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CANCER GENE THERAPY USING CYTOKINE AND CHEMOKINE GENES

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Abstract

Surgical or radiotherapy-mediated eradication of tumors gives the best opportunity for cure, if the tumor is restricted to the primary sites. Chemotherapy is employed for disseminated disease but frequently fails to give clinical benefit. Thus, with our ever expanding understanding of the regulatory mechanisms of cytokine- and/or chemokine-mediated immune reactions to tumor, these molecules have become good candidates for cancer therapy. Unfortunately, most cytokines and chemokines have very short half-lives and their systemic delivery of pharmacological doses frequently results in severe adverse effects. These circumstances prompted many investigators to evaluate the genetic delivery of cytokines and chemokines for the treatment of cancer. Here, current status of clinical and pre-clinical studies of cytokine and chemokines will be discussed, particularly focusing on those with clinical relevance.

Introduction

Since the first description of hemoglobinopathy as a molecular disease, accumulating evidence indicates that the inheritance of a single functionally defective gene can cause certain diseases. This observation has brought up the concept of gene therapy. Theoretically, diseases caused by a genetic defect can be cured by the insertion and expression of a normal copy of the mutant or deleted gene in host cells. Hence, tremendous

efforts have been made to develop gene therapy targeting inherited diseases such as cystic fibrosis and hemophilia. However, the efficiency of presently available gene transfer methods still remains too low to be applied to the treatment of monogenic hereditary diseases. Consequently, the evolution of clinical gene therapy has taken a somewhat unexpected course, and has been bound for cancer. To date, nearly 900 gene therapy trials have been approved worldwide while 601 trials have been approved in the United States alone. Among 601 gene therapy trials, 392 are focusing on cancer, and more than 250 trials are immune gene therapy against cancer (www4.od.nih.gov/oba/rac/clinical.htm).

Immune gene therapy against cancer attempts to increase the immunogenicity of cancer cells and /or enhance killing of cancer cells by gene replacement (Roth and Cristiano, 1997). These types of approaches do not require sustained and tightly regulated gene expression, which is indispensable for the gene therapy for monogenic hereditary diseases. At present, several recombinant cytokine proteins such as interferon (IFN)-α, IFN-β, and interleukin (IL)-2, are clinically used for the treatment of various cancers including melanoma, lymphoma, and renal cell carcinoma. Moreover, other cytokines and chemokines can also exert directly and/or indirectly anti-tumor activities *in vivo* and/or *in vitro*. Furthermore, cytokines and chemokines can exert their activities at extremely low concentrations (~ pM) in a paracrine and/or autocrine manner (Thomson and Lotze, 2003). Thus, even the present gene transfer method could direct the expression of a sufficient amount of cytokine and/or chemokine proteins, to achieve anti-tumor activities.

Until now, various types of gene transfer methods have been proposed. However, each method has its own drawbacks and still needs more improvement to direct the gene expression in a tissue or cell-specific manner with a better efficiency. The advantages and disadvantages of commonly-used virus-based gene transfer methods are summarized in Table I. The readers can find more extensive description on gene transfer methods in the selected book (Phillips, 2002).

It has been difficult to predict the safety of gene therapy based solely on animal models (Crystal, 1995). Thus, clinical trials have been performed mainly to establish safety, with efficacy being a secondary endpoint. However, two types of fatal adverse reactions were documented until now, although both were not for cancer treatment. The first event was the death of an adult patient with ornithine transcarbamylase deficiency. The patient succumbed to a sudden death due to acute respiratory distress syndrome, resulting from an aberrant inflammatory reaction to a high dose of adenovirus (Marshall, 1999). Some critical lessons have been emphasized: rules and guidelines, and quality assurance testing of materials, including gene delivery vectors, should be reinforced to prevent the occurrence of similar fatal adverse effects.

TABLE I
Characteristics of commonly used viral vectors.

