Progress and limitations in cancer gene therapy

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This is a brief discussion on the progress of gene therapy and the limitations of present-day gene therapy clinical trials based on a review of 464 human trial protocols from the U.S. National Institutes of Health (NIH) and the U.S. Federal Drug Administration (FDA). The paper also discusses an aspect of the gene therapy research of the author, who, together with colleagues, conducted the first gene therapy clinical trial in Korea in 1995. ***Genet Med* 2002: 4(6, Supplement):52S–55S.**

Gene therapy can be defined as a therapeutic technique in which functioning genes are inserted into the cells of a patient to correct an inborn genetic error (gene replacement therapy) or to provide a new function to the cell (gene addition therapy). Since the first clinical trial in 1990, there have been more than 400 clinical protocols. However, in 1999, there was one casualty in a gene therapy trial at the University of Pennsylvania. Since that incident, there has been a sharp decline in gene therapy clinical trials.[1–4]

To evaluate the problems regarding gene therapy clinical trials, 464 human trial protocols from the NIH and FDA that had been performed up to May 15, 2001, were reviewed. Of those, 62% (290/464 protocols) were for the control of cancer. Other categories are HIV infection, monogenic diseases such as hemophilia, and marking studies (Fig. 1). Cancer has been the most important disease for clinical trials of gene therapy worldwide because getting approval for a human trial is easier for cancer than for other genetic diseases. In Korea, there have been three clinical trials, and they involved only patients with cancer.

Current status of cancer gene therapy

Over the last 10 years, cancer has become the most important disease entity in gene therapy. Ten categories of cancer gene therapy are suggested from the NIH database. Of those, 60% of the clinical trials are the genetic modulation of the immune response (in vitro or in vivo). Other kinds of approaches are listed in Table 1.

There are two primary strategies for cancer gene therapy (Fig. 2). One is an immunologic target; the other is a molecular target. Molecular targets include oncogenes, tumor suppressor genes, and genes that regulate drug sensitivity. Two-thirds of cancer gene therapy are related to immunologic targets, such as cytokine genes, and one-third is focused on molecular targets (Table 2).

Molecular targets

It has been well hypothesized that tumor initiation and progression are based on oncogene activation or the inactivation of tumor suppressor genes. Gene therapy can down-regulate oncogenes by antisense. Antisense, the use of oligonucleotides made to be complementary to a particular mRNA in the 3'-5' rather than 5'-3' orientation, can lead to growth inhibition of cancer cell lines in animal studies. However, in human trials, antisense strategy showed only limited efficacy.

The other strategy is to reinstitute tumor suppressor genes by gene transfer. Among the tumor suppressor genes, the *p53* story is most exciting.[5–7] Clinical trials using the *p53* gene have been done in several tumors. In non–small-cell lung cancer, *p53* was introduced using an adenoviral vector with cisplatin chemotherapy. Of 24 patients in an American study, only 2 showed an objective clinical response.[6] In a European study,[7] a combination of *p53* gene therapy with cisplatin chemotherapy was compared with cisplatin chemotherapy alone. There was no difference between the two groups. At the moment, *p53* gene therapy is not effective for non–small-cell lung cancer.

Other gene therapy trials using molecular targets are in progress. The herpes simplex thymidine kinase (*HSVtk*) and cytosine deaminase (*CD*) genes are being transfected to tumor cells as drug sensitivity genes to activate prodrugs into anticancer drugs.[1,8] The transfer of the *HSVtk* gene to a tumor followed by antiviral agent therapy (a suicidal vector trial) enjoyed great success in animal trials. Culver and Blaese[8] introduced *HSVtk* genes into an experimental brain tumor model and treated the animals with ganciclovir. The brain tumor was eliminated without affecting normal brain tissue. However, other clinical trials did not confirm the NIH results.

Yet other trials include insertion of *MDR* genes into normal bone marrow to confer a drug-resistant phenotype. Gottesman's group[9] intended to treat cancer patients with high-dose chemotherapy and transplantation of autologous *MDR*-containing bone marrow without serious hematologic side effects.

