understanding the mechanism of 8-oxoG destruction is crucial for assessing the validity of a multitude of existing methods for oxidative damage in biological systems, which often rely on 8-oxoG quantification.

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**POS26-24. Analysis of DNA double strand breaks created by 60Co irradiation using Monte Carlo track simulations and H2AX immunofluorescence.** Carmen Villagrasa, G. Gruel, P. Voisin, M. Dos Santos, J.F. Bottollier-Depois, IRSN, France

Introduction: Ionizing radiation interacting with cellular systems can affect their structure, function and response to the medium. Complex damages created in the primary structure of the DNA as double strand breaks (DSB) are considered to be key lesions to determine the nature and magnitude of the final effect.

Research into the DNA damage repair has outcome the observation that H2AX undergoes extensive phosphorylation at the DSB, creating γH2AX foci that can be visualized by immunofluorescence. Moreover, the topology generated by the foci shows dependence with the radiation quality.

IRSN initiated a project, called ROSIRIS, which main issue is to correlate the initial radiation track to this early DNA damage response.

Materials and Methods: Deposited energy in the cell nucleus by 60Co gamma rays, was calculated by Monte Carlo simulation using the Geant4-9.4 code. In order to study DNA strand breaks, a nanometric track description was performed by using the Geant4-9.4-DNA processes, implementing the low energy electromagnetic package. Nevertheless, for gamma irradiation simulations, the contribution of electrons created some millimeters away from the cell nucleus must be taken into account. A detailed description of these tracks will lead to the section properties calculations. The new release of the Geant4 Monte Carlo code offers the opportunity to combine the standard processes with the Geant4-DNA processes in the same simulation depending on the problem region.

In addition, the DBSCAN algorithm (Density Based Spatial Clustering of Application with Noise) was used in order to analyze the obtained tracks and determine the cluster damages.

Besides, the first experimental results of γH2AX foci after 60Co irradiation obtained by the radiobiology department in IRSN provided some biological images of the repair.

Results: The importance of the different parameters used in the track simulation and in cluster analysis was quantified for a correct comparison with the experimental γH2AX foci. In particular, variations due to dose distribution and K-shell ionization rate were studied. It appears that the cluster size in the use of DBSCAN must be consistent with that of the foci images obtained.

Conclusion: A new methodology has been established allowing the analysis of photon interactions at the sub cellular level and their correlation to DNA double strand break repair foci.

**POS26-25. In Search of Ionizing Radiation Effect on Pyrimidine Nucleosides.** Olga Zavyalova1, S. Truszkowski1, K. Misura1, A. Chetopaeva2, O. Zavyalov1, 2: Department of Nuclear and Radiation Chemistry, Faculty of Chemistry NCU, Poland, 3: Department of Chemical Technology of Pharmaceuticals, Faculty of Pharmacy, Collegium Medicum NCU, Poland

DNA is considered to be crucial cellular target of ionizing radiations due to biological effects, such as chromosomal aberrations, mutagenesis, carcinogenesis and cell death. The deleterious effects of ionizing radiations can largely be attributed to DNA damage induced by both its reaction with hydroxyl radicals (OH·) formed by water radiolysis and direct ionization of DNA. In some respects, ionizing radiation is similar to oxidative metabolism, which contributes to the natural processes of aging and mutagenesis. The lesions may occur at every nucleotide along the DNA molecule. Extensive work has been devoted to the understanding of processes underlying the damages of DNA constituents by radiation on the nucleic acids components has been intensively investigated [1-3].

In our studies processes initiated by gamma radiation occurring in aqueous solutions of pyrimidine nucleosides were examined by means of UV spectrometry, HPLC, HPLC-MS and GC methods. Kinetic study of nucleosides disappearance and formation of radiolys products were investigated by HPLC and GC in the concentration range of 100 – 1800 µmol/l. It was found that irradiation of aqueous solutions of pyrimidine nucleosides (concentration ca.100 µmol/l) leads to their complete disappearance at dose above the level of 1.5 kGy. Chromatographic analysis showed that several main products detectable by UV are formed during irradiation in the dose range of 0.2 – 1.5 kGy. It was proved that pyrimidine nucleosides disappearance follows the pseudo-first-order rate kinetics.


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**POS26-26. Alterations of albumin in Chernobyl clean-up workers blood plasma after myocardial infarction and group with epilepsy paroxysm,** Tija Zvagule1, I. Kalma1, N. Kurjane1, I. Vanadzins1, N. Gabruseva2, J. Reste2, A. Skesters2, 1: Riga Stradins University, Institute of Occupational Safety and Environmental Health, Latvia, 2: Daugavpils University, Latvia, 3: Centre of Occupational and Radiological Medicine of Paula Stradins Clinical University Hospital, Latvia, 4: Riga Stradins University Latvia

ABM (3-aminobenzanthrone derivative) developed at the Riga Technical University, Riga, Latvia) has been previously shown as a potential probe for determination of the immune state of patients with different pathologies. It is widely accepted that the dynamics of plasma proteins (e.g. albumin) play a prime role in immune characteristics of humans. The aim was to determine the several aspects of blood plasma albumin alterations in Chernobyl clean-up workers in relation with patients having no professional contact with radioactivity. For the study were selected the 3 groups of Chernobyl clean-up workers groups (common, after myocardial infarction, and with epilepsy paroxysm) and 2 groups of patients (myocardial infarction and epilepsy paroxysm) having no professional contact with radioactivity. In Chernobyl clean-up workers groups the patterns in spectral characteristics of ABM resemble so-called N-F transition of albumin with blue shift of fluorescence maximum to 602-620 nm and increased fluorescence intensity as compared by healthy control. In groups of patients having no contact with radioactivity theABM emissions wave length was not changed and fluorescence intensity as compared with control value decrease. The levels of pathological and pharmacological metabolites (fatty acids, antioxidants, plasma levels of lipoperoxidation products etc.) balance differs in patients groups comparable to controls and hence their correlation to seizures pathophysiology and their degree. The metabolites caused conformational changes in albumin molecule and shifts in binding parameters are in agreement with results of albumin auto-fluorescence data and ABM binding sites characteristics. A result clarifies the heterogeneous nature of ABM binding on DNA and leaded to different conformational of albumin in observed groups of patients. The more pronounced albumin structural/functional alterations were observed in clean-up workers with epilepsy and after myocardial infarction. Results taking in account the negative dynamics of EEG indices/CNS pathology state in relation with disturbances in regulation mechanisms of neuroendocrine and immune
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systems. Therefore seems likely that external radiation and incorporated radionuclides predominate in alterations of albumin.

**POSS26-27. Iodine-125-labelled Triplex-forming oligonucleotides: Studies on cytotoxicity of multi-binding-site TFOs and on specific gene expression alterations caused by single-binding-site TFOs.** Volkert Dahmen, R Kriehuber, Department of Safety and Radiation Protection, Forschungszentrum Jülich, D-52425 Jülich, Germany

Introduction: Triplex-forming oligonucleotides (TFOs) are able to bind DNA in a sequence specific manner and are a promising tool to manipulate genes or gene regulatory units in a cellular environment. TFOs might have also therapeutic potential e.g. as a carrier molecule for Auger-Electron-Emitter (AEE) to target specific DNA structures of tumour cells. We established a method for the effective labelling of TFOs with the AEE iodine-125 (I-125) and studied the influence of labelled TFO with regard to cell survival and appearance of DNA Double-Strand-Breaks (DSB). Furthermore the ability of TFOs to alter gene expression of targeted genes was examined.

Methods: TFO specific for the genes BCL2, GAPDH and BRCA1 were designed employing TFO Target Sequence Search (Univ. of Texas). TFO labelling with I-125 was performed using the primer extension method. Formation of DNA triplexes was visualized with MS Imaging Plates employing a FLA-5000 Imaging System (Fujiﬁlmo) and electrophoretic mobility shift assay (EMSA). Cell survival and DNA DSB frequency in SCL II cells after transfection with an I-125-labelled Multi-Binding-Site (MBS) TFO (~7000 binding sites) were analyzed with the Colony-Forming Assay (CFA) and the 53BP1-Foci Assay. SCL-II cells transfected with TFOs binding to single DNA targets in specific genes were analyzed for gene expression alterations of the targeted genes with qRT-PCR on a 7500 Real Time PCR System (Applied Biosystems).

Results: The MBS I-125-TFO transfected SCL-II cells showed a reduction of colony forming ability of ~45 % and the number of 53BP1-Foci was ~1.5 times increased when compared to sham-transfected negative control. The transfection with single binding site I-125-TFOs lead to a 1.7-times increased expression for BCL2 and a 0.5-times reduced expression for GAPDH. No altered gene expression was detected for BRCA1.

Conclusions: I-125-labelled MBS TFOs have a pronounced cytotoxic effect and induce DNA DSB in SCL-II cells. Single gene targeting TFOs can alter gene expression in a gene-specific manner.

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**POSS27 Normal tissue damage**

**POSS27-01. Markers of oxidative and nitrosative stress in response to developing radiation pneumonitis.** Andrea Babicova1, Z. Havlinova1, J. Chladek1, M. Rezacova1, M. Hroch1, J. Pejchal1, J. Vavrova2, 1: Charles University, Prague, Faculty of Medicine in Hradec Kralove, Department of Medical Biochemistry, Czech Republic, 2: Charles University in Prague, Faculty of Medicine in Hradec Kralove, Department of Pharmacology, Czech Republic, 3: University of Defence, Faculty of Military Health Sciences, Centre of Advanced Research, Czech Republic, 4: University of Defence, Faculty of Military Health Sciences, Department of Radiation Biology, Czech Republic

The purpose of study was to mark monitors of oxidative and nitrosative stress during the development of radiation pneumonitis (RP) after thoracic irradiation of Wistar rats. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine in Hradec Kralove and University of Defence, Hradec Kralove, Czech Republic.

Female, specific pathogen free Wistar rats (Velaz s.r.o., Prague, Czech Republic), weighing 205-245g, were divided into 3 groups of 6-10 animals each. Lung gamma irradiation at doses of 15 and 20 Gy was performed with the help of a 60Co γ-ray source (Chirana, Prague, Czech Republic) using a dose rate of 1 Gy/min. Control rats (C) were treated under the same conditions as irradiated groups (IG). Exhaled nitric oxide (NO), the putative predictive marker of radiation pneumonitis, was measured at regular intervals over a 7-week period using a chemiluminesence analyzer CLD 88 (Ecomedics, Duernten, Switzerland).

At the study end, blood was collected from the abdominal aorta under anaesthesia and rats were sacrificed by bleeding out. Bronchoalveolar lavage fluid (BAL) was obtained by instilling 10 mL 0.9% saline solution through the tracheostomy. The tissue for determination of airiness and inflammation of the lungs was collected and put into 10% formalin for immediate fixation. Levels of malondialdehyde (MDA) were determined in the plasma and BAL by HPLC.

No differences between IG and C were observed in ENO. At week 7, there was a trend towards a dose-dependent increase of MDA in BAL by 41% and 73% in IG as compared to C, but the differences did not reach significance (P=0.06) Parameter of airiness was decreased in both IG (p<0.001; 81%, 69%). A dose-dependent decrease of airiness after irradiation was observed. Markers of inflammation were increased in both IG (p<0.001; 164%, 473%).

The development of radiation pneumonitis was demonstrated histologically. Increased MDA levels in BAL documented mild oxidative stress in the airways. However, eNO levels were not increased as would be expected due to ongoing inflammation.

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**POSS27-02. Radiation-induced heart disease and the kallikrein-kinin pathway.** Marjan Boerma1, V. Sridharan1, S. Sharma1, E. Moros1, P. Corry1, E. Kaschina2, T. Unger1, M. Hauer-Jensen1, 1: University of Arkansas for Medical Sciences, USA, 2: Charite - University Medicine, Germany

Background. Radiation-induced heart disease (RIHD) is a potentially severe side effect after radiation therapy of thoracic and chest wall tumors. We have previously shown that mast cells protect against cardiac function loss and adverse structural remodeling after local heart irradiation in the rat. Mast cells are known to interact with the kallikrein-kinin-pathway. In this pathway, kininogen serves as the precursor for kinins, small peptides that have pro-inflammatory properties, but may also show some cardioprotective effects. The current study addresses the role of the kallikrein-kinin system in RIHD.

Methods: Male adult kininogen-deficient Brown Norway Katholiek (BNK) rats and wild-type Brown Norway (BN) rats received localized heart irradiation with a single dose of 18 or 24 Gy, or received sham-irradiation. Cardiac functional and structural changes were assessed with small animal echocardiography, Langendorff-perfused rat heart preparations, histology and immunohistochemistry.

Results: Localized heart irradiation resulted in dose-dependent changes in cardiac function and structure. A single dose of 18 Gy resulted in increased diastolic wall stress in wild-type BN rat hearts, but not in kininogen-deficient BNK rat hearts. In addition, at 3 months after a single dose of 24 Gy, radiation-induced changes in echocardiographic parameters were more severe in BN rats compared to BNK rats.

Conclusions: These results demonstrate that the kallikrein-kinin pathway contributes to functional and structural radiation injury in the heart. Further studies to elucidate the interactions between mast cells and the kallikrein-kinin system are ongoing.

**POSS27-03. Delayed effects of acute radiation exposure (DEARE) in the murine intestine following exposure to high-dose irradiation.** Catherine Booth1, G. Tudor1, T. MacVitte2. 1: Epistem Ltd, UK, 2: University of Maryland SOM, USA

The acute effects of irradiation on the gastrointestinal (GI) system are well documented (weight loss, diarrhea, dehydration, susceptibility to infection). Histopathology reveals an initial shortening of the crypt and villi (as cell division is interrupted) followed by crypt loss and ulcer development at times coincident with the onset of diarrhea (day 4 post-irradiation in C57Bl/6 mice). This occurs before the symptoms of bone marrow (BM) toxicity, but requires higher doses of irradiation (beyond the LD<sub>10</sub> for BM toxicity). However, in a nuclear terrorist scenario radiation exposure will be non-uniform, often resulting in low levels of bone marrow shielding.

We have established models of partial-body irradiation (PBI) with both high (40%; head, thorax, forelimbs shielded) and low (5%; tibiae, fibulae, ankles, feet shielded) BM sparing. These both allow the study of GI effects with maintained haematological involvement. Irradiations at ~0.7Gy/min used a 300kVp X-ray, 10mA, with filtration giving a radiation quality of 2.3 mm Cu HVL. Mice husbandry used acid water and filter-top cages.

Following both types of PBI a prolonged GI syndrome was seen at doses >12Gy, with a dose related increase in observed pathologies. After 20 days mice developed structures similar to early adenomas. By day 75 most mice had adenomas in both the small and large
intestine, and became moribund. The villi also appeared fragile and Paneth cells were extremely prominent. The histology of the mature cells in the large intestine was also changed.

However, when mice were maintained on ciprofloxacin they continued to thrive for >200 days. In these mice the crypts became smaller and villi larger. Abnormally high levels of apoptotic and mitotic cells were present, along with the early adenomas, both suggesting deregulated cell turnover. A larger, inflamed and potentially neoplastic fibrous, submucosa developed. These observations are similar to those seen in geriatric animals, suggesting that DEARE may induce a premature aging of the GI.

This phenomenon requires further investigation but it is the first recorded evidence of DEARE in the gut and may provide a model for evaluating potential therapeutics for this phenomenon. Further, anti-fibrotic DEARE are seen in the lung, there is the opportunity to link the two model systems using the 5% BM-shielding method.


Whole brain irradiation (WBI) at doses as low as a few Gy are sufficient to disrupt granule cell neurogenesis within the rat dentate gyrus, and this disruption has been associated with an impairment of hippocampal plasticity and cognitive function. Anti-inflammatory agents have previously been shown to mitigate decreases in granule cell neurogenesis following WBI. Here we have investigated the mitigating effects of Ramipril, an angiotensin converting enzyme (ACE) inhibitor on object recognition following whole brain radiation and a new model combining a sublethal dose of total body irradiation immediately followed by an equal dose of whole brain irradiation.

Groups of male Fischer 344 rats received WBI doses of 0 (control), 12 Gy or 6 Gy plus 6 Gy WBI using a Cs-137 irradiator. Ramipril (1.5 mg/kg/day, oral in drinking water) therapy was initiated 24 hours post-irradiation and continued for 6 months until sacrifice. Rats were acclimated to the testing chamber on three consecutive days and allowed to become familiar with two similar objects. On the third day after acclimation, one of the familiar objects was replaced with an unfamiliar, novel object. The percentage of time within a three minute duration that the rats spend exploring the novel object was measured.

Unirradiated control rats spent an equal amount of time with two familiar objects whereas unirradiated control rats spent 80 +/- 9% more time in the proximity of a novel object. Irradiated rats, either 12 Gy total brain or 6 Gy total brain plus 6 Gy WBI spent an equal amount of time between familiar and novel objects indicating unirradiated rats without drug treatment were not motivated to explore the novel object. Irradiated rats receiving Ramipril spent 90% +/- 13% more time in the proximity of a novel object indicating rats receiving Ramipril retained curiosity, motivation, inquisitiveness and memory of novel objects. The cognitive studies confirmed the results of previous and ongoing studies using a variety of structural and functional endpoints that demonstrate Ramipril mitigates sub-acute and late radiation injury to the CNS.

ACE inhibitors, FDA approved for other uses and with a proven safety record, can be administered post-irradiation to provide significant mitigation of undesirable radiation injury to the brain. Supported by NIAID U19 AI067734 (JHK).

**POS27-05. Regional Thoracic Irradiation in Mice to Avoid Pleural Effusions and Resolve Genetic Differences in Radiation Lung Damage.** Julian Down1, I. Jackson2, Z. Vujaskovic2, 1: Massachusetts Institute of Technology, USA, 2: Duke University Medical Center, USA.

Historical comparisons among nine different mouse strains receiving whole thorax irradiation (WTI) have shown a diverse progression of injury that limits survival. This encompasses variations in sensitivity and latency of severe compressive pleural effusions as well as of pulmonary pathology (pneumonitis and fibrosis). In many mouse strains (BALB/c, A/J, C57/BR, WHT, TO and C57BL/6), the effusions appear to resolve more rapidly at the time of pneumonitis and prevent the complete evaluation of lung pathology (Jackson et al. Radiat. Res. 173:10-20, 2010; Radiat. Res. 175:510-518, 2011). While the complicating effects of pleural effusions after WTI in these strains may not satisfy the requirements of the FDA “Animal Rule” prior to approval of new models combining lung and cardiac injury, it remains desirable to develop a model in which the genetic susceptibility towards radiation pneumonitis and fibrosis can be resolved for development of predictive assays and targeted therapeutics. There is also a need to address conditions relevant to localized thoracic radiotherapy where the compensatory role of unirradiated lung tissue is expected to provide recovery from injury. Earlier studies showed that localizing the irradiation over a range of doses to the right hemithorax (HTI) of CBA mice avoids the effusions and enables long-term survival with non-invasive evaluation of lung injury using the breathing rate assay (Down et al. Radiother. Oncol. 64:3-50, 1986). Moreover, we have observed precedence for mitigating lung damage using high resolution micro-CT among three mouse strains (CBA, C57L and C57BL/6) receiving 12.5-17.5 Gy HTI and revealed large differences in both the sensitivity and timing of radiological density changes within the irradiated lung and without lethality. Other experiments involving partial irradiations to bilateral lung volumes (≥50%) that include the base of the thorax have, however, produced morbidity in C3H mice and where pleural effusion was often found in the sick animals (Liao et al. JROBP 32:1359-1370, 1995). The availability of a high precision image-guided irradiation platform enables more accurate localized dose delivery to different regions of the thorax and may show the location of radiation damage that leads to effusions as well as enabling regional dose-volume relationships that determine pulmonary tolerance.

**POS27-06. Understanding the Mechanism of Mitigation of Radiation Nephropathy by ACE Inhibitors.** Brian Fish, J. Moulder, E. Cohen, K. Potempa, M. Medhora, Medical College of Wisconsin, USA.

We have shown that the ACE inhibitor (ACEi) captopril is effective in the mitigation of radiation-induced renal injury in humans and rodents. However, the mechanism of action remains unknown. Four ACEi have been tested and are effective in mitigation of radiation nephropathy. We cannot assume that the mitigation efficacy is directly related to suppression of ACE, since ACE inhibitors have other effects (e.g., on bradykinin and on the hematopoietic cytokine, AcSDKP). In addition, captopril contains a reducing sulphydryl (-SH) group, which may be effective against oxidative stress induced by radiation. We are therefore testing 3 ACE inhibitors (ramipril, fosinopril and enalapril) each, which have different chemical structures, head-to-head against captopril for the mitigation of radiation nephropathy. We are using doses that are equally effective for suppression of the systemic renin-angiotensin system in these rats (as measured the raising of plasma renin activity, PRA). If the four ACEi are equally effective, it would imply that their mitigation efficacy is directly tied to a common mechanism such as systemic suppression of the renin-angiotensin system.

In normal rats we tested the four ACEi’s for their effects on plasma renin activity (PRA) and systemic blood pressure (BP). Captopril at 300 mg/L, enalapril at 30 mg/L, fosinopril at 50 mg/L or ramipril at 1.2 mg/L, given in drinking water, all raised PRA 3 fold. Normal rats have a PRA of 7-10 ng/mg/hr. At these doses the four ACEi had a small effect on systemic blood pressure making normal rats slightly hypotensive.

Mitigation of radiation nephropathy is being tested in our rat model of total body irradiation with bone marrow transplant (TBI). The ACEi’s are being delivered in the same manner in irradiated and control rats. Serial serum samples will be assayed for BUN, a proven marker of renal injury and PRA levels. Rats will be followed until moribund. We will determine whether ACEi have equal mitigation benefit at doses that raise the PRA to the same degree, to correlate mitigation with suppression of the renin-angiotensin system. Funding: NIAID U19 AI067734.

**POS27-07. NRF2 deficiency reduces life span of mice administered thoracic irradiation.** Michael Freeman1, E.L. Travis2, G. Rachakonda1, K.R. Mukhtar1, M.L. Freeman1, 1: Vanderbilt University School of Medicine, USA, 2: The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Subsets of cancer survivors who have been subjected to thoracic irradiation face the prospect of developing pulmonary injury. Radiation induces a fibrotic reaction and an inflammatory response that presents 6 to 24 months after irradiation and continues to progress over a period of years. TGF-β and reactive oxygen species contribute significantly to the pathogenesis of this injury. The transcription factor NRF2 controls antioxidant gene expression and therefore regulates mitogens for burden. This work demonstrates an additional paradigm for NRF2: suppression of TGF-β-mediated induction of PAI-1n fibroblasts, measured using GMSAs, ChIP, and
CAGA-directed reporter assays. Thoracic irradiation of Nfe2l2 (-/-) mice resulted in rapid expression of PAI-1 and FSP-1 compared to irradiated wild type mice. Examination of lung tissue 16 weeks after thoracic irradiation of Nfe2l2 (-/-) mice revealed the presence of distended alveoli and decreased numbers of alveoli compared to wild type mice. Suppression of NRF2 expression shortened life span in mice administered 16 Gy to the thorax. Nfe2l2 (+/-) and (-/-) mice exhibited a mean life span of 176 days compared to wild type mice that lived an average of 212 days. These novel results identify NRF2 as a susceptibility factor for development of late tissue injury.

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**POS27-08. Heart-Lung interaction in the development of radiation pneumonitis.** Ghazaleh Gobahi, S.J. van der Veen, B. Bartelds, R.A. de Boer, J.R. de Jong, S. Brandenburg, R.M.F. Berger, J.A. Langendijk, R.P. Coppes, P. van Luikj, 1: Department of Cell Biology, Section of Radiation and Stress Cell Biology, University Medical Center Groningen, Netherlands, 2: Center for Congenital Heart Disease, Beatrix Children Hospital, University Medical Center Groningen, Netherlands, 3: Department of Cardiology, University Medical Center Groningen, Netherlands, 4: Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, Netherlands, 5: Kernfysisch Versneller Instituut, University of Groningen, Netherlands, 6: Department of Radiation Oncology, University Medical Center Groningen, University of Groningen, Netherlands

The risk of early radiation pneumonitis limits the radiation dose and efficacy of radiotherapy of thoracic tumors. Besides lung dose, co-irradiation of the heart was shown as a risk factor of radiation pneumonitis (2,3). However, classically the heart is regarded a late responding organ. Aim: Investigate potential early pathophysiologic cardiac changes after heart and lung irradiation. Methods: Rats’ heart and/or lung were irradiated to 20 Gy using high-precision proton beams. Subsequently until 8 weeks post-irradiation cardio-pulmonary performance was assessed by left/right-sided cardiac catheterization and FDG-PET scans. Heart and lung tissue were evaluated using histopathology. Results: Already 8 weeks after irradiation of the heart (+ive/negative) 25% of the lung) left ventricle (LV) end-diastolic pressure and relaxation time increased from 3.8 ± 0.6 to 11.3 ± 1.8 mmHg and 8.7±0.2 to 11.6±0.4 msec, n=5 respectively indicating early LV diastolic dysfunction. Moreover, pronounced LV perivascular fibrosis (19% of vascular surface area) and pulmonary perivascular and interstitial edema in non-irradiated lungs were observed. Interestingly, besides inducing irradiated-volume dependent changes in pulmonary artery pressure, lung irradiation alone also increased the relaxation time of LV to 12±1 mmHg, n=5 and decreased LV stroke volume (40%). Moreover, combined irradiation of lung and heart reduced LV volume parameters and cardiac output even further. In addition to LV diastolic irradiation of 80% irradiated mice revealed the presence of 18±1 to 30±3 mmHg, n=4, lung + heart irradiation also induced a pronounced right ventricle diastolic dysfunction indicated by increased right ventricle end-diastolic pressure from 0±2 to 22±1 mmHg, n=4. Conclusion: Heart and lung irradiation independently impair the same cardiac diastolic performance parameters through different mechanisms. Contrasting the classical view of the heart as a late responding organ, in our rat model heart irradiation may induce early subclinical pathophysiological changes that, if combined to lung irradiation may manifest as an enhanced risk and severity of radiation pneumonitis. 1. van Luikj P, Cancer Res. 2005. 2. van Luikj P, Int. J. Radiat. Oncol, Biol. Phys. 2007 3. Huang EX, Acta Oncol. 2011.

**POS27-09. Inter-individual variations in the human transcriptome following in vivo exposure to low dose ionizing radiation.** Albrecht Huguette, M. Saiproon, D. Blythe, K. Karen, R. David, University of California Davis, Medical School, Department of Public Health Sciences, USA

Human exposure to low dose ionizing radiation (LDIR), defined as equal or lower than 0.1 Gy, can occur through a variety of sources, including natural (cosmic rays, radionuclides in Earth’s crust), medical (diagnostic imaging and therapy), occupational (nuclear facilities and radionuclides), and accidental (nuclear catastrophe, terrorist act). However, the biological effects and molecular mechanisms involved in the human response to LDIR are still poorly understood. Since the effects of radiation in humans are best studied in humans, our laboratory has pioneered analysis of human skin following in vivo exposure to LDIR. In essence, normal skin from consenting men undergoing radiotherapy for early stage prostate cancer is used. Studying the effects of IR in human skin is highly relevant since skin is the most abundant organ in humans, and the first organ exposed to IR during occupational, therapeutic and accidental exposures.

Methods: The present study included 7 patients and a total of 4 skin punch biopsies were collected per patient: one prior IR exposure and one each at 3, 8 and 24 hours following exposure to 0.01 Gy, as determined by MOSFET (metal-oxide semiconductor field-effect transistor) dosimetry. Transcriptional changes in duplicate samples (obtained by splitting each biopsy) of control skin and skin exposed skin were assessed by global gene expression analysis using Illumina Human Ref-8 beadchips (24,526 RefSeq curated gene probes). While the same original data set has been previously analyzed to identify common biological responses following in vivo exposure to a 0.01 Gy dose, for the Xrays, here we demonstrated individual variations in the inter-individual variability in response to LDIR. Linear mixed effects modeling was used to select genes with the most variability between patients in response to radiation over time. Genes were classified into functional groups with DAVID (http://david.abcc.nig.ac.jp/), and MetaCore by GENEGO was used to map differentially expressed genes to functional pathways.

Results: Following background subtraction, probes with a detection P-value < 0.05 for all arrays were selected, resulting in a data set with a total of 21145 probes. Out of these, 2592 probes (12%) had a raw P-value < 0.05 for the likelihood ratio test of a patient-treatment interaction effect (indicating significant variability among patients in response over time), and 493 (2%) had a false discovery rate (FDR)-adjusted P-value < 0.05. For most probes, the variability in gene expression between patients at a given time point was greater than the mean change in expression between time points. Genes and pathways represented in these inter-individual variable gene expression profiles will be discussed.

Analysis of the inter-individual transcriptional changes induced by in vivo exposure to LDIR is expected to contribute to a better definition of the biological effects of LDIR in humans, and thereby enable risk assessment to assist with public policy decisions.

**POS27-10. Functional Mitigation Following Whole Brain Irradiation.** Kenneth Jenrow, S. Brown, K. Lapanawski, H. Naei, A. Kolozsvary, J.H. Kim, Henry Ford Hospital, USA

Whole brain irradiation (WBI) doses of 10 Gy or less are sufficient to impair neurogenesis in the rat dentate gyrus, along with hippocampal plasticity and cognitive function. Impaired neurogenesis reflects both the acute loss of neural progenitors via apoptosis and a more gradual disruption of neurogenic signaling via inflammation. Anti-inflammatory drugs have been shown to prevent radiation-induced cerebral injury when administered prior to irradiation. Here we report that minozac, a selective inhibitor of pro-inflammatory microglial cytokines, can mitigate these deleterious effects and restore measures of hippocampal function to levels comparable to those of unirradiated controls. Male Fischer 344 rats received WBI doses of 0 and 10 Gy using a Cs-137 irradiator. Minozac therapy (5 mg/kg/day,IP) was initiated 24 hours post-WBI and continued for 4 weeks post-WBI. As measures of hippocampal function, in vivo long-term potentiation (LTP) and behavioral assays were performed at 5, 6, and 9 months post-WBI. Rats were sacrificed by transcardial perfusion/fixation and brains processed for paraffin embedding and immunohistochemical staining for BrdU, Ki67, DCX, NeuN, Ox-6, and Arc. Cells expressing these markers were quantified within the dentate gyrus in multiple sections along the anterior-posterior axis.

Minozac significantly improved the consolidation of LTP in the dentate gyrus and the consolidation of hippocampus-dependent spatial memory at 3, 6 and 9 months post-WBI. These persistent mitigating effects were correlated with immunohistochemical measures of neuroinflammation and activity-induced neurogenesis and protein expression.

The mitigating effects of minozac in this context are consistent with reduced neuroinflammation, preservation/restoration of neurogenic signaling and activity-dependent gene/protein expression and structural plasticity in the dentate gyrus. The persistence of these mitigating effects after therapy withdrawal suggests that the pathological skin associated with radiation-induced cognitive impairment may be initiated by pro-inflammatory cytokines during a relatively brief interval post-WBI.
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POS27-11. Mechanisms of thymic recovery and T cell reconstitution following sublethal ionizing radiation. Zuna Kepuska, G. Sempowski, Duke University, USA

Background and Hypothesis: Proper thymic function is important for maintaining lifelong peripheral homeostasis of naive and memory T cells. Age-induced thymic involution is a general phenomenon, and does not reverse itself. Acute thymus involution in settings of stress can spontaneously recover. We hypothesize that thymic involution associated with ionizing radiation is acutely driven by intrathymic factors similar to age-induced involution. Specific Aims: The overall goal of this research is to define mechanisms of thymic damage and recovery following acute stress (irradiation). Our specific aims are: 1) to define the cellular and molecular mechanisms that drive thymic damage and recovery associated with ionizing irradiation, 2) to determine if inhibition of thymosuppressive cytokines coupled with thymostimulatory agents can enhance immune recovery following exposure to ionizing irradiation and 3) to determine whether similar mechanisms of damage and recovery function in aged.

Methods: Young and old BALB/c mice were sublethally irradiated (≤ 125 rad). Thymus function was monitored by thymus weight, cellularity, and TCR gene re-arrangement (mTREC). In old, irradiation significantly reduced thymocyte number and depleted mTREC levels by day 7, followed by full recovery (day 42). In old, radiation did not cause further decrease in thymocyte number or mTREC compared to that induced by age. To define mechanisms of thymic involution/recovery expression of thymostimulatory factors and indicators of healthy thymus were analyzed. While IL-7 and FoxN1 mRNA levels were not impacted in the young, KGF and AIRE mRNA expression levels were significantly reduced during irradiation-induced involution. These levels restored to baseline 21 days prior to full thymus functional recovery. Thus, suggesting a critical role of thymic-stromal derived KGF in promoting stroma/thymocyte crosstalk to mediate thymus recovery.

Conclusions: Overall, these findings may lead to a better understanding of mechanisms involved in thymus damage and recovery, and development of strategies to restore thymic function in the aged.

POS27-12. Plerixafor, a CXCR4 Antagonist, Mitigates Skin Radiation-Induced Injury in Mice. Jae Ho Kim, A.J.J. Kolozsvary, K.A. Jenrow, S.L. Brown, Henry Ford Hospital, USA

Skin injury limits the achievable dose during radiotherapy and is of concern in the context of radiological terrorism or accident since skin irradiation lowers the lethal dose of whole body radiation. Our working hypothesis is that radiation-induced skin injury originates from a loss of stem and progenitor cells, accompanied by excessive thymic cytokine production and excessive thymocyte apoptosis in Medaka pre-maturation. Plerixafor (AMD3100) is a CXCR4 antagonist, one of the most efficient bone marrow stem cell mobilizers and these studies were designed to experimentally assess the potential of Plerixafor to reduce skin radiation injury. The right hind legs of groups of C57BL/6 mice were exposed to radiation alone or in combination with Plerixafor. Plerixafor was administered either intraperitoneally or subcutaneously at a dose of 5 mg/kg given in two doses separated by two days started either on day 0, 4, 7, 15, or 24 after radiation (typically 25 Gy). The primary endpoint was skin injury assessed three times a week for at least 2 months using a semi-quantitative scale (and pooled data from two independent observers). Secondary endpoints measured at selected time points included histology (primarily H&E), tissue thickness measurements obtained using a 13MHz ultrasound real time B scan, and cytokine levels (TGF-beta and TNF-alpha). The late skin injury in mice receiving Plerixafor was highly dependent on the timing of the administration of the drug. The maximum benefit was observed when the drug was started one week AFTER the radiation exposure; earlier or later administrations of drug decreased its efficacy. Secondary endpoints of damage assessed histologically, tissue thickness and cytokine levels provided confirmatory observations. In an attempt to understand mechanism of action, the pre-mitotic CXCR4, stromal derived factor, SDF-1, was measured. Expression of SDF-1 monitored in skin as a function of time after a 10 Gy radiation exposure suggested a strong correlation between timing of administration of Plerixafor and expression of SDF-1 in irradiated skin: optimum drug administration timing coincided with maximal SDF-1 expression in the skin of irradiated mice. The report presents the first observation that CXCR4 antagonist improves both acute and late skin response to radiation exposure.

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POS27-13. p53 Controls Radiation-Induced Heart Disease by Regulating Mitotic Death in Endothelial Cells. Chang-Lung Lee1, K. Cuneo1, J. Sullivan1, E. Moding1, L. Jefodirs1, R. Rodrigues1, Y. Ma1, C. Kontos1, Y. Kim2, D. Kirsch1. 1: Duke University, USA, 2: North Carolina State University, USA

Radiation therapy plays an important role in the curative treatment of cancer patients. However, late effects of radiation therapy are frequently dose-limiting in the clinic. One well-described late effect of radiation therapy is damage to the heart and subsequent myocardial necrosis. However, the molecular mechanisms that control radiation-induced damage to the myocardium and loss of the myocardial capillaries are not well understood. Here, we show that Tie2Cre; p53fl/fl mice, in which p53 is deleted in endothelial cells, are susceptible to radiation-induced myocardial necrosis, which is preceded by changes in microvessel permeability and capillary loss in the myocardium. Experiments with primary cardiac endothelial cells from Tie2Cre; p53fl/fl mice irradiated in vitro demonstrate that p53 functions to protect endothelial cells from radiation by preventing premature entry into mitosis and mitotic catastrophe. Furthermore, mice lacking the p53 transscriptional target cyclin-dependent kinase inhibitor p21, are also susceptible to radiation-induced myocardial necrosis. Therefore, our results demonstrate that p53 functions in endothelial cells to control radiation-induced damage to the heart to a least partially through p21.

POS27-14. Angiogenesis Gene Expression Profiling in Irradiated Skin. Argelia Lopez1, A. Schock1, N. Duncan1, E. Olasz2, W. Kittipongda1, S. Doctrow2, B. Fish1, J. Moulder1, Z. Lazaro1. 1: Medical College of Wisconsin, USA, 2: Boston University, USA

Angiogenesis plays a pivotal role in the normal wound healing process, requiring endothelial cell proliferation and migration and aiding fibroblast proliferation. Skin exposure to ionizing radiation suppresses angiogenesis and delays wound healing. To identify gene expression changes potentially involved in radiation-induced wound healing, microarray analysis was performed on dorsal skin collected at 30 days following a 30 Gy single-dose skin irradiation using an angiogenesis pathway microarray. Male WAG/Rij/Cmr rats (n=10) were divided into irradiated and non-irradiated groups (5 rats per group). The dorsal skin was exposed to a 15kVp x-ray beam with a steep dose gradient. Rats were irradiated without anesthesia and control rats were sham irradiated. At the peak of radiation dermatitis, the animals were euthanized. Total RNA was isolated from the skin using RNeasy Mini Kit (Qiagen, Valencia, CA), pooled and RT2 Profiler PCR Arrays on Rat Angiogenesis (SA Biosciences, Frederick, MD) were performed. There was an up regulation of 36 genes and down regulation of 10 genes in irradiated skin compared to controls. The key up regulated genes were matrix metalloproteinases 2, 3, 9, 19 and chemokine ligand (C-X-C motif) 2. Among the down regulated genes were fibroblast growth factor 2, fibroblast growth factor receptor 3 and interferon beta 1 suggesting a dominant role of dermal fibroblasts in the pathomechanism of radiation induced skin damage. This work was supported by NIAID cooperative agreement AI067734.

POS27-15. Heavy ion radiation effects on spermatogenesis in Medaka (Oryzias latipes) p53 nonsense mutant. Hiroshi Mitani1, S. Oda1, Z. Li1, Y. Kimon2, H. Yasuda1, T. Fujisawa-Ishikawa1, T. Todo1, T. Yasuda1. 1: Graduate School of Frontier Sciences, The University of Tokyo, Japan, 2: National Institutes of Natural Sciences, Japan, 3: National Institute of Radiological Sciences, Japan, 4: Graduate School of Medicine, Osaka University, Japan

Medaka (Oryzias latipes) is a useful experimental fish and precise system to measure germ cell mutation induction by specific locus test was established and found that the spontaneous and γ-ray irradiation mutation rates are very similar to those of mice (Shima and Shimada1991). Kuwahara et al (2004) examined radiation induced apoptosis in Medaka pre-meiotic spermatogonial stem cells and spermatogonial stem cells (SSCs) and suggest that after irradiation most surviving spermatogonial cells, except for the SSCs, are prematurely eliminated from the tests by spermatogenesis acceleration. Taniguchi et al. (2006) showed that p53 also plays a general role in tumor suppression. To investigate the function of p53 mutation in irradiated equatorial spermatagonia, we established p53 nonsense mutant strain with inbred HdrR background to examine the role of p53 on radiation induced
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histological change including apoptosis in testis. The spermatogenesis in p53 nonsense mutant is almost normal but small number of cells with large nuclei are observed. At 24 hours after 5Gy γ-irradiation, there were a lot of pycnotic cells in the isogenic wild-type testes. In p53 mutants, a lot of pycnotic cells appeared after 6 days after irradiation but the increase in somatic cells and the acceleration of spermatogenesis was also observed like in wild-types. The number of cells with large nuclei was increased and their size becomes as big as immature sperm. We also observed that RBE of Fe ions for histological damage in testis is similar to that for germ cell nuclei.

POS27-16. Changes in hippocampal gene expression 48 hours and 2 months after fractionated whole-brain irradiation of the young adult male rat. Elizabeth Moore, D. Schloesser, L. Miller, M. Robbins, Wake Forest University School of Medicine, USA

Background: In 2011 ~200,000 patients will receive partial or whole-brain irradiation (WBI) for the treatment of primary and metastatic brain tumors in the USA. Radiotherapy has been shown to cause damage to the normal brain; as a result the risk of damage to the normal brain limits the dose that can safely be delivered to the tumor, compromising therapeutic effectiveness. Preclinical studies demonstrate a marked and persistent radiation-induced reduction in neurogenesis as well as neuroinflammation in the hippocampus, however, the molecular mechanisms involved remain ill-defined. We used a microarray approach to identify putative mediators/signaling pathways modulated in the rat hippocampus following fractionated (f)WBI.

Methods: Groups of young (12 week old) adult male Fischer 344 x Brown Norway rats received a total dose of 40 Gy (WBI (5 Gy fractions, 2x/week for 4 weeks); the hippocampi were isolated at 48 hours (h, acute) and 2 months (m, delayed) postirradiation and mRNA samples analyzed using Rat Genome 230 2.0 microarray chips. Results: We observed two distinct radiation response signatures. Upregulation at 48 h was followed by downregulation 9 months post WBI, but in the CA1 (FAS), TNF-α (ARC, HEXB) and Erk1/2 (JUNB, EGR2, SGK1, CSPG) signaling pathways was observed 48 h after WBI. In addition, we observed induction of genes involved in p53-mediated apoptosis (AEN) and cell cycle arrest (Mgnt). At 2 m after WBI we observed upregulation of a different series of genes involved in NF-κB (CD36, HLA-C, CXCL16), TNF-α (CCl4, CLEC7A) and Erk1/2 (CD14, PLAU) signaling pathways. Only Mgmt and CDK1/Nap2/p21, genes involved in cell cycle arrest and DNA repair, were both increased 48 h and 2 m after WBI. Homeri1 a gene involved in synaptic transmission was upregulated at 48 h but downregulated at 2 m after WBI. Similar to our previous study, Akt dependent phosphorylation of MAPK kinases Erk1/2, but not JNKs or p38 was significantly elevated in the irradiated RLE-6TN cells. Radiation also induced a time-dependent inactivation of glycogen synthase kinase-3β (GSK-3β), an endogenous inhibitor of Snail transcription, by increasing phosphorylation at serine 9 residue. A marked increase in phosphorylation of MAPK kinases Erk1/2, but not JNKs or p38 were observed in irradiated cells. Silencing of the Erk1 and Erk2 genes using lentiviral delivery of shRNAs and inhibition Erk1/2 activity using the specific inhibitor U0126 attenuated the radiation-induced phosphorylation of GSK-3β, activation of Snail, and altered protein levels of α-SMA and E-cadherin. Taken together, our findings reveal, for the first time, that radiation-induced alveolar EMT is mediated by the ERK/GSK-3β/Snail pathway.


Children exposed to therapeutic radiation for either cancer or bone marrow transplantation often demonstrate long-term neurocognitive deficits. Studies using animal models have identified a dose-dependent correlation between irradiation and declining neurocognitive performance. These studies often target the hippocampus, given its role in neurogenesis and memory formation. In order to further elucidate behavioral and molecular phenotypes following neonatal whole-body radiation exposure, 4-day-old C57BL/6 mice were treated with 2.5 or 5 Gy gamma radiation. Irradiated and unirradiated control mice underwent fear conditioning to assess hippocampal-dependent and -independent learning at 2, 3, 6, and 12 months. Upon completion of the memory test, all mice were sacrificed and brains harvested to investigate dose- and time-dependent changes in neurogenesis and brain morphology. Substantial memory deficits were observed in 5 Gy irradiated mice at all time points. To assess hippocampal neurogenesis, brain sections were stained for doublecortin (DCX) and Ki-67. Irradiated neonatal mice had reduced numbers of DCX+ and Ki-67+ cells, especially with 5 Gy of radiation. Moreover, the dentate gyrus, which is still developing at postnatal day 4, displayed significant volumetric shrinkage at both 2 and 9 months following 5 Gy irradiation. The CA1 and CA3 regions of the hippocampus also had reduced volume at 2 months. After 9 months, the role of hippocampal development in radiation-induced CNS sequelae, additional groups of mice were irradiated at postnatal day 7 and 14, separately, and sacrificed 3 months post-irradiation. Analyses of memory functioning, neurogenesis, and hippocampal volume are currently underway. These studies will provide a model of neonatal radiation injury for future investigations of possible radiation mitigating agents. This work was supported by NIH grants: U19 AI067733, U19 AI091036, T32 NS05152-05, and the NIH Postbaccalaureate R25GM64133.


It is known that p53 protein expression patterns due to some stresses show different dynamics in some cultured cells over time (1). In addition, p53 protein expression patterns after whole body irradiation might also show different dynamics in some organs over time. It is important to determine p53 protein expression patterns for each organ to point out the differences between Western blot and immunofluorescence analysis. Studies using animal models have identified a dose-dependent correlation between irradiation and declining neurocognitive performance. These studies often target the hippocampus, given its role in neurogenesis and memory formation. In order to further elucidate behavioral and molecular phenotypes following neonatal whole-body radiation exposure, 4-day-old C57BL/6 mice were treated with 2.5 or 5 Gy gamma radiation. Irradiated and unirradiated control mice underwent fear conditioning to assess hippocampal-dependent and -independent learning at 2, 3, 6, and 12 months. Upon completion of the memory test, all mice were sacrificed and brains harvested to investigate dose- and time-dependent changes in neurogenesis and brain morphology. Substantial memory deficits were observed in 5 Gy irradiated mice at all time points. To assess hippocampal neurogenesis, brain sections were stained for doublecortin (DCX) and Ki-67. Irradiated neonatal mice had reduced numbers of DCX+ and Ki-67+ cells, especially with 5 Gy of radiation. Moreover, the dentate gyrus, which is still developing at postnatal day 4, displayed significant volumetric shrinkage at both 2 and 9 months following 5 Gy irradiation. The CA1 and CA3 regions of the hippocampus also had reduced volume at 2 months. After 9 months, the role of hippocampal development in radiation-induced CNS sequelae, additional groups of mice were irradiated at postnatal day 7 and 14, separately, and sacrificed 3 months post-irradiation. Analyses of memory functioning, neurogenesis, and hippocampal volume are currently underway. These studies will provide a model of neonatal radiation injury for future investigations of possible radiation mitigating agents. This work was supported by NIH grants: U19 AI067733, U19 AI091036, T32 NS05152-05, and the NIH Postbaccalaureate R25GM64133.
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peaks nearly corresponded to that of p21 protein expression for the same period. However, a clear peak was not shown for p53 or p21 protein in the skin. Because numerous high radiation sensitive cells are included in the spleen, these cells may respond to irradiation at the same time, and our results suggested such a response pattern. In contrast, there are few cells that respond to radiation in the skin, which precluded detecting a clear peak. Spleen cells’ apoptosis was attuned to the first p53 peak and the p21 protein, but did not correspond with the second p53 peak. We considered that the first peak corresponded to the cascades of the so-called apoptosis or G1 arrest system induced by the p53 protein, but the second peak was not associated with the apoptosis cascade. These results suggest that the dynamics of the p53 and p21 protein expression patterns over time after whole body irradiation differ within organs. The second peak in the spleen may indicate that the p53 protein has some roles for tissue recovery after apoptosis or cell repair.


POS27-20. Sphingosine-1-phosphate, a new class of tissue radiation protector. François Paris, Inserm, France

Protecting the vasculature from radiation-induced death is a major concern in tissue radioprotection (Paris et al, Science 2001). Premitotic apoptosis and mitotic death are 2 prevalent cell death pathways induced by ionizing radiation. Endothelial cells (EC) undergo apoptosis after radiation through generation of the sphingolipid ceramide. However, direct involvement of mitotic death in proliferating ECs sensitive to mitotic inhibition has not been clearly demonstrated. We proved that proliferating human microvascular EC undergo 2 waves of death after 15 Gy: an early ceramide-dependent premitotic apoptosis and a delayed DNA damage-induced mitotic death. The fact that Sphingosine-1-Phosphate (S1P), a ceramide antagonist, protects EC only from membrane-dependent apoptosis, but not from DNA damage-induced mitotic death proves the independence of the 2 pathways (Bonnaud et al Cancer Res. 2007). Adding nocodazole, a mitotic inhibitor, to S1P blocked the 2 cell death and fully prevented radiation-induced death. Segregation between ceramide-mediated apoptosis and DNA damage-induced mitotic death may give the opportunity to define a new class of radioprotectors for normal tissue where quiescent endothelium represent the most sensitive target, while excluding malignant tumor containing proliferating angiogenic EC, sensitive to mitotic death.

To validate its potential radioprotection activity, S1P was injected in mice exposed to 15 Gy, a dose-inducing acute gastrointestinal (GI) syndrome within 10 days. S1P injection before irradiation prevented GI syndrome by inhibiting endothelium collapse (Bonnaud et al Cancer Res. 2010). We defined endothelium as a specific therapeutic target because only these cells and not intestinal epithelial cells, or B and T lymphocytes, were protected. Pharmacologic approaches using AKT inhibitor and pertussis toxin established that S1P affords endothelial cell protection in vitro and in vivo through a mechanism involving AKT and 7-pass transmembrane receptors coupled to Gi proteins. Our results provide strong pharmacologic and mechanistic proofs that S1P protects EC against acute radiation enteropathy.

POS27-21. Expression of TGF-β1 in rat colon enterocytes after whole body γ-irradiation. Jaroslav Pejchal1, V. Maláňík1, J. Österreich1, A. Tichý1, J. Vávrová2, Z. Sinkrová2, L. Zárybnická2, E. Novotná2, A. Babicová1, J. Chladek1, 1. Faculty of Military Health Sciences, Czech Republic, 2: Department of Radiology Biology, Faculty of Military Health Sciences, University of Defence, Czech Republic, 3: Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic

Purpose: The purpose of our study was to examine early expression of TGF-β1 in rat colon enterocytes after irradiation and to assess the contribution of this protein to pathology of intestinal radiation disease. Material and methods: Male Wistar rats were randomly divided into 28 groups and irradiated with whole body γ-irradiation of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 Gy. Samples were taken 4 and 24 h after irradiation, immunohistochemically stained and TGF-β1 expressions were measured in enterocytes using computer image analysis. In selected groups (0, 0.5, 1, 3, 5, 8, and 10 Gy), morphometric parameters, mitosis and apoptosis were evaluated.

Results: TGF-β1 expression increased in apical enterocytes 4 h after irradiation by 0.5–2, 4, 5 Gy and 24 h after irradiation by 6–10 Gy. In crypts, no changes of TGF-β1 expressions were observed. The length of crypts decreased 24 h after irradiation by 8 and 10 Gy. In apical enterocytes, the length of basal lamina decreased 24 h after irradiation by 0.5 and 1 Gy, while extension of basal lamina was observed 24 h after irradiation by 3, 5, 8, and 10 Gy. In crypts, we observed extension of basal lamina 24 h after irradiation by 5, 8, and 10 Gy. In apical enterocytes, TUNEL positivity increased 4 h after irradiation by 8 and 10 Gy, while decreased 24 h after irradiation by the same doses.

In crypts, apoptotic index of cryptal enterocytes increased 4 h after irradiation by 0.5, 1, and 5 Gy, and 24 h after irradiation by 3, 5, 8, and 10 Gy. Mitotic index was significantly decreased 4 h after irradiation by 3, 5, 8, and 10 Gy and 24 h after irradiation by 5, 8, and 10 Gy.

Conclusion: This study reveals that in vivo there is a chronological and dose-dependent order of TGF-β1 expression. Increased TGF-β1 expression seems to protect apical enterocytes in vivo after high dose irradiation.


Computational models of hematopoiesis, used in conjunction with hazard prediction models, allow planners to estimate the time-phased casualties and patient streams associated with specific radiation exposure scenarios. Previous efforts have produced models for lethality from hematopoietic syndrome (MarCell, Jones et al. Radiat. Res 128: 1991) and hematopoiesis (Smirnova 2011, Fliedner et al. Health Phys 70: 1996). MarCell describes time-dependent changes in an unspecified bone marrow population. Differential equations describe the kinetics of cell killing, cell injury, repair of injured cells, death of injured cells, and repopulation of the normal population of cells. The rate constants computed from experimental mortality data in animals predicted a radiation sensitivity of the critical cell that was more consistent with stromal cells than progenitor cells. Recent literature supports this possibility, demonstrating that bone marrow proliferation is dependent on the viability of osteoblasts in the bone marrow niche.

Smirnova’s models include lymphopenia, thrombocytopenia, and neutropenia associated with chronic radiation exposures and can be readily adapted for acute exposures. At least three populations of cells are considered: the dividing bone marrow precursor cells; a non-dividing, maturing cell in the bone marrow; and mature cells in circulation. Cells in tissue are also considered for granulocytes. In addition, an inhibitory mediator (chalone) produced by all cell populations is modeled to simulate feedback regulation of the rate of reproduction of the dividing cells. Most of the rate constants are based on data, were protected. We have implemented these models and made comparisons of the predicted time course with experimental data. We find that these mathematical models provide an excellent starting point for prediction of hematopoietic consequences of radiation exposure. We are working to integrate these hematopoietic modules with other modules (e.g., for inflammation or infection) to enable pathophysiologically based predictions of clinical endpoints, in addition to cytopenias and hematopoietic death following radiation exposure. The ability to make casualty predictions from complex dose and dose-rate exposures will be useful for consequence planning.


Radiotherapy for head & neck cancer often results in complications related to parotid gland (PG) dysfunction. To reduce the risk of such dysfunction the mean dose to the whole PG is currently minimized.
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However, regeneration of the PG depends on which part of the gland is irradiated and on the number of surviving stem cells. Therefore we investigated the relationship between the localisation of PG stem cells and late PG dysfunction after irradiation. In human and mouse PG c-kit stem cell marker-positive cells were found only in ducts. More intense c-kit expression in larger ducts suggested that these cells are predominantly located in the major ducts. To test this, the rat PG was split in a central part containing the major ducts and in the outer parts, dispersed mixed cultures as stem cells containing salivaryspheres. Indeed significantly more spheres could be grown from the central than from the outer parts of the gland, suggestive of a higher number of more stem cells in the central of the PG. High-resolution proton irradiation of this central part in vivo indeed resulted in an excessive reduction of salivary production. In contrast minimal effects were observed after irradiating of the other parts of the gland. To test whether this local sensitivity is also observed in 36 patients treated in the British Columbia Cancer Agency (BCCA) saliva production 1 year after radiotherapy was related to dose to specific sub-volumes of the PG. Excitingly, dose to the cranio-ventral extension of the gland containing the major ducts was most predictive of salivary production. In addition, we investigated if this part of the gland can be spared during radiotherapy and found that ~50% dose reduction can be achieved without increasing the mean dose to the whole PG or other critical structures in the head and neck region, and without compromising adequate target coverage. Based on the results of the BCCA cohort this is expected to prevent parotid gland dysfunction in all 36 patients.

In conclusion, the response of the parotid gland to radiation critically depends on the dose to its stem cells, located in the major ducts. These can easily be spared with current treatment modalities, expected to significantly reduce complications in radiotherapy for head and neck cancer.

**P0S27-24. Necropsy Findings in C57Bl/6 Mice Exposed to the LD80/30 Dose of Ionizing Radiation: Further Characterization of a Murine Model of the Hematopoietic Syndrome of the Acute Radiation Syndrome.**

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In response to the threat of radiation exposure from terrorist acts and nuclear disasters, our laboratory has developed a murine model of the Hematopoietic Syndrome of the Acute Radiation Syndrome (HS-ARS) for testing potential radiomitigators. Herein we report necropsy results of select tissues from mice exposed to the LD80/30 dose of radiation (810cGy; 155Cs, 62cGy/min, n=12 mice/time point) on days 3, 8 and 17 post-exposure, or from moribund mice sacrificed between days 10-23. Husbandy included acidified water, filter-togaping, and no antibiotics. Histology of small intestines at all time points showed no gross abnormalities, inflammation, ulceration, or bleeding. Interestingly, although villi blunting and modest crypt loss was evident. Crypt regeneration was observed relative to non-irradiated controls. These findings are consistent with modest GI damage within the LD80/30 range of HS-ARS. Histology of the thymus and bone marrow (BM) on d3 revealed classical effects of whole body irradiation: thymic atrophy, absence of cells in the cortex, and marked BM hypocellularity. Few myeloid cells were present in the BM, and sinusoids were dilated with red cells. Foci of myeloid cells appeared in the BM by d8 and 17, sinusoidal congestion diminished, and adipocytes became numerous. On d8, the spleen showed small germinal centers with indistinct mantle zones, which became prominent with discernible mantle zones by d17, along with foci of extramedullary hematopoiesis. Considerable interanimal variability in the number of hematopoietic foci in spleen and BM was noted. Seminiferous tubules were degenerated with necrosis in males on d8 and d17. Finally, microbiological analysis revealed that septicaemia was common in moribund animals. The percentage of septic mice was 0% on d8, 42% on d17, and 96% among moribund mice. The percentage of mice that tested positive for bacteria in any tissue was: 30% on d8, 83% on d17, and 100% among moribund mice. *Klebsiella oxytoca* was the most prominent organism isolated, followed by *Enterococcus faecalis*. These results characterize the differential effects of lethal radiation on various organ systems and illustrate a clear association of microbial presence and moribund status of irradiated mice, supporting the notion that neutropenia and infection are among the leading causes of mortality in HS-ARS.

**P0S27-25. Molecular and Histopathological Changes in Mouse Intestinal Tissue Following Whole-Body Proton- or Gamma-Irradiation.**


Crew members face potential consequences following exposure to the space environment and in particular including the acute radiation damage to radio-sensitive tissues, and cancer. The space radiation environment is ample with protons. Knowledge is limited, however, regarding their effects on mammalian systems. For this research, BALB/c mice underwent whole-body exposure to 250 MeV of protons at doses of 0, 0.1, 1, and 2 Gy and the gastrointestinal (GI) tract of each animal was dissected four hours post-irradiation. Standard H&E staining methods to screen for morphologic changes in the tissue showed an increase in apoptotic lesions for even the lowest dose of 0.1 Gy, and the percentage of apoptotic cells increased with increasing dose. Results of gene expression changes showed consistent up- or down-regulation of a number of genes across exposure doses that may play a role in proton-induced apoptosis including Bok/Casp1 after 0.1 Gy and Tsc22d3 after 2 Gy. A separate study in C57BL/6 mice using the same four hour time point but whole-body gamma-irradiation showed damage to the small intestine with lesions appearing at 0.1 Gy. Furthermore, increased dose with increasing absorbed dose. Quantifications of lesions in the duodenum of the small intestine were compared between the two types of radiation and the two strains of mice. Expressions of genes associated with oxidative stress processes were analyzed at several time points after exposure to gamma rays. We saw a much greater number of genes with significant up- or down-regulation twenty-four hours post-exposure as compared to the four hour time point. At both four hours and twenty-four hours post-exposure, Duox1 and Mpo underwent up-regulation for one or both of the higher doses of irradiation, 6 Gy and 6.05 Gy. Both protons and gamma rays lead to significant variation in gene expressions and these changes may provide insight into the mechanism of injury seen in the GI tract following radiation exposure. Astronauts experiencing exposure to protons may face biological consequences that will impact a mission’s success. We will continue this work by quantifying and comparing damage due to protons and gamma rays in the small intestine in a time-dependent manner.

**P0S27-26. In vivo astatine-211 exposure reveals distinct absorbed dose-dependent gene expression profiles in mouse thyroid tissue.**

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The alpha particle emitting At-211 has almost optimal LET for creating DNA double strand breaks, and is thus proposed for radionuclide therapy. Free At-211 is, similar to iodide, accumulated in the thyroid gland. Understanding of basal radiation-induced changes of biological processes in the thyroid is to a high degree unknown and would be most valuable. Female BALB/c nude mice were i.v. injected with 0.064-42 KBq of At-211, giving an absorbed dose to the thyroid of 0.05-32 Gy. Total RNA was extracted from pooled thyroides that were removed 24 hours after injection and processed in triplicate using Illumina MouseRef-8 Whole-Genome Expression Beadchips. Nexus Expression 2.0 was used for data analysis. Analysis of At-211 irradiated thyroid tissue revealed distinct gene expression profiles compared to non-irradiated controls. The transcriptional signature displayed a higher number of differently expressed genes at low absorbed doses (0.05 and 0.5 Gy) compared with intermediate (1.4 Gy) and high absorbed doses (11 and 32 Gy). Down-regulation was more common at low absorbed doses compared to higher absorbed doses. Furthermore, the absorbed dose-dependent genes increased with decreased absorbed dose. The difference in number of affected genes between low and intermediate absorbed doses might be the result of increasing heterogeneous irradiation with decreasing absorbed dose below 1.4 Gy, indicating the presence of a bystander effect. Among the genes differently expressed in 1.4 Gy often had an opposite regulation as compared to the other absorbed doses. Seven main topics were found to represent changes in...
biological processes: 1) cellular processes, 2) immune response, 3) metabolic processes, 4) transport, 5) response to stimuli, 6) system processes, and 7) developmental processes. Only 0.05 and 11 Gy had any impact on the immune system and no inflammatory effects were seen in any group. Absorbed doses of 0.5, 1.4 and 11 Gy affected the cellular response to stimuli, where 1.4 Gy had an impact on processes related to outer stress. Also, 1.4 and 11 Gy demonstrated effects on processes connected to cell cycle regulation and proliferation.

In conclusion, these results indicate that the cellular response to ionizing radiation is complex and differs with absorbed doses.

POS27-27. The acute effects of proton and gamma radiation on peripheral blood cell counts in ferrets. Jenifer M. Sanzari1, G. Krugsfeld2, D.S. Grudley1, A.R. Kennedy1, 1: University of Pennsylvania School of Medicine, USA, 2: Loma Linda University, USA

At the Center of Acute Radiation Research (CARR), the ferret model is currently used to assess the emetic potential in response to ionizing radiation using doses and dose-rates expected during a solar particle event (SPE). A separate area of the CARR investigates the effects of SPE-like radiation on hematopoietic cells. Complete blood cell counts were analyzed in ferrets exposed to proton or gamma radiation at doses of 0.75 Gy, 1.0 Gy and 2.0 Gy delivered at either a high dose rate (HDR) of 0.5 Gy/min or a low dose rate (LDR) of 0.5 Gy/h. Blood cell counts were obtained prior to radiation exposure, and 4 hours and 48 hours post-radiation exposure, The most significant results were observed in the white blood cell (WBC), lymphocyte, and neutrophil counts. Results indicate a dose-dependent decrease in WBC and lymphocyte counts from exposure to either proton or gamma radiation. A dose-rate effect was also observed in the WBC and lymphocyte counts, with the HDR resulting in lower counts than the LDR. In regards to neutrophil counts, there was no dose-dependent correlation observed, however a dose-rate effect was observed. From these data, relative biological effectiveness values were determined. In conclusion, SPE-like radiation induces significant changes in peripheral cell counts in the ferret model.

POS27-28. PPARγ-mediated modulation of radiation-induced inflammatory responses in microglia. Caroline Schnegg, M. Kooshki, M. Robbins, Wake Forest University, USA

Approximately 200,000 patients a year receive partial or fractionated whole-brain irradiation (WBI) for the treatment of brain tumors. Radiation-induced cognitive impairment, which can progress to dementia, will occur in up to 50% of these patients. Although the exact mechanisms underlying radiation-induced late effects remain unclear, microglia, key mediators of neuroinflammation, are hypothesized to play a critical role. Peroxisomal proliferator-activated receptor γ (PPARγ) is a potent mediator of inflammatory responses. Thus, we hypothesize that PPARγ activation will modulate radiation-induced inflammatory responses in microglia. Irradiating murine BV-2 microglial cells with a single dose of 10 Gy of 137Cs g rays led to increased i) ROS generation, ii) Cox-2, MCP-1, and iNOS protein, iii) IL-1β and TNF-α mRNA, and iv) NF-κB activation. Prior incubation with the PPARγ agonist, L165041, prevented these radiation-induced changes. In contrast, incubating BV-2 cells with L165041 and GS90660, a PPARγ antagonist, failed to prevent the radiation-induced increase in inflammatory mediators. Furthermore, overexpression of PPARγ prevents the radiation-induced increase in Cox-2 expression, while shRNA knockdown of PPARγ led to an increase in radiation-induced Cox-2 and MCP-1 expression. PPARγ may modulate radiation-induced inflammatory mediators, in part, by decreasing activation of MEK1/2 and ERK. Pre-incubating BV-2 cells with L165041 reduced the radiation-activation of MEK1/2 and ERK. Moreover, pretreating BV-2 cells with the MEK1/2 inhibitors, U0126 or PD98059, prevented the radiation-induced increase in Cox-2, MCP-1, TNF-α, and IL-1β.