	Retrovirus vector	Lentivirus vector	Adenovirus vector	Adeno-associated virus (AAV) vector
Pathogenicity of wild virus	+	+/-	+	-
Virus genome	RNA	RNA	Double-stranded DNA	Single-stranded DNA
Packaging capacity	Medium, 8 kb	Medium, 8 kb	Medium, <7.5kb	Low, <4kb
Virus particle	Unstable	Stable	Stable	Very stable
Gene transfection to replicating cells	Possible and suitable	Possible	Possible but unsuitable	Possible but unsuitable
Gene transfection to un-replicating cells	Impossible	Possible and suitable	Possible and suitable	Possible and suitable
Transfection efficiency				
Adherent cells	Efficient	Efficient	Very efficient	Efficient
Non-adherent cells	Efficient	Efficient	Rather inefficient	Inefficient
In vivo gene transfer	Unsuitable	Suitable	Suitable	Suitable
Integration to chromosome	Random integration	Random integration	No integration	Random integration
Vector preparation	Established	In development	Almost established	In development
Gene expression	Stable	Stable	Transient	Relatively stable
Clinical trials	+	-	+	+

The second event was the development of acute leukemia in X-linked severe combined immunodeficiency patients treated with the gene therapy using a retrovirus to deliver common γ chain gene (Hacein-Bey-Abina, Von Kalle *et al.*, 2003). This treatment has given long-term clinical benefit to most of the treated patients, but acute leukemia developed in some of them, due to retrovirus integration in proximity to LMO2 proto-oncogene promoter, and induced aberrant transcription and expression of LMO2, thereby resulting in the development of leukemia. This event illustrates both potential benefit and risk of gene therapy.

In this review, I will discuss cytokine- and chemokine-based gene therapy, which may be useful for cancer gene therapy and finally future perspective to overcome hurdles for its clinical application.

Interleukin (IL)-2

IL-2 has a wide variety of actions on T lymphocytes and natural killer cells, including augmentation of their proliferation and their cytolytic activities, and induction of production of other cytokines such as IFN-γ, tumor necrosis factor (TNF)-α, and TNF-β (Baigent, 2002). There are a number of completed and ongoing clinical trials using recombinant IL-2 protein for cancer. A high dose of IL-2 caused clinical responses in patients with renal cell carcinoma and melanoma, but could frequently cause severe toxicity, capillary leak syndrome (CLS), characterized by low blood pressure and high cardiac output. Thus, considerable care and meticulous monitoring of patients are required for this life-threatening complication (Schwartz, 2002). In contrast, low doses of IL-2 have shown clinical activity in renal cell cancer, with low objective response rates of 18 to 23 %, but without any signs of CLS (Tourani, Lucas *et al.*, 1996).

Peritumoral administration of mouse fibroblasts transfected with the human IL-2 gene, inhibited the growth of human tumor xenografts in nude mice (Bubenik, Voitenok, *et al.*, 1988). This observation has promoted the development of clinical trials using irradiated tumor cells and fibroblasts, which were transfected *ex vivo* with the human IL-2 gene, in order to treat malignant melanoma or solid tumors with distant metastasis. These clinical trials did not cause any severe adverse effects (Osanto, Brouwenstyn *et al.*, 1993; Sobol, Shawler *et al.*, 1999) and even the treatment with the IL-2 gene-transduced xenogeneic fibroblasts was well tolerated (Rochlitz, Jantscheff *et al.*, 1999). Several lines of evidence demonstrated that the treatment with the IL-2 gene-transduced autologous tumor cells increased the frequency of specific cytotoxic T lymphocytes (CTL) against the tumor (Sobol, Shawler *et al.*, 1999; Palmer, Moore *et al.*, 1999), induced the production of T and natural killer cell-derived cytokines such as IL-2, IL-4, and IFN-γ (Tartour, Mehtall *et al.*, 2000), and generated specific antibodies against tumor-specific antigens (Maio, Fonsatti *et al.*, 2002). However, only a minor portion of patients exhibited a partial response to the treatment (Rochlitz, Jantscheff *et al.*, 1999; Palmer, Moore *et al.*, 1999).

In addition to *ex vivo* IL-2 gene transduction, several groups performed clinical trials to transfer IL-2 gene directly into tumors using either adenovirus vector (Stewart, Lassam *et al.*, 1999), plasmid DNA/cationic lipid complex (Galanis, Hersh *et al.*, 1999), or replication-restricted vaccinia virus vector (Mukerjee, Haenel *et al.*, 2000). In a clinical study using vaccinia virus vector, the IL-2 gene was successfully expressed inside the tumors without any severe adverse reactions despite a progressive increase in the antibody titers against viruses. In some of patients, the infiltration of T lymphocytes, particularly CD8-positive cells, was observed, but clinical response was marginal.

