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James F. Curtin
Dublin Institute of Technology, james.curtin@dit.ie

Marianela Candolfi
Cedars-Sinai Medical Center

Weidong Xiong
Cedars-Sinai Medical Center

Pedro Lowenstein
Cedars-Sinai Medical Center

Maria Castro
Cedars-Sinai Medical Center

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Turning the gene tap off; implications of regulating gene expression for cancer therapeutics

James F. Curtin[†], Marianela Candolfi, Weidong Xiong, Pedro R. Lowenstein, and Maria G. Castro

Board of Governors Gene Therapeutics Research Institute, Cedars-Sinai Medical Center, and Departments of Molecular and Medical Pharmacology and Medicine, Jonsson Comprehensive Cancer Center; Brain Research Institute, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, California

[†]Current Address: School of Biological Sciences, Dublin Institute of Technology, Dublin 8, Ireland

Abstract

Cancer poses a tremendous therapeutic challenge worldwide, highlighting the critical need for developing novel therapeutics. A promising cancer treatment modality is gene therapy, which is a form of molecular medicine designed to introduce into target cells genetic material with therapeutic intent. Anticancer gene therapy strategies currently used in preclinical models, and in some cases in the clinic, include proapoptotic genes, oncolytic/replicative vectors, conditional cytotoxic approaches, inhibition of angiogenesis, inhibition of growth factor signaling, inactivation of oncogenes, inhibition of tumor invasion and stimulation of the immune system. The translation of these novel therapeutic modalities from the preclinical setting to the clinic has been driven by encouraging preclinical efficacy data and advances in gene delivery technologies. One area of intense research involves the ability to accurately regulate the levels of therapeutic gene expression to achieve enhanced efficacy and provide the capability to switch gene expression off completely if adverse side effects should arise. This feature could also be implemented to switch gene expression off when a successful therapeutic outcome ensues. Here, we will review recent developments related to the engineering of transcriptional switches within gene delivery systems, which could be implemented in clinical gene therapy applications directed at the treatment of cancer.

Introduction

In the United States alone, cancer accounts for ~ 23% of all deaths yearly, ranking only second to heart disease (1). This highlights the critical need for the development of novel therapeutic approaches to reduce the public burden of cancer. One promising cancer treatment modality is gene therapy, which is a form of molecular medicine designed to introduce into target cells genetic material with therapeutic intent. Worldwide, nearly 1,000 gene therapy clinical trials have been or are being conducted, and of these, two thirds are for treating cancer (2); in 2004, the first gene therapeutic product consisting of a replication-deficient adenovirus encoding p53 (Ad-p53; Gendicine) was approved for commercial use by China's State Food and Drug Administration for head and neck squamous cell carcinoma. Some of the best outcomes have been observed when Gendicine has been used in combination with conventional treatments, that is, radiation to treat nasopharyngeal cancer (3), or with transcatheter hepatic arterial chemoembolization to treat hepatocellular carcinoma. Recently, the first oncolytic adenoviral vector, H101, was approved by the State Food and Drug Administration as a commercial gene therapeutic product (4), which is used in combination with local heat treatment and

Requests for reprints: Maria G. Castro, University of California-Los Angeles and Cedars Sinai Medical Center, 8700 Beverly Boulevard, Davis Building 5090, Los Angeles, CA 90048. Phone: 310-423-7303; Fax: 310-423-7308. E-mail: castromg@cshs.org.

chemotherapy for late-stage refractory head and neck cancers.[1] Interestingly, although gene therapy remains, in the western markets, a promising therapeutic approach, in China it is currently being implemented in the clinic. The only two companies with commercial gene therapy products are Chinese. Their gene therapy vectors have been in the market for several years without reported deleterious side effects. The reasons why the first commercial gene therapy treatment got produced and approved in China could be due to the fact that the prospect of a one-time treatment, simple to administer is very compelling; also, due to the large population in China, it is possible to recruit enough patients for a clinical trial in a short timeframe and generate statistically significant clinical data in a timely fashion. Importantly, China has not been affected by adverse events, as in the United States, with the death of Jesse Gelsinger of a serious adverse event due to gene therapy for an inherited metabolic disorder (5) and more recently in Europe with adverse events reported in the X-linked, severe combined immunodeficiency syndrome trials (6). Finally, the Chinese regulatory authorities may be more receptive to this technology. The translation of these novel treatment modalities from the preclinical setting to the clinic has been driven by encouraging efficacy data and advances in gene delivery technologies. One area of intense research involves the ability to accurately regulate the levels of therapeutic gene expression to achieve enhanced efficacy and provide the capability to switch gene expression off completely if adverse side effects should arise. Here, we will review some of the recent developments related to the engineering of transcriptional switches within gene delivery systems, which could be implemented in clinical gene therapy applications.

