**Gene Therapy of Bladder Control**

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**Background:** The incidence of bladder cancer has been steadily increasing and, despite improvements in treatment, many patients will not survive five years. Gene therapy is a promising approach that may improve the management of this disease.

**Methods:** Gene therapy involves two steps: (1) selection of an appropriate gene and (2) transfer of that gene to the target cells.

**Results:** Five broad categories of cancer gene therapy are undergoing clinical evaluation: (1) immunological augmentation by cytokine or foreign gene transfer into tumor cells, (3) insertion of a suicide gene into tumor cells, (4) tumor suppressor gene reconstitution, and (5) bone marrow protection by insertion of a multidrug resistance gene.

**Conclusions:** Although many gene transfer approaches have great intuitive appeal, several constraints, including problems with methods of gene transfer, must be overcome before this biologic approach can reach its potential.

**Introduction**

Approximately 11,700 Americans will die of bladder cancer in 1996.[1] Less than 60% of patients with locally advanced bladder cancer will survive three years, despite aggressive, multimodal treatment with combinations of radiation, chemotherapy, and surgery.[2] Clearly, new approaches to the treatment of bladder cancer are needed.

Advances in molecular biology in the last decade have led to a better understanding of the cellular events underlying tumor development and progression. Specifically, potential targets for gene therapy of cancer have been identified in tumor immunology, drug resistance, tumor suppressor gene and oncogene function, genetic control of cell cycle, the mechanisms of apoptosis, and many other cellular systems. Simultaneous advances in gene transfer technology have allowed the transfer of genetic information to specific cells in living animals. The fusion of gene transfer techniques with the new targets has led to the emergence of cancer gene therapy. In this article, the principles of cancer gene therapy are outlined, and the current approaches to gene therapy of bladder cancer are reviewed.

**Principles of Cancer Gene Therapy**

Cancer gene therapy focuses on transferring an exogenous gene into a somatic cell to increase secretion of a specific protein or to replace an abnormal or defective protein. The information transferred will not pass to future generations (germline gene therapy). Although germline gene therapy has the potential to treat some inherited cancer syndromes (defective tumor suppressor genes such as retinoblastoma and Li-Fraumeni syndrome), it poses considerable ethical dilemmas. Successful somatic gene therapy depends on two principal elements: the selection of an appropriate gene and an effective gene transfer system.[3]

**Gene Selection**

Cell survival and multiplication depend on regulated gene expression. A multiplicity of different genes are expressed and actively interact to form either malignant or normal tissue. Interruption or alteration of any cell pathway may produce profound effects on the cell phenotype. The ability to introduce genes into cells allows a gene therapist to control parts of these molecular cascades, and there are many potential target genes for gene therapy of cancer within these pathways (Figure). Active clinical protocols for cancer gene therapy can be divided into five groups.[4,5]

**Category 1: Insertion of a Cytokine Gene Into Tumor Cells In Vitro**

This system uses a gene-modified vaccine in an attempt to induce specific antitumor cytotoxicity. Activated CD-8 T lymphocytes are the primary antitumor effector cells of the immune system. Activation of the CD-8 T lymphocyte requires antigen presentation in the context of a major histocompatibility complex-class I receptor and binding of cytokines (eg, interleukin [IL]-2, IL-4, and interferon-gamma) secreted by CD-4 T-helper lymphocytes.[6] Tumor vaccine gene therapy attempts to augment this system by engineering tumor cells to secrete cytokines, thus bypassing the need for CD-4 T-helper lymphocytes. Studies in animal tumor models have demonstrated that gene-modified vaccines can produce potent, specific, and long-lasting antitumor immunity.[7] Several cytokines, including IL-2, IL-4, IL-7, tumor growth factor-beta 2, interferon-gamma, and granulocyte-macrophage colony-stimulating factor (GM-CSF), are currently undergoing phase I and II clinical trials.

Most clinical protocols involve harvesting live tumor cells from either a primary or a metastatic tumor. The tumor cells are then grown in culture. When a sufficient number of tumor cells is obtained, the cytokine gene is transferred into the tumor cells by either physical or viral methods of gene transfer. The tumor cells are then assayed for production of the cytokine. Once an adequate level of gene expression is achieved, the tumor cells are irradiated to prevent tumor formation at the injection site and are then injected subcutaneously into the patient. Preclinical studies demonstrated the feasibility of this approach in renal cell carcinoma and prostate carcinoma.[8] A phase III trial of GM-CSF-modified tumor vaccine gene therapy has been completed at Johns Hopkins University, but the results of this study have not yet been reported.

