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REVIEW ARTICLE

Gene Therapy for Cancer:
From the Laboratory to the Patient

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Gene therapy is a new form of therapeutic intervention with applications in many areas of medical treatment. There are still many technical difficulties to be overcome, but recent advances in the molecular and cellular biology of gene transfer have made it likely that gene therapy will soon start to play an increasing role in clinical practice and particularly in the treatment of cancer. The first clinical gene transfer in an approved protocol took place exactly 10 years ago, and it was for the transfer of gene-marked immune cells into patients with advanced cancer. Now there are 218 active clinical protocols in the United States, and they have involved over 2000 patients worldwide. Among the conditions and diseases for which gene transfer is being tried as treatment, cancer comes first with 130 clinical trials. Fundamental research in the mechanisms of cancer and the development of molecular biology tools are crucial for the success of the treatments in the future. The identification of tumor rejection antigens from a variety of cancers and the immune response that is defective in cancer patients are important topics for future studies. The evaluation of gene therapy combinations involving use of tumor suppressor genes and constructs that inactivate oncogenes is also another important area for future research. The future improvement of present viruses as well as the use of new viral vectors will likely expand the applicability and efficacy of gene therapy. During the next decade technological developments, particularly in the areas of gene delivery and cell transplantation, will be critical for the successful clinical practice of gene therapy.

KEY WORDS: gene therapy; cancer; vector.

Conventional cancer therapy aims to destroy the tumor, while leaving as much as possible of the normal host tissue intact. The problem with both surgery and radiotherapy is tumor invasion and spread outside areas directly accessible to these treatments; and the problem with chemotherapy is its low therapeutic ratio for many tumors and the fact that drug resistance may rapidly develop or even exist from the start. Gene therapy is a new form of therapeutic intervention at a molecular level with applications in many areas of medical treatment ranging from specific correction of single-locus inherited genetic defects through immunization to treatment of infectious disease and cancer (1–4). Genes can selectively inhibit the metastatic potential of cells, examples being nm23, β-actin, fibronectin receptor, connexin, and E-cadherin (5). With this therapeutic technique, a functioning gene is inserted into the cells of a patient to correct an inborn genetic error or to provide a new function to the cell. Although this type of intervention has only recently become technically feasible, applications to a variety of clinical settings are already being pursued.

The past decade has seen the use of molecular biology to isolate genes and then to produce, in pharmaceutical quantities, cytokines, many of which are involved in the control of the immune system. Some of these, such as the interferons and interleu-

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Table 1. Characteristics of Most Commonly Used Vectors

	Insert size	*Genome*	*Cell division*	*Expression*	*Advantages*	*Disadvantages*
Retrovirus	5–7 kb	RNA	Necessary	Permanent	Potential to integrate into genome of target cell	Insertional mutagenesis
Adenovirus	7–35 kb	DNA	Not necessary	Transient	Relatively high transduction efficiency into normal and tumor cells; easy production and at high titers	Local tissue inflammation and immune response
Adeno-associated-virus	2–4 kb	DNA	Not necessary	Long-term expression	High transduction efficiency into muscle and brain	Insertional mutagenesis; difficulties with production; do not work in all organs
Nonviral vectors	No limitation	RNA or DNA	Not necessary	Transient	Safe and cheap	Low efficiency

kins, have been demonstrated to have limited efficacy against specific human tumors (6), although the mechanism of action is unclear (7). Gene therapy strategies that have been explored include cytokine gene transfer and tumor cell vaccination (8), selective prodrug activation by so-called suicide genes (9), inhibition of activated oncogenes by antisense mechanism (10) and transfer of a tumor suppressor gene (11). There are still many technical difficulties to be overcome, but recent advances in the molecular and cellular biology of gene transfer have made it likely that gene therapy will soon start to play an increasing role in clinical practice, particularly in the treatment of cancer. Molecular biology was believed to offer little to the practicing clinician (12). Eventually, gene therapy could have a profound impact on the treatment of diseases, and it may alter day-to-day clinical practice. Therefore, practicing physicians who fail to realize the importance of this rapidly changing and expanding area of research in gene therapy will lose the train of progress in order to deliver good care to their patients.