Interleukin IL-4

Previously known to be B-cell activating and differentiating factor-1, IL-4 acts on B cells to induce activation and differentiation, leading in particular to the production of IgG_1 and IgE. It also acts on T cells as a growth and activation factor and promotes Th2 differentiation. It also induces the expression of major histocompatibility (MHC) class II antigen on macrophages, thereby enhancing cell-mediated immunity (Okada and Kuwashima, 2002). Moreover, IL-4 can inhibit corneal neovascularization induced by basic fibroblast growth factor and inhibit the migration of cultured bovine and human microvascular cells (Volpert, Fong *et al.*, 1998). Thus, IL-4 may reduce tumorigenicity by exerting anti-angiogenic activities. However, the overall response rate was only 3% in clinical trial with recombinant IL-4 protein for disseminated malignant melanoma (Whitehead, Unger *et al.*, 1998).

On the contrary, the transfection of the IL-4 gene was shown to inhibit tumor formation in several murine models by inducing either eosinophil and/or CD4-positive cell infiltration (Tepper, Coffman, and Leder, 1992; Yu, Wei *et al.*, 1993; Giezeman-Smits, Okada *et al.*, 2000). These preclinical results were quickly translated into a vaccination trial using the IL-4 gene transduced allogenic melanoma cells (Arienti, Belli *et al.*, 1999). The vaccination resulted in the generation of specific antibodies and specific T cell response against tumor antigens in 2 and 7 out of 11 cases, respectively. Although both systemic and local toxicities were mild, only minor clinical benefits were observed.

Interleukin IL-12

IL-12 is a heterodimeric molecule comprising of 35 and 40 kDa chains linked by disulfide bonds. Its most distinctive activity is its ability to promote Th1 responses by enhancing the production of IFN-γ (Gately, Rensetti *et al.*, 1998). Moreover, IL-12 can enhance the lytic activity of NK and lymphokine-activated killer cells, promote specific CTL responses, and act as a short-term growth factor for activated T and NK cells. Based on these biological activities, a pre-clinical study using the recombinant protein has done immediately. This study demonstrated that IL-12 exerted potent anti-tumor and anti-metastatic activity in several mouse tumor models (Bruda, Luistro *et al.*, 1993). These results further aroused expectation among tumor immunologists that this cytokine would be an effective agent against tumors.

The protocol in Phase I study consists of a single injection of a low dose of IL-12 before consecutive dosing, and Phase I study did not cause any severe adverse effects, further nurturing enthusiasm. However, the excitement has been tempered by severe adverse effects in Phase II study using recombinant IL-12 protein (Leonard, Sherman *et al.*,

1997). In this Phase II study, 17 patients received consecutive and daily IL-12 administration (500 ng/kg), and 12 patients were hospitalized and two patients died with aberrant IFN-γ production. A single injection of a low dose of IL-12 before consecutive dosing was excluded from Phase II study. Without this pre-treatment, IL-12 induced the production of aberrantly high levels of IFN-γ, which may account for severe adverse effects observed in the Phase II clinical study.

In parallel with this clinical study using recombinant IL-12 protein, several groups demonstrated the efficacy of the IL-12 gene therapy against various types of mouse tumor models (Tahara and Lotze, 1995). These pre-clinical studies have now been translated into clinical studies. Autologous melanoma cells were transduced with the IL-12 gene and these transfected melanoma cells were injected as a tumor vaccine. The treatment did not cause any major toxicity except for mild fever and were well tolerated by all six patients enrolled in this study (Sun, Jurgovsky *et al.,* 1998). Upon the completion of the vaccination, tumor-reactive proliferative and cytolytic cells were increased in two patients. Moreover, two patients developed delayed type-sensitivity reaction against autologous melanoma cells and one had a minor clinical response. Furthermore, a heavy infiltration of CD4-positive and CD8-positive cells was observed at the metastatic sites. Similarly, peritumoral injection of the IL-12 gene-transduced autologous fibroblasts caused transient but clear reduction in tumor sizes at the injected sites in four of nine cases, and at non-injected distant sites in one melanoma patients without causing any severe adverse effects (Kang, Park *et al.,* 2001). These results suggest that IL-12 gene therapy against cancer will be well tolerated without causing severe adverse effects and may be effective to induce specific immune response to tumors.

