Review

Cytokine and Immuno-Gene Therapy for Solid Tumors

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Despite recent progress in our understanding of cancer biology and in many areas of cancer treatment, the success rate for cancer therapy remains dismal. Immunotherapy for cancer has long been an exciting field for many cancer researchers due to the possibility to mobilize the body's own immune system to eradicate cancer not only locally but also systemically. Since its initial discovery, cytokine-based immunotherapy has been vigorously and extensively investigated for cancer treatment due to the perception of it as a relatively easily purifiable, injectable form of cancer treatment agent. However, so far most cytokine-based therapy trials have fallen short of expectations. One of main obstacles is the difficulty to achieve therapeutically relevant dosage in patients without generating excessive normal tissue toxicity. The emergence of novel gene therapy approach to deliver therapeutic cytokine to tumors locally generated great excitement since it has the potential of generating sustained high local concentration of immunostimulatory cytokine without raising the systemic levels of the cytokines, which is responsible for most of the observed toxicity. In this review, we will attempt to provide an overview of the field and discuss some of the problems associated with cytokine-based immuno-gene therapy and potential solutions. ***Cellular & Molecular Immunology*****. 2005;2(2):81-91.**

Key Words: cytokine, gene therapy, cancer, immunotherapy

Introduction

As a potential treatment for cancer, immunotherapy was initially experimented in the 19th century by New York Surgeon William Coley, who made the observation that in rare cases of spontaneous tumor regression, the patients often suffered from episodes of infections (1). Needless to mention that Coley's attempts to mobilize the body's immune system through the injection of bacterial extracts met with only limited success.

A fundamental question in tumor immunology is whether the body's immune system can recognize tumor cells, which mostly arise from the body's own normal cells. In 1909, Paul Erlich was the first to propose in theory that the body's own immune system had the potential of fighting against cancer cells (2). However, in the ensuing years, it was not possible to experimentally examine his theory due to the complete lack of understanding of the molecular and cellular details of the immune system. Therefore, little progress was made in the area of tumor immunology.

It was not until 50 years later when Thomas (3) and Burnet (4) put forward the so called "cancer immunosurveillance" theory (5). In this theory, which was based on increasing understanding of the biology of tissue transplantation (6, 7) and immunity against chemically induced tumors (8-10), it was suggested lymphocytes have the capability of surveying and destroying newly arising tumorigenic cells that are continuously being generated in the body. It is only when the system fails that tumors form.

Some key experiments in the 1970s appeared to discredit cancer immunosurveillance theory (11). In these experiments, nude mice, which had atrophic thymus and therefore were largely deficient in T-cell production, did not possess increased incidence of tumors. Specifically, the CBA/H strain of nude mice did not develop increased incidence of chemically induced or spontaneously arising tumors. Nor did they show any significant decreases in reduced latency for developing tumors. These experiments appeared to have made the tumor immunosurveillance theory obsolete (12).

However, it was later realized that the data obtained in nude mice suffer from some deficiencies. First, nude mice

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still possess some small but detectable amount of T-cells (13, 14). Second, nude mice have normal amount of the natural killer (NK) cells (15), which can have a profound effect on tumor growth. The residual T cells and intact innate immune system may function to control the incidences of spontaneous or induced tumors in the nude mice. The availability of transgenic knockout mice with deficiencies in specific effector cells or genes makes it possible to re-examine the validity of the cancer immunosurveilance theory with greater confidence and clarity of the results. These studies largely confirm the existence of the immunosurveilance theory (16). For example, in mice with genetic disruption of the RAG-2 (recombination activating gene) gene, which causes the lack of re-arrangement of the lymphocyte antigen gene and resultant complete lack of the T, B and NKT cells (17), there is clear increase in both chemically and spontaneous arising tumors compared with wild type control (16).

The re-validation of the cancer immunosurveillance theory engenders great confidence among researchers engaged in tumor immunotherapy research. It re-affirms the feasibility of mobilizing the body's own immune system to eradicate malignant tumor growth in the body.

In addition to the cancer immunosurveillance theory, some other key landmark events that play pivotal roles in the field of tumor immunology include: 1) The identification and purification of immunostimulatory cytokines (18-23) that enhances the body's immune response to cancer cells in experimental tumor models (24-28); 2) The ability to selectively expand specific immunoeffector cells such as the tumor-specific killer T or NK cells for immunotherapy (29-37); 3) The molecular characterization of various tumor-specific antigens such as *neu* (38, 39), CEA (40-43), PSA (44-46), MUC (47-50), etc.; 4) The identification of dendritic cells (DCs) as key antigen presenting cells (APCs) (51-53); 5) The realization that tumor cells have developed various approaches that evade the immune system (54, 55). On the other hand, the immune system plays significant roles in "editing out" tumor cells with immuno-stimulating antigens and therefore select for tumor cells that appear silent to the immune system (16, 56, 57).