Tumor vaccine therapy using IL-2 gene-modified tumor cells has induced tumor regression and increased survival in a mouse bladder cancer model. Animals that became disease-free were resistant to subsequent challenge with highly tumorigenic parental cells. This suggests that long-term immunological memory develops in a subset of animals.[9] The encouraging animal results suggest that tumor vaccine therapy may be beneficial in the treatment of human bladder cancer. Human trials have
not yet been initiated. 

Category 2: Direct Injection of a Gene Therapy Vector Expressing a Foreign HLA Antigen Into Tumor Tissue In Situ 

The primary goal of this therapy is to initiate an immune response to the tumor by injection of a gene therapy vector that will express a foreign HLA antigen directly into the tumor mass. HLA-B7 is the most common gene used in current protocols. A proportion of the tumor cells will take up the DNA and begin to express the foreign antigen. An immune response will develop against the tumor cells that express the foreign antigen. Simultaneously, it is hoped, an immune reaction will be generated to other foreign surface antigens expressed by the tumor cells and will generate a subsequent systemic response to tumor cells elsewhere in the body.[10] Preliminary reports suggest that this approach is associated with low toxicity. Some partial responses have been seen in patients with metastatic melanoma,[11] but similar studies have not been reported in bladder cancer.

Category 3: Insertion of a Suicide Gene Into Tumor Cells 

The rationale is to express genes in tumor cells that will selectively activate nontoxic prodrugs. Toxic metabolites will be generated and will cause tumor cell death. The most commonly used gene is that coding for the herpes simplex virus thymidine kinase (HSV-tk). Mammalian cells contain a thymidine kinase gene that is unable to phosphorylate a thymidine nucleoside base. However, the HSV-tk gene can phosphorylate a nucleoside base. Therefore, any cell into which the HSV-tk gene is transferred can phosphorylate the prodrug ganciclovir. This will generate abnormal nucleoside bases that enter and block the DNA synthesis pathway and result in cell death. A similar system using the enzyme cytosine deaminase, which selectively converts the antifungal 5-fluorocytosine to the antimetabolite 5-fluorouracil, also has been used in some gene therapy protocols.

The suicide gene system has been studied extensively as an approach to treat malignant brain tumors. Ongoing gene therapy trials consist of direct injection of the gene therapy vectors into the tumor mass or into residual tumor after incomplete resection and then followed by ganciclovir therapy. Incorporation of the gene into every tumor cell is not necessary for effective therapy with the suicide gene system; complete tumor responses have been reported in animals when less than 20% of the cells express the transferred gene. This phenomenon is known as the bystander effect.[12] However, the suicide gene system requires accurate targeting since gene expression in normal cells followed by exposure to ganciclovir will result in cell death. This problem has been addressed by placing the viral thymidine kinase gene under control of a tissue-specific (or preferentially a tumor-specific) promoter so that the gene will be expressed only in a select population of targeted cells. The alphafetoprotein promoter that is preferentially activated in hepatoma cells is an example of such an approach.[13] A preliminary report suggests that an adenoviral-mediated gene delivery of the HSV-tk gene into mouse bladder tumor (MBT-2) cells growing in a subcutaneous location resulted in significant inhibition of tumor growth.[14] The inability to selectively deliver the gene to tumor cells in vivo remains a problem with this approach in bladder cancer.

Category 4: Insertion of a Multidrug-Resistance Gene Into Bone Marrow Cells 

A major limitation of current chemotherapy regimens is bone marrow toxicity. The multidrug-resistance (MDR-1) gene codes for P-glycoprotein, a membrane-bound protein that acts as an energy-dependent drug efflux system. Thus, MDR-1 expression pumps chemotherapeutic drugs out of the cell and decreases intracellular drug concentrations to below the toxic threshold.[15] Transfer of the MDR-1 gene into the normal bone marrow cells of mice allows administration of higher doses of chemotherapy without the associated bone marrow toxicity.[16] Several clinical studies are underway to test the efficacy of bone marrow protection after MDR-1 gene transfer.