We have started to assess the anti-inflammatory actions of PPARγ in vivo. Following a single dose of 10 Gy 137Cs g WBI, young adult C57BL/6 mice exhibited a significant increase in the number of activated microglia (CD68+ cells) in the dentate gyrus region of the hippocampus one week post-WBI. Administration of the PPARγ agonist, GW0742 (200 ppm), prior to, during, and after WBI prevented this increase. These data support the hypothesis that PPARγ activation may attenuate radiation-induced inflammation and may be a therapeutic target to prevent WBI-induced brain injury, thereby improving patients’ quality of life. (Supported by CA112593)

The immature brain in the central nervous system is sensitive to ionizing radiation and develops, at a later adult stage, disorders including mental retardation, attention deficit-hyperactivity disorder and cognitive dysfunction. The relationship between an impairment of spatial cognition and a merge of ectopic neurons in the dorsal hippocampus was investigated in adult rats that were prenatally exposed to X-ray irradiation. Adult rats irradiated at embryonic day 15 (E15) showed significant learning disability in the water-maze task. According to the mean value of the swimming time, we categorized the irradiated adult rats into the following three groups: slightly damaged group, mildly damaged group and severely damaged group, based on significant difference in the brain weight was found between the three categorized groups. Ectopic neurons appearing at abnormal places were prominently observed in the dorsal hippocampus of the severely damaged group with a remarkable learning disturbance. Furthermore, to test whether the morphological deficiency of hippocampus in the severely damaged group affects hippocampal function, we examined neural activity in hippocampal slices prepared from prenatal exposure to X-rays in rats. The spatial pattern of neural activity toward and through CA1 of the severely damaged group was similar to that of the control rat, the response was distinctly weaker, producing less voltage spread and amplitude. These findings suggest that the cognitive dysfunction induced by prenatal exposure to X-ray irradiation may be attributable to ectopic neurons of the hippocampus and the reduction of hippocampal neural activity.

POS27-32. Characterization of damage to cardiac endothelial cells induced both in vitro or in vivo by ionizing radiation. Anna Walaszczynski1, K. Jelenok1, D. Gabryl1, M. Pietrowska1, C. Kantkou2, P. Widiak1,1: Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland, 2: Cancer Research UK Tumour Microcirculation Group, University of Sheffield, UK

Introduction: Cardiovascular disease associated with radiotherapy is an important clinical problem. However, only few radiobiological models relevant for assessment of cardiotoxic effects of ionizing radiation are available at the moment; Here we described isolation from mouse primary cardiac endothelial cells, possible target for cardiotoxic effects of radiation, and tested them using different radiosensitivity assays.

Materials and methods: Cells were isolated from hearts of adult animals (8-weeks-old), both control and irradiated with a 2 or 8 Gy doses, at different times after irradiation (up to 60 weeks). In addition, cells isolated from hearts of juvenile mice were cultured and irradiated in vitro.

Results: A dose-dependent formation of histone gammaH2AX foci was observed after in vitro irradiation of cultured cells. However, such cells were resistant to radiation-induced apoptosis. A high dose of 16 Gy did not increase permeability of monolayers formed by endothelial cells. However, increased levels of actin stress fibres were observed in the cytoplasm of cardiac endothelial cells either irradiated in vitro or isolated from irradiated animals (up to 20 weeks after irradiation). Up-regulated expression of Vcam1 and Sele genes was detected after 8 Gy irradiation in vitro and in cells isolated few days after irradiation in vivo. In addition, elevated expression of Hsp70 gene was observed as a long-term effect of radiation (up to 40 weeks after the in vivo treatment).

Conclusions: Radiation-related changes observed in isolated cardiac endothelial cells within 2 weeks after irradiation, i.e. increased levels of actin stress fibres and elevated expression of Hsp70 gene, might be relevant for cardiotoxic effects of ionizing radiation.

POS27-33. Endothelial cell loss initiates the development of early pulmonary radiation-induced vascular remodeling. S.J. van der Veen1,2, G. Gillaert1, G. Bosman1,2, J. Langendijk1,2, P. van Luijk1, R. Coppes1,2, P. van Luijk1,1: Department of Cell Biology, Section of Radiation and Stress Cell Biology, University Medical Center Groningen, University of Groningen, Netherlands, 2: Department of Radiation Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Introduction: The radiation dose that can be delivered to thoracic tumors is limited by the risk of radiation pneumonitis (RP). Recently in rats we found that early radiation-induced loss of pulmonary function, peaking at 8 weeks post-irradiation, coincides with radiation-induced vascular remodeling, pulmonary hypertension (PH) and right ventricle hypertrophy. Interestingly, vascular damage was found to be present not only in irradiated but also in shielded lung tissue. In PH models vascular remodeling is thought to be initiated from endothelial cell (EC) loss. Therefore, also in lung irradiation ECs may be the main target leading to radiation-induced vascular changes. Purpose: Investigate the possible initiative role of EC loss and the effect of irradiated dose and volume on the development of radiation-induced vascular remodeling. Methods: To induce different levels of vascular injury, 8 Gy X-rays at 50% or 75% or 100% rat lungs were irradiated to 12, 17 and 20 Gy respectively using high-precision protons. Verhoeff’s elastica stain, rat specific HIS52 and TUNEL staining were used to assess pulmonary vascular remodeling, EC loss and apoptosis respectively in lung tissue samples of irradiated and shielded lung tissue before and at the peak of early lung dysfunction. Results: Already 8 hours post-irradiation apoptosis of ECs was observed in the irradiated lung tissue. 2 weeks post-irradiation, EC loss was observed both in irradiated and shielded tissue for all irradiated volumes. At 8 weeks after irradiation, the peak of early lung dysfunction, different features of vascular remodeling such as muscularization of vessel media, thickening of vascular wall, and apoptosis respectively in lung tissue samples of irradiated to 12, 17 and 20 Gy respectively using high-precision protons. Verhoeff’s elastica stain, rat specific HIS52 and TUNEL staining were used to assess pulmonary vascular remodeling, EC loss and apoptosis respectively in lung tissue samples of irradiated and shielded lung tissue before and at the peak of early lung dysfunction. Results: Already 8 hours post-irradiation apoptosis of ECs was observed in the irradiated lung tissue. 2 weeks post-irradiation, EC loss was observed both in irradiated and shielded tissue for all irradiated volumes. At 8 weeks after irradiation, the peak of early lung dysfunction, different features of vascular remodeling such as muscularization of vessel media, thickening of vascular wall, and apoptosis respectively in lung tissue samples of irradiated and shielded tissue, increasing with irradiated volume. This in- and out-of-field response indicates a global pulmonary vascular response rather than a response confined only to the area where the radiation dose is deposited. Conclusion: Early radiation-induced vascular remodeling function loss is determined by irradiated-volume dependent global pulmonary vascular remodeling which seems to be initiated by acute EC loss. As such, repairing endothelial cell injury with stem- or progenitor cells may be a promising strategy to reduce the pulmonary complications induced by radiotherapy to the thoracic area.


Background: Thrombomodulin (TM) is an endothelial cell surface glycoprotein that plays a pivotal role in the regulation of coagulation and inflammation. We have previously shown, both in clinical and in pre-clinical studies, that localized irradiation downregulates endothelial TM in intestinal microvessels in a time- and dose-dependent manner. The present study was performed to examine whether recombinant, soluble TM (solulin) would attenuate early radiation-induced intestinal injury in a rat model of radiation enteropathy.

Methods: Male rats underwent fractionated X-irradiation (5Gy x 9) of a 4-cm loop of small bowel. The animals were randomly assigned to receive daily subcutaneous injections of vehicle or solulin (3mg/kg/day or 10mg/kg/day) for 27 days (from 4 days before, 9 days during, until 14 days after irradiation). Intestinal radiation injury was assessed 2 weeks after irradiation by quantitative histology, morphometry, and immunohistochemistry. Myeloperoxidase (MPO) activity was assessed using luminol bioluminescence imaging. Results:Solulin administration ameliorated radiation injury score, reduced MPO activity, attenuated deposition of collagen I, attenuated intestinal serosal thickening, and reduced TGF-b1 immunoreactivities. On the other hand, solulin did not affect intestinal mucosal surface area, total intestinal wall thickness, or proliferating cell nuclear antigen (PCNA) immunoreactivity.

Conclusions: Solulin appears to ameliorate the early intestinal radiation response and should be explored as a target for prophylactic and/or therapeutic intervention. Further studies are needed to determine whether solulin also protects against delayed radiation enteropathy.

POS27-35. Radiation impairs IGF-1 signaling and cartilage formation from chondrocytes. Jeffrey Willey, D.L. Long, R.F. Loeser, Wake Forest School of Medicine, USA

The effects of therapeutic radiation on articular (joint) cartilage are poorly understood. The late joint deterioration at sites absorbing dose during cancer treatment suggests radiation can negatively affect
cartilage. This evidence indicates an early loss of compressive stiffness of cartilage after exposure. IGF-1 is an important growth factor for articular cartilage matrix production, stimulating proteoglycan (PG) and collagen synthesis through activation of the PI3-kinase-Akt pathway. We examined the effects of a 10 Gy single dose (137) Cs gamma-rays on cartilage degradation, PG synthesis, and IGF-1 signaling in articular chondrocytes and explants using tissue harvested from distal femoral condyles of 6-month old pigs. Chondrocytes were isolated from a palate and a cranial chondral cartilage. Confluent cultures were irradiated, then stimulated with IGF-1 after 1 hour. Akt phosphorylation was measured by immunoblotting at 1 hour and 1 day after IGF-1 stimulation. Proteoglycan synthesis via S0 uptake was quantified from cells 24 hours after IGF-stimulation. Whole cartilage explants were also cultured. Proteoglycan content of explants was quantified at 4 days post-exposure using a DAB assay. Likewise, PG content was determined from conditioned media of explants at day 2 and 4 after exposure. Immunoblotting of media measured matrix degrading enzymes MMP-3 and ADAMTS5 produced from cells and explants at day 1. Cell viability was unchanged after irradiation.

Radiation stimulated increased MMP-3 and ADAMTS5 (day 1) as well as PG release (day 2) into conditioned media, suggesting early degradation of cartilage. Irradiation also reduced PG content in explants day 4. IGF-1 stimulation increased Akt phosphorylation at the 308 and Ser 473 and increased PG synthesis by control chondrocytes, whereas radiation inhibited IGF-induced Akt activation and PG synthesis. Radiation appears to impair normal IGF-1 signaling in porcine chondrocytes: exposure reduced an IGF-1-mediated increase in Akt phosphorylation and PG synthesis. Early loss of cartilage matrix coupled with lower matrix production could decrease compressive strength. Radiation may ultimately cause a functional decline of cartilage health in joints after exposure, perhaps contributing to debilitating skeletal deficiencies observed at joints after treatment.

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Stereotactic body radiotherapy (SBRT) is a highly successful technique for the treatment of lung tumors. One clinical concern is the exquisite sensitivity of normal lung parenchyma to radiation injury. However, lung injury due to SBRT has been difficult to study due to a lack of a model system to irradiate small lung volumes.

We developed a model SBRT (mSBRT) technique to deliver high-dose, small volume, single-fraction radiation with sharp dose gradients between irradiated and non-irradiated tissue in the mouse lung. Dose measurements were made with a phantom, a parallel plate, and a mouse thoracic cavities. Pneumonitis-prone (C3H) mice were irradiated using 3 mm or 5 mm collimation with doses of 20, 40, or 80 Gy to the right lung parenchyma. Animals were sacrificed at selected time-points post-irradiation, and lungs were dissected and examined histopathologically. For 3 mm collimator delivered a median dose rate of 137 cGy/min to a region measuring a median full width half maximum of 2.96 mm, with a median 80-20 penumbra width of 0.37 mm. Distant scatter was 4% and non-target scatter within 3 mm of the beam edge was less than 11% of the target dose. The 3 mm collimator delivered a median dose rate of 143 cGy/min to a region measuring a median full width half maximum of 5.82 mm, with a median 80-20 penumbra width of 0.65 mm. Distant scatter was 8% and non-target scatter within 3 mm of the beam edge was less than 16% of the target dose. Regional histopathological changes were noted in lungs 1 year post-irradiation. Evidence of chronic inflammation, including alveolar septal interstitial pneumonitis, was noted. All lungs showed evidence of hemosiderin-ladain intra-alveolar macrophages was demonstrated regardless of dose level that correlated with collimator size. Pulmonary tissue outside the region of radiation did not show such abnormalities. An easily reproducible in vivo system was designed to model SBRT for future clinical trials. The model may be prompted to mimic SBRT based on small volume and high dose irradiation, sharp penumbra, and minimal scatter to non-target tissue. Histopathological findings confirm localized radiation injury correlating with the focal areas receiving mSBRT. The mouse model appears viable for future studies on normal tissue recovery, pulmonary immune response, and possible mitigators of lung radiation injury to SBRT.

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Senescence or biological aging is a consequence of the accumulation of unrepaired DNA damage. Exposure to ionizing radiation (IR) causes oxidative stress, DNA damage, inflammation, cellular apoptosis and senescence. In the present study, we evaluated the features of human hematopoietic stem and progenitor cells (HSPC) and bone marrow microenvironment (hematopoietic niche) osteoblast cells in response to IR. We found that γ-irradiation (up to 8 Gy) induced premature senescence of human CD34+ cells in vitro and in human mesenchymal stem cells, whereas it caused massive apoptotic cell death in primary human HSPC CD34+ cells after 2 Gy, suggesting that the osteoblast cells are relatively radiation-resistant. The PS in irradiated hHOB cells were characterized by significant inhibition of clonogenicity from 450 ± 30 (0 Gy) to 90 ± 5 (4 Gy) and 5 ± 6 after 8 Gy irradiation, activation of senescence biomarker SA-β-gal 72 h after 8 Gy, and the senescence-associated cytokine secretory phenotype (SASP) as shown by secretion of interleukin (IL)-6, IL-8, granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) into hHOB cell conditioned medium (CM) after 4 or 8 Gy irradiation. We further demonstrated that stress response gene REDDI1 (regulated in development and DNA damage responses 1) was highly expressed in mouse BM osteoblasts and human hHOB cells but not in human CD34+ cells after γ-irradiation. Interestingly, differentiated hematopoietic cells from 14-day cultured CD34+ cells showed radiation resistance and increased endogenous REDDI1 expression. Knockdown of REDDI1 with siRNA decreased hHOB cell numbers from 1.7 ± 0.2 x 103 (control-siRNA) to 1 ± 0.8 x 102 (REDD1-siRNA) (p<0.05) with enhanced IL-6 levels in CM regardless of radiation. In contrast, overexpression of REDDI1 significantly suppressed IL-6 and IL-8 secretion and reduced SA-β-gal in the IL-6, IL-8 double-positive senescent cells from 62 ± 11% to 35 ± 7% in 8 Gy irradiated cells (p<0.01). Moreover, immunoblotting results showed that over-expression of REDDI1 in hHOB cells suppressed two senescence regulators, mammalian target of rapamycin (mTOR) and cyclin-dependent kinase inhibitor p21, expression without suggesting the host defense effects of REDDI1 in irradiated hHOB cells. In conclusion, our study demonstrated that REDDI1 is a...
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POS28 Microdosimetry

POS28-01. Effect of Distance between Decaying Iodine-125 Atom and DNA on Auger-Electron Induced Double-Strand Break Yield. Pichumani Balagurumoorthy, K. Wang, S.J. Adelstein, A.I. Kassiss, Harvard Medical School, USA

We are interested in understanding the biophysical mechanisms underlying the production of double-strand breaks (DSB) by Auger electrons following the decay of iodine-125 (125I). Our previous work has shown that several factors (e.g., DNA topology, O2 scavengers) affect the DSB yields in plasmids exposed to 125I decays.

To determine the possible effects of distance (125I-DNA helix) on DSB yields and assess the nature of the mechanisms by which this Auger electron emitter imparts biological damage, we have synthesized a series of 125I-labeled Hoechst (H) derivatives (125I–H, 125IB–H, 125I-C3-H and 125I-C12-H). While all four molecules share a common DNA minor groove binding benzimidazole motif, they are designed to position 125I at varying distances from the DNA helix. Each Hoechst derivative was irradiated at 4C in PBS (pH 7.4) together with supercooled (SC) HpUC19 plasmid DNA (ratio 3:1) ± the O2 scavenger DMSO (0.2 M). Aliquots were drawn over time and analyzed on agarose gels. DNA dissociated radioactivities (125I) were quantified and the DSB yields per decay of 125I atom determined.

In the absence of DMSO, the results show that the DSB yields decrease monotonically as the 125I atom is distanced – a few Angstroms from the DNA helix (125I–H: 0.52±0.01; 125IB–H: 0.24±0.03; 125I-C3-H: 0.18±0.02; 125I-C12-H: 0.10±0.00). In the presence of DMSO, DSB yields for 125I–H (0.2±0.04) remain the same (i.e., DSBs are entirely produced by direct effects). However, no DSBs were detected when SC plasmid DNA was irradiated under similar conditions with 125I–C3-H or 125I–C12-H (i.e., DSBs are entirely produced by indirect effects). These results suggest that at a critical distance between the 125I atom and the DNA helix, DSB production switches from an “all” direct to an “all” indirect mechanism, a situation that is similar to the decay of 125I bound to DNA vs. free in solution. These experimental findings will be correlated to theoretical microdosimetric calculations and expectations.

POS28-02. Numerical models of energy deposition in microscopic volumes for electrons from 100eV to 10MeV. Jing Chen, Health Canada, Canada

Integration of radiation dosimetry with microdosimetry is of great importance for the improvement of various dosimetry systems in practical radiation protection as well as clinical uses. Compared to linear energy transfer (LET), the microdosimetric parameter, dose mean lineal energy is a better parameter for describing radiation action at nanometre level is proposed. Experiments with Auger electrons emitted by 125I decays. The results serve as a convenient database for anyone performing microdosimetric calculations in radiation fields of electrons. To convert the results into a user-friendly format, mathematical models were developed to best fit the proximity functions, so that dose mean lineal energies can be readily calculated with numerical forms similar to the computation of LET.

POS28-03. Nanodosimetry of Auger electrons. Stanislaw Pszona, A. Bantsar, The Andrzej Soltan Institute for Nuclear Studies, Poland

Introduction: It is well established, that ionizing radiation induces radiation damages to a living tissue through initiation stages, which occur at the DNA levels. DNA molecules in the forms of short segments, nucleosomes, chromatin strings are the nanometer sized targets and due to this, the initiation of radiation damage is caused, predominantly, by single particle interactions. The modern approach to targetted radiotherapy of the cancer diseases is to use the radionuclides, emitting Auger electrons (i.e. I-125), which energy spectrum below 3 keV are predominant and which is vectorized to the cell nucleus. An intense interactions of these short ranged low energy electrons (up to tens for a single decay) with the DNA in cell nucleus create enough damages to kill a cell. Experiment and Results In view of theses facts, the needs for the adequate description of radiation action of Auger electrons on nanosized biological targets seems to be unquestionable. Experimental microdosimetry at nanometer scale for Auger electrons has been accomplished with a set up called JET COUNTER, JC. The later consists of a pulse operated valve which injects an expanding nitrogen jet into an interaction chamber where a gaseous sensitive volume of cylindrical shape is created. The cluster size spectra for the sensitive volumes corresponding to 2 nm and 5 nm in diameters (in unit density) irradiated by electrons emitted by I-125 source were studied and analysed. Afterward the first moments of the cluster size distributions as well as the cumulative distribution function of cluster size greater than or equal to 2 were derived and compared with the corresponding Monte Carlo simulation. The set of the new descriptors of radiation quality describing radiation action at nanometre level is proposed.

POS29 Physical dosimetry

POS29-01. Upgrade of a Bonner Sphere Spectrometer with High-Pressure 3He counters to Measure Secondary Neutrons from Cosmic Radiation Close to the North Pole. Ulrich Ackermann, C. Poch, V. Mares, W. Rühm, Helmholtz Zentrum München, Germany

Since 2005, we run a Bonner Sphere Spectrometer at the environmental research station “Schneefernerhaus” at the Zugspitze mountain, Germany, at an altitude of 2650 m. A second identical spectrometer is being operated since 2007 at the Koldewey Station on Spitsbergen, at sea level. Both spectrometers detect secondary neutrons from cosmic radiation by means of 3He proportional counters (SP90 from Centronics; diameter: 3.30 cm; gas pressure: 2 bar). Because the count rates obtained with the current spectrometer at Spitsbergen is about 7 times lower than at the “Schneefernerhaus”, due to more effective shielding of the atmosphere, we have decided to use new 3He counters with higher pressure (10 bar) and larger diameter (5,08 cm) from LND, INC. The response functions of the Bonner spheres with the LND2705 detectors were simulated using the GEANT4 tool kit. First measurements with the upgraded spectrometer were performed on the “Schneefernerhaus” and reasonable agreement was found with the unfolded neutron spectrum compared to that measured with the conventional spectrometer installed there. It is concluded that the new counters can be used to upgrade the BS5 spectrometer on Spitsbergen, which finally will lead to a factor of about 4 higher count rates and correspondingly, to improved statistics.

POS29-02. A new amino acid-based material for EPR dosimetry. Reza Amraei1, G. Bagherzadeh2, G. Raisali1. 1: Radiation Applications Research School, Iran 2: Department of Chemistry, School of Sciences, Birjand University, Birjand, Iran

In this study, ability of synthesized 2-(4-(diethylamino)-6-methyl-5-nitropyrimidin-2-yl) amino) propanoic acid was investigated as a new material for dosimetry. Powder samples of the material were irradiated by 60Co gamma rays with a dose rate of 4.26 Gy/s at doses between 0.5 to 45 kGy. Irradiated samples were analyzed with electron paramagnetic resonance (EPR) spectrometry. It is worthwhile to mention that EPR is the best technique to detect free radicals produced in an irradiated material. Results indicate a good linearity between the absorbed dose and the EPR intensity of the signals at doses between 0.5 to 25 kGy. With the increase of the absorbed dose calibration curve shows a non-linearity behavior (2nd order polynomial curve). It is to be pointed out that all the EPR measurements were performed at room temperature. The results obtained hitherto indicate that this
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material can be used as a suitable dosimeter for gamma radiation in the range of 0.5 to 45 kGy.

POS29-03. Neutron Spectrometry and Dosimetry Study at two Research Nuclear Reactors Using Bonners Sphere Spectrometer (BSS), Rotational Spectrometer (ROSPEC) and Cylindrical Nested Neutron Spectrometer (NNS), Jovicu Atanackovic¹, S. Witharan⁵, J. Duboub⁴, W. Matysiak³, L. Aslam³, 1: Atomic Energy of Canada Limited, Chalk River Laboratories, Canada, 2: DEtec, Gatineau, Canada, 3: McMaster University, Hamilton, Canada, 4: University of Ontario Institute of Technology, Oshawa, Canada

Three different neutron spectroscopy instruments were used in order to assess the neutron spectral and dosimetric information at the McMaster Nuclear Reactor (MNR) at McMaster University, Hamilton, ON and National Research Universal (NRU) Reactor at the Atomic Energy of Canada, Chalk River Laboratories, Canada. MNR is the university research reactor with nominal power of 3 MW, while NRU is also a research reactor with nominal power of 100 MW.

The instruments under investigation were the traditional a BSS[1], a ROSPEC[2] and an NNS[3]. At the same time, this opportunity was used to evaluate the relative response of these instruments in occupational neutron radiation fields. Three measurements were taken at MNR: inside beam port #3 and between beam ports #1 and #2, where there is a high degree of occupancy due to neutron radiography work. Also, measurement was taken at the top plate of the NRU reactor vessel.

The dose rates measured ranged from 3 mSv down to several µSv per hour. The BSS and NNS spectral unfolding was performed using STAY'SL code, while ROSPEC unfolding was done using the unfolding program. The unfolded neutron fluence rates were then folded with different dose coefficients in order to obtain dosimetric information for the characterized fields. The dosimetric and neutron fluence comparison was done for three energy ranges: thermal to 1eV, 1 eV to 50 keV and 50 keV to 4.5 MeV. Since the BSS and NNS have identical energy binning, a graphical comparison was done for these instruments as well.

Also, measurement was taken as the organ dose. Readouts were obtained on a solid water tank filled with water. The pillbox was covered contained some holes that allowed accommodation of TLDs. Lithium fluoride thermoluminescent dosimeters (TLD-100, Harshaw) were used to determine the absorbed dose of critical organs of tissue equivalent random phantom (Alderson research industries, Inc, Stanford, Conn, U.S.A).

The plan was accepted for treatment only if the fraction of points passing the criterion was higher than 95%.

Results: In our group of patients about 25 % of evaluated plans was rejected. Most of the rejected cases required reoptimization using the treatment planning system. A relatively high fraction of rejected plans was observed despite of performing extended and precise quality check measurements and tuning of the planning system at the initial stage, before moving into IMRT routine. The fraction of plans passing the verification remains stable over the time.

Conclusions: The verification study presented here serves as an independent check of monitor units calculation of a plan which has to be accepted for treatment. This is specially important for the patient safety since in the IMRT there is no well established method of in vivo dosimetry.

POS29-06. Evaluation of Absorbed Dose of Critical Organ in Rando Phantom Under Head, Abdomen and Pelvis Spiral CT Scanned by Thermoluminescent Dosimeter (TLD), Gholamhassan Haddadi¹, M. Haddadi², S. Meh dizadeh³, 1: Fasa University of Medical Sciences, Iran, 2: School of Electrical and Computer Engineering, University of Tehran, Iran, 3: Armed Forces Radiation department, School of Engineering, Shiraz University, Iran

Computed tomography (CT) represents 11% of all diagnostic radiology procedures but it contributes to almost 67% of the total effective dose to the human population. In head and neck CT which consist of 1/3 of total CT scans, other critical organs such as lenses and thyroid are in the radiation field. Also, in the abdomen and pelvis scan, irradiation of ovaries is unavoidable. Because of high sensitivity of these organs, the probability of abnormality and cancer in these organs has increased. Therefore the dose assessment in these organs is very important. The aim of this study is to estimate the absorbed dose in critical organ of patient undergoing common head, neck, abdomen and pelvic spiral CT scan.

In this study, Lithium fluoride thermoluminescent dosimeters (TLD-100, Harshaw) were used to determine the absorbed dose of critical organ of tissue equivalent random phantom (Alderston research industries, Inc, Stanford, Conn, U.S.A).

The mean absorbed dose received by the right and left eye lenses from computed tomography was 7.35 mGy. The mean scattered dose received by the right and left lobes of thyroid gland were 0.5 mGy and 0.54 mGy respectively. The mean absorbed dose received by ovaries

Introduction: The IMRT is a complex procedure usually applied to radiotherapy of irregular shape tumours of located in the vicinity of organs at risk. The aim of this work is to evaluate the absorbed dose to ovaries before the actual treatment. In most radiotherapy facilities such verification is limited to the fluence measurements for single fields. A system of the verification of total dose distributions is presented here.

The absolute film dosimetry as well as the ionization chamber point dose measurement procedure for total planned dose distribution was evaluated. The gamma evaluation concept was used for processing the plan verification results.

Material and methods: More than 400 IMRT plans were verified using CarPet phantom (ESTRO Quasimodo project). The plans, mainly for head/neck and pelvic regions, were generated using Eclipse (Varian) treatment planning system. Field settings and numbers of monitor units were copied from the patient plan to the phantom CT images and the dose distributions were recalculated. Kodak EDR2 films were placed inside the phantom. The films in the phantom were irradiated using all fields of the plan for recording total absolute dose distribution. For each plan the measured and the calculated dose distributions were compared using in house developed software IMRtCompare. The 3 mm in spatial domain and 3 % of the planned isocenter dose was taken as parameters for the gamma evaluation. The fraction of points passing the gamma < 1 criterion from rectangular region of interest was recorded for each plan. The plan was accepted for treatment only if the fraction of points passing the criterion was higher than 95%.

Results: In our group of patients about 25 % of evaluated plans was rejected. Most of the rejected cases required reoptimization using the treatment planning system. A relatively high fraction of rejected plans was observed despite of performing extended and precise quality check measurements and tuning of the planning system at the initial stage, before moving into IMRT routine. The fraction of plans passing the verification remains stable over the time.

Conclusions: The verification study presented here serves as an independent check of monitor units calculation of a plan which has to be accepted for treatment. This is specially important for the patient safety since in the IMRT there is no well established method of in vivo dosimetry.
and uterus from primary beam of abdomen and pelvic CT scan was 6.42mGy and 7.55mGy respectively.

International Commission on Radiological Protection has recommended a lifetime risk for cancer induction of 0.75x10^{-2} per Gy delivered by the radiosensitive gland. To estimate the risk for cancer induction in each sensitive organ, we multiplied this coefficient by organ absorbed dose.

The absorbed dose of critical organ resulting from head and abdomen CT scanning is significant, and it can lead to a low risk for development of malignancies. Therefore, further research is required to investigate whether modification of the parameters used during routine spiral CT scan can be limited absorbed dose of critical organ without a significant loss of image quality.


Cosmic radiation is the major source of occupational exposure of air crew to ionising radiation. In an effort to complement cosmic ray dose calculations done at our institute with the EPCARD code, we aim at a detailed experimental characterisation of the radiation field at mountain altitudes. For this reason, cosmic radiation and other natural sources of radiation are continuously being measured at the environmental measurement station “Schnepfauhoch” at an altitude of 2650 m using a variety of detection techniques and instruments. Results obtained with a high-pressure ionisation chamber and a plastic scintillation detector for dose rate measurements, a NaI detector for gamma spectrometry, several rem counters for neutron dose rate measurements, and instruments for radon gas detection are presented in the present paper. For example, typical dose rates measured with the high-pressure ionisation chamber and the plastic scintillation detector range between 0.010 and 0.015 mR/h or 0.10 and 0.15 μSv/h, respectively, with occasional short-time variations that might for example be due to increased local radon progeny concentrations in air. This is confirmed by time-dependent measurements of photons emitted by radon progeny 214Bi and 214Po using the NaI detector.

POS29-08. Angular responses of RPL dosemeter to photons and beta radiation calculated with MCNPX. Nora Hocine, Institut de Radioprotection et de Sûreté Nucléaire, France

At the IRSN (Institute of Radioprotection and Nuclear Safety-France), the standard dosemeter used for the monitoring of the external dose of radiation on workers is the radiophotoluminescent (RPL) glass dosemeter (Chiyoda Technol Corporation-Japan). This dosemeter consists of a plastic badge (61 x 30 x 8 mm³) with aluminium, tin and plastic filters that ensure energy compensation in all directions through the RPL glass (35 x 7 x 1.5 mm). The RPL dosemeter is designed to measure beta particles in the 100 keV to 3 MeV energy range and photon energies between 10 keV to 10 MeV. The angular response of the RPL dosemeter to photons as well as to beta particles is investigated. The RPL dosemeter and the phantom were rotated in the horizontal and vertical planes from a variety of angles of interest. The aim of this work is to determine the theoretical responses of the RPL dosemeter in terms of the personal dose equivalents H_{10}(0,07) and H_{10}(10) with the Monte Carlo transport code MCNPX. The calculated dose equivalent response as a function of angle has been examined for the RPL dosemeter that was exposed to Narrow Series X-ray, N-60, N-80, N-100, N-150, N-200, N-250, N-300, photon sources 57Co and 137Cs and beta-ray emitter 208Tl (3.63 MeV). The results were compared with the experimental data. A good agreement was found between the measured and calculated values of the relative dose equivalent angular responses of the RPL dosemeter for the angles ranging from 90° to 90°. Measured results from experimental testing of the dosemeter validated the calculated data and, consequently, served as additional confirmation of the MCNPX simulation method used.

POS29-09. Assessment of doses and neutron spectra induced by a high energy medical Linac. Fang-Yuh Hsu, J. Lin, National Tsing Hua University, Taiwan

The medical linear accelerator (Linac) is the main therapeutic equipment used in modern radiotherapy. For the energy of photon beam produced by a Linac is higher than 10 MeV, probability of photonuclear reactions increases, therefore, neutron particles will be induced. To assess the induced neutron dose, dual-thermoluminescent dosimeter (dual-TLD, TLD600/700) method is frequently used. However, the accuracy of dual-TLD method depends on the energy spectrum of the induced neutrons. The sensitivity of TLD-700 chip to low energy neutron is negligible, but it will increase and become significant relative to TLD-600 chip as the neutron energy is higher. To estimate the neutron doses correctly, measurements of energy spectra of neutrons are necessary. This paper, hereby, assessed and discussed the energy spectra and doses of neutrons induced by a Linac used in radiotherapy.

Gold foils inside the Bonner spheres with different diameters (2", 3", 5", 8", 10" and 12") were used to estimate the neutron spectra induced by a medical Linac (with max. energy of 15 MV X rays). Response functions of the gold foil were calculated with Monte Carlo simulation Code (MCNP). By means of the response functions, activated-gamma counting rates of the gold foils in different size of spheres and the unfolding UMG 3.3 code, neutron spectra were estimated. Besides, the doses and neutron spectrum at different depths (from 0 to 20 cm depth) of solid water phantom were also estimated. The doses contributed from X rays and thermal neutrons and fast neutrons were assessed by using dual TLD, 600/700 method. Two algorithms, considering (depending on the assessed neutron spectrum) and ignoring the dose responses of TLD-700 chips to higher energy neutrons were applied and discussed.

Two main peaks, thermal (0.1eV) and fast (about 1 MeV) were showed in the assessed neutron spectra. For shallow depth, fluence of high energy neutrons is higher, ignoring the dose responses of TLD-700 chips to higher energy neutrons would overestimate the dose of thermal neutrons.

POS29-10. Application of Medipix detector for radon, thoron and their progeny measurement. Miroslav Janík, O. Ploc, L. Pinsky, J. Jakubek, Y. Uchihori, T. Ishikawa, 1: National Institute of Radiological Sciences, Japan, 2: University of Houston, Texas, USA

Radon and thoron which are decay products of the radium decay, and their short-lived progenies are the most important elements for public exposure. Methods of the radon, thoron and decay products measurement are still developed. Currently, only the etch-track method (CR-39 or LR-115) is used for the passive long-term measurement of radon/thoron progeny. The result of measurement is available after exposition and chemical treatment. In this work new method for passive but “on-line” alpha particles registration based on semiconductor pixel radiation detector systems is present. The hybrid silicon pixel device of Medipix type developed at CERN was originally designed for position sensitive single X-ray photon detection. This device can provide information about energy and position of charged particles.

Preliminary energy calibration for radon, thoron and their progenies was performed using NIRS (National Institute of Radiological Sciences, Chiba, Japan) sources of radon and thoron gases and 226Ra and 228Ra contaminated from X rays or 222Rn. The good energy resolution and the linear dependency between channel number and energies with the coefficient of determination \(R^2 \approx 0.98\) in the range from 5 to 9 MeV were observed.

POS29-11. Dosimetry comparison of conformal techniques used in stereotactic radiotherapy. Marzena Janiszewska, M. Raczkowski, T. Tokarz, Dolnośląskie Centrum Onkologii, Poland

Introduction: In radiation therapy the correctness of planned dose delivery is of fundamental importance. In stereotactic radiotherapy the quality control is usually limited to accelerator control, that is dose rate control, and control of geometry. Wherefore, at the DCO the verification system has been extended with the control of treatment planning system. Calculated dose distributions have been compared with the measurement reality with the medipix accelerator reality.

Objective: The objective of the article is an evaluation of the dose calculation correctness for conformal techniques 3D and conformal arc.

Material: The verification has been performed for conformal technique 3D and conformal arc in planning systems (Plan for MLC120 and micro m3, for energy 6.10. 18MV in Eclipse system for MLC120, for energy 6 and 18MV. The 2-Gy dose has been calculated for 5 cm. Calculations for 120MCL, for photons 6 and 18MV have included square fields and rectangular fields in the scope from 10x10 cm to 1x1 cm and irregular fields MLC with field size 4x4 cm. In arc technique two arcs in gold foils range 3.0 and 4.45 have been chosen, for a combination of 13 fields from 10x10 to 1x2 cm. For micro m3 small fields scope has been extended to 0.6x0.6 cm.
Method: The measurements have been performed in water phantom with two chambers type Pin Point PTW TM 31014 (0.015 cm²) and TM 31016 (0.016 cm²). For both above mentioned chambers the calibration factor cross for reference set has been indicated: chambers PTW TM 30013 (0.6 cm²) and dosimeter UNIDOS T10008. Dose measurements included all calculated fields combinations with irradiation times determined from the calculations. Results: Average and maximum differences between planned dose in iPlan system and measured dose for 3D technique were: for MLC120: 6MV (\(\Delta L=0.3\%\), \(\Delta A=1.8\%\)), 18MV (\(\Delta L=0.5\%\), \(\Delta A=5.5\%\)); for MLC50m: 6MV (\(\Delta L=0.7\%\), \(\Delta A=2.3\%\)), 10MV (\(\Delta L=0.5\%\), \(\Delta A=6.5\%\)). For the differences in iPlan system were: for MLC120: 6MV (\(\Delta L=0.4\%\), \(\Delta A=1.9\%\)), 18MV (\(\Delta L=1.1\%\), \(\Delta A=3.1\%\)); for MLC50m: 6MV (\(\Delta L=0.3\%\), \(\Delta A=4.5\%\)), 10MV (\(\Delta L=0.7\%\), \(\Delta A=5.6\%\)).

Conclusion: The verification of planning system is a fundamental element of quality control in radiation therapy. The high level of conformity of all measured cases with the measurement reality on accelerator enables a safe realisation of procedures without delivered dose to be controlled in real time. At the DCO in Wroclaw the planning of head stereotactic surgery is performed only in iPlan system, whose correctness of working has been shown for 3 energies, 2 types of collimators. Plans for extracranial stereotactic surgery can be additionally calculated in Eclipse system. A mutual conformity of these systems in fields scope above 3 cm allows the use of two independent calculation methods. The incompatibilities in Eclipse system for fields bellow 3 cm result from interopolation of the field factors table. Wherefore extracranial stereotactic surgery for small areas has to be planned only in system iPlan.


Abstract: In relative dose measurement, we have finished developing a 3 dimensional auto-scanning measuring dose system. The water tank dimensions (LxWxH): 638mm×630mm×555mm ;Scanning volume (LxWxH): 400 mm x 400 mm x 360 mm;Position resolution: 0.1 mm;Position reproducibility: ± 0.1 mm. We used the 3D auto-scanning system to scan the X-rays fields and electron fields from the linear accelerators and compare with a IBA Blue Phantom 3D waterphantom, the X-rays energies are two ranges, 6MV and 15MV; the electron energies are 6 ranges, 4MeV,6MeV,9MeV,12MeV, 16MeV and 20MeV. The compared results with IBA 3D waterphantom coincide very well. The performance of 3D auto-scanning measurement is reliable.

POSTER 13. Alkaline/ESR Dosimetry for Electron Intra-Operative Therapy (ELIOT). Maurizio Marralle1, A. Longo1, G. Russo1, C. Casarino2, G. Cândamo3, M. Brai1, 1: University of Palermo, Italy, 2: Fondazione Istituto San Raffaele G. Giglio and IBFM-CNR, Italy, 3: LATO s.r.l, Italy

The Electron Intra-Operative Therapy (ELIOT) is a radiotherapeutic technique employed to deliver a single dose of radiation directly to the tumour bed or to the exposed tumour during the surgery. The objective is to achieve higher doses (20-22 Gy) to the target volume, equivalent to the total dosage (60 Gy) usually delivered during external fractionated radiotherapy. In case of breast treatment, this technique allows to treat only the involved quadrant of the breast, thereby shortening the radiotherapy course from 6 weeks to a single 2- min session during surgery[1]. ELIOT is performed using a mobile linear accelerator located directly in the operating room.

In this work we report the analysis response of the alkaline/ESR dosimetric system to the electron beams used for ELIOT treatment. In particular, we study the dependence of the alkaline ESR response for different dose rates and energy beams. Our results are compared with those reported in a previous work by De Angelis et al. (2006)[2]. The linearity of the dose response is investigated in the dose range below 40Gy. The irradiation of the alkaline samples was carried out with a mobile linear accelerator (NOVAC7) installed at the Laboratory of Oncological Technologies (LATOT-Fondazione Instituto San Raffaele G. Giglio) in Cefalù (Italy), specifically used for breast cancer treatment.

Furthermore, to test the anilale/ESR dosimetric system, we compared the measured dose distributions with those obtained with Monte-Carlo simulations, in the case of a aluminium-lead protection disc (usually used in surgery to minimize the irradiation of the thoracic wall) positioned in the suitable phantom.

References:

POSTER 29. Can two dimensional ionization chamber systems (Matrixx) overtrop the film in the dosimetry for quality assurance of IMRT plans? Hajime Monzen1, M. Hira1ta2, T. Suzuki2, Y. Miyabe3, S. Sato1, N. Nakamura1, T. Shinoki1, T. Mizowaki1, M. Hiraoka1, 1: Kyoto University Graduate School of Medicine, Japan 2: Otsu Red Cross Hospital, Japan

Purpose: Comparisons between measured data and calculated data on a radiotherapy treatment planning system (RTPS) are indispensable in quality assurance (QA) of intensity modulated Radiation Therapy (IMRT) plans. As for relative dose comparison, film dosimetry (FD) has been mainly used. However, it is not economical or easy to use. Therefore, development of any alternative method has been desperately needed. Method: IMRT treatment plans of 8 patients with prostate cancer were retrospectively analyzed. We evaluated the effectiveness of a two-dimensional ionization chamber system (Matrixx) in actual measurements of IMRT plans by comparing dose distributions among Matrixx, FD and RTPS based on the gamma index.

Result: In the Bland-Altman analysis, the gamma index (3 mm / 3%) for FD was favorably agreed with those calculated on the RTPS than those measured with the Matrixx. Mean percentage of points satisfying the constraint was 94% versus 90%. Errors detected with Matrixx in areas where the dose gradient was steep were larger than those detected by the FD. On the other hand, Matrixx was superior to FD in measuring doses in low dose regions.

Conclusion: In conclusion, Matrixx would potentially replace with FD in routine-based IMRT QA, although it holds disadvantage of low-spatial resolution.

POSTER 29. Measurements of low rate neutron fluence using gamma spectrometry. Kinga Polaczek-Greliek, University of Silesia, Medical Physics Department, Poland

The neutron radiation field around radiotherapeutic high-energy linacs is strongly dependent mainly on an accelerator type (beam type, operation energy, material composition of the head construction) and a topology of the facility (room area, wall composition, maze shape and length, position of the control panel). Therefore the data found in literature cannot directly serve in occupational neutron dose assessment in a particular case. The major sources of neutron dose uncertainties are associated with the sensitivity of a method of indirect neutron counting and the poor knowledge of neutron spectra. The purpose of this work is to check whether, and in what extent, gamma ray spectrometry could be applicable in clinical conditions for occupational neutron radiation monitoring, including fluence and dose assessment.

Prompt gamma neutron activation analysis (PGNAA) has been used for determination of photon/neutron fluence outside the treatment room. In situ portable spectrometry system based on semiconductor high purity germanium (HPGe) detector was used. Neutron capture reactions as well as inelastic scattering processes on germanium crystal were observed. Cross sections of these interactions are strong energy-dependent. Therefore the analysis was based on photopeaks count rates selected from registered spectra. Results obtained using
equations of neutron activation analysis have been compared with those received on the base of semiempirical formulae [Skoro et al. (1992); Wordel et al. (1996)]. Application of neutron energy moderators [Chao, Niu (1997)] allowed for roughly estimation of photon/neutron spectra outside the entrance of the high-energy medical accelerator rooms, distinguishing the slow and fast components of neutron flux. Ambient dose equivalent H*(10) 50 cm away from the door, estimated with the use of fluence-to-dose conversion coefficients of ICRP 74 (1996) were found to be 3.7·10⁻³ microSv/MU± 31% for Clnac 2300 20 MV. This is in good agreement with results obtained by others with different methods [Chen et al. (2006); Donadille et al. (2008)]. Obtained results have shown that PGNAA of HPCe with information on the neutron field inside the linac room. Although it appears to be useless in mixed photon/neutron field inside the linac room.

POSI29-16. EURADOS - The European Radiation Dosimetry Group, Werner Ruehm, Helmholtz Center Munich, Germany
W. Ruehm on behalf of European Radiation Dosimetry Group e.V.
EURADOS, the European Radiation Dosimetry Group EURADOS, the European Radiation Dosimetry Group, is a network of more than 50 European institutions and 200 scientists. EURADOS promotes research and development and European cooperation in the field of the dosimetry of ionizing radiation, and maintains a network which includes experts, reference and research laboratories, and dosimetry services.
Areas of activity include individual monitoring for external and internal exposure, retrospective dosimetry, environmental radiation monitoring, diagnostic and interventional radiology, nuclear medicine and radiation therapy, and computational dosimetry. The paper gives a brief overview on the EURADOS network.

POSI29-17. Mini-TEPC for Deep Space Dosimetry, Tore Straume1, L. Braby2, T. Lushby3, T. Borak4, H. Tran4, D. Perez-Nunez2, 1: NASA Ames Research Center, USA, 2: Texas A&M University, USA, 3: Colorado State University, USA
As humans travel beyond the protective shield of the Earth’s magnetosphere for extended periods, there will be a need for compact, low power, active radiation monitors. Such monitors must be capable of measuring the total dose-equivalent rate from galactic cosmic radiation during ambient conditions and also record the dose and issue a warning during the initiation of a high intensity solar particle event (SPE). It would be desirable if the instrument could be multi-use, i.e., be configured to serve as a compact portable radiation monitor inside the spacecraft as well as in a base camp or on a surface rover. We are developing a small tissue-equivalent proportional counter (TEPC) with modern microelectronics to measure dose-rate and quality factor in real time using only about 1 W of power and weighing only about 300 g. The monitor includes a TEPC sensor, a pre-amplifier with charge integrator, and a compact electronics package with power supply and data processor. The charge integrator and the algorithm programmed into the data processor provide the capability to perform real-time variance-covariance calculations using a single detector. For our variance-covariance approach, the output voltage of the charge integrator is proportional to the energy deposited in the detector and the specific energy for successive integrals is obtained by subtracting successive voltage values. The average of the specific energy is proportional to the absorbed dose rate and the variance is proportional to the dose mean lineal energy, which is related to the mean quality factor. This approach provides the dose equivalent without a multichannel analyzer using a single TEPC detector. The innovations involved in this characterization of low rate neutron radiation field. Although it appears to be useless in mixed photon/neutron field inside the linac room.

For neutron detection the PIN diodes were covered with converter materials, where the neutrons produce heavy charged particles such as protons, tritium and a particles detected by the PIN diode. In order to keep the sensitivity for gamma rays as low as possible, a active layer with a thickness of 55 mm was chosen. For thermal and epithermal neutrons the PIN diode was covered by a LiF converter inside a cadmium encapsulation with calibrated aperture, while for fast neutrons (MeV) the PIN diode was covered by a polyethylene-wax converter inside a lead encapsulation. These sensors (PIN diode + converter + encapsulating material) proved to be robust, simple to handle, and produced at reasonable costs. The sensitivity of the sensors for thermal and epithermal neutrons is about 150 counts per cm² and µSv, while that for fast neutrons is about 3 counts per cm² and µSv. Thirteen neutron dosimeter prototypes were built recently, and results of first calibration measurements using an AmBe neutron source are presented.
For radon gas detection, the a particles emitted by radon progeny are detected directly by the PIN diode. Here an active layer of 110 mm was chosen, to ensure a particles with energies up to 10 MeV to be stopped, and a low sensitivity for g rays. The active area of about 5 cm² required for a reasonable detection limit was achieved by connecting 4 PIN diodes (size: 12.4 mm x 12.4 mm) in parallel, resulting in low production costs. The PIN diodes were mounted inside a small aluminium box which also includes detector electronics. The device detects about 1 count per hour in a radon concentration of 30 Bq/m³. At present, about 25 prototypes are available. Examples are shown that demonstrate the reproducibility of the calibration and the use of the instruments to quantify individual radon exposures.

POSI29-20. ESRI dosimetry study for the residents of Kazakhstan exposed to radioactive fallout on 7, August 1962, Kassym Zhumadilov1, A. Ivanov2, D. Zharylganova3, D. Zhumadilov4, S. Toyoda5, M. Hoshi3, 1: Research Institute for Radiation Biology and Medicine, Hiroshima University, Japan, 2: Medical Radiological Research Center, Obninsk, Russian Federation, 3: Astana Medical University, Astana, Kazakhstan, 4: Dental clinic, Kurchatov, East-Kazakhstan, Kazakhstan, 5: Nazarbayev University, Life Sciences Center, Astana, Kazakhstan, 6: Department of Applied Physics Faculty of Science Okayama University of Science, Japan
A new project entitled “Construction of natural radiation exposure study network” commenced in 2009. Nine institutions are now being involved in this project and this project will be finished in March 2012. The aim of the project is to assess the dose for natural radiation exposures using state-of-the-art measurement techniques in four Asian countries (China, India, Korea, and Thailand) and their outcomes will be distributed worldwide. Conventional measurement techniques have been improved and been optimized. Since internal dose assessment is more difficult than the external one, in particular, some new technologies were introduced in this research field. For instance, a discrimination measurement technique of radon(²²²Rn) and thoron(²²⁰Rn) was introduced in some epidemiological area together with a passive thoron progeny measurement technique. Although insole size distribution is still not sufficient so far, a simple but effective device was developed so as to measure the particle size distribution for further understanding on the internal dose assessment.
The present study demonstrates the present status on the on-going project and its tentative results.

Electronic active dosimeters that provide online information on individual dose are important for avoiding unnecessary high radiation exposures. Here we report on recent developments at the Institute of Radiation Protection, Neuherberg, Germany, that aim at the production of small and light instruments for neutron and radon detection. All instruments include silicon-based PIN diodes developed at the Institute of Electron Technology, Warsaw, Poland.
For neutron detection the PIN diodes were covered with converter materials, where the neutrons produce heavy charged particles such as protons, tritium and a particles detected by the PIN diode. In order to keep the sensitivity for gamma rays as low as possible, an active layer with a thickness of 55 mm was chosen. For thermal and epithermal neutrons the PIN diode was covered by a LiF converter inside a cadmium encapsulation with calibrated aperture, while for fast neutrons (MeV) the PIN diode was covered by a polyethylene-wax converter inside a lead encapsulation. These sensors (PIN diode + converter + encapsulating material) proved to be robust, simple to handle, and produced at reasonable costs. The sensitivity of the sensors for thermal and epithermal neutrons is about 150 counts per cm² and µSv, while that for fast neutrons is about 3 counts per cm² and µSv. Thirteen neutron dosimeter prototypes were built recently, and results of first calibration measurements using an AmBe neutron source are presented.
For radon gas detection, the a particles emitted by radon progeny are detected directly by the PIN diode. Here an active layer of 110 mm was chosen, to ensure a particles with energies up to 10 MeV to be stopped, and a low sensitivity for g rays. The active area of about 5 cm² required for a reasonable detection limit was achieved by connecting 4 PIN diodes (size: 12.4 mm x 12.4 mm) in parallel, resulting in low production costs. The PIN diodes were mounted inside a small aluminium box which also includes detector electronics. The device detects about 1 count per hour in a radon concentration of 30 Bq/m³. At present, about 25 prototypes are available. Examples are shown that demonstrate the reproducibility of the calibration and the use of the instruments to quantify individual radon exposures.
In the period from 1949 to 1962, 125 nuclear tests (including 25 near-surface nuclear tests) were conducted at the Ground Zero technical site in the territory of the Semipalatinsk Nuclear Test Site (SNTS), Republic of Kazakhstan. The method of electron spin resonance (ESR) dosimetry has been applied to human tooth enamel to obtain individual absorbed doses of residents of settlements in vicinity of the central axis of radioactive fallout trace from the contaminating surface nuclear test in 7 August 1962. Most of settlements (Kurchatov, Akzharr, Grachi, Semenovka, Begen, Mayaksoe) are locating from 70 to 100 km to the North from epicenter of explosion at the SNTS. This region is basically agricultural region. It was found that the excess doses obtained after subtraction of natural background radiation ranged up to about 100 mGy all for residents in this region. Totally about 60 teeth samples were measured. Average excess doses in the settlements are consistent with estimations based on the official registered data indicating high levels of fallout in the period 1949-1962. Kokpekty settlement was chosen as a control and not subjected to any radioactive contamination and located 400 km to the Southeast from SNTS.

**Thursday**

**POS30 Systems biology**

**POS30-01. Radiation induced genomic instability: mechanistic modelling as a tool for data integration and quantitative analysis.**

Sergey Andreev, Y. Eidelberg, Institute of Biochemical Physics, Russian Academy of Sciences, Russian Federation

Genomic instability (GI) following radiation exposure is recognised as an important step in neoplastic transformation process. Although GI is in a focus of radiation and cancer research, the mechanisms are still obscure, data are often contradictory and their quantitative interpretation is extremely complicated. This is due to, in particular, the use of different experimental models, methods, etc. The aim of this report is to demonstrate an opportunity for integrative GI analysis on the basis of quantitative mechanistic modelling approach.

The modelling includes the Monte Carlo based multilevel simulation of possible cellular processes involved in GI, as to DNA/chromosome breakage and repair, damage transmission through the cell cycle, chromosome bridge-breakage-fusion cycle, etc. The approach presented generalises the recent model of radiation induced chromosomal instability [Radiat. Prot. Dosim. 2011, v.143, p.270].

The modelling approach predicts the wide spectrum of delayed effects of radiation serving as markers of GI, namely dicentrics, nonclonal translocations, chromatid exchanges, fragments. The quantitative modelling demonstrates that the manifestation of various GI endpoints depends on the cell’s choice of DNA DSB repair pathway, the cell-cycle phase at the time of the damage generation, DNA/chromosome breakage into account ensures an advantage in estimating the contribution of different DNA damage response pathways to GI, nonhomologous vs homologous recombination repair mechanisms, the role of DSB at telomeres or interstitial chromosomal sites, etc. The preliminary estimates show that both telomeric and non-telomeric DSB interactions are involved in delayed effects of radiation.

Since the present approach incorporates chromatin/chromosome structure, it opens the new perspectives for evaluation of different pathways involved in development of radiation induced GI, delayed chromosome damage (chromosomal instability) and chromatin modifications/remodelling (epigenetic changes).

**POS30-02. Evaluation of NF-kB signaling in a possible bystander effect scenario.**

Gabriele Bahini, D. Cappelletti, D. Aloni, L. Mariotti, D. Volpi, A. Ottolenghi, 1: University of Pavia, Dipartimento di Fisica Nucleare e Teorica & INFN, Italy, 2: Università di Pavia, Dipartimento di Fisica Nucleare e Teorica & INFN 3: University of Pavia, Dipartimento di Fisica Nucleare, LENA, Italy, 4: Università di Pavia, Dipartimento di Fisica Nucleare e Teorica & INFN, Italy 5: University of Pavia, Dipartimento di Fisica Nucleare, CRUK/MRC, Gray Institute for Radiation Oncology & Biology, University of Oxford, UK

Our understanding of how radiation kills normal and tumor cells has been based on an intimate knowledge of the direct induction of DNA damage and its cellular consequences. What has become clear is that, besides responses to direct DNA damage, effects also on cells not irradiated mediated through gap junctions and inflammatory molecules, known as bystander effect, may have an important role in the response of cells and tissues to radiation exposure[1].

Extensive experiments using ELISA assay, immunocitochemistry and Western Blot have been done on cytokines, Reactive Oxygen Species (ROS) and Nitric Oxide Synthase (NOS) enzymes concentration on cells (AG1252) exposed to low doses of ionizing radiation[2]. In order to look at the precursor mechanisms underpinning the different release of these molecules, the evaluation of nuclear NF-kB activity was performed.

NF-kB family has an important role in a myriad of physiological functions, like inflammatory and environmental stress. It’s activated by IκB Kinase (IKK) in response to extracellular stimuli, being therefore modulated by a wide range of spatiotemporal concentration gradients of signaling molecules[3]. Once activated, it moves into the nucleus to induce the expression of specific target genes, including its own inhibitors, before being rapidly inactivated due to negative feedbacks.

Using the same experimental techniques adopted for signaling proteins evaluation, NF-kB modulation was measured for different conditions such as change of the medium, irradiation with different gamma-rays doses, presence of scavengers of ROS and NOS pathway (i.e. c-PtIO, DMSO), showing a typical response of negative feedback loops. Data were analysed on the basis of the models, reported in literature ([4],[5],[6]), in order to understand how the interaction network leads to radiation induced NF-kB concentration experimentally observed. These studies suggested a key role of IKK activation, and the subsequent NF-kB nuclear import and gene transcription, due to radiation exposure.


This work was partially supported by the European Commission (EC Contract FP7 EURATOM project ‘‘EPIRABIBIO’’ and “DOREMI’’)

**POS30-03. Chromosomal aberrations after G1 or G2 irradiation of HRR or NHEJ deficient mutant cells.**

Joel Bedford, H. Nagasawa, P. Wilson, J. Brogan, T. Wade, J. Little, 1: Colorado State University, USA, 2: Brookhaven National Laboratory, USA, 3: Harvard School of Public Health, USA

We have studied the contributions of homologous recombination repair (HRR) and non-homologous end joining (NHEJ) systems involved in the processing of ionizing radiation damage leading to the formation of chromatid aberrations following exposure of cells in G2 or late S phases of the cell cycle [Nagasawa, et al, Mutation Research 701, 12-22 (2010)] or in G1/G0 (current study). The approach in both cases was to compare aberration yields in Chinese hamster wild-type vs mutant cells deficient in HRR or NHEJ. We found that for cells irradiated in late S or G2, a deficiency in NHEJ led to a large increase in the yield of chromatid deletions but a very minimal or no increase in chromatid exchanges. This would be in accord with the idea that to form exchanges a rejoining would be necessary, so a lack of rejoining would then produce more deletions and less exchanges. For HRR deficient cells irradiated in S or G2 there was an increase in both deletions and exchanges. This also seems to fit with the above idea, because the HRR deficient cells are proficient in NHEJ but lack contributions of a repair system that only operates on a large scale “post-explication”. The result for irradiation of these cell lines in G0/G1 was that for NHEJ deficient cells relative to wild-types there was an increase in both chromosome breaks and exchanges. In absolute terms for the same doses the yield of chromatid breaks per cell was much higher after S or G2 than chromosome breaks after G0/G1 irradiation, likely due to the increased amount of time during progression to mitosis for the latter. The effect here was similar to what had been reported by us and others on previous occasions [Reviewed in J. S. Bedford, W. C. Dewey, Radiat. Res. 158, 251 (2002)]. For the HRR mutant cells irradiated in G0/G1, there was very little increase in either breaks or exchanges in the irs-1 (Xrc2 deficient) or irs-3 (Rad51 deficient) cells, but there was a significant increase in both breaks and exchanges in the irs-1/SF cells. However, the increase in breaks and exchanges in these G0/G1 irradiated irs-1/SF cells which are deficient in Xrc3 (but proficient in NHEJ) is almost entirely due to increased levels of chromatid-type breaks and exchanges. Supported by grant DE-FG02-07ER64350 from the DOE Low Dose Radiation Research Program
Lung cancer is the leading cause of cancer-related mortality worldwide. Radiation therapy is often applied for treating unrespectable lung cancer, whereas the molecular mechanisms of differential radiosensitivity in lung cancer still remains unclear. Recently, it has been demonstrated that microRNAs have pivotal roles in cancer initiation, progression and metastasis. Previous studies showed that two lung adenocarcinoma cell lines (CL-1.0 and CL-1.5) derived from same parental cells had different metastatic ability and radiosensitivity. The surviving fractions of highly aggressive CL-1.5 were eight times lower than that of non-aggressive CL-1.0 after 10 Gy irradiation. Yet, little is known about the underlying mechanism and the dynamic response after radiation. Therefore, in order to explore possible regulatory machineries of lung tumor cells in response to radiation, both CL-1.0 and CL-1.5 were treated with 10 Gy radiation, and were harvested respectively at 0, 1, 4, and 24h after radiation exposure. The genomic profiling of microRNAs following irradiation was examined using Illumina Human microRNA BeadChips. Fold changes and t-tests were applied for selecting radio-responsive microRNAs. The preliminary results showed that 30 microRNAs were identified in response to 10 Gy radiation in CL-1.0 or CL-1.5. The kinetics of microRNA profiling to radiation in CL-1.0 and CL-1.5 were very different. In CL-1.5, most of microRNAs were differentially expressed at 1 or 4h after radiation, whereas more differentially expressed microRNAs were observed at 24h in CL-1.0. Overexpression and knockdown of selected microRNAs (e.g., miR-141, and miR-923) are currently being performed to examine whether radiosensitivity can be regulated by these microRNAs.

Gamma rays was analyzed by Ultra Performance Liquid Chromatography coupled to Time-of-Flight-Mass-Spectrometry (Waters). Multivariate data analysis was performed with the chemometric software SIMCA-P+ (Umetrics) and the machine learning algorithm Random Forests. We identified metabolites that were differentially expressed for LPS or IR exposure, and were common to the two perturbations. These markers were validated through tandem mass spectrometry against pure chemicals. Five metabolites, cytosine, cortisol, adiponectin, O-propanoylcarnitine, and isothionic acid showed increased excretion levels at twenty-four hours after LPS treatment. Of those, cytosine, adiponectin, and O-propanoylcarnitine showed specificity to LPS treatment when compared to IR. On the other hand, increased excretion of cortisol by LPS and IR treatments indicated a rapid systemic response to inflammatory agents, concurrent with the state of the treatments.

Isotonic acid excretion, however, showed elevated levels not only after LPS, but also following a very high dose of 15 Gy. Taken together, these results indicate a strong potential for using urine metabolomics biomarkers to distinguish between inflammatory responses arising from different perturbations and aid in rapid and reliable assessment of medical conditions in a mass casualty radiological scenario and effective triaging. Metabolomics on easily available biofluid samples has the potential to provide rapid identification and distinction between stressors and inflammatory states. In the event of a radiological event, individuals with underlying medical conditions could present with similar symptoms to radiation poisoning, prominently nausea, diarrhoea, vomiting, and fever. The purpose of this study was to compare and contrast the metabolic profiles of two inflammatory agents, lipopolysaccharide (LPS) and ionizing radiation (IR). LPS treatment leads to a severe inflammatory response and a cytokine storm, events similar to radiation exposure, and LPS can mimic many of the responses seen in sepsis. Mouse urine from controls, LPS and irradiated mice with 3, 8, and 15 Gy of
established methods of high dimensional data analysis could not. This work was supported by the NIAID Grant SU19AR067773.

POS30-08. Gene expression analysis in Jurkat cells after exposure to I-123-iododeoxyuridine, γ-rays and α-particles. Marcus Unverricht-Yebboh, S. Boldt, U. Giesen, E. Pomplun, O. Wolkenhauer, R. Kriehuber, 1: Department of Safety and Radiation Protection, Forschungszentrum Jülich, Germany, 2: Department of Computer Science, Systems Biology and Bioinformatics Group, University of Rostock, Germany, 3: Physikalisch-Technische Bundesanstalt (PTB), Braunschweig, Germany

Introduction: In order to develop a gene expression profile-based method for biodosimetry purposes we assessed the human p53-deficient T-lymphoma Jurkat cell line to study whether gene signatures exist allowing the discrimination of radiation quality as well.

Methods: Equi-effect doses, i.e. radiation doses and exposure conditions causing the same biological effect level, were determined with regard to micronucleus formation, γ-HAX foci intensity and apoptosis induction for the radiation qualities of γ-rays (Cs-137) and α-particles (Am-241) as well as for the Auger electron emitter I-123. Prior to the DNA-microarray based gene expression experiments, Jurkat cells were either irradiated with 0.8 and 5 Gy γ-rays, respectively with 0.1 and 0.5 Gy α-particles or were exposed to 4 - 200 kBg γ-rays or 111In-iododeoxyuridine (I-123-UdR) per 106E6 cells. I-123-UdR was incorporated into the DNA of synchronized cells for 20 h. After quantification of the cellular uptake the accumulated decays were calculated and the absorbed radiation dose was assessed after 3 - D geometry analysis of the cells. RNA-isolation was performed always 6 h after exposure. Whole human genome DNA-microarrays (Agilent) were processed and expression profiles were analyzed. Genes showing significant expression changes after irradiation were identified by one-way ANOVA and Tukey-HSD post-hoc testing. The biological functions of significantly regulated genes were further investigated.