Today most tumor immunologists agree that there is no doubt that the immune system plays critical roles in tumor development and may play pivotal roles in future cancer therapy. It is clear that tumor cells present various unique antigens that allow them to be recognized by the immune system. It is also clear that tumor cells possess various approaches that can evade immune system's attack in tumor cells. The challenge for tumor immunologists is to understand the molecular intricacies of the interactions between tumor cells and the immune system and to design novel approaches that can tip the balance in the immune system's fight against tumor cells.

The discovery of cytokines heralded a new era in immunology and cancer immunotherapy

Cytokines are a large family of intercellular signaling peptides that consist of more than 160 members. Many of the cytokines, such as the interleukins, have functions in regulating cancer immuno-response and are therefore studied intensively for cancer therapy.

The discovery of IL-2 in 1976 (18) signals the beginning of cytokine-based cancer immunotherapy. The use of cytokines in cancer therapy gained momentum in the 1980s when the interleukin-2 gene was purified in sufficient quantities. Great excitement was generated when it was realized that IL-2 was both a growth factor and an activator of T-cells and natural killer (NK) cells, which are both important in the body's fight against tumor cells. Initial efforts were mostly focused on the use of the cytokine to expand the so-called lymphokine-activated killer (LAK) cells (29, 58), which are mostly NK cells being induced into a hyperactivated state. The infusion of the LAK cells into the body was largely infective despite its efficacy in mouse tumors (59, 60). However, this was the beginning of the so-called adoptive immunotherapy, which usually involves the infusion of autologous or allogenic immunoeffectors that have been activated *in vitro* to eradicate tumors. The advantage of such approaches is that it is possible to obtain large quantities of immunoeffectors *ex vivo*. A later version of this approach is the transfusion of *ex vivo* expanded, tumor infiltrating lymphocytes (TILs) (34, 36). The use of TILs led to some remarkable early responses in melanoma and renal cell carcinoma patients. However, randomized clinical trials failed to show benefit over IL-2 alone (61). Both the LAKs and TILs were pioneered by Steven Rosenberg and his colleagues at the National Cancer Institute in the United States.

In addition to being a boon for adoptive immunotherapy, the advent of IL-2 also ignited widespread interests for it to be a pharmacological agent to be directly administered into the patients' body. Such an approach is called active immunotherapy. Most initial efforts focus on the systemic infusion of IL-2 *in vivo* to stimulate the general immunity against cancer cells. This was based on experiments in murine tumor models that direct infusion of IL-2 had significant anti-tumor effects (62-65).

Clinically, IL-2 is now approved for treating renal cell carcinoma in the USA, Canada, and the European Union. The approvals are based on modest but statistically response rate in clinical trials (66-70).

Systemic toxicity has been a serious issue that limits the widespread application of many cytokines in humans. Despite the initial enthusiasm that were associated with the discovery of IL-2 and numerous preclinical data that indicated the potential anti-tumor efficacy, clear clinical benefits were only seen for a limited set of patients with renal cell carcinoma and malignant melanoma. In addition to the lack of efficacy, a major factor that limited the application of IL-2 was its toxicity when applied systemically. These toxicities included hypotension, vascular leak, and respiratory insufficiency (71, 72). Less severe but nonetheless treatment-limiting side effects included nausea, emesis, diarrhea, myalgias, arthralgias, skin erythema, and pruritus. In addition, less common toxicities included myocardial infarction,

myocarditis, infection, renal failure, bowel infarction, and death.

Tumor necrosis factor α (TNF-α) provides another example for systemic application of anti-cancer cytokine therapy. It was identified in the early 1970s by virtue of its potent tumoricidal activity against murine tumors (73). It was cloned in 1980s (74, 75) and has since been tested in a number of Phase I & II clinical trials for its efficacy against a variety of human tumors (76-78). There are at least three mechanisms by which TNF-α can eradicate tumors (79). First, it has direct cytolytic activity against the tumor cells. Second, it can kill tumors by selectively destroying tumor neovasculature and causing hemorrhagic necrosis (80, 81). Third, it can stimulate T-cell-mediated immunity to tumor cells. Therefore, it possesses potent anti-tumor activities even in tumors that are not susceptible to direct cytotoxic action of TNF-α.