Regulating Gene Expression for Cancer Therapy

For gene therapy to become a successful and widely used clinical modality, it will be critical to regulate the expression of the therapeutic transgenes according to clinical needs and also to curtail any putative adverse side effects of the therapy. A promoter that is sensitive to changes in the environment of cells/tissues is the basis for achieving regulatable therapeutic gene expression. Inducible gene transfer vectors encode promoters that are regulated by transcription factors sensitive to physiologic changes (heat shock, metal ions, IFNs, and dsRNA) or exogenous chemicals (rapamycin and steroids; Table 1; ref. 7). Coexpression of both the regulated transcription factor and the inducible promoter within the same vector improves specificity of gene expression and allows using a greater range of promoters, even those that are not normally expressed in the target cell, such as a mutated steroid receptor with high affinity for the antagonist mifepristone, the tetracycline-dependent system (Tet system) and the insect steroid hormone ecdystone receptor system (Table 1;ref. 7). The Tet system has several advantages, given that tetracycline and its analogues have been proven to be nontoxic in human patients, the promoter has negligible leakiness in the “off” state (8,9), has rapid induction and repression kinetics in vivo (9), and is not expressed normally in mammalian cells, which gives it higher specificity over steroid receptor-based systems. Also, the small size of the expression cassette of the Tet system (~ 3 kB) allows it to be encoded within most viral vectors (7). The potential disadvantage of using prokaryotic transcriptional systems is that they might be immunogenic in mammalians. However, we have shown that the components of the Tet-On regulatable system are weakly immunogenic, and pre-exposure to the proteins encoded, that is, rtTA2S-M2 and tTSKid, do not significantly affect transgene expression in the central nervous system from Tet-regulated adenoviral vectors in preclinical models (10). Considering that the Tet-On system is the most widely used regulatable system in preclinical cancer research, and other regulatable systems have been reviewed in detail elsewhere (7), we will focus on the Tet-On regulatable system throughout this review, although many of the principles discussed can also be applied to other regulatable systems. Please see Table 1 for a summary

1http://www.oralcancerfoundation.org/news/story.asp?newsId=1033

description of other commonly used regulatable systems, including advantages and disadvantages for each one.

Tet System through the Ages: Progress Made Since the 1980s

The first Tet system was described in Escherichia coli, where the Tet repressor protein inhibits the transcription of genes in the tetracycline resistance operon on the Tn10 transposon by docking to the Tet operator sequences in the absence of tetracycline (11). The engineering of the Tet repressor protein over the following 20 years led to several systems with improved inducibility, stringent regulation of transgene expression, and negligible leakage. The first-generation Tet system, i.e., the Tet-Off switch, drive transgene expression in the absence but not in the presence of tetracycline. In this system, the Tet repressor protein was fused to a viral protein domain VP16, a eukaryotic transactivator derived from HSV-1, converting the Tet repressor protein from a repressor to a transactivator (tTA). The tTA is constantly expressed under the control of a constitutive promoter but induces the activity of the TRE promoter only in the absence of tetracycline. The TRE promoter is composed of seven recurring Tet operator sequences and the minimal human cytomegalovirus promoter, which ultimately drives therapeutic transgene expression. The TRE promoter activity is triggered when the transactivator tTA binds to Tet operator in the absence of tetracycline, inducing therapeutic transgene expression. Tetracycline and its analogues bind to the tTA and hinder the capacity of the tTA to become docked to the Tet operator sequences within the TRE, inhibiting the transcriptional activity of the promoter. Thus, addition of tetracycline results in inhibition of therapeutic transgene expression. The Tet-Off switch has been encoded within an adenoviral vector expressing the proapoptotic protein Bax to induce apoptosis of human lung cancer cells; its effects can be blocked by addition of tetracycline (12). Regulated delivery of tyrosine hydroxylase was implemented in preclinical models of pituitary adenomas using adenoviral vectors encoding the Tet-Off system (13). Although tyrosine hydroxylase expression leads to successful regression of pituitary hyperplasia and normalization of serum prolactin levels, chronic overexpression of tyrosine hydroxylase could elicit pituitary insufficiency. This adverse effect could be reverted by switching off tyrosine hydroxylase expression. Although the Tet-Off system has been criticized because it exhibits 1% to 10% leakage in the “off” state (12), it still allows to substantially reduce transgene expression when side effects arise. For instance, the severe side effects associated to the systemic administration of interleukins (IL) for the treatment of colon cancer led to the construction of adenoviral vectors expressing IL-12 driven by the Tet-Off system, which yields high levels of IL-12 that are inhibited 99% in the presence of doxycycline (14). Retroviruses encoding the Tet-Off system also produce high levels of thymidine kinase, with <0.5% basal expression in the “off” state (15). A potential pitfall of the Tet-Off system is that inhibition of transgene expression after regression of the tumor requires chronic administration of tetracycline.

Mutations to the tTA led to a novel transactivator, rtTA, which binds the TRE promoter in the presence of tetracycline. This system, that is, Tet-On, has the advantage of driving therapeutic transgene expression only in the presence of the inducer, remaining inactive in its absence. However, the rtTA transactivator has residual affinity for the TRE in the absence of the inducer, exhibiting some degree of basal transgene expression (16–18). This drawback was overcome by engineering the rtTA and the TRE promoter, leading to negligible background expression in the absence of the inducer (19–21). Further mutagenesis of the rtTA generated a mutant transactivator, rtTA2s-M2, which yields higher levels of transgene expression in the presence of the inducer and virtually negligible basal expression in its absence (19). To this end, regulation of oncolytic adenoviral replication in lung cancer cells was achieved by expressing the E1 gene, essential for adenovirus replication, under the control of the TRE promoter (22). Cell type specificity of replication was accomplished by encoding the transactivator rtTA2s-M2 under the control of a lung cancer cell promoter. Engineering of the TRE promoter reduced

10-fold the basal expression from adenoviral vectors encoding the highly cytotoxic FasL under the control of the Tet-On system, leading to stringent regulation of cell death in lung cancer cells (23). To further reduce the already very low basal levels of expression from the second-generation Tet-On system, encoding the novel rtTA2S-M2 transactivator, a transrepressor (tTSKid) was developed that binds and represses the TRE promoter in the absence but not in the presence of tetracycline (24). Addition of the inducer, in turn, prevents binding of tTSKid relieving repression, and promoting binding of transactivator to the TRE promoter, leading to nonleaky transgene expression. Another advantage of the transrepressor is that its presence inhibits the ubiquitin-dependent proteosomal degradation of the transactivator, leading to increased amounts of transactivator available for activation of the TRE when the inducer is added, which in turn increases the levels of transgene expression in the “on” state (18,25).