Results: Preliminary results of the gene expression analysis after exposure to the three investigated radiation qualities indicate that the expression of more and different genes is significantly altered after exposure to I-123-UdR when compared to γ- and α-irradiation. The functional analysis of significantly changed genes reveals that apoptosis relevant genes are enriched after exposure to I-123-UdR in comparison to γ- and α-irradiation.

Conclusions: I-123-UdR induces pronounced alterations in gene expression when compared to γ-rays and α-particles. Changes in the gene expression of p53-dependent apoptosis-related genes were observed after I-123-UdR exposure suggesting p53-independent back-up pathways for apoptosis signalling in Jurkat cells.

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POS30-09. Ciprofloxacin inhibits radiation combined wound trauma-induced ATP loss by preserving pyruvate dehydrogenase. Joshua Swift, J.T. Smith, J.G. Kiang, Armed Forces Radiobiology Research Institute, USA

Background: Ionizing radiation combined with wound injury (RCI) increases animal mortality more than ionizing radiation alone. Ciprofloxacin (CIP) is a fluoroquinolone, a synthetic antibiotic, found in the national stockpile for emergency use and known to inhibit bacterial sens. The purpose of this study was to evaluate the efficacy of CIP as a countermeasure to RCI mortality and determine the signaling proteins involved in energy machinery.

Methods: B6D2F1/J female mice were randomly assigned to receive either 9.75 Gy Co-60 gamma radiation followed by skin wounding (RCI) or skin procedures (SHAM). Either CIP (90 mg/kg q.d.) or vehicle (VEH: water) were administered orally to these mice starting 1 hr after wounding and thereafter daily for 10 days. Determination of ileum ATP was conducted, and immunoblotting for signaling proteins involved in ATP machinery was conducted.

Results: RCI resulted in 60% survival after 10 days as compared to 100% survival in the SHAM group. Furthermore, RCI caused significant reductions in ileum ATP concentration (~82%) as compared to SHAM. CIP administration after RCI resulted in 100% survival and increased ATP (+5-fold) as compared to RCI. Protein levels of heat shock protein 70 kDa (HSP70), a chaperone protein involved in ATP synthesis and pyruvate dehydrogenase (PDH; an enzyme complex crucial to conversion of pyruvate to acetyl CoA for entrance into TCA cycle) were significantly lower in RCI group (vs. SHAM). Using immunoprecipitation and immunoblotting, HSP70-PDH complex was found to be present in the ileum tissue of RCI mice treated with CIP.

Conclusion: These data suggest that CIP administration following RCI may increase animal survival by maintaining ileum ATP synthesis by preserving PDH. Furthermore, our findings imply that CIP treatment may be a valuable therapeutic treatment for RCI.

POS31 Modulation of radiation damage

POS31-01. γ-Tocopherol-N,N-dimethyglycine ester is a potent radiation mitigator against bone marrow death of mice induced by total body irradiation of X-rays or carbon-beams. Kazunori Anza1, M. Ueno2, K. Matsumoto2, Jiro Takata1, 1: Nihon Pharmaceutical University, Japan, 2: National Institute of Radiological Sciences, Japan, 3: Fukkou University, Japan

γ-tocopherol-N,N-dimethyglycine ester (g-TDMG) is a novel water-soluble vitamin E analog. Since topical application of γ-TDMG protected mice against UV-induced skin damage by preventing inflammation, g-TDMG may also prevent damages induced by ionizing radiations. In the present study, we examined radiation mitigation activity of γ-TDMG against bone marrow death of mice induced by total body irradiation of X-rays or carbon beams. All animal experiments conducted to institutional guidelines and were approved by the Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences. γ-TDMG was suspended in 0.5% methyl cellulose solution. Mice (C3H, 10 weeks, male) were injected intraperitoneally (i.p.) with γ-TDMG after total body X-irradiation at 7.5 Gy or carbon-beam-irradiation at 6.0 Gy. The mitigation activity was evaluated as the 30-day survival rate of mice after the irradiation. γ-TDMG showed potent mitigation activity by post-administration both for X-rays and carbon-beams. For X-rays, the optimal concentration of γ-TDMG was 50–100 mg/kg body weight (bw). The 30-day survival rate of the mice was 98% (n=42) when γ-TDMG (100 mg/kg bw) was i.p. administered immediately after the X-irradiation. When administered at 1 h, 10 h and 24 h post-irradiation, the survival rate was 86, 75, and 40%, respectively. These results showed that it was effective even for the administration at 24 h after X-irradiation. When mice were i.p. injected with γ-TDMG (100 mg/kg bw) immediately after 6.0 Gy of carbon-entuning sunburn cell formation, lipid peroxidation, and beam irradiation (290 MeV, 6 cm SOBP), the survival of mice was significantly increased from 5% to 88% at day 1. The LD50 value was 6.39 Gy, which gives DRF value of 1.15 compared to control. This DRF value was similar to that obtained for X-rays. In conclusion, γ- TDMG, a vitamin E analog, is a potent radiation mitigator, which is very effective even by the administration after exposure to X-rays and carbon-beams. 1) Kobayashi, S., Takagakazu, S., 126, 677-693, 2006.

POS31-02. Screening and identification of ELAVL4 as a modulator of radiation sensitivity. Sangwoo Bae, K.J. Choi, KIRAMS, South Korea

Identification of modulators of radiation sensitivity provides important clues to study cellular responses to ionizing radiation. We combined DNA microarray assay and viability assays to identify modulators of radiation sensitivity in A549 lung cancer cells. Up-regulated genes following irradiation in A549 lung cancer cell line were selected from microarray. Real-time RT-PCR analysis confirmed increased RNA expression levels of the genes such as ELAVL4, TMRPS57, PPE5CA and D1D.

Cell viability assays such as clonogenic assay, MTT and FACs analysis of cell death, identified ELAVL4 gene as a novel modulator of radiation sensitivity. ELAVL4 expression was induced following ionizing irradiation. Depletion of ELAVL4 gene in relation to radiation sensitivity of A549 cells and showed decrease in surviving cell fraction following irradiation in clonogenic assay. Enhanced radiation sensitivity of ELAVL4-depleted cells was attributable to decreased cell proliferation as well as increased apoptotic cell death following irradiation. Thus the function of ELAVL4 gene in relation to radiation sensitivity might be regulation of cell proliferation and death. By contrast, depletion of TMRPS57, another gene that showed higher expression following irradiation, did not affect radiation sensitivity in that colony forming ability of depleted cells showed little difference when compared to 

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control cells. Thus it appears that not all genes that show up-regulation after irradiation serve as modifier of radiation sensitivity. This approach to identification of modifiers of radiation sensitivity has several advantages in terms of functional selectivity, stringency and time. Further analysis of the modifiers such as ELAVL4 should find potential use as radiation biomarkers as well as modulators of cellular radiation responses.

POS31-03. TGFβ inhibition radiosensitizes multiple cancer cell lines in vitro. Mary Helen Barcellos-Hoff, S.F. Bouquet, M.E. Hardee, A.E. Marciscono, S. Du, S.C. Formenti, New York University School of Medicine, USA

Transforming growth factor β (TGFβ) is a pleiotropic cytokine in the tumor microenvironment that can promote malignant behaviors, including invasion and motility. Clinically, TGFβ is often elevated in the plasma of patients with breast and lung cancer and glioblastoma, which are routinely treated with radiation, a known activator of TGFβ. We have shown that inhibiting TGFβ activity or signaling prior to irradiation inhibits the DNA damage response and increases radiosensitivity of non-malignant epithelial cells via blockade of ataxia telangiectasia mutated kinase activity (Cancer Res 66:10861, 2006). If cancer cells are similarly regulated, then TGFβ inhibition could improve the therapeutic effect of radiotherapy. We screened human breast cancer cell lines (MDA-MB-231, MCF7, murine 4T1), human non-small cell lung cancer cell lines (NCI-H1299 and NCI-H292), and glioma cell lines (human U251 and murine GL261) for TGFβ sensitivity. Among the cancer cell lines, only Hs578T and NCI-H292 cells were growth-inhibited by TGFβ (500 pg/ml). Moreover, application of this compound starting on each of the three days related to the injection of LLC cells resulted in the reduced number of pulmonary tumour colonies. In contrast, no such effects were observed in mice given NA. Conclusion: The obtained results suggest that NAc, but not NA, may exert radio-protective, radio-remedial, and anti-metastatic activities. Mechanisms underlying these potential effects should be elaborated in future studies.

POS31-04. No protection of the neurogenic niche with systemic treatment of amifostine during cranial radiation in rat. Malin Bloomstrand T, Björk Eriksson K, Blomgren H, 1: Centre for Brain Repair and Rehabilitation, Sweden; 2: Department of Oncology, University Hospital Lund, Sweden

Radiation to the growing brain causes considerable late toxicity. Children that have been exposed to cranial radiation suffer from cognitive and learning disabilities throughout life. There has long been a debate whether the well-known radioprotector amifostine can protect the brain during radiation. Amifostine penetrates the blood-brain-barrier (BBB) poorly, but delayed CNS toxicity may be caused not only through direct damage to the cells in the CNS, but also damage to endothelial cells and through sustained inflammation. Some animal studies have demonstrated ameliorated tissue damage and improved cognitive function after treatment with amifostine before irradiation.

Our aim was to test amifostine on very young animals, since their brains are the most sensitive to cranial radiation, particularly the stem cell-rich neurogenic regions. Postnatal day 9 male Wistar rats were treated with either 200mg/kg amifostine or saline and exposed to 6 Gy cranial irradiation (IR) (4MV photons). Controls were exposed to sham IR. The animals where sacrificed 2 weeks post IR, a time point when the neurogenic granule cell layer (GCL) of the hippocampus has reached its full size. After IR, the GCL growth is arrested. The results showed that amifostine treatment did not reduce the size of the GCL, even though there was a tendency toward a reduced number of proliferating neurons (Ki67 +) in the GCL volume measured using a modified Nissl stain and common stereological principles.

Results: We found no radioprotection of amifostine on proliferating neural stem and progenitor cells, as judged by GCL volume.

Conclusion: Administration of amifostine in tolerable doses seems not to protect the neurogenic niche from damage during cranial irradiation.

POS31-05. Effect of nicotinamide and nicotinic acid on survival of the sublethally irradiated mice and on the development of induced metastases. Aneta Cheda, E.M. Nowosielska, J. Wrembel-Wargocka 1, J. Gębicki 2, A. Marcinek 2, S. Chłopicki 2, M.K. Janik 1, 1: Military Institute of Hygiene and Epidemiology, Poland; 2: Institute of the Applied Radiation Chemistry, Technical University of Lodz, Poland; 3: Chair of Pharmacology, Jagiellonian University, Poland

Objectives: Instigation and evolution of radiation disorders as well as of primary and secondary tumours are often associated with inflammation and thrombosis. Nicotinamide (NA) and nicotinic acid (NAC) are metabolised in the body to L-methylthionitroamide (MNA) which exerts both anti-inflammatory and anti-thrombotic activities based on its effects on the vascular endothelium. Since NA and NAC possess vitamin properties their usage should not evoke any serious side effects. In view of the above, the aim of the present study was to assess potential radio-protective, radio-remedial, and/or anti-metastatic effects of NA and NAC.

Methods: The 30-day survival of BALB/c mice was assessed after whole body irradiation of the animals with 7.5 Gy γ-rays. The numbers of macroscopic tumour colonies were counted in C57BL/6 mice which were intravenously injected with Lewis Lung Carcinoma (LLC) cells. NA or NAc were given to the animals in drinking water at 100 mg/kg/day during 7 days before, on the day of, or 7 days after the irradiation or the injection of the LLC cells and continued until death of the animals or end of the observation.

Results: The survival of mice from groups in which administration of NA started 7 days before, on the day of, and 7 days after the irradiation was increased. Also, application of this compound starting on each of the three days related to the injection of LLC cells resulted in the reduced number of pulmonary tumour colonies. In contrast, no such effects were observed in mice given NA.

Conclusion: The obtained results suggest that NAc, but not NA, may exert radio-protective, radio-remedial, and anti-metastatic activities. Mechanisms underlying these potential effects should be elaborated in future studies.

POS31-06. Inhibition of macrophages by triptolide mediates mitigation of radiation induced pulmonary fibrosis. Chun Chen, S. Yang, M. Zhang, S.B. Zhang, X. Wang, Y. Guo, Y. Tian, L. Zhang, Y. Cao, P. Okunieff, L. Zhang, University of Florida Shands Cancer Center, USA

Macrophages play a central role in the development of ionizing radiation (IR)-induced pulmonary fibrosis. Our previous work demonstrated that triptolide (TPL) mitigated IR-induced pulmonary fibrosis in C57BL/6 mice, which was related to a decreased number of macrophages in lung tissue. To determine the effect of TPL on macrophages, RAW264.7 cells (a murine macrophage cell line) and primary cultured alveolar macrophages from bronchoalveolar lavage fluid (BALF) of normal mice were used in several assays. 1) Since the migration of macrophages into lung tissue is essential for IR-induced pulmonary fibrosis, we performed the Boyden chamber assay. The results showed that monocytic chemotactic protein-1 (MCP-1)-induced primary alveolar macrophage migration was significantly inhibited by TPL. 2) Since cytokine production plays an important role in the processes of pulmonary fibrosis, we performed the enzyme-linked immunosorbent assay (ELISA) for several cytokines in cultured media. The results showed that TPL significantly down-regulated the levels of tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein-2 (MIP-2), interleukin-6 (IL-6), and transforming growth factor beta 1(TGF-β1) secreted by Raw264.7 cells. 3) Since fibrosis results from the interaction between macrophages and fibroblasts, a co-culture of RAW264.7 cells with NIH3T3 (a murine fibroblast cell line) J1, J2, set up in transwell plates. Raw264.7 cells were seeded into the top culture insert of 12-well plates, and NIH3T3 cells were seeded in the bottom of the wells. This arrangement allowed for the exchange of factors between the 2 types of cells without direct cellular contact. We used the Sirius red method to assess the resulting collagen production. The results showed that TPL inhibited the collagen production of NIH3T3, which could be inhibited by pretreatment of Raw264.7 with TPL. This inhibitory effect on collagen production was related to the inhibition of cytokine production by TPL. We concluded that: A) macrophages induced collagen production of fibroblasts through cytokines and growth factors; B) TPL inhibited the migration of alveolar macrophages being recruited into irradiated lung
tissue; C) TPL decreased collagen production by reducing cytokines and growth factors secreted by macrophages.

POS31-07. Potentiation of acute radiation-induced pneumonitis by depletion of TGFβ is associated with upregulation of IL17 + T cells. Simon Cheng, Columbia University, USA

Our findings implicate an unexpected role of TGFβ in suppressing radiation-induced pneumonitis, which might be mediated by inhibiting inflammatory cytokine IL17 production from gammadelta T cells. This study suggests immunomodulation as a potential therapeutic option for radiation-induced lung injury.


Tempol is a stable piperidine nitroxide that has been shown to function as a reactive oxygen species (ROS) scavenger and a superoxide dismutase (SOD) mimic. Tempol has been researched extensively for both radioprotective and anticancer properties due to these known mechanisms of action. Exposure to radiation can come in the form of radiation therapy for cancer treatment, which is used in greater than 60% of all anticancer therapies, but which poses a risk to normal cells. Additionally, accidental radiation exposure risks from environmental accidents or acts of terrorism have recently garnered much concern, and therapies that could efficiently and effectively mitigate these damages are greatly desired. ROS stress is derived from the mitochondria, it was hypothesized that by targeting tempol directly to the mitochondria, these anticancer and radioprotective properties might be enhanced. Mito-tempol (Mito-T) is a tempol moiety conjugated with a triphenylphosphonium group that serves to efficiently transport Mito-T into the mitochondria. We have examined the anticancer properties of Mito-T using both in vitro cellular based assays as well as in vivo animal studies. We have also investigated whether Mito-T is cardioprotective from chemotherapeutic treatments in animals. Finally, cellular based assays have been used to assay the general radioprotective potential of this compound. We will present results that indicate that Mito-T should be investigated in future in vivo studies for its abilities to mitigate the deleterious effects of both accidental and deliberate exposures to radiation. Future work on Mito-T will focus on determining the utility and mechanism of this compound.

POS31-09. Characterization of a Non-pharmacological Radiation Countermeasure. Joseph Dynlacht, J. Garrett, C. Orschell, M. Mendonca, J. Lopez, H. Chin-Sinex, Indiana University School of Medicine, USA

The detonation of an improvised nuclear device during a radiologic terrorist attack could result in the exposure of thousands of civilians, first responders, or military personnel to lethal or potentially lethal doses of ionizing radiation (IR). Unfortunately, most radiation countermeasures are generally ineffective at mitigating the effects of IR if administered after exposure, or are effective only within a narrow dose range. There are also logistical problems associated with the treatment of mass casualties using existing approaches. Thus, there is an ongoing effort to develop countermeasures that would be effective if administered after irradiation, are non-toxic, and involve easy deployment and use protocols for troops in the field as well as civilian casualties. We have identified a non-pharmacological strategy that is effective at mitigating the lethal effects of IR in a mouse model if administered after exposure. We have shown that the creation of a small, non-debilitating, subcutaneous (SC) wound, if administered shortly after irradiation, is a highly effective strategy for preventing death in ~80-day old female mice that have received an LD50/30 dose of x-rays (6.9 Gy total-body exposure). While only 54% of mice that did not receive a wound survived 30 days after the exposure, 91% of mice that received a SC wound after irradiation survived. Although modulation of the radioresponse by wounding is not entirely without precedent, the data in the literature are somewhat equivocal, and the wounds that must be produced are large and require an accompanying infection in order to effect mitigation of lethal radiation effects. However, mitigation of radiation lethality after SC wounding does not appear to be due to an immunological response to infection. Likewise, an evaluation of hematopoietic damage and regeneration of blood-forming elements as a function of time post-irradiation suggests that the increase in survival in wounded mice cannot be entirely attributed to an enhanced recovery of hematopoietic elements. Rather, our preliminary studies suggest that mitigation of radiation lethality may be related to the specific temporal pattern of changes in the levels of a select group of cytokines.

POS31-10. T-type calcium channel inhibitor mibefradil sensitizes glioblastoma cells to ionizing radiation. Barbara Dziegielewska, N.C.K. Valerie, S. Anaganti, J. Dziegielewski, J.M. Larner, University of Virginia, USA

Glioblastoma multiforme (GBM) is a highly infiltrative high-grade central nervous system glial malignancy, characterized by resistance to both radiation and chemotherapy. T-type calcium channels are a type of voltage-gated calcium channel crucial for normal cell proliferation and differentiation; however, they could be also over-expressed or aberrantly activated in several human cancers, including GBM. We hypothesized that blocking calcium channels with Mibefradil, an FDA-approved selective T-type calcium channel blocker, would sensitize GBM cells to ionizing radiation. Human GBM cell lines U251, U87 and M059K were treated with Mibefradil (0-1 and 10µM-24h), irradiated (0-6 Gy) and analyzed for cell proliferation, cell cycle distribution, DNA damage response, and cell death (clonogenic survival). Our results show that Mibefradil induces cell cycle arrest in a dose- and time-dependent manner in GBM cells expressing T-type calcium channels. Unexpectedly, the arrest occurs not only in G1 phase (as described previously), but also in G2/M phase. In addition to cell cycle arrest, Mibefradil alone at higher concentrations induced significant apoptosis in the most cells significantly affected. While the latter results suggest a potential use of Mibefradil (or related calcium channel inhibitors) as a novel therapeutic approach for the treatment of GBM patients.


Developing beneficial radiation protectors and mitigators in response to potential nuclear accidents and bioterrorist actions is a continuing priority of numerous federal initiatives. The primary focus of the UR Center for Medical Countermeasures Against Radiation (CMCR) is in drug development, specifically investigating agents targeted at late endpoints following irradiation in four critical organs and four tissues: lung, brain, skin, and bone marrow. Central to our goal of evaluating such agents is the implementation of improved methods for quantifying pathophysiological alterations, based on immunohistochemical paraffin sections. The Imaging Core of the UR CMCR has integrated several sophisticated technologies: 1) the Nuance™ multispectral image acquisition system to obtain high-resolution “image cubes” that incorporate optical spectra acquired over wavelengths of 420-720 nm at every pixel of each image. 2) the inForm™ pattern recognition software to combine machine learning with object recognition and analysis tools, and 3) customized ImagePro™ image processing software to automate, batch process, and further process the resultant pseudo-color images. Examples of several indices will be presented encompassing a variety of tissues: 1) In bone marrow, we quantify percentage areas of six tissue categories: bone matrix, adipocytes, red blood cells, myeloid/erythroid tissue, megakaryocytes, and vascular space, as well as megakaryocyte and adipocytes population densities. 2) In skin, we measure dermal and epidermal thickness as well population densities of neutrophils, mast cells, and T-cells within each sublayer. 3) In lung, we quantify resident and recruited macrophage subpopulations in bronchioles versus airways. Each of these methodologies will be presented in greater detail for each of the normal tissues, directly contrasting control and irradiated animals. In summary, this combination of sophisticated techniques provides a precise, nonbiased, and reproducible level of quantification previously unavailable in immunohistochemically stained sections. Supported by NIH/NIAID U19AI091036
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POS31-12. Modulation of radiation-induced damage by tetrahydrobiopterin: Role of endothelial nitric oxide synthase. Sarita Garg, P.G. Biju, W. Wang, K.S. Kumar, M. Hauer-Jensen, Division of Radiation Health, University of Arkansas for Medical Sciences, USA. 2: Armed Forces Radiobiology Research Institute, Uniformed Services University, Bethesda, MD, USA, 3: Division of Radiation Health, University of Arkansas for Medical Sciences, Central Arkansas Veterans Healthcare System, Little Rock, USA

Background: In models of vascular disease, BH4 (tetrahydrobiopterin) supplementation improves endothelial function by maintaining eNOS (endothelial nitric oxide synthase) coupling and NO (nitric oxide) production. Gamma-tocotrienol (GT3), a powerful radioprotector, reduces vascular peroxynitrite production, an effect dependent on inhibition of HMG-CoA reductase and thus likely dependent on modulation of eNOS activity that requires the cofactor BH4. With the current study we aimed to investigate the extent to which eNOS is required for the protective hematopoietic effect of GT3 or BH4 following total body irradiation (TBI).

Methods: Male C57BL/6J (WT) and eNOS knockout (KO) mice were exposed to various doses of TBI using a 17Gy Cs-137 irradiation source. GT3 (200mg/kg body weight) was administered as a single subcutaneous dose 24 hours prior to TBI. Another set of mice were administered twice daily either BH4 or NH4 (tetrahydrodopamine) at a dose of 10mg/kg body weight. Peripheral blood samples were collected 10 days post-TBI (8.5 Gy). Groups of mice were euthanized at 0h, 1d, 7d, and 4d and segments of proximal jejunum, lung, and peripheral blood samples were procured. Lethality, vascular peroxynitrite, peripheral blood counts, protein nitrotyrosine levels, and soluble markers of endothelial dysfunction were measured.

Results: Interestingly, survival studies in eNOS-deficient and control mice revealed that eNOS-/− exhibit decreased radiosensitivity. Protection by GT3 was partly eNOS-dependent. At d1 post-TBI, BH4 and NH4 significantly reduced vascular peroxynitrite production in KO, whereas, GT3 was effective in both WT as well as KO mice. Overall, peroxynitrite production was reduced in KO. Significant increase in circulating PAI-1 was noted at d1 post-TBI, while levels of E-selectin, MMP-9 and VCAM-1 showed reduction by d4 in both KO and WT.

Conclusion: Enhancing BH4 bioavailability reduces post-irradiation vascular oxidative stress. These findings suggest BH4 as a potential therapeutic target in the regulation of eNOS in radiation-induced vascular dysfunction.

POS31-13. AEO1-10150 (C48H56C15MnN12) as a Mitigator of Radiation-Induced Lung Damage in a Rhesus Macaque Model. Michael Garofalo, A.M. Fares, A.A. Ward, C.L. Taylor-Howell, M.V. Cohen, A.M. Gibbs, T.J. MacVittie, University of Maryland School of Medicine, USA

Purpose: To determine whether post-exposure administration of AEO1-10150 (an antioxidant and redox-modulating Mn porphyrin) is capable of mitigating potentially lethal radiation-induced lung injury (RILI) in the nonhuman primate (NHP), Macaca mulatta.

Methods: A total of 13 NHP were exposed (thorax only) to a dose of 11.5 Gy, utilizing an average 2MV photon radiation delivered in anteroposterior/posteroanterior technique as a single fraction prescribed to midplane in the thorax. Following exposure, the experimental NHP (n=7) received subcutaneous injections of AEO1-10150 at 5mg/kg initiated at 24hrs post-exposure and continued once daily for 28 days. Controls (n=6) received no drug. All NHP received supportive care (including dexmethasone) for a planned in-life study of 180 days. The primary endpoint of the study was mortality. Secondary endpoints included respiratory rate (RR), oxygen saturation of peripheral blood (SpO2), and radiographic lung injury as quantified on serial chest radiographs.

Results: All animals developed clinical and radiographic evidence of RILI. Two out of 7 (28.6%) of the AEO1-10150-treated NHP survived (180d), whereas no control NHP survived. All lethality observed in the study was a consequence of RILI. Radiographic analysis demonstrated that on average, the AEO1-10150-treated NHP developed 28% less volume of pneumonic, non-fibrotic fibrosis than did the control NHP. RR and SpO2 data reflected greater RILI in the controls and correlated with radiographic findings. The total dexmethasone support required per animal, per day alive, was less in the AEO1-10150-treated cohort (0.134mg/kg/d) than in the controls (0.200mg/kg/d).

Conclusions: The results of this pilot study suggest that the catalytic antioxidant AEO1-10150 may be effective as a mitigator of potentially fatal RILI following a thoracic midplane exposure of 11.5Gy. Administration of AEOL-10150 resulted in 28.6% survival of the treated cohort following exposure to a dose that was uniformly lethal (LD50) in the controls. Secondary clinical and radiographic endpoints support the efficacy of AEOL-10150 as a mitigator of RILI. The whole-thorax lung irradiation model is a requisite to independently study the delayed pulmonary effects of acute radiation exposure and assess the efficacy of candidate medical countermeasures.

POS31-14. The absence of radiation-induced pulmonary injury mitigation by two redox active agents. Leo Gerweck1, P. Leblanc2, P. Biggs1, M. Pozansky2, K. Held1, 1: Department of Radiation Oncology, Massachusetts General Hospital, USA, 2: Department of Medicine, Massachusetts General Hospital, USA

The potential efficacy of two novel therapeutic agents for mitigation of radiation-induced pulmonary injury was investigated. NOV-002 increases intracellular BH4 and has been reported to reduce PtO2 toxicity. NOV-205 is an anti-inflammatory and anti-fibrosis agent with efficacy in hepatotoxicity studies in rodents and is in clinical trials in hepatitis patients. Both compounds are proprietary formulations of oxidized glutathione.Adult C57BL/6J mice were irradiated in specially designed micro-isolator cages and housed in a barrier facility for the post-irradiation, with all surviving mice being sacrificed. Anesthetized mice were treated with single doses of 0 and 10 to 19 Gy 60Co radiation in 3 Gy increments, with 10-11 mice per unique radiation dose ± drug combination. The superior inferior dimensions of the irradiation field were 17 mm at full width half-max and the 90/50 penumbra was 1.3 mm. Definition of the thorax and field alignment were established and confirmed by radiographic thoracic simulation. Animals were irradiated at a nominal dose rate of 2 Gy/min at 70 cm SAD. Within 4 hours following irradiation, and then thrice weekly until death, mice were subcutaneously injected with drug in saline or saline alone. Endpoints evaluated ± drug were: radiation dose resulting in 50% lethality (LD50), lung weight, heart weight, lung hydroxyproline content and histologically estimated inflated lung air space. Prior to day 415 post-irradiation, neither the rates of death nor the LD50 values significantly differed between mice treated with saline vs. mice receiving NOV-002 or NOV-205. Although significant lung injury was histologically apparent in dying mice, no consistent differences were noted between drug and saline treated groups. For surviving mice sacrificed at day 415, no significant differences were noted between lung weight, lung hydroxyproline, heart weight, or inflated lung airspace in mice receiving drugs vs. saline. The redox active compounds used in this study have been shown to substantially increase intracellular reduced and oxidized glutathione. They are without effect as mitigators of radiation-induced lung injury in this study. This work was supported by NIAID grant # 1RC1 AI081282.

POS31-15. The PPARα agonist fenofibrate prevents fractionated whole-brain irradiation-induced cognitive impairment in young adult male rats. Dana Greene-Schloesser, A. Peiffer, W. Payne, F. Hsu, M. Robbins, Wake Forest Baptist Health, USA

BACKGROUND: Fractionated partial or whole-brain irradiation (WBI) is routinely used for the treatment of primary and metastatic brain cancer. However, more than 50 percent of patients will experience some form of cognitive impairment 6 months to years after WBI. Previously we have shown that administration of the peroxisomal proliferator-activated receptor (PPAR) agonist, fenofibrate, to mice treated with a single dose of 10 Gy WBI prevented the radiation-induced decrease in hippocampal neurogenesis and inhibited microglial activation (Sriram et al Int J Radiat Oncol Biol Phys 2009;75:870–877). However cognition was not assessed in this study. In the current study we independently study the delayed pulmonary effects of acute radiation exposure and assess the efficacy of candidate medical countermeasures.

METHODS AND MATERIALS: Eighty young adult male Fischer 344 x Brown Norway (F344xBN) rats, 12–14 weeks old, received either: (1) WBI: 40 Gy of 6 MeV photons in 4 fractions, (2) sham-irradiation; (3) WBI plus fenofibrate (20 mg/kg soft meal diet) starting 3 days prior, during, and for 28 weeks post-irradiation; and (4) sham-irradiation plus fenofibrate. Non-hippocampal- dependent cognitive function was assessed using the novel object recognition (NOR) task; hippocampal-dependent cognitive function was assessed using the Morris water maze (MWM) task and a
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delayed-match-to-place version of the MWM task, 26 weeks post-irradiation.

RESULTS: FWI led to a significant decline in cognitive performance in the NOR task (p<0.05). Fenofibrate prevented the FWI-induced decrease in cognition (no significant difference between sham groups and FWI+fenofibrate group, p>0.05). There was no significant difference between any of the groups in the standard MWM task at any time point.

CONCLUSIONS: These data provide further support for a role of the PPARα agonist, fenofibrate, in preventing FWI-induced decline in cognitive function. Of interest, no difference was seen in the hippocampal-dependent MWM between any of the sham and irradiated groups; indicating that hippocampal dysfunction may not be the impetus for FWI-induced cognitive decline. Fenofibrate is currently approved for the treatment of hypercholesterolemia and hypertriglyceridemia and is well tolerated in humans, making it an ideal candidate for rapid translation into the clinic as a therapeutic for the prevention of radiation-induced cognitive decline, thus improving the quality of life for brain cancer patients receiving FWI.

POS31-16. Immunohistochemical Studies on the Potential Effects of Phloroglucinol on Radiation-Induced Injury in Small Intestine of C57BL/6 Mice. Danhee Ha1, G. Ahn2, D.S. Kim1, M. Al-Amin1, S.J. Park1, I. Hwang1, Y. Jee1. 1: College of Veterinary Medicine, Jeju National University, South Korea. 2: Faculty of Marine Life Science, Jeju National University, South Korea

Phloroglucinol (PG) is a polyphenol compound isolated from Ecklonia cava, a brown algae abundant in Jeju island, Korea. Previous reports have suggested that PG exerts antioxidative and cytoprotective effects against oxidative stress. In this study, we confirmed that PG protected small intestines from damages caused by ionizing radiation and investigated its protective mechanism in detail. PG (10mg/kg b.w.) was injected intraperitoneally twice into mice, first at 18h and then again at 2h, before irradiation (7Gy). At 8.5 day after gamma ray irradiation, we performed microcolony survival assay on intestinal crypts to investigate the potential effects of PG on the proliferation of stem cells damaged by gamma ray irradiation. In addition, we investigated the expression level of apoptosis-related molecules such as p53, Bax, and Bak in small intestine was down-regulated and that of anti-apoptotic molecules such as Bcl-2 and Bcl-XL was augmented in PG treated group. On the histological observation of small intestine, PG inhibited immunoreactivity of p53, Bax and Bak and accelerated that of Bcl-2 and Bcl-XL. These results demonstrate the protective mechanisms of PG in mice against intestinal damages from ionizing radiation, providing the benefit of raising the apoptosis threshold of jejunal crypt cells. This research was supported by Basic Science Research Program funded by the Ministry of Education, Science and Technology (2011-0006016).

POS31-17. Short term changes in MDA and GSH following irradiation of rat’s lens. Maryam Haddadi1, A. Shirazi1, S. Jakoei2, G. Haddadi2, S.A. Kuhpaye1, M.H. Meshkibaf1. 1: Tehran University of Medical Sciences, Iran. 2: Fasa University of Medical Sciences, Iran

Morbidity of the eye is widely observed in patients receiving total-body irradiation (TBI) prior to bone marrow transplantation or radiotherapy for ocular or head and neck cancers. It is considerable that free radicals are naturally produced by some systems within the body, as well as ocular tissues, and under normal conditions the antioxidant defense system within the body can easily handle free radicals that are produced, but gamma-ray exposure causes the imbalance between free radical production and the antioxidant defense and resulting in oxidative stress conditions. The aim of this study is to investigate the effect of irradiation on lipid peroxidation in rat’s lens. Thirty Albino adult female Sprague-Dawley rats were divided into three groups. A sham radiation was performed on the rats in group I as a control group. Group II received whole cranium 5 Gy of gamma irradiation and group III was exposed as the third group, with dose of 8 Gy. Ten days after irradiation, all rats were sacrificed and their eyes were examined. We measured the levels of chemical parameters i.e. malondialdehyde (MDA) and glutathione (GSH).

The levels of MDA in the lens tissues were found to be significantly higher in irradiation, when compared to control group (p<0.05 for irradiation 5 Gy compared to control group and p<0.002 for irradiation 8 Gy compared to control group). The levels of GSH in the lens tissues significantly decreased after irradiation, when compared to the control group (p<0.05 for irradiation 5 Gy compared to control group and p<0.001 for irradiation 8 Gy compared to the control group).

These results showed that lipid peroxidation is one of the consequences of irradiation in rat’s lens. This change may disturb the function of the lens cells and lead to an increase of light scattering in the lens and formation of cataract. The decrease in lens GSH concentration after irradiation may be explained by the interaction of this antioxidant with free radicals induced through radiation.

POS31-18. The anti-fibrotic activity triggered by the combination of pentoxifylline and vitamin E is mediated by TGF-β1 and miR210 inhibition. Saad Hamama1, S. Delaman1, V. Monceau1, M. Vozenin1, 1: Institut de Cancérologie Gustave Roussy, France. 2: Hôpital Saint-Louis (AP-HP), France

Introduction: Radiation-induced fibrosis is a frequent late complication of radiotherapy. It affects the functions of the organs affected and reduces patient’s life quality. At the molecular level, the role of the TGF-β1/Smad signaling pathway in radiation-induced fibrogenesis is today well recognized. At the clinical level, Pentoxifylline-vitamin E combination has proven its anti-fibrotic activity (Delanian et al, 2003; Hille A et al, 2005). Nevertheless the mechanism of action of this combination is still unclear and constitutes our main objective. We have speculated that this combination could modulate the activity of TGF-β1/Smad signaling pathway. SMAD proteins play a dual role as TGFβ1’s signal transducers and transcription factors. In addition recently, in a model of vascular smooth muscle cells, Davis et al. showed that SMAD proteins control DROSHA-mediated microRNA maturation. When showing that the combination effectively inhibited TGF-β1/Smad signaling pathway we have opted to study the effect of Pentoxifylline-Vit E treatment on microRNAs expression profile.

Materials and Methods: Primary human smooth muscle cells issued from radiation-induced fibrosis and healthy tissues were treated with Trolox, a hydrophilic analogue of Vitamin E, Pentoxifylline or the combination of both of them (10 and 50 µg/ml). Levels of microRNA expression were analyzed by qRT-PCR using specific primers. Results were normalized to the 18S expression. To study the transcription activity of TGF-β1 promoter, cells were transiently transfected with luciferase reporter construct containing TGF-β1 full-length promoter and a random control vector by electroporation. Protein expression of TGF-b target genes was studied by western blot. Finally, microRNAs study was realized using Agilent Human v3 (G4471A) miRNA array according to manufacturer’s protocol.

Results: Combining Pentoxifylline-Trolox reduced the level of miRNA of genes involved in fibrosis and equally known as targets of TGF-β1/Smad signaling pathway such as Collagen Iα1, Fibronectin and PAI-1. The combination reduced the level of mRNA of TGF-β1 itself at earlier time point and studying luciferase activity of TGF-β1 promoter shows that this effect occurs at transcriptional level. In every case, Pentoxifylline-Trolox combination was more effective than individual treatments. Interestingly, only one miRNA was shown to be negatively modulated by Trolox or Pentoxifylline-Trolox treatments: this was miR-210, a well-known hypoxia induced miRNA whereas HIF1a level remained unchanged.

Discussion: Our aim was to decipher the molecular mechanisms involved in the anti-fibrotic action of Pentoxifylline and Trolox combination. The present results show that the combination trigger transcriptional inhibition of TGF-β1 associated with alteration of its signaling pathway and ultimately inhibition of genes know to be involved in the fibrogenic process including ECM-related genes and PAI1. Pentoxifylline and Trolox appear to enhance the activity of each other; and the effect of their combination was more potent than any of the individual treatments even with higher doses. This synergy offers a molecular rational for the clinical use of Pentoxifylline-Vitamin E combination. In addition our study for the first time, miR-210 was shown to be involved in radiation-induced fibrogenic process. Trolox and combination treatments indeed downregulate mir-210, a unique microRNA robustly and ubiquitouslly induced by hypoxia (Kulshreshtha, Ferracin et al. 2007; Chan, Zhang et al. 2009), suggesting that Trolox combination with PTX/Trolox combination induces a normalization of hypoxic zones in fibrotic tissue. Further
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Investigations are ongoing to decipher the role of mir-210 in RIF in relation with hypoxia.

POS31-19. Studies of the effect of a radiation mitigator "NorLeu-3-A (NLE)" gel applied to thermal burns after total body irradiation exposure to 2 Gray. Colin Hill\(^1\), K. Rodgers\(^2\), T. Espinosa\(^1\), N. Roda\(^1\), J. Arangüa\(^1\), C. Meeks\(^1\), G. diZerfa\(^1\), 1: USC Keck School of Medicine, USA, 2: USC School of Pharmacy, USA

Studies were performed using a Guinea-Pig model to ascertain the effect of a renin angiotensin system agonist as a mitigator of the combined effects of local thermal burns and whole body radiation (TBI). 6 to 8 weeks old Guinea Pigs are given 2 Gray TBI of gamma rays from an AEC1 cesium 137 irradiator. One to two hours later with the Guinea-Pig under light anesthesia two, 18 mm diameter partial thickness burns are induced on shaved skin on the back of the animal. Treatments with NLE gel (10 or 100 μg per wound) are begun immediately after the burn or at 1, 2, 3 and 4 days later and continued on an ongoing basis for 14 days or until the animal succumbed. Mortality of the guinea pigs is analyzed by Kaplan-Meier plots with time for 14 days. The animals that survived were sacrificed at day 14 and histological sections made of the area in the thermal wound zone. These were stained with an antibody for Ki67 and the positive cells enumerated in the basal epithelium and hair follicles in both the burn site, and in the edge of the burn zone. The results show that the combination of TBI and two limited size and thickness burns cause considerable mortality in the Guinea Pigs perhaps due to infections (more than 80% of combined exposure control animals died by day 14). Treatment with the gel reduced mortality and improved recovery in the burn site. The best results were found for a treatment with 10 μg/wound given between 1 and 3 days after exposure (less than 40% of the animals treated with gel one day after exposure died by day 14). The Ki67 results showed improved recovery in epithelial cells in the burn site and in the edge of the burn site. The results will be discussed in the context of mitigating combined thermal and radiation injury both from a mechanistic point of view and from the potential use of such a gel after a radiological accident or act of terrorism.

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POS31-20. Survival-modulating effects of meloxicam, a cyclooxygenase-2 inhibitor, administered before or after exposure of mice to lethal doses of ionizing radiation. Michal Hofer, M. Pospšíšil, L. Wetterová, Z. Hoferová, Institute of Biophysics, v.v.i., Academy of Sciences of the Czech Republic, Czech Republic

Cyclooxygenase inhibitors (inhibitors of prostaglandin synthesis, non-steroidal anti-inflammatory drugs /NSAIDs/) have been repeatedly designated to represent a group of interesting modifiers of acute radiation damage because of their ability to stimulate hematopoiesis. However, administration of classical NSAIDs, indomethacin, diclofenac, or ibuprofen, inhibiting non-selectively both cyclooxygenase-1 and cyclooxygenase-2 (COX-2), is accompanied by a high incidence of undesirable side effects on the gastrointestinal tract causing, among others, impaired survival of experimental mice exposed to lethal doses of ionizing radiation. Therefore, selective COX-2 inhibitors, retaining protective operation of prostaglandins in the gastrointestinal tissues, have been synthesized and have partially replaced classical NSAIDs in clinical practice. Recent animal studies have confirmed that meloxicam, a selective COX-2 inhibitor, retards hematopoiesis-stimulating effects of classical NSAIDs in sublethally irradiated mice. The aim of the experiments reported here was to evaluate efficiency of meloxicam in modulating survival of mice exposed to lethal doses of ionizing radiation. It has been found that administration of meloxicam improves survival if the drug is given in a protective regime 1 hour before irradiation or in a therapeutic treatment regime when given within the very early, i.e. 1 hour after irradiation. Administration of meloxicam 24 hour after irradiation has been observed to be ineffective and five daily (1 day to 5 day after irradiation) injections of meloxicam have been found to be deleterious. These results emphasize the timing of treatment with COX-2 inhibitor. Favorable outcomes of treatment with meloxicam close to the irradiation suggest that the drug affects some very early mechanisms of the radiation damage, e.g. apoptosis.

The work was supported by the Grant Agency of the Czech Republic (grants nos. 305/08/0158 and P303/11/0128).

POS31-21. Jeju Water Containing Vanadium Induced Immune Activation on Peripheral Immunocytes of Low Dose Gamma Rays-Irradiated Mice. Insun Hwang\(^1\), D. Ha\(^1\), H. Jo\(^1\), L. Ahn\(^1\), H. Kim\(^1\), J. Won Hyun\(^1\), Y. Je\(^1\), 1: College of Veterinary Medicine, Jeju National University, South Korea, 2: School of Medicine, Jeju National University, South Korea

Vanadium, a dietary micronutrient, has been reported to present interesting biological and pharmacological properties, including superoxide and nitric oxide scavenging effects. Low-dose ionizing radiation (LDR) triggers DNA damage and causes apoptosis of peripheral immunocytes by inducing reactive oxygen species (ROS). Here, we elucidate the capacity of immune activation of Jeju water containing vanadium against immunosuppression caused by LDR. We examined the ROS production, DNA damage, cell apoptosis and proliferation of peripheral immunocytes in irradiated mice drinking Jeju water of different vanadium concentrations for 180 days; V\(_{1}\)(vanadium 0 μg/L, control), V\(_{15}\)(vanadium 15–20 μg/L) and V\(_{20}\)(vanadium 20–25 μg/L). Compared to V\(_{0}\)control, the ROS production was attenuated in peripheral immunocytes of irradiated mice drinking V\(_{1}\) and V\(_{2}\) DNA damage of peripheral immunocytes caused by LDR was significantly increased in mice drinking V\(_{1}\) whereas V\(_{2}\) dramatically induced the reduction of DNA damage. Also, V\(_{1}\) and V\(_{2}\) showed the potency to reduce the number of apoptotic cells whereas irradiated mice drinking V\(_{0}\) exhibited raised number of apoptotic cells. In addition, Jeju water containing vanadium (V\(_{1}\) and V\(_{2}\)) enhanced cell proliferation of peripheral immunocytes, which was suppressed by LDR as shown in V\(_{0}\) control. Jeju water containing vanadium reduced DNA damage and apoptosis and induced the stimulatory potential on immunocytes. These results suggest that Jeju water containing vanadium sustains immune activities under immunosuppression by LDR. This research was supported by the Ministry of Knowledge Economy (MKE), Korea Institute for Advancement of Technology (KIAT) and Jeju Leading Industry Office through the Leading Industry Development for Economic region.

POS31-22. Hypo-CpG methylation controls PTEN expression and cell apoptosis in irradiated lung. Isabel Jackson, X. Zhang, Z. Rabbani, P. Xu, C. Hadley, Z. Vujaskovic, Duke University Medical Center, USA

Introduction: Thoracic exposure to accidental or therapeutic radiation can result in debilitating or life threatening pulmonary injury. The underlying mechanisms associated with radiation-induced lung injury are unclear, however, our recent study has shown PTEN-P13/Akt signaling plays an important role in stimulating parenchymal cell death in lung tissue post-exposure. The purpose of this study was to determine the mechanism by which radiation induces PTEN-mediated apoptosis.

Methods: Female C57BL/6J mice were irradiated with 15 Gy to the whole thorax and euthanized at pre-determined time points of 1 and 3 days, 1, 3, and 6 weeks and 6 months. RNA and genomic DNA were isolated from snap frozen lung tissue and evaluated for Nox4 and PTEN mRNA expression and DNA methylation of the 5’ terminal sequence of the PTEN gene (-809 to +59) using the EZ methylation kit. Paraffin embedded tissue was evaluated for apoptosis (TUNEL), 8-hydroxy-2-deoxyguanosine (8-OHdG), and histopathology. Western blot was performed for Nox4, PTEN, phospho-PTEN, and DNA methyltransferases (Dnmt1, 5, 3, and 3B. In vitro experiments were performed on cultured endothelial (HUVEC) and bronchial epithelial cells (BEAS-2B) for detection of DNA damage (8-OHdG), global methylation of genomic DNA, and Nox4 and PTEN mRNA/protein expression following treatment with H\(_{2}\)O\(_{2}\).

Results: Data show continuous and progressive apoptosis in irradiated lung tissue across all time points. This correlated to an increase in PTEN mRNA and protein levels. The ratio of phospho-PTEN/total PTEN was only slightly decreased suggesting the increase in PTEN protein was the result of increased promoter activity rather than post-translational stabilization. Increased expression of PTEN was associated with hypo-methylation of the PTEN promoter which did not correlate to changes in DNA methyltransferase (Dnmt1, Dnmt3a, Dnmt3b) expression, suggesting another mechanism by which PTEN promoter activity was increased. An increase in Nox4 and 8-OHdG was observed which corresponded to changes in PTEN methylation. HUVEC and epithelial cells treated with H\(_{2}\)O\(_{2}\) show an increase in 8-OHdG, hypomethylation, and an increase in PTEN activity.

Conclusions: In irradiated lung tissue, oxidative DNA damage via Nox4 derived H\(_{2}\)O\(_{2}\) impairs the ability of DNA methyltransferases to cause methylation of cytosine bases in the PTEN promoter. This leads
to persistent upregulation of PTEN, which antagonizes the PI3/Akt signal resulting in cell death in the days, weeks, and months after thoracic irradiation. The linkage between apoptosis and development of radiation-induced pneumonitis and fibrosis will be evaluated in future studies.

POS31-23. Effect of HMGB1 on Radiation-Induced DNA Damage Repair in Human Fibroblast Cells. Yang Jiao, L. Wang, S. Fan, Soochow University, China.

Objective: HMGB1 (high mobility group box 1) is a non-histone nucleoprotein, which is known as an important cytokine mediator of inflammation and is involved in the maintenance of chromatin stability. In this study, we investigated the effects of HMGB1 on the ionizing radiation (IR)-induced DNA damage repair in human fibroblast GM0639 cells. Methods: Human fibroblast GM0639 cells were transfected with HMGB1 siRNA and control siRNA (as a negative control). Clonogenic assay was performed to determine cell survival. Double strand breaks were detected by γ-H2AX in-situ immunohistochemistry staining. The DSB repair efficiency was examined by chromatin assembly assay. Protein expression was detected by Western blot assay. Results: Decreased expression of HMGB1 by HMGB1 siRNA resulted in a decreased sensitivity of GM0639 cells to γ-ray irradiation, the N value in GM0639 cells with Control siRNA and GM0639 cells with HMGB1 siRNA was 2.35 and 3.94, respectively. γ-H2AX foci formation induced by γ-ray irradiation significantly decreased in HMGB1 siRNA cells compared to the control cells. An increased capability of DSB repair was observed in HMGB1 siRNA cells, compared with an up-regulation of the DNA-PKcs, Ku70, Ku80, and XRCC4 protein expression. Conclusion: These studies indicate HMGB1 may be involved in IR-induced DNA DSB repair, associated with the regulation of γ-H2AX foci formation and DNA repair protein expression, making HMGB1 as an attractive therapeutic target for cancer radiotherapy in clinic.

POS31-24. Lasting impairment of Th1-related immune responses by ionizing radiation and its modulation with HemoHIM. Sung-Kee Jo, H. Park, N. Choi, U. Jung, S. Yee, S. Kim1, 1: Korea Atomic Energy Research Institute, South Korea; 2: Sunchon National University, South Korea.

We previously reported that the single or fractionated γ-irradiation induced the imbalance of helper T (Th) 1- and Th2-related immune responses that are associated with many diseases. This study evaluated the possibility of HemoHIM to ameliorate an immunological imbalance that persisted long-term in fractionated-ionizing radiation (IR) exposed mice. The mice were exposed to γ-rays twice a week (0.5 Gy fractions) for a total dose of 5 Gy. The experiments were performed 4 and 6 months after their first exposure. HemoHIM ameliorated the lasting imbalance of Th1- or Th2-related immune responses that showed up in the fractionated IR-exposed mice. Namely, HemoHIM restored the lowered production of interferon (IFN)-γ and immunoglobulin (Ig) G2a, whereas restored the high levels of interleukin (IL)-4, IL-5, IL-13, and IgE in irradiated mice with or without antigen immunization. The NK cell activities as well as the percentages of natural killer (NK) cells in the spleen were greatly decreased in the fractionated IR-exposed mice. HemoHIM administration in the irradiated mice restored the NK cell activities despite not changing the percentages of NK cells. Also, the lasting low levels of IL-12p70 in the irradiated mice were ameliorated by HemoHIM administration. HemoHIM enhanced the phosphorylation of STAT4 that was decreased in the irradiated mice. In conclusion, our findings suggest that HemoHIM, a new herbal composition, ameliorated the lasting impairment of Th1-like immune responses by regulating IL-12/IL-12 receptor-pSTAT4/IFN-γ signaling pathway in irradiated mice and could also be a good recommendation for the alleviation of long-term complications after radiotherapy. [This study was supported by the Nuclear R&D program of MEST(Grant No. 2007-2000091)].


Cellular senescence, a phenomenon in which isolated cells demonstrate a limited ability to divide in a culture is the state or process of aging. Ionizing radiation (IR) is well known to induce cellular premature senescence and cause a decline in the ability to respond to stress, increasing homeostatic imbalance and the risk of disease in vitro and in vivo. In this study, we aimed at searching medicinal herbal extracts that are effective in reducing radiation-induced cellular senescence in BMR-90 human lung fibroblast cells. First, 759 samples of water or ethanol extracts of medicinal plants were tested for inhibitory activities against ionizing radiation-induced senescence by fluorescein di-b-D-galacto-pyranoside(FDG) assay, which is a fluorimetric method for the quantitative measurement of senescence-associated β-galactosidase(SA-β-gal) activity in 96 well plates. Through this screening, 18 samples with high inhibitory activity against SA-β-gal were selected and their effects were further confirmed through X-gal staining method. Next, 18 selected samples were examined for their inhibitory effects on radiation-induced mdDNA common deletion and p21 expression. Taken together, 5 samples including Polygalae Radix and Acanthopanacis Cortex showed the highest inhibitory effects on SA-β-gal activity, mdDNA common deletion, and p21 expression in radiation-induced senescence. Therefore, these results suggest that these selected extracts may be good candidate agents for the inhibition of IR-induced premature senescence. [This study was supported by the Nuclear R&D program of MEST(Grant No. 2007-2000091)].
after irradiation. Antioxidant levels were quantified 6 hours after 4 Gy irradiation by a commercial kit (Northwest Life Science Specialties). Results: Autophagy promoting drugs CBZ, lithium and valproic acid were compared. The latter two did not protect or mitigate irradiation damage in vitro. 10 μM CBZ before irradiation increased the 8 from 5.4 ± 0.9 to 11.1 ± 0.2 (p = 0.0287) in Atg5+/+ MEFs and from 4.6 ± 0.7 to 11.6 ± 2.6 (p = 0.0002) in Atg5-/- MEFs. 10 μM CBZ after irradiation increased the 8 in Atg5+/+ and Atg5-/– MEFs to 8.8 ± 0.2 (p = 0.0119) and 7.5 ± 0.03 (p = 0.0035) respectively. Incubation with 10 μM CBZ decreased a decrease in antioxidant levels independent of irradiation (50.0% decrease after 4 Gy irradiation and 84.0% decrease without irradiation). In contrast, antioxidant levels in Atg5-/- cells were at a lower baseline but were not significantly reduced after incubation with CBZ.

Conclusion: CBZ functions as a radiation dose modifier by an autophagy independent mechanism.

Acknowledgement: This project was supported in part by NIH T32AG21885 and NIAID/NIH Center for Medical Counter Measures (CMCR) Grant 1U19 A168021.

POS31-28. A novel small molecule activator of Nfr2: A promising pharmacological countermeasure to radiation injury. Junghyun Kim1, S. Kumar2, W. Cui3, V. Kumar1, S. Malliotra, T. MacVitte1, R. Thimmulappa1, S. Biswal1, 1: Johns Hopkins University, USA, 2: Greenbaum Cancer Center, University of Maryland, USA, 3: SAIC Frederick, Inc, National Cancer Institute, USA

Background: Exposure to lethal ionizing radiation causes death by damaging hematopoietic and gastrointestinal system. Ionizing radiation induces hematopoietic injury via oxidative stress induced cell death of hematopoietic stem and/or progenitor cells. Nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) is a redox sensitive transcription factor that protects from oxidative stress by upregulating a pleiotropic cytoprotective defense program including antioxidants. Recently, we identified 2-trifluoroethyl-2'-methoxychalcone (TMC) to be a novel potent small activator of Nrf2 pathway. The objective of the current study is two-fold: first, investigate whether Nrf2 is a potential drug candidate to mitigate radiation induced mortality and second, evaluate therapeutic potential of TMC for mitigating ionizing radiation induced mortality.

Methods: C57BL/6 mice (Wild-type and Nrf2-deficient mice) were exposed to total body irradiation (TBI, 7.0Gy (LD30/30) and 7.3Gy (LD70/30)) using an AECL Gamma cell 40 irradiator. To test the therapeutic potential, mice were treated with TMC or vehicle 1h or 24 h after irradiation and thereafter every 4th for 15 days by gavage. Survival and hematopoietic reconstitution were evaluated over 30 days post-irradiation. Nrf2 signaling was assessed by measuring expression of Nrf2 regulated antioxidant genes in small intestine at selected time periods after TBI.

Results: TBI caused early and greater mortality in Nrf2 deficient mice compared to wild-type mice. Nrf2 activity as assessed by expression of Nrf2 regulated antioxidant genes, NADPH:quinone reductase and glutamate-cysteine ligase were significantly reduced in small intestine of mice by 24h and beyond in irradiated mice compared to sham. The administration of TMC 1h or 24h after irradiation significantly improved 30 day survival of mice compared to vehicle: CBC analysis showed a significant recovery of WBC in irradiated mice treated with the TMC.

Conclusion: Nrf2 is critical for survival after irradiation. Activation of Nrf2 by our novel small molecule improves survival after lethal irradiation.

POS31-29. Suppression by X-ray-induced non-homologous end joining of homologous recombination repair can explain radio- sensitization by post-irradiation treatment with a camptothecin derivative. K. Hirasawa1, N. Kumagai1, T. Terada2, S. Shinozara2, T. Takenoto2, Y. Ezuma2, M. Kitano1, 1: Shiga University of Medical Science, Japan, 2: Prefectural University of Hiroshima, Japan

X-irradiation causes DNA double strand breaks (DSBs) in mammalian cells. There are two distinct mechanisms for repair of X-ray-induced DSBs, homologous recombination (HR) and non-homologous end joining (NHEJ). SN38, an active metabolite of irinotecan which is derived from camptothecan has inhibitory effects on DNA topoisoerasernase I and interferes with DNA replication forks to result in DSBs. We report here that sensitivity of Balb/c 3T3 cells to X-rays was enhanced when SN38 was administered for 4 hr after X-irradiation. On the other hand, no radio-sensitization was found in DNA dependent protein kinase- (DNA-PK) deficient SC1K cells. As it has been shown that camptothecin-induced DSBs are repaired by HR, and that DNA-PK is responsible for NHEJ, we concluded that the radio-sensitization by SN38 may be due to changes in a balance between HR and NHEJ, complementary systems for DSB repair.

We examined the amount of DSBs, activities of HR and activities of NHEJ, using histochemical focus formation assay with antibody to g-H2AX, Rad51 and pDNA-PK, respectively. Total DSBs at 4h after X-irradiation ± 1.5 (p = 0.0019) reflected the radio-sensitization by SN38. Administration of X-rays in combination with SN38 caused more DSBs than administration of X-rays solely in Balb/c 3T3 cells. There were no such increases in DSBs in SC1K cells. On the other hand, activities of NHEJ (obtained histochemically by counting the number of foci sensitive to NHEJ) were reduced in SC1K cells to a half of those in Balb/c 3T3 cells, reflecting DNA-PK-deficient characteristic of SC1K cells. On the contrary, there were less activities of HR in Balb/c 3T3 cells than those in SC1K cells. These facts may mean that NHEJ induced by X-rays in Balb/c 3T3 cells suppress HR, hence suppressing recovery from SN38 induced DSBs. On the other hand, in SC1K cells, X-rays may not have induced enough amount of NHEJ to reduce amount of HR, thereby DSBs produced by SN38 could have been repaired efficiently. It is thus possible that X-ray-induced NHEJ suppress HR, a repair mechanism responsible for SN38-induced DSBs, to cause radio- sensitization by post-irradiation treatment with SN38 in Balb/c 3T3 cells.

POS31-30. The effects of antioxidants on radiation-induced chromosomal damage in cancer and normal cells under radiation therapy conditions. Maria Konopacka1, J. Rogoñitski2, K. Słosearek3, Maria Sklodowska-Curie Memorial Institute of Oncology, Branch Glówice, Poland

We studied the modulating effects of vitamin C, vitamin E and ferulic acid on clastogenic activity of ionizing radiation in lung cancer and normal bronchial epithelial cell lines. The study was used a water phantom to model radiotherapy of lung tumour in humans, with cancer cells located in a beam and receiving a 5 Gy dose, and normal cells located outside of the radiation field and receiving 0.2 Gy of scattered radiation. The clastogenic effect of radiation was assessed using the cytokinesis-block micronucleus test. Results indicated that treatment with either ferulic acid or vitamin E reduced micronuclei frequency in normal cells irradiated outside the beam, while of the same time increased micronuclei frequency in directly irradiated cancer ones. The effect of vitamin C was concentration-dependent and did not vary between normal and cancer cells. The results indicate that the use of vitamin E and ferulic acid may augment the efficacy of radiation therapy by enhancing the response of cancer cells to the radiation and simultaneously the substances can protect the normal cells exposed to the low dose of scattered radiation outside the radiation field during radiotherapy.

POS31-31. Investigation of the capability of ursolic acid on tumor radiosensitization and radio-protection of benign cells. Yuan-Hao Lee1, R.D. Glickman2, 1: Radiology Dept., UTHSCSA, USA, 2: Ophthamology Dept., UTHSCSA, USA

Radiation-induced tumor resistance has been known to reduce the efficiency of radiotherapy. Also, radiation can cause malignancy through energy deposition to biomolecules. To ameliorate or prevent the adverse effects of radiation, ursolic acid-stimulated immunomodulation is investigated. In this study, UV-VIS light and/or-irradiation were employed to investigate the efficacy of ursolic acid in enhancing tumor radiosensitivity while conferring radioprotection to benign cells. Two populations of human p53-reactive cells, retinal pigment epithelium (RPE) and skin melanoma cell lines were used, comparitively. On the aspect of parametric tests, radiation-induced DNA damage was demonstrated by Comet assay; cellular oxidative stress, DNA synthesis and apoptosis were assessed with DCFH (2,7'-difchlorofluorescin) oxidation as assessed by HPLC, BrdU (bromodeoxoyuridine) staining and apoptosis assay, respectively. On the aspect of mechanistic experiments, immunoprecipitation and electrophoresis have been conducted for protein expression analysis. Nuclear protein expression in response to UV-VIS irradiation was specifically studied in RPE cells, while the cytoplasmic protein expression in response to dry-ray irradiation was studied in both RPE and skin melanoma cells.
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The insulins of UV-VIS radiation can lead to DNA breakage and cellular apoptosis based on the experimental results using RPE cells. Through BrdU staining and DCFH fluorometry, ursoic acid was found to modulate the cell cycle and inhibit oxidative reactions of RPE cells. Remarkably, UV-VIS induced cell death was prominently facilitated by ursoic acid treatment of skin melanoma cells. The treatment of ursoic acid enormously increased the level of p53 and decreased the level of phosphorylated p65 in skin melanoma cell line. In addition, the inhibition of ursoic acid on NF-κB activation appears to be correlated with the increase of oxidative stress in RPE cells.

In conclusion, we have found differential signaling modulation of ursoic acid on two cell models. Based on the findings of this study, we speculate that ursoic acid exerts radiation-induced genotoxicity on tumor cells while potentiating a radiosensitizing effect on benign cells.

PO31-32. Impact of Alcohol on Radiosensitivity of Cancer Cells. Zhao Lin, Y. Jiao, X. Xu, S. Fan. Soochow University, China

Objective: To investigate the effects of alcohol on radiosensitivity of human cancer cells. Methods:Clonogenic assay was used to determine cell growth; flow cytometry was employed to analyze cell cycle progression; DNA damage and repair were determined by DNA fragmentation assay. Results: Alcohol at ≤100 mmol/L exhibited no significant effects on cell growth in both human breast cancer MCF-7 cells and human cervical cancer HeLa cells. Compared to c-ray alone, the more cell survival was observed in the cells exposed to pre-treatment with alcohol at ≤100 mmol/L and X-ray irradiation in both cell lines. X-ray caused G2/M arrest and DNA damage repair was enhanced by pretreatment with alcohol, accompanied with an increased radiation-induced apoptosis and a decrease of radiation-induced DNA damage.

Conclusion: Our present studies suggest that alcohol at non-cytotoxic doses reduces decrease radiosensitivity of cancer cell by mediating cell cycle arrest, apoptosis, DNA damage and repair.

PO31-33. The lethal dose response relationship and time course of morbidity and mortality in a nonhuman primate (NHP) model of the acute gastrointestinal (GI) sub-syndrome of the acute radiation syndrome (ARS), Thomas MacVittie1, A.M. Fairese2, A. Bennett3, E. McFarland1, G. Tudor4, J. Tudor5, T. Shea-Dohnoue6, W. Jackson III, 1: University of Maryland, School of Medicine, USA, 2: University of Maryland, USA 3: APG, 4: Epistem Ltd., UK

There is no well characterized model of the GI-ARS in the non-human primate (NHP), thens macaque, relevant to the human radiation response and treatment. A GI-ARS model is a prerequisite for the successful development of medical countermeasures (MCM) under the criteria of the U.S. FDA “animal rule”. All NHP (n=69), were successful development of medical countermeasures (MCM) under response and treatment. A GI syndrome of the acute radiation syndrome (ARS) and at necropsy. A subset of NHP post TBI. The lethal dose response relationship and time course of morbidity and mortality in a NHP model of the acute gastrointestinal sub-syndrome of the acute radiation syndrome (ARS) is still unclear and therefore the treatment or prophylaxis of this is unknown. To disclose the mechanism and to establish the treatment strategy, we describe here the possible role of vascular endothelial growth factor (VEGF) in ARS.