Although current trials do not target bladder cancer for this type of gene therapy, advanced bladder cancer is sensitive to chemotherapeutic regimens that include doxorubicin, vinblastine, and paclitaxel. These drugs are transported out of the cell by P-glycoprotein, the protein expressed by the MDR-1 gene.

Category 5: Tumor Suppressor and Anti-oncogene Therapy 

Loss of expression of tumor suppressor genes and overexpression of oncogenes are important factors in the initiation and progression of certain tumors. Gene therapy has the potential to replace lost tumor suppressor genes or to down-regulate the action of oncogenes. Thus, tumor suppressor genes and oncogenes are attractive targets for gene therapy. Clinical studies have been initiated in acute myelogenous leukemia, non-small cell lung carcinoma, and head and neck cancer to test the ability of reintroduction of the p53 tumor suppressor gene to inhibit tumor progression.

Tumor suppressor gene replacement therapy is a particularly promising method of treatment for human bladder cancer. Several studies have shown that loss of expression of the p53 and retinoblastoma (Rb) tumor suppressor genes is common in locally advanced bladder cancer and portends a poor prognosis.[17,18] Studies in our laboratory have demonstrated the feasibility of Rb replacement therapy for the treatment of bladder cancer in animal models.[19] Encouraging results have been seen with the use of a modified Rb gene that has a broad spectrum of antitumor activity by inhibiting cell growth in tumor cells that not only have lost expression of the Rb gene, but also maintain normal Rb expression.[20]

Gene Transfer Systems 

Gene therapy can be successful only when the gene is effectively transferred to the appropriate cell population. A number of systems can be used to achieve successful gene transfer in vitro, but successful gene transfer in vivo is more difficult to achieve. Despite advances in gene transfer technology in the past five years, gene transfer remains the Achilles' heel of gene therapy. Gene transfer techniques can be broadly divided into physical and viral methods (Table). This review focuses on cationic liposomes as an example of a physical method of gene transfer and retroviruses and adenoviruses as examples of viral methods of gene transfer.

Physical Methods of Gene Transfer 

Cationic liposomes, the most frequently used physical method of gene transfer, usually consist of a cationic amphiphile (eg, DOTMA, DOSPA, and DMRIE) and a neutral helper lipid (the most common being DOPE) that are sonicated to form small, unilamellar liposomes. The liposomes form complexes with DNA through charge interactions. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Intracellularly, the endosome is destabilized by the liposomes' releasing the DNA for transcription.[21] Advantages of using liposomes for gene therapy are the relative simplicity of the technology, the ability to effectively transfect a wide variety of tumor cells in vitro, and the low potential for both immunological reactions and contamination of the helper virus. Disadvantages include variable transfection efficiency, direct toxicity of the liposomes on target cells, and disappointingly low in vivo transfection efficiency.

Two approaches for liposome-mediated gene transfer are being used in current cancer gene therapy trials. (1) Liposomes are being tested for gene transfer of cytokine genes into tumor cells in vitro for gene-modified tumor vaccine therapy of metastatic melanoma (Category 1), and (2) liposomes are being used to transfer the HLA-B7 gene into melanoma tumor nodules in vivo by direct injection of DNA liposome complexes (Category 2). Liposome-mediated transfer of the IL-2 gene into bladder...
tumor cells in vitro to create a gene-modified tumor cell vaccine prolongs survival in a mouse model of bladder cancer.[22] Also, liposome-mediated transfer of tumor suppressor genes into the transitional cell epithelium of mice has been reported. However, gene transfer efficacy is low and variable, possibly due to the presence of glycoaminoglycans lining the transitional cell epithelium. Glycoaminoglycans are significant inhibitors of liposome-mediated gene transfer. We have attempted to improve gene transfer into transitional cell epithelium in vivo by chemically stripping the glycoaminoglycan layer with little success (JDS, Xu HJ, unpublished data).