Most clinical trials failed because of serious systemic toxicity associated with TNF-α. Because of the side effects, such as hypotension, vascular leak, fever, and neurotoxicity, an effective anti-tumor dose can not be reached in most cases (82). In fact, humans can only tolerate only 2% dose/kilogram necessary to cause tumor regression in mice (82, 83). However, in instances where TNF-α can be localized to certain organs or compartments of the body and an effective dose can be reached, it is clearly effective in killing human tumors (84, 85). A good example is the use of TNF-α in treating osteosarcomas and metastatic melanomas by limb perfusion (86).

Both of the above examples indicate that for cytokine-based immunotherapy to succeed, the toxicity/side effects have to be resolved.

There are inherent problems with systemic infusion of cytokines for anti-tumor therapy in addition to toxic side effects.

In addition to the toxicity issue, there are additional problems with systemic infusion approach for cytokine cancer therapy. These problems are multifold: 1) It often leads to artificially high concentration of the cytokines that are orders of magnitude higher systemically, which in most cases leads to unwanted side effects such as toxicities as described in the previous section, or even lethality; 2) The high systemic concentration, while orders of magnitude higher than what the body is used to, is usually far below what is needed at the site where the activation of the immune system is needed, such as within a tumor mass; 3) The bolus infusion of the cytokines usually causes only a transient increase in the level of cytokine, which is usually rapidly cleared out of the body by liver or kidney. Therefore, there is insufficient time for the mobilization of the immune system against the tumor cells.

To activate the immune system against the tumor cells with the cytokines, it is necessary to re-visit how our body's own immune system is activated against what it deemed as foreign antigens such as those from bacteria or viruses. Usually such antigens enter into the body at various defined locations. The immune system, through its various components, such as macrophages, dendritic cells, NK cells, etc., recognizes such antigens and creates localized "danger" signals, among which high concentrations of cytokines provide the paracrine signals that attract various immuno-effector cells to the site. This local elevation of cytokines and attraction of immunoeffector cells create an amplification loop that facilitates the elimination of the foreign invasion and in many cases the generation of memory T cells that allow the immune system to initiate swift and effective attacks when foreign organisms with the same antigens invade next time.

The important lesson drawn from how the body's own immune system mounts an attack on the invasion of a foreign organism is that local, sustained high levels of cytokines are necessary for the activation of immune system. With systemic infusion, the required local high concentrations of cytokines are never achieved, even at the systemic levels that are often toxic or lethal to the host.

Gene therapy approaches significantly improved the prospects for the use of cytokine cancer immunotherapy

The emergence of gene therapy in the early 1990s opened up various novel approaches/opportunities for the delivery of cytokines to the body for the purpose of anti-tumor therapy. A key characteristic of various gene therapy approaches is the use of gene therapy "vectors" to deliver therapeutic genes locally (87-89). These vectors consist of viral and non-viral vehicles that are engineered, through recombinant DNA technology, to carry genes that are usually delivered to the disease sites, such as the site(s) of tumor growth, through local injections (90). In the case of tumor gene therapy, the rationale is that such local delivery will generate local cytotoxicity (with cytotoxic genes) or immunity against tumor cells.

When used to deliver immunostimulatory cytokines, the distinct advantages of such local gene delivery approaches are: 1) The ability to generate locally high concentrations of cytokines, similar to the body's own response against foreign antigens; 2) The ability to provide sustained high levels of cytokines with robust paracrine effects that activate the immune system.

Many different gene therapy vectors have been used for the delivery of tumor cytokine therapy. A large variety of viral and non-viral vectors have been adopted for delivery of gene therapy experimentally. Among the ones that have been used to deliver cytokines are murine retrovirus vectors (91), human or feline lentiviral vectors (92, 93), adenovirus vectors (94), adeno-associated virus vectors (95), herpes simplex virus vectors (96, 97), vaccinia virus vectors (98), Fowlpox virus (99, 100), Semliki Forest virus (101), Naked plasmid DNA virus vectors (102, 103), and plasmid DNA vectors in combination with "gene guns" (104, 105).