Table 2 shows that the evolution of the Tet-On system, from the first-generation switches encoding the rtTA to the third-generation switches comprising the rtTA2S-M2 and the transrepressor tTSKid, resulted not only in enhanced levels of expression in the “on” state but also in negligible transgene expression in the “off” state. This third-generation Tet-On switch has been shown to be very effective in achieving tight regulation of transgene expression in mice, rats, and nonhuman primates (9,17,26). Figure 1 shows the virtual absence of leakage from regulated high-capacity adenoviral vectors expressing different transgenes, such as a cytoplasmic enzyme (β-galactosidase; Fig. 1A) and a secreted cytokine (Flt3L; Fig. 1B). This third-generation Tet-On system also exerts tight regulation and strong induction of transgene expression bidirectionally yielding isomolar production of IL-13 and IL-4 when encoded by an adenoviral vector expressing these transgenes under the control of the bidirectional TRE promoter (Fig. 1C).

Considering the prokaryotic origin of the Tet-On switch components, rtTA2S-M2 and tTSKid, the likelihood of immune responses against these exogenous proteins has to be taken into account when using these inducible systems for therapeutic transgene expression. After systemic administration of high doses of high-capacity adenoviral vectors (27), immune responses against the components of the Tet-On switch are triggered, causing a reduction in the longevity of transgene expression. This has been overcome by reducing the dose of the vector (27) or by expressing the Tet-On switch under the control of a cell type–specific promoter, which inhibits expression of the Tet-On components in professional antigen-presenting cells (27). In the brain, however, the systemic immune status against the components of the Tet-On switch seems to have less of an effect on the longevity and stability of transgene expression from high-capacity adenoviral vectors. Pre-exposure to the Tet-dependent regulatory proteins does not severely compromise regulated transgene expression from high-capacity adenoviral vectors delivered in the brain, with expression remaining detectable for up to 7 weeks postdelivery of the high-capacity adenoviral vector into the brain parenchyma (10). Thus, the regulatory switch composed of rtTA2S-M2 and the tTSKid appears as a safe and very useful tool for regulating gene expression in the brain.

In summary, the latest-generation Tet-dependent transcriptional regulatory system that comprises the transactivator rtTA2S-M2 and the transrepressor tTSKid exhibits all the features of an ideal transcriptional regulatory system: high levels of transgene expression in the induced state, negligible transgene expression in the repressed state, quick response to the administration or removal of the inducer, and negligible cytotoxic or inflammatory responses associated with the regulatory elements within the switch system or with the inducer. Also, the possibility of encoding the Tet-dependent transactivators under the control of cell type–specific promoters makes this system very versatile to target transgene expression to specific cancer cells. The advantages and disadvantages of the Tet regulatable system compared with other commonly used regulatable systems are outlined in Table 1.

Regulating Gene Expression for Cancer Therapeutics: Seeing the Light at the End of the Side Effects Tunnel

Cancer arises after accumulation of mutations in the genome, which predispose daughter cells to the acquisition of traits that favor uncontrollable cell proliferation at the expense of other cells in the body. These mutations generally occur in a certain order and this genetic pathway to cancer has been most fully investigated to date in colon cancer (28). Several genetic mutations in p53, ATM, NF-1, P16, and Rb have also been identified that predispose individuals to central nervous system cancers, including glioma and other cancers (29). Strategies that are currently used to treat cancer in the clinic and in preclinical models usually attempt to kill or otherwise incapacitate cancer cells while sparing normal cells. Seven hallmarks of cancer have been identified, that is, insensitivity to proapoptotic stimuli, increased angiogenesis, deregulation of cell cycle control, activation of growth factor signaling, mutation of oncogenes/tumor suppressors, increased tissue invasion, and evasion of the immune system (30). By specifically targeting one or several of these pathways, it should theoretically be possible eradicate cancer cells from the patient while minimizing adverse toxic events.

In the clinic, several key issues need to be considered before chemotherapeutic/oncolytic agents can be administered to patients with a high degree of safety while retaining their efficacy. These include the following: (a) toxicity: relates to uncovering putative adverse side effects arising in response to acute or chronic exposure to the drug. (b) Route of administration: local versus systemic. (c) Pharmacokinetics and pharmacodynamics: relate to determining the half-life and clearance of drugs; establishing the duration and frequency of administration (that is, single versus multiple dosage) required to achieve therapeutic benefits without adverse effects. (d) Adverse drug reactions: identifying and avoiding unwanted interactions with other therapies. To address these issues, novel drug formulation (that is, slow release), changing the frequency or route of administration, increasing or lowering the dose, and halting the treatment are all strategies that can be employed to enhance efficacy while minimizing adverse events. In the following sections, we will discuss how these issues affect the safety and efficacy of anticancer therapeutics and how regulatable expression cassettes can be used to optimize gene therapy strategies for cancer treatment/management.

