Gene Therapy for Lung Cancer

Lung cancer is the leading cause of cancer death in American men and women, and with current trends in cigarette smoking among teenagers, there is little doubt that it will remain unchallenged as the leading cause for the foreseeable future.

Only 13% of people who develop lung cancer survive 5 years. This poor survival can be explained by the high frequency of advanced disease discovered at diagnosis, meaning that surgery, the best hope of a curative intervention, is not an option for these patients. Other treatments, including radio- and chemotherapy, are not satisfactory for most patients and offer only palliation.

In recent years, our improved understanding of tumor biology has formed a foundation for the development of new therapies that exploit the molecular mechanisms of cancer formation. These biological therapies, including gene therapy, have the potential to overcome some of the limitations of standard cancer therapies and ultimately may lead to more specific and effective treatment.

The lung has several advantages as a target organ for gene therapy. The availability of effective imaging techniques allows precise tumor localization, and tumors often are accessible for safe direct injection either through the chest wall or via the bronchial tree during bronchoscopy. The lung also is highly vascular, receiving all of the cardiac output, which permits rapid distribution of agents delivered by the intravenous route. Alternatively, gene therapy agents could be inhaled and thus deposited on the surface of the bronchial tree, an ideal route for interventions to target premalignant lesions.

The desirable features for a cancer therapeutic agent include specific and high-level delivery to cancer cells, effective spread through tumor tissue, specific killing of cancer cells, and induction of an immune response against cancer cells. Several different gene therapy approaches have been investigated to these ends for the treatment of lung cancer.

Although many vector systems, including retroviruses, herpesvirus, adenovirus-associated virus, and nonviral vectors, are being investigated to deliver genes, adenoviral vectors are currently the preferred system for gene therapy applications.They offer ease of production and modification, a relatively high efficacy of gene transfer (compared to other vectors), as well as a highly favorable safety profile.

To achieve specific delivery, cancer cells must selectively express a receptor or surface molecule that can be targeted. Several methods to form a link between the adenovirus capsid and a specific receptor or surface molecule on the target cell have been explored. These approaches have included the use of bi-specific (specificity to virus and target) chemical conjugates, fusion proteins, as well as polymers that link viral particles to specific ligands.

Adenovirus capsid proteins are amenable to genetic modification to change the tropism of the virus from native liver to cancer cells. It is probable that this "genetic" retargeting will prove to be advantageous. Although this area of gene therapy has not been extensively exploited for lung cancer, potential targets with selective expression on cancer cells include the epidermal growth factor receptor for non-small cell lung cancer and NCAM-1 for small cell lung cancer.

To achieve specific cancer cell killing, the approaches studied have included the use of "suicide genes" that can activate prodrugs and the exploitation of deranged molecular pathways in cancer cells.

Suicide gene therapy has shown encouraging preclinical results using genes that encode an enzyme to convert a nontoxic prodrug into an active tumoricide. Under conditions that facilitate infection of most cells, this approach has been quite effective. In addition, there is bystander effect on surrounding nontransduced cells.

However, for in vivo treatment of established tumors with this approach, gene delivery is dependent on injection into or around the tumor tissue, a limitation that may result in insufficient transduction efficiency. Clinical trials in which an adenovirus expressing herpes simplex virus thymidine kinase (HSV-tk) was directly delivered into the pleural space of patients with mesothelioma demonstrated that first-generation adenoviral vectors are not capable of infecting a sufficient amount of the tumor mass to be clinically effective.

Many genes and cancer pathways are being investigated to enable more targeted therapy, but most data exist with *p53*. The central role of p53 protein in cell cycle control, DNA repair, and, in particular, induction of programmed cell death (apoptosis) has been known for a number of years. In virtually all lung cancers, the p53 pathway is nonfunctional, either