Results: In all survival NHP, the NHP (n=69), were exposed to one of eight radiation dose levels ranging between 10.0 Gy to 14.0 Gy. Midline tissue (thorax), bilateral, total-body irradiation (TBI) was delivered with average 2MV LINAC-derived photons at approximately 0.80Gy/min. Following TBI, all NHP received supportive care comprised of fluids, antibiotics, anti-emetics, analgesics, nutrition and whole blood transfusions as per approved clinical trials. Hematopoietic and histological (cort and vili) parameters and plasma-based biomarkers were determined during the 15 day in-life period of the GI-ARS and at necropsy. A subset of NHP were terminated at day 7 (n=4) and day 10 (n=4) and day 15 post-TBI and non-irradiated NHP (n=4) for jejunum crypt and villus analyses. Gross and histological evaluation of both small and large intestine was performed. Mortality within the first 15 days post TBI defined the acute GI-ARS. Mortality consequence to TBI resulted in a dose response relationship defined by an LD30/15, LD50/15 and LD70/15 of 10.8Gy, 11.3Gy and 11.9Gy, respectively. Crypt and villus loss were dose- and time-dependent. The apparent nadir occurred within days 6-7 with noted recovery and crypt proliferation occurring within 15 days post TBI. Severe myelosuppression was evident in all NHP. Febrile neutropenia was noted in 71.2% of the dose cohorts from 10.0 to 12.5 Gy. These infections were administered in 50% of the NHP. Plasma citrulline levels decreased in all cohorts to approximately 25% of normal within 7-9 days post TBI. The GI-ARS model defines the dose response in the rhesus macaque and may be used to evaluate MCM to treat the acute GI-ARS.

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PO31-34. Inhibition of constitutive and radiation-induced NF-κB with DMAPT inhibits cell growth and increases fractionated X-ray induced cell killing of pancreatic cancer cells, Marc S. Mendonca1, H. Chin-Sin2, J. Scheerle3, M. Kikuzure4, R. Shapiro5, P. Crooks2, C.J. Sweeney1, 1: IU School of Medicine, USA, 2: University of Kentucky, USA, 3: Dana Farber Cancer Center, Harvard University, USA

Pancreatic cancer has a survival rate of less than 5% five years after diagnosis. Treatment for pancreatic cancer consists of surgery, chemotherapy, and radiation therapy. Despite this multimodality approach local regional recurrences of pancreatic cancer is common. We hypothesize that we could increase the effectiveness of the X-ray arm of treatment through inhibition of the transcription factor NF-kappaB. We propose that suppressing the activation of NF-kappaB after radiation exposure with DMAPT prevents the repair of DNA double strand breaks which leads to increased cell death in pancreatic cancer cells. In summary, we found that DMAPT is an active chemotherapeutic agent for pancreatic cancer that increases X-ray-induced cell death.

PO31-35. Mechanism and treatment strategy of radiation necrosis in the brain. Shin-Ichi Miyatake, Osaka Medical College, Japan

Purpose: Symptomatic radiation necrosis (RN) is a serious problem in the radiotherapy for brain tumors and head and neck cancers. However, the pathophysiology of this is still unclear and therefore the treatment or prophylaxis of this is unknown. To disclose the mechanism and to establish the treatment strategy, we describe here the possible role of vascular endothelial growth factor (VEGF) in RN in the brain using clinical specimens.

Methods and Materials: 18 surgical specimens of symptomatic RN in the brain were retrospectively reviewed. These cases included different original histological tumor types and were treated with different radiation modalities. Histological analyses were performed using hematoxylin and eosin (H&E) staining, anti-VEGF and anti-hypoxia inducible factor (HIF)-1α immunohistochemistry.

Results: In all surgical specimens, irrespective of original tumor histology and radiation modalities, H&E staining showed marked angiogenesis and reactive astrocytosis at the boundary between the apparent necrotic area and the normal brain. We described this border zone as the ‘peri-necrotic’ area. The most prominent vasculatures in this area consisted of a thin endothelium, mimicking venules, which is identified as telangiectasia. Immunohistochemistry indicated that HIF-1α was predominantly expressed in peri-necrotic area and the large majority of VEGF-expressing cells were the reactive astrocytes intensively distributed in this area.

Conclusions: VEGF produced by the reactive astrocytes localized mainly in the peri-necrotic area might be a major cause of both the angiogenesis and the subsequent peri-tumoral edema typically found in RN of the brain. The benefits of anti-VEGF antibody (bevacizumab) treatment in RN maybe in inhibiting VEGF secreted from the peri-necrotic tissue and that surgery would remove this tissue hence both result in effective reduction of edema associated with RN. Based on these observation, now we are performing multi-centric prospective clinical trial “Intra-venous administration of bevacizumab for the treatment of radiation necrosis in the brain” as Investigational Medical Care System approved by Japanese Government. In the presentation, let me show the drastic effects of bevacizumab on severe RN.

PO31-36. Potential radio-protective, radio-remedial, and anti-neoplastic activities of 1-methylisocitomide, Ewa M. Nowosielska1, A. Cheda1, J. Wrobel-Warczak1, P. Gębic1, S. Chlopicki1, M. K. Janiak1, 1: Military Institute of Hygiene and Epidemiology, Poland, 2: Institute of the Applied Radiation
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Chemistry, Technical University of Lodz, Poland, 3: Chair of Pharmacology, Jagiellonian University, Poland

Objectives: Disorders instigated by absorption of moderate-to-high doses of ionizing radiation as well as development of neoplastic tumours are often associated with primary and secondary thrombosis and inflammation. 1-methylnicotinamide (MINA) – a primary metabolite of nicotinamide – exerts anti-thrombotic and anti-inflammatory effects based on its ability to act on the vascular endothelium, the dysfunction of which contributes to evolution of the above disorders. Since MINA is non-toxic and very well tolerated its usage, even at elevated doses, should not evoke any serious side effects. In view of the above, the aim of the present study was to compare prophylactic, radioprotective, radioameliorative, and anti-metastatic activities exerted by various doses of MINA.

Methods: The 30-day survival of BALB/c mice was assessed after whole body irradiation of the animals with 7.5 Gy γ-rays. The numbers of macroscopic tumour colonies were counted in C57BL/6 mice which were intravenously pre-injected with syngeneic Lewis Lung Carcinoma (LLC) cells. MINA was given to the animals in drinking water at 100, 300 or – for the survival assay only - 500 mg/kg b.w. daily starting 7 days before, on the day of, or 7 days after the irradiation or the injection of the LLC cells and continued until death of the animals or end of the observation.

Results: The survival of mice from groups in which administration of MINA at all the three doses started 7 days before or 7 days after the irradiation was increased. Likewise, MINA applied at 100 and 300 mg/kg b.w. exerted a significant anti-metastatic activity when given 7 days before and 7 days after injection of the tumour cells.

Conclusions: The radionuclide results suggest that MINA applied at 100 and 300 mg/kg b.w. exhibits significant radio-protective, radioameliorative, and anti-metastatic activities. Mechanisms underlying the demonstrated effects of MINA should be elaborated in future studies.

POS31-37. Survival Efficacy of G-CSF and GM-CSF Pegylated using a Unique 40kDa Peg Moiety to Increase Half-Life in a Murine Model of the Hematopoietic Syndrome of the Acute Radiation Syndrome. Christie M. Orschell1, P.A. Plett1, C.H. Sampson1, H.L. Chua1, M. Joshi1, S.D. Kane2, R.S. Nelson3, G.N. Cox1, 1 Indiana University School of Medicine, USA, 2: Bolder Biotech Inc., USA.

The growing threat of radiation exposure from terrorist activities and increasing use of nuclear power underscores the need for countermeasures against ionizing radiation. Hematopoietic growth factors (HGF) are recommended therapy for persons exposed to high dose radiation, but unfavorable administration schedules involving early and repeat dosing limit the logistical ease with which they can be widely used. The current study evaluated two new pegylated (PEG) HGF developed by Bolder Biotechnology, Inc., PEG-G-CSF analog BBT-015 and PEG-GM-CSF analog BBT-007. These HGF mimetics are unique in that their large PEG moiety (40 kDa) imparts a longer half-life and more potent hematopoietic properties than HGF modified with smaller PEGs. To evaluate survival efficacy of these PEG-HGFs in a mouse model of Acute Radiation Syndrome, C57BL/6 mice were exposed to the LD70/30 or LD30/30 dose of radiation (150 Gy, 62Gy/min) and were injected every other day for 9 doses between days 1-17 with either 100ug/kg or 300ug/kg of PEG-G-CSF BBT-015 or murine PEG-GM-CSF BBT-007. Thirty day survival of mice treated with either dose of Peg-G-CSF BBT-015 was significantly increased (70.0% and 62.5%, respectively) compared to vehicle-treated mice (28.2% survival, p<0.001), whereas 30 day survival of mice treated with murine PEG-GM-CSF BBT-007 was only enhanced with the 300ug/kg dose (90.0% vs. 73.7% in controls, p=0.05), but not the 100ug/kg. To determine if changes in neutrophil nadir or recovery correlate with survival, subgroups of mice were bled on day 10 (during nadir), and on days 20, 25 and 30 (in recovery phase). Treatment with either HGF did not affect nadir values, but did effect significant increases in recovery of neutrophils, erythrocytes, and platelets compared with controls (p<0.034). Interestingly, in another study documenting survival efficacy of a single dose of Peg-G-CSF BBT-015 given 24h post-irradiation, recovery of neutrophils at day 30 following irradiation was less than that observed when 9 doses were given. These data demonstrate the potential of Peg-G-CSF BBT-015 and Peg-GM-CSF BBT-007 to serve as effective medical countermeasures against lethal radiation exposure, and begin to illustrate the impact that differing administration schedules of HGF can have on neutrophil recovery.

POS31-38. FTS (Fused Toes Homolog) modulates radiation resistance in uterine cervix cancer cells. Woo-Yoon Park1, A. Anandharaj1, S. Cinghu1, W. Kim1, J. Yu1, 1: Changbuk National University College of Medicine, South Korea, 2: Konkuk University College of Medicine, South Korea

Radiotherapy is the major treatment modality for uterine cervix cancer, but in some cases, the disease is radioresistant. Defining the molecule to radiosensitivity and progression of cancer are of critical importance. Here we evaluated the role of FTS (Fused Toes Homolog) in radiation resistance of cervix cancer. Immunostaining of cervix cancer cells and tissues revealed that FTS localization and expression was changed after radiation. Targeted stable knockdown of FTS in HeLa cells led to the growth inhibition after irradiation. Radiation induced AKT mediated cytoprotective effect was countered by FTS knockdown which leads to PARP cleavage and caspase-3 activation leading to cell death. FTS knockdown promotes radiation induced cell cycle arrest at G0/G1 and apoptosis of HeLa cells with consistent alterations in the display of cell cycle regulatory proteins. This study revealed FTS is involved in radioresistance of cervix cancer. Targeted inhibition of FTS led to the shutdown of key elemental characteristics of cervix cancer and could lead to an effective therapeutic strategy.

POS31-39. Characterization of transgenic Gfrp knock-in mice: implications for BH4 in modulation of radiation response. Rupak Pathak1, S. A. Pawar1, P. Gupta2, Q. Fu3, M. Berbé6, B. Iruji3, S. Garg4, H. Hendrickson5, M. Hauer-Jensen1,2, and K. See Kumar1, 1: Division of Radiation Health, University of Arkansas for Medical Sciences, Little Rock, USA, 2: Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, USA, 3: Armed Forces Radiobiology Research Institute, USUHS, Bethesda, USA, 4: Surgical Service, Central Arkansas Veterans Healthcare System, Little Rock, USA

Background: Tetrahydrobiopterin (BH4) serves as an essential cofactor for all three isoforms of nitric oxide synthase (NOS) as well as for aromatic amino acid hydroxylases. BH4 bio-availability is decreased under oxidative stress conditions, as well as in vascular dysfunction both commonly associated with acute radiation injury. The committing step of BH4 biosynthesis via the de novo pathway, the conversion of GTP to 7,8-dihydroyoquinine triphosphate, is catalyzed by GTP cyclohydroxylase I (GTPCH-I). GTPCH feedback regulatory protein (GFRP) inhibits GTPCH-I activity via the end-product of the de novo pathway, BH4, in a negative feedback fashion. We have previously shown that BH4 levels diminish significantly at day 3.5 after total body irradiation (TBI) and that exogenous administration of BH4 alleviates TBI-induced vascular oxidative stress and peroxynitrite formation. Modulation of the BH4 biosynthetic pathway is thus an attractive target for ameliorating IR-induced injury. In this study, the generation and characterization of Gfrp transgenic founder lines and their response to TBI will be presented.

Methods: The flox-stop vector containing Gfrp cDNA was used to generate the transgenic Gfrp “knock-in” founder lines in a C57BL/6 background. The Gfrp transgene was expressed by crossing the Gfrp positive founders with Ela-cre mice. Gfrp expression in lung tissue was verified by qPCR analysis. BH4 levels in plasma and lung tissue from the Gfrp transgenic lines were measured by HPLC-MS. Thirty day survival was monitored after exposing different lines of Gfrp-transgenic mice as well as control mice to 8.5 Gy TBI.

Results: Difference in Gfrp mRNA and BH4 correlated with survival for the survival assay only -3: Chair of Radiation Health, University of Arkansas for Medical Sciences, South Korea, 4: onions of cervix cancer. Targeted inhibition of FTS led to the shutdown of key elemental characteristics of cervix cancer and could lead to an effective therapeutic strategy.

POS31-40. Erythropoitin mutation significantly improves recovery of the erythroid lineage following sublethal total body irradiation. Scott Peslak1, J. Wenger1, J. Bemis2, J. Palis1, 1 University of Rochester, USA, 2: Litron Laboratories, USA

The massive steady-state output of the erythron makes the erythroid lineage exquisitely sensitive to clastogenic injury; however, the mechanisms underlying recovery of the erythron following sublethal total body irradiation (TBI) remain poorly defined. We sought to
elucidate these mechanisms to provide insight into potential therapeutic strategies for treatment of anemia in acute radiation syndrome. Following 4 Gy TBI in C57BL/6 mice, functional colony assays were used to study the erythroid progenitor compartment, consisting of immature day 7 erythroid burst-forming units (BFU-E) and more mature day 3 BFU-E and erythroid colony-forming units (CFU-E). Imaging flow cytometry and automated blood analysis were used to quantify erythroid precursors and circulating erythrocytes, respectively. By 2 days following 4 Gy irradiation, greater than 95% of erythroid progenitors and precursors in the marrow were lost. Following this initial injury, erythroid repopulation was centered on specific expansion and maturation of later erythroid progenitors (day 3 BFU-E and CFU-E). Erythropoietin (EPO) is known to be the primary regulator of the erythroid lineage, and day 3 BFU-E and CFU-E form the EPO-responsive compartment of the erythron. As a 13-fold increase in endogenous plasma EPO levels directly preceded the day 3 BFU-E/CFU-E expansion seen at 4-6 days post-radiation, we hypothesized that EPO directly stimulates day 3 BFU-E/CFU-E expansion and subsequent erythroid recovery from radiation injury. Mitigation of irradiated mice with 1000 U/kg EPO at 1 hour following 4 Gy TBI advanced the timing of day 3 BFU-E/CFU-E expansion in the bone marrow by 2-3 days. In addition, the accelerated synchronous wave of recovery following EPO mitigation very closely mirrored the physiological wave of recovery during the endogenous EPO response and more closely correlated the radiation-induced anemia. The in vitro studies, taken together, indicate that EPO mitigation at 1 hour following sublethal 4 Gy TBI significantly improves recovery of the erythron by initiating robust expansion and maturation of day 3 BFU-E/CFU-E. A better understanding of the role of EPO during recovery from radiation damage may ultimately lead to improved clinical therapies to protect and mitigate the hematopoietic system from the effects of acute radiation syndrome.

**POS31-41: Suppression of inflammation by apigenin given to mice after irradiation.** Kanokporn Rithidech, P. Phongpatthanaphong, L. Honikel, S.R. Simon, 1: Pathology Department, Stony Brook University, New York, USA; 2: Department of Applied Radiation and Isotopes, Kasetsart University, Bangkok, Thailand

Inflammation is one of the major detrimental consequences of exposure to radiation. Nuclear factor-kappa B (NF-kB) is a key transcription factor known to play a pivotal role in inflammatory responses. Hence, reducing levels of pro-inflammatory mediators or cytokines by biological countermeasures that target the NF-kB pathway would protect exposed individuals from radiation-induced inflammation. A great deal of work has been done on the development of agents that can protect exposed populations. However, most currently known radioprotectors must be applied prior to irradiation, making them unsuitable for use in unplanned exposure. Consequently, there is an urgent need to find new agents that are more efficient than the currently available compounds and that can be used after exposure to radiation. We determined the efficacy of apigenin (AP, a flavonoid compound) against radiation-induced inflammation in mouse bone-marrow-derived-macrophages (BMDMs) at days 3 and 10 post-irradiation. Various concentrations of AP (0, 10, 20, and 40 mg/kg body weight) were given (by a single intra-peritoneal injection) to mice 3 hr after exposure to 0 or 3 Gy of 137Cs gamma rays. Mice received no radiation and no AP served as sham-controls. Levels of NF-kB activation and the expression of selected NF-kB target pro-inflammatory cytokines (i.e. Interleukin 1-beta (IL-1 beta), IL-6, and tumor necrosis factor-alpha (TNF-alpha)) were measured in BMDMs. We found that AP (given to mice 3 hr after gamma-irradiation) significantly reduced levels of activated NF-kB and selected pro-inflammatory cytokines (i.e. TNF-alpha, IL-6 and IL-1 beta) in BMDMs even 10 days post-irradiation. Such beneficial effects were AP-concentration dependent. This exceptional anti-inflammatory capability suggests that AP could be highly valuable as a therapeutic agent against inflammation induced by radiation. Further, the findings of such novel mitigative effects of AP in BMDMs provide an impetus for the use of AP in counteracting the injury induced by higher levels of radiation in other tissue/cell types of exposed individuals. It is also important to determine the therapeutic efficiency of AP with different intervals between radiation exposure and AP treatment. Research funded by School of Medicine, Stony Brook University, Stony Brook, New York.

**POS31-42: Single Injection of Maxy-G34 is as Effective as 2 or 3 Injections to Increase 30-Day Survival of Lethally-Irradiated Mice?** Carol Sampson, 1, A. Plett, 1, H.L. Chua, 1, M. Joshi, 1, G. Yonehoro, 1, B. Devens, 1, K. Lenden, 1, B. Katz, 1, T.J. MacVittie, 1, A.M. Plett, 2, C.M. Orschell, 1, 1: Indiana University School of Medicine, USA; 2: Maxyx Technologies, LLC.

Radiation-induced neutropenia and the ensuing risk of life-threatening opportunistic infection are major concerns driving the development of effective medical countermeasures (MCM) to prevent lethality from lethal radiation exposure. Filgrastim (Neupogen®, Amgen, Inc., Thousand Oaks, CA) has demonstrated efficacy to improve neutrophil recovery and increase survival in mice and non-human primate exposed to lethal radiation doses resulting in the hematopoietic sub-syndrome of the acute radiation syndrome (H-ARS) [Farese et al, Rad. Res. Soc. (meeting abstract), 2009]. However, prolonged daily injection of filgrastim will be challenging in a mass-casualty emergency scenario warranting the development of an alternative MCM for H-ARS with an improved administration schedule. Maxy-G34 (Isotopes, Kasetsart University, Bangkok, Thailand), a pegylated G-CSF compound designed specifically to reduce clearance and extend half-life, has been evaluated for survival efficacy in a murine model of lethal H-ARS. Initial studies demonstrated that 2 injections of 1mg/kg Maxy-G34 given subcutaneously (SC) on days 1 and 7 post lethal radiation exposure (137Cs, 60 Gy/min, 796Gy/Gy) were effective as 3 injections given on days 1, 7, and 14 to increase 30-day survival [Plett et al, Rad. Res. Soc. (meeting abstract), 2008]. We now report that a single injection of 1mg/kg Maxy-G34, delivered SC 24hr post-exposure, resulted in 100% survival of mice exposed to 796Gy of gamma irradiation (p<0.005 when compared to survival of vehicle-treated mice, 73.3%). Mice were provided acid water and wet feed in petri dishes in filter-top cages and were not administered antibiotics. These data illustrate the potential of a single injection of Maxy-G34 as an effective MCM against H-ARS and warrants further development for use in the event of radiological device detonation or nuclear accident. [Funded by NIAID, contract # HS02626020050043C]
diffusible water radicals (indirect effect). Double-strand breaks (dsb) and clustered DNA damages are considered specific effects of ionizing radiation creating deleterious biological consequences. The most important tasks of radiobiology are radioprotection of normal cells and tissues, and radiosensitization of tumors. The comet assay (single-cell gel electrophoresis) is a simple, generally acknowledged method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells. The aim of the study was to analyze the genotoxic effect of micronuclei formation in the presence of four biologically active, low molecular mass compounds: MG132, lactacystine, celastrol and geldanamycin. MG132 and lactacystine are proteasome inhibitors, already applied in cancer therapy. Celastrol is a natural product with antioxidant properties whereas geldanamycin, possesses antibiotic and anti-tumor activity. We measured the levels of blood mononuclear cells after in vitro gamma irradiation with doses ranging from 0.5 to 8 Gy in the presence of different concentrations of each of the compounds and found that each substance in certain concentration reduced the genotoxic effects of ionizing radiation.

POS31-45. Quinic Acid Derivatives as Radiomodulators: Mechanism of Action of KZ-41 and Identification of Potential Derivatives. Karin Thompson1, P.S. Make1a, K. Zeng2, J. Pagadala1, S. Sinclair3, D.D. Miller4, C.R. Yates5, 1: The University of Tennessee HSC, USA 2: Kronos Science Laboratory, USA

Purpose: We have previously reported that the novel quinic acid derivative KZ-41 is a safe and effective radiomodulator eliciting activity by inhibiting activation of Caspase-3 and/or Caspase-7. Our aim was three-fold: 1) Further evaluate the radiomodulating effect and mechanism of KZ-41, 2) Investigate the enhanced radiomodulatory potential of an anti-oxidant linked KZ-41 ester in a high-throughput screen, and 3) Determine if ester lability is related to radiomodifying efficacy.

Methods: Compounds were screened (1 µM) using a previously described U937 cell conditioned medium (CM) transfer experiment. CM was collected 24 hours after irradiation of 7Gy, and transferred onto proliferating cells ± treatments for 24 hours. Cells were washed and analyzed. Western blots were prepared to evaluate total and cleaved Caspase-3 (KZ-41 treated). Caspase-3/7 and Caspase-8 were measured using a luminescent based assay. In vivo 30-day mortality was evaluated in irradiated mice (female, 10-12 weeks, 6.5 Gy, KZ-41 treated). Quinic acid ester (QAE) derivatives were tested as intact molecules and broken at the ester bond. Chemical and enzymatic stabilities were evaluated in buffer and plasma respectively, and quantified via HPLC and/or LC-MS/MS.

Results: Radiation caused a time-dependent increase in cleaved Caspase-3. Treatment with KZ-41 reduced radiation-induced cleaved Caspase-3 in a dose dependent manner (1-100 µM) which is due in part, to inhibition of Caspase-8. KZ-41 (100 mg/kg, SC, +24 hrs) is also a potent radiomodulator in vivo with a 40% survival advantage over vehicle 30 days post lethal total body irradiation (LD100 = 5.5 Gy, 107Cs). QAE derivatives showed a range of efficacy in inhibiting activation of Caspase-3/7. The position of the ester bond affected the anti-apoptotic activity in vitro as well as the chemical and enzymatic lability of the compounds.

Conclusions: Our latest data with KZ-41 confirms a mechanism of action disrupting the caspase-mediated apoptotic pathway upstream of Caspase-3, and demonstrates in vivo efficacy (~40% survival advantage). Quinic acid derivatives provide an attractive scaffold for radiomodulator development, and the addition of antioxidant activity offers the potential for enhanced efficacy. Based on these findings, we hypothesize that the release kinetics of the quinic acid derivative from the antioxidant moieties can be modulated, effectively enhancing radiomodulating activity after a single dose. In vivo pharmacokinetic studies are underway to verify that the stability phenomenon translates to a physiological system.

POS31-46. Administration of histone deacetylase inhibitor 4-Phenylbutyrate ameliorates radiation-induced lung injury. Lei Wang, N. Devipnya, T. Melo, W. Zhao, Wake Forest School of Medicine, USA

Lung cancer is a major health problem worldwide and is one of the leading causes of cancer-related deaths in men and women. About 225,000 new cases of lung cancer are anticipated in 2011, and ~160,000 deaths occur annually in the USA. Although considerable effort has been devoted to developing strategies for modulating radiation-induced lung injury, concerns about tumor protection and toxicity have limited their clinical application. Recent studies indicate that HDAC inhibitors, a class of anti-cancer drugs, have therapeutic benefits for treating inflammatory diseases and fibrotic lesions. In our studies, we have determined the effect of thoracic irradiation on the lungs of FVB/N mice. Survival data showed a dose-dependent increase in morbidity following thoracic irradiation (WTI) with single (11-13 Gy) and fractionated doses (18-36 Gy) of 107Cs g-rays. Histological examination showed a thickening of vessel walls, accumulation of inflammatory cells, collagen deposition in lungs at 4.5 months post irradiation with a single dose of 11 Gy. To test if administration of histone deacetylase (HDAC) inhibitors can prevent radiation-induced lung injury, 8 weeks old female FVB/N mice were divided into 3 groups, i) non-irradiated controls, ii) irradiated with a single dose of 11 Gy WTI, and iii) 11 Gy WTI + 20 mg/kg 4-Phenybutyrate (PBA). PBA was given daily by subcutaneous injection starting 3 days prior to irradiation and continuing for 28 days postirradiation; WTI and sham- WTI control mice received an equal volume of saline injected daily. At 2 to 4.5 months postirradiation, breathing rate was measured using a Mouse-OX device. WTI led to a significant and persistent increase in breathing rate starting from 3 to 4.5 months postirradiation. Administration of PBA effectively ameliorated radiation-induced increase in breathing rate. PBA treatment also decreased the hydroxyproline levels in the lungs of irradiated mice when compared to non-irradiated control, determined at 4.5 month postirradiation. Our data suggest that HDAC inhibitors can prevent/ameliorate late radiation-induced lung injury.

POS31-47. 1,4-Dimethylpyridine and 1-methyl-3-acetylpyridine as potential radio-protective, radio-remedial, and anti-metastatic agents. G. Chetta1, E.M. Nowosadzka2, J. Gębicki3, S. Chlopicki1, A. Marcinek2, M.K. Janiak3, 1: Military Institute of Hygiene and Epidemiology, Poland, 2: Institute of the Applied Radiation Chemistry, Technical University of Lodz, Poland, 3: Chair of Pharmacology, Jagiellonian University, Poland

Objective: Disorders caused by the short-term absorption of more than 1 Gy of ionizing radiation and development of tumor metastases are both associated with primary and secondary inflammation and thrombosis. 1,4-Dimethylpyridine (1,4-DMP), a pyridinium salt formed during combustion, and 1-methyl-3-acetylpyridine (1,3-MA), a synthetic analogue of NAD+, exert both anti-inflammatory and anti-thrombotic properties based on their effects on the vascular endothelium. In view of the above, the aim of the present study was to assess potential radio-protective, radio-remedial, and/or anti-metastatic effects of 1,4-DMP and 1,3-MA.

Methods: The 30-day survival of BALB/c mice was assessed after whole body irradiation of the animals with 7.5 Gy γ-rays. The numbers of macroscopic tumour colonies were counted in C57BL/6 mice which were intravenously injected with Lewis Lung Carcinoma (LLC) cells. 1,4-DMP or 1,3-MA were given to the animals in drinking water at 100 mg/kg/day, daily starting 7 days before, on the day of, or 7 days after the irradiation or the injection of the LLC cells and continued until death of the animals or end of the observation.

Results: Administration of 1,4-DMP significantly increased the survival of mice from all the three tested groups, but the effect was most pronounced when the administration started on the day of the irradiation, whereas application of 1,3-MA significantly increased the survival of mice when the administration started 7 days after the irradiation. In contrast, the anti-neoplastic activity of 1,4-DMP was significantly expressed only when the compound was given 7 days before the injection of LLC cells, whereas similar activity of 1,3-MA was significant in mice from all the three tested groups.

Conclusion: The obtained results suggest that 1,4-DMP exerts radio-protective, radio-remedial, and anti-metastatic activities, whereas 1,3-MA shows only radio-remedial and anti-metastatic activities. Mechanisms underlying the demonstrated effects of 1,4-DMP and 1,3-MA should be elaborated in future studies.

POS31-48. Coming to a Consensus on Selecting the Most Appropriate Mouse Models for Studying Medical Countermeasures against Radiation Injury to the Lung. Zeljko Vujaskovic1, LLC. Jackson1, 1: Duke University Medical Center, USA, 2: Harvard–Massachusetts Institute of Technology Division of Health Sciences and Technology, USA

The development of animal models is a key element in the successful implementation of medical countermeasures (MCMs) directed against radiological threats. In 2002, the FDA amended its regulations (21 CFR 314.600 for drugs and 21 CFR 601.90 for biologics), commonly
referred to as the „Animal Rule,” to permit approval or licensure of MCs in the U.S. based on substantial evidence of effectiveness in animals. Because the inference of efficacy in humans is based on data derived from animals, it is of paramount regulatory importance that radiation injury reasonably reflects the pathogenesis, pathophysiology and delayed effects of radiation exposure as anticipated in a human population. Wide-field radiotherapy and accidental nuclear incidents have already shown that the human lung is very intolerant to radiation and white blood cell inflammatory pneumonitis reaction begins particularly life-threatening at 2 to 6 months when substantial volumes of pulmonary tissue are exposed. A large body of data has now been accumulated employing whole thorax irradiation of mice to reveal a very diverse progression of lethal injury among different genetic strains that encompasses variations in sensitivity and latency, diverse compressive pleural effusions as well as of pulmonary pathology (pneumonitis and fibrosis). In many mouse strains (e.g. BALB/c, A/J and C57BL/6), the effusions can interfere with evaluation of direct lung pathology and negates the usefulness of such mouse strains in meeting the FDA “Animal Rule”. CBA and C3H strains, on the other hand, appear to be adequate models for evaluating radiation pneumonitis, accepting that late pleural effusions prevent long-term evaluation of chronic injuries and may be brought forward in time on raising the radiation dose. Studies on the C57L mouse strain have the advantages by exhibiting salient pneumonitis at lower radiation doses that are closer than predicted for our own species and enable a subsequent monitoring of pulmonary fibrosis without effusions. A major disadvantage is their limited availability thus a compromise may be required that involves prudent use of the C57L strain in essential studies with supporting data coming from either CBA or C3H mice. The usefulness of genetic crosses that retain preferred radiobiological phenotypes from C57L mice remain to be explored.

POS31-49. Order effect of base excision processes to repair clustered DNA damage. Akinori Yokoya1,2, I. Shirahishi1, N. Shikazono1,2,1. Japan Atomic Energy Agency, Japan, 2. Ibaraki University, Japan

In a living cell, a multiply damaged site consisting of SSBS, nucleobase lesions and AP sites is thought to be repaired by several different repair pathways simultaneously or sequentially. Under this situation the final cellular response to the lesion cluster might depend on the order of repair processes because the configuration of the lesions will be modified by the reaction of the initial repair protein, affecting the DNA-linking or lesion-excision activities of the latter repair protein (interference effect of DNA repair processes). For example, a cluster comprised of an AP site or SSBS and base lesions is formed after one of the nucleobase lesions in a nucleobase cluster is excised by a glycosylase protein. Theoretical molecular dynamics simulation study showed that SSBS proximately located 8-oxo-guanine could inhibit the binding activity hOGG1 (Higuchi and Pinak, 2010). In this study, we investigate how the initial enzymatic activities. Nfo recognizes and converts an AP site t

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POS32-01. UV-induced replication fork collapse in DNA polymerase η deficient cells is independent of the MUS81 endonuclease. E. Higuchi1,2, I. Elvers1, F. Johansson1,2, N. Schultz1, A. Wójcik1, T. Helleday1, 1. Department of Genetics, Microbiology and Toxicology, Stockholm University, Sweden, 2. Gray Institute for Radiation Oncology & Biology, University of Oxford, UK

The MUS81 endonuclease was initially identified in response to UV and MMS lesions, and has been implicated in replication fork collapse after exposure to cross-linking agents. After stalling of replication forks by hydroxyurea treatment, the forks collapse independently of MUS81 but the endonuclease is required for replication fork restart. However in cells deficient in the Werner helicase, MUS81 is needed for collapse of replication forks after hydroxyurea treatment, indicating that the endonuclease might play a role in replication fork collapse in cells deficient in helicase activity. UV induced DNA damage that physically block replication fork elongation may be bypassed by translesion synthesis polymerases. Here we have investigated the role of MUS81 after UV irradiation of human fibroblasts deficient in Polh, and restored (wild-type) cells. We show that depletion of MUS81 does not affect survival after UV irradiation. However in Polh deficient cells, MUS81 depletion further lowers the survival after exposure to UV. In spite of this, replication forks collapse in UV irradiated Polh deficient cells independently of MUS81.

POS32-02. The Role of Tight Junction in Electromagnetic Pulse-Induced Blood-Brain-Barrier Opening. Gui-Rong Ding, L. Qiu, Y. Zhou, X. Wang, Y. Li, G. Guo, Fourth Military Medical University, China

Electromagnetic Pulse (EMP) is a short high-voltage pulse with an extremely fast rising time and a broad bandwidth. This kind of signal can be generated by nuclear bomb explosion. EMP signals also exist in certain occupational conditions, for example, Pulse Power Technology Lab, in which the strong electrical field apparatus such as high pressure gas switch and Tesla transformer can generate EMP. The unusual properties of EMP have raised concerns about their biological effects and possible health hazard to humans, especially to some workers or researchers who work with or can be exposed to this kind of electromagnetic field in their working environment. In our previous study we found that brain is one of the sensitive target of EMP, under certain conditions, EMP exposure could increase the permeability of rat Blood-Brain Barrier (BBB). However, the mechanism is unclear. Since Tight Junction ( TJ) between brain endothelial cells plays an important role in maintaining the integrity of the BBB. In this study, we investigated the role of TJ in EMP-induced BBB opening and its mechanism. Adult male SD rats were sham or whole body exposed to EMP at 200 kV/m for 200 or 400 pulses. The permeability of BBB in rat cerebral cortex was examined by using Evans Blue (EB) and lanthanum nitrate as vascular tracers. The localization and expression of TJ proteins (ZO-1, occludin and cytokeratin protein (actin) were assessed by Western Blotting and immunofluorescence analysis, respectively. It was found that comparing to sham group the BBB permeability began to increase at 0.5 h after EMP exposure, and reached the peak at 3h after EMP exposure, then began to recover at 6 h, and finally recovered to sham levels at 24 h after EMP exposure. An electron microscopic (TEM) examination showed that the predominant route of lanthanum extravasation was by the interendothelial pathway. In addition, we found that the expression level of ZO-1 in both cerebral cortex homogenate and cerebral cortex microvessel homogenate was significantly decreased at 1 h and 3 h after EMP exposure. The immunofluorescence assay showed that the staining intensity for ZO-1 appeared to be reduced after EMP exposure, and this is consistent with Western Blotting data. In addition, the alterations in ZO-1 protein localization occurred in cerebral cortex microvessel after EMP exposure. In sham animals, ZO-1 showed a predominant pattern of continuous staining along the margins of cell–cell contact. However, at 3 h after EMP exposure, ZO-1 lost its distribution in continuity and well defined filaments. Immunofluorescence and Western-Blotting analysis documented no significant alterations in the immunoreactivity or the expression of occludin and actin in cerebral cortex of rats exposed to EMP. Therefore, the data obtained from our investigation indicated changes in both ZO-1 expression and the cellular localization were associated with increased BBB permeability after EMP exposure.

It was reported that PKC was associated with BBB permeability as well as BBB TJ proteins translocation under pathological conditions. To determine the role of protein kinase C signaling in EMP-induced BBB permeability change in rats. The protein level of total PKC and two PKC isoforms (PKC-α, and PKC-β II ) were determined in brain cerebral cortex microvessels by Western Blotting after exposing rats to EMP at 200 kV/m for 200 pulses with 1 Hz repetition rate. It was found that the protein level of PKC and PKC-β II (but not PKC-α) in
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cerebral cortex microvessels increased significantly at 0.5 h and 1 h after EMP exposure compared with sham-exposed animals and then recovered at 3 h. A specific PKC antagonist (H7) almost blocked EMP-induced BBB permeability change. EMP-induced BBB tight junction protein ZO-1 translocation was also inhibited. Our data indicated that PKC signaling was involved in EMP-induced BBB permeability change and ZO-1 translocation in rat.

Matrix metalloproteinases (MMPs), in particular gelatinases (MMP-2 and MMP-9), play a key role in degradation of tight junction proteins, are known mediators of BBB compromise. We hypothesized that the EMP-induced BBB disruption contributed to the degradation of ZO-1 by gelatinases. To test this hypothesis, levels of MMP-2, MMP-9 and tissue inhibitor of metalloproteinases (TIMP-1 and TIMP-2) were detected in rat cerebral cortex after exposure rats to EMP at 200 kV/m for 200 pulses. It was found that the protein levels of MMP-2 and MMP-9 significantly increased at 5 h and 0.5 h respectively. However, TIMP-1 (inhibitor of MMP-2) and TIMP-2 (inhibitor of MMP-2) only moderately increased after EMP exposure. In addition, in situ zymography results showed that the gelatinase activity increased in cerebral microvessels at 3 h after EMP exposure. When rats were treated with gelatinase inhibitor (SB-3CT) before EMP exposure, the EMP-induced BBB disruption was attenuated and the ZO-1 degradation was reversed. Our results suggested that gelatinase mediated EMP-induced BBB disruption by degrading ZO-1.

In general, these results indicated that EMP played an important role in EMP-induced BBB opening; changes in both ZO-1 expression and the cellular localization were associated with increased BBB permeability after EMP exposure; PKC signaling was involved in EMP-induced BBB permeability change and ZO-1 translocation in rat; gelatinase mediated EMP-induced BBB disruption by degrading ZO-1.

**POST32-03.** The study of biological action of infrared light on mice and their offspring. Aslu Dyukina, S. Zaichkina, O. Rozanova, S. Romanchenko, G. Apitkea, S. Sorokina, Institute of Theoretical and Experimental Biophysics of RAS, Russian Federation

In the last decade, the phenomenon of adaptive response (AR) has attracted considerable attention of investigators. It is considered as a form of cell defense from mutagenic agents and factors. Therefore the search for adaptogens of physical and chemical nature which are able to transform the organisms to a new adapted state similarly as low doses is an actual problem. Various devices, based on the action of electromagnetic waves of the infrared subspectrum are currently used in clinical practice.

The aim of the present work was to investigate biological action of infrared light (IRL) (850 nm, 22 mW/cm², 101 Hz) on the induction of adaptive response in hemopoietic organs (bone marrow cells, thymus) and Ehrlich carcinoma growth rate on mice and their offspring (F1, F2).

White mongrel SHK male mice were irradiated by IRL at a wavelength of 850 nm, modulated by a frequency 101 Hz (22 mW/cm²). To induce the AR used the standard scheme of irradiation (0.1 GY + 1.5 GY). The level of cytogenetic damage was assessed in bone marrow cells using a micromass test. The influence of the adaptive exposures on the tumor growth was estimated by measuring the size of the tumor at different times after the inoculation of ascitic cells into the femur.

Our study demonstrated that exposure of mice to IRL as well as low doses of X- and γ-rays, induced AR in bone marrow cells obtained by micromass test, remained unchanged thymus weight after irradiation with a challenging dose (1.5 GY) and decreased tumor growth rate.

In mice of the F1 and F2 generations born from males that were irradiated of IRL radiosensitive to influence of a high dose decreased in bone marrow cells and AR was absent. Thymus weight decreased on the thymus right level of mice exposed only to a dose of 1.5 GY. In two generations produced from males that were irradiated of IRL the tumor growth rate did not differ from that of the offspring from unirradiated males.

The obtained experimental data demonstrate the protective effects of infrared light on the immune parents and genomic instability induction on offspring at least two generations.

**POST32-04.** The nucleotide pool sanitization enzyme hMTH1 reduces the level of 8-oxo-dG in the nucleotide pool and protects UVA exposed cells from mutations. Asal Fotouhi, S. Skild2, M. Harms-Ringdal1, A. Shakeri Manesh2, S. Osterman-Golkar1, A. Wójcik, D. Jenssen2, M. Xrysovergis1, N.D. Xrysovergis1

Department of Genetics, Microbiology and Toxicology, Stockholm University, Sweden

The exact mechanisms by which UVA induces mutations are not known. The aim of this project was to investigate the protective ability of the human mutT homolog (hMTH1), a nucleotide pool sanitization enzyme with 8-oxo-dGTPase activity, against the mutagenic effect of UVA. TK6 (human lymphoblastoid cell line) cells were transfected with short hairpin RNA (shRNA) against hMTH1. The Western blot technique was applied to investigate the expression level of hMTH1 in the transfected and non-transfected cells. Clonogenic survival, mutant frequency as well as the extra-cellular and intra-cellular level of 8-oxo-dG and dG was investigated in UVA-irradiated transfected and non-transfected cells. The extra-cellular and intra-cellular 8-oxo-dG levels (after conversion of 8-oxo-dGTP/dGDP/dGMP to 8-oxo-dG) levels were measured using ELISA while intracellular dG (after conversion of dGTP/dGDP/dGMP to dG) levels were measured using HPLC. The results from clonogenic survival show that the transfected cells were slightly more sensitive to UVA exposure as compared to the non-transfected cells. In transfected cells, a significantly increased level of mutations as well as of intracellular 8-oxo-dG was observed in the transfected cell lines when exposed to UVA, while the level of extra-cellular 8-oxo-dG decreased. The results indicate that the nucleotide pool is a significant target for UVA induced mutations and that hMTH1 plays an important role in protecting cells from UVA induced oxidative stress.

**POST32-05.** Short term and long term exposure of mice to wireless detct base or mobile phone radiation affects brain proteome. Adamanita F, Fragopoulou A, Samarak, H. Antonelou1, A. Papadopoulou1, E. Anastasiadou2, D.J. Stravopodis1, G.T. Tsangaris1, L.H. Margarits1, 1: Department of Cell Biology and Biophysics, Faculty of Biology, Athens University, Greece, 2: Genetics and Gene Therapy Center, Center of Basic Research II, Biomedical Research Foundation of the Academy of Athens, Greece, 3: Proteomics Research Unit, Center of Basic Research II, Biomedical Research Foundation of the Academy of Athens, Greece

The extended use of wireless devices has triggered the interest of the scientific community to the investigation of possible health hazards caused by electromagnetic fields (EMFs). The objective of this study was to investigate the effects of two sources of EMFs on the proteome of cerebellum, hippocampus and frontal lobe in Balb/c mice following short term and long term whole body irradiation.

Two series of experiments have been performed:

The first -short term irradiation- comprised of three groups of animals (6 animals/group); the first group (A1) was exposed to a conventional mobile phone, at a SAR level range of 0.17-0.37 W/kg for 3 hours daily for 8 months. The second group (A2) was exposed to a wireless Digital Enhanced Cordless Telephone (DECT) base at a SAR level range of 0.012-0.028 W/kg for 8 hours/day for 8 months. The third group (A3) comprised the sham-exposed animals. The second -short term irradiation- comprised of three groups of animals (8 animals/group); the first group (B1) was exposed to a wireless DECT base for 8hr/day at a SAR level of 0.006 W/kg for 1 day, the second group (B2) was exposed to the same conditions but for 7 days and lastly the B3 group comprised the sham exposed group.

Comparative proteomics has revealed that long term irradiation from both EMF sources altered significantly (p<0.05) the expression (overexpression up to 114 fold or downregulation down to 300fold) of several neural function related proteins, heat shock proteins, cytoskeletal proteins and proteins of the brain metabolism to nearly all brain regions studied. Western blotting on selected proteins confirmed the proteomics data.

The short term irradiation preliminary data showed similar hippocampal related proteome changes, as the long term irradiation, which are under further evaluation.

The observed protein expression changes may be related to brain plasticity alterations and could underlie the symptoms reported so far, such as headaches, sleep disturbance, fatigue, memory deficits and long term brain tumour induction under similar irradiation conditions.

This study was supported by the Special Account for Research Grants of the University of Athens to the Research Group of Professor L.H. Margaritis. AFF is a scholarship recipient by the Hellenic State Scholarship Foundation – “N.D. Xrysovergis” Bequest (PhD fellowship).
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**POS32-06. Effects of electromagnetic pulses on the blood-brain barrier of rats.** Guoqun Guo, the Fourth Military Medical University, China

Aims: To study the bioeffects of Electromagnetic Pulse (EMP) on Blood-Brain-Barrier (BBB). Methods: Animals were exposed to EMP with field intensity 25, 50, 100, 200 and 400 kV/m, and 25, 50, 100 and 200 pulses; duration between every two pulses is 2x. Firstly, Evans blue (EB) and fluorescent microscope were used to observe the changes in the permeability of BBB after exposed to different combinations of the parameters. Secondly, electron microscope was used to investigate the effects of EMP on the ultrastructure of different area in hippocampus and the marginal division of the brain after injection of lanthanum nitrate. Results: The permeability of BBB of rat can be changed after exposed to EMP with a significant dose-effect relationship. With the increasing of field intensity and rising of pulse number, the number and the area of spots increased so as to the intensity of fluorescent colorations. Morphological changes in BBB and nervous tissue after EMP exposure also showed dose-effect relationship. The lanthanum nitrate granules were appeared in the tissue of cortex, hippocampus and hypothalamus in the EMP exposed group. The process of the granules crossing through the tight junction of capillary vessels and the phagocytes of the granules in the endothelium cells were presented. Conclusions: Exposure to EMP can lead to increased permeability of BBB and could damage the endothelium cells of the capillary, resulting in increases of the perivascular space of capillary.

**POS32-07. Biological effect of megahertz ultrasound on human lymphoma U937 cells.** Wakoaka Hiraoka, R. Fuji, Y. Odate, Meiji University, Japan

Megahertz ultrasounds are now attracting attention for its therapeutic potential. Medical treatment by MHz ultrasound is expected for a profound therapeutic effect on an affected area with less injury on normal tissues. However, for detailed biological effect of MHz ultrasound, it has not yet been clarified. Moreover, it is not enough to estimate both biological safety and dose limitation in the clinical use of high-frequency ultrasound. Here we have studied the biological effect of continuous ultrasonic MHz frequencies on cell cultures, and we have further assessed biological safety of MHz ultrasound. For an ultrasound apparatus, piezoceramic transducers of 1.0 MHz (for production of 1 MHz) and 2.4 MHz (for production of 2.4 - 7 MHz) were used and placed in the bottom of water tank made of brass. A glass tube with the diameter of 1 cm containing 2 ml sample solution for irradiation was set on the transducer just above the water level. The intensity of ultrasound for the frequencies ranging from 1 to 7 MHz at the position of placed tube was determined by calorimeter and Kl dosimetry, which was also verified with ultrasonic hydrophone (NH114, Toray Engineering). For the study of biological effect, human lymphoma U937 cells were irradiated and then assayed for membrane damage, reproductive cell death, cell cycle and apoptosis. Membrane damage was determined with propidium iodide (PI) uptake immediately after irradiation. The effect of ultrasound on the rate of cell proliferation, i.e. reproductive cell death, was evaluated using a particle counter 72 hours after irradiation. Apoptotic cell death was examined with annexin V-FITC and PI. Change of cell cycle after irradiation was studied with PI for assayng DNA content by flowcytometry. Each effect on cells was compared at the constant value of ultrasound intensity (0.5 - 2 W/cm²) with 10-seconds exposure in all frequencies. As a result, exposure to 1 - 3.4 MHz produced membrane damage, apoptosis and reproductive cell death. Exposure to 5.7 - 7 MHz at high power brought on membrane damage, apoptosis and the reduction of cell viability. However, low power irradiation with 5.7 - 7 MHz showed not cell death but the delay of cell cycle. These results indicate the safety limit and the benefit of MHz ultrasound on clinical usage.

**POS32-08. No Effect of Extremely Low Frequency Electromagnetic Field on Cell Growth and ROS Production in Human Keratinocytes Cell Line (HaCaT).** Chao-Yin Huang, C. Chung, L. Lin. Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Taiwan

Human exposes to extremely low frequency electromagnetic field (ELF-EMF) from electric appliances. There is growing public concerns on the biological effects, especially the possible cancer risk of exposure to ELF-EMF. Several epidemiological results suggested carcinogenic potential of environmental exposure to ELF-EMF specifically around 50 or 60 Hz. In this study we investigated whether a 60 Hz ELF-EMF can affect growth, viability, cell cycle distribution on Human Keratinocytes Cell Line (HaCaT). Additionally, we examined extents of reactive oxygen species (ROS) potentially influenced by ELF-EMF. HaCaT was exposed to 4-96 h to 1.5 mT uniform 60 Hz ELF-EMF which was generated by a Helmotz coil system. Control cell cultures were placed in the same incubator outside the Helmotz coil system and shielded by mu-metal. Our results revealed no differences of ELF-EMF induced in cell viability, cell cycle distribution, and amount of ROS between exposed and control cells. From results we concluded that exposure for 4-96 h to 1.5 mT at 60 Hz ELF-EMF did not affect growth and oxidative stress of HaCaT cell.

**POS32-09. Electromagnetic pulse inhibits chemotaxis of murine T lymphocytes.** Junye Liu, H. Zhang, Y. Zhou, J. Li, Y. Chen, K. Li, G. Ding, Y. Li, G. Guo, Department of Radiation Medicine, Fourth Military Medical University School of Preventive Medicine, Shaanxi, China

Background and Aim: It has been found in our previous study that electromagnetic pulse (EMP) could increase the lymphocyte number in murine spleen and inhibit proliferation of splenocytes, which suggests EMP induced accumulation of lymphocytes in spleen. This study was aimed to evaluate the effects of EMP on T lymphocyte migration.

Methods: Balb/c mice were exposed to EMP radiation. The in vitro migration directed by chemokines SDF-1 and CCL5 of T lymphocytes from spleen, thymus and lymph nodes was evaluated with transwell assay. The in vivo migration was determined with localized Shwartzman reaction model.

Results: The mice received 200 times of irradiation of 200 kV/m EMP and sacrificed at designated time points. Both in vitro and in vivo migration assays revealed that EMP inhibited migration of splenocytes, thymocytes and lymph-node cells to SDF-1 and CCL5. The inhibitory effects of EMP emerged 2 h after radiation, reached to its peak at 48 h and sustained even 72 h after EMP radiation. In another experiment, the mice were exposed to 200 times of EMP radiation at different field density (25-400 kV/m) and migration was performed at different frequencies of the radiation. It was shown EMP could suppress T lymphocyte migration in a dose-dependent manner with the most profound effect at 50 kV/m.

Conclusion: EMP radiation could inhibit chemotaxis of T lymphocytes in time-dependent and dose-dependent manners, suggesting that EMP could suppress the recruitment of T lymphocytes to inflammation sites and thus influence the adaptive immunity. It will be interesting to further explore the underlying mechanisms.

Keywords: electromagnetic pulse (EMP); chemotaxis; lymphocyte; SDF-1; CCL-5

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**POS32-10. Membrane and biochemical effects of total body exposure of rats to low intensity 900-MHz microwaves.** Margarita Malakyan1, S. Bajinyan1, H. Aghijoyan1, D. Yeghisharayan2, L. Abrahamyan1, L. Pogosyan2, Z. Mirzchyany2, M. Gazaranyan2, L. Nersesova1, J. Akopyan1, 1: Scientific Centre of Radiation Medicine and Burns, Yerevan, Armenia, 2: Institute of Molecular Biology, NAS RA, Armenia

Membrane and biochemical effects of animal total body exposure to low intensity radio-frequency electromagnetic waves (RF-EMW) were studied. Adult white inbred rats were exposed to 900-MHz microwaves produced by X1-42 generator (1-2 mWt output power; 7 μW/cm²/power density). Two schemes of exposure were used: a) single 2-hour exposure; b) fractional exposure during 4 consecutive days for 0.5 hour daily. On days 1 and 5 after the exposure rats were sacrificed. The following indices of blood were analyzed: membrane potential, permeability for K⁺ ions and functional state of Ca²⁺-activated K⁺-channels of erythrocytes; the intensity of lipid peroxidation (LPO) in erythrocytes and blood plasma; activity of creatine kinase (CK), purine nucleoside phosphorylase (PNP), serum basic phosphatase (BP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Indices of intact rats served as Norm.

According to findings, there were no significant perturbations in K⁺ outflow from erythrocytes of animals of both groups. However, on day 1 after single 2-hour exposure the significantly raised activity of Ca²⁺-dependent K⁺-channels was registered. Then, on day 5, as well as
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on days 1 and 5 after fractional exposure the activity of Ca2+-dependent K+-channels was essentially attenuated, perhaps, due to exhaustion of K+ ions in cells. This phenomenon was anticipated to be reflected in the decrease of electronegativity of erythrocyte membrane. Indeed, the reduced membrane potential was registered by us. Both single long-lasting and multiple short-term exposure to RF-EMW facilitated the significant intensification of LPO processes in erythrocytes. However, LPO activation in blood plasma was observed only on day 1 post single 2-hour exposure, while on day 5 LPO activity was fallen almost twice in comparison with Norm. In case of fractional exposure the LPO intensity in blood plasma was drastically low on both days 1 and 5. A sharp reduction of CK activity and a slight increase of BP activity along with non-significant changes in AST and ALT were registered at both schemes of irradiation. The most expressed changes were stimulated by RF-EMW in PNP activity: a fall of the activity on day 1 and a sharp increase on day 5 after 2-hour exposure. Fractional exposure of rats to RF-EMW facilitated the complete suppression of PNP activity.

POS32-11. The role of cytoskeleton filaments polarity in the interaction with external radiofrequency field. Ivan Pavicic, I. Trosic, Institute for Medical Research and Occupational Health, Croatia

The study was carried out on the purpose to get insight into the response of cytoskeleton filaments of different polarity after external radiofrequency (RF) radiation. Polar and non-polar cytoskeleton network filaments were observed after modulated RF radiation. Continuous V-V wave line was exposed to modulated RF radiation frequency of 915 MHz, electric field strength of 10, 20 and 30 V/m. Average specific absorption rate (SAR) was calculated at 0.23, 0.8 and 1.6 W/kg. Cell exposure treatment lasted for 1, 2, and 3 hours. Experimental cell groups were matched with negative- and positive control groups. Exposure set-up consisted Gigahertz Transversal Electromagnetic Mode chamber (GTEM), generator, power amplifier and signal modulator. Rhodamine-phalloidin fluorescent technique was used to mark polar actin microfilament structure. Cytoskeleton vimentin filaments were determined by indirect immunocytochemistry method. Both actin and vimentin structures were inspected within the five hundred cells which were observed under the fluorescent microscope, respectively. In comparison with negative control cell samples, actin microfilaments were significantly altered after three hour of exposure to 30 V/m electric field strength. Otherwise, vimentin filaments did not respond to applied radiation in any other way, i.e., it’s remained unchanged throughout the course of experiment. In conclusion: Contrary to non-polar cytoskeleton vimentin filaments, external modulated RF radiation frequency of 915 MHz might significantly damage the polar actin filament structures.

POS32-12. The influence of static magnetic fields on reactive oxygen species - time as a confounding factor in research. Piotr Politanski, M. Zmyslony, Nofer Institute of Occupational Medicine, Poland

The aim of this study was to investigate the effect of time as an confounder in reactive oxygen species (ROS) research. Authors discuss methodology of research involving influence of weak (0 - 7 mT) static magnetic fields (SMF) on lipid peroxidation in rat lymphocytes in vitro with or without additional stimuli (e.g. X- radiation or FeCl3 addition) with various ways of including time factor into data analysis. Shown results also fully confirm the hypothesis about SMF affecting ROS levels in rat lymphocytes in vitro by radical pairs mechanism. It has been particularly interesting to note, after elimination of time confounder, the effect of SMF in non-stimulated lymphocytes. Such effect has been for some time already obvious in theory, but it is the first time it has been confirmed in an experiment. 


Background: The worldwide dramatic increase in mobile phone use has generated great concerns about the detrimental effects of microwave radiations emitted by these communication devices. Reaction time plays a critical role in performing tasks necessary to avoid hazards. As far as we know, this study is the first survey of the effects of exposure to radiations emitted by a high specific absorption rate mobile phone on human reaction time. It is also the first study in which previous history of mobile phone use is taken in to account. Objective: The aim of the study was to assess both the acute and chronic effects of electromagnetic fields emitted by mobile phones on reaction time in university students. Methods: Visual reaction time (VRT) of young university students was recorded with a simple blind computer-assisted visual reaction time test, before and after a 10 minute real/sham exposure to electromagnetic fields of mobile phones. Participants were 50 right-handed university students aged 18-30. To assess the effect of chronic exposures, the reaction time in sham exposed phases were compared among low level, moderate and frequent users of mobile phones. Results: The mean±SD reaction time after sham exposure and sham exposure were 292.51 ±/-. 55.73 seconds and 294.21 +/- 54.36 seconds, respectively. The age of students did not significantly alter the reaction time but gender significantly affected the reaction time. The mean reaction time in male students was than that of females. Conclusions: The students’ visual reaction time was not affected by exposure to electromagnetic fields emitted by a mobile phone. It can be concluded that these exposures do not cause increased reaction time to different hazards that might lead to higher chances of human errors and fatal accidents.

POS32-14. Permeability of blood-brain barrier in male and female rats under radiofrequency radiation. Bahreir Sirav1, N. Seyham2, 1: Gazi University Faculty of Medicine, Department of Biophysics, Turkey, 2: Gazi University, Turkey

During the last several decades, numerous studies have been performed aiming at the question on whether or not exposure to radiofrequency radiation (RFR) influences the permeability of the blood-brain barrier (BBB). In the present experiment, we investigated the effect of RFR on the permeability of BBB in male and female Wistar albino rats. Right brain, left brain, cerebellum and total brain analyzed separately in the study. Rats were exposed to 0.9-GHz and 1.8-GHz continuous-wave (CW) RFR for 20 min (at SARs of 4.26 mW/kg and 1.46 mW/kg, respectively) while under anesthesia. Control rats were sham-exposed. Disruption of BBB integrity was detected spectrophotometrically using the Evans-blue dye, which has been used as a BBB tracer and is known to be bound to serum albumin. Right brain, left brain, cerebellum and total brain were evaluated for BBB permeability. In female rats; no albumin extravasation was found in in the brain after RFR exposure. A significant increase in albumin was found in the brains of the RF-exposed male rats when compared to sham-exposed male brains. These results suggest that exposure to 0.9-GHz and 1.8-GHz CW RFR at levels below the international limits can affect the vascular permeability in the brain of male rats. The possible risk of RFR exposure in humans is a major concern for the society. Thus, this topic should be investigated more thoroughly in the future. 

POS32-15. Consequences of low-intensity RF radiation on the intracellular macromolecular structures. Ivanca Trosic, I. Pavicic, Institute for Medical Research and Occupational Health, Croatia

The option that radiofrequency (RF) radiation might induce undesired alterations at cellular level is a general scientific and public concern. The reason is extensive and growing development of different radiation resources including equipment for telecommunications and broadcasting systems, domestic devices, and medical apparatus. This study was carried out on rationale to evaluate the biological consequence of low-intensity RF radiation on intracellular macromolecular structures; DNA and cytoskeleton protein network. The study was performed both in vivo and in vitro; in particular the experimental animals and continuous culture of cells were used. In vivo experimental design incorporated exposed and unexposed control rats. Animal groups were divided into the subgroups in order to be sacrificed on 2, 8, 15 and 30 experimental day after irradiation daily treatment of two hours each. Rats were irradiated with 2450 MHz RF waves and an average power density of 7.5 W/kg, SAR=0.2 W/kg. In vitro design included continuous cell culture of lung fibroblasts exposed to a 915 MHz RF field and 10 V/m electric field strength, SAR=0.2 W/kg. Exposure lasted 1 h, 2, h and 3 h. Micronucleus test (MN) on polychromatic erythrocytes (PCE) in vitro was performed. The in vivo and in vitro exposures performed aiming at the question of whether or not exposure to radiofrequency (RF) radiation influences the permeability of the blood-brain barrier (BBB). In the present experiment, we investigated the effect of RFR on the permeability of BBB in male and female Wistar albino rats. Right brain, left brain, cerebellum and total brain analyzed separately in the study. Rats were exposed to 0.9-GHz and 1.8-GHz continuous-wave (CW) RFR for 20 min (at SARs of 4.26 mW/kg and 1.46 mW/kg, respectively) while under anesthesia. Control rats were sham-exposed. Disruption of BBB integrity was detected spectrophotometrically using the Evans-blue dye, which has been used as a BBB tracer and is known to be bound to serum albumin. Right brain, left brain, cerebellum and total brain were evaluated for BBB permeability. In female rats; no albumin extravasation was found in in the brain after RFR exposure. A significant increase in albumin was found in the brains of the RF-exposed male rats when compared to sham-exposed male brains. These results suggest that exposure to 0.9-GHz and 1.8-GHz CW RFR at levels below the international limits can affect the vascular permeability in the brain of male rats. The possible risk of RFR exposure in humans is a major concern for the society. Thus, this topic should be investigated more thoroughly in the future. In vivo experimental design incorporated exposed and unexposed control rats. Animal groups were divided into the subgroups in order to be sacrificed on 2, 8, 15 and 30 experimental day after irradiation daily treatment of two hours each. Rats were irradiated with 2450 MHz RF waves and an average power density of 7.5 W/kg, SAR=0.2 W/kg. In vitro design included continuous cell culture of lung fibroblasts exposed to a 915 MHz RF field and 10 V/m electric field strength, SAR=0.2 W/kg. Exposure lasted 1 h, 2, h and 3 h. Micronucleus test (MN) on polychromatic erythrocytes (PCE) in vitro was performed. The in vivo and in vitro designs were applied on 18-20 university students aged 18-20. The age of students did not significantly alter the reaction time but gender significantly affected the reaction time. The mean reaction time in male students was than that of females. Conclusions: The students’ visual reaction time was not affected by exposure to electromagnetic fields emitted by a mobile phone. It can be concluded that these exposures do not cause increased reaction time to different hazards that might lead to higher chances of human errors and fatal accidents. In vivo experimental design incorporated exposed and unexposed control rats. Animal groups were divided into the subgroups in order to be sacrificed on 2, 8, 15 and 30 experimental day after irradiation daily treatment of two hours each. Rats were irradiated with 2450 MHz RF waves and an average power density of 7.5 W/kg, SAR=0.2 W/kg. Exposure lasted 1 h, 2, h and 3 h. Micronucleus test (MN) on polychromatic erythrocytes (PCE) in vitro was performed. The in vivo and in vitro designs were applied on 18-20 university students aged 18-20. The age of students did not significantly alter the reaction time but gender significantly affected the reaction time. The mean reaction time in male students was than that of females. Conclusions: The students’ visual reaction time was not affected by exposure to electromagnetic fields emitted by a mobile phone. It can be concluded that these exposures do not cause increased reaction time to different hazards that might lead to higher chances of human errors and fatal accidents.
intermediate filaments. Actin proteins were labeled with phallolin- rhodamine complex. One thousand cells per slide were analyzed using a fluorescent light microscope (400 x magn). The MN test showed significantly more PCE+ with micronuclei in rats irradiated for 16 h that is on 8th day of experiment. The in vitro study confirmed the significantly higher occurrence of micronucleated cells as well as damage to polar microtubules and actin filaments, which correlated with the duration of radiation exposure. The intermediate filaments remained intact. In our in vivo and in vitro results confirm the possibility of unfavorable effects of low-intensity, radiofrequency radiation on intracellular macromolecular structures.

PO332-16. Exposure to low level 2.45 GHz microwave radiation causes DNA single strand break and altered the DNA band pattern in testis and ovary of Sprague Dawley rats. Mojisola Usikalu, O. Obembe, M. Akinyemi, 1: Covenant University, Nigeria

The genotoxic effects of 2.45 GHz microwave (MW) radiation on the testis and ovary of Sprague Dawley rats were investigated. The animals were exposed to various level of Specific Absorption Rate (SAR) which were 0 (control), 0.48, 0.95, 1.43, 1.91, 2.39, 2.90, 3.40, 3.80 and 4.30 W/kg respectively using the microwave generator, model ER660E. Serial No MX704CCR from Toshiba UK Ltd for maximum period of ten minutes. The induction of DNA damages was assessed in the testis and ovary of the rats using DNA direct amplification of length polymorphisms (DALP), re-affirmed with single cell gel electrophoresis (SCGE) comet assay for same cells at SAR 2.39 W/kg and histopathological study on the same organs was conducted. The results shown that after exposure to 2.45 GHz radiation the band patterns of the DNA extracted from the animals were distinctly altered in the range of 40 – 120 bp compared with the control and in their tail DNA before exposure, as shown in the densitometry gel analysis. There are statistical significant differences in the Olive moment and % DNA in the tail of the exposed animals compared with control (p < 0.05).