Currently there is no consensus on the optimal vector to use for cancer gene therapy as each vector has its advantages or disadvantages. However, among these vectors, the murine

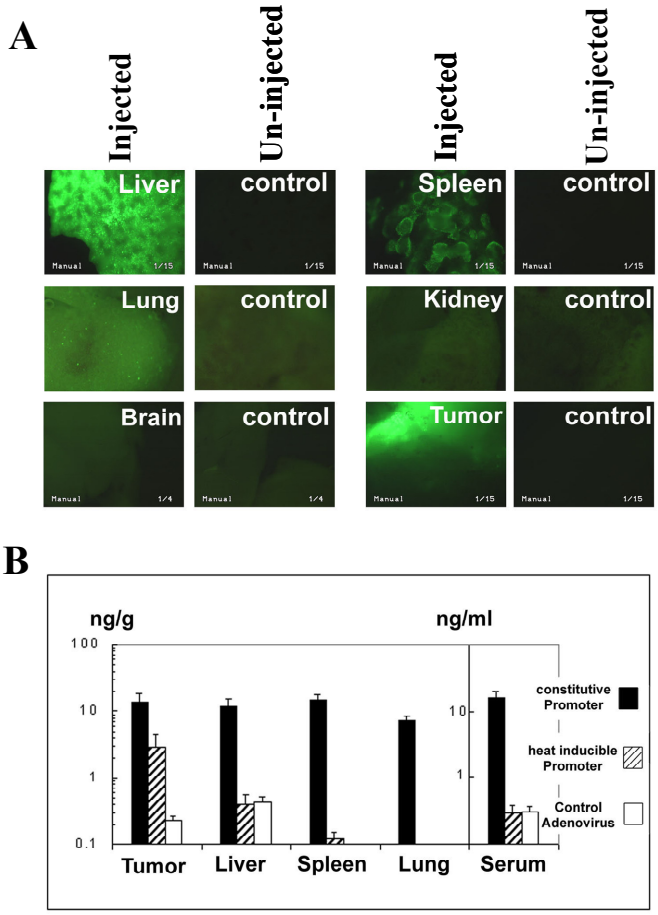

Figure 1. (A) Systemic dissemination of adenovirus after intratumoral injection. About 3×10^8 pfu (plaque forming units) AdGFP/tumor was injected into the center of tumors through syringes with 30-gauge needles. Twenty-four hours later, the tumors and organs of injected mice were harvested, cut, and mounted in aqueous solution for fluorescence microscopy. Expression of green fluorescence protein (GFP) in tumors and organs of tumor bearing C57BL/6 mice after intratumoral injection of adenovirus constitutively encoding GFP (AdGFP) as compared to non-injected control animals (magnification 20 ×). (B) Intratumoral and systemic expression of murine interleukin 12 (mIL-12) after intratumoral vector injection. About 1×10^8 pfu of AdmIL-12 was injected into the center of tumors. Intratumoral and systemic expression of murine interleukin 12 (mIL-12) after intratumoral injection of either control adenovirus (AdGFP used as control in this experiment), adenovirus constitutively expressing mIL-12 (AdCMVIL-12) or adenovirus expressing mIL-12 controlled by a heat inducible promoter (AdhspIL-12) combined with heat treatment in subcutaneous B16.F10 melanomas. Animals were sacrificed 24 hours after heating. The results are plotted as mean ± range of two to four animals per data point (reproduced from reference 124 with permission from AACR).

retrovirus vector, the adenovirus vector, herpes vectors and plasmid DNA virus vectors are the most frequently studied due to their ease of manipulation and preparation. They are also the ones that were the first to be evaluated in the human clinic therapy.

A large number of cytokine genes have been evaluated in preclinical and clinical trials for their anti-tumor efficacy. A list of some of the more common ones includes IL-2, IFN-α, β, γ, IL-12, IL-15, GM-CSF and TNF-α.

In terms of delivery approach, there are mainly two different approaches: 1) The direct injection of gene therapy vectors into the tumor mass or the periphery of the tumor mass (106); 2) The implantation of *ex vivo* cytokine-modified autologous or allogenic fibroblasts, stem cells, or other normal cell types into or in the vicinity of the tumor mass (106).

GM-CSF gene-modified vaccine has shown early promise in clinical trials

In addition to the two approaches for delivery of cytokine gene therapy, a third mode of gene/cytokine therapy is the use of *ex vivo* modified autologous or allogenic and lethally irradiated tumor cells as vaccines. Various immunostimulatory cytokine genes are transduced into tumor cells, which are subsequently processed (mostly through irradiation) as vaccines to be injected into cancer patients. This mode of therapy is in fact the powerful combination of the cytokine gene therapy approach and the conventional tumor cell-based vaccine approach. These two approaches in history have been developed along their own tracks without crossing each other until the mid 1990s.