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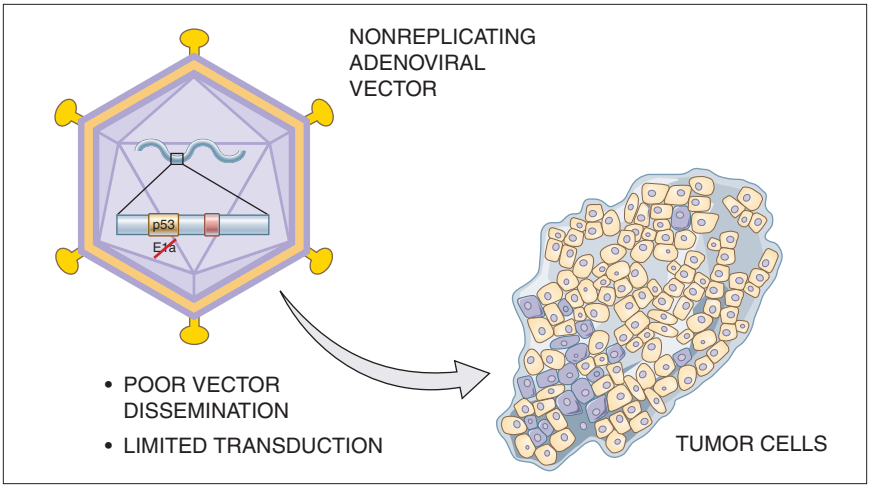
Early constructs of the p53-expressing adenoviral vectors inserted the p53 gene in place of the viral replication gene *E1a*. In human trials, this nonreplicating vector showed successful gene transfer, but the treatment response was minimal. The lack of efficacy probably was due to poor dissemination of vector through the tumor mass and poor transduction efficiency.



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by mutation of *p53* itself or by p53 inactivation through overexpression of mdm2 (an E3 ubiquitin ligase).

It has also been established that expression of p53 in normal cells is nontoxic, whereas restoration of p53 function in cancer cells leads to cell death. Thus, p53 is an obvious target for gene replacement therapy, and studies with nonreplicating first-generation adenoviral vectors expressing p53 have advanced into clinical trials.

In preclinical testing, these vectors successfully transduced and lysed tumor cells. In several clinical trials in patients with lung cancer, single-agent treatment with direct injection of vector into the tumor demonstrated very little toxicity and evidence of *p53* gene transfer. However, the treatment response was minimal. The lack of efficacy was most likely related to the poor tumor cell accessibility and poor transduction efficiency seen with current replication-defective adenoviral vectors.

Inactivation of *p53* in cancer cells leads to resistance to conventional chemo- and radiation therapies, a phenomenon that provides strong rationale for combined treatment of *p53* replacement gene therapy with conventional treatment. A recent single-arm phase 2 study combining radiation with *p53* gene replacement therapy showed some tumor response. However, a larger phase 2 study failed to show an advantage of combined chemotherapy plus *p53* gene replacement therapy compared with chemotherapy alone. The lack of efficacy again was most likely related to poor tumor cell accessibility and transduction efficiency seen with current replication-defective adenoviral vectors.

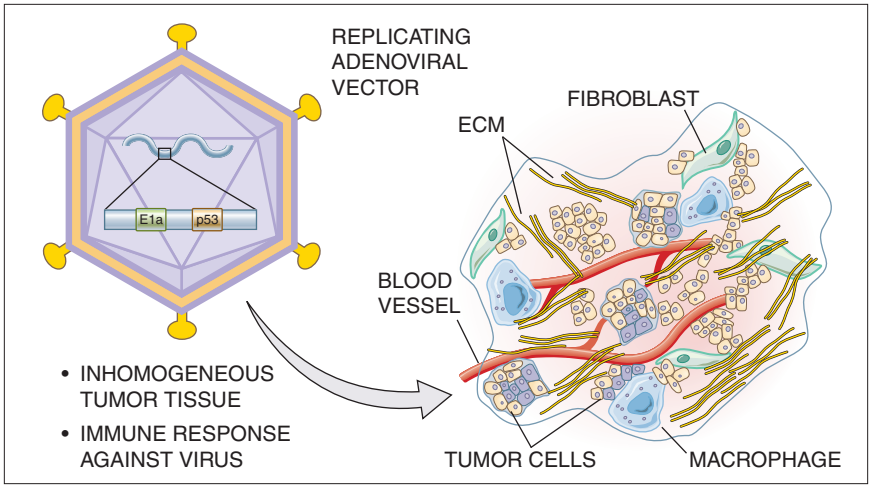
In attempts to overcome the limited delivery and dissemination of first-generation adenoviral vectors to tumor tissue, replicating vectors have been studied. Replication-competent adenoviral vectors have the capacity to multiply up to a thousand-fold in the target cell, induce cell lysis, and then spread to neighboring tumor cells. However, a major consideration when using replicating viruses is to restrict viral replication and toxicity to tumor cells. This concern has been addressed in two main ways, by transcriptional targeting and by the mutation of viral genes that are essential for the viral life cycle in normal cells but not in tumor cells.

By replacing the promoter of essential viral genes with a promoter element from a gene that is selectively expressed in cancer cells, it is possible to achieve tumor-specific transcription (and therefore replication and toxicity). No lung cancer-specific promoters have been identified so far, but several promoters that are active in many cancer types, such as the telomerase promoter or the E2F promoter, can be applied to lung cancer.