**Viral Methods of Gene Transfer**

**Retroviral vectors** are modified RNA viruses in which the genes encoding for the retroviral proteins are removed and replaced by the gene to be transferred. As the normal viral genes are deleted, cells infected by the modified virus express only the transferred gene and are unable to generate further infectious virus. For this reason, retroviruses are useful for gene therapy as they are replication-defective retroviral vectors. They are produced in special packaging cell lines that contain the deleted viral genes integrated into their genome. The advantage in using retroviruses for gene therapy is the high efficiency of gene transfer and integration into dividing cells. The disadvantages of retroviruses are the inability to infect nondividing cells, the potential for helper virus (replication-competent virus) formation, and the possibility of cellular oncogene activation by insertional mutagenesis.[23]

Retroviral vectors were the first vectors approved for human gene therapy and have been used extensively as a gene transfer vector in many trials of cancer gene therapy. Retroviruses were used in the first human gene therapy trial to transfer a neomycin-resistant marker gene into tumor-infiltrating lymphocytes. Since that time, retroviruses have been used for the creation of gene-modified vaccines (Category 1), for HSV-1k gene transfer (Category 2), for drug resistance gene transfer (Category 4), and for replacement therapy for lost tumor suppressor genes (Category 5). Initial gene-modified vaccine experiments in mouse models of bladder cancer used retroviral gene transfer.[9]

**Adenoviral vectors** are modified adenoviruses (adenovirus type 5 is the most common vector currently in use) in which part of the E1A and E1B regions of the viral DNA has been replaced by the gene to be transferred. As the E1A and E1B regions are absent, cellular infection by the virus results in gene expression without viral multiplication. The virus will replicate only in a packaging cell line that has the complete E1A and E1B sequences incorporated into the genome. Advantages of adenoviral vectors include the ability to infect nondividing cells, the ease of preparation in a high titer, rare integration into the target cell genome, and effective gene transfer in vivo. The major disadvantage of adenoviral vectors is their induction of an immunological reaction that complicates repeated virus application.

Because of their high transduction efficiency, adenoviruses are being increasingly studied as gene therapy vectors. Currently, eight clinical trials (with one in most of the categories of gene therapy) use an adenovirus as the prime method of gene transfer. Adenoviral vectors can effectively transfer marker genes to the lining of the bladder in vivo and can transduce a variety of bladder tumor cells in vitro.[19,24] A preliminary report suggests preferential marker gene expression in rat transitional cell carcinomas compared with normal epithelium after intravesical injection of adenoviral vector.[25] Successful gene therapy for bladder cancer has been reported in a variety of murine bladder tumor models following adenoviral gene transfer.

**Future Directions**

The ideal cancer therapy would selectively eliminate cancer cells without damaging surrounding tissue. To achieve this goal, a gene therapy vector will need to (among others things) concentrate in the tumor, be selectively activated in the tumor, selectively kill tumor cells, have no adverse effect when expressed in normal cells, and be deactivated once the therapeutic goal is achieved. Bladder tumors have been reported to overexpress the epidermal growth factor receptor alpha 6 beta 4 integrin, and autocrine motility factors.[26] Targeting gene therapy vectors to these receptors may allow selective gene accumulation in bladder cancer cells. The uroplakin gene, which codes for a transitional cell epithelium membrane protein, has been reported to be selectively expressed in bladder epithelium. The promoter region of the uroplakin gene has been recently cloned.[27] The use of a uroplakin promoter in gene therapy constructs may allow selective gene expression in transitional cell carcinoma. Loss of expression of tumor suppressor genes is important in the genesis and progression of bladder cancer. Thus, identification of tumor suppressor genes lost in specific tumors may allow selective gene reconstitution and cause cell death in tumor cells but not in cells normally expressing the gene. Several inducible promoter systems have been described that allow selective gene activation and inactivation.[28] Inclusion of such promoter systems in the gene therapy vector may allow selective control of the therapeutic effect. The development of effective gene therapy for bladder cancer is dependent on the combination of these and other elements in the gene therapy constructions.

**Conclusions**

The first trial of gene transfer in humans was initiated in 1989. In 1996, 51 active trials of cancer gene therapy are being conducted in the cytokine/immunotherapy area alone. The technology of gene therapy has progressed remarkably over the past seven years. However, significant problems must be overcome before gene therapy becomes a standard part of the oncologist’s armamentarium. As the molecular biology of cancer is unraveled and as biotechnology improves, the identification of new gene targets and the development of new vector systems will allow accurate and effective gene therapy of cancer.

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**References**