Interferon (IFN)

There are three types of IFN; α, β, and γ. IFN-α is encoded by a family of about 20 genes on chromosome 9, while IFN-β and IFN-γ are encoded by a single gene on chromosomes 9 and 12, respectively (Soos and Szente, 2003; Schrieber and Schreiber, 2003). Most types of cells can produce IFN- α and β (called also as type I IFN), upon viral and bacterial infection. In contrast, IFN-γ (called also as type II IFN) is secreted by Th1 cells, CD8-positive T cells, and NK cells, when these cells are activated as part of an immune response, particularly in response to IL-2 and IL-12. Type I IFN's and to a lesser degree, IFN-γ, exhibit anti-viral activities. IFN-γ and to a lesser degree, type I IFN's, enhance the expression of major histocompatibility (MHC) class I and II antigen and activate the activities of NK cells against tumor cells as well as virus. IFN-γ can promote Th1 differentiation, leading to the activation of macrophages (Schrieber and Schreiber,

2003). Moreover, type I IFN's and to a lesser degree, IFN-γ, are able to arrest the growth but generally do not kill many type of cells in culture, including transformed cell lines. These biological properties prompted many investigators to use these molecules for immunotherapy against cancer.

Among recombinant IFN's, only IFN-α has established its roles in cancer therapy. The application of IFN-α is now a standard practice in the treatment in kidney cancer, with a response rate in the 5 to 10 % range. IFN-α has been approved by US Food and Drug Administration for the treatment of patients with hairy cell leukemia and acquired immunodeficiency syndrome-associated Kaposi's sarcoma (Kirkwood, 2002). Moreover, Phase II clinical trials using IFN-α were done for metastatic melanoma, with response rates of 20% (Dorval, Palangie *et al.,* 1987). However, there was no evidence that IFN-α improves survival in stage IV melanoma. IFN-α is effective as single agents in certain diseases described above, its therapeutic potential may be further enhanced in combination with other chemotherapeutic agents. For example, combined treatment with IFN-α and 5-fluorouracil showed increased efficacy against colorectal carcinoma, compared with IFN-α alone.

IFN-α gene transfer has been evaluated in pre-clinical models (Ahmed, Johnson *et al.,* 2001; Heller, Ingram *et al.,* 2002) and demonstrated the ability to reduce tumor growth and induce complete tumor remission, when the gene was transferred into tumors by using adenovirus vector or electrical delivery system. Moreover, a single IFN-α gene transfer by adenovirus vector was as effective against murine tumor models as weekly injection of polyethylene glycol modified IFN-α, which has a longer half-life than ordinary recombinant one (Demers, Johnson *et al.,* 2002). Furthermore, the same group developed a tetracycline-inducible adenovirus vector encoding IFN-α. IFN-α expression was augmented in mice by addition of doxycycline to their drinking water and resulted in 85 % reduction in tumor growth compared with animals that were not given doxycycline. This approach may be feasible in clinical situations.

The administration of recombinant IFN-β demonstrated anti-proliferative activity in preclinical studies. However, the activity relies on IFN-β concentrations that cannot be achieved by parental administration due to its rapid clearance and severe systemic adverse effects (Salmon, Le Contonnec *et al.,* 1996). Thus, IFN-β gene delivery has been tried by several groups.

Tumor formation was blocked by *ex vivo* IFN-β gene transduction by adenovirus vector even if as few as 1 % of implanted cells successfully expressed IFN-β transgene (Qin, Tao *et al.,* 1998). The same group further demonstrated that direct *in vivo* IFN-β gene delivery into established tumors generated high local IFN-β concentrations, inhibited

tumor growth, and in many cases caused complete tumor regression in immune-deficient mice, indicating that the anti-tumor effect was primarily through direct inhibition of tumor cell proliferation and survival. Subsequent studies demonstrated that IFN-β gene delivery exhibited potent anti-tumor activities by inhibiting angiogenesis (Dong, Greene *et al.*, 1999) and enhancing specific immune responses to tumors (Lu, Fidler, and Dong, 1999; Natsume, Tsujishima *et al.*, 2000). One Phase I clinical study for malignant gliomas has been approved in the United States (Eck, Alavi *et al.*, 2001). In Japan, a pilot clinical study has been done to evaluate the safety and effectiveness of IFN-β gene transfer against patients with malignant gliomas (Yoshida, Mizuno *et al.*, 2003). In this study, five patients were treated with intratumoral IFN-β gene transfer by using cationic liposomes. The therapy was well tolerated. Two patients showed a partial response with more than 50 % reduction in tumor sizes and two others had stable disease even at 10 weeks after beginning therapy. One patient could not be evaluated because of previous treatment with γ-knife therapy. Thus, IFN-β gene therapy may be feasible and an effective treatment option for patients with malignant glioma, an otherwise incurable condition.