Although several reviews of gene therapy research have been published, none has focused on the impact of gene therapy in medical practice. In this review, I have attempted to present the current application of gene therapy for cancer in clinical trials and discuss the future developments and problems in that field.

GENE TRANSFER VECTORS

Vectors are the vehicles used to carry the genetic material. Gene therapy will have a major impact on the health care of our population only when vectors are developed that can safely and efficiently be injected directly into patients as drugs. Vectors need to be engineered that will target specific cell types, insert their genetic information into a safe site in the genome, and be regulated by normal physiological signals (13). When efficient vectors of this type are produced, then gene therapy will probably have a profound impact on the practice of medicine (14). To date, the perfect vector does not exist, because there are different vector requirements depending on the specific aims of the investigator. Thus, some approaches may require permanent gene expression, while others may require transient expression. Broadly, gene transfer vectors are classified as either nonviral or viral (15). The characteristics of the most commonly used vectors are summarized in Table 1.

The majority of approved gene transfer protocols use retroviral vectors. The propensity of the retrovirus to integrade into dividing cells appears to be an advantage for cancer applications. Retrovirus attaches to the cell surface of host cells via the envelope surface protein (16). Following attachment, the virus enters the cell by receptor-mediated endocytosis (17). To provide a means of infecting most cells of interest, researchers have developed specialized cell lines (termed packaging cells) that permit the production of high titers of replication-defective recombinant virus, free of wild-type virus (18). However, there are several potential limitations for retrovirus (19). The size of the gene inserted in wild-type retroviral vectors is limited to up to 8 kb (20). The currently achivable titers (10^7) are low compared with what will be needed for the treatment of large tumors, and large-scale production is difficult (19). Finally, the host range of some retroviruses may be limited.

Retroviral vectors derived from the Moloney murine leukemia virus (MMLV) are currently the gene transfer vehicles of choice for the majority of approved clinical trials. They have the potential to inte-

grate into the genome of the target cell, and these viruses can only insert their genes into the genome of actively dividing cells.

In the last few years, vectors derived from lentiviruses, such as human immunodefficiency virus type 1 (HIV-1), have been shown to offer a great advantage over their oncoretroviral counterparts, because they can transduce nondividing cells, a crucial asset for genetically modifying tissues considered the main potential targets of gene therapy, such as the brain, muscle, liver, lungs, and hematopoietic system (21). The biosafety of HIV-based vectors requires extremely careful evaluation, considering the pathogenicity of the parent virus. Recently, the resulting third-generation lentivirus gene delivery system, which conserves only three of the nine genes of HIV-1 and relies on four separate transcriptional units for the production of transducing particles, has been shown to offer significant advantages in its predicted biosafety (22). This eliminates the possibility that a wild-type virus will be reconstituted through recombination.

Adenoviral vectors for gene therapy are adenoviruses (AdVs), which have been genetically modified by introducing deletions of the viral genome to create space for a foreign gene (transgene) to be inserted and to render the virus replication-defective (23). They can easily be rendered replication-defective by deletion of critical regulatory genes, and they have a relatively high transduction efficiency into a wide variety of normal and tumor cells. They do not require actively proliferating target cells and, from a practical standpoint, recombinant AdVs can be prepared and purified at high titers ($>10^{11}$). Finally, they do not incorporate into the genome. This can be advantageous because there is no risk for insertion mutagenesis; however, multiple injections into the target tissue may be required (24). *In vivo* transgene expression declines after about two weeks and becomes negligible by four weeks. Although first-generation recombinant AdVs are replication-deficient, they are, unfortunately, not replication-absent. Second-generation recombinant AdVs have been developed by taking advantage of temperature-sensitive mutations in the E_2 gene, to blunt the host immune response against viral proteins and to increase the duration of therapeutic gene expression (25, 26). Another approach to this problem is to use an AdV deleted of all sequences, leaving only the absolutely required terminal repeats and packaging sequences. The deleted viral genome enables accommodation of a very large expression cassette (28 kb), and abolishes viral gene expression (27, 28). Two regions of the viral genome (E_1 and E_3) have been used to make insertions or substitutions of DNA to generate helper-independent recombinant viruses. Combined deletions in E_1 and E_3 allow insertion of up to approximately 8 kb of foreign DNA into vectors. Recently, the wild-type AdVs have had all their structural genes removed, allowing proviral DNA of up to 35 kb to be inserted.