Hyperchromasia was observed in the ovary of the animals exposed to MW radiation. Also, there was reduction in the number of germ cells and cell disorganization observed from the testis of exposed group. The degree of alteration reduction in the number of the germ cells varies with SARs, highest reduction was observed in the group V exposed to 2.39 W/kg which suggest that MW radiation has the potential to affects both male and female fertility adversely.

PO332-17. Addressing a Critical Problem: How Safe is the Use of 3D Wire Panels for Reducing the Magnetic Flux Density in Homes near High Tension Power Lines? Zainat Zatrini, S. Namazi, S. Ensaf, S. Mortazavi, Shiraz University of Medical Sciences, Iran

Background: In Larestan, a city in Fars province in south Iran, due to insufficient land, some houses are being constructed in close vicinity of high tension power lines. AC power lines which employ voltages ranging 66 kilovolts to several hundred thousand kilovolts are the only high voltage transmission lines in cities and have led to great concerns regarding the biological effects of power line induced- high intensity electromagnetic fields. It has been usually accepted that the easiest intervention forreducing the magnetic flux density levels inside the houses.

Methods: A 50x 50x 50 cm model house was built from conventional 3D wire panel. This panel is constructed from a central foamed metallic, mesh screen on the roof and in the walls of the dwellings. Objective: The aim of this study was to assess if the mesh wire routinely used in 3D panels can minimize the magnetic flux density levels inside the houses.

Results: At a distance of 20 m from the powerline, the means+/−SD magnetic flux density levels, outside the model house that was placed at the ground level along the x, y and z axes were 5.77+/− 0.12 , 1.6 +/− 0.1 and 10.67 +/− 0.15mg, respectively. On the other hand, flux density levels, inside the model house along the x, y and z axes were 3.55 +/− 0.07, 1.55 +/− 0.07 and 10.45 +/− 0.21mg, respectively. The overall magnetic field intensity that was measured as the square root of the sum of the squares of magnetic field intensity in x, y and z planes for outside and inside were 12.24 and11.93mg, respectively.

The difference between the magnetic flux density levels outside and inside the model house was not statistically significant. While the 3D panel was unable of blocking the power line induced magnetic fields, it significantly blocked both the FM radio waves and 900 MHz GSM signals.

Conclusions: Based on the findings obtained in our study,3D wire panels cannot serve as the metallic mesh screens for protecting the residents of these dwellings against high intensity electromagnetic fields. Our in vivo and in vitro results confirm the existence of unfavorable effects of low-intensity, radiofrequency radiation on intracellular macromolecular structures.

PO333-01. The role of lysosomes in radiation induced genomic instability. Scott Bright, S.L. Irons, S. Vaughan, M. Kadhim, SoLF Oxford Brookes University, UK

Radiation-induced genomic instability (RIGI) has been described comprehensively in the past two decades. The response manifests itself as the appearance of various types of damage such as gene mutations, delayed reproductive cell death and chromosomal damage that appear in the descendants of the irradiated population. Various factors effect RIGI induction including radiation type, dose and cell genotype. As yet the exact mechanisms for induction of genomic instability remain elusive, though experiments have shown induction of reactive oxygen species and cell signalling through cytokines are involved. Such events have been implicated on a cellular level, however disrupted sub-cellular organelles, such as lysosomes, and their role in RIGI is not well documented. This project focuses on radiation induced lysosomal damage and its role in RIGI. Lysosomes contain numerous acid hydrolases capable of degrading cellular components. Enzymes of particular interest include DNase II, which degrades DNA, as well as LAMP-1, important to the membrane and structure of lysosomes.

In order to test the hypothesis that ionizing radiation induces RIGI through lysosome damage, several methods were used to determine lysosomal membrane permeability, in combination with other techniques to measure basic lysosomal properties such as size. Lysosomal ultra-structure is also currently being examined through electron microscopy in order to identify any abnormalities in shape and integrity. In parallel, lysosomal rupture was examined for its ability to induce early and delayed chromosomal aberrations following treatment with sphenosine as a positive control.

Preliminary results indicate that radiation may subtly alter lysosome size and permeability particularly in the early stages post radiation, approximately one hour after exposure. This may suggest that the resulting release of acid hydrolases could induce and contribute to observed cell death through apoptosis and necrosis. However, if rupture is only partial without full release of the acid hydrolases held within, it could be speculated this may only result in cell damage and potentially chromosomal instability (CIN). These results and observations will be discussed in detail.

*ARR early career investigator award

PO333-02. Bio-signature of Radiation Response in Aging Metabolome. Amrita K. Cheena1, J.B Tyburski2, S. Strawn3, G. Kaur, A.J. Fornace Jr.1,2, 1: Department of Oncology, Georgetown University Medical Center, Washington D.C., USA, 2: Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington D.C., USA

The elderly population (65 and older) is projected to increase to 72 million in the USA by 2030 and will comprise approximately 25% of the total projected U.S. population. There is a critical need to better understand biological aging through the characterization of molecular changes associated with the aging process, so that potential targets for prevention and therapeutics can be more thoroughly explored. Exposure to stressors appears to be an important factor in promoting biological aging, and the corresponding stress responses are known to be affected by age. Since chronological age is the best predictor of biological age and age-related diseases, we probed the metabolic differences between young and old populations of wild type C57BL/6 mice to study the impact of ionizing radiation on the study population.UPLC–QTOFMS (Ultra-performance liquid chromatography – Quadrupole Time of Flight Mass Spectrometry) based metabolic analysis was performed for global profiling of
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C57BL6 mice pre and post radiation treatment (3 Gy) for three age groups namely 6 weeks, 12 months and 24 months, followed by multivariate data analysis and functional pathway analysis to identify significantly altered metabolites for further characterization. Our overall goal was to elucidate and integrate the metabolic alterations associated with aging for the purpose of developing biomarkers associated with this process and also those which promote aging augmented by stress. Our results indicate perturbations of pathways related to aging and cell metabolism. The results will be discussed in the context of the aging phenotype.

PO533-03. Molecular mechanisms for induction of sustained elimination of low dose hyper-radiosensitivity, Nina Edin1, J.A. Sandvik1, C. Cheng1, H.S. Vollan1, K. Reger1, A. Görlach1, L. Bergersen1, E.O. Pettersen1, 1: University of Oslo, Norway, 2: Akershus University Hospital, Norway, 3: German Heart Center Munich, Germany

We have previously found that protracting a priming dose of 0.3 Gy over 1 hour resulted in permanent elimination of low dose hyper radio-sensitivity (HRS) instead of the transient elimination by an acute priming with the same dose. The cells exposed to the low dose-rate priming secreted a factor into the medium, which removed HRS transiently in recipient cells. The factor could also be induced by low-dose-rate induction of cell conditioned medium, but only when a serum was present in the medium during conditioning. We have now identified the serum factor and the factor secreted by the low dose-rate primed cells and found evidence for a self-sustaining molecular mechanism responsible for permanent elimination HRS transmitted to the progeny. The phenomenon was induced by low-dose-rate using the colony assay as well as anti-phospho-histone H3 staining. Cytoplasmic levels of TGFβ3 were assessed using post embedding immunogold electron microscopy. Superoxide was measured by EPR and gene expression by microarray analysis. Two cell lines known to display HRS were used, T-47D breast cancer cells and T98G glioblastoma cells. The change to a HRS-negative phenotype was found to be transiently induced by extracellular TGFβ3, which could be activated through iNOS activity by low-dose-rate irradiation (0.2-0.3 Gy/h for 1 h) of cell conditioned medium. However, direct cell irradiation at low dose-rate induced a permanent elimination of HRS by activation of a self-sustaining mechanism found to depend on iNOS activity and resulting in continuously elevated cytoplasmic levels of activated TGFβ3. The HRS-negative phenotype was reversed by iNOS inhibitor 1400W. The serum factor was identified as interleukin-13. The effect of low dose-rate irradiation could be mimicked by high dose-rate irradiation or reoxygenation after hypoxia in combination with NO. In addition to supporting our proposed model for a self-sustaining molecular mechanism, the data contributes to the understanding of the importance of distinguishing between dose-rates in relation to radiation protection issues. They indicate that the effects induced by prolonged low-dose rate irradiation and irradiation of a permanently sensitized cell was maintained and exhibited a permanent sensitization.

PO533-04. Current Status of Microbeam Irradiation System, SPICE-NIRS, Terunai Konishi1, M. Oikawa1, T. Ishikawa1, M. Isom0, N. Shiomori1, T. Maeda1, N. Suya1, 1: National Institute of Radiological Sciences, Japan, 2: Tokyo Metropolitan University, Japan

Single-cell microbeam irradiation systems have become significant tools in the field of radiation biology and have discovered much important evidence that have never been described in the study using conventional broad beam irradiation. Single Particle Irradiation System to Cell, SPICE at National Institute of Radiological Sciences (NIRS) is a proton microbeam irradiation system developed for low dose radiation effect studies, such as for the cellular response of targeted and non-targeted effects. SPICE provides 3.4 MeV proton microbeam by using two slit system and a mono-bloc triplet Q lens so as to exclude such low-energy particle components by scattering seen with the collimation method. Approximately, 2 μm in a diameter beam are routinely available. A cell dish is placed on the voice coil stage of the microscope system, which also contains a fluorescent microscope and a CCD camera. As a routine procedure, cell nuclei are dyed with 1 μM Hoechst 33342, and the X-Y coordinates of the cell position in the dish are calculated automatically corresponding to the obtained fluorescent images. Each nucleus can be followed with the precision of proton beam probability of 99.6% accuracy, and will be irradiated according to the calculated coordinates with a maximum speed of 400 cells per minutes by controlling the voice coil motor stage. Approximately, two thousand cells in 25 mm² area per dish can be irradiated within 10 minutes including image capturing, cell recognition, and irradiation. In this presentation, collaborative researches such as targeted and non-targeted effects in mammalian cells and also in vivo studies performed using SPICE will be introduced.

Electrostatic accelerator facility of NIRS received a severe damage from the Tohoku-Kanto earthquake on March 11th, 2011, and currently, SPICE is under construction. Hopefully, SPICE will be renewed, and resume its operation by the beginning of April of 2012.

PO335-05. Nitric Oxide is a Key Molecule Serving as a Bridge between Radiation-Induced Bystander and Adaptive Responses, Hideki K. Oikawa1, M. Shiomori1, 1: National Institute of Radiological Sciences, Japan, 2: German Heart Center Munich, Germany, M. Hasunaa1, 1: Division of Oncology, Biomedical Imaging Research Center, University of Fukui, Japan, 2: Radiation Safety Research Center, Central Research Institute of Electric Power Industry (CRIEPI), Japan, 3: Research and Development Department, The Wakasa Wan Energy Research Center (WERC), Japan

Possible risks from exposure to low dose ionizing radiation (below 100 mSv) are estimated by extrapolating from data obtained after exposure to higher doses of radiation, using a linear no-threshold (LNT) model. However, the validity of this dose-response model is controversial because evidence accumulated over the past two decades has indicated that living organisms, including humans, respond differently to low-dose/low-dose-rate radiation than they do to high dose/high dose-rate radiation, which cannot be explained by the classical “target theory” of radiation biology. These important responses to low dose-rate radiation are the radiation-induced adaptive response, the bystander response, low-dose hypersensitivity, and genomic instability. The phenomenon, “radiation-induced adaptive response” was described by Sheldon Wolff and his colleagues who studied chromosomal aberrations in human lymphocytes after irradiation. Their findings indicated that the harmful effects of radiation received as a challenging dose may be reduced by a prior priming or conditioning low dose. In contrast, among reports describing cellular responses to low dose/low fluence radiation, a number of investigations have shown since the 1990s that the harmful effects of radiation may be amplified due to induced “bystander responses”. Radiation-induced bystander responses have been observed in a range of cell types and following both high- and low-LET radiation. Radiation-induced bystander responses have resulted from some types of communication or signaling between the irradiated cells (targeted cells) and nearby unirradiated cells (non-targeted or bystander cells) through direct physical connections between cells such as gap-junction intercellular communication (GJIC) or through diffusible factors released in the culture medium. Moreover, correlations between the radiation-induced adaptive and bystander responses have been recently discussed in several reports reporting that the key signaling molecules serving as a bridge between the two phenomena are reactive nitrogen species (RNS). In the present talk, we sum up findings describing the radiation-induced adaptive and bystander responses, and discuss the contribution of the latter to the former through production of RNS.

PO533-06. Cell survival and DNA damage responses following exposure to modulated radiation fields, Colman Trainer1, K.T. Butterworth1, C.K. McGarry1, F. Liberante1, J.M. O'Sullivan1, A.R. Honnessell1, K.M. Prise1, 1: Centre for Cancer Research & Cell Biology, Queens University Belfast, UK, 2: Radiotherapy Physics, Northern Ireland Cancer Centre, Belfast Health and Social Care Trust, UK

The purpose of this investigation was to determine cell survival and DNA damage responses following exposure to modulated radiation fields in cells with differing radiosensitivity. Cell survival was determined in primary human fibroblast (AG0-1522B), human breast cancer (MDA-MB-231), human prostate cancer (DU-145) and human glioma (T98G) cells following exposure to either a uniform or modulated field delivered using either a 160 kV X-ray set or a 225 kV X-ray set. Modulated exposures were delivered by shielding 50% of the flask during irradiation. The DNA damage response was determined within AG0-1522B and DU-145 cells following exposure to a modulated field. Cells were fixed at a range of time points following exposure and assessed for the presence of strand breaks using comet assay. 225 kV X-ray set and the levels of DNA damage marker 53BP1 were measured.
Following modulated exposure a significant loss of survival (p < 0.05) was observed within the out-of-field areas of flasks lower than predicted from the linear quadratic model. The out-of-field response was cell type dependent and positively correlated with radiosensitivity. The observed decrease in cell survival out-of-field was abrogated by physical inhibition of cellular communication between the in and out-of-field regions. The level of cell survival within the out-of-field region was shown to increase following pre-treatment of cells with Aminoguanidine, DMSO or cPTIO that are known to inhibit factors involved in mediating the radiation induced bystander effect. An increase in 53BP1 was observed within the out-of-field region up to 1 cm from the centre of the flask 30 minutes following exposure to a modulated field. Decreased levels of 53BP1 were observed in-field 30 minutes following modulated exposure when compared to 53BP1 levels following a uniform exposure. These data indicate that cellular communication between the in and out-of-field regions has an important role on the cellular response following exposure to a modulated field. Within all cell lines investigated a significant decrease in survival was observed within the out-of-field region. The cellular radiosensitivity is observed to be an important determinant of the magnitude of response. Investigations into DNA damage have outlined a complex response following modulated exposure that suggests a potential role for intercellular communication.

**POSS3-07. Premature senescence in Human Umbilical Vein Endothelial Cells (HUVEC) induced by chronic low-dose gamma radiation - a proteomic approach**, Venkata Ramesh Yentrapalli1, O. Azimzadeh1, Z. Barjaktarov1, A. Wójcik1, M. Harms-Ringdahl1, M.J. Atkinson1, S. Haghdoot2, S. Tapiero1, 1: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Biology, Neuherberg, Germany, 2: Center for Radiation Protection Research, Department of Genetics, Microbiology and Toxicology, Stockholm University, Sweden

An increased risk for circulatory diseases is prevalent in radiation-related epidemiological studies, recent data suggesting causality even at low doses of ionizing radiation. Endothelial cells form a unique permeable single-layered lining of the cardiac vessels with the main task of transporting nutrients from blood. Endothelial dysfunction plays an important role in the initiation and progression of the formation of atherosclerotic plaques. Consequently, the endothelium is one of the main targets affected in radiation-induced circulatory diseases in general and in cardiovascular diseases in particular.

The aim of this investigation was to study the dose- and dose-rate-dependent influence in the progression of cellular senescence in HUVEC cells and to analyze the corresponding changes in protein expression profiles. The HUVEC cells were chronically exposed to 4.1 mGy/h in a cell culture incubator equipped with a 137-Cs source. Our results indicate an induction of premature senescence by chronic low-dose-rate irradiation, verified by the loss of growth potential and early appearance of senescence-associated markers (S-β-gal).

We optimized protein extraction for different downstream proteomics approaches. For the quantification of the differentially expressed proteins after different time points of exposure (1, 3, 6, and 10 weeks), we applied 2D-DIGE (Differential In-Gel Electrophoresis) and ICPL (Isotope-Coded-Protein-Labeling) methodologies. In both approaches, labeled proteins isolated from sham and irradiated cells were mixed and separated on SDS-PAGE before quantification. The bands or spots corresponding to the differentially expressed proteins were digested, and identified and quantified using tandem mass spectrometry.

The proteomic analysis was performed of cells exposed to a chronic dose rate of 4.1 mGy/h and analyzed after week 1, 3 and 6 with cumulative doses of 0.68 Gy, 2.06 Gy, and 4.1 Gy, respectively. Differentially expressed proteins were involved in cell-to-cell signaling and interaction, growth and proliferation, RNA post-transcriptional modification and cellular movement. The protein network analysis was performed by the Ingenuity software. Proteins deregulated at all time points included annexin A1, histone H2A type 1C, non-POU domain containing octamer-binding protein, lamin-A/C, H4 nucleosomal protein and vimentin.

**POSS3-08. Ionising radiation induces damages at molecular level in mammalian cells that results in biological responses mediated by proteins. Complex DNA damages induced by high-LET radiation appear more difficult to repair and little is known how the complexity of the DNA damage triggers the response pathways engaged in preserving genomic stability.**

Ionising radiation induces damages at molecular level in mammalian cells that results in biological responses mediated by proteins. Complex DNA damages induced by high-LET radiation appear more difficult to repair and little is known how the complexity of the DNA damage triggers the response pathways engaged in preserving genomic stability. Cellular models with low LET radiation appear more predictable. Ionising radiation induces changes in protein expression in whole cell and nuclear lysates identified a total of 13 differentially expressed proteins that were analyzed by mass spectrometry. Evaluation of protein expression results by orthogonal partial least square discriminant analysis revealed significant differences in altered protein spots for different endpoints, e.g. doses, irradiations, and only few spots were in common for those endpoints. The profile of certain proteins changed when exposure was performed in the presence of DMSO. The results demonstrate that unique patterns of up or down regulated proteins are produced in response to different complexity of DNA damages and that the indirect effect of radiation for both low- and high-LET causes different cellular response.

**POSS3-04. Differentially expressed proteins were involved in cell motility and trafficking, cell cycle, cell communication and free radical scavenger dimethyl sulphoxide (DMSO). Proteins were extracted from cells 3 hours after exposure and used for protein expression analysis by two-dimensional polyacrylamide gel electrophoresis. Significantly altered protein spots were analyzed by mass spectrometry.**

Radiation Protection Research, Department of Genetics, Microbiology and Toxicology, Stockholm University, Sweden

Ionising radiation induces damages at molecular level in mammalian cells that results in biological responses mediated by proteins. Complex DNA damages induced by high-LET radiation appear more difficult to repair and little is known how the complexity of the DNA damage triggers the response pathways engaged in preserving genomic stability. Cellular models with low LET radiation appear more predictable.
Introduction: The study was aimed to detect features of human serum proteome that were associated with exposure to ionizing radiation.

Materials and methods: Analyzed group consisted of 46 patients treated with radical radiotherapy because of larynx cancer; patients were irradiated with total doses in a range from 51 to 72 Gy. Three consecutive blood samples were collected from each patient: before the start, 2 weeks after the start, and 1-2 months after the end of radiotherapy. The low-molecular-weight fraction of the serum proteome (2,000-13,000 Da) was analyzed by the MALDI-ToF mass spectrometry.

Results: Proteome profiles of serum samples collected before the start of radiotherapy and during early stage of the treatment were similar. In marked contrast, mass profiles of serum samples collected several weeks after the end of therapy revealed significant changes. We found that 41 out of 321 registered peptide ions changed their abundance significantly when serum samples collected after the final irradiation were compared with samples collected at two earlier time points. We also found that abundances of certain serum peptides were associated with total doses of radiation received by patients.

Conclusions: The results of this pilot study indicate that features of serum proteome analyzed by mass spectrometry have potential applicability as a retrospective marker of exposure to ionizing radiation.

PO534-04. Proteomic Analysis of Radiation-inducible Phosphorylation Profiles for Identification of Molecular Targets in Prostate Cancer Cells. A. Adeola Makinde1, D. Cerna2, S.T. Palayoor3, M. Aryanakallyi4, E.F. Petricic5, L.M. LaToia6, C.N. Coleman2. 1: National Institutes of Health/NCI, USA; 2: George Mason University, USA

The fate of cells exposed to radiation is dependent on their response to the resulting damage, which is a function of their capacity to repair and extent of the damage. Higher doses cause overwhelming DNA damage and cell death. On the other hand, exposure to sublethal doses increases the chances of repair and survival. We previously showed that in comparison to single dose, fractionated radiation induced robust changes in mRNA and miRNA in prostate cancer cells. In addition to gene expression analysis, profiling phosphorylation patterns would facilitate identification of potential druggable targets. Protein phosphorylation was analyzed in PC3 and DU145 exposed to 10Gy radiation, either in a single dose(SD), or 10 fractions of 1Gy(MF). Some cleavage-activated proteins were also analyzed. Lysates were collected at 6 timepoints (0-24h) after concluding radiation. 73 proteins were analyzed by reverse-phase protein microarray and analyzed with MicroVigene image analysis software, ver. 2.2 and Microsoft Excel 2000. Phosphorylation patterns were different with both radiation regimens. An overall increase in the phosphorylation/cleavage status of cell death associated proteins was observed with both radiation exposures (markedly increased CASP9 cleavage, decreased p4EBP1); while increased PARP cleavage (survival/repair) was observed only after MF in DU145. pSRK3 (survival/proliferation) was elevated in PC3 following MF. PC3 and DU145 exhibited different phosphorylation patterns in response to radiation. In DU145, exposure to radiation, led to a decrease in pAKT and pBCL2-2 along with an increase in pBAD, while pAKT, pBAD and pBCL2 was increased in PC3. Elevated CASP9 cleavage was also observed in PC3 but not in DU145. Our data show response profile of PCa cells to fractionated radiation in comparison to single dose. A vital question in this study is "does fractionated radiation alter the protein phosphorylation profile in tumors enough to make the pathways targetable?" Further analysis is currently in progress, testing some identified potential targets with clinically applicable inhibitors. Modulation of the cellular pathways in the surviving cancer cells exposed to fractionated radiation (repeated exposure to sublethal doses) could provide molecular targets and exploitable pathways for improved therapy.

PO534-05. Radiotherapy-induced changes in serum proteome of patients with head and neck cancer; correlation with radiation doses given to different tissue volumes. Tomasz Rutkowski1, A. Wygodza1, M. Sklodowska2, K. Sklodowska3, P. Wasiak4, R. Rutkowski1, 1: Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland; 2: Silesian University of Technology, Poland

Background: Technologically advanced techniques of radiotherapy (RT), e.g. IMRT, allow precise delivery of high doses to the gross tumor volume (GTV). However, such techniques also involves relatively large volumes of normal tissues irradiated with low doses, which biological significance is not well established so far. Exposure to ionizing radiation is likely to induce changes in serum proteome, which can be assessed by proteomics. Establishing the correlation between specific changes in serum proteome and irradiation doses/volumes would help to identify novel predictive and prognostic markers of tolerance/toxicity of RT and its distant results. Aim: To identify specific radiation-induced changes in serum proteome related to various tissue volumes and radiation doses. Particular interest is given to large volumes of normal tissues receiving relatively low radiation doses. Material and methods: One hundred twenty five patients treated with RT due to squamous head and neck cancer were included into the study. Overall treatment time (OTT) was in the range of 21-58 days. All patients were treated exclusively with RT to a total dose (D) of 51-72 Gy given to GTV, 60-66 Gy given to the clinical target volume (CTV1; defined as a volume of considerable risk of subclinical disease marginal to GTV), and 45-55 Gy given to CTV2 (defined as a volume of low risk of subclinical disease). Volume of tissue receiving at least 1 Gy was also calculated as a total irradiated volume (IV). Due to various fractionation schedules dose intensity (DI) was calculated as a D/OTT. Four consecutive blood samples were collected from each patient: (1) before RT, (2) 2 weeks after RT start, (3) 1-2 months after RT completion and (4) 6 months later. The low molecular weight fraction of the serum proteome (2-14 KDa) was analyzed by MALDI-ToF mass spectrometry and specific features of mass profiles identified in registered spectra. Features of proteome profiles were correlated with known prognostic factors related to patient, tumor, and treatment.

Results: Changes in serum proteome profiles specifically related to irradiation (doses and irradiated volumes) were observed. Identified peptide signatures, which reflect response of patient’s organism to irradiation, are of potential prognostic or predictive value in assessment of RT tolerance and efficacy.

PO535 Radiation carcinogenesis


Background: As the human body is continually exposed to various environmental carcinogens, radiation and other environmental carcinogens are considered to act cooperatively in inducing cancer. We previously showed that the combined effect on the induction of thymic lymphoma after exposure to X-rays and N-ethyl-N-nitrosourea (ENU) was dependent upon dose of carcinogen and order of treatment. The aim of this study was to clarify the effect of various ENU treatments following an X-ray exposure during the pubertal period; particularly we focused on the interval between X-rays and following ENU treatment.

Materials and Methods: Four-week-old female B6C3F1 mice were exposed to X-rays (1.0 Gy per week) for four consecutive weeks, and then were treated with ENU (200 ppm) in drinking water for 4 weeks started from 8, 10, 12, or 16 weeks of age (i.e., the interval was 0, 2, 4, and 8 weeks, respectively). The mice were observed until moribundity and their lifespan and the incidence of thymic lymphoma were analyzed. The subtype of thymic lymphoma was analyzed by flow cytometry and their DNA, RNA, and proteins were analyzed.

Results and Discussion: The incidence of thymic lymphoma after X-irradiation was 13% at the 300 days after irradiation. The incidence after ENU treatment was dependent on the age at the time of treatment which was 20%, 22%, 12% and 12% after the treatment at 8, 10, 12 and 16 weeks of age, respectively. The incidence of thymic lymphoma was extremely increased by the combined treatment with an interval of 0 or 2 weeks (94% or 98%, respectively), whereas that with an interval of 4 or 8 weeks resulted in the incidence of 20-40%. Thus, the interval period drastically affected the incidence of thymic lymphoma.

Conclusion: The combined exposure to X-rays and ENU increased the development of thymic lymphoma in a supra-additive manner. The increase was higher with a short interval (≤2 weeks) than with a long
interval (≥4 weeks) between the exposures, suggesting that the influence of past radiation exposure persists at least 8 weeks after the exposure.

POS35-02. Cellular Automata Model to Predict the Number of Transformation cells in Lung Tissue Induced Radon Progeny. Samaneh Baradaran1, S. Setayeshi, M.R. Kardani1, 2. National Radiation Protection Department, Iranian Nuclear Regulatory Authority, Iran, 1: Medical Radiation Department, Amirkabir University of Technology, Tehran, Iran, 3: Nuclear Radiation Application School, Nuclear Sciences and Technology Research Institute, Tehran, Iran

According to the scientific reports about the potential health hazards of Radon and its products, in homes and working places, Radon is the second leading cause of lung cancer after smoking in the USA. This relationship has prompted concern to estimate this health risk by using various methods.

In this study, cellular automata model was used to predict the number of transformable cells that their nucleus was passed by alpha particles decay from 222Po and 218Po. The direct alpha particles hit can transform the DNA of cell nuclei and can trigger of cancer. Simulation of human respiratory system and lung cancer induced inhaled radon are required long partial differential equations and many parameters. The cellular automata is a suitable tool for optimization complex systems. This method by using simple functions and local convergence while taking neighboring effects into account could result accurate global solution.

The transition rule (function) of this model was derived from experimental in vitro data for CSH 10T1/2 mouse cells. This model results the number of transformation cells in different cumulative exposure of Radon (WLM) based on Cellular Automata model that was illustrated in figures and they are in excellent agreement with experimental data.

POS35-03. The contribution of delta rays to cancer induction in rat skin. Fredric Burns, M.E. Tang, F. Wu, NYU School of Medicine, USA

A plausible, but still unproven, hypothesis of radiation carcinogenesis is that an inappropriate joining of DNA double strand breaks (DSBs) can create a critical initial lesion that disposes the cell to carcinogenic progression. Many such joinings occur but the cancer-relevant ones destabilize the cellular genome without compromising long-term cellular viability. Along the track of heavy ion particles, delta rays (electrons) are ejected with sufficient energy to produce ionizations of their own. A direct contribution of these delta rays to cancer induction by heavy ions is unlikely because of their comparatively wide spacing along the track and their radial orientation away from the track core. However as the ion flux increases, the ion tracks become more tightly packed, and the space between them becomes filled with a field of electron radiation, which is expected to have the same carcinogenicity as a field of pure electron radiation. Consequently any cancers that might be derived from this field are expected to occur in proportion to the square of the delta ray dose, i.e. Cancer Yield (Deltad > BD2, as established for a pure electron beam. Since nearly all the energy of protons above about 200 MeV is dissipated in the delta rays, for practical purposes such protons are carcinogenically-equivalent to a pure electron beam. Based on the above, a dose can be calculated where the heavy ion track and the delta rays contribute equally to overall cancer induction, i.e. \[ D_{eq} = \frac{CL}{B}, \] where C and B are empirical parameters from cancer induction results in rat skin and L is the mean LET of the radiation. For several ion beams with different LET values (56Fe, 40Ar, 29Ne, 1H), each \( D_{eq} \) value corresponded to a consistent ion track separation distance of 0.67 microns. These findings strongly support the idea that delta ray overlap is a significant source of cancer induction for heavy ion radiations particularly at low doses above \( D_{eq} \) and adds independently to the carcinogenicity of the central ion track.

POS35-04. Harderian Gland Tumorigenese: Low-Dose-, Low Dose-Rate- and LET-Response. Polly Y. Chang1, E.A. Blakeley2, 1: SRI International, USA, 2: Lawrence Berkeley National Laboratory, USA

Increased cancer risk remains one of the primary concerns for travel into deep space and may preclude manned missions to Mars due to the large uncertainties that currently exist in estimating cancer risk from the spectrum of radiation types found in space with available human epidemiological radiation-induced cancer data. Existing data on human risk of cancer from exposures to X- and gamma-rays must be scaled to the many types and fluxes of radiations found in space using radiation quality factors and dose-rate modification factors, and assuming linearity of response since the shape of the dose-response at low doses below 100 mSv is unknown. The murine Harderian gland tumorigenesis studies represent the most complete set of experimental observations and dose dependence available to assess the relationship of RBE to LET for risk estimation of space (1-2).

However these data are lacking information on low dose responses below 0.5 Gy, at chronic low dose rate, and data in the LET region between 25 and 190 keV/µm. The goal of our project is to reduce uncertainties in the estimation of particle radiation carcinogenesis by use the existing data on Harderian gland tumorigenesis as reference, and extending the database using the same animal model to obtain Harderian gland tumor data for heavy ion beams not previously reported to fill the gaps in the LET range to improve our understanding of the dose-response curve at low doses (<50 cGy) and at low dose-rate (<60 cG/yr), and to test for deviations from nonlinearity [3]. The hypothesis to be tested is that filling these data gaps will delineate more comprehensively the space radiation risk of radiation-induced tumorigenesis, and in particular to provide data that will allow a test for a role of targeted versus non-targeted effects in cancer risk. Studies using low energy Silicon ions with LET’S at ≤50 keV/µm, and at low dose-rate (≤60 cG/yr), and to test for deviations from nonlinearity [3].

Reference:


We apply a biologically motivated mechanistic model to describe radiation-induced leukemia incidence in survivors of the atomic bombs on Hiroshima and Nagasaki. The model yields risk estimates that can be compared with results from epidemiological studies, but more importantly, it provides insight into the impact of radiation action on leukemogenesis and forms a biologically based motivation for the transfer of risks from acute exposures of the Japanese population to chronic, low dose exposures of any population.

Inputs for the model are individual data on exposure to both gamma radiation and neutrons and on the incidence as well as mortality of leukemia in the Life Span Study cohort. Data were available for 112,932 individuals, constituting approximately 3.5 million person-years. These data are combined with biologically based information from the literature on the form of the mathematical equations that describe DNA damage by ionizing radiation and on the cells that are the target for radiation induced leukemogenesis. Parameters related to both radiation action and baseline leukemia incidence are determined with a maximum likelihood method using a simulated annealing routine.

Baseline cancer rates vary across populations, but since the spontaneous incidence of cancer is described separately from radiation action in the model, risk transfer to populations where leukemia incidence is different is feasible: values for the parameters that describe baseline cancer incidence can be determined by fitting the model to mortality or incidence data of national cancer registries. We will present preliminary fitting results, with corresponding derived risk estimates and will show how these estimates translate to low-dose leukemia risk in a general population. These results provide important input for radiation protection measures.

POS35-06. Cells in the thymus of the gamma-radiation target and of origin in lymphoma in Bcl11b heterozygous mice. Ricka Go, R. Kominami, Niigata University, Japan

Mutations or deletion of BCL11B gene were found in 16% of human T-cell acute lymphoblastic leukemias (T-ALLs) and a half of the TLX1 oncogene-induced T-cell leukemia. T-cell lymphomas harbored Bcl11B genetic changes, indicating Bcl11B as a tumor suppressor. Bcl11b was originally isolated by our group from the
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analysis of γ-ray induced mouse thymic lymphomas and found to act as a haploinsufficient tumor suppressor gene. We previously showed that loss of one Bcl11b allele plays a key role in clonal expansion of premalignant thymocytes in irradiated Bcl11b<sup>−/−</sup> mice. However, what cells in the thymus are radiation targets leading to malignancy and how Bcl11b heterozygosity affects the irradiated cells remain open. In this study, we investigated what type of cells in thymus were targets of radiation and cells of origin in thymic lymphomas. Thus, we developed a novel mouse model, in which Bcl11b heterozygosity occurs only from a certain C57CD8 double-negative stage of thymus. Such Lek-Cre;Bcl11b<sup>−/+</sup> mice were subjected to γ-irradiation of 3 Gy at 8 weeks of age and their thymocytes were characterized of cell number, clonal expansion and differentiation. We found clonal expansion of thymocytes at a high frequency at 60 days after irradiation and premalignancy started mostly in thymocytes at a mature type of CD8 single-positive cells bearing highly expressed TCRβ. These results suggest that cells of radiation target are differentiated cells in the thymus that derived from thymocytes in the bone marrow. However, these premalignant thymocytes tended to have characteristics of more immature thymocytes and were retained within the thymus. On the other hand, most of the overt thymic lymphomas had acquired the capability to emigrate to the periphery, forming lymphoblastic leukemias. These results may help understand which type of cells can be the radiation target and cells of origin in cancer.

POS35-07. Noninvasive monitoring of radiation-induced mouse thymic lymphoma by positron emission tomography and magnetic resonance imaging, Sumiaka Hayeogawa, T. Morokoshi, T. Furukawa, T. Saga, National Institute of Radiological Sciences, Japan

Here we show in vivo imaging of radiation-induced mouse thymic lymphoma (TL) by positron emission tomography (PET) and magnetic resonance imaging (MRI). Molecular imaging using PET and MRI are widely used not only for clinical cancer diagnostics but also for animal models of cancer. The aim of this study is to establish a method for monitoring mouse thymic lymphoma (TL) development induced by ionizing radiation. For radiation lymphomagenesis, we used fractionated whole body X-ray irradiation protocol and thus irradiated 4 weeks-old C57BL/6 mice at 1.2 Gy weekly for 4 consecutive weeks (total dose: 4.8 Gy). Three to six months after irradiation, affected mice showed body weight loss and difficulty in breathing. To assess the size of thymus of these affected mice, T- weighted images were acquired by high magnetic field animal 7T-MRI scanner. PET with [F]fluoro-2-deoxyglucose (FDG) was also applied to mice with an enhanced thymus. FDG (~4MBq) was administrated into an affected mouse intravenously and a 10-min emission scan was started at 50 min after injection using Siemens Inveon small animal PET systems. FDG was highly accumulated in the affected thymus, suggesting that the lesions were metabolically active and malignant. Histopathological studies confirmed that these lesions were T-cell lymphomas. Molecular imaging using PET and MRI allows us to trace the development and progression of mouse TL in living animals. Therefore, we propose that these techniques are powerful tools for detecting radiation-induced thymic lymphoma and would offer a new opportunity to understand the molecular and cellular mechanism(s) of radiation-induced mouse thymic lymphoma development.

POS35-08. Mammary carcinogenesis after exposure of fetal, neonatal, juvenile and adult rats to γ-rays and carbon ions, Tatsuhiko Imaoka<sup>1</sup>, M. Nishimura<sup>2</sup>, K. Daino<sup>1</sup>, D. Iizuka<sup>2</sup>, T. Kokubo<sup>1</sup>, Y. Nishimura<sup>1</sup>, T. Okutani<sup>1</sup>, M. Takabatake<sup>1</sup>, S. Kakinuma<sup>2</sup>, T. Takabatake<sup>2</sup>, Y. Shang<sup>1</sup>, Y. Shimada<sup>1</sup>, 1: Radiobiology for Children’s Health Program, Research Center for Radiation Protection, National Institute of Radiological Sciences, Japan, 2: Department of Molecular Radiobiology, Research Institute for Radiation Biology and Medicine, Hiroshima University; Radiation biology for Children’s Health Program, Graduate School of Biomedical Sciences, Hiroshima University, 3: University of Tokyo, 4: Department of Radiobiology, Graduate School of Medicine, Chiba University; Radiation biology for Children’s Health Program, Research Center for Radiation Protection, National Institute of Radiological Sciences, Japan, 5: Department of Radiological Sciences, Graduate School of Human Health Sciences, Juntendo University; 6: Department of Radiation Emergency Medicine, Research Center for Radiation Emergency Medicine, National Institute of Radiological Sciences, Japan, 7: Department of Radiobiology for Children’s Health Program, Research Center for Radiation Protection and Medical Exposure Research Project, National Institute of Radiological Sciences, Japan, 8: Radiobiology for Children’s Health Program, Research Center for Radiation Protection and Medical Exposure Research Project, National Institute of Radiological Sciences, Japan.

Background: The risk of developing secondary cancer after radiotherapy, especially after the treatment of childhood cancers, has been a matter of great concern. Epidemiologically, there have suggested that the breast is one of the most susceptible organs to radiation-induced carcinogenesis. However, little information is available on the effect of carbon ion radiation on breast carcinogenesis in children.

Experimental procedures: Experiment 1. Female ICR-SD Sprague-Dawley rats were whole body-irradiated with 1 Gy of Cs-137 γ-rays or a monoenergetic carbon ion beam (290 MeV/u, having linear energy transfer [LET] of ~13 keV/μm) at various ages from the embryonic stage to adulthood. Experiment 2. Neonatal, juvenile and young adult rats (1, 3 and 7 weeks of age, respectively) were irradiated with γ-rays or monoenergetic carbon ions at various doses between 0.2 and 2 Gy. In both experiments, the rats were observed until 90 weeks of age and incidence of mammary carcinoma was analyzed.

Results: The incidence of mammary carcinoma was increased in groups of rats irradiated with 1 Gy of either γ-rays or carbon ions between 1 and 7 postnatal weeks: irradiation of fetal rats with either radiation did not increase the incidence. The dose response showed an irregularity at 2 Gy, which may be due to early cessation of the estrous cycle caused by the damage to the ovarian follicles. Dose responses of γ-rays below 1 Gy were similar among the groups of rats irradiated at 1, 3 and 7 weeks of age. The effect of heavy ions tended to increase along with the age at the time of irradiation, indicating small relative biological effectiveness (~2).

Conclusion: Irradiation of immature mammary gland with carbon ions with LET of ~13 keV/μm has a small carcinogenic effect, compared to our previous result on the spread-out Bragg peak beam having LET of 40–90 keV/μm (Int J Radiat Oncol Biol Phys 69:194).

POS35-09. Lifespan shortening after exposure of mice at fetal, childhood and adulthood periods to gamma-rays and carbon ions. Shizuko Kakinuma<sup>1</sup>, Y. Shang<sup>2</sup>, Y. Amasaki<sup>3</sup>, S. Hirano<sup>2</sup>, T. Sawai<sup>1</sup>, M. Nishimura<sup>2</sup>, T. Takabatake<sup>1</sup>, K. Yamauchi<sup>1</sup>, A. Nakata<sup>1</sup>, Y. Sawa<sup>1</sup>, T. Imaoka<sup>1</sup>, Y. Shimada<sup>1</sup>, 1: National Institute of Radiological Sciences, Japan, 2: National Institute of Radiological Sciences, Juntendo University, Japan, 3: National Institute of Radiological Sciences, Chiba University, Japan.

Background: There are insufficient data at present on cancer risk after exposure of heavy ions, to the fetal and childhood periods. Using animal models, we studied the age-at-exposure effects of heavy ions on cancer induction and lifespan shortening for radiation protection for fetuses and children.

Materials and Methods: Fifty female and male B6C3F1 mice per group were exposed to gamma rays (57Co) or carbon ions (13 keV/μm) at various ages from fetal to mature adulthood periods. Mouse ages at the time of irradiation included pre-implantation (3 days post-conception (dpc)), major organogenesis (13 dpc), late fetal (17 dpc), neonatal (1 week after birth), infantile (3 weeks), young adulthood (7 weeks) and mature adulthood stages (15 weeks). The doses ranged between 0.2 and 4 Gy for gamma rays and 0.2 and 2 Gy for carbon ions. The mice were observed until moribund or death and their lifespan and the developed cancer were recorded.

Results and Discussion: Our study indicated that female mice appeared to be more susceptible to radiation-induced lifespan shortening than male mice. Effect of gamma-rays on lifespan shortening was more manifest when irradiated at neonatal than adult stage. Surprisingly, irradiation with gamma rays at the late fetal stage had little influence on lifespan shortening compared to infant and adulthood exposures. On the other hand, carbon ions were more potent in reducing lifespan than gamma rays when female neonatal mice were exposed. When carbon ions were exposed, however, fetuses were as susceptible as infants. The results on the lifespan shortening suggest that for fetuses of carbon ions, radiation at the earlier stages of the rapidly dividing cell population may reduce the tumor-free survival rate.
for fetuses, suggesting that RBE of 13keV/μm for cancer induction is 1.0–1.5 irrespective of age-at-exposure.

Key words: radiation carcinogenesis, lifespan shortening, age-at-exposure, RBE.

POS35-10. Role of mutagenic 8-nitroguanine in estrogen-dependent radiation-induced mammary tumorigenesis of rats. Shosuke Kawanishi1, N. Ma2, M. Onoda1, H. Inano1, S. Ohnishi1, M. Murata2, I. Fujita2, 1: Faculty of Pharmaceutical Sciences, Shizuoka University, Japan, 2: University of Hyogo, Japan. In this study, we examined 8-nitroguanine formation in mammary tumorigenesis induced by radiation with steroid analogue in rats. Wistar-MS rats were exposed to whole-body irradiation with gamma-rays (1.5 Gy) at day 21 of lactation and then implanted with a pellet of diethylstilbestrol (DES, a tumor promoter) in the interscapular area at one month after irradiation. After 1 year for the development of mammary tumors, mammary tissues were obtained, fixed with formalin, embedded in paraffin, and followed by immunohistochemical analyses. Wistar-MS rats were primed by implantation with 17beta-estradiol (E2) pellet. After 2 weeks of priming the mammary glands were isolated, diced into 5-mm cubes and cultured with a medium of diethylstilbestrol (DES, a tumor promoter) in the interscapular area at one month after irradiation. After 1 year for the development of mammary tumors. The overall miRNA screening identified a number of miRNAs altered in radiation induced carcinogenesis and probably involved in the development of this neoplasia. Specifically, we found that four miRNAs, namely miR-467a, miR-762, miR-455 and miR-714, were among the miRNAs regulated in radiation-induced thymic lymphoma tissues (P<0.001, up-regulated >4 fold). Pathway Prediction by an informatics method found that these four miRNAs may potentially target the apoptosis pathway (P<0.0001). In particular, we found that miR-467a had a strong anti-apoptotic function, and may play a unique role in directly targeting 11 apoptosis related molecules. We identified two direct targets of miR-467a, the pro-apoptosis molecules Fas and Bax, which were significantly down-regulated in radiation-induced thymic lymphoma tissue samples. Furthermore, knockdown of miR-467a suppressed the growth of murine thymic lymphoma EL4 cells as well as primary radiation induced thymic lymphoma cells in vitro and in vivo, indicating that miR-467a may be a novel therapeutic target for radiation-induced thymic lymphoma. Finally, we showed that miR-467a can be stable in serum and serve as a novel biomarker of radiation carcinogenesis. Taken together, these data strongly suggest that miR-467a is associated with the pathogenesis of, and useful in the diagnosis of radiation-induced thymic lymphoma in BALB/c mice, and may be a novel therapeutic target for radiation induced thymic lymphoma.

Table 1. The selected pathway prediction result: Apoptosis pathway. The table lists important molecules in the apoptosis pathway that the 4 validated, highest up-regulated miRNAs (miR-762, miR-455 & miR-467a) may potentially target. This table also indicated that miR-467a may target 11 apoptosis related molecules.

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POS35-11. Reversible knockdown of the tumor suppressor p53 during total-body irradiation prevents radiation-induced lymphomagenesis. David Kirsch1, C. Lee1, J. Blum1, J. Sullivan1, L. Jeffords1, Y. Kim1, D. Duke University, USA, 2: North Carolina State University, USA

Radiation therapy can cause acute toxicity and long-term side effects including radiation-induced cancer. Because part of the short-term side effects of radiation are due to p53-mediated apoptosis, blocking p53 during radiation can protect some normal tissues from acute radiation injury and might improve the therapeutic ratio of radiation therapy. However, temporarily blocking p53 could also increase late effects of radiation because mice in which p53 is permanently deleted are sensitized to radiation-induced carcinogenesis. Experiments using a mouse model where p53 can be temporarily turned on during irradiation suggest that radiation-induced apoptosis does not contribute to p53-mediated tumor suppression. Here, we perform reciprocal experiments and temporally turn p53 off during total-body irradiation (TBI) using transgenic mice with reversible TBI interference. We found that p53 knockdown during and permanently after TBI sensitized mice to radiation-induced carcinogenesis, which recapitulates the tumor spectrum of p53−/− mice exposed to irradiation. In contrast, we found temporary knockdown of p53 during TBI prevented lymphoma development. Because temporary knockdown of p53 during TBI suppressed cell death in hematopoietic progenitors, our results indicate that ameliorating p53-mediated hematologic radiation toxicity prevented lymphoma formation. Taken together, we show that although p53 is indispensable to suppress tumor formation after radiation, p53 functions during TBI to promote radiation-induced lymphomagenesis.

Dysregulation of certain microRNAs (miRNAs) has been shown to promote tumorigenesis. However, the functions and targets of only a few mammalian miRNAs are known. In particular, the miRNAs that participate in radiation induced carcinogenesis remain undefined. In this in vivo BALB/c mouse study, we found that miRNAs were aberrantly expressed in radiation-induced thymic lymphoma tissues. The overall miRNA screening identified a number of miRNAs altered in radiation induced carcinogenesis and probably involved in the development of this neoplasia. Specifically, we found that four miRNAs, namely miR-467a, miR-762, miR-455 and miR-714, were among the most up-regulated miRNAs in radiation-induced thymic lymphoma tissues (P<0.001, up-regulated >4 fold). Pathway Prediction by an informatics method found that these four miRNAs may potentially target the apoptosis pathway (P<0.0001). In particular, we found that miR-467a had a strong anti-apoptotic function, and may play a unique role in directly targeting 11 apoptosis related molecules. We identified two direct targets of miR-467a, the pro-apoptosis molecules Fas and Bax, which were significantly down-regulated in radiation-induced thymic lymphoma tissue samples. Furthermore, knockdown of miR-467a suppressed the growth of murine thymic lymphoma EL4 cells as well as primary radiation induced thymic lymphoma cells in vitro and in vivo, indicating that miR-467a may be a novel therapeutic target for radiation-induced thymic lymphoma. Finally, we showed that miR-467a can be stable in serum and serve as a novel biomarker of radiation carcinogenesis. Taken together, these data strongly suggest that miR-467a is associated with the pathogenesis of, and useful in the diagnosis of radiation-induced thymic lymphoma in BALB/c mice, and may be a novel therapeutic target for radiation induced thymic lymphoma.

Table 1. The selected pathway prediction result: Apoptosis pathway. The table lists important molecules in the apoptosis pathway that the 4 validated, highest up-regulated miRNAs (miR-762, miR-455 & miR-467a) may potentially target. This table also indicated that miR-467a may target 11 apoptosis related molecules.
POSTER PRESENTATIONS

POSTER 13

H.G. Modulates the radiosensitivity of human neoplastic cells. Severino Michelin1, C. Gallegos1, S.B. Trase1, D. Dubner1, B. Favier1, E.D. Carosella1, 1: Nuclear Regulatory Authority, Argentina, 2: CEA, France.

H. G. molecule is characterized by low polymorphism and tissue-restricted expression. It can be induced in transplantation, inflammatory diseases, cancer, multiple sclerosis, and viral infections. The expression of H. G. gene is regulated through epigenetic mechanisms, and occurs at the transcriptional and post-transcriptional level. The H. G. primary transcript yields the production of 7 isoforms: 4 membrane-bound (H. G.1 to -4), and 3 soluble (H. G.6 to -9). Functionally, H. G. inhibits the cytolytic function of NK cells and T lymphocytes, the alloproliferative response of CD4+ T cells, the ongoing proliferation of T cells and NK cells, the maturation of dendritic cells and induces regulatory T cells. H. G. mediates this inhibitory action by binding to the inhibitory receptors ILT2, ILT4 and KIR2DL4.

Clinically, the expression of H. G. has been correlated with the tolerance of the fetus by its mother, the acceptance of solid organ transplants, and the immune escape of tumors and virus-infected cells. It has been determined that gamma radiation modulates H. G. expression at the plasma membrane of human melanoma cells. However its role in tumoral radiosensitivity has not been demonstrated yet. The objective of this work was to determine if the radiosensitivity of human neoplastic cell lines cultured in vitro was mediated by H. G. expression. For this, 5 Gy gamma irradiation was performed on H. G.-negative (M8 G) and H. G.-positive (M8 G+ human melanoma cells and in H. G.-negative and H. G.-positive erythroleukemia cells (K562). The M8 G+ survival frequency, evaluated by clonogenic assay 20 days post irradiation was diminished by 40% respect to the M8 G- cell line. No significant differences were observed in the apoptosis level or cell cycle profile between both M8 G+ and M8 G- cell lines. H. G.-1 surface expression was decreased in irradiated M8 G+ cells (~ 20% diminution 24 h post irradiation) and was accompanied by the concomitant increase in H. G-1 levels in the culture medium. The survival of the K562 G+ cell line was diminished by 20% with respect to K562 G- cells, 5 days pos-irradiation, suggesting that this response is not exclusive of the melanoma cell line. In summary, our results indicate for the first time that H. G. confers higher radiosensitivity to human neoplastic cells, and would be a useful contribution for the optimization of radiotherapy protocols.

Keyword: radiosensitivity; H. G.-neoplastic cells; gamma radiation

POSTER 14


Background: Epidemiological studies indicate that breast is one of the most susceptible organs to radiation-induced carcinogenesis. Most studies, however, have failed in identifying clear genetic alterations in radiation-induced breast/mammary cancers. We therefore aimed at identifying genomic changes in the radiation-induced rat mammary cancers.

Experimental procedures: F1 hybrid rats were generated by crossing mammary cancer susceptible Sprague-Dawley females with resistant Copenhagen males. They were exposed to 4 Gy gamma-rays at 7 weeks of age and underwent autopsy at the time of spontaneous death. Genome-wide DNA copy number was analyzed by array comparative genomic hybridization. Loss of heterozygosity (LOH) and quantitative RT-PCR analyses were also performed.

Results: Copy number losses were identified in small regions of chromosomes 1q52, 2q12-15 and 3q31–36. These correspond to the syntenic regions of human chromosomes, 10q23, 5q11.2 and 1p13–14, respectively, where genomic loci is frequently reported to be deleted. LOH was observed frequently in chromosomes 1 and 2; the loss was either paternal or maternal depending on the chromosome. The lost region of chromosomes 1–3 contained breast cancer–related genes including Pten, Pik3r1, Fas, Map3k1l, Wt1, Ijlst, Rad51, Bcl2l11 and Tp53bp. Among these genes, expression of Fas and Ijlst was significantly lower than that in the normal mammary gland.

Conclusions: The loss of above chromosomal regions may be causative in radiation induction of rat mammary carcinoma through abrogation of apoptosis and interleukin 6 pathways.

POSTER 15

Dynamics of delayed p53 mutations in mice given whole-body irradiation at eight weeks. Ryuji Okazaki1, A. Ootsuyama1, Y. Mabuchi1, Y. Matsuoka2, Y. Michikawa2, T. Ima1, 1: University of Occupational and Environmental Health, Japan, 2: Keio University, Japan, 3: National Institute of Radiological Sciences, Japan.

Purpose: Ionizing irradiation might induce delayed genotoxic effects in a p53-dependent manner. However, there are a few reports that show a p53 mutation as a delayed effect of radiation. In this study, we investigated the p53 gene mutation by the translocation frequency in chromosome 11, loss of p53 alleles, p53 gene methylation, p53 nucleotide sequence and p53 protein expression/phosphorylation in p53+/- and p53-/- mice after irradiation at a young age.

Materials and Methods: p53+/- and p53-/- mice were exposed to 3 Gy of whole-body irradiation at eight weeks of age. Chromosome instability was evaluated by FISH analysis, p53 allele loss was evaluated by PCR, and p53 methylation was evaluated by methylation-specific PCR. p53 sequence analysis was performed. p53 protein expression was evaluated by western blotting.

Results: The translocation frequency in chromosome 11 showed a delayed increase after irradiation. In old irradiated mice, the number of mouse that showed p53 allele loss and p53 methylation increased compared to these numbers in old non-irradiated mice. In two old irradiated p53-/- mice, the p53 sequence showed hetero-mutation. In old irradiated mice, the p53 and phospho-p53 protein expressions decreased compared to old non-irradiated mice.

Conclusion: We concluded that irradiation at a young age induced delayed p53 mutations and p53 protein suppression.

POSTER 16

Molecular and clinic-pathological analysis of sporadic pediatric thyroid cancers in Belarus. Tatiana Rogounovich1, V. Saenko1, S. Mankovskaya1, M. Fridman1, M. Mutsue2, N. Mitsuake2, Y. Demidchik2, Y. Yamashita1, 1: Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, Japan, 2: Department of Helath Risk Control, Atomic Bomb Disease Institute, Nagasaki University, Japan, 3: Institute of Physiology, NAS of Belarus, 4: Republican Centre for Thyroid Tumors, Minsk, Belarus, 5: Belarusian Medical Academy for Post Graduate Education, Minsk, Belarus.

Mutational and clinical features in pediatric patients with papillary thyroid carcinoma (PTC) in Belarus have been mostly studied in radiation-related series after the Chernobyl accident. Comparative characteristics of currently diagnosed childhood PTCs remain poorly addressed. In this investigation we analyzed the prevalence of BRAF T1799A point mutation, RAS gene family mutations, and RET/PTC and AKAP9/BRAF rearrangements in 34 sporadic PTCs diagnosed in Belarusian children who were born 2 - 16 years after the Chernobyl accident and therefore were not exposed to radioactive fallout. Mean age at the time of operation was 12.4 ± 2.4 y.o.; range 5 - 15 y.o. Sex distribution in the analyzed group was: males – 12/34 (35.3%), females – 22/34 (64.7%); sex ratio 0.55. Tumor size varied from 6 to 45 mm, mean 16.1 ± 9.0 mm. We found 10/34 (29.4%) of tumors with RET/PTC rearrangements (4 of 10 (40%) with RET/PTC1 and 6 of 10 (60%) with RET/PTC3 rearrangements) and 5/34 (14.7%) tumors with BRAF mutation, RET/PTC rearrangements and BRAF mutation did not coexist in any tumor. No mutations in codons 12, 13 and 61 of K-, N- and H-RAS, or AKAP9/BRAF rearrangement were detected. The subgroups of patients harboring different genetic abnormalities did not differ significantly in terms of age, tumor size and morphology, perhaps due to relatively small sample size. Mutational frequencies in this series, although being somewhat lower for RET/PTC rearrangements and slightly higher than expected for BRAF T1799A, did not differ significantly from the previously reported prevalence of these mutations in radiation-induced and sporadic pediatric PTCs. Clinical and pathomorphological characteristics, such as extrathyroidal invasion, lymph node involvement and distant metastases, tumor stage, association of the conventional papillary variant of PTC with RET/PTC3 parallel those described in radiation-induced pediatric PTCs. The obtained results demonstrate that sporadic childhood PTCs display clinical features similar to those of radiation-induced PTCs, and therefore they require the same treatment strategy.
Iozizing radiation is a well-recognized etiological factor that increases the risk of the development of papillary thyroid carcinoma (PTC) in exposed individuals. The purpose of this work was to determine whether inherited genetic variability may play a role in susceptibility to radiation-related PTC after Chernobyl.

To identify genetic factors, a large-scale case-control association study was undertaken. A total of 667 patients diagnosed for PTC during 1989–2009 and 827 healthy individuals from Belarus, and 448 population controls from Russia were enrolled. A genome-wide association study (GWAS) performed in two phases was followed by a validation study involving an additional independent set of cases and controls.

Our GWAS identified four SNPs in strong linkage disequilibrium (LD) with each other at chromosome 9q22.33 significantly associated with the disease. A validation analysis for one of these SNPs (rs965513) returned an overall P=4.8E-12 by meta-analysis with an odds ratio of 1.65 (95% confidence interval: 1.43–1.91). This SNP is located within an LD block centromeric to the FOXE1 gene which encodes a thyroid-specific transcription factor TTF2. Rs965513 was recently shown by GWAS to be the strongest genetic marker of sporadic thyroid cancer in individuals of European descent. Another study, using a target gene approach, reported a functional SNP (rs1867277) within the LD block spanning FOXE1 as associating with sporadic PTC. Interestingly, rs944289 on chromosome 14q13.3 in the proximity of the NKX2-1 gene (encodes a thyroid-specific TTF1 transcription factor) showing strong association with sporadic PTC in Europeans, was not significant in our results (P=0.17). Instead, we observed a strong tendency for two other SNPs on chromosomes 4q and 5q associating with disease risk, but genotyping of additional samples, currently underway, is necessary to validate their significance.

We conclude that among the genetic factors affecting risk for developing radiation-induced PTC, the strongest is the same which confers predisposition to the sporadic form of this cancer and that the effect of putative radiation-associated markers, in terms of its strength, comes next to and after the general susceptibility to thyroid cancer.

PO353-18. Radiation-induced marked reduction in hematopoietic progenitor cells in infant leukemia prone C3H/He mouse, Yoshia Shimada1, K. Ariyoshi1, S. Kakinuma2, T. Takabatake3, M. Shinagawa2, K. Kadono2, M. Nishimura1, 1: National Institute of Radiological Sciences, Japan, 2: National Institute of Radiological Sciences, Hiroshima University, Japan

Background: Age–at–exposure is a critical factor that influences the risk of radiation leukenogenesis. Whereas adult C3H/He mice are prone to develop myeloid leukemia after ionizing-radiation (IR) exposure, fetal and neonatal mice are resistant. Nakano et al. reported that dose response of chromosomal translation in hematopoietic cells was not observed in mice irradiated in utero or soon after birth. They hypothesized that the fetal or neonatal hematopoietic stem cells are genetically highly sensitive to ionizing radiation, so that the aberrant cells disappear soon after IR exposure. The purpose of this study was to determine the sensitivity to IR of developing hematopoietic cells both in vitro and in vivo.

Experimental procedures: After in vivo gamma-ray irradiation to the 1 week-old to 14 week-old C3H/He mice, the survival of hematopoietic progenitor cells was determined by both colony forming assay in vitro using MethoCult kit and spleen colony formation assay in vivo. The gene expression analysis of bone marrow cells from 1 week-old and 8 week-old mice were also examined.

Results: A marked reduction in the number of colony forming cells was observed after in vivo irradiation in the 1 week-old mice; the colony forming unit-granulocyte macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E) and colony forming unit-spleen (CFU-S) of irradiated 1 week-old mice were more radiosensitive than those of older ages. Interestingly, in vitro irradiation did not show age difference between 1 week-old mice to 14 week-old mice. This suggests that in vivo microenvironment and/or its response to IR critically affects the radiation sensitivity of hematopoietic progenitor cells.

Further, the gene expression profiles of bone marrow cells revealed that expression of cytokines and chemokines which can stimulate hematopoietic progenitor cell growth or survival, were reduced in 1 week-old mice compared to those of 8 week-old mice.

Conclusion: These results demonstrate that hematopoietic progenitor cells of neonatal stage are radiation sensitive, which may associate the lack of expression of survival cytokine and chemokine after ionizing radiation.

PO353-19. Analysis of Non-Tumor Dose for Radiation-Induced Cancer as a Function of Dose-Rate, Hiroshi Tanooka, Radiation Effects Association, Japan

Radiation-induced cancer risk depends not only on the total dose of radiation delivered, but also on the dose-rate and the whole-body or partial body exposure conditions. To demonstrate the dose-rate effect on the radiation-induced cancer risk, non-tumor dose (Dn), defined as the highest radiation dose at which no statistically significant increase of tumors is observed above the control level, was surveyed on the dose-response curves for radiation-induced cancers of experimental animals and humans in the literature, and plotted as a function of dose-rate, ranging from 2x10^8 Gy/min, an environmental level, to 10^13 Gy/min, A-bomb survivors level. Dn values were grouped in four categories, i.e., for low and high LET and for whole-body and partial-body exposures and the regression line was drawn for each exposure condition. For whole-body radiation with low LET, Dn appeared to be constant for dose-rate from 10^5 down to 1 Gy/min, below which Dn increased with decreasing dose-rate up to 20-fold at an environmental level. Partial-body radiation exhibited 10-fold higher Dn with similar dose-rate dependence. High LET radiation also exhibited the dose-rate dependence of Dn at a 10-fold lower level as compared with low LET. The discrepancy in the dose-response between leukemia incidence in A-bomb survivors and bone sarcoma incidence in radium painters in the literature can be explained by a difference in Dn in the regression line between exposure conditions, i.e., acute whole-body and chronic partial-body exposures.

In conclusion, the non-tumor dose Dn inversely varies with the dose-rate of radiation, more than 100-fold depending on exposure condition, and serves as a measure of the safety level for cancer risk of radiation at different exposure patterns.


PO353-20. Opposite modifying effects of HR and NHEJ deficiency on cancer risk in PTC1 heterozygous mouse cerebellum, Mirella Tannol1, A. Saran1, E. Pasquali1, S. Leon1, S. Lopriore1, V. Di Majo1, G. Tacciali2, J. Essers3, R. Kanaar4, L.H. Mullenders5, M.J. Atkinson6, M. Mancuso7, S. Pazzaglia8, 1: Laboratory of Radiation Biology and Biomedicine, Agenzia Nazionale per le Nuove Tecnologie, l’Energia e lo Sviluppo Economico Sostenibile (ENEA) CR-Casaccia, Rome, Italy, 2: Department of Radiation Physics, Università degli Studi Guglielmo Marconi, Rome, Italy, 3: Goldman School of Dental Medicine, Boston University, USA, 4: Cancer Genomics Center, Netherlands, 5: Erasmus Medical Center, Rotterdam, Netherlands, 6: Leiden University Medical Centre, Leiden, Netherlands, 7: Helmholtz Zentrum München-German Research Center for Environment and Health, Neuhemberg, Germany

Recent evidence has linked DNA damage and DNA repair alterations with brain tumors. Glialia is associated with germline abnormalities manifested as either gene polymorphisms or hereditary mutations of DNA repair genes. Moreover, various combinations of targeted deletions in genes controlling cell cycle checkpoints, apoptosis and DNA repair result in medulloblastoma (MB) in mice. Non-homologous end joining (NHEJ) and homologous recombination (HR) contribute to genome stability, and deficiencies in either pathway predispose to genome rearrangements. We tested a role of defective HR or NHEJ in tumorigenesis in a well established MB mouse model, the Patched1 heterozygous (Ptc1+/+), mice, in which neonatal exposure to ionizing radiation dramatically increases the frequency and shortens the latency of MB. Loss of the normal remaining Ptc1 allele is characterized by forming unit-granulocyte macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E) and colony forming unit-spleen (CFU-S) of irradiated 1 week-old mice were more radiosensitive than those of older ages. Interestingly, in vitro irradiation did not show age difference between 1 week-old mice to 14 week-old mice. This suggests that in vivo microenvironment and/or its response to IR critically affects the radiation sensitivity of hematopoietic progenitor cells.