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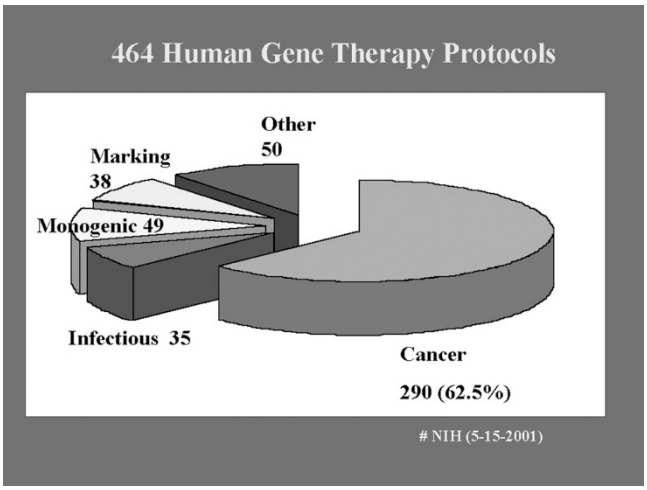

Fig. 1 Categories of clinical trials of gene therapy (based on http://www4.od.nih.gov/oba/rac/hgtprep.asp).

Table 1
Categories of cancer gene therapy

Category	Quantities: *n* (%)
Antisense	6
Chemoprotection	12 (4)
Immunotherapy/in vitro	81 (28)
Immunotherapy/in vivo	100 (34)
Pro-drug/HSVtk	37 (13)
Tumor suppressor gene	34 (12)
Single chain antibody	2
Oncogene down-regulation	7
Vector-directed cell lysis	10 (3)
Dominant negative mutation	1

Information derived from http://www4.od.nih.gov/oba/rac/hgtprep.asp.

The problems were low transfection efficiency and risk of transfection of the *MDR* gene into contaminating tumor cells.

The major limitations of in vivo nonimmunologic approaches are targeting selectivity and transfection efficiency. Not only is it very difficult to attack tumor cells without affecting normal cells, but less than 5% of tumor cells can be transfected after the addition of foreign genes.

Immunologic targets

Because of theoretical limitations and poor clinical results with cancer gene therapy with molecular targets, most clinical trials concentrate on immunologic targets. Among many strategies, genetic modulation of the immune response is the hottest area of clinical cancer research.

The first clinical trial using gene therapy strategy was with tumor-infiltrating lymphocytes (TILs) transduced with the gene for TNF-α. The approach was designed to use TILs for the

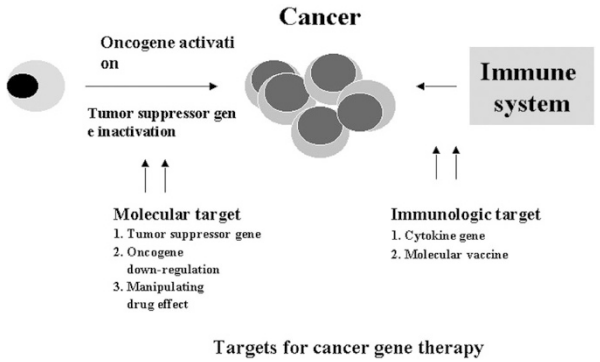

Fig. 2 Cancer gene therapy strategies.

Table 2
Classification of cancer gene therapy based on targets

A. Molecular targets
   1. Tumor suppressor gene therapy
   2. Down-regulation of oncogenes
   3. Manipulating drug effects (sensitivity/resistance)

B. Immunologic targets
   1. Passive immune modulation—immune effector cells transfected with cytokine genes
   2. Active immune modulation
      a. Genetically modified cells—tumor cells transfected with cytokine genes
      b. Molecular vaccine—tumor antigens (MART-1, gp100, CEA, PSA)

purpose of effective production of TNF-α at the tumor site without systemic side effects. Effective systemic TNF-α blood levels (400–500 μg/kg/day in mice) were unattainable because of systemic side effects (maximum tolerated dose in human trial: 8 μg/kg/day). However, problems with the TILs' inadequate expression of the transduced TNF-α gene (expression blockade) and ineffective delivery of TILs to the tumor site (traffic) limited the effectiveness.

