p53 Tumor Suppressor Gene Therapy for Cancer

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Gene therapy has the potential to provide cancer treatments based on novel mechanisms of action with potentially low toxicities. This therapy may provide more effective control of locoregional recurrence in diseases like non–small-cell lung cancer (NSCLC) as well as systemic control of micrometastases. Despite current limitations, retroviral and adenoviral vectors can, in certain circumstances, provide an effective means of delivering therapeutic genes to tumor cells. Although multiple genes are involved in carcinogenesis, mutations of the p53 gene are the most frequent abnormality identified in human tumors. Preclinical studies both in vitro and in vivo have shown that restoring p53 function can induce apoptosis in cancer cells. High levels of p53 expression and DNA-damaging agents like cisplatin (Platinol) and ionizing radiation work synergistically to induce apoptosis in cancer cells. Phase I clinical trials now show that p53 gene replacement therapy using both retroviral and adenoviral vectors is feasible and safe. In addition, p53 gene replacement therapy induces tumor regression in patients with advanced NSCLC and in those with recurrent head and neck cancer. This article describes various gene therapy strategies under investigation, reviews preclinical data that provide a rationale for the gene replacement approach, and discusses the clinical trial data available to date. [ ONCOLOGY 13(Suppl 5):148-154, 1999]

Introduction

The concept of gene therapy to treat human disease originally developed as a potential treatment for inherited monogenic disorders.  In theory, a disease caused by the absence or mutation of a single gene, such as cystic fibrosis or Gaucher’s disease, could be treated and potentially cured by inserting a normal copy of the mutant or deleted gene into a renewable population of host cells, such as bone marrow stem cells. While conceptually simple, this strategy of gene replacement therapy is proving to have practical complexities that make its clinical implementation more difficult than had been anticipated. Currently available vectors have been unable to sustain high enough levels of gene expression over long enough periods of time.

A major focus in gene therapy has been the treatment of cancer. Approaches have included transferring cytokine genes to stimulate an antitumor immune response, delivering genes that express prodrugs to tumors, and transferring genes to protect stem cells during high-dose chemotherapy. This review will focus on restoring the function of tumor suppressor genes. In the context of cancer, transient gene expression that triggers cancer cell death may be enough to mediate a therapeutic effect. Expression of the transgene in only a fraction of tumor cells also may not be limiting because these cells may alter the growth of adjacent cells.

Genetic Basis of Carcinogenesis

The gene families implicated in carcinogenesis include dominant oncogenes like ras and tumor suppressor genes like p53. [4-6] Proto-oncogenes (normal counterparts of oncogenes) ordinarily participate in such functions as signal transduction (relaying information from the outer cell membrane to the nucleus) and gene transcription. An abnormality (point mutation, amplification, translocation, or rearrangement) in only one of the two alleles of a proto-oncogene is sufficient to convert it into an active oncogene, that is, to dysregulate its function and lead to malignant transformation.

In contrast, a single normal tumor suppressor gene in a cell is typically sufficient to perform the normal function of the gene, but loss of function of both alleles, by mutation or deletion or a combination thereof, leads to dysregulation of cellular growth. In addition, some tumor suppressor gene mutations [for example, some p53 mutations] act in a dominant negative manner, that is, a mutation in one allele can lead to production of a mutant p53 protein that binds to, and thereby inactivates, the structurally normal protein encoded by the opposite allele. Viral proteins also may bind and functionally inactivate p53. [7]
Rationale for Restoring p53 Function in the Cancer Cell

All cancers are thought to contain multiple abnormalities in a variety of genes that control various aspects of cell growth and development. Thus, correcting all the genetic abnormalities in the cancer cell might seem necessary to reverse the malignant process. Correction of all genetic abnormalities would be an impossible task, however, particularly since some of these abnormalities have not yet been identified. Moreover, individual patterns of expression would need to be assessed for each gene in every patient. Fortunately, correcting a single genetic abnormality is enough in some cases to induce tumor cell death by apoptosis.[8-10]

A number of in vitro studies that used cultured cancer cell lines demonstrated that eliminating the expression of a single dominant oncogene (\textit{ras}) or adding a normal copy of a tumor suppressor gene (p53 or the retinoblastoma [Rb] gene) to cells that had deleted or mutated copies of these genes reduced or even abolished critical aspects of the malignant phenotype, such as tumorigenicity in animals or anchorage-independent growth.[10-12] Because of this, the problem of simultaneously correcting multiple functional genetic defects in the cancer cell did not appear as daunting.

