Gene Therapy for Lung Cancer and Mesothelioma*

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The idea of using gene therapy began with the straightforward paradigm of treating genetic diseases in which one gene was missing. Although this strategy seems conceptually simple, it has proved to be difficult to implement in practice because the vectors currently available have been unable to provide sufficient levels of gene product over long enough periods of time. Accordingly, most current gene therapy trials are aimed at acquired diseases (such as cancer) in which long-term gene expression is not necessarily required. Recent advances in the understanding of growth factors, molecular oncology, and tumor immunology have provided the rationale for several strategies (Table 1). Many of these approaches have been applied to thoracic malignancies in animal and/or human trials.

STRATEGIES FOR ANTICANCER GENE THERAPY

Molecular Chemotherapy

One of the most well-developed approaches to the treatment of cancer using gene therapy is based on the use of “suicide genes” in which DNA that encodes an enzyme capable of generating a toxic metabolite is transferred to tumor cells followed by administration of the nontoxic enzyme substrate. The two systems that are currently most well developed use the bacterial cytosine deaminase gene and the herpes simplex thymidine kinase gene (HSVtk). The gene-encoding cytosine deaminase converts 5-fluorocytosine to the cytotoxic antimetabolite fluorouracil (5-FU). The HSVtk gene converts the normally nontoxic nucleoside analog ganciclovir (GCV) into a monophosphorylated form that is then converted by the normal mammalian thymidine kinase enzymes into a triphosphorylated form that is extremely toxic and leads to cell death.

Both of these approaches are bolstered significantly by the presence of the “bystander effect,” a phenomenon that involves the transfer of toxic metabolites from transduced cells to nontransduced cells allowing for amplification of cytotoxic activity. Enhanced immune responses to the dying cells may also play a role in vivo. Thus, relatively complete cell killing has been observed with as few as 10% of the tumor cells expressing the HSVtk gene or with only 2 to 10% of cells using the cytosine deaminase gene.

The use of suicide genes is currently limited to treatment of localized malignancies in which reasonable levels of gene transfer can be expected. A number of approaches have been used to introduce suicide genes into tumors, including injection of “producer” cell lines that secrete retrovirus containing the suicide gene, systemic injection of retroviral vector, direct intratumoral injection of the suicide gene complementary DNA (cDNA), or direct injection of an adenoviral vector containing the suicide gene (see below). Injection of producer cell lines that secrete retrovirus containing the HSVtk gene followed by treatment with GCV has been used successfully in animal models of brain tumor, ovarian cancer, and hepatic carcinoma (reviewed by Moolten). The use of the HSVtk/GCV system has been explored more recently using an adenoviral vector to directly transduce malignant mesothelioma, lung cancer, colon carcinoma, hepatocellular carcinoma, brain tumors, and melanomas.

Because of its localized nature, extremely poor prognosis, and lack of effective therapy, malignant mesothelioma has been identified as a reasonable target for localized thoracic cancer gene therapy. Replication-deficient adenovirus efficiently transduced mesothelioma cells both in tissue culture and in animal models, and infection with an adenovirus containing the HSVtk gene driven by the Rous sarcoma virus promoter (AdRSVtk) rendered human mesothelioma cells sensitive to doses of GCV that were 2 to 4 logs lower than the doses required to kill cells infected with control virus. Based on these results, the AdRSVtk vector has been used to treat established human mesothelioma tumors and human lung cancers growing within the peritoneal cavities of severe combined immunodeficient (SCID) mice. Following GCV therapy, macroscopic tumor was eradicated in 90% of animals and microscopic tumor was undetectable in 80% of animals. Tumor reduction was accompanied by a significant increase in survival. Marked decreases in tumor size have also been seen in a rat model of syngeneic mesothelioma cells growing in the pleural space; however, survival increases have been more modest in this model.