Toxicity

The toxic dose (determined in phase I trials) and the effective dose (characterized in phase II and III trials) of any new drug must be determined during controlled, regulated clinical trials in a relatively small number of patients. Any new therapy will have a threshold above which toxic effects can be routinely observed. The doses are adjusted to determine therapeutic efficacy at doses that do not cause severe (grade 3 or 4) side effects. Increased toxicity usually correlates with decreased specificity of the therapeutic anticancer strategies. As an example, we will discuss the use in clinical trials of a peptide that inhibits the serine protease urokinase plasminogen activator (uPA) receptor activity (A6) tested in patients with gynecologic cancers in a phase I trial (31). Expression of uPA receptor or the ligand uPA strongly correlates with metastatic disease and it was proposed that uPA and uPA receptor inhibitors would limit tissue invasion and metastasis. In this trial, in one patient who received the highest dose of A6 (300 mg/d by s.c. injection), mild neurologic disorders developed (oropharyngeal hypoesthesia, sensory neuropathy, and dizziness). It was also determined that moderate muscle weakness and progressive abdominopelvic disease detected on computed tomographic scans that were most probably due to A6. These symptoms became evident 3 months after beginning therapy and were resolved after stopping the therapy for 2 weeks. When the therapy was restarted, the symptoms developed again 1 month later (31). Another example of toxicity was evident when depleting antibodies were used to reduce expression of vascular endothelial growth factor (VEGF), a potent angiogenic molecule implicated in tumor maintenance and progression.

When antibodies against VEGF that hamper its binding to the VEGF receptor were administered i.v. to breast cancer patients, 40% of the patients developed grade 3 hypertension and 100% if the patients exhibited hemorrhagic episodes (32), almost certainly related to the blocking of endogenous VEGF vasodilator and procoagulant effects. After the administration of this treatment to patients with brain tumors, four patients developed grade 4 thromboembolic complications resulting in the death of two patients (33). Another example of toxic side effects arising from lack of specificity is the implementation of a chimeric toxin composed of IL-13 fused to Pseudomonas exotoxin (Cintredekin Besudotox hIL13-PE) for the treatment of patients with glioblastoma multiforme (34). hIL-13-PE was not designed to treat brain tumors but rather to compare its efficacy with an IL-4-based cytotoxin in renal carcinoma (35). Nevertheless, a high proportion of human glioblastoma multiformes overexpress a mutant IL-13Rα2 receptor, which can be targeted using this chimeric cytotoxin approach (34,36). In a phase III clinical trial for glioblastoma multiforme, dose-related neurologic side effects arose in most of the patients after the intracranial administration of hIL13-PE (34). IL-13 can also bind to the physiologic IL13/IL4 receptor expressed in normal cells. Therefore, the toxic side effects encountered with this therapeutic approach could be due to the lack of specificity of the targeted ligand (that is, IL13). The targeting of IL-13Rα2 would be greatly improved by using the mutated form of IL-13, which has higher specificity for the IL13Rα2 receptor expressed on a significant proportion glioblastoma multiforme cells and negligible binding to the normal IL13/IL4 receptor expressed in normal brain cells (36). The severity of the side effects induced by these drugs underscore how clinical trials are an essential part of testing new therapies to identify potential problems in small numbers of patients before testing for efficacy in larger cohorts of patients, also highlighting how the ability to regulate the levels of the active therapeutic compound could aid in minimizing putative adverse events.

Route of Administration

The accumulation of chemotherapeutic drugs in different body compartments, hence the toxicity of the drugs, varies depending on the route of administration. Ideally, drugs would be delivered locally to the tumor that would limit the toxic side effects associated with the treatment regimen. However, local administration of chemotherapeutic agents can be costly, inefficient, or inconvenient. Gene therapy vectors can express highly toxic genes from within the tumor, that is, locally. One of the advantages of using gene therapy is that the expression of the therapeutic genes from within tumor cells might avoid the more serious side effects associated with systemic administration. To exemplify the scenario described in the section above, adenoviral vectors expressing antisense uPA receptor and uPA inhibited invasion and induced regression of human brain tumor xenografts in mice (37). Expression of the serine protease inhibitor Maspin (SERPINB5) using adeno-associated virus inhibits prostate cancer growth when delivered using adeno-associated virus vectors (38). VEGF receptor signaling has been similarly blocked in preclinical gene therapy trials with the adeno-associated virus–dependent delivery of soluble VEGF receptors that inhibited angiogenesis and metastasis and induced regression of several tumor mouse models with no adverse events (39). However, in the clinical scenario, in spite of local delivery of the gene therapy vectors into the tumor mass, the potential for the development of adverse events encourages the use of regulatable gene expression systems. For instance, local delivery of oncolytic adenoviruses (ONYX-015) in the tumor bed after surgical resection in glioblastoma patients showed that the treatment is well tolerated in a phase I clinical trial (40) and this virus is already approved for the treatment of head and neck cancer in China (4). However, in case of tumor regression, it would be highly desirable to inhibit viral replication that could affect normal cells. ONYX-015 has a deletion in the E1-B region, allowing replication in p53 null tumor cells, but replication fails to proceed in p53-competent normal cells. Addition of regulated and cancer cell-specific promoters to replication-competent viruses could further improve the safety of these vectors by reducing the chance of replication in normal cells. In fact, regulated replication of oncolytic adenovirus

was achieved in lung cancer cells by expressing the E1 gene under the control of the TRE promoter (22), whereas cell type specificity of replication was carried out by encoding the transactivator under the control of a lung cancer cell promoter.

