Thoracic and Cardiovascular Surgery

Gene Therapy for Lung Cancer

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Over the past three decades, the molecular biology of lung cancer has been progressively delineated. Concurrently, gene therapy techniques have been developed that allow targeting or replacement of dysfunctional genes in cancer cells, such as activated tumor-promoting oncogenes, inactivated tumor-suppressing, or apoptosis-promoting genes. This article will review the therapeutic implications of molecular changes associated with non-small cell lung cancer and the status of gene therapy.
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It is recognized that novel, systemically active therapeutic agents are needed to improve current cure rates for non-small cell lung cancer (NSCLC). In addition, the utilization of less toxic agents would allow for more comprehensive and effective treatment of lung cancer patients. This article will review the status of gene therapies for treatment of NSCLC.

Gene Therapy Strategies

The design of any successful cancer gene therapy protocol requires the identification of the correct gene to be transferred, the gene delivery method, and the target cells to be modified. Several technical issues must be considered, such as transduction efficiency, specificity of cell targeting and gene expression, level and duration of gene expression, effect on normal cells, and ability to establish systemic immunity. While the overall goal is to eradicate all malignant cells, low transduction efficiency may be acceptable if either a local bystander effect or systemic immunity can be achieved.[1,2]

The specificity of cell targeting and gene expression is important when the transferred genes would be toxic to normal cells, especially during systemic administration. A high level of gene expression may be more important for secreted gene products than for ones that remain within the tumor cells. Transient gene expression may also be acceptable if the duration of gene expression exceeded the time period required to kill all tumor cells. Toxicity to normal cells may be avoided with the use of tumor-specific promoters. Finally, achievement of systemic tumor immunity, for example, by stimulation with cytokine-producing tumor cells, may produce a more durable antitumor effect than strategies with antisense constructs or with suicide genes.

Choice of Target Cells

The possible target cells include not only the tumor cells and the immune cells but also surrounding normal tissue. Gene therapy of tumor cells could result in correction of their abnormal growth and reestablishment of apoptosis, or in increased drug or radiation sensitivity of the tumor cells. Gene modification of tumor cells could also enhance their immunogenicity. Immune cells, such as dendritic cells (DCs), could be gene-modified to increase their capacity to induce lung-cancer-specific T-cells. Clinical trials of gene therapy for lung cancer showed the feasibility of delivering a variety of agents as well as highlighted problems with the delivery of therapeutic constructs, which have caused some to consider initial results of these novel therapies to be disappointing but underscored the complexity of these approaches and the likelihood that these approaches will be effective only when used in a coordinated fashion in the proper clinical context.[3]

To improve tumor-specific transgene expression, tissue-specific and tumor-specific promoters and enhancers can be used to control transgene expression. For example, survivin is expressed in 81% of NSCLC tumors but not in normal lung tissues.[4] The survivin promoter, driving the luciferase gene, was more highly activated in cancer cell lines than in normal and immortalized normal cell lines and, when delivered intravenously by DNA:liposome complexes, more than 200 times more cancer-specific than the cytomegalovirus promoter in vivo.[4] In a novel lung cancer system, human telomerase reverse transcriptase promoter and human surfactant

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protein A1 promoter showed much higher promoter activity in lung cancer cells compared with other kinds of cancer and normal lung cells, including stem cells.[5] Moreover, insertion of negative glucocorticoid responsive elements in the system allows it to be drug controllable.

Physical Delivery Methods

Various physical and biological methods are available to deliver foreign genes into target cells. The delivery method chosen depends on the local, regional, or systemic route of administration chosen or needed to reach the tumor.[6] Physical methods, such as calcium phosphate precipitation, electroporation, direct microinjection, and particle bombardment (or "gene gun"), may be suitable for introducing naked DNA into established cell lines in vitro, but are generally of low efficiency and are often impractical for in vivo applications. Nevertheless, gene delivery to lung tumors by aerosolization of adenoviral (Ad) vectors incorporated into calcium phosphate precipitates resulted in much greater expression in tumors than in normal lung tissue.[7]