Gene products from the E_4 region of the AdV genome shut off host protein synthesis, stabilize viral mRNAs, and regulate viral DNA replication (29). The doubly-defective E_1–E_4 deleted vector also results in markedly reduced viral DNA replication and protein synthesis (30), prolonged transgene expression, and strikingly reduced chronic inflammation in the livers of immunocompetent mice (31). A vector combining the existing E_1 deletion, a temperature-sensitive E_2 mutation, and an E_4 deletion may offer additional benefits (32). Adeno-associated viruses (AAVs) are unique among animal viruses in that they normally require coinfection with an unrelated helper virus (ie, AdV) for productive infection in cell culture. Without a helper virus, AAVs integrate into the host genome and remain as provirus. Once incorporated into the genome, transcription is undertaken with the assistance of genes derived from the adenovirus. As a result, this virus has less chance to produce an immune response and is capable of infecting both dividing and nondividing cells. There are many attractive advantages of AAV as a gene transfer vector. Wild-type AAVs do not require target cell replication to infect the cell and are capable of infecting all human cell cultures tested so far. The inability to provide a high-titer-producing packaging cell line continues to be a limiting factor for the efficient use of this system.

The herpes simplex virus (HSV) has received attention because of its ability to establish latent infection in the brain. Consequently, it has been used in some initial applications to deliver therapeutic genes to some forms of brain cancer (33). The primary problems with HSV-mediated gene transfer are: (1) the cytopathic nature of HSV; (2) the difficulty in maintaining long-term expression of inserted genes—introduced genes are generally shut down within weeks of infection; and (3) infection efficiency is relatively low compared to other viral systems (34–36). Vaccinia viruses, poxviruses, and baculoviruses have also received recent attention for use in the delivery of genes for therapeutic purposes (37, 38). The further improvement of those viruses as well as

the use of new viral vectors will likely expand the applicability and efficacy of gene therapy.

Nonviral vectors have been another promising area of vector development. They consist of liposomes, electroporation, and naked DNA delivered by mechanical methods (39–42). Liposomes have been the method of choice for the current clinical trial delivering foreign MHC genes (43) and will efficiently deliver genes to a wide variety of tissues *in vivo* (39).

Electroporation is an important method that allows the passage of naked genetic material into the target cell by using electrical currents to disrupt the cell membrane. It is applicable to a wide variety of cell types, including hematopoietic cells and stromal elements (44, 45). It also allows the use of naked DNA, eliminating the need for viral packaging systems. The DNA is not incorporated into the genome, and hence long-term expression is not achieved.

Direct injection of naked DNA into tumors by using mechanical methods has been shown to result in gene transfer and expression, with an efficiency that can be of therapeutic benefit (46). The direct injection of naked DNA into muscle has led to DNA delivery and expression *in vivo* (47, 48). However, this method of delivery is limited only to cells near the injection site acquiring the DNA, with no tissue targeting. Another problem is the inability to transduce a large number of cells (47, 48). Recently, this technology has been applied to cancer for the generation of cancer vaccines, and the related studies have resulted in at least three clinical protocols for the generation of antitumor immunity against colon cancer and melanoma (49). Problem is that the DNA is only transiently maintained following physical uptake. This may be of less concern in cancer gene therapy than in other gene therapy applications. The delivered gene only needs to be expressed for sufficient time to cause the death of its target cell in tumors, whereas lifetime maintenance of the inserted gene is often desirable for the correction of inherited genetic disorders. It is also possible that this might be overcome by the incorporation of viral sequences that lead to episomal genome maintenance, such as those of EBV or papilloma viruses.