Pharmacokinetics and Pharmacodynamics

The route of administration also greatly determines many of the pharmacokinetic and pharmacodynamic properties of a drug. These values must be empirically derived from clinical trials and preclinical studies to identify the best route of administration and dose of a new therapy. Many of the recombinant proteins that are now being tested in the clinic have very short half-lives. A good example of cytokines that have a half-life of <1 h includes soluble TRAIL with a half-life in plasma of 32 min (41). Similarly, tumor necrosis factor-α (TNF-α), IL-1, IL-2, and IL-6 all have half-lives following i.v. delivery of <15 min. Targeted immunotoxins conjugated to cytokines are also rapidly eliminated from the body. The chimeric toxin composed of IL13 and Pseudomonas exotoxin (Citredekin Besudotox) has a half-life of 2 h after intratumoral administration (42); thus, high doses and repeated, prolonged administration, typically 4 to 6 days, were required to maintain therapeutic levels of the protein into the tumor mass (42). Peptides have very short half-lives because their small size and relative hydrophilicity increase the rate of filtration by the kidneys. The uPA receptor inhibitor A6 has a half-life of <2 h (31) and uPA inhibitor WX-671 has a half-life of 5.8 h when given orally (43). The advantage of using drugs with short half-lives is that they can be rapidly removed from the patient should adverse reactions develop. Unfortunately, the therapy must usually be administered either at relatively high concentration and/or frequency to maintain an effective dose. Generally, altering the route of delivery or the formulation of a new drug can increase the half-life of these therapeutic compounds. Formulation of recombinant Flt3L in a sustained release Poloxamer-407-based matrix increased the half-life from 5.2 to 11.7 h in mice (44) and i.p. delivery increased the half-life of recombinant glycosylated GMCSF to 25 h when the half-life was only 3 min when administered i.v. (45). A disadvantage of changing the route of administration is that maintaining the effective concentration of the drug at the tumor may not be possible. Gene therapy can overcome disadvantages of frequent dosing schedules and routes of administration by expressing de novo therapeutic proteins from within the tumor. It is usually difficult, however, to adjust the concentration of the therapeutic gene product when constitutive promoters are used. Instead, the common practice is to administer a defined number of gene delivery vectors/particles to the patient to try to induce gene expression within a therapeutic “range.” Regulatable gene expression vectors allow the concentration of a gene product to be adjusted, in addition to switching on or off gene expression if adverse effects should ensue. This can be achieved by alternating the dosing schedule of the small-molecule inducer, thus altering the concentration of the therapeutic gene product to a desired level between minimum and maximum expression levels.

Adverse Drug Reactions

Perhaps the most difficult toxic effect to predict is the adverse pharmacologic interactions that occur between a new therapy and existing therapies. Adverse pharmacologic interactions are the most common cause of therapy-induced death in patients, accounting for 160,000 hospital deaths each year (46). Flavopiridol, an inhibitor of the cyclin-dependent kinases CDK2 and CDK4, was tested in a phase I clinical trial and was shown to be well tolerated in patients (47). Flavopiridol was found to be safe in combination with cisplatin, docetaxel, and irinotecan, but unexpected severe toxicity was noted when flavopiridol was combined with a DNA alkylating agent, carboplatin, resulting in pulmonary embolism and death in one patient (47). Regulatable gene therapeutic vectors, either using small molecule–sensitive promoters such as the Tet-On system or suicide genes such as thymidine kinase to kill cells expressing the therapeutic gene, would be critical to reduce or eliminate the expression of the therapeutic gene if adverse reactions are observed.

Is Regulation a Must for Gene Therapy Applications?

Ongoing research is harnessing the immune system as a means to eliminating tumors. These immunotherapies are administered to cancer patients either alone or (most often) in combination with established therapeutic regimens. As outlined below and reviewed elsewhere (48), several promising gene therapy strategies have been developed in preclinical models to stimulate adaptive immune responses against tumors. However, tumors share many antigens with normal healthy tissue; therefore, a potential drawback with the use of immunotherapy is the development of autoimmunity (Fig. 2). Nonregulated immune-mediated gene therapy could promote the progression and the severity of the autoimmune response and timely elimination of gene expression would be essential to limit any potential long-term damage. Thus, regulatable gene expression would be desirable in situations where long-term adverse side effects might develop, in response to immunotherapeutic strategies or cytotoxic cytokines that can enter the systemic circulation. To promote immune responses against tumor antigens, approaches such as administration of cytokines that drive infiltration of immune cells into the tumor are being pursued. These strategies elicit the presentation of tumor-derived antigens to T cells or the transient depletion of regulatory T lymphocytes to mobilize systemic immune responses that specifically target tumor cells (Fig. 2).