Because IFN-γ is a potent activator of NK cells and CTL (Schreiber and Schreiber, 2003), various clinical trials have been performed to evaluate the efficacy of recombinant IFN-γ against tumors. IFN-γ was marginally effective to improve progression-free survival at 3 years and 3-year overall survival after the operation among patient with ovarian cancer (Windbichler, Hausmaninger *et al.*, 2000). However, other clinical studies have failed to show its clinical efficacy in other types of cancers including lung cancer, metastatic renal cell carcinoma, and advanced malignant melanoma.

The effects of IFN-γ gene transfer have been extensively studied in many tumor systems. IFN-γ gene-transduced melanoma cells exhibited enhanced expression of HLA molecules (Ogasawara and Rosenberg, 1993; Abdel-Wahab, Osanto *et al.*, 1994). Moreover, these cells augmented the cytokine production capacity and the cytolytic activity of autologous tumor-infiltrating lymphocytes and/or peripheral blood lymphocytes. These observations have been translated into clinical studies of IFN-γ gene transfer against melanoma. The injection of IFN-γ gene-transduced autologous melanoma cells induced specific anti-melanoma antibody generation in a small proportion of patients, accompanied with a discernible tumor regression (Abdel-Wahab, Weltz *et al.*, 1997). However, due to the difficulties in obtaining a sufficient number of melanoma cells for *ex vivo* gene transfer, intratumoral IFN-γ gene transduction has been tried by using either retrovirus (Fujii, Huang *et al.*, 2000) or adenovirus vector (Khorana, Rosenblatt *et al.*, 2003). Multiple injections of IFN-γ-expressing retrovirus vector induced a clinical response, which correlated well with the titers of the induced specific antibodies to melanoma-associated

antigens. However, it still remains unknown on the long-term effects of IFN-γ gene therapy against melanoma.

Granulocyte-macrophage (GM)-colony stimulating factor (CSF)

GM-CSF facilitates the formation of granulocytes and macrophages in bone marrow, thereby increasing the circulating white blood cells in a dose and schedule-dependent manner, when administered parentally. Due to this pharmacological effects, recombinant GM-CSF has been used clinically for treatment and/or prevention of fever and neutropenia after myelosuppressive chemotherapy (Rubenstein, 2000). The use of GM-CSF has been explored also for collection of peripheral blood progenitor cells from healthy donors but there is still a need to develop improved procedure before its clinical application (Fischmeister and Gadner, 2000).

Evidence is accumulating to indicate that GM-CSF has a crucial role in the differentiation and maturation of dendritic cells, potent antigen-presenting cells (Strobl, 2003). This was mirrored by the observation that injection of irradiated melanoma cells, which were transduced with GM-CSF gene, induced a very efficient specific immunity, accompanied with an intense local reaction consisting of dendritic cells, macrophages, and granulocytes (Dranoff, 2003). Moreover, as compared with other cytokines, which can induce the differentiation and maturation of dendritic cells, GM-CSF has several advantages. First, GM-CSF can elicit a subset of dendritic cells that are superior for the phagocytosis of particulate material, such as apoptotic tumor cells. Second, GM-CSF can evoke higher levels of co-stimulatory molecules on dendritic cells, leading to more efficient T cell stimulation. Finally, GM-CSF can promote uniformly high levels of CD1d, non-classical MHC class I molecule that presents lipid antigens and stimulates invariant NKT cells. Based on these observations, tumor vaccination therapy has been tried for prostate cancer, melanoma, and renal cell carcinoma by injecting irradiated autologous tumor cells, which have been transduced with the human GM-CSF gene.