For the vaccine approach, there have been a few studies that directly compared the effectiveness of a large cohort of cytokines in eliciting a prophylactic anti-tumor responses in the same tumor models (107, 108). One cytokine stands out as the most effective: GM-CSF. Because of these pre-clinical results, quite a few human clinical trials have been or are being conducted in various solid and hematogenic tumor types that included prostate cancer, lung cancer, pancreatic cancer, leukemia and myeloma. The chief sponsor of GM-CSF-transduced vaccine trials is the US based company Cell Genesys, which trade-marked their GM-CSF-based vaccines as the GVAX™ series cancer vaccines. So far GVAX vaccines have enjoyed successes in early clinical trials (109-112). Among these, prostate cancer vaccines have progressed to phase III clinical trials while all the others (lung, pancreatic, leukemia, and myeloma) have obtained positive phase II results. These results are encouraging since most of the patients were late-stage, high risk cases that had failed or likely to fail conventional therapies. It is possible that the GVAX vaccine will be available to some cancer patients in the next few years. If true, it would be the first commercially available gene modified vaccine for cancer.

A potentially serious problem of intratumoral injection of viral vectors is virus "leakage" out of the tumor mass

In theory, cytokine-encoding gene therapy vectors injected

Table 1. Heat-induced cytokine gene expression in recombinant adenovirus infected cells or tumors as measured by ELISA[a, e]

AdV construct	*In vitro* measurement			*In vivo* measurement[c]		
	No HT	HT[b]	Fold increase	No HT	HT[d]	Fold increase
AdhspTNF-α	< 2.5 pg/ml	1.85 μg/ml	> 6.8×10^5	9.5 pg/g	7.94 ng/g	835
AdhspmIL-12	< 5 pg/ml	68 ng/ml	> 13,600	0.18 ng/g	6 ng/g	33

[a]See details in reference 124; [b]42ºC for 30 minutes; [c]Production of IL-12 converted into amount/per gram of tumor tissue; [d]42.5ºC for 40 minutes; [e]Reproduced from reference 124 with permission from the AACR.

intratumorally would mostly be deposited into the tumor mass. This is one of the most important advantages that gene therapy mediated cytokine delivery have over traditional infusion-based cytokine delivery. However, evidence is accumulating those even local injections of adenoviral vectors are not localized to the site of injections. Indeed, intratumoral injections have been shown to be very leaky. Injections of adenovirus vectors intratumorally led to virus leakage into the liver, kidney, and lungs (113, 114) (Figure 1).

The leakage of virus vector seriously undermines the advantage of the gene therapy based cytokine delivery as most of the leaked vectors are likely to be absorbed by liver and other normal organs. The immune response against the vectors and the systemically elevated levels of cytokines can seriously elevate toxic side effects or even mobidity in hosts (115). Therefore, for gene therapy mediated cytokine therapies to be successful, effective approaches have to be taken to ensure that cytokine expression is restricted to the tumor mass.

Tumor-specific gene expression is possible through physical and biological approaches

One of the very effective approaches to restrict gene therapy to the tumors is the use of tumor-specific 'switches' or promoters that can limit gene expression to tumors. Examples of these include: 1) Biologically based cancer-specific promoter activation mechanisms. Examples include: CEA promoter, which is active in colon and other cancer types (116, 117); Erb-B2 promoter, which is active in breast and other cancer types (118); α-fetoprotein promoters, which are active in liver cancers (119, 120); prostate specific antigen (PSA) gene promoter, which is active in prostate tissues (121); telomerase promoter, which is active in over 90% of all tumor types and in active in over 99% of normal tissues (122). 2) Physically based inducers. Examples include ionizing radiation (123); heat (124, 125).

Biologically based gene therapy strategy usually depends on the identification of disease-specific gene activation. This approach has been successful in some experimental applications. However, a major obstacle is the requirement to identify tumor cell specific promoters that are activated to very high levels for individual tumors. A further impediment to these systems is the lack of a means to create temporal control of gene expression since they can not be switched on or off.

External physical agents possess distinct advantages in controlling gene expression both spatially and temporally. Ionizing radiation has been used with success to activate TNF-α gene expression with some spatial and temporal control (123). The main advantage of ionizing radiation is the precision with which the diseased area can be targeted. However, an apparent disadvantage of ionizing radiation is the lack of naturally existing promoters that can respond to ionizing radiation consistently and at a sufficiently high level. Published data suggest that a 20 Gy single dose γ-irradiation induced a 9-fold increase in reporter gene expression under the control of a commonly used radiation-inducible EGR (early growth response) gene promoter (126).