Other gene therapy approaches have exploited the properties of death receptor ligands, such as TNF-α, which bind to receptors on tumor cells and induce apoptosis via conserved intracellular signaling pathways. TNF-α receptor is expressed on the majority of cells in the body, so regulation of TNF-α expression is required to reduce toxicity. A vector called TNFerade was developed by Genvec to regulate expression of TNF-α using an early growth response 1 promoter activated by ionizing radiation. This leads to high expression of TNF-α in the area receiving radiation and allows down-modulating transgene expression if severe systemic side effects arise. Importantly, radioinducible promoters allow spatial and temporal control of transgene expression for cancer therapeutics. Initial phase I results confirmed that regulated TNF-α expression was well tolerated with no dose-limiting toxicities observed (49). TNFerade is currently undergoing evaluation in a multi-institution randomized phase II/III trial for patients with locally advanced pancreatic cancer. Genvec have recently announced that the median survival was 19.3 months for patients treated with TNFerade in combination with standard of care compared with 11.1 months for patients receiving standard of care alone (50).

Regulation of gene expression may not be required for every gene therapeutic target. For example, restoration of p53 using adenoviral vectors (INGN 201) has been conducted in phase III clinical trials in patients with advanced carcinoma (51) with very limited side effects due to p53 overexpression. This is because p53 is expressed intracellularly and is not toxic when overexpressed in normal healthy cells. Other oncogenes have been similarly targeted using adenoviral vectors addicted to certain transcription factors. Most notable of these is ICOVIR-5, an adenovirus that requires the absence of the tumor suppressor gene Rb to drive its life cycle. In preclinical models, ICOVIR-5 shows potent antiglioma effects alone and in combination with the mTOR kinase inhibitor RAD001 or the alkylating agent temozolomide (52). In these situations, local delivery of adenoviral vectors into the tumor is usually sufficient to limit any side effects that are determined to be caused by the transgene. Signal transduction from oncogenes can also be inhibited in tumor cells using short hairpin RNA or kinase inactive mutants. Intratumoral delivery using viral vectors is generally sufficient to limit toxicity associated with inhibiting growth factor signaling in normal cells. Some gene therapies, such as HSV-1 thymidine kinase, require the administration of a prodrug that is metabolized into a toxic product by the enzymatic activity of the gene therapy. Phase II clinical trials using adenoviral vectors constitutively expressing HSV-1 thymidine kinase were recently completed and a randomized phase III clinical trial has just commenced to treat glioblastoma multiforme

(53). Regulatable gene expression would not be necessary when using these conditional cytotoxic anticancer approaches because withdrawal of the prodrug will limit therapy-associated toxic events.

The ideal scenario is that regulatable systems will become accessible for use in most gene-based anticancer therapeutics as the inherent ability to switch a gene “off” or modulate the intensity of expression has advantages over constitutive expression. However, due to considerations of the extra time and cost to develop these regulatable switches for gene therapy applications, it is likely that in the near future regulatable cassettes will be primarily developed to express highly toxic gene products. On a longer timescale, we expect that as regulatable cassettes are further developed, more investigators will choose to place the therapeutic gene (s) of choice under the control of regulatable promoters to improve the efficacy and safety of the therapies.

Conclusions and Future Prospects

The ability to tightly regulate therapeutic gene expression for the treatment of cancer is critical to achieve greater treatment efficacy and also, very importantly, to minimize any putative adverse events. This is an area of very active research both in basic developments of novel, more effective, less immunogenic switches and in the preclinical testing to drive expression of anticancer target genes in relevant animal models of cancer. In this review, we have discussed the advantages of being able to regulate the expression of genes that have the potential of providing novel therapeutic targets and improved strategies for the treatment of cancer. The fact that several signal transduction pathways, such as growth factor receptor and apoptotic signaling pathways, as well as angiogenesis and cell motility, are mostly affected in cancer cells provides a unique opportunity for therapeutic intervention. Nevertheless, these signaling cascades and growth factor receptors can also be present in normal, noncancerous cells; therefore, treatments aimed at these targets can also adversely affect the functioning of normal tissues. This poses a critical need to develop and test regulatable expression systems, especially in vivo, in appropriate preclinical models, to limit any potential systemic toxicity before we can actually embark on translational testing in human clinical trials. Further, these gene therapeutic approaches could be used in conjunction with other chemotherapeutic agents to overcome drug resistance. Finally, the ability to modulate the expression of anticancer genes may be useful at safer doses for the regression of primary cancers and for the prevention of recurrences and metastatic disease.