Restoring normal gene function to every cancer cell, which is beyond the capabilities of the vectors currently available for use in gene therapy, was also thought to be necessary at one time. However, transduced cells expressing a toxic transgene are now recognized to alter the growth of adjacent nontransduced cells. This has been termed the bystander effect.[3]

While multiple genes offer potential targets for gene therapy in several common malignancies, our group has focused on the goal of replacing normal p53 function. Many of the identified tumor suppressor genes and proto-oncogenes encode proteins that are components of a network that converges on the protein encoded by the Rb gene.[13,14] Phosphorylation of the Rb protein increases transcription of other genes and protein synthesis, leading to cell growth. Phosphorylation of Rb is controlled by a multimolecular complex of proteins, containing cyclins and cyclin-dependent kinases as well as a potent inhibitor of most cyclin-dependent kinases, termed p21.[15,16] In turn, one of the many functions of the p53 protein is regulating p21 function.[17,18] Thus, p53 plays a central role in regulating the cell cycle because it indirectly regulates the function of Rb. When p53 function is normal, this pathway is tightly regulated; however, when p53 mutates or is absent, uncontrolled cell growth reflects lost control of the pathway.

Moreover, p53 plays a central role in other metabolic pathways, including, importantly, control of apoptosis.[19] In response to various toxic insults to cells, such as exposure to ionizing radiation or chemotherapy, normal cells either pause in their cell cycle long enough to repair DNA damage or, in other cases, undergo apoptosis; repair and programmed cell death both prevent the damaged DNA from being passed along to the cell’s progeny. When normal p53 function is absent, however, damaged DNA is much more likely to be passed along.[20]

Selecting the Tumor Model

Non–small-cell lung cancer is a logical target for novel therapeutic strategies for several reasons. It is the leading cause of cancer death in the United States, and the median survival of patients who present with stage III or IV disease, as the majority of patients do, is measured in months. For most patients who are not potentially curable by surgery, other standard therapies are relatively ineffective. Radiotherapy, which offers the best chance for locoregional control of disease in patients with surgically unresectable disease, is successful in only 20% of cases, and local primary failure or recurrence may be the only site of failure in up to one third of patients.[21]

Mutation or inactivation of p53 occurs in a high proportion of nearly all common human cancers, including non–small-cell lung cancer.[21-23] In view of the key role of p53 in cell-cycle regulation and apoptosis and the role of defective p53 function in carcinogenesis, attempting to replace p53 represents a logical gene replacement strategy. My colleagues and I have therefore studied this approach in patients with non–small-cell lung cancer. Our initial approach has been based on injecting the primary tumor with a vector expressing wild-type p53, with the aim of improving the locoregional control of non–small-cell lung cancer.

Selecting an Efficient Vector

Most available vectors were inefficient in transducing genes into cancer cells. Thus, the next experimental goal was to develop an efficient vector to deliver a therapeutic gene into cancer cells. Studies of models of human tumors in vitro and in nude (immunooincompetent) mice showed that uptake of retroviral vectors that contained wild-type p53 or antisense \textit{ras} were efficient enough to
mediate a therapeutic effect. In contrast, control vectors carrying cancer-associated p53 mutations did not suppress cell growth, indicating that the therapeutic effect was a direct result of normal p53 expression and not a nonspecific effect caused by transduction of the vector or the p53 sequences.[24]

These experiments and many other early studies, including our first clinical trial of p53 gene replacement therapy, used a retroviral vector that contained wild-type p53 cDNA linked to a b-actin promoter. While retroviral vectors yield levels of gene expression sufficient to demonstrate a biologic effect, vectors derived from adenoviruses can achieve much higher levels of gene expression, which should offer greater therapeutic potential. Further, adenovirus vectors have the advantage of infecting both dividing and nondividing cells, in contrast to retroviral vectors, which infect only actively dividing cells. Adenovirus vectors do not integrate into the genome, so expression is transient. This is not necessarily a disadvantage in cancer patients, however, since prolonged expression is not required or even necessarily desirable after the tumor cell kill.