Heat-based gene regulation is a powerful approach to deliver cytokine-based immuno-gene therapy

The heat-based gene regulation approach is based on the ubiquitous heat shock response/stress response. In mammalian cells this response is usually activated when the cells are exposed to environmental temperatures that are 3-7ºC higher than physiological norms (127). During the heat shock response, the cells shut down the majority of protein synthesis and initiate the synthesis of a class of proteins called the heat shock proteins (hsps). Mammalian cells may devote as much as 90% of their protein synthesis machinery on the production of the heat shock proteins (127). Many of the proteins carry out protective functions such as keeping other key proteins from being denatured by the elevated temperature. Some of the proteins are normally expressed at very low levels and their expression increases to very high levels during the heat shock response. The induction can range from hundreds to thousands of fold over background levels. Much of the induction is mediated at the transcriptional level through the promoters of these genes.

As an example, a heat shock protein 70 promoter (hsp70B) which we have used was used to control the expression of a reporter gene (EGFP). The expression is induced over 1,000 folds by hyperthermia (Figure 2). When cytokine genes encoded in recombinant adenovirus vectors were used, inductions of the TNF-α gene reached 6.8×10^5 folds *in vitro* and 835 folds *in vivo* for TNF-α encoding virus

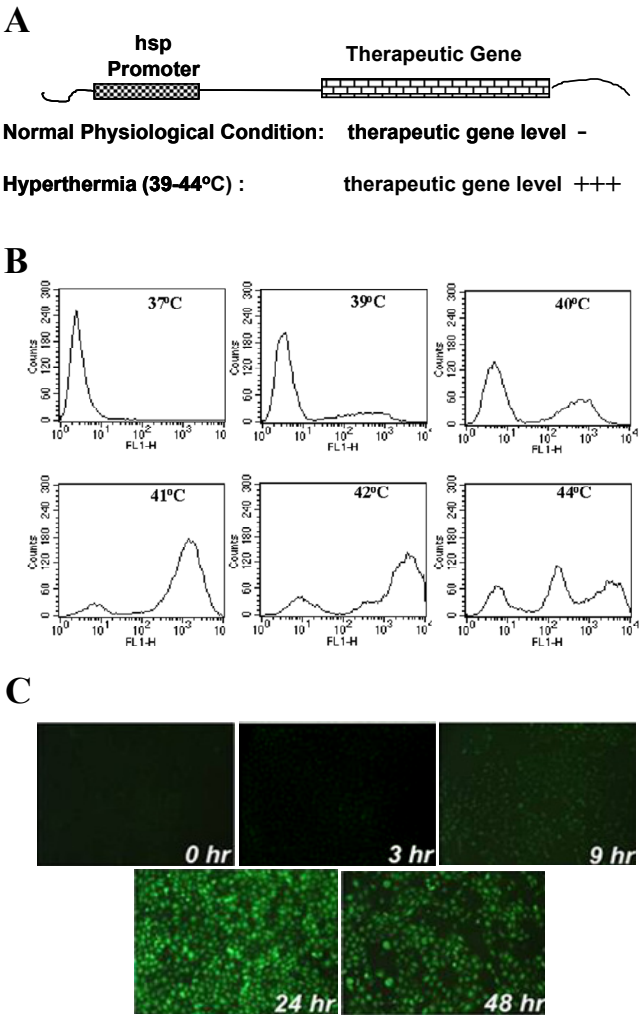

Figure 2. Proof of principal for heat induced gene therapy. (A) A graphic representation of hyperthermia-regulated gene therapy. (B) Flow cytometric analysis of 4T1 cells infected with an adenovirus carrying a heat-inducible green fluorescence protein (GFP) gene. The x-axis represents fluorescent intensity and the y-axis represents cell numbers. It is clear that GFP is induced at 39°C. (C) The time course of heat-induced GFP expression in 4T1 cells that have infected with an adenovirus encoding a heat-inducible GFP gene.