In the clinical study on prostate cancer, eight patients were treated with irradiated autologous tumor cells, which were genetically engineered to secrete GM-CSF (Simons, Mikhak *et al.,* 1999). The injected sites manifested infiltrates of dendritic cells and macrophages along with prostate tumor vaccine cells. Delayed hypersensitivity reactions against autologous tumor cells were evident in two of eight patients, while sera from three patients contained newly-generated antibodies recognizing protein components in prostate cancer cells.

Injection of GM-CSF gene-transduced melanoma cells resulted in an increased infiltration of dendritic cells in regional lymph nodes 7 days after the injection in all five

patients enrolled in the study (Chang, Li *et al.,* 2000). When lymph node cells from four patients, were secondarily activated and expanded *ex vivo* in the combined stimulation with anit-CD3 monoclonal antibody and IL-2, the resultant T cells manifested diverse responses in terms of cytokine production *in vitro* when exposed to the tumor antigen. Moreover, one patient had a durable complete remission of metastatic tumor, when these secondarily activated cells were adoptively transferred.

Seven immuno-competent patients with surgically incurable cutaneous melanoma underwent treatment with twice-weekly intratumoral injections of vaccinia virus vector which direct GM-CSF expression (Mastrangelo, Maguire *et al.,* 1999). Chronically treated lesion showed a dense infiltration of CD4-positive and CD8-positive lymphocytes and eosinophils with GM-CSF mRNA expression, despite the presence of anti-vaccinia virus antibodies after the repeated injection. One patient had a complete remission and one had a partial response. In contrast, three patients had mixed responses, with regression of treated sites and progression of elsewhere, while the two patients with the largest tumor burdens failed to respond. Thus, these experiences suggest the potential efficacy of gene therapy using the GM-CSF gene against melanoma.

Chemokines.

A wide variety of tumors can produce various chemokine constitutively or in response to various stimuli. Then, chemokines can recruit various types of leukocytes including monocytes/macrophages, T lymphocytes, NK cells, and dendritic cells, thereby host responses to tumors (Brault and Kurt, 2003). Moreover, several chemokines exert positive and negative effects on angiogenesis, an indispensable step for tumor growth, directly and/or as a consequence of leukocyte infiltration and/or induction of growth factor production (Bernardini, Ribatti *et al.,* 2003). Furthermore, chemokines can guide the growth and the motility of tumor cells, thereby accelerating tumor progression (Homey, Muller, and Zlotnik, 2002).

CXCL10/interferon-inducible protein-10 (IP-10) and CXCL9/monokine induced by interferon γ (Mig) are potent chemoattractants for Th1 cells and exhibit potent anti-angiogenic activities (Mukaida, Ketlinsky, and Matsushima, 2003). The growth of metastatic hemangiosarcoma, which was explanted into immunocompetent mice, was suppressed by intratumoral and intraperitoneal injection of CXCL10-expressing parvovirus vector, accompanied with the generation of specific immunity (Giese, Raykov *et al.,* 2002). Moreover, established tumors was completely regressed with a concomitant generation of specific CTL against the tumors, by intratumoral co-injection of adenovirus vectors expressing CXCL-10 with IL-12 or IL-18 (Narvaiza, Mazzolini *et al.,* 2000; Liu,

Huang et al.., 2002). Similar effects were also observed with CXCL9 gene transfer (Addison, Arenberg *et al.,* 2000).

CCL2/monocyte chemoattractant protein-1 (MCP)-1 can augment the cytotoxic activity of monocytes/macrophages and chemoattract NK cells (Mukaida, Harada, and Matsushima, 1998). CCL2 gene-transduced human lung cancer cell lines, enhanced antibody-dependent cellular cytotoxicity reaction accompanied with augmented macrophage infiltration, when injected into nude mice (Nishioka, Yano *et al.,* 1997). Moreover, CCL2 gene-transduced human lung adenocarcinoma cells suppressed their systemic spread by augmenting NK cell activity (Nokihara, Yanagawa *et al.,* 2000). In contrast, intratumoral injection of CCL2-expressing adenovirus vector alone, had only marginal effects on the growth of hepatocellular carcinoma cells, which were transplanted into nude mice (Sakai, Kaneko *et al.,* 2001). However, anti-tumor effects of herpes simplex thymidine kinase/ganciclovir system was enhanced synergistically by codelivering CCL2 gene, probably because CCL2 induced macrophages to phagocytoze tumor cells, which became apoptotic by the combined treatment with thymidine kinase and ganciclovir. Similarly, a suboptimal dose of cisplatin reduced markedly the tumor formation rates of CCL2 gene-transduced human cervical cancer cells, which were injected into nude mice (Nakamura, Kyo *et al.,* 2004).