Based on these studies, a phase I clinical trial for patients with mesothelioma began in November 1995 at the University of Pennsylvania Medical Center in conjunction with Penn’s Institute for Human Gene Therapy. The purpose of the phase I trial was to determine the maximal tolerated dose of AdRSVtk virus instilled into the pleural space, to evaluate the biological effects of therapy, and to evaluate, in preliminary fashion, any response rate. The eligibility criteria for this trial are detailed in Table 2. The protocol is summarized in Table 3. Patients with a presumed diagnosis of malignant mesothelioma had a chest tube placed and the virus instilled into the pleural space. Three days after viral instillation, another biopsy specimen was obtained and IV GCV therapy was started for 14 days. The patients were carefully evaluated for evidence of toxic reactions, viral shedding, immune responses to the virus, and radiographic evidence of tumor response.

Currently, eight patients have been treated: three patients at the initial dose of virus (1×10^5 plaque-forming units [pfu]), the next two at 1×10^5 pfu, and the last three at 1×10^6 pfu. Although analysis is still ongoing, clinical
Table 1—Gene Therapy Approaches for Treatment of Cancer

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Molecular Mechanism</th>
<th>Examples of Therapeutic Gene*</th>
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<tr>
<td>Molecular chemotherapy</td>
<td>Delivery of enzyme capable of generating toxic metabolite</td>
<td>Herpes simplex thymidine kinase bacterial cytosine deaminase</td>
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<td>Genetic immunopotentiation</td>
<td>Augmentation of immune response to tumor</td>
<td>Cytokines (IL-2, GM-CSF, etc)</td>
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<td>Mutations compensation</td>
<td>Inhibition of dominant oncogenes; augmentation of tumor suppressors; disruption of autocrine loops</td>
<td>Foreign MHC class I</td>
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<td>Normal tissue protection</td>
<td>Augmentation of resistance of bone marrow to chemotherapy</td>
<td>Costimulatory molecules (B7)</td>
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<td>Replicating virus</td>
<td>Replication restricted virus capable of lysis tumor cells</td>
<td>Antisense TGF-β</td>
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<td></td>
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<td>Antisense K-ras</td>
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<td></td>
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<td>Wild-type p53</td>
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<td></td>
<td></td>
<td>Intracellular single-chain antibody</td>
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<td></td>
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<td>Fragment directed against erb B2</td>
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*GM-CSF = granulocyte macrophage colony-stimulating factor; MDRI = multidrug-resistant 1.

Toxicity has been limited to fever (maximum temperature of 39.4°C) approximately 12 h after instillation of the virus and asymptomatic and transient increases in levels from liver function tests. Gene transfer has been visualized by in situ hybridization in a small percentage of cells in one of the patients receiving the high dose of virus. High titers of neutralizing antibodies and CD4+ T-cell proliferative responses to adenoviral structural proteins have been observed within 1 to 2 weeks of viral administration in both blood and pleural fluid. Clinical observation is still ongoing; however, there have been no deaths or evidence of tumor progression noted so far.

**Genetic Immunopotentiation**

Using gene therapy to augment the immune response to tumors is another active area for cancer gene therapy research. This strategy is based on two principles: first, that the immune system has the capacity to recognize and destroy tumor cells through diverse mechanisms (ie, cytotoxic T lymphocytes, natural killer cells, macrophages, and eosinophils), and second, that the tumor cells have somehow escaped from immune surveillance. Based on these principles, a number of approaches have been taken (Table 1), including the following: (1) introduction of various cytokine genes into tumor cells with the intent of stimulating T-cell and natural killer cell proliferation and activity, augmenting antigen presentation, or attracting effector cells; (2) introduction of genes to augment major histocompatibility complex (MHC) class I antigen presentation or direct introduction of foreign MHC class II molecules; (3) introduction of genes that provide costimulatory signals to augment T-cell proliferation; (4) use of agents that inhibit the production of suppressive factors (such as transforming growth factor-β [TGF-β] or interleukin [IL]-10); and (5) active vaccination with tumor—specific” antigens. In most studies to date, tumor cells have been transfected with the gene of interest in culture and then reinjected into the host animal. More recent protocols have attempted to directly transduce tumor cells in situ.