Further, the gene expression profiles of bone marrow cells revealed that expression of cytokines and chemokines which can stimulate hematopoietic progenitor cell growth or survival, were reduced in 1 week-old mice compared to those of 8 week-old mice.

Conclusion: These results demonstrate that hematopoietic progenitor cells of neonatal stage are radiation sensitive, which may associate the lack of expression of survival cytokine and chemokine after ionizing radiation.

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Molecular epidemiology study of Chernobyl thyroid cancer, Vladimir Saenko1, M. Takahashi2, T. Rogovinovitch3, T. Kawaguchi4, V. Drozd4, N. Akulevic4, L. Damlova4, Y. Demidchik4, M. Lischchuk4, N. Mitoutake4, R. Yamada5, M. Latrop5, F. Matsuoka6, S. Yamashita1, 1: Department of Health Risk Control, Atomic Bomb Disease Institute, Nagasaki University, Japan, 2: Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Japan, 3: Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, Japan, 4: Belarusian Medical Academy for Post Graduate Education, Minsk, Belarus, 5: Centre National de Genotypage, Institut Genomique, Evry, France

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PKcs alleles were monitored for MB development. We also examined the effect of Rad54- or DNA-PKcs-deletion on the processing of endogenous and radiation-induced double-strand breaks (DSBs) in neural precursors of the developing cerebellum, the cells of origin of MB. We found that, although HR and NHEJ collaborate in protecting cells from DNA damage and apoptosis, they have opposite roles in MB tumorigenesis. In fact, while Rad54 deficiency increased both spontaneous and radiation-induced MB development, DNA-PKcs disruption suppressed MB tumorigenesis. Our data provide the first evidence that the distinct activities of DSBs repair pathways may differentially affect cancer risk by radiation.

POS35-21. RBE of mammography X-rays at the level of DNA dsb and chromosomal damage. Bert Thieryns, A. Baert, V. Vandersickel, J. Depuydt, A. Vral, University of Gent, Belgium

Introduction: Breast cancer (BC) affects approximately 1 in 9 women. In many countries, BC screening programs based on periodic mammography exist for women aged between 50–70 years to diagnose BC in an early stage. Although breast doses are low in mammography (typically 4 mGy two-view mammography), the risk for radiation-induced BC cannot be neglected in view of the large population size and the repetitive character involved in this type of asymptomatic screening. Furthermore, recent studies pointed to a significantly higher radiobiological efficiency of low-energy X-ray beams used for mammography (30kV) compared to conventional higher kV X-rays. In view of the importance of a correct calculation of the detection over induction ratio in the screening population more in vitro radiobiological studies are needed to investigate the damaging effect of mammography X-rays at DNA and chromosomal level.

Methods: Blood of 5 healthy donors was irradiated in vitro with mammography X-rays and Co-60 g-rays with doses ranging from 5 to 2000 mGy. The gH2AX-foci technique was used to quantify the DNA double strand breaks (dsb) 30 min after irradiation in lymphocytes. The resulting chromosomal damage, after repair/misrepair of the DNA dsb, was quantified with the micronucleus (MN) assay.

Results: For both detection qualities and endpoints the threshold detection dose, leading to a significant increase compared to sham-irradiated controls, was determined. For the gH2AX-foci test a threshold dose of 10 mGy was obtained for mammography X-rays compared to 100 mGy for g-rays. For the MN-assay a threshold dose of 50 mGy was obtained for mammography X-rays compared to 200 mGy for g-rays. Calculation of RBE values for mammography X-rays resulted in RBE values higher than 1 for both endpoints. In the low-dose range (0-100mGy) an RBE of 1.8 was obtained with the gH2AX-foci assay. For the MN-assay the RBE is dependent of the dose and ranges from 5.7 to 4.2 in the dose range of 50-100 mGy.

Conclusion: Our results confirm literature data regarding the higher RBE of mammography X-rays. Comparison of the RBE values obtained with both endpoints shows that mammography X-rays not only induce more dsb compared to g-rays but also have a higher mutagenic potential probably because of the more complex DNA damage relative to the higher LET nature of mammography X-rays.

POS35-22. Sex-Dependent Differences in Intestinal Tumorigenesis induced in Apc1638N/+ Mice by low- and high-LET Radiation. Daniela Tranii, B. Mooni, B.V.S. Kallakuryii, D.P. Hartmannii, K. Dattai, A.J. Fornace Jrii, 1: Department of Biochemistry and Molecular & Cell Biology and Lombardi Comprehensive Cancer Center, Georgetown University, Washington DC, USA; 2: Georgetown University Medical Center, Washington DC, USA

Introduction: Studies on the Childhood Cancer Survivor Study (CCSS) and the Life Span Study (LSS) of the A-bomb survivors have shown an association between radiation exposure and cancer risk, including small intestine and colorectal (CRC) cancer. Although multiple published studies have described that exposure to ionizing radiation (IR) significantly enhances intestinal tumorigenesis in adenomatous polyposis coli (APC) mutant mouse models, previous work has focused only on effect of high radiation doses (≥2 Gy) and low-linear energy transfer (LET) radiation. Furthermore, no investigations have assessed sex-differences in the effects of MB induced by ionizing radiation. In this study, we aimed to assess the effect of low-radiation doses and different LETs, and to investigate in vivo the interplay of sex and radiation with regard to intestinal tumorigenesis.

Methods: Six- to eight-week old female and male C57BL/6J-Apc1638N+ mice were exposed whole-body to either 5 Gy or 1 Gy of g-rays, or equitoxic doses of 1 GeV/n Fe ions and protons. Exposed and age-matched control mice were euthanized when irradiated animals were moribund. Intestinal tissues were fixed and tumor number, distribution and grade were assessed, along with molecular parameters relevant to proliferation, sex-hormone receptors expression and Wnt/Catenin signaling. Molecular differences were investigated also by Western Blot of protein lysates from intestinal full-thickness or epithelial cells only.

Results: Female mice showed a significant protection from low-LET radiation induced tumorigenesis compared to males: at 5 Gy, a ~10-fold increase was observed in intestinal tumor burden for male mice compared to ~3-fold in females. When molecular analyses were performed, we found in irradiated males long-term changes in both normal epithelium and adenomas/carcinomas. These sex-dependent differences were modified by exposure to high-LET radiation.

Conclusions: Our data provide the first in vivo evidence that exposure to low doses (≤ 1 Gy) of low- and high-LET radiation produced a significant increase in intestinal tumor multiplicity in Apc1638N/+ mutant mice, both in small and large intestine. Furthermore, we also showed that existing mouse models can be employed to dissect a potential role of sex-hormones in radiation-induced intestinal tumorigenesis.

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POS36-01. Can radiation-induced transcription of human endogenous retrovirus-K (HERV-K) contribute to enhancing immunogenicity of prostate cancer? Lorenzo Agou, I. Lenzii, H. Zhang1, C. Guah1, 1: Pathology Department, Albert Einstein College of Medicine of Yeshiva University, USA; 2: Genetics Department, Albert Einstein College of Medicine of Yeshiva University, USA; 3: Radiation Oncology Department, Albert Einstein College of Medicine of Yeshiva University, USA; 4: Pathology Department and Radiation Oncology Department, Albert Einstein College of Medicine of Yeshiva University - Montefiore Medical Center

Ionizing Radiation (IR) has been shown to increase the immunogenicity of cancer cells by modulating various cellular processes, including antigen presentation by MHC. Previous experiments in our laboratory detected HERV-K-derived peptides exclusively from irradiated LNCaP cells upon proteomic LC-MS/MS analysis of peptides eluted from MHC-I complexes. We hypothesized that IR could reactivate subsets of Human Endogenous Retroviruses (HERVs), which are retrovirus integrated into the human genome, remnants of ancient retroviral infections, and that HERV-derived peptides, the final product of HERVs transcription, will increase the diversity of the antigen repertoire presented by irradiated cancer cells and may thus enhance anti-tumor immune responses. HERVs are known to be transcribed in numerous diseases including cancer, HIV infection, and autoimmune disorders. HERV-K is the most recently integrated and thus intact HERV family, with approximately 350 loci in the human genome, 98 to 99% identical. To test the influence of IR on HERV-K transcription, four prostate cancer cell lines (LNCaP, DU145, PC3 and VCaP), a normal prostate epithelium cell line (RWPE-1) and a normal fibroblast cell line (BJ) were analyzed by RT-PCR and qRT-PCR before and after 20 Gy γ-irradiation. HERV-K transcription was identified in all cell lines studied. After IR, HERV-K transcription was increased by 3- to 10-fold in cancer cells and decreased at least 10-fold in normal cells. Individual active HERV-K loci were identified by cloning and sequencing: seven different loci were identified among the cell lines tested, each cell line preferentially activating specific loci. IR was found to influence the activation or suppression of specific loci, two being activated (22q11.23 and 5p13.3) and two others inactivated (21q21.1 and 19q13.12) after IR. Most of the sequenced transcripts contained open reading frames compatible with translation to proteins. These findings show that HERV-K is actively transcribed in prostate carcinoma cell lines. The IR-dependent and cancer-selective increase in HERV-K transcription and HERV-K-derived MHC-I peptide presentation likely enhances antigen repertoire diversity, may contribute to the immunomodulatory properties of IR, and offers attractive targets for the development of novel IR-enhanced immunotherapies for cancer.

POS36-02. Correlation between miRNA expression and the radiation response in breast cancer cell lines. Nataša Anastasović, I. Höfging, S. Winkler1, M. Aubelé, M.J. Atkinson, 1: Helmholtz Zentrum München, Institute of Radiation Biology, Germany; 2: Helmholtz Zentrum München, Institute of Pathology, Germany
MicroRNAs (miRNAs) modulate gene expression and they are implicated in the regulation of cellular sensitivity upon radiation therapy. To test this hypothesis we analyzed the miRNA changes induced by radiation in breast cancer cell lines in correlation with protein expression changes of major breast cancer tumour markers (HER 2, HER 3, p21, PR and ER). Increased HER2 / HER3 receptor and p21 expression at protein levels was detected 24 hours after irradiation (60 Gy). In MDA-MB-361 cells, a different response to 2.5 and 5.0 Gy irradiation after cell cycle, cell proliferation and colony formation assay analysis. Therefore, 24 hours after 5.0 Gy irradiation we screened 364 miRNAs for expression changes using a TaqMan-based assays. Twenty two miRNAs showed increased expression and 13 miRNAs showed reduced expression in T47D cells. In MDA-MB-361 cells increased expression was detected for 6 miRNAs and 23 miRNAs showed reduction. Furthermore, reduction in miR-21 expression was characterized with single TaqMan miRNA assays 4 hours and 24 hours after 2.5 and 5.0 Gy irradiation. Additionally miR-21 was upregulated and downregulated using lentivirus vectors and effects on HER receptor expression and downstream signalling molecules activation were analyzed. The results confirm that miRNA expression is influenced by radiation and is accompanied by changes in tumour marker expression (HER 2, HER 3 and p21). Based on these results we conclude that miRNAs have a role in HER receptor signaling pathways regulation and downstream targets activation. Additional analysis will show if there is an association between changed miRNA expression and tumour resistance response after radiotherapy.

Pos36-03. Epidermal Growth Factor Receptor Inhibition Improves the Tumor Reoxygenation by Modulating Vascular Response to Fractionated-radiotherapy. Fanghsin Chen¹, S. Fiu¹, C. Chiang², C. Wang², J. Hong², 1: National Tsing Hua University, Taiwan, 2: Chang Gung Memorial Hospital, Taiwan

Gefitinib is a potent inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase and has been demonstrated to treat advanced or metastatic non-small cell lung cancer (NSCLC) after failure of first-line chemotherapy. However, little is known about its effects when combined with radiotherapy. In the present study, we addressed whether inhibition of EGFR tyrosine kinase activity by gefitinib during daily radiotherapy affected tumor microenvironment with respect to tumor growth, hypoxia, number of vessels, and distribution of tumor-associated macrophages (TAMs). A syngeneic TRAMP C-1 tumor model of mice was generated and treated with either gefitinib, 60 Gy irradiation in 15 fractions, or combination of gefitinib and irradiation. Tumor necrosis, hypoxia as well as vascular numbers were quantified by image analysis of tumor sections. Quantitative data demonstrated gefitinib alone did not significantly change tumor microenvironment, but fractionated-irradiation decreased tumor necrosis, hypoxia and number of vessels comparing to size-matched control tumors. Besides, irradiation caused vascular dilatation and improved the pericytes adhesion to vascular wall. Avascularized hypoxic regions became infiltrated with aggregates of CD68 positive TAMs, reaching a maximum at the end of irradiation. Combination with gefitinib protected tumor vessels from irradiation damage and resulted in elevating vessel numbers with good perfusion but rare adhesion of pericytes. Tumor necrosis and hypoxia further decreased in combination treatment tumors and aggregation of TAMs in hypoxia region was also interrupted which rendered TAMs to distribute randomly among tumors. These results suggest that inhibition of EGFR activity in tumors could modulate vascular response to irradiation and improve tumor reoxygenation, thus provides a potential strategy to enhance drug delivery during fractioned-irradiation procedure.

Pos36-04. TM4SF4 affects the radiation resistance and cell migration in non small cell lung cancer. Soo Im Choi¹, T.L. Kim¹, M.J. Kim¹, I.G. Kim¹, 1: Department of Radiation Biology, Environmental Radiation Research Group, Korea Atomic Energy Research Institute, South Korea, 2: University of Science and Technology (UST), Daejeon, South Korea

Purpose: The aim of this study is to investigate new important radiation resistance genes that make cells resistant to γ-radiation, anticancer drugs and toxic chemicals.

Experimenter Human lung adenocarcinoma A549 cells were obtained from the American Type Culture Collection and cultured in RPMI-1640 media at 37°C in a 5% CO₂-humidified incubator. For colony-forming assays, cells were plated in 35-mm culture dishes at a density of 5 x 10³ cells/plate and after 24, they were immediately irradiated with a single exposure to a dose of 2Gy (10Co γ-ray source; dose rate, 0.2 Gy/min).

Summary: TM4SF4, membrane protein of the tetraspanin, is involved in the signaling pathways of development, growth and motility of cells. To investigate and identify the function of γ-radiation resistance-related genes, gene expression pattern in A549 cells highly resistant to radiation and H460 cells showed low extent of resistance were compared using DNA chip analysis. It was observed that the expression of TM4SF4 in A549 cells significantly increased when compared to H460 cells. In this study, we showed that TM4SF4 suppression with siRNA in A549 cells induced the decrease of radiation resistance and cell growth inhibition. On the other hand, TM4SF4 overexpression resulted in increment of radiation resistance and cell growth. Moreover TM4SF4 affected significantly cell migration. TM4SF4 activated insulin growth factor receptor (IGFR)-b and subsequently AKT phosphorylation that affect radiation resistance and cell growth. In conclusion, regulation of cell growth and radiation resistance by TM4SF4 may result from IGFR-b-AKT signaling pathway

Conclusion: This study provides the first demonstration that TM4SF4 participates in novel mechanisms regarding resistance to a γ-radiation and cell growth. [The authors wish to acknowledge the financial support of the Ministry of Education, Science and Technology Nuclear Research & Development Program (of the Republic of Korea).


The aim of this study was to evaluate the impact of radiation treatment on tumor microvessels integrity using contrast-enhanced microCT in Glioblastoma Multiforme (GBM). Orthotopic U87-MG GBM xenografts (n=50) were established in female nu/nu mice and treated daily with 2 Gy X-irradiation (RT) for 7 consecutive days to a total of 14 Gy using two treatment regimes (single 200 cGy fraction or 10 pulses of 20 cGy each). In a preclinical study, we addressed whether pulsed radiotherapy has a protective effect with respect to microvessel integrity immediately irradiated with a single exposure to a dose of 2Gy (10Co γ-ray source; dose rate, 0.2 Gy/min). Tumor response was volumetrically determined using contrast-enhanced microCT (Omnipaque 350) using a GE FLEX Triumph® combined PET-SPECT-CT system with CT energy set to 80Kvp250mAs. Raw microCT data were reconstructed to enhance tumor contrast and density values were converted to Houndsfeld Units (HU) for densitometric analysis using the OsiriX imaging suite. Control animals and those treated with conventional RT, with or without TMZ, caused a pronounced increase in tumor contrast enhancement at the time of sacrifice. The pulsed RT regimen, with or without TMZ, resulted in a substantial reduction and contrast enhancement, suggesting that microvessel integrity was maintained. These observations were confirmed with H&E histology. This pre-clinical study demonstrated that pulsed radiotherapy has a protective effect with respect to microvessel integrity, as compared to controls and conventional chemoradiotherapy. Moreover, contrast-enhanced CT has proven to be a reliable surrogate for vascular integrity since contrast-enhancement is significantly seen in areas of damaged vasculature. Such an imaging protocol could be integrated with evolving technologies that permit proper adaptive radiation treatment planning using a collimated beam for small animal work, based on microvessel integrity.

Pos36-06. The pO2 fluctuation pattern and cycling hypoxia in human cervical carcinoma and melanoma xenografts. Christine Ellingsen¹, K.M. Øvrebo², K. Galappathi², B. Mathiesen², E.K. Ro frostad², 1: The Norwegian Radium Hospital, Norway, 2: Oslo University Hospital, Norway

Purpose: The blood perfusion in tumors is spatially and temporally heterogeneous, resulting in local fluctuations in tissue oxygen tension (pO2) and tissue regions showing cycling hypoxia. In this study, we investigated whether the pO2 fluctuation pathways showed the extent of cycling hypoxia may differ between tumors having developed blood vessels that are located within broad bands of connective tissue (cervical carcinoma xenografts) and tumors having developed blood vessels that are not surrounded by connective tissue (melanoma xenografts).

Methods and materials: Two cervical carcinoma lines (CK-160, TS-415) and two melanoma lines (A-07, R-18) transplanted into
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BALB/c nu/nu mice were included in the study. Tissue pO2 was measured simultaneously in two positions in each tumor by using a two-channel OxyLite fiberoptic oxygen-sensing device. The extent of acute and chronic hypoxia was assessed by combining a radiobiological and a pimonidazole-based immunohistochemical assay of tumor hypoxia.

Results: The proportion of tumor regions showing pO2 flucutations, the pO2 fluctuation frequency in these regions, and the relative amplitude of the pO2 fluctuations were significantly higher in the melanoma xenografts than in the cervical carcinoma xenografts. The cervical carcinoma and the melanoma xegrafts did not differ significantly in the fraction of acutely hypoxic cells or the fraction of chronically hypoxic cells. However, the ratio of the fraction of acutely hypoxic cells to the fraction of chronically hypoxic cells was significantly higher in the melanoma than in the cervical carcinoma xenografts.

Conclusions: The temporal heterogeneity in blood flow and tissue pO2 in tumors may depend on the tumor histology. Connective tissue surrounding microvessels may stabilize the blood flow and pO2 and thus protect the tumor tissue from cycling hypoxia.

POSS6-07. Manipulation of lactate metabolism and radiation resistance in two human ovarian cancer cell lines. Christian G. Fabian, A. Siebers , W. Müller-Klieser , U.G.A. Sattler, Institute of Physiology and Pathophysiology, University Medical Center of the Johannes Gutenberg University Mainz, Germany

Malignant transformation is associated with an increase in glycolytic flux and an enhanced accumulation of lactate even under normoxic conditions. This phenomenon, termed “Warburg effect”, is mainly caused by an upregulation of numerous glycolytic and glycolysis-related genes in the majority of human tumors. Therefore, manipulation of the glycolytic pathway may alter tumor cell metabolism and thereby influence its radiobiological properties.

In the present experimental study, two human ovarian cancer cell lines IGROV-1 and OC316, were characterized according to their metabolic and radiobiological properties with and without addition of oxamate (an inhibitor of lactate dehydrogenase). The influence of 20-60 mM oxamate was investigated using the Seahorse extracellular flux analyzer XF24-3 which allows for simultaneous measurements of extracellular oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) in vitro. Colony forming assays were accomplished from cells irradiated with X-ray doses from 0-8 Gy with and without oxamate, γH2AX foci were counted by counting the number of foci per nucleus 1 and 24 h after irradiation via a computer based analysis software.

Both cell lines showed a significant decrease (p < 0.000001) of ECAR and an increase of OCR 100 min after addition of oxamate. OC316 cells revealed a 50% reduction in ECAR and an increase to 174% in OCR. ECAR and OCR of IGROV-1 were decreased by 69% and increased to 154%, respectively. Dose response curves illustrated a significantly higher radiation resistance of untreated IGROV-1 cells compared to OC316. Clonogenic cell survival increased up to 155% or 146% after 4 Gy X-ray irradiation with oxamate compared to control cells. IGROV-1, respectively. 6 Gy potentiated the oxamate effect up to a 7- and 5-fold increase of clonogenic cell survival, respectively. For both cell lines the number of γH2AX foci was elevated dose-dependently. Consistent with their higher radiation resistance, IGROV-1 showed enhanced reduction of γH2AX foci 24 h after irradiation. Further experiments regarding the influence of oxamate to double strand break formation after irradiation are in progress. (Supported by the Deutsche Forschungsgemeinschaft SA 1749/3-1).

POSS6-08. Radiosensitization of tumors by 2-DG: Cross talk among different immune components. Abdullah Farooque1, L. Alagh1, P. Chhabra 1, Deepi 1, J.S. Adhikan2, F. Afrin3, A. Verma3, S. Khanna3, B.S. Dwarkanath3, 1: INMAS, India, 2: Department of Biotechnology, Lovely Professional University, Jalandhar, India, 3: Department of Biotechnology and Bioinformatics, Dr. D.Y. Patil University, Pune, India, 4: School of Biosciences and Technology (SBST), VIT University, Vellore, India, 5: Division of Radiation Biosciences, INMAS, Delhi, India, 6: Department of Biotechnology, Jamia Hamdard University, Delhi, India

The glycolytic inhibitor, 2-deoxy-D-glucose (2-DG), has been found to enhance the effects of radiation and chemotherapeutic drugs in vitro, while a heterogeneous response has been reported for the local tumor control in vivo. Since immune system is one of the important factors that contribute to the systemic responses, we investigated the effects of the combined treatment (2-DG+ Radiation) on different immune components in responders and non responders. Local tumor control was also investigated in nude mice to establish the role of immune system in the radiosensitization of tumors by 2-DG.

Ehrlich ascites tumors were grown in the hind leg of Strain A mouse and focally irradiated (10 Gy) immediately after 2-DG administration (2mg/kg b.w, intravenous). Blood profiling, antibody classes and cytokine levels of certain detection factors were analyzed by ELISA and CBA, flow Cytometry and western blotting respectively.

Animals with complete tumor regression (complete responders; CR) showed maximum lymphopoenic condition at 24 h after treatment as compared to the partial responders (growth delay; PR) after the combined treatment, which is a suggestive of the removal of tumor tolerant immune cells. Restoration of CD4/CD8 ratio and decrease in T-regulatory cells was observed in CR as compared to PR. Decreased Fox P3 and GATA3 localization was observed in CR at later point (21 days). Furthermore, increase in Th1 cytokines such as IFN-gamma and TNF-alpha and decrease in Th2 cytokines such as IL-4 and IL-10 was observed only in responders. These findings were further strengthened by the antibody class switching from IgE and IgA to IgG2a in responders, although total IgG was reduced in the responders. The combined treatment did not elicit complete response in any of the tumor bearing nude mice, suggesting that immune modulation plays a vital role in determining local tumor control.

These results emphasize the role of immune system in influencing the local tumor control following the combined treatment (2-DG + Radiation) and provide insight into the molecular targets determining the efficacy facilitating the individualization of therapy.

POSS6-09. In vitro studies on phosphatidylinositol-3-kinase-Akt pathway inhibition and radiation in malignant glioma. Carlos Alexandre Pedregal1,2, B. Garicóchea, G.J. Peters3, B. Slotman4, J. van den Berg5, L. Stappers1, B. Baumert4 and P. Sminia1, 1: Pontificia Universidade Católica do Rio Grande do Sul; Brazil, 2: Medical Oncology VU University Medical Center, Amsterdam, Netherlands, 3: Radiation Oncology, VU University Medical Center, Amsterdam, Netherlands, 4: Department of Radiation Oncology, Academic Medical Center, Amsterdam, Netherlands, 5: Department of Radiation Oncology, Maastro Clinic, Maasstricht, Netherlands

Purpose: Glioblastoma multiforme (GBM) is the most common, invasive and deadly primary type of malignant brain tumor. Current therapy consists of surgery, radiotherapy (IR) and chemotherapy with temozolomide (TMZ). The phosphatidylinositol-3-kinase pathway is commonly overexpressed in GBM, leading to activation of the downstream serine/threonine kinase Akt. The pathway is involved in apoptosis, cell proliferation, differentiation, migration and metabolism of the cell, and is often associated with resistance to chemotherapy and IR. In the present study, we evaluated the effect of the specific Akt pathway inhibitor MK-2206 and IR on glioma cells in vitro.

Materials and Methods: Experiments were performed on a panel of six GBM cell lines (U251, U251-TMZ resistant variant-, T98, D384, U87, VU-122). Cells were treated with the allosteric pathway inhibitor MK-2206 alone or in combination with irradiation. Endpoints: cell survival (clonogenic assay), cell migration (scratch wound assay), cell invasion (transwell Boyden chamber technique) and expression of the key proteins PTEN, Akt and pAKT (western blot).

Results: MK-2206 was cytotoxic for all glioma cells in the dose range between 1 – 10μM for 24 hours. In addition, 8 hours exposure to 10μM of drug inhibited cell migration ranging from 26 to 68%. The invasion capacity was assessed at increasing doses of 1μM, 5μM and 10μM MK-2206 for 24 hours. A dose-dependent increase in inhibition of invasion was noticed for all but one of the cell lines. Irradiation (4 Gy) alone increased the expression of γH2AX, which was inhibited (30min, 1h, 2h and 4h) following pre-incubation with MK-2206 (1μM and 10μM for 1h).

Conclusion: Targeting of the PI3Kinase-Akt pathway is a promising tool in GBM therapy and might counteract resistance to irradiation.


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POSS6-10. Chronological responses of CD11b+ Gr-1+ cells in irradiated tumors; early recruitment and subsequent aggregation at necrotic regions caused by chronic hypoxia. Sheng Yung Fu1, C.S. Chiang1, F.H. Chen1, C.C. Wang2, J.H. Hong2, 1: Department of
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CD11b+ Gr-1+ cells, known as myeloid derived suppression cells, are heterogeneous groups including CD11b+Ly6G-CD16+ neutrophil-like cells and CD11b+Ly6GLy6C+ monocytic-like cells that can mediate tumor growth, angiogenesis and immune suppression, but their function and behavior in irradiated tumor are still unclear. The murine prostate cancer cell line, TRAMP-C1, were inoculated at shank muscle of right leg. When tumor diameter reached 4 mm, tumor was irradiated by single dose of 25 Gy. The distribution pattern and percentage change of CD11b+ Gr-1+ cells in tumor in short-term (4hr), long-term (2 weeks) were determined via IHC staining and flow cytometry. The short-term irradiated tissues did not have any significantly histological changes, such as the ratio of hypoxic or necrotic regions, but the numbers of CD11b+ Gr-1+ cell was increased and were random-distributed within tumors. Both CD11b+Ly6G-CD16+ neutrophil-like cells and CD11b+Ly6GLy6C+monocytic-like cells were increased in post-48hr irradiated tumor as well as in the bone marrow harvested from the non-irradiated left leg. In long-term irradiated tumor tissues, the percentage of CD11b+ Gr-1+ cells were higher than that in control tumor. These cells were aggregated at central necrotic region within chronic hypoxia caused by vessel insufficiency. In summary, we found that local tumor irradiation not only increase the number of CD11b+ Gr-1+ cells in irradiated tumors, but also increase their number in the bone marrow of non-irradiated leg. This indicates that local irradiation has a mechanism to drive the differentiation of CD11b+ Gr-1+ cells. The recruitment of these cells into irradiated tissues is associated with tumor re-growth after radiation therapy. To disrupt the recruitment pathway of CD11b+ Gr-1+ cells may benefit radiation therapy.


Purpose: The aim of this study was to compare the effects of X-ray or carbon-ion (C-ion) irradiation on invasive potential of human pancreatic cancer cell line, MIAPaCa-2.

Experimental procedures: The human pancreatic cancer cell line, MIAPaCa-2 was irradiated with either X-ray (0, 2, or 4 Gy) or C-ion (0, 0.5, 1, 2, and 4 Gy). We examined the migration, invasion, MMP expression and invasiveness in response to MMP inhibitors and/or ROCK inhibitors of irradiated cells, and the activation of Rac1 and Rho.

Summary of data: X-ray irradiation stimulated MIAPaCa-2 invasiveness via induction of MMP-2 activity, whereas C-ion irradiation reduced invasion with decreased expression and activation of MMP-2. The use of MMP-2 inhibitor (MMPI) reduced X-ray induced invasiveness suggested that MMP-2 has role in X-ray induced invasion. However the single use of MMPI did not reduce basal level of invasiveness. ROCK inhibitor in addition to MMP-2 inhibitor was needed to suppress X-ray-induced and basal level of invasiveness indicated that both mesenchymal and amoeboid modes of motility were functioned in MIAPaCa-2 invasion. Thus, we next hypothesized that both modes of motility were inhibited in the C-ion irradiated cells, causing the effective reduction of invasiveness. In fact, the activation of Rac1 and Rho, the key factors functioned in the signal transduction pathway involved in mesenchymal and amoeboid modes of motility, were both diminished in C-ion-irradiated cells, whereas those activations were not reduced in X-ray irradiated cells.

Conclusions: X-ray and C-ion irradiation show differential effects on the invasive potential of MIAPaCa-2 cells with corresponding alterations in the MMP-2 activity and also the activation of Rac1 and Rho signaling. Unlike X-ray irradiation, C-ion irradiation effectively suppresses the invasive potential of MIAPaCa-2 cells.

POS36-12. Clinically Relevant Radiosensitive Cells are Established through Acquired Radiosensitivity by Exposure to Long-Term Fractionated X-ray Radiation. Manabu Fukumoto, Y. Kuwahara, T. Shimura, Dept Pathology, IDAC, Tohoku University, Japan

Although radiotherapy is one of the major therapeutic modalities for eradicating malignant tumors, the existence of radiosensitive cells remains one of the most critical obstacles. Standard radiotherapy consists of fractionated radiation (FR) of 2-Gy X-rays once a day, 5 days a week, over 60 Gy in total. To understand the characteristics of radiosensitive cells and to develop more effective radiotherapy, we established novel radiosensitive cell lines by long-term (> 5 years) exposure to fractionated X-rays. While all the parental human cancer cells (HepG2, HeLa, SAS and KB) ceased, their radiosensitive derivatives continue to proliferate with daily exposure to 2-Gy FR for more than 30 days. Those cells are coined as “clinically relevant radiosensitive” (CDR). While CDR cells retained the phenotype of parental HepG2, the CDR HepG2-8960-R induced by FR were less than those in parental HepG2. Flow cytometric analysis revealed that the proportion of cells in S- and G2/M-phase of the cell cycle was higher in HepG2-8960-R than in HepG2. We have shown that the suppression of autophagic cell death but not apoptosis was mainly involved in cellular radiosensitivity. Therefore, the enhancement of autophagy may have a considerable impact on the treatment of radiosensitive tumors. We again performed FR experiments to prove reproducibility of establishment of CRR cells. We showed that long-term FR exposures for more than 31 days conferred radiosensitivity to parental HepG2 and HeLa with overexpression of cyclinD1. Radiosensitivity was stably maintained in the tumor cells even on 31 days after the cessation of irradiation. A feedback loop was responsible for the cyclinD1 overexpression in which constitutively active AKT was involved. AKT is known to inactivate glycosyn thase kinase-3beta (GSK-3b), which constitutes the negative feedback of cyclinD1. As the result, cyclinD1 overexpression led to production of DNA double strand breaks to activate DNA-PK which further activated the AKT/GSK-3b pathway, thus promoting the loop of cyclinD1 overproduction. Inhibition of the AKT/GSK-3b/cyclinD1 pathway suppressed irradiated cells of long-term FR cells. Present observations give a mechanistic insight for acquired radiosensitivity of tumor cells through long-term FR exposure, and provide novel therapeutic targets for radiosensitization.


The cytotoxic effects of ionizing radiation (IR) and endoplasmic reticulum (ER) stress-inducing agents are associated with the promotion of an anti-tumor immune response via the induction of immunogenic cell death (ICD) of cancer cells. ICD promotes the cross-presentation of tumor-derived antigens by dendritic cells (DCs) to T cells (Semin Immunol 22, 113-124, 2010; Calreticulin (CRT), an ER chaperone protein) redistribution to the surface of tumor cells acts as a potent “eat me” signal for DCs involved in tumor associated antigen processing, thereby serving as a key step in ICD. In the classical setting, IR or ER stress alone may not quantitatively and/or qualitatively achieve cancer cell death in a manner sufficient to induce ICD. The combination of IR, when combined with thapsigargin (Tg, an ER stress inducing agent) triggers immune-mediated tumor rejection. Thus, we hypothesized that IR, when combined with thapsigargin (Tg, an ER stress-inducer via sarcoplasmic/ER calcium ATPase inhibition), may intensify CRT translocation to the cell surface. To test this, we employed the poorly immunogenic 4T1 mouse breast cancer cells. 4T1 cells were treated with IR (0, 6, or 20 Gy) followed by 24 hrs culture in the presence or absence of Tg (1 mM). Thereafter, the cells were assayed either via Western blot (WB) or immunofluorescence (IF). Cytotoxicity was determined via MTT assay at 12, 24, and 48 hrs. Relative amounts of protein were determined via WB analysis with specific antibodies to phospho-EIF2a-2, caspase-8, BAP-31, and PARP. Actin was used as a loading control. CRT redistribution was determined by IF analysis. When combined, IR (6 Gy) + Tg (1 mM) triggered elevated phosphorylation of EIF2a-2 (a marker for ER stress and protein translation inhibition) in 4T1 cells. In addition, IR (6 and 20 Gy) + Tg (1 µM) increased the cleavage of the apoptotic markers Bid, BAX, BAP-31, and PARP. Finally, we observed that cell death by IR (6 Gy) in the presence of Tg (1 µM) was preceded by enhanced CRT translocation to the cell surface. In this in vitro model, IR (66 Gy) alone was unable to induce CRT redistribution. However, in the presence of Tg (1 µM), IR (66 Gy) induced CRT redistribution. Taken together, these findings suggest that IR combined with an ER stress-inducing agent is a novel application of radiotherapy that can potentially trigger ICD and serve as a strategy to promote immune-mediated tumor rejection in cancer patients.
POSTER PRESENTATIONS

POS36-14. Radiation sensitization though regulation of transcriptional factor Sp1. Yoshiho Hosoi, Research Center for Radiation Biology and Medicine, Hiroshima University, Japan
DNA-dependent protein kinase (DNA-PK) is involved in DNA double-strand breaks (DSBs) repair, and it consists of Ku70, Ku80 and DNA-PKcs. It has been shown that the promoter regions of these three genes have Sp1 binding sites, and the expression levels are correlated with that of Sp1. The purpose of this study is to clarify the contribution of Sp1 to radiation sensitivity of cells through the transcriptional regulation of DSBs-repair genes. We investigated whether Sp1 affects the protein and mRNA levels of Ku70, Ku80, DNA-PKcs, XRCC4, NBS1, MRE11 and MDC1. In addition, we examined the DSBs-repair, DNA-PK activity, cell cycle, and radiation sensitivity in Sp1-down-regulated cells. A human transformed kidney cell line 293T was transfected with siRNA vector targeting Sp1 (Sp1-siRNA) or control vector. The vector-transfected cells were selected with G418. After 7 days selection, protein and mRNA levels were evaluated by Western blotting and RT-PCR, respectively. The protein and mRNA levels of Sp1, Ku70, Ku80, DNA-PKcs, XRCC4, NBS1, MRE11 and MDC1 were down regulated by the Sp1-siRNA treatment. The DSBs-repair after 80 Gy irradiation and DNA-PK activity were suppressed by the Sp1-siRNA treatment. The surviving fraction after irradiation was also suppressed by the Sp1-siRNA treatment whereas the cell cycle was not affected by this treatment. These results suggest that Sp1 regulates the radiation sensitivity by the transcriptional regulation of DSBs-repair genes.

To determine the functions of hsa-miR-663 in HeLa cells responding to X-ray irradiation and tumor proliferation, the precursor of hsa-miR-663 was cloned into pcDNA3.1-ZsGreen/GFP-miR and the expression cassette was transferred into pT-REx-DEST30 which contains a tetracycline operator through gateway reaction. Stable inducible hsa-miR-663 expressing cell line, which is named HeLa-TetR-663, was constructed by transfecting HeLa cells stably expressing tetracycline repressor (TetR) with hsa-miR-663 and selected with G418 for 14 days. After verification of hsa-miR-663 expression by qRT-PCR, the HeLa-TetR-663 cells were injected subcutaneously into the flanks of NOD/SCID mice (n=5 flanks). After formation of tumors, the miR-663 expression was induced continuously for 7 days by injecting tetracycline subcutaneously once a day. Then the tumors were subjected to 5 Gy X-ray irradiation and the tumor volumes were assessed every 2 days post-irradiation using a caliper. The tumor growth of radiation group as well as radiation plus induction group is slower than that of control, However, the induction group obviously exhibited high tumor growth rate. In conclusion, we found that hsa-miR-663 functions as an oncogene and its high expression promotes tumor growth in vivo.

POS36-16. The Progeny of Polyploid Mitotic Catastrophe Cells Acquires Anchorage-Independent Growth Capabilities, Stem Cell-Like Properties, and the Ability to Form Xenograft Tumors in Nude Mice? Fiorenza Ianzini, E.A. Kosmacek, E. Napoli, E.A. Colwell, M.A. Mackey, University of Iowa, USA
We demonstrated that polyploid tumor cells conserve their original individual genomic integrity and can re-initiate cell division and originate viable colonies (Cancer Res 69, 2296; 2009). The role played by the colonies formed through this process in tumor progression and tumor resistance to treatment remains to be elucidated. We are conducting studies aimed at answering these questions. Therefore, we subjected MDA-MB435 cells to a single dose of 5 Gy of g-rays after which we followed the formation of polyploid polyclones. When these were formed we isolated each of them and plated each in a 30 mm diameter Petri dish and let them grow. Cells were monitored for 30 days at which time those that divided and formed a colony were detached and expanded. We have now a collection of clones from each derived from a specific polyploid cell that had escaped radiation-induced MC death that were analyzed for their tumorigenic potential both in vitro and in vivo. Our live cell imaging findings show that the clones have acquired a better proliferative capacity over control cells, that is the re-irradiated clones have both a higher yield of cell division and a lower yield of cell death compared to the control cells. Transformation assay data reveal that these clones proliferate well in soft agar, demonstrating anchorage-independent growth capability. FCM measurements of aldehyde dehydrogenase (ALDH1), a non-immunological, functional marker of human stem and progenitor cells, show high activity for this enzyme in the clones demonstrating that the clones have acquired stem cell-like properties. Finally, injection of the clone cells in the flank of nude mice induced fast growing and larger xenograft tumors than the control cells. IHC analysis aimed at testing for the presence of meiotic genes, cancer stem cell markers, and morphology are underway on the new clones to determine their tumor xenograft properties, cellular pathways of cell proliferation that might be instrumental for our understanding of how irradiated tumor cells escape death and acquire higher proliferative and tumorigenic potential compared to the untreated cell population. These findings will lend important insights into phenomena occurring during tumor resistance to treatment and tumor progression and will be instrumental for the development of new therapeutic strategies.

POS36-17. Hypoxic induction of SM22α in A549 NSCLC cells activates the IGF-IR/Akt pathway which contains cellular resistance against radiation therapy. In Gyu Kim, T.R. Kim, M. Kim, S.I. Choi, Korea Atomic Energy Research Institute, South Korea
Purpose: The hypoxic status of various solid tumors has been related to an increased resistance to radiotherapy and chemotherapy treatments in a variety of tumor types with a more malignant phenotype. The aim of this study is to investigate a new important hypoxia inducible factor that makes cells resistant to a γ-radiation, anticancer drugs and toxic chemicals.
Experimental procedure: A549 cells were subjected to hypoxia (0% and 1%) by placing them in a humidified airtight chamber and neutrons expression was analyzed by real time PCR and western blotting. Neutrons-in-overexpressed A549 cells were constructed and these cells were plated in a T25 flask (1X10^5 cells/flask), 24-48h after irradiation with a single exposure to a dose of 20 Gy on γ-ray source; dose rate, 16 Gy/min), cell viability was analyzed by flow cytometry.
Summary: Hypoxia stress-induced resistance to radiation or chemotherapy often undermines the efficiency of cancer therapy and makes tumors refractory to treatment. SM22α is an actin-binding protein found in smooth muscle, fibroblasts, and some epithelium and is possibly a marker of smooth muscle differentiation or senescence. SM22α was significantly induced in A549 non-small-cell lung carcinoma cells by hypoxic stress and SM22α overexpression improved chemoresistance and radiation resistance of the cells. Although it was induced by hypoxic stress, HIF-1α was not involved in the expression of SM22α and vice versa. SM22α overexpression enhanced tumor cell growth and markedly activated the IGF-IR/Akt pathway via direct interaction with IGF-1R. This is the first demonstration of hypoxic regulation of SM22α and suggests that SM22α could be a novel target to enhance the treatment of hypoxic human lung cancer cells.
Conclusion: This study not only provides the first demonstration of a severe hypoxic regulation of SM22α but also suggests the participation of novel mechanisms regarding resistance to a γ-radiation and anticancer drugs caused by hypoxia. The authors wish to acknowledge the financial support of the Ministry of Education, Science and Technology (Nuclear Research & Development Program) of the Republic of Korea.

Purpose: Curcumin, a major component of the plant Curcuma longa, has a potent anticancer effect on a variety of cancer cell types. However, the anticancer mechanism of curcumin for lung cancer cell lines is not yet fully determined. In the present study, we examined the anticancer mechanism and radiosensitizing effects of curcumin on cell proliferation and cell death in human lung cancer cells. Experimental procedures: Human lung adenocarcinoma A549 cells were treated with curcumin, irradiation, or their combination. The cells were pretreated with curcumin followed by exposure to clinically relevant doses of gamma rays and the effect on cell growth was determined by cell viability assay and colony formation assay. The effect of curcumin pre-treatment on the expression of cell cycle regulatory proteins and survival factors was determined by Western blot analysis.
Summary: Curcumin inhibited growth of A549 cells and augmented G2/M-phase arrest. When used in combination with radiation, curcumin further suppressed cell proliferation and induced apoptosis in A549 cells, leading to increased cell killing. Curcumin inhibited the phosphorylation of the mammalian target of rapamycin (mTOR) and its downstream targets, p70S6K kinase1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1). Additionally, curcumin pre-treatment inhibited radiation-induced phosphorylation of p53 and the p56 subunit as well as the nuclear translocation of p65 subunit of NF-κB. We also found that curcumin alone or in combination with radiation inhibited the expression of cyclin D1, cyclin E, CDK4 and CDK2, and the phosphorylation of retinoblastoma protein (pRb). Furthermore, curcumin inhibited the basal or radiation-induced motility of the cells.

Conclusions: Collectively, our results show that curcumin inhibits cell proliferation, survival and motility of lung cancer cells through targeting multiple cellular signaling pathways, and that curcumin could further enhance the antitumor activities of radiotherapy. These findings may be clinically relevant and suggest a novel therapeutic strategy for increasing the efficacy of radiotherapy.

POS36-19. Regulation of inositol polyphosphate 4-phosphatase type II in tumor resistance against radiation and anticancer drugs. Jae-Sung Kim, S. Hwang, Korea Institute of Radiological and Medical Sciences, South Korea

Although development of tumor radioresistance remains a significant impediment to successful radiotherapy, the underlying molecular mechanisms and markers are not well defined. In this study, we identified up-regulations in human type II (INPP4B) as novel marker of radioresistance by using systematically analyzing Unigene libraries for profiles derived from laryngeal cancer. INPP4B was highly expressed in radiosensitive laryngeal cancer cell lines and was induced by treatment with either radiation or anticancer drugs in various types of cancerous cells. Radiation-induced INPP4B expression was blocked by the inhibition of extracellular signal-regulated kinase-1/2. Ectopic INPP4B overexpression increased radioresistance or anticancer drug resistance by suppressing apoptosis in various types of cancer cells. Conversely, INPP4B depletion with small-interfering RNA re-sensitized the cells to radiation- or anticancer drug-induced apoptosis. Furthermore, increased Akt phosphorylation was significantly reduced by INPP4B depletion, indicating that INPP4B-mediated radioresistance is regulated by Akt activation. In conclusion, our data not only provided evidence that radiation- or anticancer drug-induced INPP4B expression is associated with the development of tumor resistance against radiation and anticancer drugs, but it also defined the protective function of INPP4B as a stress-responsive protein in cancer cells.

POS36-20. Brain tumors - an epigenetic connection: analysis of epigenetic changes in radiation-sensitive & radiation-resistant glioblastoma cells. Anna Kovalchuk1,2, J. Novak1, R. Rodriguez-Juarez3, B. Kolb2, O. Kovalchuk1, 1: Department of Biological Sciences, University of Lethbridge, AB, Canada, 2: Department of Neuroscience, University of Lethbridge, AB, Canada

Glioblastoma is the most frequent primary brain tumor occurring in adults. Its prognosis is dismal, the average survival time being often less than 12 months after diagnosis. Glioblastomas are characterized by striking radioresistance and understanding the mechanisms of radiation sensitivity of glioblastoma cells is crucial for development of novel treatment strategies.

Epigenetic changes are important in a variety of malignant tumours, including brain tumours. Epigenetic changes are meiotically heritable and mitotically stable alterations in gene expression that include DNA methylation, histone modification and RNA-associated silencing. They are important regulators of tumour treatment responses. Yet, very little is known about the roles of epigenetic changes in responses of glioblastoma cell lines to high (therapy-like) and low (diagnostic exposure-like) doses of radiation.

We profiled radiation responses of 5 glioblastoma lines: M059J and M059K, SK-N-2 and A172 and IMR-32. The rhobdomyosarcoma (RMS) cell line was used as a control for the other tissues. Amongst the tested lines, the M059J cell line is deficient in DNA-dependent protein kinase (DNA-PK) due to a mutation in PRKDC gene. Whereas, the M059K cell line, isolated from the same malignant tumour, harbours a wild-type DNA-PK, active and radiosensitive, while M059K cells are very resistant to radiation. We profiled their responses to high, therapy-like radiation doses. SK-N- BE, A172 and IMR-32 lines have been used before for the analysis of glioblastoma radiation responses and have also been proven to be good models of Alzheimer’s disease. Thus, we profiled their responses to low (diagnostic-exposure-like) doses of radiation.

We hypothesized that M059K and M059J glioma cells would exhibit significant differences in the global DNA methylation and gene and microRNA expression under normal conditions and after high dose irradiation. Furthermore, we predicted that low doses of radiation would express in some extent the epigenetic status of glioblastoma SK-N-BE, A172 and IMR-32 cells.

To study the distribution and plasticity of DNA methylation in a quantitative fashion, we used the Illumina Infinium HumanMethylation27 BeadChip Assay. Global gene expression profiling was conducted using the Illumina Whole-Genome Expression BeadChipsTM platform. MicroRNA profiling was contracted out to LC Sciences.

Here we will present a new model to explain the role of epigenetic changes in high and low dose radiation responses of glioblastoma cells.

POS36-21. Ionizing Radiation Induces Migratory and Invasive phenotypes via E-cadherin-Catenin-Small Rho GTPases axis in MCF7 Human Breast Carcinoma Cells. Amit Kumar, M. Ali, B.N. Pandey, Radiation and Cancer Biology Section, Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, India

Recently molecular and pathological evidence of epithelial–mesenchymal transition (EMT), that play a key role in the development of metastasis, has been well documented in human breast tumors. However, the effect of ionizing radiation (IR) on EMT like changes in human breast cancer cells has not been studied systematically. An in vitro study was conducted to investigate the IR induced EMT like alterations including morphology, adhesion, and migration of estrogen receptor-α-positive human breast carcinoma cells (MCF7). Cancer cells were irradiated at 2-12 Gy and after 24-72 h of irradiation, a dose-dependent change in cell morphology from cuboidal (cubblestone type) to an elongated spindle-like shape was observed by bright field microscopy. Un-irradiated control cells were found to form colonies of tightly clustered cells. In contrast, tightly clustered cells were not observed in cells treated with IR. These changes were likely accompanied by the rearrangement of cell–cell or cell–extracellular matrix (ECM) contacts. Further experiments showed that irradiated MCF7 cells (4-12 Gy/48 h) exhibited significant and dose-dependent increase in adhesion (~2-4 folds). The mesenchymal transition of MCF7 cells with surface protrusions and increased cell adhesion seems to be potentially linked with rearrangement of cytoskeleton contact. Immunostaining of irradiated cells with TRITC-conjugated phalloidin revealed significantly increased F-actin stress fibre formation. Results also showed that IR promoted E-cadherin repression and altered localization of p120 catenin. Pretreatment with latrunculin-A, an inhibitor of actin polymerisation or CT04, a specific inhibitor of Rho protein prevents both the cytoskeletal reorganization and filopodial/lamelipodial structures. Bright field microscopic images of scratch motility assay showed IR increased MCF7 cell motility. Collectively, these results show that IR induces E-cadherin-catenin mediated Rho-dependent actin stress fibre formation that seems to be involved in the acquisition of invasive and migratory phenotype of MCF7 human breast carcinoma cells.

POS36-22. In vivo global transcriptional response to hypoxia in human tumor xenografts predicts treatment outcome. Gloria C. Li, A. Minami, F. He, V. Seshan, Q. Chen, C.C. Ling, Memorial Sloan Kettering Cancer Center, USA

Tumor hypoxia, present in many human cancers, causes radio- and chemo-resistance, a more aggressive phenotype, and is prognostic of treatment outcome. Recently, we generated a human tumor (colorectal carcinoma) xenograft model (HT29-HRE) in which the dual reporter fusion gene (HSV1-TK and eGFP) was under the control of hypoxia-inducible promoter (HRE). One promising aspect of this model is the ability to separate tumor cells of different hypoxia status, and then to examine their respective characteristics. We have previously demonstrated the expression of this model in vivo (Li et al., 2014). Here, we will present the hypoxia-inducible global transcriptional response in vivo. HRE tumors were grown subcutaneously in the limbs of nude mice, the M059J xenograft and the M059K xenograft. Single cell suspensions were then prepared, and sorted by fluorescence activated cell sorting (FACS) based on the level of
hypoxia-induced eGFP expression. The sorted populations (eGFP<sup>Hi</sup> vs eGFP<sup>Lo</sup>) were then analyzed by Illumina expression microarrays to assess their global transcriptional response to hypoxia. The microarray analysis revealed clear differences in gene expression profile between the two groups, i.e., the hypoxic and the aerobic cells. A differential expression signature for hypoxia was derived using a false discovery rate (FDR) of 5% and with > 1.5-fold change in mRNA expression. This gave us a gene list of 122 genes. We then evaluated the impact of our in vivo hypoxia gene signature on the treatment outcome of breast cancers, from publicly available gene expression datasets. We first clustered the patients using the in vivo hypoxia signature genes to arrive at two clusters, and then we compared the patient survival data across these two clusters by Kaplan-Meier analysis. Our study demonstrated that our in vivo hypoxia signature, derived from the human tumor xenograft model, has significant prognostic potential.

**POS36-23. Mesenchymal stem cells stimulated by γ-irradiation promote mesenchymal transformation in glioma.** Eun-Jung Lim<sup>1</sup>, Y. Suh<sup>2</sup>, C. Youn<sup>3</sup>, R. Kim<sup>4</sup>, K. Uoo<sup>5</sup>, G. Lee<sup>1</sup>, Y. Ha<sup>1</sup>, S. Lee<sup>1</sup>, S. Kang<sup>1</sup>, 1: Hanyang University, South Korea; 2: Catholic University, South Korea

The variety of stromal cells in the surrounding environment are recruited to tumors, and these not only enhance growth of the primary cancer but also facilitate its metastatic dissemination to distant organs. Mesenchymal stem cells (MSCs) have been recently described to localize to cancer, where they integrate into the tumor-associated stroma. However, the involvement of mesenchymal stem cells in brain tumor Metaphenology has not been extensively assessed. Here, we show that radiation-induced cytokines in MSCs are involved in the mesenchymal transformation of glioma cells. Exposure to ionizing radiation stimulated MSCs to secrete cytokines such as IL6 and IL8, which are key actors in a paracrine fashion on the glioma cells and enhances their motility and invasion. Moreover, the secretion of IL6 and IL8 by MSCs led to the enrichment of glioma stem-like cell population in glioma cells. Collectively, these data demonstrate that radiation-activated MSCs contribute to tumor microenvironment and facilitates to change glioma cells to more malignant cells.


Glioblastomas (GBM) are aggressive brain tumors that are highly resistant to therapy. We tested a novel radiotherapy (RT) regimen, pulsed low dose radiation (PLDRT), in combination with temozolomide (TMZ) to assess its efficacy and mechanism of action using a murine orthotopic GBM model. Treatment response was evaluated using microPET/CT imaging and immunohistochemistry. Nude mice were intracranially injected with U87MG human GBM cells and imaged weekly with a FLEX Triumph<sup>TM</sup> combined PET/CT system. 18F-FDG PET images were co-registered with high-resolution MicroCT images. The CT component of the fused images was used to contour tumor volume, which was then applied to the PET image to obtain tumor metabolic activity (SUV<sub>max</sub>). RT to the cranium was delivered to 14 Gy in 7 days beginning on day 7-10-post-implantation. TMZ (10mg/kg via oral gavage) was given 1hr before RT. Six groups (n=9 per group) were evaluated: Sham; RT alone (2 Gy x 7 days); PLDRT alone (10x0.2 Gy pulses with 3 minute inter-pulse interval x 7 days); TMZ alone; TMZ + RT; TMZ + PLDRT. Control mice exhibited neurologic death on day 17±2. PLDRT group showed an additional growth delay of 1 week compared to conventional RT group. The addition of TMZ to conventional RT led to 3 week growth delay compared to the control arm. There was no significant tumor growth delay difference between conventional RT vs. pulsed low dose RT with temozolomide. However, assessment of weekly metabolic activity indicated that TMZ + PLDRT was more efficacious than TMZ + RT arm. TMZ + PLDRT arm showed 40% decrease in SUV<sub>max</sub> activity that lasted for 2 weeks compared to only 1 week for TMZ + RT arm. The increased efficacy of TMZ + PLDRT was also evident in the tumor volume measurements at the end of treatment. In addition, TMZ + PLDRT group showed 2-3 fold reduction in normal brain tissue damage compared to the TMZ + RT group. Areas of low tumor Ki-67 tended to be associated with reduced vascularization. The high Ki-67 level and increased p53 level in well with the high tumor regrowth kinetics observed by CT imaging. Utilizing an in vivo murine orthotopic GBM model, the concurrent delivery of TMZ to RT or PLDRT resulted in additional growth delay. Duration of decline in SUV<sub>max</sub> was longer in TMZ + PLDRT group compared to TMZ + RT group and this enhanced cytotoxicity was associated with less normal brain damage.

**POS36-25. Effect of the Angiogenesis Inhibitor Sorafenib on Tumor Perfusion and Hypoxia in Cervix Cancer Patients Treated with Radiotherapy.** Michael Milosevic<sup>1</sup>, C. Townsley, S. Kim, H. MacKay<sup>2</sup>, L. MacBean, W. Levin, I. Young, M. Haider, J. Xie, R. Hill, A. Oza, A. Fyles, Princess Margaret Hospital, Canada

Background: Angiogenesis is up-regulated in many solid tumors, including cervix cancer. Pre-clinical studies have shown that inhibition of angiogenesis can improve radiation therapy (RT). The purpose of this study was to examine the effect of the angiogenesis inhibitor sorafenib on the tumor microenvironment and clinical outcome in cervix cancer patients receiving RT and cisplatin chemotherapy (RTCT).

Materials and Methods: This was a Phase I-II study of sorafenib in 13 patients with locally advanced cervix cancer receiving RTCT. Sorafenib 400 mg or 800 mg was given daily for 1 week prior to the start of RTCT in all patients, as well as during RTCT in 3 patients with high-risk disease. Biomarkers of response to sorafenib were measured at baseline, after 1 week of sorafenib alone and after 1 week of RTCT. Median follow-up was 21 months.

Results: Mean tumor volume measured using MR increased during treatment with sorafenib alone (78 to 86 cm<sup>3</sup>, p<0.01). Mean DCE MR enhancing fraction (EF) and mean relative signal intensity (RSI) both decreased (86 to 73%, p=0.02 and 1.9 to 1.7, p=0.07) in keeping with changes in vascularization. However, there was no change in mean interstitial fluid pressure (IFP: 24 to 21 mm Hg). After 1 week of RTCT, mean tumor volume had decreased (81 to 57 cm<sup>3</sup>, p=0.01) and there was a significant reduction in mean IFP (24 to 16 mm Hg, p=0.04). However, EF, RSI and median pO2 were not different from baseline. At last follow-up, 2 patients had recurred – 1 in pelvic lymph nodes and 1 at distant metastatic sites – and 1 patient had died without ever achieving disease control. Grade 3-4 late toxicity was identified in 3 patients – 1 with pelvic fistulae, 1 with rectal bleeding and 1 with anal fissure.

Conclusions: Sorafenib reduces tumor perfusion/permeability and increases hypoxia in cervix cancer consistent with an antiangiogenic effect. Although sorafenib was well tolerated in combination with RTCT, these biomarker changes suggest that the drug is unlikely to improve clinical outcomes in this patient population.

**POS36-26. Isolation of endothelial cells from cancer and normal tissue of human breasts, and the radiosensitivity of the isolated endothelial cells in vitro.** Eun-Taek Oh, M. Park, H. Lee, M. Song, B. Cho, H. Joo Park, Inha University College of Medicine, South Korea

Purpose: The purpose of present study was to compare various radiobiological aspects including clonogenic death of cancer-derived endothelial cells (ECs) and normal tissue-derived endothelial cells (NECs) in vitro.

Experimental procedures: We have developed a novel method for harvesting endothelial cells from blood vessels of freshly obtained cancer and adjacent normal tissue of human breast. When human breast tissues including cancer were embedded in Matrigel and cultured in endothelial cell culture medium (ECM) containing growth factors, endothelial cells grew out of the tissues. The endothelial cells were harvested and cultured as monolayer cells in plates coated with gelatin. The 2nd – 5th passages were exposed to various doses of radiation and the changes in clonogenic cell survival, wound healing and tube formation capacity were evaluated.

Summary: Both ECs and NECs expressed almost the same levels of surface markers CD31, CD105 and TEM-8 (tumor endothelial marker-8), which are known to be expressed in angiogenic endothelial cells, i.e., mitotically active endothelial cells. Furthermore, both ECs and NECs were able to migrate into experimental wound in the monolayer culture, and also to form capillary-like tubes on Matrigel-coated plates. However, the suppressions of migration and capillary-like tube formations by irradiation were greater for ECs than NECs from the same patients. The clonogenic survival assays also demonstrated that ECs were far more radiosensitive than NECs.

Conclusions: We have developed a simple and efficient new method for isolating endothelial cells from cancer and normal tissue, and demonstrated for the first time that endothelial cells of human breast
cancer are significantly more radiosensitive than their normal counterparts from the same patients.

**POSTER PRESENTATIONS**

**POS36-27. Differential regulation of angiogenesis by MAPK signaling pathways in irradiated human normal and cancer endothelial cells.** Moon-Taek Park, E.T. Oh, H. Lee, M.J. Song, B.H. Choi, H.J. Park, Inha University College of Medicine, Department of Microbiology, South Korea

Purpose: Despite the strong possibility that endothelial cells of tumors and normal tissues may be different in various aspects, most of the previous studies on endothelial cells have been conducted using normal endothelial cells. Therefore, we have developed a novel method for obtaining endothelial cells from blood vessels of freshly obtained cancer and adjacent normal tissue of human breast, and further investigated the role of ionizing radiation-induced cellular signaling pathways in the alternation of angiogenic process observed after the purified endothelial cells were irradiated.

Experimental procedures: When human breast tissues including cancer were embedded in Matrigel and cultured in endothelial cell culture medium (ECM) containing growth factors, endothelial cells grew out of the tissues. The endothelial cells were harvested, cultured as monolayer cells in plates coated with gelatin, and exposed to radiation, and then the changes in clonogenic survival, tube formation capacity, gene expression, and cellular signaling pathways were determined.

Summary: Cancer endothelial cells were significantly more radiosensitive than their normal counterparts. In normal endothelial cells, 4 Gy of ionizing radiation (IR) induced an increase in the capillary-like tube formation, the expression of matrix metalloproteinase-2 (MMP-2), and the activation of ERK pathway. However, in cancer endothelial cells, 4 Gy of IR significantly reduced the capillary-like tube formation, and induced the expression of angiostatin and the activation of both AKT and JNK pathways.

Additionally, inhibition of ERK with a pharmacological inhibitor or a small interfering RNA markedly suppressed the capillary-like tube formation and the expression of MMP-2 caused by IR in normal endothelial cells. In cancer endothelial cells, inhibition of either AKT or JNK with a pharmacological inhibitor or a small interfering RNA clearly attenuated the expression of the capillary-like tube formation, and the expression of angiostatin caused by IR.

Conclusion: Our results demonstrate that IR promotes the formation of capillary-like tubes via ERK-mediated MMP-2 expression in normal endothelial cells, whereas IR suppresses the formation of capillary-like tubes via AKT and JNK-mediated angiostatin expression in cancer endothelial cells.

**POS36-28. Effect of Astaxanthin on Tumor Cell Response to Ionizing Radiation.** Anne Sanders, W. Chen, V. Payne, C. McMahan, K. Barlow, M. Robbins, L. Metheny-Barlow, Wake Forest School of Medicine, USA

The survival or quality of life of cancer patients could be improved if conventional therapies could be combined with drugs that enhanced their anticancer effects or protected against normal tissue toxicity. One candidate is astaxanthin (AST), a xanthophyll carotenoid with both antioxidant and anticancer properties. Here, we tested the influence of AST on the radiosensitivity of a panel of human and rodent breast cancer cell lines. In clonogenic or MTS assays, while no protection from ionizing radiation (IR) was observed when tumor cells were exposed to AST only prior to radiation, we also did not observe any AST-mediated enhancement of radiation response in the five breast tumor cell lines tested thus far. Interestingly, we find that exposing tumor cells to AST alone after plating in a clonogenic assay significantly reduces plating efficiency of the cells. When cells were exposed to IR then treated with AST afterward, breast cancer cell lines either showed no effect or a slight protective effect was observed. However, overall clonal survival was decreased with the combination of IR and post-IR AST treatment compared to sham vehicle, suggesting that the combined treatment was more effective than either treatment alone. Using an in vivo syngeneic rat breast cancer model, we determined that Fischer 344 rats implanted with MATBIII mammary tumor cells and treated with fractionated whole-brain irradiation (FWBI) (5 Gy fractions delivered 2x/week for 4 weeks) were fed an AST diet (100mg/kg diet) initiated after FWBI, 100% of animals (n=10) were tumor free at 21 weeks postimplantation. In contrast, only 67% of tumor-bearing animals treated with FWBI alone (n=12) were still surviving at 21 weeks (p<0.05). However, animals that were fed AST during FWBI had reduced survival (median survival time 15 weeks, n=10; p<0.02 compared to AST post-IR). Together these data suggest that irradiation followed by AST treatment may enhance therapeutic response, but that AST administration during radiation treatment may be contraindicated.