Given these theoretic advantages of adenoviral vectors, we selected first-generation adenoviruses in which nonessential genes were deleted and replaced with wild-type p53 driven by a cytomegalovirus promoter (ie, adenovirus p53 [Ad-p53]). In animal studies, subsequent treatment with Ad-p53 greatly inhibited the tumorigenicity of intratracheally inoculated lung cancer cells.[25]

Preclinical Studies of p53 Gene Therapy

The demonstration of an antitumor effect for normal p53 transduced into cancer cells growing in culture notwithstanding, we were concerned that only a fraction of tumor cells would be transduced in vivo, limiting therapeutic efficacy. Thus, the discovery that viral vectors can readily penetrate 3-dimensional cancer cell matrices, indicating that they would spread beyond the site of intratumoral injection, was encouraging.[26] Furthermore, adenovirus that contains wild-type p53 is not toxic to normal bronchial epithelial cells in culture, and it is minimally toxic in mice, even at very high levels, suggesting a very favorable safety profile for clinical trials.[27] In addition to the work reported with non–small-cell lung cancer, studies in nude mouse models of squamous cell carcinoma of the head and neck have demonstrated a significant therapeutic effect for adenovirus p53, confirming induction of significant apoptosis.[28]

Despite the high efficiency of the cancer cell transduction achieved by viral vectors and the spread of injected vectors beyond the site of intratumoral injection, it is unlikely that every tumor cell will be transduced. In vivo studies of intratumoral injections of Ad-p53 into subcutaneous tumors in mice showed a degree of regression exceeding that predicted by the transduction efficiency of the vector, suggesting bystander effects. This phenomenon, which is critical to achieving a widespread antitumor effect, was first noted in vivo in brain tumor cells transduced with the herpes simplex thymidine kinase gene and then exposed to ganciclovir (Cytovene). The ganciclovir is nontoxic to normal cells, but the transduced viral thymidine kinase converts the drug into cytotoxic ganciclovir triphosphates, leading to the death of the transduced tumor cells.[3]

In contemplating gene replacement therapy with p53 or other tumor suppressor genes, demonstrating that a bystander effect similar to that seen with the thymidine kinase gene also occurred in the case of p53, was essential. Using in vivo mixing experiments, investigators demonstrated that retroviral wild-type p53-transduced cells could reduce the growth rate of nontransduced human lung cancer cells, indicating an operative bystander effect in this system.[29] Several mechanisms may mediate bystander effects, which may differ depending on the gene transduced and the specific type of tumor cell. These mechanisms include transfer of toxic metabolic products through gap junctions, phagocytosis of apoptotic vesicles of dead tumor cells by live tumor cells that mediate apoptosis, induction of an immune response against the tumor, and inhibition of angiogenesis.

The so-called death receptor, Fas, also might mediate a bystander antitumor effect following transduction of a p53 expression vector. For example, H358 lung cancer cells express abundant Fas ligand but not the Fas protein needed for apoptosis. Wild-type p53 upregulates expression of Fas protein by these cells, and because the protein is released in soluble form, it may traffic to other cells, leading to a bystander effect.[30]

Expression of the wild-type p53 gene also may downregulate the expression of vascular endothelial growth factor, which is one of the most common factors stimulating angiogenesis in human cancers. This downregulation reduces the formation of blood vessels by tumor cells, thereby possibly inhibiting the continued growth of the cancer.

In another study, the growth of lung cancer was studied using an orthotopic experimental model in
which human lung cancer cells grew as xenograft tumors in nu/nu mice. Directly administering a retroviral wild-type p53 expression vector into the tumor cells in vivo suppressed tumor growth.[31] Subsequently, we used an adenovirus expression vector to deliver wild-type human p53 cDNA to tumor cells. This p53 expression vector induced apoptosis in cancer cells carrying a mutated or deleted p53, but had little effect on the growth of cells containing wild-type p53. Similar p53-adenovirus vector constructs also inhibited growth of rat gliomas, human head and neck cancers, and human colon cancers in nude mice and can mediate p53 gene expression in bladder and liver cancers.[32] The products of other tumor suppressor genes, such as p16 and a truncated Rb gene also have been found to suppress tumor growth in animal models.[32]