Although much of the initial work with immune modulation has focused on malignant melanoma, a tumor considered traditionally to be “immunogenic,” this approach has been applied to lung cancer and mesothelioma. Evidence exists that IL-219 and the combination of IL-2 and tumor necrosis factor acetylates may induce an immune response to murine lung carcinoma. Schemes using vaccination with plasmid DNA encoding human carcinoembryonic antigen.

Table 2—Inclusion Criteria for Penn Phase I Trial of Gene Therapy for Mesothelioma

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<td>1. All patients must have histologically proved malignant mesothelioma of pleural origin that cannot be surgically resected.</td>
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<td>2. Patients must have measurable or evaluable disease.</td>
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<td>3. Patients must have Eastern Cooperative Oncology Group performance status of 1 or 2.</td>
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<td>4. Patients must have a WBC count of ≥4,000/mm³ and platelet count of ≥100,000/mm³.</td>
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<td>5. Patients must have a BUN &lt;30 mg/dL, creatinine &lt;1.3 mg/dL, and bilirubin &lt;2.0 mg/dL.</td>
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<td>6. Patients may not have received prior chemotherapy or gene therapy.</td>
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<td>7. Patients may not have received prior radiotherapy.</td>
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<td>8. Patients may not have undergone treatment of a pleural effusion with placement of a chest tube and sclerosis.</td>
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<td>9. Patients must give written informed consent.</td>
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<td>10. Pretreatment FEV₁ must be &gt;2.0 L and patient must be medically able to undergo pulmonary resection.</td>
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<td>11. Patients with a history of another malignancy within the last 5 years, other than basal or squamous cell carcinoma of the skin, or cervical in situ carcinoma will be excluded.</td>
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<td>12. Patients with brain metastasis at time of study are ineligible.</td>
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<td>13. Patients with a serious active infection or other serious illness are ineligible.</td>
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<td>14. Patients who are pregnant or lactating or fertile men or women who are not using effective contraception are ineligible.</td>
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<td>15. Patients with HIV infection are ineligible.</td>
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Table 3—Clinical Protocol for Penn Phase I Trial of Gene Therapy for Mesothelioma

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<td>Day 1. Patients with a presumed diagnosis of malignant mesothelioma are admitted to the Clinical Research Center and have a video thoracoscopy during which a chest tube is placed and biopsy specimens are taken to confirm the diagnosis of mesothelioma and to assess disease extent.</td>
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<td>Day 2. Adenovirus containing the HSVtk gene is instilled via the chest tube and the tube removed.</td>
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<td>Day 5. Patients undergo a second video thoracoscopy in which biopsy specimens are taken to evaluate the degree of inflammation and the efficiency and extent of gene transfer. Days 6-20. IV GCV therapy is begun at a dose of 5 mg/kg bid for 14 days.</td>
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<td>Day 21 and onward. The patients are discharged from the hospital, followed up closely as outpatients with clinical examinations, hematologic and biochemical blood testing, chest radiographs, and CT scans.</td>
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Erb B2: Aberrant expression of erb B2 (also called Her-2 or neu), a 185-kd transmembrane protein kinase receptor with homology to the epidermal growth factor receptor, has been shown to contribute to malignant transformation and progression in a number of epithelial-derived carcinomas, including breast, ovary, GI tract, and lung. As an alternative to using antisense constructs to downregulate this gene, Deshane et al have recently described a strategy in which a vector encoding for an intracellular single chain antibody fragment directed against erb B2 has been transfected into tumor cells. Production of this antibody fragment within the cell appears to prevent cell surface expression of the erb B2 receptor and inhibit cellular proliferation.