In addition, several promoters, including those driving the expression of surfactant protein A and B which are highly active only in normal lung cells, have been investigated in preclinical studies. A clinical trial targeting viral replication to osteogenic sarcoma metastases in the lung using an osteocalcin promoter driving *E1a* is underway. This strategy using various promoter elements also has been investigated in clinical studies in prostate cancer and hepatoma.

Various viral-deletion mutants that selectively exploit the cancer cell phenotype have been investigated for their tumor-specific oncolytic properties. There is a striking duplication of function in adenoviral-infected cells and cancer cells. In both, the Rb and p53 pathways are inactivated. Viruses that have a deletion of the gene that inactivates p53 (*E1b-55kD*) or that contain a mutation in the gene that inactivates Rb (*E1a*) are anticipated to be attenuated in normal cells. In contrast, in cancer cells with inactivated Rb and/or p53 pathways, the function of these viral genes may be dispensable, and such deletion mutants may therefore replicate selectively in cancer cells.

A virus that combines a lung-tissue-specific SP-B promoter with a deletion in the adenoviral *E1a*



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Replication-competent viruses preserve the E1a region, and they have the potential to multiply several hundred-fold within a tumor cell and then spread to other tumor cells. Tumors, however, are not composed of just tumor cells, as was believed until several years ago (*see opposite figure*). The complexity of tumor tissue poses hurdles for efficient viral spread.

gene has shown promising results in preclinical investigations to target lung cancer. Early clinical trials with replicating adenoviral vectors in a variety of cancers have demonstrated a good safety profile, yet thus far, significant efficacy has only been observed in combination with chemotherapy.

A variety of other strategies are being investigated to improve the oncolytic potency of replication-competent adenoviruses. Suicide genes have been incorporated into oncolytic viruses, with mixed results. Furthermore, *p53* replacement therapy has been combined with the advantages of a replication-competent vector system. It has also been demonstrated that the deletion of a viral gene that codes for an anti-apoptotic protein (*E1b-19K*) enhances the efficacy of the virus.

Contrary to initial expectations and despite the strong replicative capacity of the virus, it appears that spread of replicating virus in established tumors is limited. Expression of the primary adenoviral receptor (CAR) is low in several advanced tumors, potentially limiting the oncolytic potency and spread of the virus. Thus, capsid modifications to achieve CAR-independent infection have been under very active study in preclinical investigations; clinical trials have been initiated for other cancer types.

However, other factors that may limit viral spread, such as non-malignant tumor-supporting tissue, hypoxic conditions within solid tumors, and an activated immune response against the virus, also must be addressed to develop the full therapeutic potential of oncolytic viruses.

Gene therapy to induce specific antitumor immunity has the theoretical advantage of producing a systemic effect, a prerequisite for effective therapy for most lung cancers at the time of clinical presentation. Unfortunately, unlike the case with malignant melanoma, the relevant immunologically active tumor antigens in lung cancer are poorly defined.

Strategies to enhance antitumor immunity can be divided into ex vivo and in vivo approaches. One example of ex vivo immuno-gene therapy is the use of autologous tumor cells that are genetically modified ex vivo to express GM-CSF. GM-CSF is a cytokine that enhances antigen presentation and immune cell activation. Irradiated GM-CSF-expressing cells are then re-administered to the patient.

In a recent phase 1 clinical trial in patients with metastatic lung cancer, this approach resulted in the induction of anti-tumor immunity in the majority of patients and stable disease in a subgroup. A variety of other immune stimulatory cytokines, such as IL-2 and IL-7, also have been used to genetically modify autologous tumor cells, dendritic cells, or tumor-infiltrating lymphocytes.

In vivo immuno-gene therapy approaches, such as direct intratumoral injection of vector-encoded immune-stimulatory cytokines, have not yet shown effectiveness in lung cancer. However, preclinical research has validated the concepts of this strategy. For example, intracavitary IFN-β gene therapy in combination with intratumoral CD40 ligand gene therapy using adenoviral vectors has resulted in strong $CD8^+$ T-cell-mediated antitumor effects in murine models of mesothelioma.

In summary, gene therapy for lung cancer is a very active field of research. Proof of principle has been provided, but distribution of the therapeutic agent to all cancer cells remains a problem. Rapid progress in the areas of transduction targeting, immuno-gene therapy, and the use of replication-competent viruses may yet lead to substantial improvements in efficacy.

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