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**POS36-29. The role of Rev1 in radiation and chemical -induced tumor development using Rev1 Tg mice.** Megumi Sasatani1, H. Honda1, K. Hamasaki2, Y. Kusunoki1, Y. Masuda1, K. Kamuya1, 1: Hiroshima university, Japan, 2: Radiation Effects Research Foundation, Japan

Ionizing radiation is a carcinogenic agent. Radiation carcinogenesis is the result of a series of somatic mutations. However, it remains poorly understood when and how and somatic mutations are accumulated in radiation induced cancer development. Translesion DNA synthesis (TLS) carried by specialized DNA polymerases plays a critical role in mutagenesis. Rev1, which possesses deoxycytidyl transferase activity, plays the central role on the TLS and can interact with other TLS polymerases. It has been reported that absence of Rev1 sensitizes to a variety of DNA damaging agents such as ionizing radiation and alkylating agents. Furthermore, overexpression of Rev1 showed increased resistance to cisplatin known as DNA damaging agent. However, how expression level of Rev1 affects the mutagenicity and tumorigenicity in vivo treated by these DNA damaging agents is not still established. To explore this issue, we have developed Rev1 transgenic mice in the C57BL/6 strain. Both Rev1 Tg and wild type mice (C57BL/6) were exposed to radiation or treated with alkylating agent and examined their development of cancers. Radiation and chemical treatment induced thymic lymphoma in both Rev1 Tg and wild type mice, but the latency was significantly different between the two group. Our data assume that overexpressed Rev1 has the some different role in radiation and chemical induced tumor development. 1KZFI (Baro) tumor suppressor gene may be associated with these phenomena. These demonstrate that expression of Rev1 may be correlated with radiation and chemical induced tumor development.

**POS36-30. Cell-in-Cell structures in tumors.** Manuela Schwegler1, B. Abendroth1, F. Putz2, L. Distel1, 1: Universitätssklinikum Erlangen, Germany, 2: Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Tumor cells have an increased potential for the incorporation of cells. This phenomenon is called cell cannibalism or cell-in-cell structures (CIC). CIC have been found in tumor tissue sections and in cell cultures. CIC is regarded to be one type of cell death. Our question was whether CIC formations is common among tumor cells and whether cells from normal tissue are able to form CIC? The precondition for cell uptake is the adhesion of two tumor cells. For quantification of adhesion two samples of tumor cells were dyed with two different membrane-permeable dyes, mixed and analyzed by flow cytometry before and after 90 minutes of coincubation. One of the so dyed samples was pretreated to stimulate incorporation. Microscopic analysis followed 4 hours of coincubation. This experiment was repeated 3 times with 5 tumor cell lines, one primary tumor cell line, one lymphoblastoid cell line and 3 primary skin fibroblasts cell lines. Additionally the process of incorporation was imaged via live-cell microscopy. The incorporation of tumor cells was tried to inhibit by ionizing radiation (50 Gy) or exposition to ultra-violet light.

Flow cytometry analysis showed a significant increase of adhesion of tumor cells between untreated and pretreated-samples. All cell lines were able to form CIC structures. Highest rates were among the tumor cell lines, lower rates in the fibroblasts and lowest in the lymphoblastoid cell line. The number of cell-in-cell structures found via microscopy correlated with the degree of aggregation. CIC structure has a characteristic appearance. The cell is filled with a cell inside the nucleus makes a large deformation to one side. The amount of detected E-Cadherin and β-Catenin was reduced in the pretreated cells compared to the untreated cells. Exposure to UV light resulted in a small decrease of the incorporation ratio, whereas irradiation did not change the incorporation rates. Via westernblot analysis, phosphorylation of Ikapos (AKT) in heat-treated cancer cells could be detected. Caspase-dependent cell death was not induced by pretreatment as activated caspase 3 was not detected.
In conclusion not only tumor cells but even normal tissue cells seem to be able to form CIC. CIC seems to be common to a variety of cell types and may be an underestimated type of cell death.

**POS36.31.** High interstitial fluid pressure is associated with different vascular abnormalities in human melanoma xenografts. Trude G. Simonsen, J. Gaustad, M.N. Leinaas, E.K. Rofstad, Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Norway

Purpose: Interstitial fluid pressure (IFP) is highly elevated in many solid tumors and has been shown to be associated with radioresistance in human melanoma xenografts and poor survival after radiation therapy in clinical melanoma cancer patients. Abnormalities in tumor vascular networks have been identified as an important cause of elevated tumor IFP. The aim of this study was to investigate the relationship between elevated tumor IFP and the functional and morphological properties of tumor vascular networks.

Methods and materials: A-07-GFP and R-18-GFP human melanomas growing in dorsal window chambers in BALB/c nu/nu mice were used as preclinical tumor models. Blood supply time, plasma velocity and morphological parameters of the vascular network were assessed from first-pass imaging movies and vascular maps recorded after intravenous bolus injection of 155-kDa tetramethylrhodamine isothiocyanate-labeled dextran. IFP was measured in the center of the tumors using a Millar catheter.

Results: High IFP was associated with low angiogenic activity, i.e. low growth rate and low vascular density in A-07-GFP tumors. Conversely, high IFP was associated with high angiogenic activity, i.e. high growth rate and high vascular density in R-18-GFP tumors. IFP was associated with blood flow resistance in both A-07-GFP and R-18-GFP tumors. High IFP correlated with a high ratio between the plasma velocity in the tumor arterioles and the plasma velocity in the tumor venules.

Conclusions: High IFP in human melanoma xenografts was associated with high blood flow resistance. Different vascular abnormalities caused high blood flow resistance in A-07-GFP and R-18-GFP tumors.

**POS37 Radiation chemistry: basic problems**

**POS37.01.** Postirradiation chemical processing of DNA damage generates DSBs in cells already engaged in repair. George Blakis, S. Singh, M. Wang, C. Staudt, 1: University of Duisburg-Essen, Medical School, Germany

In cells exposed to ionizing-radiation (IR), double-strand-breaks (DSBs) form within clustered-damage sites from lesions disrupting the DNA sugar-phosphate backbone. It is commonly assumed that these DSBs form promptly and are immediately detected and processed by the cellular DNA-damage-response (DDR) apparatus. This assumption is questioned by the observation that after irradiation of naked DNA, a fraction of DSBs forms minutes to hours after exposure as a result of temperature-dependent, chemical processing of labile sugar lesions. Excess DSBs also form when IR-exposed cells are processed at 50°C, but have been hitherto considered method-related artifact. Thus, it remains unknown whether DSBs actually develop in cells after IR exposure from chemically labile damage. Here we show that irradiation of “naked” or chromat-in-organized mammalian DNA produces lesions, which evolve to DSBs and add to those promptly induced, after 8-24h in-vitro incubation at 37°C or 50°C. The conversion is more efficient in chromat-in-associated DNA, completed within 1h in cells and delayed in a reducing environment. We conclude that IR generates sugar lesions within clustered-damage sites contributing to DSB formation only after chemical processing, which occurs efficiently at 37°C. This subset of delayed DSBs may challenge DDR, may affect the perceived repair kinetics and requires further characterization.

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**POS37.02.** Excited States of p-Terphenyl in Selected Ionic Liquid under Electron Pulses Irradiation. Rafał Kocia, J. Grodkowski, J. Markowski, Institute of Nuclear Chemistry and Technology, Poland

Room temperature ionic liquids (ILs) are regarded as low volatile and non-combustible green solvents for various reactions. Improvement of the yield of a number of reactions in the ILs, controlling outcome and stereoselectivity of products makes ILs more than just eco-friendly substitute for volatile organic solvents. The unique properties of ILs and numerous experiments provide useful applications of ILs in many fields, e.g. in nuclear industry, biochemistry, chemical engineering, energy production, etc.

For radiation chemistry the ionic liquids constitute a challenge as a new object to study in two aspects, their stability under ionizing radiation and as a new medium for basic study of mechanism of chemical reactions, some of them unique, occurring in these solvents only. The pulse radiolysis of p-terphenyl (TP) solution as a source of radiation generated intermediates gives then some insight into the nature of primary products of ionic liquid radiolysis. In the present study the formation of excited states of TP, both singlet and triplet, in the methyltributylammonium bis[(trifluoromethyl)sulfonyl]imide ([MeBu,N][NTf2]), solutions have been examined by pulse radiolysis. The pulse radiolysis of TP solution has been carried out under appropriate gases such as argon and nitrogen oxide in the presence or absence of triethylenetetramine (TEA). Additional measurements on the TP anion radical (BP) have been selected an effective sensor of excited states formations.

Fast kinetic measurements have been carried out using 10 ns, 10 MeV electron pulses from a Linear Electron Accelerator (LAE 10) delivering the dose up to 20 Gy per pulse. Measurements have been carried out using two switchable detectors: photomultiplier or ICCD camera (Intensified Charge Coupled Device). The system ICCD enables us to take whole absorption or emission spectrum from single pulses.

TP photocatalytic activity of TP was the main reason to select this compound for experiments in IL solutions. TP anion radical is a strong reductor and was used in catalyzed photochemical reduction of carbon dioxide in different solvents. The radiolysis of TP solution in IL indicated the formation of ion radicals and obviously excited species. TP anion radical is created photochemically mainly from singlet excited states by reaction with a sacrificial electron donor TEA and in radiolysis also directly in reaction with solvated electrons. Excited states of p-terphenyl in IL solution are formed by energy transfer from excited radiolysis products of IL and probably in direct TP excitation by Cerenkov light.

The singlet excited state is short lived and was investigated by observation of light emission only during the electron pulse. However, in the case of TP yield of singlet – triplet intersystem crossing is very low. To observe undoubtedly TP triplet excited state formation energy transfer from BP triplet excited state was applied. The yield of benzophenone singlet – triplet intersystem crossing is close to 1 and formation of TP triplet excited state in the process of energy transfer from BP triplet excited state was evident.

**POS37.03.** Generation of oxidizing radicals and their reactivity in ionic liquids with the same anion. Małgorzata Nyga1, G.L. Hug2, J. Mirkowski, T. Szreder1, J. Grodkowski1, 1: Centre for Radiation Research and Technology, Institute of Nuclear Chemistry and Technology, Poland, 2: Notre Dame Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana, USA

Ionic liquids (ILs) are fascinating class of salts with melting point below 100°C. Their properties can be controlled by selecting various combination of anion and cation. Some of them called room temperature ILs melt below 25°C. These liquids are compose from irregularly shaped cations and anions. Due to significantly low symmetry of ions ILs have properties which were not available before and give new possibilities to carry out experiments in conditions which were earlier impossible. ILs have a great potential for replacing non-environmental friendly classical solvents in industry. They do not evaporate and they are more stable chemically, thermally and electrochemically than volatile organic solvents.

ILs have potential to be used in nuclear power technology for production, transportation, storage and recycling of nuclear fuel. For such applications knowledge of mechanisms of ILs radiation degradation and radiation resistance thresholds are crucial.

The aim of this work is study of radiation initialized reactions in selected ILs by pulse radiolysis. The radiolysis allows to investigate short living free radicals, providing detailed information about the
spectrum, kinetics and mechanism of reactions of these species. We examined reaction:

\[ \text{IL}_{\text{vac}} + A^- + A^+ + \text{IL}, \text{ (where A is solute anion)} \]

in three ILs: N-methyltritylummonium, N,N-bis(trifluoromethylsulfonyl)imide, (MeBu,NITf2) 1-hexyl-3-methylimidazoliumN,N-bis(trifluoromethylsulfonyl)imide, (hmmNITf2) and triethylmonium N,N-bis(trifluoromethylsulfonyl)imide (Et,NHNTf2).

We determined optical and kinetic characteristic of reactions products. Two fundamental groups of radicals arise in the process of radioilysis: from the reaction of holes (centers with deficiency of electrons) and from the reaction of electrons. Oxidizing radicals which are successor of solute reaction with holes are very important individuals which play significant role in chemistry and biochemistry. In our study we focus on oxidizing reactions in ionic liquids. Knowledge of these species in ILs is still in its infancy. Due to high viscosity of ionic liquids the reactions are much slower as compared to water solutions. With very high viscosity of ionic liquids it is possible to measure investigated reactions in ways that had not been possible previously. One can observe transient spectra and identify reaction intermediates and individual steps in reaction mechanism.

All three chosen ionic liquids containing NTF2 anion. The ionic liquids with this anion were radiation resistant with low viscosity. The change of cation modified properties of ILs making possible observation of influence of this change on behavior of short lived species. We generated radical-anions (SCN-) + Br-, I-, with different reduction potential between +1.03 to +1.66 V (vs NHE, in water) and Nf3+ applying solute anions SCN- , Br- + Nf3- respectively. Collected absorption spectra indicate the presence of mentioned radical-anions. The study confirm hypothesis about different behavior of free radicals in ILs as compared to classical organic solvents and water.

**POS37-04. Electron irradiation in irradiated liquid argon:** a computational study. Mariusz Wojcik, M. Jaskolski, Inst. of Applied Radiation Chemistry, Technical University of Lodz, Poland

Because of its favorable physical properties and availability, liquid argon (LAr) has been widely used as a detection medium in high-resolution detectors of elementary particles, Important examples of such detectors are: ICARUS (Italy), DUSEL and ATLAS (part of the Large Hadron Collider at CERN, Switzerland). Electron-ion recombination processes in irradiated LAr cannot be described by the standard theories of recombination based on the diffusion equation. This is caused by a very high electron mobility in LAr, which results from infrequent electron scattering in this medium. Moreover, nonhomogeneous distributions of ionization products in radiation tracks make it difficult to track the track evolution processes analytically.

In this work, we present a computer simulation methodology which is based on realistic models of electron transport and reactions in LAr [1,2] and allows us to quantitatively reproduce the experimental data obtained from the ICARUS detector [3]. In our approach, electron trajectories in radiation tracks are directly simulated and electron collisions are modeled using energy-dependent cross sections. By introducing the concept of one-dimensional periodicity in the tracks, we are able to model very large ionization clusters. We calculate the probability of escape from recombination for radiation tracks of various initial distance (r) between the ions. The simulation results are in good agreement with experiment in the range of r up to about 30 nm. At lower ionization densities, the simulation results overestimate the experimental data. We explain this discrepancy by considering the role of delta-tracks (short tracks of secondary electrons) in electron recombination in LAr. We introduce a theoretical model that allows us to obtain quantitative agreement between the theory and experiment in the whole range of ionization density. We also compare the simulation results with the predictions of the classical Jaffe theory of columnar recombination and discuss the applicability limits of this theoretical theory.


**POS38 Radiation technologies**

**POS38-01. Towards the medical application of laser driven particle beams.** Elke Beyreuther1, M. Baumann1, T. Burris-Mog2, W. Enghardt1, L. Karisch2, S. Kraft1, L. Laschinsky3, E. Lessmann1, J. Metzkes1, D. Naumberger1, M. Oppel1, C. Richter1, U. Schramm1, M. Schüter1, K. Zeil1, J. Pavelke1,2, H. Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany, 2. OncoRay – National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden, Germany, 3. Universitätshospital Carl Gustav Carus, Dresden, Germany

During the last years, the new laser based technology of particle acceleration was developed at such a rate that medical application, i.e. for cancer therapy, becomes entirely conceivable. Promising more compact and economic proton accelerators, being suitable for existing radiotherapy institutes, the laser technology however results in ultra-short pulsed particle beams of ultra-high pulse dose and pulse dose rate. Thus, the consequences of laser particle acceleration on beam transport and radiation field formation, dosimetry and radiobiological effects have to be investigated carefully for the whole translational chain from bench to bedside.

Within the German joint research project “onCOOPtics” systematic in vitro cell experiments aiming on the influence of the ultra-high pulse dose rate were firstly established at the Jenoptik laser system JETI that provides laser accelerated electrons of some tens of MeV. Secondly, the increased laser intensity of the 150 terawatt laser system DRACO at the HZDR was applied to accelerate protons to energies of up to 20 MeV. Previous to these experiments, both laser systems had to be extensively optimized in terms of intensity, energy distribution, background reduction, spot size, stability and reliability of the particle beams. The combination of real-time monitoring of dose delivery and a precise retrospective absolute dosimetry enabled the application of defined doses, in spite of the laser based fluctuations of beam intensity and energy. For comparison, reference irradiations with conventionally accelerated continuous particle beams were performed in parallel to each laser experiment.

In consequence, all key requirements necessary for systematic in vitro cell experiments as the basic translational step towards clinical application of laser-driven particle beam have been fulfilled. Moreover, the dose response curves obtained for pulsed and continuous particle beams show no significant influence of the ultra-high pulse dose rate on the radiobiological response. As next step, animal studies that demand for the translation from 2D to 3D irradiation are in preparation.

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**POS38-02. Radiation inactivation of Paeabacillus larvae larvae and sterilization of american foul brood (AFB) infected hives using CO-60 gamma rays.** Chitho Feliciano, Philippine Nuclear Research Institute, Philippines

The effectiveness of gamma radiation in inactivating the Philippine isolate of Paeabacillus larvae was investigated. Spores of *P. larvae* were irradiated at incremental doses (0.1, 0.2, 0.4, 0.8 and 1.6 kGy) of gamma rays by a CO-60 source and the number of surviving spores was counted and used to estimate the decimal reduction (*D*0) value. A dose of 0.2 kGy was sufficient to inactivate 90% of the total recoverable spores from an initial count of 104 to 9 x 103 spores per glass plate. The sterilizing effect of high doses of gamma radiation on the spores of *P. larvae* in infected hives was determined. In this study, a minimum dose (D90) of 15 kGy was tested. Beehives with subclinical infections of AFB were irradiated and examined for sterility. All the materials were found to be free of *P. larvae* indicating its susceptibility to γ-rays. After irradiation, there were no visible changes in the physical appearance of the hives’ body, wax, and frames. Thus, a dose of 15 kGy is effective enough for sterilization of AFB-infected materials.

**POS38-03. Radioluminescence studies of bulk and nano-particle fluoroperovskites for radiation dosimetry.** Christian Gaedtke1, G.V. M. Williams1, S.G. Raymond2, J. Donaldson1, L. Greig2, J. Steel2, D. Clarke2, S. Janssens3, 1: Victoria University of Wellington, New Zealand, 2: Industrial Research Limited, New Zealand, 3: Blood and Cancer Centre, Wellington Hospital, New Zealand, 4: SCPS, Victoria University, New Zealand

Radioluminescence (RL) has recently attracted considerable interest because it can be used to determine the real-time radiation dose and dose rate for radiation protection, non-destructive testing, and in medicine for monitoring radiation dose during radiotherapy or for dose verification and validation. RL is a phenomenon that occurs from the prompt radiative recombination of charged particles after irradiation. There is a need for new RL materials that have good
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transparency, high sensitivity, and with a response to radiation that is comparable to that of tissue. We have previously shown the some fluoroperovskites (e.g. NaMgF3:Eu2+ [1], Rb:MgF3:Eu2+ [2], RSCF:Mn2+ [3,4]) have potential as optically stimulated luminescence dosimeters for monitoring the total radiation dose after irradiation. In this paper we report the results from RL measurements on bulk transparent polycrystalline fluoroperovskites and fluoroperovskite nanoparticles and discuss their potential application as real-time dosimetry. Nanoparticles have many advantages that they can be used to make dosimeters with arbitrary shapes. We will present a model that can account for the RL and methods to obtain dose history independent RL. We will also show how RL can be used to measure the dose rate in these compounds and report the results from characterizing radioactive sources and their medical radiotherapy linac operating at 6 MV. References [1] Dotzler, C., Williams, G.V.M., Rieser, U., Edgar, A. 2007, Appl. Phys. Lett. 91, 121910 [2] Dotzler, C., Williams, G.V.M., Rieser, U., Robinson, J. 2009, J. Appl. Phys. 105, 023107 [3] Dotzler, C., Williams, G.V.M., Edgar, A. 2008, Current Applied Physics 8, 447 [4] Dotzler, C., Williams, G.V.M., Edgar, A. 2007, Appl. Phys. Lett. 91, 181909


Technological advances have dramatically improved the conformity and accuracy of radiotherapy (RT) of cancer patients. Recently, microimage-guided RT (μGRT) systems have been developed that integrate small brain geometries, cone beam CT (CBCT) guidance and robotic corrections. These μGRT systems therefore bridge the technology gap between pre-clinical radiation research and clinical radiation therapy. The purpose of this work was to quantify radiation delivery accuracy of a μGRT system (X-RAD 225Xc, Precision X-Ray Inc.) in vivo by using gold nanoparticles (AuNPs), injected intracranially with U87 tumor cells, as contrast agent. AuNPs (30-100 μg/mL, 150 nm, in saline solution) embedded in gel were imaged using CBCT (40-120 kVp). AuNPs were subsequently stereotactically injected in nude mice with (n=3) or without (n=5) U87 tumor cells, 3 mm below the skullcap. CBCT scans were taken every 3-5 days with different scanning techniques for up to 2 weeks. The tumor marked by the AuNPs was targeted by selecting its position on the CBCT reconstructed scan. The computer controlled couch consequently moved to place the selected position at the isocenter, which was irradiated with a 5 mm diameter field or star shots (8 equiangular spaced beams, 1 mm diameter field). H&E and HE-staining were performed on the slices around the tumor. Adequate contrast is found in vitro for each imaging technique (maximum relative contrast C=2.4 at 40 kVp). In vivo, no signal was detected for AuNPs alone. An increased contrast of the tumor region (C=0.49 for AuNPs) is found for AuNPs at day 15 after injection. No abnormalities in mouse behaviour were observed. The presence of AuNPs increased the targeting accuracy in CBCT by 0.5 mm. The staining results showed AuNPs are confined within the volume of the tumor. Radiation damage was shown to be centered in the tumor area (0.09 and 0.47 mm deviation, with and without AuNPs respectively). AuNPs are an efficient and reliable contrast agent when injected directly in a tumor cells containing suspension, allowing tumor detection during the RT. CBCT of AuNPs in brain is a promising 3D addition to 2D bio-luminescence for tumor growth follow-up. The comparison of tumor and radiation damage positions demonstrates the high targeting accuracy of 0.1 mm of the μGRT system.


X-ray microscopy has been paid much attention as a promising analytical tool with many advantages: high resolution, long depth of focus, ability to observe samples without micrometric or heavy metal staining, and so on. Moreover, the distribution of specific elements will be visualized by the X-ray absorption differences. (J. Kirz et al., Q. Rev. Biophys. 28, pp33–130, 1995)

However, the spatial resolution of currently available devices, such as CCD and x-ray nanofocus X-ray microscope are not enough for nano-scale imaging. Hence, using high resolution resist materials as a detection layer has been studied to realize nano-scale x-ray imaging. By placing a resist behind the object and irradiating with X-rays, the object’s profile and X-ray absorption information can be recorded in the resist as the pattern and its depth. By choosing appropriate X-ray wavelengths and examining the resist surface with an atomic force microscope (AFM), nano-scale imaging and elemental mapping would be possible. The sensitivities of several kinds of resist materials have been evaluated with monochromated X-rays in our previous studies. It has been suggested that the sensitivities would vary in response to the X-ray wavelengths and the experimental results have shown that the RL characteristics depend on the resist composition. (T. Gowa et al., Radiat. Phys. Chem., 80(2), pp248-252, 2011)

In the present study, X-ray imaging of nano-particles was actually attempted. An electron beam resist ZEP7000 (ZOEK Co.) was chosen as the imaging layer because of its high sensitivity. S118 (~65 nm) and Au (~50 nm) were coated on the ZEP7000 and monochromated X-rays were irradiated. As the result, the distribution of the nano-particles was recorded on the resist surface. In addition, the images recorded with X-rays around K-shell absorption edge of nitrogen were compared, in order to evaluate the possibility of elemental mapping of nitrogen. The detailed results will be presented at the conference.

POS38-06. Development of Nanosecond and Picosecond Pulse Radiolysis System. Yuji Hosaka1, Y. Kawauchi1, R. Betto1, K. Ogata1, K. Sakaue1, R. Kuroda1, S. Kashiwagi1, K. Ushida1, M. Washio1, 1: Waseda University, Japan, 2: AIST, Japan, 3: Tohoku University, Japan, 4: RIKEN, Japan

A compact photocathode S-band RF-Gun has been developing at RISE WASEDA University, and pump-probe pulse radiolysis has been studied as application of electron beam obtained by this photocathode RF-Gun. Pulse probe absorption spectroscopy is one of the most well-known methods of pulse radiolysis. Pulse radiolysis has been used in various institutes as a powerful method to trace rapid initial chemical reactions by ionizing radiation.

In pump-probe absorption spectroscopy, probe light absorption is caused by active species formed by pump beam, and is measured at each wavelength. Therefore, both pump beam and probe light are necessary in pump-probe pulse radiolysis. About 5MeV electron beam that was obtained from compact photocathode RF-Gun was used as pump beam, and the various white light sources were used as probe light source of this experiment.

In nanosecond time resolution pulse radiolysis, the stable white light emitted from Xe arc flash lamp was used. Because high intensity, good stability and broad spectrum of white probe light are key issues for pump-probe pulse radiolysis, high-quality pulse radiolysis system would be constructible when this white light was used. Thus, nanosecond pulse radiolysis without any averaging of waveform was successfully conducted. A short pulsed white light is necessary in picosecond time resolution pulse radiolysis. In this case, supercontinuum radiation with PCF (Photonic Crystal Fiber) that was generated from IR monochromated pulsed laser is used. The IR pulsed laser is whitened and transformed into supercontinuum by nonlinear optical effect in PCF. Supercontinuum radiation with PCF is a new technique of white light generation, and is studied for various applications recently. One of the advantages of supercontinuum probe is the possibility to generate white light because of smaller core diameter of PCF, which can improve pulse radiolysis system.

The experimental procedures and results of these pulse radiolysis systems would be reported in this presentation.

POS38-07. Optimizing the experimental components required in a laser-accelerated particle cell irradiation. Nicole Humble1, K. Allinger1, J. Bin2, K. Ushida3, M. Washio3, 1: Dept Oncology Klinikum Rechts der Isar der Technischen Universität München, Germany, 2: Max-Planck-Institut für Quantenoptik, Ludwig-Maximilians-Universität München, Germany

Developments in laser-accelerated heavy-ion technologies offer much potential in radiation therapeutic applications. Before these can be realised however, preclinical cell irradiation studies must first be carried out to understand the biological effects of laser-accelerated particles against those established characteristics of particles beams already used in the clinic. The nature of laser-accelerated particle production already alludes to difficulties in establishing the necessary conditions for radiation therapy. A nonmonenergetic energy spectrum, insufficient beam energy and a pulsed rather than continuous beam are but some of the issues addressed here. Therefore preliminary in-vitro cell irradiations are
carried out with the ATLAS Ti-Sapphire laser at the Max Planck Institute of Quantum Optics using a 1 J beam in 45 fs at 10 Hz. Here we describe the methods employed in order to meet the conditions required for a laser-accelerated proton cell irradiation with beam energies between 5 and 10 MeV.

Although the beam is produced over a broad energy spectrum, this can to an extent be accounted for using two permanent quadrupole magnets. Simulations predict a narrow energy spectrum resulting in the beam not being collimated to ±100 keV. A dipole magnet later determines the energy of the beam before the beam passes through an exit window. Once the beam passes through the cell holder, allowing the cells to be in a single layer, perpendicular to the beam axis, radiochromic film is employed as a means of dosimetry directly behind the cells. The films are used here so as to offer absolute dosimetry and spatial information on the beam profile with sub-mm resolution to allow for localisation of the dose distribution and subsequent cell damage. Cell damage is analysed using the gamma-H2AX assay. The dose is delivered in one shot thus minimising uncertainties regarding dose delivery inhomogenities.

Despite the obvious limitations in such an experiment, we present here the physical problems needed to be addressed in a laser-accelerated heavy ion cell irradiation with some possible solutions. These initial experiments are a biological pre-cursor necessary before clinical applications can start to be considered.

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**POS38-08. Introduction of Education and Training of Radiation Using Natural Radiation Sources.** Takao Kawano, National Institute for Fusion Science, Japan

Potassium is contained in various materials. Naturally occurring potassium exists as three isotopes: 40K, 39K, and 41K. Of these isotopes, only 40K is a radionuclide; it emits a 1.33 MeV beta particle as a result of beta decay (89%) and a 1.46 MeV gamma radiation due to electron capture (11%). Potassium chloride chemicals, potassic chemical fertilizers and kelps are the most well known of those materials, and are often used in educational courses for illustrating naturally existing radiation and radioisotopes around us. With materials containing naturally occurring potassium-40 and radioisotopes, a method of fabricating radiation sources was recently developed and was applied to commercially available potassium chloride chemicals, potassic chemical fertilizers and kelps to fabricate radiation sources for educational use. In the present study, thus fabricated radiation sources were called natural radiation sources and were actually used in training students in a course on laboratory measurements of radiation for allowing students to easily experience radiation and better understand its characteristics. It was also found that the natural radiation sources could be handled safely and easily in radiation courses and that the data sheets were very important for carrying out measurements smoothly in practice within short periods of time.

**POS38-09. Development of a compact electron accelerator for radiation applications at Waseda University.** Yohei Kawaucchi1, Y. Hosaka2, T. Aoki1, K. Ogata1, R. Betto1, T. Yamamoto2, Y. Yokoyama1, K. Sakurai1, M. Washio1, R. Kuroda1, S. Kashigawa1, K. Usuda1, H. Hayano1, N. Toruiwa1, H. Hayano1, 1: Waseda University, Japan, 2: AIST, Japan, 3: Tohoku University, Japan, 4: RIKKEN, Japan, 5: KEK, Japan

At Waseda University, we have been developing a compact laser photocathode RF gun to generate high quality electron beam for application researches of radiation. The RF gun system is composed of BNL type S-band RF cavity with Cs-Te photocathode, a solid-state picosecond Nd:YLF laser to irradiate photocathode and an RF power source. Although our linac system is table top size (about 2m x 2.5m), electron beam generated from the gun has enough energy (~5 MeV) and charge (10 nC/beam) for applied research. As applied research, we have conducted a pulse radiolysis experiment based on pump-probe method to trace initial processes of radiation chemical reactions. The beam to pump samples is generated from the RF gun. And the white pulsed light to probe samples is generated by interaction between laser pulse with laser beam and single-pulse laser. To increase the X-ray intensity, we are developing a multiple collision system between multi-bunch electron beam and laser pulses. We have been also trying to generate micro-beam for MRT (Micro-beam Radiation Therapy), which is new technique for cancer treatment, using bremsstrahlung X-ray or electron itself. In this conference, the current status and specifications of our electron linac system, results of application researches, and future prospective are presented.

**POS38-10. Temperature is a critical factor to determine leaf senescence of spinach after gamma irradiation.** Jun-Hong Kim, M.H. Lee, Korea Atomic Energy Research Institute, South Korea

Treatment of low temperature or ionizing radiation has been considered for maintaining post-harvest quality of flowers, vegetables, and fruits. The present study aimed to reveal a relation between different temperatures and radiation doses during post-harvest period and leaf senescence of spinach (Spinacia oleracea L.) leaves. Leaf disks of 1.8 cm in diameter were excised from spinach plants, which were purchased from a local market. Then, they were incubated for 6 d at 4, 15, 22, or 30°C in darkness after exposure to gamma rays of 0.5, 1, 2, or 3 kGy for 1 h. Progress of the induced leaf senescence was evaluated by chlorophyll fluorescence parameters and photosynthetic pigment contents. Photochemical efficiency, Fv/Fm, decreased gradually in the control and irradiated leaves at 22 and 30°C during the 6-d dark-incubation. The higher radiation dose or temperature caused the more decrease of Fv/Fm. In contrast, the Fv/Fm was never or much less affected at 4 and 15°C, showing weak dependence on the radiation doses applied. Photosynthetic electron transport rate, ETR, also decreased similarly to the Fv/Fm. Total chlorophyll content was found to decrease with the increasing radiation dose and temperature, while total carotenoid content was shown to rather increase. In the latter case, the composition of total carotenoid was characterized of the higher proportion of lutein but the lower of β-carotene. Based on the obtained data, we suggest that acceleration of leaf senescence depending on the radiation dose could be almost fully prevented by incubation at low temperatures below 15°C.

**POS38-11. Development of quasi-monochromatic LCS X-ray source for novel combination cancer radiotherapy with drug delivery system.** Ryunosuke Kuroda1, H. Tsurushima1, K. Yamada1, M. Koike1, E. MIURA1, E. Yamaguchi1, 1: National Institute of Advanced Industrial Science and Technology (AIST), Japan, 2: Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan

We have developed a quasi-monochromatic hard X-ray source via laser Compton scattering (LCS) for medical uses at AIST in Japan. The X-ray source consists of a 40-MeV S-band compact electron linac and a high power laser system. We are planning to apply the quasi-monochromatic X-ray source to the novel combination cancer radiotherapy with the active-targeting drug delivery system (DDS). Our concept of the radiotherapy is proposed that the tuned quasi-monochromatic X-ray is irradiated to the high-z nanoparticles delivered to tumors by the active-targeting DDS. The high-Z element, such as gold (Z=79), in tumors can locally enhance the dose of radiation like the Bragg peak curve. According to results of our Monte-carlo simulation, the most effective X-ray energy is calculated to be about 40 keV. In this conference, we will present our latest results of our radiotherapy design using the quasi-monochromatic X-ray with the DDS and the recent status of the X-ray source development.

POSTER PRESENTATIONS

Schubert, R. Schulte, Radiation Medicine, School of Medicine, Loma Linda University, USA

Purpose: We have developed a system for targeting functional areas in the rat brain with narrow proton beams. The system utilizes either the sharp lateral dose falloff of high-energy proton beams using a cross-fire technique with multiple shoot-through beams converging on the target, or the sharp distal falloff of a proton Bragg peak to selectively irradiate specific regions in the rat brain. Verification of the spatial accuracy of dose-delivery is crucial for such experiments. We have developed and tested an immunohistochemical staining protocol based on the DNA double strand break (DSB) marker phosphorylated H2AX (γH2AX).

Materials and Methods: Male Sprague-Dawley (8 weeks, 150-190g) rats were subjected to CT-guided stereotactic proton irradiation with a single 200 MeV proton beam, delivering a dose of 10 Gy. Thirty to 60 minutes after proton irradiation, the rats were sacrificed and 4% paraformaldehyde-perfused rat brains were cryostat sectioned and slide mounted. Sections then underwent immunofluorescence detection of phosphorylated γH2AX. Radiation-induced DNA DSBs were detected with a confocal microscope as discrete or overlapping nuclear foci using specific antibodies against phosphorylated γH2AX, which were visualized by anti-mouse IgG Alexa Fluor 488 (green), while nuclei were counterstained with propidium iodide (PL red).

Results: A dose of 10 Gy resulted in multiple radiation-induced DSB foci in cell nuclei within the path of a single proton beam. Focal antibody staining of phosphorylated γH2AX demonstrated the beam profile in the irradiated hemisphere.

Conclusion: Phosphorylated γH2AX identifies the small brain regions irradiated with separate proton beams and is thus useful for verification of targeting accuracy and in-vivo dosimetry for radiosurgery procedures in small animals.

POS38-13, Quantitative Evaluation of Spatial Dose Modulation for Preclinical Radiobiology Studies. James Stewart, P. Lindsay, D. Jaffray, Princess Margaret Hospital Hospital, Canada

Purpose: The recent development of dedicated irradiation platforms specifically designed for preclinical studies brings with it the potential for developing and testing the physiologic efficacy of highly inhomogeneous dose distributions. Such systems have the ability to deliver highly modulated dose distributions with the precision necessary to determine the relative biological effectiveness of these dose prescriptions. The performance of a novel preclinical irradiation system (Xrad225Cx, Precision X-Ray, Inc., North Branford, CT) was evaluated in terms of its ability to deliver spatially inhomogeneous dose for a varying amplitude dose pattern similar to patterns characterized as 'grid' distributions.

Methods: The positioning stage on the preclinical irradiator was programmed to move between a series of individual irradiations. A pattern consisting of an 8x12 array of irradiations was delivered with a fixed 4.65 mm nominal square central collimator and a 9 mm distance between irradiation isocenters. The 96 irradiations were exposed from 0 to 190 seconds in 2 second increments (0 to 8.25 Gy) at an energy of 225 kVp and current of 13 mA. Dose delivery was measured between two 4.9 mm thick water equivalent plastic slabs using GaFchromic EB72 radiochromic film. After delivering the dose distribution, the films were scanned at a resolution of 400 DPI (~0.06 mm/pixel) for quantitative analysis.

Results: The 96 individual irradiations were delivered with 96% of the radiation isocenters within 0.1 mm of the desired 9 mm spacing. Each irradiation was geometrically consistent, with the area at half maximum dose between 4.33 mm2 and 4.83 mm2 for 91 (95%) of the irradiations. Maximum dose varied linearly with exposure time with an average dose rate across all non-zero exposure time irradiations of 2.61 +/- 0.34 Gy/s (mean +/- SD).

Conclusion: The quantitative evaluation of the delivered dose demonstrates the ability to precisely modulate dose distributions at the scale demanded by preclinical small animal irradiation studies. This dosimetric ability indicates the inherent potential for studies of the radiobiological efficacy of complex radiation delivery patterns.

POS38-14, A new microbeam facility for cell-irradiation research. Y.C. Yu1, I. Cho2, H. Niu3, 1: Institute of Physics, Academia Sinica, Taiwan, 2: Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Taiwan, 3: Nuclear Science and Technology Development Center, National Tsing Hua University, Taiwan

A 90°-scattering cell irradiation system was installed in the NTHU accelerator lab. This system is capable of providing 0.8–2.5 MeV of proton and alpha beams with 1 mm in diameter for radiobiological research [1]. In previous studies, we showed that this system was successfully applied on DNA damage formation along the particle trajectory [2]. To study the single cell or partial cell irradiation application, a new microbeam facility was developed. This study employs the ICP-RIE (Inductively Coupled Plasma Reactive Ion Etch) method to build a micro-collimator, which was fabricated by 100 um-thick silicon wafers with an aperture diameter of 10 um. Two pieces of micro-collimator were aligned and bound to each other as a double slit collimator. Shrinking beam spot size caused a decrease in the scattering solid angle and lost the exiting beam flux. Therefore, the scattering chamber was designed to compensate the loss of exiting beam flux. The system performance test was measured by using a 1.5 MeV proton. The energy spectrum of the exiting proton was found to be of 78 keV FWHM energy resolution at the mean energy 1.460 MeV. The CR-39 nuclear track detector etching study indicates that 80 % of particles-collimator were aligned and bound to each other as a double slit collimator. Shrinking beam spot size caused a decrease in the scattering solid angle and lost the exiting beam flux. Therefore, the scattering chamber was designed to compensate the loss of exiting beam flux. This results demonstrate that the beam diameter can be shrunk to the scale of micrometers by using the silicon-based micro-collimator. Certain primary measurements will be discussed.

References

POS39-01. Effects of radiotherapy on survival, comorbidity, and remission for elderly patients with lung and breast cancer in the USA. Igor Akushevich, J. Kravchenko, S. Ukraintseva, K. Arbeev, A. I. Yashin, Duke University, USA

The effects of the radiation therapy on survival, comorbidity, and remission in cancer patients are not completely clear. The majority of cancer patients are older than 65+, therefore, the Surveillance Epidemiology and End Results Registry data linked to Medicare claims could provide researchers with information on remission and comorbidity in the U.S. elderly through the reconstruction of individual disease histories for lung and breast carcinomas and comorbid conditions. Since multiple factors can influence the probability of getting radiation therapy including severity of diagnosed cancer, comorbidity, age at diagnosis, and treatment other than radiation therapy, the propensity score technique was used to provide pseudorandomization in compared groups with and without radiation therapy. Four outcomes were analyzed: total mortality, cancer-specific mortality, cases of cancer in remission, and rapid increase of comorbidity. Information on different types of radiation therapy (i.e., beam radiation, radiation implants, radioisotopes, and other radiation) and mortality of tumor/exposure (i.e., the side of paired organ where the tumor was located) were analyzed. For breast cancer, the beneficial effect of radiation therapy on both total and cause-specific mortality overcame its side effects (HR=0.33 (p<0.0001) for total death and HR=0.49 (p=0.0002) for breast-cancer death); these effects were less pronounced for lung cancer (HR=0.85 (p=0.0005) for total death and HR=0.90 (p=0.04) for lung-cancer death). For both tumors, there were no effects of radiation therapy on cancer remission rates. The modest effects were observed on comorbidity (including the effect of literacy): the risk of acute coronary heart disease and stroke increased for lung cancer patients who received radiation therapy compared with those who were treated only surgically; also, the therapy with radiation implants and radioisotopes increased the risk of chronic obstructive pulmonary disease and thyroid disease/gester in lung cancer patients, and risk of renal disease - in breast cancer patients. In summary, the radiation therapy decreased total and cancer-specific mortality, however, affecting the comorbidity of cardio- and cerebrovascular, respiratory, thyroid and renal diseases in lung and breast cancer patients.

POS39-02. Irreversible EGFR inhibitor EKB-569 targets low-LET gamma-radiation-triggered Rl orchestration and potentiates cell death in squamous cell carcinoma? Natarajan Aravindan1, J. Veeraraghavan1, S. Aravindan1, A.S. Mohan1, J.D.


EBK-569, an irreversible EGFR tyrosine kinase inhibitor has shown potential therapeutic efficiency in solid tumors. However, its synergistic/complementary cell-killing potential alongside radiotherapy, if any, and, underlying molecular orchestration remains to be explored. We investigated the effect of EBK-569 on radiation (IR) associated NFκB-dependent cell death. SCC-4 and SCC-9 cells exposed to IR (2 Gy) with or without EBK-569 were analyzed for transactivation of 88 NFκB pathway molecules, NFκB DNA-binding activity, translation of NFκB downstream (Birc1, 2 and 5) molecules and cellular outcomes, including cytotoxicity and apoptosis. Selective targeting of IR-induced NFκB by EBK-569 and its influence in cell-fate was assessed by overexpression (p50/p65) or silencing (ΔIKBκB) NFκB. QPCR profiling revealed a significant induction of 74 NFκB signal transduction molecules after IR, of which, 72 were suppressed with EKB-569. EMSA revealed a dose-dependent inhibition of NFκB DNA activity after EKB-569 treatment. More importantly, EKB-569 inhibited IR-induced NFκB in a dose-dependent manner and this inhibition remained consistent at least up to 72h. Immunoblotting revealed a significant suppression of IR-induced Birc1, 2 and 5 with EKB-569. We observed a dose-dependent inhibition of cell viability (examined by blue dye method) increased in a dose dependent manner (assessed by MTT assay) and induction of apoptosis (Acridine orange staining) with EKB-569. Evidently, EKB-569 significantly conferred IR-reduced cell viability and increased apoptosis. Blocking NFκB similarly augments IR-inhibited cell death. Conversely, NFκB overexpression inhibits complete suppression of NFκB-dependent cell survival upon EKB-569 treatment. Together, these data clearly delineates that EKB-569 in combination with IR potentiates the therapeutic efficiency for squamous cell carcinoma and further demonstrates that EKB-569 associated radiosensitization may involve selectively targeting IR-induced NFκB-dependent survival signaling.

**POSTER PRESENTATIONS**


**Purpose:** To estimate the risk of radiation-induced secondary malignancies from intensity modulated radiation therapy (IMRT) and conformal radiation therapy (CRT) for head and neck (H&N) tumours and to investigate the level of consistency between risk models.

**Background:** IMRT has been increasingly employed for treating H&N tumours due to its ability to produce concave isodose levels suitable for the complex anatomy of the H&N region. Consequently, this treatment technique has led to a reduction in the rate of acute side-effects in comparison to the more conventional CRT. However the use of more fields in IMRT to spread the dose outside the target to a larger volume has been a cause of concern from the point of view of the risk for secondary cancers from radiotherapy.

**Material and methods:** Ten H&N patients were planned both with IMRT and CRT and the resulting treatment planning data was used to calculate the risk of radiation-induced malignancies in four relevant tissues: esophagus, body and left and right parotid glands. Three risk models with biologically relevant parameters were used for calculations. The influence of head scatter radiation and repeated imaging sessions has also been investigated.

**Results:** The results showed that for each risk model the total risks from the two treatment techniques were small and comparable: 0.7-1.2% for CRT and 0.6-1.2% for IMRT. The largest contributions were from the irradiation of the body and the esophagus. The average risk for the body was higher for IMRT than for CRT, while the average risk for the esophagus was higher using CRT. Head scatter radiation and repeated use of diagnostic imaging modalities had comparatively little contribution to the total risk.

**Conclusions:** The results indicate that the redistribution of the dose characteristic to IMRT leads to a redistribution of the risks in individual patients, the body receiving a larger volume of the body to lower doses with IMRT leads to an increase of the risk in this region, but the corresponding risk from the irradiation of the esophagus decreases. Further distinction between treatment techniques is not possible given the rather large uncertainties for the parameters available. Nevertheless, the low levels of absolute risks support the use of radiation therapy as a viable treatment modality for inoperable or advanced H&N tumours.

**POS39-04. Differential transcriptomic responses of normal and tumor brain tissues after synchrotron microbeam radiation therapy (MRT).** Audrey Bouchet 1, 2, 3, 4, Martin Brown, G. Ahn, M. Koir, S. Liu, R. Alomran, Stanford University, USA

**The aim of this study was to characterize transcriptomic responses of normal brain and 9L gliosarcoma tissues after synchrotron microbeam radiation therapy (MRT), a spatial microfractionated X rays beam.** This unique radiation geometry allows very high dose depositions in tumor while covering normal brain and minimizing normal tissue damage. We have compared transcriptomic responses using microrays of normal and tumor brain tissues, 6 h after radiation. On the 28,000 probesets analysed, almost 2,000 and 3,000 were significantly modified, in tumor and normal tissues, respectively (Mann-Whitney test). 900 of these probesets common to both responses concern the inflammatory response. Moreover, histone acetylation and DNA damages. However, the major part of the response is tissue-specific.

To conclude, MRT significantly increases the MST of brain tumor-bearing rats. We have compared transcriptomic responses of normal and tumor brain tissues, 6 h after MRT irradiation, in a syngenic model. The analysis of interactions between genes would allow defining pathways specifically activated in each tissue during response to MRT.

**References:**


**POS39-05. Inhibiting Vasculogenesis Following Irradiation: A New Paradigm for Radiotherapy.** Martin Brown, G. Ahn, M. Koir, S. Liu, R. Alomran, Stanford University, USA

**The aim of this study was to characterize transcriptomic responses of normal brain and 9L gliosarcoma tissues after synchrotron microbeam radiation therapy (MRT), a spatial microfractionated X rays beam.** This unique radiation geometry allows very high dose depositions in tumor while covering normal brain and minimizing normal tissue damage. We have compared transcriptomic responses using microrays of normal and tumor brain tissues, 6 h after radiation. On the 28,000 probesets analysed, almost 2,000 and 3,000 were significantly modified, in tumor and normal tissues, respectively (Mann-Whitney test). 900 of these probesets common to both responses concern the inflammatory response. Moreover, histone acetylation and DNA damages. However, the major part of the response is tissue-specific.

To conclude, MRT significantly increases the MST of brain tumor-bearing rats. We have compared transcriptomic responses of normal and tumor brain tissues, 6 h after MRT irradiation, in a syngenic model. The analysis of interactions between genes would allow defining pathways specifically activated in each tissue during response to MRT.

**References:**


Kao, Department of Radiation Oncology, University of Pennsylvania, USA

Purpose: To investigate vascular changes induced by targeted radiation therapy within tumors in a mouse model system. Background: Stereotactic radiation therapy (RT) has become standard treatment for many patients with solid malignancies, but the mechanism(s) underlying the demonstrated clinical efficacy remains unclear. Animal models of targeted RT have been limited due to the inability of conventional laboratory irrigators to focus radiation precisely to just the tumor and not nearby normal tissues. Irradiation of adjacent normal tissues and organs confound interpretation of vascular changes within tumor. The Small Animal Radiation Research Platform (SARRP) enables accurate targeted RT to tumor alone within animals, facilitating studies on vascular effects.

Methods: Human glioma cells were grown as xenografts in the flanks of nude/athymic mice. The SARRP was utilized to deliver 20 Gy to a collimated field tightly covering each tumor. Mock-irradiated animals served as controls. All mice were sacrificed 5 days after irradiation and the tumors fixed, sectioned, and stained for CD31 (defining endothelium/vascular), phospho-histone H3 and DAPI to mark mitotic cells. The number of CD31-outlined lumen-containing vessels and mitotic cells per low power field (LPF (100X)) was counted for each tumor section. Automated nonbiased computer delineation of vessel area (total luminal space contained within CD31-positive areas) was also performed.

Results: CD31 expression was evident in all tumors. However, while control tumors showed a mean number of 33 + 14 lumen-containing vessels per LPF, irradiated tumors showed only 2 + 1 lumen-containing vessels per LPF. Total lumen area was 2735 µm² per LPF in control tumors versus a mean of 454 µm² per LPF in irradiated tumors. Consistent with RT inhibiting proliferation, control tumors showed an average of 62 + 3 mitotic cells versus 35 + 3 in irradiated tumors. All described differences between control and irradiated tumors were statistically significant (p < 0.05).

Conclusions: Targeted RT induces within days dramatic intratumoral vascular collapse, measured by two different assays. These remarkable results suggest a novel anti-tumor mechanism induced by large single fractions of RT, potentially distinct from intracellular DNA damage.

POSTER PRESENTATIONS

POS39-07. Analysis of DNA damage by γ-H2AX assay in whole blood lymphocytes of patients with neuroendocrine tumours undergoing 177Lu-octreotate (LuTate) therapy. Delphine Denoyer1, O.A. Martin1, V. Johnston1, T. Barber1, P. Jackson1, W.M. Ronnero1, R.J. Hick1, 1: Peter MacCallum Cancer Centre, Australia, 2: National Cancer Institute, USA

Ionizing radiation (IR)-induced DNA double-strand breaks (DSBs) can lead to cell death, genome instability and carcinogenesis. Upon formation of DSB, hundreds of molecules of γ-H2AX become phosphorylated and create a nuclear “γ-H2AX focus” attracting repair proteins. External IR (X-rays, y-rays)-induced γ-H2AX foci are detectable soon after irradiation in a dose-dependent manner, with maximum size attained ~30 min post-IR and disappearance within several hrs due to repair. However, radiouclide-induced DNA damage kinetics can be more complex, reflecting the range of gamma and particulate emissions, the variable biodistribution of the agent within cells and at an organ level, and the time course of radiation delivery determined by the physical decay characteristics of the radionuclide. In this ongoing study, we tested γ-H2AX as an in vivo biodosimeter after extended internal whole-body irradiation. We investigated the kinetics of γ-H2AX foci in whole blood lymphocytes after radionuclide incorporation in patients with neuroendocrine tumours undergoing up to three 177Lu-octreotate (LuTate) therapy treatments. 177Lu was received 6-11 GBq of LuTate therapy. We measured γ-H2AX foci at 0.72 hrs post-treatment, and correlated these numbers with 177Lu radioactivity clearance data in serum. Up to date, 10 patients underwent the therapy. The 177Lu activity was the highest after administration and gradually decreased with time, corresponding to biexponential disappearance of the isotope from the organism. The 90% of clearance occurred within 2 hrs, primarily by renal excretion. The radiation absorbed dose induced excess y-H2AX foci in all patients. The foci numbers were higher (0.95+0.57-0.86+0.52 vs. 0.28+0.22 baseline values) at 0.5-4 hrs and lower (0.42+0.4-0.49+0.34) at 24-72 hrs post-treatment, and were significantly associated with therapy administrations introduced even more pronounced individual-response variation, often with un-repaired damage at 72 hrs post-treatment. Thus, γ-H2AX assay can be utilized to track LuTate therapy effects. When combined with clinical markers (lymphocyte and bone marrow counts etc.), it could indicate individual radiosensitivity. These data are yet to be compared with tumour response.

POS39-08. Tumor cell repopulation in rectal cancer after preoperative short course of radiotherapy. Anna Gasinska1, P. Richter1, Z. Maleska1, 1: National Centre of Oncology, Krakow, Poland, 2: Department of General Surgery, Jagiellonian University, Poland

Purpose: Inhibition of tumor proliferation rate based on Bromodeoxyuridine labelling index (BrdUrd/LI), S-phase fraction (SPF) and MIB-1 labeling index (MIB-1LI) as an early rectal cancer response to preoperative radiotherapy (RT).

Methods and material: One hundred and twenty-two patients qualified either for short RT (5 Gy/fraction/5/6 days) and surgery about one week after RT (schedule I), or for short RT and 4 weeks interval before surgery (schedule II). Tumor samples were taken twice from each patient, before RT and at the time of surgery. In each sample, the BrdUrd/LI, SPF and MIB-1 were calculated. Early tumor response was assessed by biologist, pathologist and surgeons.

Results: Fifty-six patients were treated according to schedule I and 66 patients according to schedule II. Mean BrdUrd/LI, SPF and MIB-1 LI before RT were 8.8 ± 9.1, 21.0 ± 53.3 % and these values did not differ between the two compared groups. After RT, tumors showed statistically significant growth inhibition based on all assessed biological markers. As pretreatment assessed parameter was not prognostic, for overall survival measured a mean of 2.0 GY/m² per LPF in irradiated tumors. Consistent with RT inhibiting proliferation, control tumors showed an average of 62 ± 3 mitotic cells versus 35 ± 3 in irradiated tumors. All described differences between control and irradiated tumors were statistically significant (p < 0.05).

Conclusions: Targeted RT induces within days dramatic intratumoral vascular collapse, measured by two different assays. These remarkable results suggest a novel anti-tumor mechanism induced by large single fractions of RT, potentially distinct from intracellular DNA damage.

POS39-09. Prostate cancer radiotherapy outcome: alpha/beta = 1.4 (0.9-2.2) Gy from 5969 patients, and no dose-response for high-dose-rate brachytherapy in 1207 patients. Jolyon Hendry1, R. Miralbell1, S. Roberts1, E. Zubizarreta1, 1: Manchester University, UK, 2: Hopitaux Universitaires de Geneve, Switzerland, 3: International Atomic Energy Agency

Objective: Multiple primary datasets have collected and analysed to substantiate and elucidate reports of a high sensitivity of prostate cancer to radiotherapy dose fractionation (low a/b ratio) regarding different stages of disease, the effect of androgen deprivation, and the dose-response for high-dose-rate brachytherapy.

Material and Methods: Seven external-beam datasets were assembled from institutions worldwide. 5969 patients were treated using daily (5 per week) dose fractions in overall treatment times of 1 to 8 weeks. Standard fractionation (1.8-2.0 Gy per fraction) was used for 40% of the patients, and hypofractionation (2.5-6.7 Gy per fraction) for the remainder. Low-risk patients comprised 23% of the total, intermediate-risk 44%, and high-risk 33%. In addition, 1207 patients in 6 datasets received external-beam doses (20-39 fractions of 1.8-2.0 Gy) plus 2 to 9 high-dose-rate brachytherapy fractions over 1-7 weeks. Direct biomathematical modelling and analysis of the primary data for tumour control at 5 years was undertaken, using the Phoenix criterion of biochemical relapse-free survival (bRFS).

Results and Conclusions: With external beam alone, there was an expected tendency for a decrease in bRFS as the risk group increased from low to intermediate to high. The values of a/b for the 3 risk groups were not significantly different from that for the pooled data of 1.4 (95% CI: 0.9-2.2) Gy, and they were within the low range generally reported for prostate cancer. Androgen deprivation improved the b-RFS by about 5% for all risk groups, but it did not affect per LPF. The brachytherapy data showed no clear dose-response, which was inconsistent with predictions from the external beam data. Hence, confounding factors possibly associated with
POSTER PRESENTATIONS

POS39-10. Ascorbic Acid Enhanced Apoptosis of Human Leukemia HL60 Cells on Radiation. Yoichiro Hosokawa, S. Monzen, L. Kashiwakura, Hiroswa University and Graduate School of Health Sciences, Japan

This study was conducted to examine the combined use of ascorbic acid (ASA) and radiation to examine clinical applications. We investigated cell survival, DNA fragmentation, and caspase activation by X-ray irradiation with ASA in human leukemia HL60 cells. The number of living cells decreased with X-ray irradiation treatment combined with ASA (2 Gy + 5 mM) in comparison with X-ray irradiation (2 Gy) or ASA (5 mM) alone. In the cells treated with X-ray irradiation combined with the use of ASA, more DNA fragmentation was observed compared to X-ray irradiation alone. Caspase-3, caspase-8, and caspase-9 were highly activated following X-ray irradiation and ASA treatment, but caspase-8 activity was not markedly increased after X-ray irradiation alone. The levels of Bax in the mitochondrial membrane fraction were observed after treatment with ASA and after X-ray irradiation and ASA together. However, there was no significant increase in the levels of Bax after X-ray irradiation treatment alone. As mentioned above, this study confirmed that supplementing X-ray irradiation with ASA treatment results in increased apoptosis in HL60 cells. When considering the apoptosis-inducing factors, we hypothesized that Bax and caspase-8 were activated and treated with X-ray irradiation and ASA compared to either treatment alone.

POS39-11. The Effect of environmental pO2 and pH on the radiosensitivity of endothelial cells. So Ra Kim, E. Kim, W. Ji, Seoul National University, South Korea

Purpose: The microbeam radiation therapy (MRT) protocol has been proven to improve the therapeutic effect in brain tumor treatment by employing a radiation beam under spatial fractionation in micron scale instead of the conventional broad beam in millimeter scale. The improvement has been attributed mainly to the better sparing of normal tissue with the fractionated beam than with the broad beam. The biological mechanism for the beneficial response of normal tissue to the fractionated beam is still to be better understood. Yet, the greater repair efficiency of microvessels after being damaged from radiation exposure in normal tissue than in tumor seems to be responsible for the better sparing of normal tissue. In this study, we investigated whether the vascular endothelial cells are different in radiosensitivity in normal tissue from in tumor.

Methods and Materials: The hypoxic culture medium was prepared in a hypoxia chamber (MIC-101) via the flow of 20% CO2 and 80% N2 gas mixture. The pH of culture medium was ensured in the range of 7.2-7.4. The normaloxic and slightly alkaline culture medium was employed to simulate the microenvirionment of normal tissue whereas the hypoxic cell culture medium was made up to simulate the tumor microenvironment.

Results: When comparing the cell survival with different levels of P02, it was observed that the cell survival was better when the P02 level was lower. The cell survival was also better when the pH of the culture medium was lower.

Conclusion: The microbeam radiation therapy protocol has been proven to improve the therapeutic effect in brain tumor treatment by employing a radiation beam under spatial fractionation in micron scale. Yet, the biological mechanism for the beneficial response of normal tissue to the fractionated beam is still to be better understood. Future studies are needed to better understand the biological mechanism for the better sparing of normal tissue.

POS39-12. Influence of Fractionated Irradiation and Non-Uniform Beam Intensity on the Therapeutic Effect of Gliosarcoma and Hepatoma Treatment. Eun Hee Kim, M. Lee, Seoul National University, South Korea

Purpose: In the conventional radiotherapy employing the fractionated irradiation method, dose is delivered daily for several weeks. The fractionated irradiation is expected to provide normal cells a chance to recover from any damage that would occur during the irradiation targeting tumor cells. The chance, however, may not be taken only by normal cells but also by the targeted tumor cells. In this study, we investigated the optimal period in fractionated irradiation for gliosarcoma and hepatoma treatment. We also examined whether the non-uniform beam intensity during each fractional irradiation would bring any change in radiosensitivity to tumor and normal cells and thus improve or diminish the therapeutic effect.

Methods: The flow cytometric profiles were obtained from unsynchronized gliosarcoma cells (ATCC, CRL-2200), normal dienecphalon cells (ATCC, CRL-2005), hepatoma cells (ATCC, CRL-1830) and normal liver cells (ATCC, CRL-1638) at different time points after a single radiation exposure at 2 Gy. The cell fraction at G2/M phase was recorded as a function of elapsed time after radiation exposure. On the other hand, the radiosensitivity has been examined in terms of clonogenic survival at different radiation dose levels for all four cell lines. Each curve was approximated to the linear-quadratic function S = exp\{-a(D+bD^2)\} with different values of a and b. The cell irradiation was performed with 200 kV bremsstrahlung X-rays at a dose rate of 1 Gy/min. Fractionated irradiation was performed by having the elapsed time for the maximum G2 accumulation of cells after irradiation as the time interval between irradiations. The non-uniform beam intensity during each fractional irradiation was designed in a triangle or in a V-shape.

Results: The cell accumulation at G2/M phase was indicated at 6 hrs after 2 Gy of radiation exposure with hepatoma cells whereas no significant increase of the G2/M-phase cells was observed with normal liver cells. The same as the normal liver cells occurred to 9L gliosarcoma cells and normal dienecphalon cells. The hepatoma cells and the normal liver cells shared a quite the same a/b ratio. The dienecphalon cells, on the other hand, gave the a/b ratio at around 5, which is compared to the value of around 12 for the 9L gliosarcoma cells. The fractionated irradiation at 6-hr time interval better saved the normal liver cells as compared to the single-dose irradiation. The hepatoma cells, however, gained no benefit in clonogenic surviving from the fractionated irradiation. The V-shape of beam intensity control during each fractional irradiation led to a gradual increase in clonogenic survival of normal dienecphalon cells while bringing a negligible change to 9L gliosarcoma cells.

Conclusion: There exists a chance for the improvement of therapeutic effect in radiation treatment of hepatoma and brain tumor. The optimization of time interval between fractional irradiations would work for hepatoma treatment. Also, the V-shape of beam intensity control during each fractional irradiation would be beneficial to the sparing of normal brain cells while maintaining the brain tumor control.

POS39-13. Clinical Results of Definitive Chemo-radiotherapy for T4 Esophageal Cancer according to the 7th TNM classification (UICC 2009). Ryuta Koike, Kinki University School of Medicine, Japan

Purpose: In the new UICC TNM classification (7th, 2009), N-classification was changed from N0,1 to N0-3 according to the number of metastatic lymph nodes. Clinical results of definitive chemoradiotherapy (CRT) for T4 esophageal cancer rested according to the 2009 TNM classification.

Patients and Method: From 1998 to June, 2010, 104 consecutive patients with T4 esophageal cancer (M/F: 81/23; median age: 64, range: 38-82) were treated with definitive CRT at our hospital. Twenty-six tumors were located at Ce, 26 at Ut, 43 at Mt, 9 at Lt. Except for one tumor with adenocarcinoma and squamous cell carcinoma, 103 tumors were squamous cell carcinoma. According to the 2009 TNM staging system, 8 tumors were T4a and 96 T4b. N-factor was 9 N0, 31 N1, 32 N2, 32 N3, and 32 patient had cervical lymph nodes metastasis (M1, Stage IV). Two courses of concurrent chemotherapy (CT) were combined with RT of 60 Gy/30 fractions/7 weeks (one week split at the 6th week). For each treatment, 200-250 mg/m2 5-FU day 1 to 14 was given, while full-dose CT of cisplatin 70
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mg/m² x 1 day and 5-FU 700 mg/m² x 5 days was given for 36 patients. After CRT, 1-2 courses of adjuvant CT of cisplatin/5-FU were given for 44 patients.

Results: A full dose of 60 Gy could be delivered for 101 patients (97%), 3 patients terminated RT at 50Gy/25F. 2 courses of planned CT could be combined concurrently with RT for 84 patients (81%). Median survival time (MST) for all the 104 patients was 13.0 months, and the 2- and 5-year overall survival rates(OSR) were 32% and 21%, respectively. The MST and 2-year OSR of the 2-year cases were 84months and 53% for T4N0M0, 13.5 months and 37% for T1N1M0, 13.0months and 35% for T4N2M0, 12.0 months and 20% for T4N3M0. The MST and 2-year OSR were 14.5months and 36% for stage IIIC, while those for T4aN1M1 (stage IV) were 9.5 months and 23%, respectively, with marginal significance (M0 vs M1, p=0.0532).

Conclusions: In CRT for T4 esophageal cancer, number and site of lymph node metastases affect OSR. Patients with N0 disease showed the best 2-year OSR of 53%, while those with N3 and/or stage IV disease (neck lymph nodes metastases) showed the worst 2-year OSR of approximately 20%. The 2-year OSRs for patients with N1 or N2 disease were approximately 35%.


Purpose: Distinguishing between tumor progression and radiation necrosis after treatment in patients with brain tumors presents a clinical dilemma. There is a lack of experimental models of radiation-induced necrosis in rodents. Investigators have used dedicated and expensive irradiators to create an animal model of radiation necrosis. No studies have been reported on the use of clinical irradiators in developing a model of radiation necrosis in tumor bearing animals.

The objective of this study was to create focal radiation necrosis in rat brain bearing human glioma using stereotactic radiosurgery and confirm it by immuno-histological analysis.

Materials/Methods: Nude rats implanted with primary glioblastoma cells were irradiated using a stereotactic setup (n=3) or received no radiation (n=4). 18-24 hours after implantation, growth of the tumors was confirmed by magnetic resonance imaging (MRI). For each animal contrast enhanced CT images of 1mm slice thickness were obtained and transferred to Brain Lab Novalis treatment planning system (BrainLab, Feldkirchen, Germany). MR images was acquired from the same animal, transferred to the planning workstation, and fused with the CT data using image fusion software. The tumor was identified and delineated using the fused CT/MRI images. A radiosurgery plan was generated using a 4mm radiosurgery cone such that one portion of the tumor receives 100% dose of 75Gy sufficient to cause necrosis, whereas the tumor edge at depth receives only 50% or less dose, allowing for regrowth of the tumor. The brains were collected 10 weeks after irradiation and immune-histological analysis was performed.