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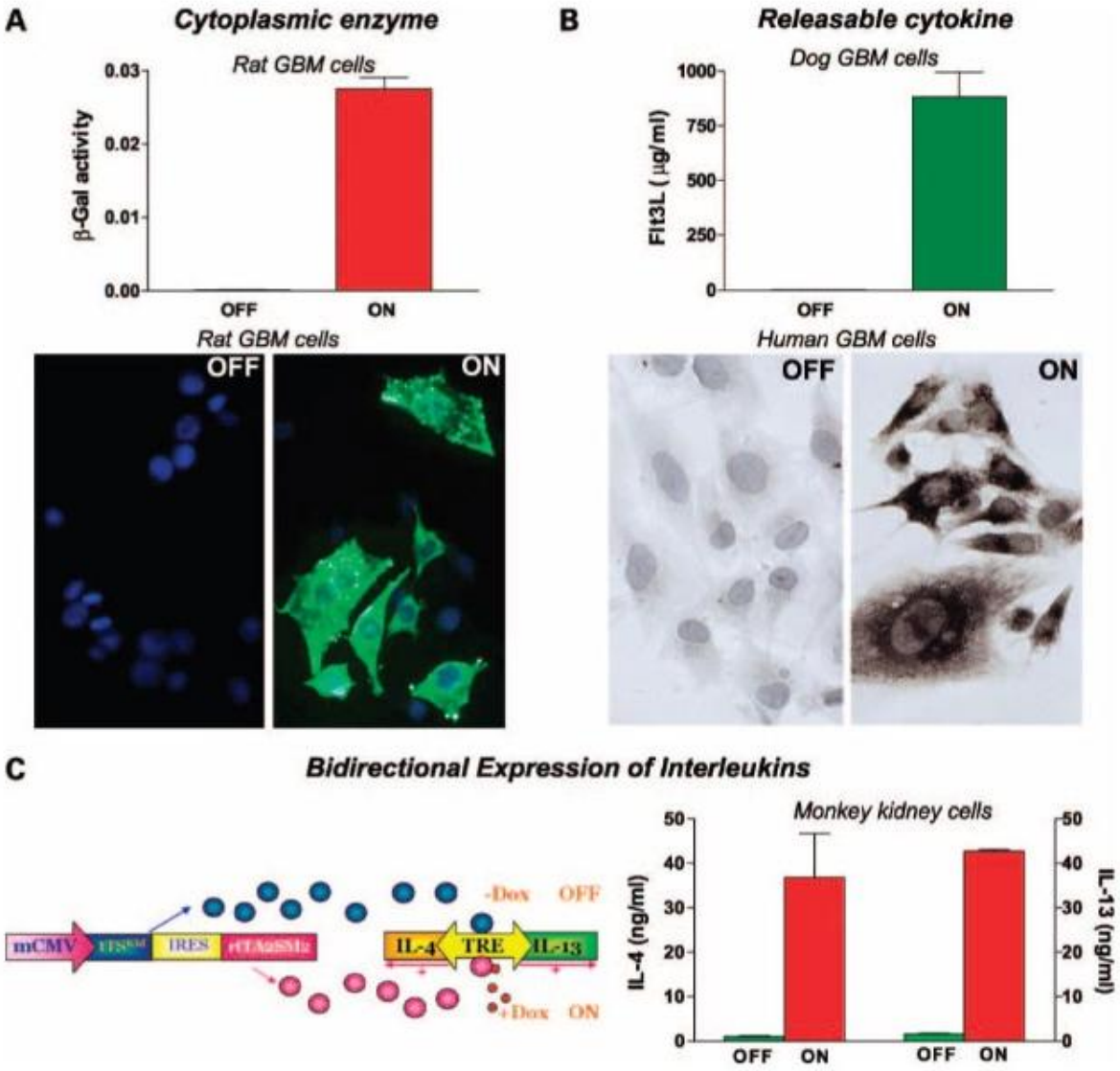

Figure 1.

Regulated transgene expression within glioblastoma cells from different species using high-capacity adenoviral vectors encoding reporter and therapeutic transgenes under the control of a third-generation Tet-On system. A, CNS-1 rat glioblastoma cells were infected with high-capacity adenoviral vectors encoding β-galactosidase under the control of a third-generation Tet-On system. Transgene expression was determined 72 h later by β-galactosidase enzymatic activity assay and immunocytochemistry. B, J3T dog glioblastoma cells and IN859 primary cultures from human glioma biopsies were infected with high-capacity adenoviral vector encoding Flt3L under the control of a Tet-On system for 72 h in the presence or absence of the inducer doxycycline (Dox; 1 μg/mL). Transgene expression was determined by ELISA and immunocytochemistry. C, COS-7 cells were infected with Ad-muIL4-TRE-muIL-13, which expresses muL-4 and muIL-13 under the control of the bidirectional TRE promoter. Transgene expression is controlled by a third-generation Tet-On system (see diagram) driven by the mCMV promoter in the presence of the inducer Dox. Transgenes were detected in the supernatant of COS-7 cells after 72 h by ELISA.