p53: One of the most intriguing, but as yet mysterious, approaches to cancer gene therapy involves introduction of the p53 tumor-suppressor gene into tumor cells. Loss or mutation of p53 is a common genetic abnormality in non-small cell lung cancer. These changes may be extremely important because the loss of normal p53 or the presence of mutated p53 (which acts in a dominant negative fashion) can have a number of effects on the malignant process, including prevention of normal apoptotic control, genetic instability leading to increased frequency of mutations, decreased sensitivity to chemotherapeutic agents, dysregulation of angiogenesis, and direct influences on normal growth control genes. The thoracic surgery group at the MD Anderson Cancer Center, headed by Jack Roth, MD, has conducted a series of experiments using wild-type p53 constructs delivered by retroviral or adenoviral vectors to affect tumor cell growth.

Transduction of wild-type p53 into lung cancer cells lines with deleted or mutated p53 increased sensitivity to the antitumor drug cisplatin. Interestingly, introduction of p53 via retroviral supernatant into animals with preinjected intratracheal tumor cells led to marked suppression of tumor growth in the animals. In contrast, direct injection of an adenoviral construct containing wild-type p53 into lung cancer cells growing as subcutaneous tumor nodules had little effect on tumor growth when administered alone, but induced marked tumor suppression when coupled with cisplatin therapy.

Since mutant p53 works in a dominant negative fashion, it remains unclear how in vitro transfection of only a fraction of tumor cells with wild-type p53 leads to such strong antitumor effects. On the basis of mixing experiments, Cai et al suggest that there may be a “bystander effect” by which supernatants from transduced cells somehow inhibit growth of nontransfected cells. In experiments performed in my lab, using an adenoviral vector expressing p53, no such bystander effect can be detected in vitro, despite experiments showing a powerful antitumor effect in a model of human malignant mesothelioma in SCID mice in which only a relatively small fraction of tumor cells are transduced. One possibility is that induction of wild-type p53 stimulates release of inhibitors of angiogenesis such as thrombospondin. In any case, human trials using retrovirus and adenovirus-mediated gene transfer of the p53 gene in conjunction with cisplatin chemotherapy have been approved by the RAC and initiated.

Mutational Compensation

Although the molecular basis of thoracic malignancies is complex, data exist that suggest it may be possible to specifically correct or alter some of the oncogenes or tumor-suppressor genes that contribute to the malignant phenotype with therapeutic efficacy. Investigators with interests in lung cancer have initially focused on the oncogenes K-ras and erb B2 and the tumor-suppressor gene p53.

K-ras: Approximately one third of lung adenocarcinomas have mutations in the K-ras gene, and constitutive expression of an activated form of this protein in these cells is thought to contribute to the malignant phenotype. Antisense constructs of K-ras have therefore been introduced into a non-small cell lung cancer cell line expressing mutant K-ras in which they inhibited cell proliferation and tumorigenicity in nude mice. Despite the reported low efficacy of in vitro gene transduction with retroviral supernatants, injection of a retroviral vector expressing antisense K-ras was reported to induce a marked reduction in tumor size and number of tumors in nude mice with established tumors nodules created by intratracheal inoculation of human lung cancer cells. A clinical protocol for the use of antisense K-ras using a retroviral vector has been approved by the US Food and Drug Administration for a clinical trial in patients with non-small cell lung cancer.
Normal Tissue Protection

Investigators have approached some malignancies by the introduction of genes that may protect normal bone marrow cells from chemotherapy or radiation. Examples include transfer of the multidrug-resistant 1 (MDRI) gene into bone marrow stem cells and manganese superoxide dismutase into lung epithelial cells. This may be a less useful approach in lung cancer since this tumor is resistant to most chemotherapeutic agents. The use of adenovirus containing a toxin gene to selectively infect and kill cancer cells in bone marrow to be used for autotransplantation has also been demonstrated in vitro.37 Again, this may become useful if bone marrow transplantation becomes a viable option in the treatment of lung cancer.