CLINICAL TRAILS IN GENE TRANSFER

In the last few years remarkable advances in recombinant DNA technology and cell biology have made it likely that gene therapy will become a reality, and furthermore, that it will not be restricted to the correction of single-gene disorders, but will have applications for many other branches of medicine (50–53). The notion that it is possible to alter an individual's genetic makeup is still repugnant to many people. However, somatic cell gene therapy does not present any fundamentally new ethical issues; essentially it is no different than any other form of transplantation. The inserted genes will, if all goes well, simply modify the genetic makeup of one organ system. Currently, several approaches to cancer therapy are being explored (54, 55). First, immune responses to tumors are being enhanced. Second, genes are being inserted into tumor cells to evoke "cell suicide." Finally, methods are being developed to modify tumor suppressor or antioncogenes.

The first unsuccessful attempt was made in 1980 to carry out gene therapy for b-thalassemia with the use of calcium phosphate-mediated DNA transfer. Retroviral-mediated gene transfer was developed in the early 1980s in animal models (56). The first clinical gene transfer in an approved protocol took place on May 1989. It was for the transfer of gene-marked immune cells [tumor-infiltrating lymphocytes (TIL)] into patients with advanced cancer. The protocol had two primary objectives: (1) to demonstrate that an exogenous gene could be safely transferred into a patient and (2) to demonstrate that the gene could be detected in cells taken back out of the patient (57). The first gene therapy protocol approved by the United States Food and Drug Administration, for correction of adenosine deaminase (ADA) deficiency began on September 1990. There were two objectives of the ADA gene therapy protocol (58). The clinical objective was to evaluate the possible therapeutic efficacy of the administration of autologous lymphocytes transduced with a normal human ADA gene in an effort to reconstitute the function of the cellular and humoral immune system in patients with ADA-deficient severe combined immunodeficiency. The scientific objective was to evaluate *in vivo* survival of culture expanded autologous T cells and the duration of expression of the inserted genes. There are now 218 active clinical protocols in the United States, and they have involved over 2000 patients worldwide. Among the conditions and diseases for which gene transfer is being used, cancer ranks first with 130 clinical trials (59). Additional protocols in various stages of development exist on the three continents.

Sixty protocols worldwide have been designed to augment the immune response against cancers by gene therapy (ie, by vaccine or direct cytokine or costimulatory molecule gene transduction). A total of 376 patients have been entered in these ongoing

Table 2. Current Clinical Studies for Cancer Gene Therapy by Gene Transfer

Primary and metastatic liver cancer
Metastatic colon carcinoma
Small-cell lung cancer
Lung cancer (NSCLC)
Malignant mesothelioma
Prostate cancer
Renal carcinoma
Squamous carcinoma
Refractory neuroblastoma
Primary and metastatic breast cancer
Bladder carcinoma
Malignant melanoma
Primary and metastatic ovarian cancer
Brain tumors
Head and neck squamous cell carcinoma
Persistent multiple myeloma
Refractory non-Hodgkin's lymphoma
Acute myelogenous leukemia
Chronic myelogenous leukemia
Acute leukemia

protocols. Major tumor regressions (defined as either complete responses or partial responses) have been observed in 15 of 237 patients with sufficient information to evaluate responses. Twenty-one protocols using drug-sensitivity gene strategy had been proposed. A total of 104 patients have been entered in these ongoing protocols. Major tumor regressions have been observed in 8 of 62 patients, with sufficient information to evaluate responses.

Approved protocols for multiple-drug resistance (MDR1) gene include protocols for the treatment of patients with breast or ovarian cancer who are receiving paclitaxel (Taxol). There are, however, some potential problems with this strategy: higher doses of chemotherapy may not translate into higher response rates, nonhematologic toxic effects may be dose limiting, and cancer cells in the marrow may be transduced with the drug-resistance gene. Eight protocols using this strategy had been proposed and are being tested in clinical trials (60–64). However, insufficient information is available to evaluate the therapeutic efficacy. The current clinical applications of cancer gene therapy are summarized in Table 2.