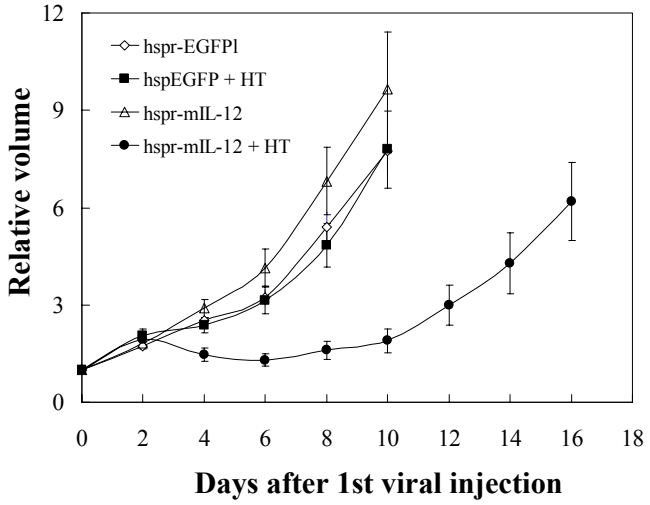

Figure 3. Adenovirus mediated, heat-regulated gene therapy in a mouse melanoma model. Experimental tumors were established in syngeneic C57BL6 black mice by implanting 10^6 B16F10 melanoma cells. Viral injections were carried out 1 week later when tumors grew to sizes of 5-7 mm in diameter. In the shown experiment, four groups of animals were included. These are mice injected with adenoviruses encoding a) a heat inducible EGFP gene alone (◇); b) a heat inducible EGFP gene with heat treatment (■); c) the murine IL-12 gene (△) alone and d) the murine IL-12 gene with heat treatment (●). There were 10 animals in each group. The error bars for all the data points represent the standard error of the mean (reproduced from reference 27 with permission from AACR).

(Table 1). Most importantly, the background activity of this promoter is extremely low. The cytokine concentration was below the level of detection when sensitive ELISA kits were used. Another study demonstrated that the activity of the promoter can be regulated multiple times after the initial treatment (128). This is critical since it indicates therapeutic gene level can be modulated by multiple rounds of hyperthermia.

The low background coupled with the high inducibility of this promoter makes it the ideal promoter to use for cytokine-based immuno-gene therapy purposes. In our past studies, we have shown that heat-inducible cancer gene therapy vector showed significant advantages over the non-regulated gene therapy vectors in this respect (113, 125). When a similar amount of viral vectors were injected intratumorally into subcutaneouly grown tumors, different levels of non-targeted gene expression were observed. For the vector where the reporter gene was under the control of a constitutively active cytomegalovirus promoter, gene expression was observed in various organs and tissues such as the liver, the lung, and the spleen (Figure 2). For the vector encoding a heat-inducible reporter (e.g., IL-12), the gene expression was only observed in the heated tumor area. In addition, when vectors encoding heat-inducible IL-12 gene were injected intratumorally (at doses of 10^8-10^9 pfu/mouse), little toxicity was observed. This is quite different from adenovirus vectors encoding a constitutively active CMV promoter controlled IL-12 gene, which causes quite serious normal tissue toxicities such as splenomegaly, lethargy, or even death. These serious side effects occurred despite the same amount of recombinant virus particles being injected intratumorally. Therefore, a heat-inducible approach for regulating gene expression has significant advantages in restricting therapeutic gene expression to targeted tissues thereby reducing unwanted normal tissue toxicity. Most importantly, despite the restricted and targeted gene expression from the heat-inducible gene expression, the efficacy of the virus was still quite impressive. It showed quite potent anti-tumor effect in reducing tumor growth when

administered with heat in a very aggressive mouse model of melanoma B16F10 (Figure 3).

A combination of cytokine gene therapy with radiation or chemotherapy is necessary to overcome the defense of solid tumors against the immune system

Despite the enormous promise of various immunotherapy strategies, little clinical success was achieved so far. Many researchers have long realized that immunotherapy alone is not sufficient in most cases to eradicate solid tumors as most well-established solid tumors have developed various mechanisms of down-regulating host immune response against tumor cells. These include: 1) Solid tumors, may be treated as immuno-privilaged sites by the host immune system (129), thereby allowing them to escape immune surveillance. Through the procedure of "immuno-editing", many tumor cells are essentially silent for the immune system, many tumors have down-regulation of immunostimulatory MHC proteins or mutating the TAP-1 genes so that they assume 'stealth' phenotypes in the presence of the immune surveillance (130, 131); 2) Tumor cells have developed a variety of defensive 'weapons' or barriers that help them to fend off host immune system. Examples include: expression of the Fas ligand on the surface of some tumor cells that can kill T-cells (132, 133); expression of the immunosuppressive IL-10 or TGF-β cytokines that down-regulate the activity of immune effector cells; 3) Many solid tumors have developed modifications of their vasculature that reduce leukocytes extravasation (134, 135), thereby avoiding activation or attack by the immune system all together.