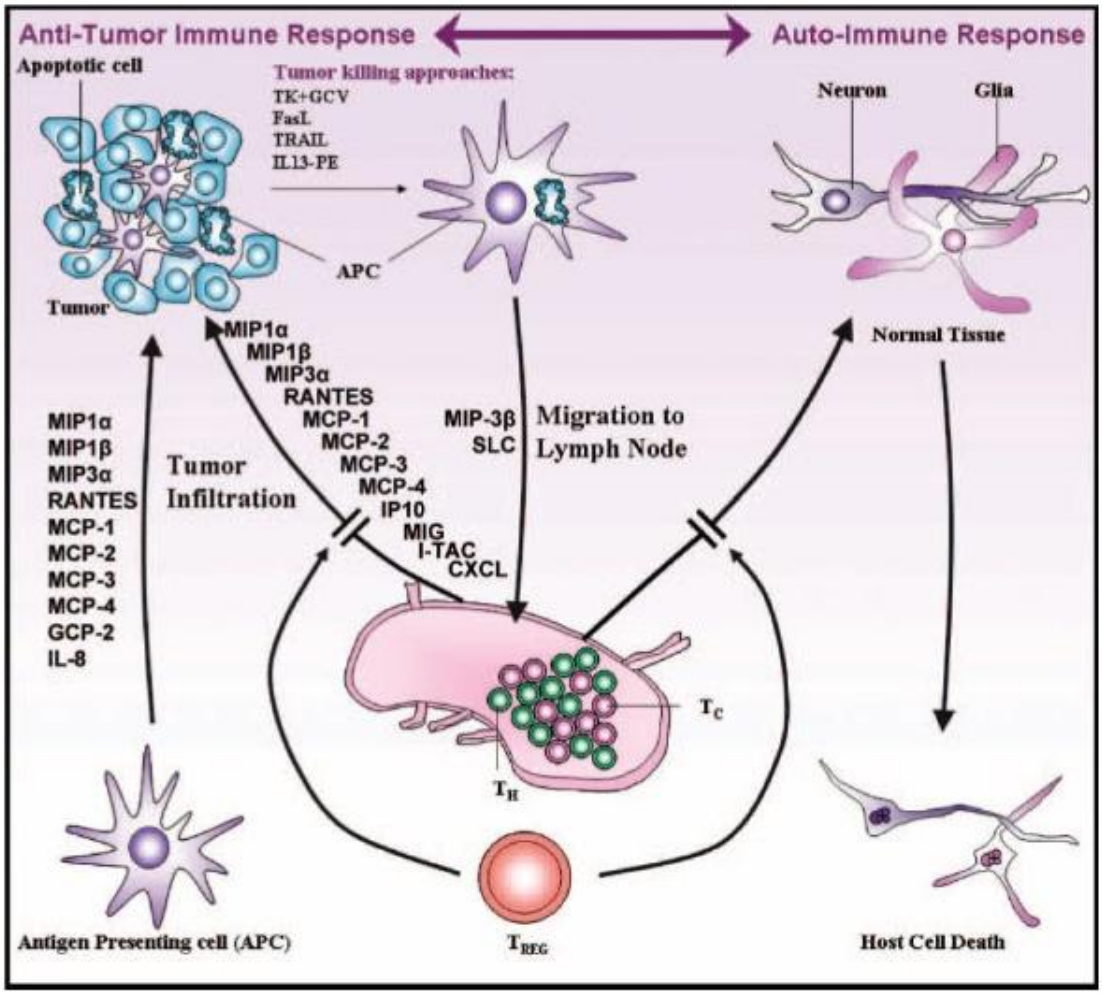

Figure 2.

Immunologic targets for cancer therapeutics. Immature dendritic cells express CCR1, CCR2, CCR5, CCR6, and CXCR1 chemokine receptors and migrate in the same direction as the concentration gradient of the corresponding cytokines. After phagocytosing tumor antigen, dendritic cells then upregulate CCR7 and migrate towards the draining lymph nodes in response to MIP3β and SLC. Dendritic cells interact with and stimulate the proliferation of tumor antigen-specific T lymphocytes. Activated helper (T_H) and cytotoxic (T_C) lymphocytes migrate into the tumor mass where they initiate tumor regression. Tumor antigens are often also expressed by normal cells; therefore, tumor antigen-specific T lymphocytes can also cause destruction of normal tissues. Tumor infiltrating regulatory T lymphocytes are essential to suppress autoimmune responses but can also down-regulate T_H- and T_C-induced tumor regression. Immune responses can be modulated using gene therapy approaches. However, it is important to tightly regulate these functions to reduce the risk of autoimmune diseases developing.

Table 1

Features, advantages, and disadvantages of commonly used regulatable cassettes

		Environment-sensitive promoters (e.g., heat shock)	Mutated progesterone receptor system	Ecdysone receptor	Tumor-specific promoter	Third-generation Tet-On
System components						
Promoter		Endogenous derived	Progesterone-responsive elements	Ecdysone-responsive element	Endogenous derived	TRE
Transcription factor		Endogenous expressed	Mutated progesterone receptor	Ecdysone receptor	Tumor specific	rtTA2^{S}-M2
Inducer		Heat, metal ions, etc.	Mifepristone (RU486)	Various ecdysteroids	N/A	Doxycycline
Cassette composition						
Regulatable cassettes encoding transgenes		Regulatable promoter	Regulatable promoter	Regulatable promoter	Tumor-specific promoter	Regulatable promoter
		Transgene	Transgene	Transgene	Transgene	Transgene
		polyA	polyA	polyA	polyA	polyA
Cassettes encoding transcription factors			Constitutive promoter	Constitutive promoter		Constitutive promoter
			Mutated progesterone R	Ecdysone R		rtTA2^{S}-M2
			polyA	polyA		IRES
						tTS^{Kid}
						polyA
Effects of inducer						
Nonspecific alteration of host genome expression		High	High	Low	N/A	None
Side effects of inducer		Intermediate-high	Intermediate	Intermediate	N/A	Low
Effects of environment						
Nonspecific activation of transgene		High	Low-intermediate	Low	Intermediate-high	Low
Antigenicity		Low	Low	Intermediate-high???	Low	Low

Table 2

Reduction of background expression from gene therapy vectors encoding late-generation Tet-On switches

	First-generation Tet-On switch rtTA (% of "ON" state)	Second-generation Tet-On switch rtTA2^{S}-M2 (% of "ON" state)	Third-generation switch rtTA2^{S}-M2-IRES-tTS^{Kid} (% of "ON" state)	Total reduction in basal expression levels	Reference
Erythropoietin	N/A	0.4	0.009	10	(26)
	5.2	0.38	N/A	15	(19)
Luciferase activity	0.2	0.02	N/A	10	(17)
	4.6	3.0	0.3	15	(18)
	10.2	1.2	N/A	10	(16)
	N/A	3.25	0.1	30	(20)
Secreted alkaline phosphatase	N/A	3.5	0.23	15	(21)
	N/A	1.3	0.003	400	(25)