**Synergistic Effect of Transduced p53 With Other Therapies**

In addition to the antitumor effect of transduced tumor suppressor genes used as single modalities in various experimental systems, preclinical studies demonstrated synergy between p53 replacement therapy and DNA-damaging chemotherapeutic agents that are useful against non-small-cell lung cancer, such as cisplatin and etoposide (VePesid).[33] Chemotherapy enhances expression of transduced genes, whether viral or nonviral vectors are used, with a range of promoters. In one model, adding cisplatin before p53 transfection increased apoptosis and suppression of tumor growth.[34] The mechanism underlying this effect is not yet known. Preclinical studies also have indicated that gene therapy may increase sensitivity to radiation.

We tested whether adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation.[35] Wild-type p53 gene transfer into the SW620 colorectal carcinoma cell line was performed using Ad-p53 to evaluate the effect of wild-type p53 expression on radiation sensitivity. Based on the fact that survival at 2 Gy was reduced from 55% to 23% (Figure 1), the results indicated that this vector sensitized the cells. Flow cytometric analysis of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay-labeled cells and in situ TUNEL staining of xenograft tumors demonstrated an increase in labeled cells after the combination treatment. Compared with p53 gene therapy alone, this combination strategy significantly enhanced the suppression of tumor growth in an animal model of subcutaneous tumor. The delay in regrowth to control tumor size of 1,000 mm³ was 2 days for 5 Gy, 15 days for Ad-p53, and 37 days for Ad-p53 plus 5 Gy, indicating synergistic interactions. These data indicate that the delivery of wild-type p53 to cells with p53 mutations increases their radiation-sensitivity and that this delivery may be accomplished by adenoviral-mediated gene therapy.

Additional experiments were conducted to ascertain whether the Ad-p53 vector also would sensitize non-small-cell lung cancer cells to radiation. Two different non-small-cell lung cancer lines were examined, H358 and H1299. Both of these cell lines have homozygous deletions of the p53 gene. The H358 line was examined in vitro. In this case, the cells growing in culture were infected with Ad-p53 at a multiplicity of infection (number of infectious viral particles per tumor cell) of 70 and the cells were irradiated 48 hours following infection. Sensitization was assessed using an in vitro clonogenic assay. Survival at 2 Gy was reduced from 70% for the cells receiving 2 Gy alone to 50% for the cells receiving both treatments. The H1299 cells were injected into the hind legs of nude mice. When the tumors reached 6 to 8 mm in diameter, they were injected with 1.5 x 10¹⁶ viral particles. Two days later, the xenograft tumors were given a single radiation dose of 5 Gy. Similar to the results with the colorectal xenografts, the non-small-cell lung cancer xenograft tumors had a dramatic response to the combined treatment (Figure 2). The delay in regrowth to a control tumor size of 1,000 mm³ was 1 day for the 5-Gy dose alone, 14 days for the Ad-p53 alone, and 36 days for the combination of Ad-p53 plus 5-Gy treatments. Thus, the results for the combination of virus and radiation appeared to indicate a synergistic interaction and validate the use of this gene therapy strategy to treat non-small-cell lung cancer.

Based on the results of recently completed preclinical studies, Ad-p53 in combination with radiation also may be a useful strategy for treating brain tumors. To determine the effects of adenovirus-mediated delivery of wild-type p53 on the radioresponse of glioma tumor cells, in vitro experiments were performed.[36] The responses of two human glioma cell lines were compared: U87MG, which has wild-type p53, and U251MG, which has a mutant p53 allele. Monolayer cultures of these cell lines were infected with Ad-p53, control vector dl312 or culture medium. The cultures were irradiated 2 days later and colony formation efficiency determined.