PROBLEMS AND FUTURE DEVELOPMENTS OF CANCER GENE TREATMENT

Fundamental research in the mechanisms of cancer and the development of molecular biology tools are crucial for the success of the treatments in the future. There are still formidable technical problems to be overcome. There is much to learn about the regulation of mammalian genes and the sequences required for their stability when introduced into foreign genomes. It is still not clear whether it is safe to incorporate genes into nuclear DNA or whether it will be possible to develop stable extrachromosomal gene transfer systems. The identification of tumor rejection antigens from a variety of cancers and the immune response that is defective in cancer patients are important topics for future studies. The products of other tumor suppressor genes, such as p16 and retinoblastoma gene (Rb), have been found to suppress tumor growth in animal models (65, 66). The evaluation of gene therapy combinations involving use of tumor suppressor genes and constructs that inactivate oncogenes is an important area for future research. Combination strategies of suicide gene transfer and cytokine immunogene therapy is another interesting approach. The success of combination therapies underlines the fact that the different approaches can be synergistic and that attacking a tumor with different weapons could be beneficial. However, enhancing the specificity of the transgene expression, as well as its regulation, are important points.

Future research should also be focused in modifying viral vectors to reduce toxicity and immunogenicity, increasing the transduction efficiency of nonviral vectors, enhancing vector targeting and specificity, regulating gene expression, and identifying synergies between gene-based agents and other cancer therapeutics (67). The future improvement of present viruses as well as the use of new viral vectors will likely expand the applicability and efficacy of gene therapy. Toxic effects associated with the use of retrovirus vectors for gene transfer in humans can likely be overcome by modifying vector structure to eliminate expression of endogenous viral genes, improving vector targeting, and using immunomodulators to reduce the immune response against the vector. Recent important advances, however, have signaled a new optimism in the field. Recombinant adenovirus (AdV) in their current form may not be the final, ideal vectors for human gene therapy. Nevertheless, recent improvements will ensure that AdV vectors play a significant role in the continued development of gene therapy for the treatment of human disease. The development of molecular conjugates is underway, consisting of protein or synthetic ligands to which a nucleic acid- or DNA-binding agent has been attached for the specific targeting of nucleic acids to cells (40, 42, 68). Once the DNA is coupled to the molecular conjugate, a protein–DNA complex results. This delivery system provides the basis for the generation of "synthetic viruses" capable of efficient

gene delivery without the detrimental effects of intact viruses. A universal gene delivery system has yet to be identified, but the further optimization of each of these vectors should result in each having a unique application.

One of the more important aspects of gene therapy continues to be the specificity of therapeutic gene expression. Two studies have shown that viral vectors can be targeted to specific cell types after attachment of ligands to the viral capsid either chemically or with antibodies (40, 69). Another study has shown that the retroviral envelope gene can be manipulated to express chimeric protein that consists of the envelope with a cell-specific ligand (70). This work provides the capability of developing targeted retroviral vectors. It may also be possible to alter binding motifs of the AdV coat proteins to enhance specific binding (71). The next level of specificity can be generated by using tissue or cell-specific promoters. The cytomegalovirus promoter and enhancer has been identified to be active primarily in rapidly dividing cells, since the enhancer is activated by transacting factors present in the nucleus (72). The duration of therapeutic gene expression is another important determinant of the effectiveness of the therapy. Nonviral systems may provide the best potential for the further development of gene constructs that mediate episomal maintenance, replication, or integration into the host genome.

CONCLUSIONS

Gene therapy has applications across many fields of medicine, particularly the treatment of cancer. Although the first clinical protocols in gene therapy began just over 10 years ago, progress has been rapid, and important observations have emerged from ongoing trials. It is obvious that gene therapy for cancer requires the cooperation of physicians and investigators in the basic sciences to develop safe systems for clinical use. Despite substantial progress, a number of key technical issues need to be resolved. Improvement in vector design that increases the duration of expression and the precision targeting and that reduces immunogenicity and toxicity should improve response rate. During the next decade technological developments, particularly in the areas of gene delivery and cell transplantation, will be critical for the successful clinical practice of gene therapy. Even now clinical attempts at gene therapy for cancer are underway and have opened new possibilities for the development of cancer treatments. As practicing clinicians we must incorporate this new information into our daily clinical practice or resign ourselves to obsolescence.

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