Another approach using chemokine genes for cancer gene therapy is dendritic cell-based vaccines, which favor dendritic cell antigen presentation and induce antigen-specific CTL response. Dendritic cells express and utilize distinct sets of chemokine receptors, depending on their maturation stages (Allavena, Sica *et al.,* 2000). Immature dendritic cells express CCR1, CCR2, CCR4, CCR5, CXCR1, and CXCR4, while mature dendritic cells express a limited set of chemokine receptors, CXCR4 and CCR7. Based on these observations, the genes of ligands for CCR2, 4, 6, and 7, have been transduced either ex vivo or in vivo to examine their efficacy in animal models.

When an adenovirus vector encoding the gene of a ligand for CCR4, CCL22/macrophage-derived chemokine, was administered into the established tumor, a marked tumor regression was observed with the CTL response, which was dependent on both CD4-positive and CD8-positive lymphocytes (Guo, Wang *et al.,* 2002). Moreover, the administration of the adenovirus vector induced the chemoattraction of dendritic cells, facilitated dendritic cell migration, and activated dendritic cells to produce high levels of IL-12. Similarly, growth of established tumors was significantly inhibited by intratumor injection of an adenovirus vector encoding CCL20/macrophage inflammatory protein-3α, a ligand for CCR6 (Fushimi, Kojima *et al.,* 2000). Moreover, dendritic cells were accumulated inside the tumors and eventually elicited tumor specific CTL activity. Similar

effects were also observed by intratumoral injection of an adenovirus vector encoding CCL21/secondary lymphoid tissue chemokine (SLC), a ligand for CCR7 (Kirk, Hartigan-O'Connor *et al.,* 2001). Antitumor activity by intratumoral CCL21 gene transduction was further augmented by co-delivery of the gene of an accessory molecule, CD40L (Tolba, Bowers *et al.,* 2002).

Chemokines have complicated roles in tumor biology. For example, CCL21, a candidate chemokine for cancer gene therapy, exhibits chemotactic activities for some of human breast cancer cell lines, which express CCR7 (Muller and Homey *et al.,* 2001), suggesting that CCL21 can promote metastasis of breast cancer cells. These observations illustrate that more detailed understanding of the biology of each chemokine is mandatory before clinical application of chemokine gene therapy for cancer treatment.

Future Perspectives

The target of cytokine- and chemokine-based cancer gene therapy is patients with widespread metastasis and/or progression, who cannot be treated successfully with surgery and/or irradiation. In order to be effective against these conditions, cytokine and/or chemokine genes should be expressed persistently at appropriate levels and at the right place.

In most cases, the expression levels are not still sufficient to induce effective antitumor effects. At present, adenovirus vector system can yield vectors with a higher titer and is used widely for in vivo gene transfer (Volpers and Kochanek, 2004). However, adenovirus is not inserted into the host chromosome and therefore repeated administration is required for continuous transgene expression. Moreover, the remaining virus gene products induced additional immune responses, thereby further reducing transgene expression. "Gutless" or helper-dependent adenovirus vector system has been designed to circumvent these pitfalls.

Some therapeutically relevant cell types express only low levels or completely lack expression of the primary adenovirus receptor, resulting in low levels of transgene expression. Until now, several methods have been successfully employed in order to improve adenovirus vector-mediated gene delivery to those cell types, including genetically encoded or chemically engineered structural modification of the capsid and the use of bispecific adapator molecules. These approaches can expand or shift the adenovirus vector tropisms. Moreover, the use of tissue-specific promoters has been shown to be able to confine the transgene expression to certain tissues.

The utilization of recombinant proteins and gene-modified animals has helped remarkably to clarify the in vivo biological roles of cytokines and chemokines. However,

due to species differences between humans and rodents, we cannot completely understand their kinetics and roles in humans under physiological and pathological conditions. Cytokine- and/or chemokine-based cancer gene therapy is still in its infancy, but present human clinical studies will help to obtain this type of knowledge, which is fundamental to design more effective cancer gene therapy.

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