In contrast, in vitro modulation of tumor cells using cytokine genes gave us the new possibility of a tumor vaccine (tumor-cell directed lymphokine gene therapy). Among the strategies to activate host immune response to cancer, cytokine gene therapy has been evaluated most extensively.[10,11]

Various cytokine genes (interleukins, interferons, GM-CSF, etc.) have been introduced into tumor cells to increase immunogenicity (increase immune recognition by immune effector cells). Data from animal experiments indicate that this approach can offer systemic antitumor immunity based on the observation of decreased tumorigenesis of genetically modified tumor cells and the rejection of subsequent injection of unmodified tumor cells. However, it is not clear whether this approach can eradicate established tumors in tumor-bearing animals.

We have explored the possibility of genetic modulation of the immune response against cancer. Among cytokine genes,

the introduction of both GM-CSF and IFN-γ genes into tumor cells showed synergistic effects in vitro and in animal experiments.[12] Although there have been numerous clinical trials using cytokine genes, there is still no convincing evidence to support the clinical efficacy of cytokine gene therapy.[13,14]

The first clinical trial of human gene therapy in Korea was conducted using the HLA-B7/β2-microglobulin gene. Intratumoral injection of the therapeutic gene induced two clinical responses out of nine patients. All patients showed an increase in natural killer (NK) activity in their circulating peripheral blood lymphocytes.[15]

The purpose of the study was to assess the therapeutic potential of injecting the gene for HLA-B7/β2-microglobulin into the subcutaneous metastatic nodules of patients who are refractory to conventional treatments (Fig. 3). The nine patients evaluated were divided into three groups and given escalating doses of DNA (20, 40, and 100 μg of the HLA-B7 plasmid DNA/lipid complex for each group) every 2 weeks. Biopsy specimens from the treated tumor nodules of all nine patients were positive for the presence of DNA and for HLA-B7 mRNA expression. Moreover, in six of the nine patients, immunohistology of tumor biopsy samples revealed the expression of recombinant HLA-B7 protein. Also, all nine patients showed an increase in NK activity in their circulating peripheral blood lymphocytes. In two lung cancer patients, one partial response and one mixed response were observed after gene transfer. These responses were confined to the treated nodules and the untreated locoregional lymph nodes; the lung masses showed no regression. Remission durations were 14 and 6 weeks, respectively, and in a total of 35 cycles, no significant toxicities were observed. Immunohistologic analysis revealed an increased infiltration of CD4+ T cells, macrophages, and NK cells after therapy. In the two responding cases, direct intratumoral injection of an allogeneic class I gene could elicit an antitumor response in locoregional areas, possibly through the activation of NK cells.[15]

Genes for tumor-specific antigeneic determinants like CEA (carcino-embryonic antigen), PSA (prostate-specific antigen), MART-1, or gp100, are being explored for therapy.[16]