Results: H&E staining showed central liquefaction necrosis in the high dose region consistent with radiation necrosis and viable tumor in the peripheral low dose region. Both PCNA and Ki-67 staining showed high proliferative tumor cells surrounding the necrotic parts of the tumor. No necrosis was observed in control tumors.

Conclusions: We have developed a novel animal model of radiation necrosis using human gliomas in rat brain using stereotactic radiosurgery. Our long-term goal is to use the model to differentiate radiation necrosis from tumor recurrence by using non-invasive imaging parameters obtained using dynamic contrast enhanced CT and MRI.

POS39-15. Evaluation of Premature Senescence for Single and Fractionated Irradiation in vivo and in vitro. Jae-Seon Lee¹, B.C. Kim¹, H.J. Yoo¹, S. Park¹, Y. Ji¹, Y. Lee¹, 1: Korea Institute of Radiological and Medical Sciences, South Korea, 2: College of Pharmacy and Division of Life Science and Pharmaceuticals, Ewha Womans University, South Korea

Radiotherapy is one of the best therapeutic strategies for cancer treatment. The cellular response to ionizing radiation (IR) is varied ranging from senescence to apoptosis. Recently, it has been reported that the induction of premature senescence could be a promising strategy for cancer treatment. The aim of this study was to elucidate whether the premature senescence could be contributed to the outcome of radiotherapy in vivo and in vitro. Either cultured human cancer cell lines or xenografted mice were exposed to single radiation (2, 6, or 12 Gy; SR) or fractionated radiation (3 x 2 Gy or 6 x 2 Gy; FR) of IR. Induction of premature senescence was assessed using variety of senescence-associated biomarkers including senescence-associated β-galactosidase activity (SA-β-Gal), eukaryotic translation elongation factors (eEF1A1, eEF1B2); cathepsin D (CD), decoy receptor 2 (DcR2), and DEC1. We found that both SR and FR treatment effectively induced premature senescence in H460 and MCF7 cancer cell lines and tumor xenografts. The 2-year overall survival rates for stage IIIC were 84months and 53% for T4N0M0, 13.5 months and 37% for T1N1M0, 13.0months and 35% for T4N2M0, 12.0 months and 20% for T4N3M0. The MST and 2-year OSR were 14.5months and 36% for stage IIIC, while those for T4aN1M1 (stage IV) were 9.5 months and 23%, respectively, with marginal significance (M0 vs M1, p=0.0532).

In cancer treatment, apoptosis is a well-recognized cell death mechanism through which cytotoxic agents kill tumor cells. Here we report that dying tumor cells use the apoptotic process to generate potent growth-stimulating signals to recruit other tumor cells undergoing apoptosis. The apoptotic cells were enriched with cell-free factors (e.g. sFasL, CD95L, TNF-α, GM-CSF, IL-1β), which can potently stimulate growth of surviving tumor cells. We propose the existence of a “Phoenix Rising” pathway of cell death-induced tumor repopulation in which caspase 3 plays key roles.

POS39-17. The Impact of Repeat Stereotactic Radiosurgery on The Management of Brain Metastasis with Maintaining Brain Function. Yauoshi Matiya¹, G. Sekizawa¹, Y. Matsuoka¹, I. Kashiwakura², 1: Iwate Prefectural Central Hospital, Japan, 2: Hiroasaki University Graduate School of Health Sciences, Japan

To control tumor with maintaining brain function is regarded as important in the recent radiotherapy against brain metastases. From this viewpoint, we investigated the utility of repeat stereotactic radiosurgery (RSRS), assisted with a careful monitoring by MRI, in the management of brain metastases from various types of malignancies. Between 2004 and 2011, 37 patients harboring brain metastasis received RSRS using linear accelerator. There were 21 men and 16 women, and the median age was 66 (range, 49-79). The number of patients with baseline performance status 0, 1, 2 and 3 was 18, 13, 5 and 1, respectively. Site distribution for the primary included the following: lung 27, breast 6, rectum 2, thyroid 1 and vulva 1. The total number of RSRS sessions ranged from 2 to 5. The total number of target lesions in one patient ranged from 1 to 6, which showed that RSRS to the same site was performed in a part of patients. The number of patients with active extracranial disease (AECD), including previously untreated stage IV disease, and that without AECD was 25 and 12, respectively. The 2- and 5-year overall survival rates were 72% and 46%, respectively, and the median survival time was 50 months. There were 14 long term survivors, who survived more than 2 years. Cause of death was as follows: 8 extracranial disease progression and 1 progressed brain metastases. Neurological decline, which was defined according to the criteria by Bhatnagar et al. (2002), based on the serial change of 3 neurological symptoms: seizures, focal deficits, and headaches due to uncontrolled brain lesions, was identified in 11 of 37. The remaining 26 (70%) patients did not show neurological decline. Further, for 15 patients, neurocognitive function was examined using the revised version of Hasegawa’s dementia scale (HDS-R). The most recent HDS-R scores of the patients revealed that 13 of 15 (87%) were not associated with impaired recognition. In conclusion, RSRS can be offered as a preferred option to manage brain metastases from various types of malignancies, leading to a long survival with a relatively well preserved brain function.

In vitro investigation of the dose-rate effect on the biological effectiveness of megavoltage X-ray radiation doses.
Sianne Oktaria 1,2,3, S. Corde 1,4, M. Lerch 1, A. Rosenfeld 1, M. Teheri 1,3, 4: 1: Centre for Medical Radiation Physics, University of Wollongong, NSW, Australia, 2: Centre for Medical Bioscience, University of Wollongong, NSW, Australia, 3: Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia, 4: Department of Radiation Oncology, Prince of Wales Hospital, Randwick, NSW, Australia, 5: School of Chemistry, University of Wollongong, NSW, Australia

Modern external radiation therapy technologies enable delivery of X-ray with higher dose-rate to allow quicker treatment times for patients treated with highly complex fluence-modulated techniques (e.g. intensity modulated radiation therapy or volumetric modulated arc therapy). Conventional 3D conformal technique still being delivered with these linear accelerators, it is important to study dose-rate effects on the biological effectiveness of the delivered physical radiation doses.

We examined in vitro the response of the radiosensitive human breast carcinoma MCF-7 and the radioresistant rat gliosarcoma 9L cell lines after single exposure to 10MV X-rays radiation doses up to 8 Gy, delivered at low dose-rate (LDR), 0.5Gy/min compared to 10-fold higher dose-rate (HDR), 5Gy/min. The biological effects of the radiation were then assessed by colony-forming assay and survival curves fitted with the linear quadratic model analyzed.

We observed that two cell line evaluated different biological response with dose-rate variation, with LDR irradiation resulting in decreased clonogenic survival of MCF-7 compared to HDR irradiation whereas 9L cell line did not demonstrate any noticeable dose-rate effect. Thus, the preliminary results of this study support the concept that different tumor cell lines have different sensitivities to the radiation dose-rate, depending on their relative radioresistance. The traditional key characteristic of the dose-rate effect, which is the biological effect of the radiation is decreased as the dose-rate is lowered, is inverted in this study with LDR irradiation can be as effective as HDR irradiation or more in tumor cell killing.

POS39-19. Differential effect of whole tumor or spatially fractionated single dose radiotherapy of murine breast tumor on myeloid cells and cytokines in peripheral blood. Beata Przybyla, S. Sharma, J. Penagaricano, J. Webber, N. Koonce, P. Corry, E.G. Moros, R.J. Griffin, University of Arkansas for Medical Sciences, Department of Radiation Oncology, USA

Purpose. Spatially fractionated single dose radiotherapy (GRID) increases tumor control and therapy outcomes in clinic. Yet systemic and local molecular changes associated with this therapy are not fully defined. The goal of the presented study was to evaluate the level of leukocytes (CD8+, CD4+ and CD11b+) and level of 9 cytokines in peripheral blood at 8 h post-GRID therapy with a single dose of 20 Gy and compared to 20 Gy whole tumor radiotherapy in mice model of breast cancer.

Experimental procedures. SCK murine breast tumors were grown in A/J mice. An average volume of treated tumors was 132 mm³. During the spatially fractionated therapy the 20Gy peak dose was delivered, using an integrated robotic-based irradiation system for small animal research housed in our department. The GRID pattern consisted of an array of 7 parallel beams of 1 mm in diameter. Whole tumor therapy was performed using a cabinet X-ray system where 20 Gy was delivered over 22.7 minutes. Blood was collected 8 hours after radiation. Populations of leukocytes and cytokines were compared among 3 groups: no treatment, GRID and whole tumor radiation. Flow cytometry was used to evaluate levels of CD8+, CD4+ and CD11b+ leukocytes cytokine levels were measured using an antibody 9-plex array.

Results. Eight hours after spatially fractionated single dose radiotherapy (20 Gy) there were 3 times more CD11b cells in the leukocytes population in circulation than in a group treated with whole tumor 20 Gy radiotherapy. GRID therapy was also associated with a significant decrease of the chemokine RANTES level in peripheral blood as compared with no treatment control.

Conclusion. Inhibition of RANTES, a known factor involved in tumor progression, may be an important benefit of the GRID therapy. A role of early increase of CD11b in circulation following GRID therapy is not clear. Since CD11b+ cells can participate in antigen presentation, it is conceivable that CD11b may enhance innate immunity and in consequence. Convincational 3D conformal radiotherapy improves of greater immunity. Further studies are needed to define important effects of GRID therapy on enhancement of antitumor immunity and potential involvement of myeloid cells.

POS39-20. 1H NMR spectra mobile lipid signals and proliferative status of C6 glioma cells after X-ray irradiation. Waldemar Przybyzewski 1, L. Matulewicz 2, A. Cichon 1, M. Sokol 1, M. Głowala-Kosińska 1, M. Gibeau 3, 1: Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland, 2: Silesian University of Technology, Gliwice, Poland

The appearance of mobile lipid (ML) signals in 1H NMR spectra have been associated with numerouscellular effects including cell proliferation or apoptosis. Despite of ionizing radiation is widely used in cancer therapy, little information on their dependence of 1H NMR to irradiated cells in vitro have been known. The aim of our study was to investigate the biochemical response of living model of glioma C6 cell line to X-ray radiation. The rat C6 glioma cells were routinely maintained as a monolayer culture in DMEM, supplemented with 12% fetal bovine serum. Irradiation was performed at room temperature with photons X generated by a linear Clinac-600C/D accelerator (Varian). C6 glioma cell cultures received a one-time dose of 3.8 Gy (D0, 37% clonogenic survival dose at a dose rate of 8.8 Gy/min) Metabolic activity of the cells was determined using MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay).

Distribution of cell cycle population was analysed by flow cytometry using an antibody 9-plex array based irradiation system for small animal.

Conclusion. Spatially fractionated single dose radiotherapy specifically correlated with cell death or proliferation status but rather in vitro.

POS39-21. Effect of dose rate on response of cells irradiated during radiotherapy. Jacek Rogoliński, M. Konopacka, K. Słośarek, A. Rusin, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland

Introduction: Cancer radiotherapy regimens use radiation of varying dose rates. At the MSC Memorial Cancer Center in Gliwice a commonly used dose rate is 3 Gy/min (under standard conditions). New irradiation techniques use different dose rates; for example rotational techniques with dynamic change of irradiation field generates beam rates of 600 MU/min. Cellular response depends not only on the magnitude of absorbed dose but also on dose rate, its fractionation, positioning of cells with respect to irradiation field, etc. It has been demonstrated that for low dose rates (below 1 GY/min) biological response of cells is closely linked to dose rate whereas it has not been sufficiently investigated in case of high dose rates. Aim: To compare biological responses of cells to a 5 Gy dose delivered at two different rates: 100 and 600 MU/min.

Methods: The study was carried out using several cancer cell lines and one normal line (BEAS-2B). As a radiation source Clinac 2300 accelerator was used, delivering photon radiation (6 and 20 MV), 5 Gy dose was used (at 100 and 600 MU/min dose rate); cells were placed in a water phantom at two depths (3 or 15 cm), either within or outside of the irradiation field. Biological damage was assessed as: micronucleus frequency, apoptosis induction, cell survival, and cell senescence.

Results: Significant differences were observed in biological response of cells to varying irradiation conditions. Dose rate: The irradiation, at the same dose, when delivered at a lower dose rate, induces a higher degree of biological effects (apoptosis, increase in S-phase, decrease in G0/G1) compared to irradiation at a high dose rate. This relationship is observed only within the beam field. Depth: A greater depth more cytogenetic damage is observed for the same dose.
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as compared to smaller depths. Positioning with respect to the radiation beam: Cells placed outside of the irradiation field are damaged to the same extent irrespective of depth and dose rate. Type of cells: These observations pertain to nontoxic and normal cell types.

Conclusions: It was found that biological response of cells depends on dose rate of radiation used in cancer radiotherapy. Since dose rates other than 3 Gy/min. are more and more commonly used the explanations of observed relationships becomes justified. The observations presented herein can be used in the future for radiotherapy planning.

POS39-22. True Beam1 high dose rate flattening-filter-free fractionated irradiation on human cancer cell lines? Peter Sminia1, J. van den Berg1, B.J. Slotman1, W.F.A.R. Verbeke2. 1: Department of Radiation Oncology, Section of Radiobiology, VU University Medical Center, Netherlands 2: Department of Radiation Oncology VU University Medical Center

Purpose: New external beam precision irradiation techniques have prompted the discussion about radiobiological effects of high and ultra-high dose-rates. With flattening-filter-free beams, the average dose rate is increased by a factor of 1.5 to 4 relative to irradiation using flattened beams. In the present study, clonogenic cell survival was determined using single dose and fractionated irradiation using both flattened and flattening filter free beams.

Materials and methods: The human astrocytoma cell line D384 and the human lung carcinoma cell line SW1573 were either irradiated at single dose (0 – 8 Gy) or in a fractionated protocol (5 daily fractions of 2 resp. 3 Gy). Cells were irradiated with a homogenous dose distribution created by means of a sliding window multi leaf collimator technique of a single posterior field using (1) flattened beam (6 MV, 600 Monitor Units/min.) and (2) flattening-filter-free beam (True Beam, Varian Medical Systems) (10 MV, 2400 Monitor Units/min.)

Cell survival was determined by clonogenic assay. In addition, in the fractionated irradiation set-up, the number of clonogenic cells was estimated by including tumor cell proliferation during the 4 days overall treatment time in the analysis.

Results: Equal cell survival was found following irradiation, either using the flattening-filter-free 10 MV beam with high dose rate or the flattened 6 MV beam. This was observed after single dose exposure (0 – 8 Gy) as well as after fractionated irradiation with five daily 2 Gy (D384 cells) or 3 Gy (SW1573 cells) fractions. With the fractionation set-up, a difference in fraction size as small as 0.05 Gy; i.e. a dose deviation of 2.5%, could be discriminated using the number of clonogenic cells as endpoint.

Conclusion: Two human cancer cell lines showed no difference in cell survival following fractionated irradiation with flattened beams or flattening-filter-free beams at an approximately four times higher average dose rate.

POS39-23. Fractionated-radiation enhances mesenchymal transformation of glioma cells via Nitric Oxide synthesis. Youngsoo Sul1, R. Kim1, C. Yoon1, K. Yoo1, E. Lim1, G. Lee1, Y. Ha1, M. Kim2, S. Lee1. 1: Hanyang University, South Korea, 2: Korea Atomic Energy Research Institute, South Korea

The major limitations of glioma treatment are the prevalence of recurrence after surgery and radiotherapy, infiltration into surrounding regions, and intrinsic or acquired resistance to chemo- and radiotherapy. However, the mechanisms by which glioma relapse after treatment and diffuse to other regions of brain remain unknown. In this study, we observed that fractionated radiation, commonly adapted for treatment of cancers, increases malignant properties of glioma cells. Also, we investigated the mechanisms by which glioma cells acquire malignant properties such as migration and invasion by exposure to ionizing radiation. When U87 and U373 glioma cells were exposed to gamma-irradiation (3 x 2 Gy), their migration and invasion behavior were increased, compared to non-irradiated cells. Moreover, Snail and Slug transcription factors, known as repressors of mesenchymal transformation were decreased in glioma cells by fractionated radiation. Notably, fractionated radiation induced Nitric Oxide (NO) synthesis in glioma cells and ablation of NO by treatment with small interfering RNA or inhibitor targeted to NO synthase decreased radiation-induced mesenchymal transformation. Taken together, our study suggests that radiotherapy could induce NO synthesis, a critical regulator for glioma malignancy. Radiotherapy accompanying with targeting to NO synthesis may lead to more effective glioma treatment.


Men with localized cancer of the prostate (stage T1c) have access to an abundance of treatment options including several modes of radiation therapy including conventional teletherapy, intensity modulated teletherapy (IMRT), proton therapy, permanent implant brachytherapy and high dose-rate brachytherapy. In vitro cancer cell survival data were applied to a typical prescription in each case, and final cell survival was calculated using the Payne-Garrett formalism, namely -lnS = CD + AR[1 + (DR/AR) + exp(-DR/AR)] where S = tumor cell surviving fraction, D = absorbed prescription dose or dose fraction in Gy, R = instantaneous dose rate in Gy/min, t = time to deliver dose or dose fraction D, t =sublethal damage recovery mean time, 69 min for the in vitro cells, C = coefficient for single-event cell killing, 0.223 Gy-1 for photons, 0.36 Gy-1 for protons, and A = coefficient for sublethal damage induction, 0.060 Gy-1 for photons and 0.042 Gy-2 for protons. Cell multiplication during the irradiation period was assumed negligible. The following table lists for each modality studied, a typical dose prescription, the calculated tumor cell survival values, and equal number of surviving tumor cells based on one gram (109 cells) of tumor cells in the treatment zone.

<table>
<thead>
<tr>
<th>MODALITY</th>
<th>TOTAL DOSE,Gy</th>
<th>FRACTIONATION</th>
<th>CELL SURVIVAL</th>
<th>RESIDUAL CELLS</th>
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<tbody>
<tr>
<td>PHOTONS</td>
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<td>2 Gy/day x 37</td>
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<tr>
<td>IMRT 3D-CRT</td>
<td>86.4</td>
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<td>1.5 x 10^6</td>
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<tr>
<td>PROTONS</td>
<td>79.2</td>
<td>1.8 Gy x 44</td>
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<tr>
<td>BRACHY- THERAPY</td>
<td>124</td>
<td>17-d half-life</td>
<td>7.3 x 10^6</td>
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<tr>
<td>HDR BRACHY</td>
<td>43.5</td>
<td>7.25 Gy x 6</td>
<td>2.2 x 10^6</td>
<td>22</td>
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</tbody>
</table>

These predictions may hold implications for current trends in prostate cancer treatment, such as hypofractionation and combined modalities. Research supported by USPHS and DOE. Participation of Carter Schroy, Paul Furcintti, Harvel Wirt, Adolf Wainson, Andreas Koehler, John Little, and Michael Gotzin is acknowledged.

POS39-25. Local effect of three-dimensional conformal radiotherapy for patients with hepatocellular carcinoma. Koh Tusig1, M. Kawaguchi1, T. Tanaka1, H. Tamahata1, 1: Minami Wakayama Medical Center, Japan, 2: Kishiwhada Tokushukai Hospital, Japan

Purpose: Though transarterial chemoembolization (TACE) is effective for hepatocellular carcinoma (HCC), we frequently experience local recurrence, or ineffective cases. It is sometimes difficult to treat HCC by TACE for some reasons. Three-dimensional conformal radiotherapy (3D-CRT) will be effective treatment option in these cases. Local effect of 3D-CRT for such patients is presented.

Materials and methods: From Oct 2009 to Mar 2011, 12 consecutive patients with HCC recurred after or ineffective or difficult to treat with TACE were registered. Cases with Child-Pugh C or PS 3-4 were excluded from the study. Age of the patients ranged from 68 to 85 years old. Eight male cases and 4 female cases were included. Eight cases were treated with TACE while 4 cases (each case with portal venous invasion, abdominal aortic aneurysm, severe arteriosclerosis or dementia) were not. All lesions outside the gross tumor volume (GTV) were excluded from the study. The GTV volume ranged from 0.01 to 142cc. In these cases, Local effect of 3D-CRT will be a useful therapeutic option in the treatment of HCC in curative intent.

<table>
<thead>
<tr>
<th>MODALITY</th>
<th>TOTAL</th>
<th>FRACTIONATION</th>
<th>RESIDUAL</th>
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<tr>
<td>PHOTONS</td>
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<td>43.5</td>
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</tr>
</tbody>
</table>

Conclusion: Though transarterial chemoembolization (TACE) is effective for hepatocellular carcinoma (HCC), we frequently experience local recurrence, or ineffective cases. It is sometimes difficult to treat HCC by TACE for some reasons. Three-dimensional conformal radiotherapy (3D-CRT) will be effective treatment option in these cases. Local effect of 3D-CRT for such patients is presented.

POS39-26. Roles of tumor-secreted SDF-1 on glioma response to radiation therapy. Shu-Chi Wang1, J. Hong2, C. Chiang1, 1: National
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Tsing Hua University, Taiwan, 2: Chang-Gung Memorial Hospital, Taiwan

We have been found that tumor-associated macrophages (TAMs) can specifically co-localize with hypoxic tumors after radiation therapy (RT) in an ectopic prostate cancer murine model. In this study, we used the common used GL261 cells and a new murine astrocytoma-ALTS1C1 cell to examine whether TAMs could also accumulate in the hypoxic region of brain tumors after RT. Intracranial implantation of the ALTS1C1 astrocytoma has the similar medium survival days as the GL261 tumor. However, these two tumors display distinct tumor microenvironments. Compared to GL261 tumors, the ALTS1C1 represents good vessel density and networks and less of hypoxia region. In addition, only the ALTS1C1 tumors have significant aggregation of CD68+ TAMs in hypoxic region after RT 2 weeks but this phenomenon does not appear in the GL261 tumors. SDF-1 plays a critical role in regulating revascularization, which is highly expressed on ALTS1C1 cells. To further define the roles of SDF-1 in ALTS1C1 tumors, the lentiviral shRNA particle was used to silence SDF-1 expression. Intracranial implantation of ALTS1C1-SDF46-cell decrease microvascular density, resist to RT, and diminish the aggregation of TAMs into hypoxic region after RT 2 weeks compared to undisturbed tumors. This study demonstrated that SDF-1 expression by ALTS1C1 cells is critical for tumor vessel formation, the aggregation of TAM into hypoxic regions, and the response of brain tumor to RT.


Chung-Fang Yu1, C. Lin1, S. Wang1, P. Hong1, C. Chang1, 1: Department of Biomedical Engineering and Environmental Sciences, NTHU, Taiwan, 2: Department of Radiation Oncology, Chang Gung Memorial Hospital, Taiwan

The recurrence of brain tumors after radiation therapy (RT) is frequently reported clinically. To investigate the behavior of recurrent glioma after RT, we used a murine astrocytoma, ALTS1C1, growing from pre-irradiated (pre-IR) brain tissues as a pre-clinical brain tumor recurrent model. Mouse brain was irradiated by 8 or 15 Gy prior to tumor implantation. The results showed that the prolonged survival time of tumor-bearing mice was only seen in mice receiving 15 Gy, but not 8 Gy of pre-irradiation. However, the decrease of tumor vessel density and the increase of astrocytosis were found in both groups in a dose dependent fashion. We were surprised how ALTS1C1 cells in 8 Gy pre-IR tissues could have the same growth rate as in control tissues, while 8 Gy pre-irradiation significantly decreased the vessel number. When the tissues were examined by confocal imaging for the co-staining of GFAP and CD31 antibodies, more double positive cells were found in tumors growing from 8 Gy pre-IR tissues than that from control tissues. This indicates that the vessels in tumor growing from pre-IR tissues might have better function than those from control tissues. In summary, we found that 8 Gy of pre-irradiation activated astrocytes, which might consequently restore the vessel function and promote tumor re-growth. This study suggests that the control of radiation-induced astrocystosis is a potential approach for preventing tumor recurrence.

POS40 Radiation biophysics


Lei Chang, W. Hu, Institute of Modern Physics Chinese Academy of Sciences, China

Purpose: To provide a feasible way to examine nucleoli changes in a real-time manner after exposure to ionizing radiation (IR).

Materials and methods: We constructed NPM gene into pEGFP-N2 vector and subsequently transfected it into human melanoma 92-1 cells.

Results: Successfully we established a cell line stably expressing NPM-pEGFP fusion protein by selecting with G418, which might be a powerful tool for studies on the nucleoli in responding to exogenous stresses.

Conclusions: Our data shows that both 3H+ ions and X-ray irradiation could cause nucleoli to change their morphological structure, size etc. This result indicates that nucleoli changes may have some relationship with cells response to radiation. So, the further study of nucleoli structure and constitutive proteins changes could show us a new way to study molecular mechanism of cell biological effects of radiation.

Keywords: NPM, irradiation, 3H+ ions, X-ray.

POS40-02. Unraveling complexity of complex chromosome aberrations.

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One of the key recent findings in radiation cytogenetics revealed by mISH technique is high complexity of radiation-induced chromosomal aberrations (CA). The existing mechanistic models of CA formation fail to explain this phenomenon. The aims of the present report are a) the extension of the previous work [Edelman and Andreev, Radiat. Prot. Dosim. 2011, v.143, p.202] considering CA formation by interaction of damaged chromosome subunits via random contacts by addition of the alternative pathway, interactions on nuclear centers; b) the analysis of “rejoining cycles” of complex aberrations.

The types and frequencies of simple and complex CA induced by low LET radiation were predicted on the basis of the direct simulation of pathways mentioned above. Structure and dynamics of interphase chromosomes, chromosomal contacts distribution, damage of chromosomal subunits, including simulation of DSB induction/repair, were simulated for the model of human lymphocyte interphase nucleus developed previously. Damaged subunits of different chromosomes can be highly localised in space due to their attachment to nuclear centers. They undergo multiple interactions resulting in complex CA. The rest of the damaged loci in the nucleus, not attached to nuclear centers, form CA by "random contacts" pathway. For each complex aberration its cycle structure was determined.

The calculated dose response curves depend on which of the mechanisms considered prevails. For the nuclear centers pathway the complex aberrations are more frequent and more "complex" in terms of higher order of joining cycles than for the “random contacts” pathway. The comparison between the theoretical results and the mISH data shows that the formation of CA through involvement of random contacts of damaged chromosomes is insufficient to explain both high ratio of complex to simple CA and high frequency of complex CA with high-order rejoining cycles. Incorporation of the “nuclear centers” pathway allows to explain both of these phenomena.

POS40-03. Radioprotective properties of fullerol C60(OH)26

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The aim of our work was to assess radioprotective properties of fullerol C60(OH)26 in two biological systems.

The solutions of alcohol dehydrogenase (ADH) in a 20 mM Na phosphate buffer (pH 7.4) with or without 75g/ml of fullerol (FulOH) were exposed to 0-15 Gy of X-rays 0-0.5 h under air. The activity of ADH was measured after 1 and 24 hours of incubation at 37°C and the radiation yield of inactivation of ADH was calculated.

Human erythrocytes (hematocrit of 2%) suspended in PBS were incubated with FulOH at concentration 150 mg/ml at 37°C for up to 3 hrs. Erythrocytes with FulOH (150 mg/ml) were irradiated with electron pulses from a linear electron accelerator ELU-6 with the dose of 650 Gy. Potassium efflux, lactic dehydrogenase (LDH) release, acetatecholimesterase (ACHE) kinetics and membrane fluidity were determined.

Our results showed that fullerol protected ADH against radiation-induced damage. The radiation yield of inactivation was about 25% lower for ADH irradiated in the presence of fullerol.

The increase of potassium efflux and LDH release are thought as parameters indicating a plasma membrane disruption preceding hemolysis. The measurements of ACHE activity and kinetics give valuable information about the structural changes in the erythrocyte membrane under actions of various factors e.g. irradiation, chemicals. Fullerenol protected erythrocytes from radiation-induced potassium efflux. Extracellular concentration of K+ was about 10% higher in the samples irradiated without fullerol comparing to those with fullerol at 150 mg/ml. Furthermore, the LDH release was also decreased. However, the ACHE activity did not change either after fullerol or radiation or FulOH and radiation treatment. But the substrate affinity of ACHE decreased after irradiation. FulOH preserved that parameter. Moreover, the substrate affinity was increased slightly by FulOH itself.
AChE activity is modulated by the hydrophobic environment of the membrane and depends on the membrane fluidity and surface charge. FuOH decreased slightly the fluidity of erythrocyte membrane and therefore could change the substrate affinity of AChE. These results confirm that fullerol could prevent isolated enzymes and the intact cells from radiation-induced damage.

**POSTER PRESENTATIONS**


The Auger effect is not yet fully understood in respect to the average numbers of emitted Auger electrons per decay and consequently to the deposited energy as well as to the dose rate. Therefore, we studied the Auger electron emitter (AEE) I-123 and I-125 which are characterized by a different half-life (13.2 h vs. 59.4 d) and by different average numbers of Auger electrons emitted per decay (ratio I-123/I-125 ~ 2:1). The biological response of mammalian cells labelled with various activity concentrations of I-(123)iodine-2'-deoxyuridine (I-123-UdR) and (125)iodine-2'-deoxyuridine (I-125-UdR) was thoroughly investigated to further elucidate the biological effectiveness of these particular electron emitters.

**POS40-05. Numerical model of mutation induction by α-radiation in the bronchial epithelium.** Balázs Madas, I. Balásházy, Hungarian Academy of Sciences KFKI Atomic Energy Research Institute, Hungary

Radiation is taught to be causing mutations mainly due to its DNA damaging effect. However, densely ionizing radiation can be underestimated. Investigating the dose dependence, three ranges can be distinguished. At low doses (below ~350 mGy) the curve has a convex shape, then mutation induction depends linearly on tissue dose, while beyond ~2.3 Gy, where the turnover rate reaches its maximum, the slope of the curve is lower. In the later range, the normal number of progenitor cells is no more sufficient for the maintenance of tissue homeostasis, i.e. there is a chronic need for these cell types which can result a hyperplasia of cells capable of dividing. Modeling efforts presented here help us to understand the role of different processes in mutagenesis.

**POS40-06. Chromatin structure and radiation-induced intrachromosome exchanges.** Lingegowda Mangalag, Z. He, Y. Zhang, M. Hada, F.A. Cucinotta, L. Rohde, H. Wu, 1: NASA Johnson Space Center, USA, 2: University of Houston Clear Lake, USA

To investigate the relationship between chromosome aberrations induced by radiation and chromatin folding, we measured the physical distances between different regions of the chromosome during metaphase. Previously, we have investigated the location of breaks involved in intrachromosomal type exchange events in human chromosome 3, using the multicolor banding in situ hybridization (mBAND) technique. In human epithelial cells exposed to both low- and high-LET radiations in vitro, we reported that intrachromosome exchanges occurred preferentially between a break in the 3p21 and one in 3q11 region, which could be observed between a break in 3p21 and one in 3q26, but few exchanges were observed between breaks in 3q11 and 3q26, even though the two regions are located on the same arm of the chromosome. Here, we report the physical distances measured between these regions of chromosome 3 in human epithelial cells during the G1 phase of the cell cycle. We further analyzed fragile sites on the chromosome that have been identified in various types of cancers. Our results demonstrated that the distribution of breaks involved in radiation-induced intrachromosome aberrations depends upon both the location of fragile sites and the folding of chromatin.

**POS40-07. Irradiation of tumour cell lines with very high-LET particle beams accelerated at Naples Tandem facility.** Lorenzo Manti, L. Campajola, I. Improta, F.M. Perozziello, G. Porzio, G. Grossi, 1: Department of Physical Sciences, University of Naples Federico II, Italy, 2: National Institute for Nuclear Physics (INFN), Naples Section, Italy, 3: Centre for Radiation Protection and Health Physics (CRAFS), University of Naples Federico II, Italy

The impending National Centre for Oncological Hadrontherapy (CNAO) in Italy is due to start in vitro radiobiological evaluation of tumour treatment by charged particles. Ongoing experiments at other Italian facilities have already shown a gain by 12C ions (LET ~ 200 keV/micron) in terms of cell killing in glioma cell lines otherwise resistant to low-LET radiation. There is, however, increasing interest for the therapeutic use of heavier ions, such as oxygen and neon. At our Department we have recently produced oxygen beams at a 3 MV TTT Tandem accelerator by degrading a sputtering source where oxygen is continuously sprayed on a tantalum cathode. We aim at evaluating the biological effectiveness of such particles at tumour cell inactivation using a panel of glioma cell lines. Cells will be grown on 1.5 micron-thick mylar and irradiated in purposely built Perspex vessels. A novel experimental irradiation set-up is being developed in that vessels are vacuum-held at the channel beam exit with the mylar base facing the beam. This arrangement significantly reduces hitherto occurring energy losses downstream since the pristine 21-MeV beam is degraded solely through the vessel’s mylar. Therefore, ions will be able to reach and traverse the cell monolayer, with an estimated incident LET of ~1,000 keV/micron. Energy spectra and dosimetric measurements will be performed by means of Si-based detectors as well as CR-39 detectors. Irradiated cells will be allowed to form colonies for up to 2 weeks and clonogenic survival will be measured. The results will be compared with those obtained from previous 12C ion irradiations.

**POS40-08. Nanodosimetry: a novel dosimetric concept for radiation biophysics.** Heidi Nettelbeck, M. Bug, G. Hilgers, H. Rabus, Physikalisch-Technische Bundesanstalt (PTB), Germany

**Aim:** This work is an overview of recent progress in nanodosimetry to establish novel dosimetric concepts based on measurable track structure properties of ionizing radiation. **Methods:** Nanodosimetry is the development of experiment and numerical techniques for characterising particle track structure based...
on the formation of ionisation clusters in targets comparable to DNA. Ionisation cluster size distributions (ICSDs) are measured in macroscopic gas targets with an ion-counting nanodosimeter, since detection of these distributions in nanometric volumes of condensed matter is not possible [1]. Details of the particle track structure in condensed matter are obtained by means of a dedicated Monte Carlo simulation code. A theoretical scaling relation is used to relate the measured and simulated ICSDs in different materials and volumes [2]. Simulation of the energy and angular distributions of the DNA–substitute tetrahydrofuran (THF) were also performed to study the accuracy of using water to approximate biological matter.

Results: Measured ICSDs were used to validate the PTB code and the scaling procedure for these distributions in different gases. Comparison of nanodosimetric parameters with experimental data shows the potential for nanodosimetry to provide meaningful definitions of concepts describing radiation damage to biological targets.


POS40-09. Microdosimetric Kinetic Analysis of the Lesions in Bio-Cells by Radiation Exposure, Yousuke Ohtsubo1, K. Tsurumi2, K. Sasaki2, K. Waku1, H. Date1, Graduate School of Health Sciences, Hokkaido University, Japan, 2: Faculty of Veterinary Medicine, Hokkaido University, Japan, 3: Graduate School of Engineering, Kyoto University, Japan

The effect of ionizing radiation on bio-cells has been evaluated by RBE (Relative Biological Effectiveness) that is described as a function of LET (Linear Energy Transfer) in radiotherapy. The LET is usually treated as a constant value averaged over the energy for the radiation particle. However, the energy transfer from the particle to bio-cells differs depending on the “moment to moment” of the particle energy in the path, yielding different effects on local regions in the cell volume. In this study, we employ the Microdosimetric Kinetic (MK) model in order to take account of the lesions induced locally by the passage of electrons, for estimating the parameters associated with lethal lesions and clarifying the relation between the electron energy distribution and cell survival under photon beam irradiation.

In the MK model, the volume of the nucleus is divided into smaller subunits called “domains” so that local damage along the passage of ionizing radiation can be evaluated. A set of rate equations for the number of lesions in the domain per cell nucleus is solved. We apply the solution to cell survival data from the literature, and various parameters describing the behavior of the lesions are deduced. In particular, we focus on the parameter (γ) that represents the difference of the energy distribution in the photon.

The parameters in the MK model were determined for CHO K1 cells exposed to 200 kV X-rays and 60Co γ-rays (1.17 & 1.33 MeV). The γ-parameter for 200 kV X-rays was found to be larger than that for 60Co γ-rays. Since γ renders the dose mean specific energy in the microscopic region of the cell nucleus, the result means that the local effects by electron passage vary according to initial energy of the electrons generated by the photon beam. In general, the stopping power of an electron has a peak around the end of the range, enhancing the ionization and excitation processes which may increase the damage. Therefore, the number of the lesions (e.g., double strand breaks) and cell lethality may be related with the number of the endpoints of electron tracks, which is governed by energy distribution of the electrons generated.

In this study, the damage of cells exposed to photon beams was evaluated by the MK model. It was shown that the surviving fraction of the cells depends on the electron energy induced by photon irradiation.

POS40-10. The analytical model of chemical phase and formation of DSBs in chromosomes by ionizing radiation, Hana Pisakova1, J. Barilla1, M. Lokajicek1, P. Simr1, 1: Institute of Physics AS CR, v.v.i., Czech Republic, 2: J.E. Purkinje University in Usti nad Labem, Faculty of Science, Czech Republic.

The irradiation of biological systems causes a sequence of different processes, which can be separate into physical, biological and chemical phase. Physical phase involves interactions between ionizing radiation and atoms in cells, chemical phase is characterized by rapid chemical reaction of ionized atoms and molecules and finally the biological phase is related to response of cell to caused damages. To distinguish chemical and biological phase in cells the corresponding damage degree should be estimated independently. The analytical model of chemical phase describes the processes running in individual radical clusters after irradiation. The formation of chromosome double strand breaks in the case of low-LET radiation will be presented.

The chemical processes and diffusion characteristics of radical clusters have been described with the help of parameters taken from the literature; only several additional free parameters having been established. The concept using the Petro nets enables to consider the role of diffusion process and chemical reactions of radicals concurrently has been used in this simulation. The model may be easily extended by including the effects of various radiomodifiers present in medium during irradiation.

The model enables to distinguish chemical and biological phases in cell on the basis of determination of DSB amount in DNA and thus to help to establishfulness repair capability of a given cell. Possible applications of the proposed model in hadron radiotherapy will be discussed.


New experimental data show how chromosomal aberrations for low- and high-LET radiation are dependent on DSB repair deficiencies in wild-type, AT and NBS cells. We simulated the development of chromosomal aberrations in these cells lines in a stochastic track-structure-dependent model, in which different cells have different kinetics of DSB repair. We updated a previously formulated model of chromosomal aberrations, which was based on a stochastic Monte Carlo approach, to consider the time-dependence of DSB rejoining. The previous version of the model had an assumption that all DSBs would rejoin; therefore, we called it a “time-independent” model. The chromosomal-aberrations model takes into account the DNA and track structure for low- and high-LET radiations and provides an explanation and prediction of the statistics of rare and more complex aberrations. We compared the program-simulated kinetics of DSB rejoining to the experimentally-derived bimodal exponential curves of the DSB kinetics. We scored the formation of translocations, dicentrics, acentric fragments, and other aberrations.

The model of DSBs participating in aberrations was studied in relation to the rejoining time. Comparisons of simulated dose dependence for simple aberrations to the experimental dose-dependence for HF19, AT and NBS cells will be made.

POS40-12. New radiation-dosimetry 'targets' in marrow for assessing the risk of childhood leukemia and bone cancer. Richard Richardson1, R. Kramer1, 1: AECL, Canada, 2: Federal University of Pernambuco, Brazil

The International Commission on Radiological Protection (ICRP) assesses the risk of radiation-induced leukemia and bone cancer by calculating the dose to ‘target’ tissues in the bone marrow of trabecular bone cavities (Int J Radiat Biol 87, 343, 2011). However, these ICRP targets, which are reassessed in this paper, are based on marrow physiology and data that are more applicable to adults rather than fetuses, infants and children. Current ICRP targets do not account for hematopoietic and mesenchymal stem cells being comprised of active and quiescent subpopulations that occupy niches with different radiosensitivity. The biological reality is that, when quiescent, pre-cancerous cells, stem cells and perhaps cancer stem cells, reside in a hypoxic, radioprotective microenvironment such as the endosteum. These same stem cells when active reside in peri-vascular niches that are 2-3 fold more radiosensitive than the endosteum.

For leukemia, rather than assess the radiation dose to whole marrow as recommended by the ICRP, a more meaningful risk assessment is achieved by estimating and weighting the doses to marrow partitioned into its quiescent and active portions. The greater proportion of active
Interrelation between characteristics and composition of the liposome lipids and the radiation action. Lyudmila Shishkina,1 M. Klimovich, M. Kozlov, D. Paramonov, V. Trofimov,1: Emanuel Institute of Biochemical Physics of Russian Academy of Sciences, Russian Federation, 2: Scientific and Technological Center "Lekbiotech", Russian Federation

Earlier it is established that the physicochemical characteristics and the composition of the natural lipids play the important role in the liposome forming from them. The aim of this work is to reveal the most important parameters among the initial characteristics of the liposome lipids which are due to the formation of consequences after their irradiation. Liposomes were formed by ultrasound dispersant from the native and oxidized lecithin-standard and the liver and brain lipids of outbred mice. Physicochemical characteristics of the liver and brain lipids are modified by carrying out experiments in different seasons. g-Irradiation was performed at the dose rate of 27.6; 26.4 and 29.9 Gy/min for liposomes from lecithin and the murine liver and brain lipids correspondingly. The range of doses is from 0 to 7 Gy. The stage changes in dependence on the radiation dose were revealed for all investigated parameters of liposomes formed from lecithin: the TBA-reactive substances and peroxide amounts in lipids, the relation of phospholipid (PL) fractions and PL proportion in the total lipid composition (%PL), the content of the diene conjugate (DC) and ketoide (KD) in lipids. However, the stage changes in liposomes formed from the organ lipids of mice were only obtained for %PL, the relation of PL fractions and antiperoxide activity (APA) of lipids. In all cases the diminution of the medium pH was found when the radiation dose increased. While the common direct correlation between the DC and KD content in liposomes formed from any intact lipids was found, the similar correlations had the different scale for liposomes formed from lecithin and the organ lipids of mice in dependence on the radiation dose. The initial values of DC and KD content cause the difference in scale and direction of correlations between the medium pH, APA or the DC and KD content and the radiation dose. Irradiation results to the alteration of interrelation between the molar ratio [sterols]/[PL] and the TBA-reactive substances amount in liposomes formed from the organ lipids of mice. Data obtained allow us to conclusion that the initial extend of the lipid unsaturation and the sterol presence play the important role for the formation of consequences after the radiation action.

Interactions of Some Porphyrins and Chlorin e6 with Human Serum Albumin or DNA. Tadeusz Strzóz, M. Wolszczak, M. Hilczer, Technical University of Lodz, Institute of Applied Radiation Chemistry, Poland

Photodynamic therapy (PDT) is the treatment of malignant lesions with visible light following the systemic administration of tumour-localizing photosensitizers, e.g., porphyrins or chlorins. Under certain conditions, porphyrins can also act as radiosensitizers. Thus, a reasonable combination of radiotherapy and PDT might be really promising treatment. We have conducted pulse radiolysis and flash photolysis studies to determine properties of one-electron-reduced and one-electron-oxidised forms of porphyrins and near chlorin e6. Studies within porphyrin standard generated the reactive oxygen species with light and pulse radiolysis in order to determine absolute rate constants of free-radical reactions and to collect spectral information about transient species produced by one-electron reduction or oxidation. Experiments were conducted in selected homogeneous solvents as well as in the presence of biologically important molecules, such as human serum albumin (HSA) or DNA.

We have found that charges present in side chains of porphyrins are able to influence the localization of porphyrins in HSA and DNA. The cationic type porphyrins (e.g., meso-tetraphenyl-4-N-methylpyridyl)porphine and meso-tetraakis(4- (dimethylammoniumyl)phenyl)porphine) are effectively intercalated within DNA helix. The negatively charged porphyrin (meso-tetraphenyl(4-sulfonatophenyl)porphyrin) or chloride e6 penetrate into protein structure. From pulse radiolysis studies we concluded that reaction rate of the hydrated electron (e-?) with intercalated porphyrins is reduced tenfold in respect to that for free porphyrins. We did not observe the electron transfer along DNA helix, from DNA base radical anions to porphyrins. In our opinion it is due to low driving force of such a process. The laser flash photolysis experiments showed that the electron transfer occurs between the triplet state of porphyrin and another intercalator of high electron affinity (e.g., 1-nitro-9-aminocaridine) within DNA. The electron transfer distance was restricted to less than four base pairs.

Detoxification of α-radioactive solutions by humic substances. Tatiana Rozhko1, N. Kudyashyeva2, A. Bolsunovskyy2, O. Mogilnaya3, G. Vdvydrovaya1, 3: Siberian Federal University, Russian Federation

Ecological biomonitoring is based on the use of the living organisms. All biological assay systems (bioassays) are nonspecific and integral. The use of microorganisms, of marine luminous bacteria in particular, is currently favored. Bioluminescent bioassays are characterized by simplicity, high rate of analysis, and by relatively low prices. The aim of the research was the investigation of detoxification of radioactive solutions of low activity by humic substances (HS) by bioluminescent assay systems. The bioluminescence intensity of the luminescent bacterium P. phosphoreum on time of exposure to radioactivity in the presence and in the absence of HS was determined. This effect is a demonstration of detoxifying properties of HS in the radioactive solution. We found that HS (C=0.25 mg/ml) do not change time-decay of bioluminescent intensity under all concentrations of Uranium. (C=10−3, 2×10−3 M).

The studies on localization of americium in cell structures of intact bacteria in the absence and in the presence of HS were carried out. The introduction of HS was found to affect the distribution of 241Am in cells. Electron-microscopy study showed that HS decrease damage of the bacterial cells in the radioactive solutions. The effect of HS was shown to depend on a radionuclide concentration, as well as on the complexity and integrity of bioluminescent assay system. The study demonstrated a high potential of the bioluminescent assay to monitor detoxification efficiency of HS in solutions of radioactive compounds. HS (C = 0.25 mg/ml) detoxified solutions of Americium and did not detoxify solutions of Uranium.

Track structure

Track structure analysis can be utilized to refine the uncertainties in estimating the biological risks of heavy ions. The spatial distribution of energy deposited in cell nuclei depends on an ion’s charge and velocity. Ions of equal linear energy transfer (LET) have largely varying velocities and produce a significantly different spectrum of secondary particles. LET is an average value, ignoring track structure on the cellular scale. Variations in the particle trajectories of different ions cause a change in energy deposition and the resulting target effects. GCR have vastly different velocities and produce secondary electrons that can travel several millimeters away from the primary ion’s track to affect many more cells. Damage to a cell can be described by the energy imparted to a cell nucleus by a known number of particle traversals. Using FLUKA charged particle transport modeling, the microdosimetry of heavy ions traversing biologically
relevant targets can be simulated. This approach models the dose and particle fluence for individual cell nuclei making up a larger target volume. The damaging ability of an ion can be compared with other ions having equal LET by assessing the number of particle crossings and dose to each cell. This study presents the dose characteristics of Carbon, Oxygen, Magnesium, Silicon, Calcium, and Titanium, having an LET of 100 keV μm⁻¹. Ion kinetic energies range from 6.8 MeV n⁻¹ to 1582 MeV n⁻¹, and are calculated to have a stopping power of 1000 keV μm⁻¹ in water by the Bethe-Bloch approach to charged particle energy loss. Relative damaging ability is established by comparing the microdosimetric quantities determined by FLUKA simulation.

**POSTER PRESENTATIONS**

**POS41-02. First comparison of nanodosimetric measurements of protons and deuterons.** Hilgiers Gerhard, H. Netteltbeck, H. Rabus, Physikalisch-Technische Bundesanstalt (PTB), Germany

One of the main aims of experimental nanodosimetry is to investigate the initiation of radiation damage due to direct ionization processes in biological targets of the type of DNA segments, as well as for other macromolecules. This work presents an investigation of fundamental aspects of nanodosimetry. The electronic stopping power of a substance depends on the type, charge state and energy of the ionizing particle being stopped. According to Bethe's theory, the stopping power is identical for different isotopes of the same charge and identical velocity. In the field of nanodosimetry, the first moment of the frequency distribution of the number of ionizations, that is the mean ionization cluster size \( M_i(Q) \), is expected to be proportional to the stopping power. Therefore, \( M_i(Q) \) should also be identical for ions which are isotopes (i.e. identical atomic number \( Z \), but different mass number \( A \)), if the charge states and particle velocities are the same.

Ionization cluster size distributions from monoenergetic proton and deuterons beams in the energy range from 0.1 MeV to 3.3 MeV were measured in CH₄ and N₂ at a pressure of 1.2 mbar with an ion-counting nanodosimeter. For both gases, the mean ionization cluster size \( M_i(Q) \) derived from the measured cluster size distributions for proton and deuterons shows a similar dependence on the particle velocity. As regards to absolute values, however, the measurements for deuterons are systematically smaller than those for protons. The relative deviations between the two isotopes are in the order of 20% to 45% and are significant with respect to the measurement uncertainties. The deviations and uncertainties associated with these preliminary experimental data suggest that further measurements are needed to improve the comparison of the mean ionization cluster size \( M_i(Q) \) for different isotopes.

**POS41-03. Calibration of CR-39 using stochastic simulation for 10B measurements in autoradiography.** Seyed Behnamedin Jameie¹, A. Paziранده¹, S. Goodarzi², N. Baghban Khojasteh¹, 1: Basic Science Department, Faculty of Allied Medicine, Teheran University of Medical Science (Hemorheology and Nuclear Engineering), Science and Research Branch, Islamic Azad University, Iran

To determine boron concentration as a function of ion tracks number per unit area of a polycarbonate surface, an experiment was performed. Eleven CR-39 polycarbonate samples covered by H₃BO₃ solution with known amounts of ¹⁰B concentration were irradiated in a thermal neutron flux of 8x10⁹n.cm⁻².s⁻¹ in the nuclear facility of Tehran Research Reactor (TRR). Alpha and lithium tracks were produced on CR-39 surface as a consequence of ¹⁰B (n, α) Li reactions. After etching process using a solution of ethanol 30% and KOH 70%, the revealed tracks were counted under an optical microscope. After etching process using a solution of ethanol 30% and KOH 70%, the revealed tracks were counted under an optical microscope. Model: 3100.5000 Triton-II with 500times as much optical magnification. The experiment was also modeled using a stochastic simulation considering the random characteristic of the boron number capture reaction. Comparing experimental and theoretical results, the calibration factor, i.e., the ratio of counted tracks number in the experiment to the number of produced ions in simulation was obtained. Furthermore, the absorbed dose due to ion particles LET was quantified by means of the absorbed dose curve, which correlates with boron concentration in the samples. The later curve was also obtained by modeling the experiment using FLUKA code simulation.

**POS41-04. Analytical differential cross sections for ejection of electrons in ionization of water by protons in the plane-wave Born approximation (PWBA) and the energy-loss, Coulomb-deflection, perturbed-stationary-state and relativistic (ECPSRR) theory.**

Gregory Lapicki, Department of Physics, East Carolina University, Greenville, USA

Single-differential cross sections (SDCS) with respect to the energy transferred to ejected electrons are required in detailed modeling of interaction of ionizing radiation with matter; in particular, in proton bombardment of biological materials of which water is a prime constituent. The existing SDCS calculations are typically developed in the PWBA of the first Born approach (FBA), fortified with semi-empirical corrections for the breakdown of the FBA with the decreasing proton energy and performed numerically [1-3]. Our analytical PWBA based on the atomic and molecular composition of water is comprehensively compared with measured SDCS in ionization of water vapor by 0.015-4.2 MeV protons [4]. Analytical corrections of the ECPSRR theory [5], that goes beyond the FBA, do not remove existing discrepancies with data for the slowly ejected electrons ionized by slow protons. It is for these less than several hundred keV-protons, however, that this theory predicts increasingly smaller cross sections with the increasing energy of the ejected electrons. This could be a significant change as an input for Monte Carlo track structure codes in water and other biologically relevant materials [6].

After adjustments for its physical state as in [2], our analytical PWBA and ECPSRR calculations are done for the liquid water SDCS, which despite their usefulness in radiation biology are practically unobtainable experimentally. Restrictions on the range of momentum transfer in the derived formulas will be proposed for solid-state water, as ice measurements in conjunction with vapor data may support a bridge over troubled liquid water.


**POS41-05. A theoretical study of positive ion production by partially dressed ion impact on water.** Thansins Liansmsusan, H. Nikjoo, Group, Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

We investigated fragmentation of a water molecule induced by partially dressed ions C⁺ and C²⁺ of energies 10 keV/u to 1 MeV/u. The cross sections for positive ion production were calculated from the sums of partial interaction cross sections weighted by the branching ratios for breaking down of a water molecule by each interaction. Ionisation and electron capture from the 5 energy levels of water were taken into account. In this work, the interaction cross sections were obtained from the Classical Trajectory Monte Carlo method [1]; and the asymptotic branching ratios for fragmentation of water into H₂O, OH⁻, H⁺, and O²⁻ were taken from the derivation related to a set of experimental dipole oscillator strengths [2]. The cross sections for positive ion production are compared with available experimental data for C⁺ and C²⁺, as well as with the data for other ion species (H⁺, H⁺, and He⁺). The contributions of ionisation and electron capture are presented for selected projectile energies. The results presented give insight into physical processes leading to breaking down and dissociation of water molecules. This work is of relevance for studies of biological damage induced in the Bragg peak of a carbon ion beam. The data presented in this study have also been implemented in a Monte Carlo track structure code for a full slowing-down carbon ion track in water.

References
**POSTER PRESENTATIONS**


**POS41-06. Consequences of track structure for chemical stage of radiation action and DNA damage.** Václav Štěpán, M. Davidková, Department of Radiation Dosimetry, Nuclear Physics Institute ASCR, Czech Republic

Initial track structure is one of the key factors determining damage to biomolecules by ionizing radiation. Theoretical model RADAMOL was applied to highlight differences in time-space track evolution for different types of charged primary particles in water. The RADAMOL model follows radiation action from initial track structure of charged ionizing particles in water through physico-chemical and chemical stage. Resulting damages of a biological target, DNA oligomer or DNA-protein complex in water, can be scored. RADAMOL was built as an extension to codes STOCHECO and RADACK, which provide description of water radiolysis and interactions with target molecule. Initial track structures are generated using Monte Carlo code TRIOL. Time-space evolution of the track is followed up to 1 ms. Yields of simple and complex damages are evaluated by default; task-specific damage clusters can be followed as necessary. Complex DNA damages are considered closely related to cellular effects of ionizing radiation; more densely ionizing particles are assumed to cause proportionally more complex DNA damages. Using RADAMOL code we modelled an in vitro situation when DNA oligomers are irradiated in water solution without radical scavengers. To assess the effect of track structure, three data sets were computed for each particle/energy combination. (1) Full simulation with track structure taken into account. (2) Case when positions of radiolytic species are randomized after pre-chemical stage. (3) Case when absorbed energy is converted into randomly placed radicals using escape radical yields for low-LET radiation and only chemical stage is modelled. Yield kinetics of chemical species and consequences on DNA damage will be discussed for selected energies of electrons, protons and alpha particles.

**POS41-07. Computational Approach for Determining the Properties of Low-Energy Electron Track.** Yuji Yoshi1, K. Sasaki2, K.L. Sutherland3, H. Date4, 1: Education and Instrumentation Center, Sapporo Medical College, Japan, 2: Graduate School of Engineering, Kyoto University, Japan, 3: Graduate School of Medicine, Hokkaido University, Japan, 4: Faculty of Health Sciences, Hokkaido University, Japan

Introduction: Local energy deposition of electrons generated in bio-tissues exposed to radiation is crucial in the processes leading to cell damage. The purpose of this study is to make clear the agglomerative properties of electron processes (ionization and excitation) along low energy (below a few keV) electron tracks contributing to cell damage such as double strand breaks (DSBs) of DNA.

Methods and Materials: The Monte Carlo technique was used for determining the spatial location of the ionization and excitation events by electrons with incident energies ranging from 100 eV to 1 MeV in liquid water. The aggregation degree of the events per track was evaluated by calculating the distance distribution between two arbitrary events. The distance frequency distributed below 3.4 nm (corresponding to 10 bp in DNA) was considered to correlate with the induction of DSBs, which we call “aggregation index” (AI). The group size of the events was classified by the distance frequency and the isolated pattern configuration to find the minimal element as a cluster of the events for inducing DSB.

Results: The simulation results show that the value of AI increases proportionally with the incident electron energy above a few keV while it surpasses the linear trend (in convex shape) below a few keV energies. Low energy electrons (particularly around sub-keV) have a tendency to induce ionization and excitation events densely in local volumes as clusters even though the number of the clusters is few. In this case, DSB may be generated efficiently.

Conclusions: The computation of AI and clustering number is useful for evaluating DNA damage such as DSBs. The initial energy of electron track affects the cluster formation leading to DNA damage.
General information

The venue. The congress rooms can be accessed from the East through the main entrance to the Palace of Culture and Science (PKiN) or from the West through the doors leading to LR1 Kongresowa. Name badges must be shown upon entering the building. Address of the venue is: PKiN, plac Defilad 1, 00-901 Warszawa.

PowerPoint presentations. Please hand in your presentation directly to the operator in your lecture room. Please do this either in the morning or during the coffee/lunch break preceding your session.

Posters. The poster size is 240 x 95 cm (94 inches by 37 inches). Poster walls will be standing in rooms PR1 Starzyński and PR2 Broniewski. Authors are asked to be at their posters during the times given in the program (see description of the poster sessions). Posters will be exposed during the whole meeting.

Coffee and lunches. Coffee and lunches are included in the registration fee and will be served in CR1 Marmurowa and CR2 Korczak.

Internet access. Free wireless internet will be available in all rooms. Access passwords will be provided at the registration desk.

Printer. A desk computer with a printer will be available at the registration desk. Please consult the crew of the registration desk for access.

Some information about the venue

The Palace of Culture and Science (PKiN). The Palace was a gift of the Stalinist Soviet Union to the Polish People’s Republic. The main architect was Leo Rudniew. It was erected between 1952 and 1955. With its 230.68 m the Palace still remains the highest building in Poland. It has 42 storeys and 3,288 rooms and halls, some of which are listed below with the origin of their names explained.

LR1 Kongresowa. Designed as a congress and conference hall, but also used on numerous occasions as a concert and theatre hall. Famous performers include Marlene Dietrich, Ella Fitzgerald, Duke Ellington, and the Rolling Stones. It can accommodate 2,880 people.

LR2 Ratuszowa. The English translation is “town hall room”. It is identical in size and shape to CR1 Marmurowa. The walls are covered by marble and the floor is a stone mosaic.

LR3 Skłodowska. Named after Maria Skłodowska-Curie, who does not need to be introduced here. If you want to learn more about her – listen to the lecture by her granddaughter Hélène Langevin-Joliot on Thursday 1 September 14:00 (PL 7).

LR4 Warszawska. The English translation is “Warsaw room”. It routinely serves as the meeting place of the Senate of the city of Warsaw.

LR5 Mikołajksa. Named after Halina Mikołajksa, who was perhaps the best Polish theater actress of the 20th century. Mikołajksa was also deeply engaged in resistance against the communist regime. She died in 1989.

LR6 Kruczkowski. Named after Leon Kruczkowski, a Polish writer and publicist. His works include novels and theatre plays. He was an active and vocal supporter of the new communist order in Poland and, being a major literary figure, is recognized as having a significant influence on the post-war Polish cultural policy. He died in 1962.

PR1 Starzyński. Named after Stefan Starzyński, a Polish politician, economist, writer, and statesman. He was the mayor of the capital city before and during the Siege of Warsaw in 1939. Captured by the Nazis he died in 1943 in the Dachau concentration camp.

PR2 Broniewski. Named after Władysław Broniewski, a Polish poet and soldier. He is considered as one of the most important representatives of revolutionary lyrics and socialist realism. He was also a talented translator of poetry and prose from Russian and German. He died in 1962.

CR1 Marmurowa. The name means “marble room”. It is a twin room to LR2 Ratuszowa. Both rooms are perhaps the most representative chambers of the Palace and were meant to resemble the interior of a renaissance palace.
**CR2 Korczak.** Named after Janusz Korczak (actually Henryk Goldszmit), who was a Polish-Jewish pedagogue, children's author, and paediatrician. Korczak was passionately engaged in fighting for children’s rights during the period before the World War II. He was the director of an orphanage and was killed by the Nazis in 1942, together with his children, in the concentration camp in Treblinka.

**MR1 Rudniew.** Named after Lev Vladimirovich Rudniev, the main architect of the palace. Rudniev was a Russian architect and a leading practitioner of Stalinist architecture. He died in 1956.

**MR6 Puszkln.** Named after Alexander Sergeyevich Pushkin (1799 – 1837) who was a Russian author of the Romantic era and who is considered to be the greatest Russian poet and the founder of modern Russian literature.

**Gagarin.** Named after Juri Alexeyevitch Gagarin, a Russian test pilot and cosmonaut. In 1961 he became the first man in space and a hero of the Soviet Union. He died in an aeroplane crash in 1968.

**Tiereszkowa.** Named after Valentina Vladimirovna Tereshkova, a retired Soviet cosmonaut, who in 1963 became the first woman in space.

**Trojka.** (Threesome in English) was initially a restaurant with the same name. Today, Trojka is used as a corridor to connect CR1 Marmurowa and LR2 Ratuszowa.

**Mickiewicz.** Named after Adam Mickiewicz, who lived between 1798 and 1855. He was a Polish-Lithuanian poet of the Romantic period. He is regarded as a national poet of Poland and perhaps the greatest poet in the Polish literature.

**Sienkiewicz.** Named after Henryk Sienkiewicz, who lived from 1846 to 1916. He was one of the most popular Polish writers at the turn of the 19th and 20th centuries, and received the Nobel Prize in Literature in 1905 for his outstanding merits as an epic writer. One of his novels, *Quo vadis*, was filmed in 1951 in Hollywood, starring Robert Taylor, Deborah Kerr, and Peter Ustinov. A Polish film based on the novel appeared in 2001.

**Pen Club.** The name comes from the fact that this room is used as a meeting place by the Polish Pen Club.

A map of the Warsaw city centre with the location of the palace (in the centre) is shown below.
Plan of lecture rooms – main room complex

4th floor

 Warszawska annexes
 MR3  MR4  MR5
 Gagarin
 PR1 Starzyński
 PR2 Broniewski
 MR2 (Rudniew annex)
 MR1 Rudniew
 LR4 Warszawska
 LR3 Sklodowska

to LR1 Kongresowa

2nd floor

 LR5 Mikołajska
 CR2 Korczak
 CR1 Marmurowa
 Sienkiewicz
 Trojka
 LR2 Ratuszowa

Main street entrance
(entrance side of the Palace)
GPS: 52.1354 N, 21.0728 E

to LR1 Kongresowa

Note: rooms Gagarin, Sienkiewicz and Trojka can be accessed, but no events are planned in them.
Plan of lecture rooms – LR1 Kongresowa and adjacent rooms

2nd floor

1st floor

Note: rooms Tiereszkowa, Mickiewicz, Kopernik and Pen Club can be accessed but no events are planned in them.
Conference gala dinner, Tuesday 30 August

The gala dinner will take place on Tuesday 30 August, starting at 19:30. It will be a seated banquet with wine served at an open bar. A multi-course dinner will be served from a buffet. A six-person music band whose original repertoire ranges from jazz to Latin-American tunes will make the evening even more attractive. A dance floor will also be available for those who like dancing.

The venue of the gala dinner is EXPO XXI, one of the most modern event halls in Poland. EXPO XXI is located ca 7 km west of the conference venue. Transport to EXPO XXI will be provided – detailed information will be available at the registration desk. Please organize your own return transport by taxi or bus (see below).

EXPO XXI can also be reached by the public bus line 109 (heading for OS GORCZEWSKA) The route of this bus is marked black on the map above. Circles represent bus stops. Please get off at bus stop "SZPITAL WOLSKI" and walk to the EXPO entrance as marked by the dotted line on the map above and on the satellite picture on the left. The walking distance is about 500 m. The whole trip may take ca 40 min.

You can take the same bus line back to the palace. The destination is "DW CENTRALNY". The last bus will leave "SZPITAL WOLSKI" at 23:06. It is also possible to take the night bus line N43 destination "DW CENTRALNY". The bus leaves at 23:19, 23:49, 24:19, 24:49.

Address of EXPO XXI: ul. Prądzyńskiego 12/14, 01-222 Warszawa.
GPS coordinates: 52°13'25"N, 20°58'2"E.
**List of meetings parallel to ICRR2011**  
(only for involved participants)

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