Future Approaches

A number of major challenges face gene therapy for thoracic malignancy today. The first is the incomplete understanding of the molecular pathogenesis and immunobiology of lung cancer and mesothelioma. Discoveries about the basic nature of oncogenesis and tumor immunology, as well as more focused studies on how these alterations specifically relate to thoracic oncology, will be needed to identify ideal therapeutic target genes. The second major challenge is that of optimal gene delivery. Current vectors are quite limited in their ability to transduce all (or most) tumor cells in a localized malignancy and are even less capable of efficiently delivering genes to disseminated tumors. One way around this problem is the development of more effective immunotherapy. Another approach, especially the treatment of localized disease, is the use of genes that engender stronger bystander effects.

A more radical strategy is the development of replicating viral vectors.38 A number of clinical trials using such viruses were conducted in the 1950s and 1960s with some success. Interest in the use of viral-based cancer therapy waned thereafter because of the reliance on naturally occurring viruses that were limited in their efficacy and safety. However, advances in virology and molecular biology have now allowed the engineering of viruses with specific properties99 that make the idea of viral-based cancer therapy more attractive.

One promising virus in this regard is the herpes simplex virus type 1 (HSV-1). A number of naturally occurring and artificially engineered HSV-1 mutants have recently been identified that appear to replicate preferentially in rapidly dividing transformed cells. Initial experiments utilizing HSV-1 mutants with deletions in the thymidine kinase gene showed dose-dependent improvement in the survival of nude mice bearing human brain tumors both in vitro and in tumor-bearing nude mice.40,41 More recently, additional “replication restricted,” nonneurovirulent mutants of HSV that contain the HSV-TK gene (a potential safety factor that would allow elimination of virus by treatment with the drug GCV), but lack other HSV genes, have been developed and have shown even more promise.42 One of these mutants is HSV-1716, a virus that contains a 759 base pairs deletion in the genes coding for infected cell protein 34.43 This mutation, through mechanisms that are still unclear, severely attenuates the ability of HSV-1716 to replicate in normal tissues but does not appear to affect the virus' ability to replicate in rapidly dividing malignant cells.

Since the wild-type HSV-1 is able to infect and lyse a wide variety of cell types, we therefore reasoned that a mutant HSV virus, such as HSV-1716, might be efficacious in the treatment of localized, non-CNS malignancies such as mesothelioma.44 Human malignant mesothelioma cells supported the growth of HSV-1716 and were rapidly lysed in vitro. Intraperitoneal injection of HSV-1716 into animals with established tumor nodules reduced tumor burden and significantly prolonged survival. Importantly, the HSV-1716 mutant was replication-restricted to malignant cells in that it did not disseminate or persist after intraperitoneal injection into SCID mice bearing human tumors. These findings suggest that this virus may be efficacious and safe for use in localized human malignancies of nonneuronal origin such as malignant mesothelioma.

Ultimately, the development of new and improved vector technology will be required for the treatment of disseminated disease. However, as vectors are developed that have the ability to transduce larger numbers of cells, issues of tissue specificity will have to be addressed. Promoters that selectively activate genes only within tumor cells will thus become increasingly important. In this regard, experiments have been undertaken to evaluate tissue-specific promoters such as the promoter from the secretory leukoprotease inhibitor found in epithelial cells.45 Even more potentially useful will be true tumor specific promoters, such as the promoters of the erb B2 gene or the CEA gene that are overexpressed in many lung tumors.46,47 Another intriguing area of research in this area is exploration of promoters that are activated by ionizing radiation.48

Conclusions

Cancer gene therapy is still in its infancy. Even though clinical trials have begun, it is important to realize the very preliminary nature of these studies. Although it is very unlikely that any of these early trials will result in practical therapies for advanced tumors, well-designed trials that are aimed at testing specific hypotheses and generating useful information about issues such as gene transfer and immune responses will be important first steps that must be taken for the field to move onward. As more information is obtained about tumor immunology and biology, and as better vectors are developed, gene therapy will almost certainly play a key role in the treatment of cancer in the next decade.