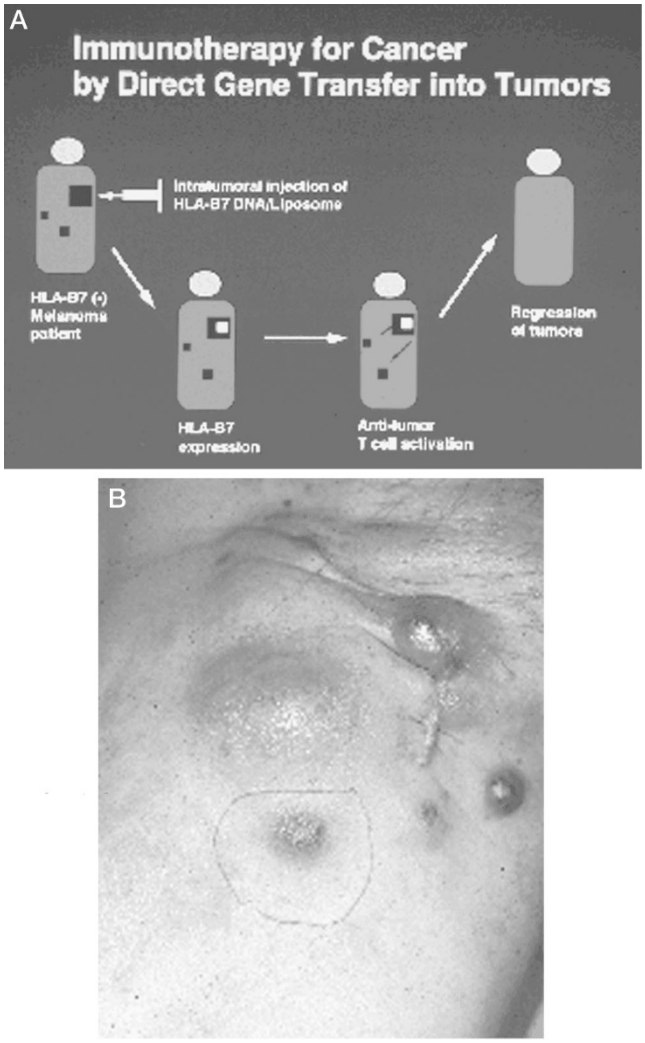

Fig. 3 A: strategy of intratumoral injection of HLA-B7/β2-microglobulin gene in patients with cancer. B: subcutaneous metastatic nodules in a melanoma patient.

Limitations and prospects

Despite some promising results in cancer gene therapy, there are many limitations to overcome.[17–19] Only a limited number of therapeutic genes can be used in clinical trials.

Vectors are not efficient in vivo. Although the high transfection efficiency with adenovirus in vitro is well documented, it is still not clear whether adenoviral vectors are effective in vivo in solid tumor models. In our experiment, transduction of tumor tissue was limited to just around the injection site after intratumoral injection of the adenoviral vector.[19] In our experiment, tumor cells showed depressed expression of the CAR (coxsackie-adenovirus receptor) gene for adenoviral vector in comparison to normal cells.

The basic elements of gene therapy consist of therapeutic genes, vectors, and strategies (Fig. 4). As more information about cloned genes becomes available through various pro-

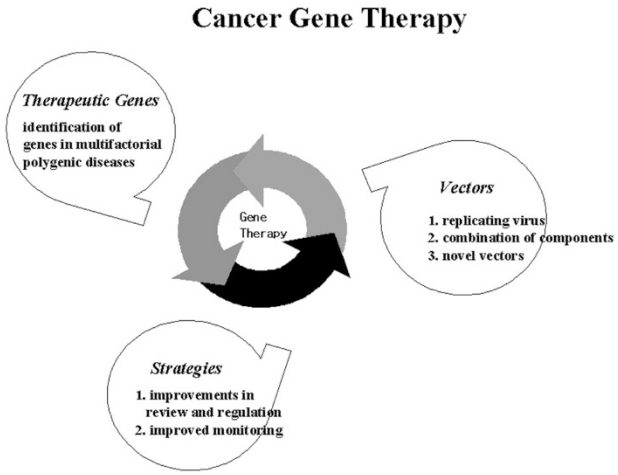

Fig. 4 Three essential elements for gene therapy.

grams, such as the Human Genome Project, the potential of gene therapy expands. Genes of interest can be inserted into various target cells, such as immune effector cells, tumor cells, and hematopoietic stem cells.

Recent trials with an oncolytic virus showed promise.[20] A phase II trial in recurrent head and neck cancer demonstrated three complete and two partial remissions out of 40 patients with replication-competent adenoviral vector (ONYX-015).[21] A phase III clinical trial is presently going on.

There have been remarkable developments in our understanding of the molecular basis of human cancer, and the enormous potential of the use of gene therapy to not only cure cancer, but to also prevent it, is anticipated. However, clinical trials have so far showed no definite advantages of gene therapy over conventional modalities. To make gene therapy a standard treatment modality for cancer patients, more improvements are needed in areas such as therapeutic genes and vectors. Many ethical and technical hurdles still remain.

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