Transfection with p53 had only a minor effect on the plating efficiency of unirradiated U87MG cells but significantly enhanced the radiosensitivity of these cells. The surviving fraction at 2 Gy was reduced from 0.61 in controls to 0.38 in p53-transfected U87MG cells (Figure 3). The control vector,
dl312, did not influence radiosensitivity. The Ad-p53 vector was toxic to the U251MG cell line and, therefore, its influence on radiosensitivity could not be determined. A flow cytometric analysis of TUNEL-stained cells demonstrated that the Ad-p53 vector sensitized the U87MG cells to radiation-induced apoptosis; the percentage of TUNEL-positive cells was increased following 9 Gy from about 3% in the controls to 19% in the Ad-p53 plus 9-Gy–treated cells. These data suggest that adenovirus-mediated p53 may enhance the radioresponse of brain tumor cells with wild-type p53 and that this radiosensitization may at least partially involve a restoration of the propensity for apoptosis. Additional support for this concept is derived from a study showing that radiation improves immediate transduction efficiency and duration of transgene expression from an adenoviral vector.[37]

Clinical Studies

In our initial human study, nine patients with non-small-cell lung cancer received a retroviral vector containing the wild-type p53 gene under the control of a b-actin promoter.[38] Other treatments had failed in these patients, all of whose tumors had been documented as having a p53 mutation. Since transduction via retrovirus was known to yield low titers of the transduced gene, the vector was injected into the tumor on 5 consecutive days, using either a bronchoscope or, in the case of chest wall lesions, a percutaneous needle. Regression of the injected lesion was observed in three patients, while disease stabilized in three others. One patient who died of a progressive kidney metastasis showed no evidence of viable tumor at the treated site at autopsy 4 months following the injection. One patient’s primary disease progressed, and two patients were inevaluable (one could not tolerate the general anesthesia and so did not complete treatment, and the other died within 3 weeks of treatment). Polymerase chain reaction (PCR) and/or in situ hybridization studies of posttreatment biopsies from eight patients before treatment effects were evaluated showed that tumor cells had indeed integrated vector DNA sequences in up to 20% of cells in certain areas of the tumors. An important finding was that TUNEL staining (which detects DNA nicking) of the posttreatment biopsy samples showed that apoptosis had increased following treatment compared with the pretreatment baseline.

No side effects attributable to the p53-vector sequences occurred in any patient, although bronchoscopy-related complications occurred in three patients. We found no evidence of retroviral sequences in DNA extracted from lymphocytes, sputum samples, or various nontumor tissues obtained at autopsy on three patients. These results confirmed a high safety profile, successful delivery of normal p53 sequences to tumor cells, and a biologic effect of the transduced gene. These promising results have been supported by a more recent two-arm study in which a wild-type p53 gene was given to 52 patients with non–small-cell lung cancer using an adenovirus vector. Patients received Ad-p53 either alone or preceded by cisplatin, 80 mg/m² over 2 hours, 3 days before p53 injection (cisplatin was chosen because of preclinical evidence of a synergistic effect with p53 gene replacement). A single intratumoral injection of p53 was given, either bronchoscopically or guided by computed tomography, once per month for up to 6 months. Most patients had received previous chemotherapy, in some cases with cisplatin, and all had tumor progression during conventional therapy before entry into the study.

As with the study using the retroviral vector, both clinical and laboratory evidence of p53 expression were seen. Ad-p53 alone (26 evaluable patients) mediated two partial responses and stabilized disease in 16 patients. A higher dose of Ad-p53 increased progression-free survival. Ad-p53 plus cisplatin (23 evaluable patients) mediated a partial response in two patients who had been treated with cisplatin previously. One additional patient achieved a partial response but did not have the required follow-up documentation to confirm the response. Ad-p53 plus cisplatin prolonged progression-free survival compared with Ad-p53 alone. A majority of patients also had evidence of vector DNA by PCR of posttreatment biopsies. Although antiadenovirus antibodies were detected in all patients after one treatment, no anaphylaxis or other toxicities except transient fever accompanied subsequent treatments. Perhaps surprisingly, despite high levels of serum antiadenovirus antibody, p53 transgene expression occurred in the tumor cells, and clinical responses were maintained.