Acknowledgments: The author would like to acknowledge the many collaborators and colleagues whose work was described herein. This includes past and present members of the Thoracic Oncology Laboratory (Drs. Larry R. Kaiser, Kunjilata Amin, Leslie Litzky, Cathy Molnar-Kimber, Roy Smytho, Harry Hwang, Ashraf Elshami, John Kuchareczuk, Daniel Sterman, and Nabil Rizk), members of the Penn Institute for Human Gene Therapy (Drs. Stephen Eck and James Wilson), members of the Penn Comprehensive Cancer Center (Dr. Joseph Treat, Adri Riccio, and Dr. John Glick), and Drs. Nigel Fraser and Bruce Randazzo of the Wistar Institute.
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Carboxypeptidase M

Variable Expression in Normal Human Lung and Inactivation in Lung Cancer

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(CHEST 1997; 111:1498)

Carboxypeptidase M (CPM) is a cell surface peptidase which hydrolyzes bioactive peptides such as bradykinin (BK) and epidermal growth factor (EGF). CPM is normally expressed in the lung on type I alveolar epithelial cells. Since BK and EGF are mitogenic for lung cancer cell lines, we hypothesized that CPM could be inactivated in lung cancer, which could increase BK and EGF availability for receptor binding.

In order to investigate the CPM expression in normal lungs vs lung cancer, we assayed CPM protein expression, activity, and mRNA by immunohistochemistry, enzyme assay, and reverse transcriptase polymerase chain reaction (RT-PCR) in small cell lung cancer (SCLC) and non-SCLC and compared them to uninvolved lung and bronchial epithelial cells. CPM activity was variably expressed in 12 normal human lung specimens and in BAL fluid.

In normal bronchial epithelial cells, CPM activity was 14.46±1.25 nmol/mg/h (n=2) and among the lung cancer cell lines, CPM activity was 2.32±0.63 nmol/mg/h (n=6) in SCLC cell lines, 0.57±0.58 nmol/mg/h (n=2) in large cell lines, and 20.9±10.4 nmol/mg/h (n=3) in adenocarcinoma cell lines. Two SCLC lines demonstrated absence of CPM activity. CPM activity was measured in lung tumors and compared to adjacent uninvolved lung from the same patient. CPM activity was significantly lower in tumors (10.27 nmol/mg/h) than in adjacent normal lung (21.97 nmol/mg/h) (p=.0078). We then examined whether low CPM activity was a result of low mRNA. CPM mRNA was assessed by RT-PCR in 24 lung cancer cell lines; CPM products were detected in 6 of 17 SCLC, 3 of 6 adenocarcinoma, 3 of 5 large cell, 0 of 1 mesothelioma, 1 of 4 squamous cell, and 0 of 1 carcinoid cell lines as compared to 5 of 5 normal bronchial epithelial cells. RT-PCR was also performed on cDNA from lung tumors and adjacent uninvolved lung. While CPM mRNA was expressed in 5 of 10 adjacent lung specimens, only 2 of 8 lung tumors expressed CPM message by RT-PCR. mRNA quality was verified by RT-PCR amplification of the housekeeping gene (HPRT). Sequence analysis of representative PCR products was consistent with the sequence for CPM.

We conclude that there is variable expression of CPM in normal human lung and BAL fluid. However, there is low expression of CPM in lung cancer, especially SCLC, which may be important in the pathogenesis of lung cancer. Reconstitution of CPM by gene therapy may be a therapeutic option.

Conference Summary

Gene-Based Therapies for Inherited and Acquired Disorders of the Lung

David T. Curiel, MD

(CHEST 1997; 111:1498-1525)

I would like to first thank the Aspen conference organizers. This was my first trip to Aspen. To paraphrase one of the other speakers, "We also have mountains in Birmingham, but we don't have all the other stuff!"

*From the Gene Therapy Program, University of Alabama at Birmingham.