Liposomes, composed of cationic lipids, envelope DNA and allow for more highly efficient DNA entry on fusion with the cell membrane both in vitro and in vivo. Transgene expression using an improved liposomal formulation was significantly increased in human tumor cells in vitro, due to increased uptake of the liposomal–DNA complex by tumor cell phagocytosis, compared with normal human cells, and was also greater in lung tumors than in surrounding normal tissues in vivo.[8] An innovative alternative for high-efficiency gene transfer into specific cells exploits the affinity of certain ligands for cell-surface receptors. The gene, conjugated to the ligand to make a ligand–DNA complex, enters the cell on internalization of the ligand–receptor complex. However, the internalized conjugates tend to be trapped within the endosomes and are rapidly degraded. More recently, polylysine–DNA conjugates that also incorporate Ad capsid proteins, which have endosomolytic activity to allow the DNA to escape degradation and enter the nucleus to be expressed, resulted in fourfold higher reporter gene expression in up to 99% of cells both in vitro and in vivo.[9]

Biological Delivery Methods

Biological vectors in the form of genetically modified, replication-defective viruses exploit their natural tropism for mammalian cells and biological life cycles to achieve much more effective gene transfer and gene expression. Retroviral vectors can infect a variety of cell types and integrate into the target cell genome. However, because retroviral-mediated transduction might result in permanent integration of the foreign gene into the target cell, the promoter used to drive the transcription of the foreign gene must be carefully selected. Retroviral vectors, however, are generated at low titer, infect only dividing cells with low-transduction efficiency, and result in variable expression levels. The addition of a selectable marker, such as the hygromycin resistance gene, during retrovirus vector construction is useful in vitro by allowing selection of transduced cells despite the low gene transfer efficiency of retrovirus vectors. The ability for retroviruses to transfer genes only into dividing cells, such as tumor cells, may protect surrounding quiescent normal cells when used in vivo, but the proportion of actively dividing tumor cells within a given tumor during retroviral vector administration would likely be small.[10]

Low gene transfer due to a combination of factors in vivo is the most substantial hurdle in the practical application of gene therapy. Adeno-associated virus (AAV) vectors, small parvoviruses that are ubiquitous but not pathogenic to humans, are able to frequently integrate multiple concatemeric copies of itself into the target cell genome. These AAV vectors are trophic for a wide variety of cell types and have successfully transduced lung cancer cells in vitro.[11] However, the full potential of these vectors for in vitro and in vivo gene transfer has not been determined. Many current AAV vector generation methods are cumbersome and require the development of packaging cell lines and better purification techniques, which could make AAV the vector of choice in future gene therapy trials. A variety of other viral vectors, such as lentiviral vectors based on the human immunodeficiency virus type 1 (HIV-1) and the vaccinia virus, are also under investigation; however, viral-mediated cancer gene therapy is limited by the inability to deliver viral vectors to every tumor cell and is limited to treating localized tumors due to host-immunity against the gene delivery vector and the transgene.[12,13] Therefore, there is a tremendous effort to develop and test alternate gene delivery vectors that are efficient, nonimmunogenic, and applicable for systemic therapy.

Choice of Therapeutic Gene

Therapeutic genes include the following classes: antisense RNA interference (RNAi); tumor suppressor gene replacement; suicide genes; cell-surface antigens; cytokine genes; and multiple drug-resistance genes. Oncogene inhibition or tumor suppressor gene replacement could correct the abnormal malignant phenotype. Suicide genes would provide transduced tumor cells with enzymatic machinery to convert otherwise nontoxic substances into toxic metabolites. Similarly, the transferred gene could render drug-resistant tumor cells more sensitive to cytotoxic drugs. Delivery of genes that encode tumor-specific antigen, major histocompatibility, adhesion, costimulatory, or cytokine molecules would result in better tumor/immune cell interaction and in stimulation of the immune response.

Oncogene inhibition therapy can be performed using antisense, RNA interference (RNAi)–ribozyme sequences against oncogene transcripts. The most common approach to inhibit endogenous oncogene expression is to introduce into tumor cells either single-strand antisense oligonucleotides (ASO) or else plasmid or viral vectors containing cDNA constructs, which encode for single-strand antisense RNA molecules, that are complementary to oncogene (sense) mRNAs.[14,15] The antisense and sense sequences then bind, which inhibits translation and mutant protein production by blocking ribosome access to the mRNA or by bringing about