Although these studies corrected only one of the many genetic abnormalities present in non–small-cell lung cancer, evidence suggests an antitumor effect based directly on p53-mediated apoptosis, as shown in the retroviral p53 trial. The two trials provided no evidence for the contrary view that tumor stabilization and regression resulted from a nonspecific immune reaction. In the Ad-p53 study, PCR detected vector DNA in the majority of patients, but fewer patients showed
evidence of gene expression. Detection of gene expression following transfer of wild-type p53 in vivo is difficult because successful transfer and expression of wild-type p53 in a tumor may destroy evidence of gene expression if apoptosis is induced and the cells die. However, in those patients whose serial gene expression could be quantitated by immunohistochemistry, it was clear that expression of the transgene occurred despite the presence of high titers of antiadenovirus antibody. It is possible that serum antibodies have little effect due to poor penetration of solid tumors as a result of high interstitial fluid pressure. Whether suppressing the antiadenovirus immune response would further enhance levels of transgene expression in the tumor is not known. In any case, repeated intratumoral injections of adenoviral p53 appear safe despite increases in antiadenovirus antibodies.

A similar strategy has been reported for treating recurrent head and neck squamous cell carcinomas.[39] Head and neck cancer patients may benefit from improved local control, as the principal cause of death for these patients is locoregional recurrence. In a phase I dose-escalation study, 33 patients were injected with doses of up to 1011 plaque-forming units. Dose-limiting toxicity or serious adverse events were not observed. Gene expression in tumor tissue was documented. Two of the 17 patients who had unresectable disease had a partial response and six had stable disease for up to 3.5 months. One of the patients with resectable disease was noted to have a pathologic complete response at the time of resection. Infectious Ad-p53 was detected in sputum and urine at the highest doses of 1011 infectious particles. Interestingly, the excretion time course did not change in duration or amount with subsequent injections despite the presence of high titers of antiadenovirus antibody. This raises the intriguing possibility that the antibody may not limit the bioavailability of systemically administered Ad-p53.

**Future Research and Conclusions**

The first patient treated with retroviral p53 received general anesthesia, but outpatient treatment is now given using only topical anesthesia. Injection takes about 20 minutes. The simplicity of the treatment procedure suggests that gene transfer using Ad-p53 may provide highly cost-effective therapy. Given the promising results of these phase I studies, phase II trials in patients with non-small-cell lung cancer or head and neck cancer are now in progress, and plans also are under way to expand these studies to include patients with locally recurrent breast, bladder, and ovarian tumors who may benefit from more aggressive treatment of locoregional disease. Based on the promising preclinical data indicating that Ad-p53 and ionizing radiation are synergistic in causing cancer cell apoptosis, a phase II trial has been initiated in patients with unresectable, untreated non-small-cell lung cancer combining Ad-p53 given by intratumoral injection with external-beam ionizing radiation therapy.

The low viral titer achieved with the retroviral p53 vector and its inhibition by circulating complement make it unsuitable for systemic (rather than intratumoral) use. These limitations do not apply to the adenovirus vector, but its use systemically would require enhanced transgene expression, low vector toxicity, and, ideally, elimination of the immune response to the vector. Modification of the vectors used may reduce expression of endogenous adenoviral proteins, leading to a diminished anti-adenoviral immune response.[40] The addition of agents that enhance transgene expression may increase the effectiveness of low levels of transgene expression sufficiently to render them therapeutic.

Also useful would be a method of targeting delivery to the tumor. Early clinical work has suggested that some systemic effect occurs from adenovirus shed from the intratumoral injection site, as autopsy evidence has indicated that vector DNA was detected in distant tumor deposits. Other areas of current research include methods of increasing levels of p53 expression and suppression of the immune response via concomitant administration of low-dose etoposide.[32]

In conclusion, clinical trials have shown that repeated intratumoral administration of Ad-p53 is well tolerated and not associated with major toxicity. Evidence supports both gene transfer and gene expression in the majority of the tumors. Progressively growing tumors refractory to conventional treatments have shown objective clinical responses to Ad-p53.

In the near future, potentially promising areas for gene replacement therapy include the treatment of locally advanced disease, adjuvant and neoadjuvant treatment of patients with resectable tumors, the use of gene therapy to sensitize tumors to radiotherapy and chemotherapy, and, potentially, the treatment of localized premalignancy. Gene replacement therapy also may come full circle since basic and clinical research in cancer also may help solve the obstacles that have inhibited the application of gene therapy to monogenic inherited diseases. Given the rapid pace of recent
progress and the explosion of interest in gene therapy, we envision that in the near future, gene therapy willtake its place alongside chemotherapy, radiotherapy, and surgery as one of the tools routinely available to help treat patients with cancer.

**References:**


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