With the multitude of problems, it would be very difficult for any form of immunotherapy, no matter how potent it may be, to get rid of the solid tumor burden completely by itself, especially when the solid tumor is at an advanced stage.

Fortunately, there are help for immunotherapy. One such help is radiation therapy, which has shown strong potency in eradicating local tumor growth. One approach is to combined radiation therapy and cytokine-based immunotherapy. There are several rationales for doing this. First, radiation can 'knock out' the defensive weapons of tumors that are directed against the immune system by killing off the majority of tumor cells in a 'carpet bombing' fashion. Second, radiation can induce increased expression of immunostimulatory genes such as MHC-I, MHC-II, ICAM-I, etc., which can facilitate the extravasation and activation of immuno-effector cells (136, 137). Third, radiation-induced death through apoptosis/necrosis may provide a rich source of antigens that may stimulate the anti-tumor immune response (138, 139). Fourth, the stimulation of the immune system by the combined treatment is likely to help in the prevention of recurrent tumor growth from the local site and in the eradication of metastatic diseases.

Indeed, our own data have lent strong support for such a combined therapeutic strategy. When adenovirus-mediated interleukin 12 (IL-12) gene therapy was combined with radiation therapy, a significantly stronger anti-tumor effect was seen compared with either forms of therapy alone (140) (Figure 4).

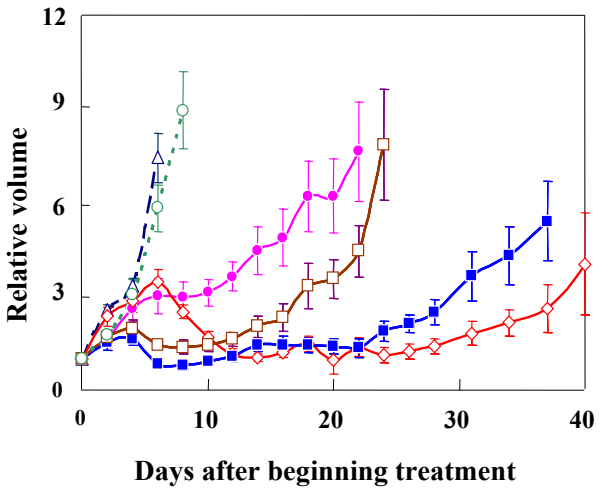

Figure 4. Synergistic anti-tumor efficacy when an adenovirus encoding constitutively expressed IL-12 and B7.1 (AdIL-12/B7.1) was administered after radiation therapy (RAD). (△) untreated control; (○) injection of control AdGFP on day 7 after transplantation (a. Tx.), no radiotherapy (RAD); (□) injection of AdIL-12/B7.1 on day 7 a. Tx., no RAD; (●) initiation of RAD on day 7 a. Tx., injection of AdGFP after the last (3rd) RAD fraction; (■) initiation of RAD on day 7 a. Tx., injection of AdIL12/B7.1 after the 1st RAD fraction; (◇) initiation of RAD on day 7 a. Tx., injection of AdIL12/B7.1 after the last (3rd) RAD fraction. The virus dose injected was 3×10^8 pfu (in 50 μl PBS) except for the injection after the 3rd radiation fraction in 4T1, when 3×10^7 pfu was used. Error bar represents the mean relative tumor volumes (± standard error (SE)) for different combinations of radiotherapy and adenovirus gene therapy in B16.F10 tumors in C57BL/6 mice. See reference 140 for details.

The rationale for the combination of chemotherapy and immunotherapy is less obvious. This is because chemotherapy target proliferating cells systemically and preferentially, including various immuno-effector cells. Therefore, in general, chemotherapy is thought to suppress the immune system. However, there have been published literature reporting that synergistic efficacy exists between chemotherapy and cytokine-based immunotherapy (141-143), especially at the lower doses. The exact mechanisms are not clear.

Summary

Cytokine-based immuno-gene therapy is entering into an very exciting phase with many studies indicating its potential in numerous preclinical as well as clinical studies. It is quite possible that it will become a routinely available form of cancer therapies in the not too distant future. However, continued studies are clearly needed both to optimize the delivery approach and to clarify the right circumstances for

its application. It is likely that most cytokine-based gene therapy will be delivered as an adjuvant therapy (in combination with surgery, chemotherapy, or radiation therapy) with the main goals of eradication and prevention of cancer metastases and recurrences.

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