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Gene Therapy Approaches for Lung Cancer

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Introduction

Lung cancers exhibit multiple genetic lesions that can be detected even in histologically normal bronchial mucosa from individuals with a smoking history. These genetic abnormalities provide an array of targets for therapy. Tobacco smoke has over 100 carcinogenic agents, and the specific interactions of specific carcinogens with genes that suppress tumors and repair DNA have been identified.[1] Dysfunctional tumor suppressor genes are the most common genetic lesions identified to date in human lung cancers. Functional copies of tumor suppressor genes can be introduced into cancer cells by gene transfer.

The *p53* tumor suppressor gene appears to play a central role in lung cancer development and was the initial focus of gene therapy approaches to lung cancer. This approach has been extensively studied in the clinic with intratumoral injection of a replication-defective adenovirus that expresses *p53* (Adp53). Overexpression of *p53* in cancer cells induces growth arrest and apoptosis. Injections of Adp53 have an excellent safety profile and have mediated tumor regression and growth arrest as monotherapy or have overcome resistance or increased the effectiveness of radiation therapy and chemotherapy. Expression of the *p53* transgene has occurred at high levels and is associated with activation of other genes in the p53 pathway. These studies indicate proof-of-principle for tumor suppressor gene therapy and represent a new paradigm in targeted therapy.

Mechanism of *p53* Tumor Suppression and Rationale for *p53* Gene Therapy

Expression of some gene products, including growth factors, oncogenes, cyclins, and cyclin-dependent kinases (Cdks) stimulate cell proliferation. Expression of tumor suppressor genes and other inhibitors of Cdks induce cell cycle arrest, thus limiting the cell proliferation. Two interconnected pathways, the retinoblastoma (Rb) pathway and the p53 pathway, which are both, in turn, regulated at the protein level by oncogenes and other tumor suppressor genes, contribute to the regulation of cell proliferation. The Rb protein regulates maintenance of, and release from, the G1 phase. The p53 protein monitors cellular stress and DNA damage, either causing growth arrest to facilitate DNA repair or inducing apoptosis if DNA damage is extensive.[2] When a cell is stressed by oncogene activation, hypoxia, or DNA damage, an intact p53 pathway may determine whether the cell will receive a signal to halt at the G1 stage of the cell cycle, whether DNA repair will be attempted, or whether the cell will self-destruct via apoptosis.

The *p53* gene is central in the processes of apoptosis, DNA repair following various cell stresses, and regulation of the cell cycle. Apoptosis plays a key role in numerous normal cellular mechanisms, from embryogenesis to DNA damage control due to random mutations, ionizing radiation, and DNA damaging chemicals, and has more recently been implicated as a major mechanism of cell death due to DNA-damaging cancer therapies such as chemotherapy and radiation. The observation that expression of a wild-type *p53* gene in a cancer cell triggers apoptosis was the seminal observation that lead to *p53* gene therapy approaches.[3] Prior to this it was thought that gene therapy could not replace all the damaged genes in a cancer cell and thus would not have an effect. The requirement for restoring only one of the defective genes to trigger apoptosis suggests that the DNA damage present in the cancer cell may prime it for an apoptotic event that can be activated through a single pathway.

The major functional role for the *p53* gene product is that of a transcription factor.[4] A group of genes whose expression is in part regulated by *p53* are the apoptosis genes. The balance between two proapoptotic versus prosurvival (antiapoptotic) signals, often compared to a rheostat, determines whether or not apoptosis will be

induced. While these signals determine *p53*'s actions, expression of many of the genes that generate these critical signals is, in turn, regulated by the activation status of *p53,* forming a complex feedback loop. *p53* carries out its housekeeping duties by downregulating the "prosurvival" (or antiapoptotic) genes, including the antiapoptotic genes *Bcl-2* and *Bcl-xL* and upregulating the proapoptotic genes *Bax*, *Bad*, *Bid*, *Puma*, and *Noxa.*[5] Available transcripts of each of the pro- and antiapoptotic genes with *Bcl-2* homology-3 domains interact with one another to form heterodimers, and the relative ratio of proapoptotic to prosurvival proteins in these heterodimers determines activity of the resulting molecule, thereby determining whether the cell lives or dies. *p53* also targets the death-receptor signaling pathway, including DR5, and Fas/CD/95, the apoptosis machinery including caspase-6, Apaf-1, and PIDD, and may directly mediate cytochrome c release. Thus apoptosis is an important mechanism by which *p53* mediates its tumor suppressor function.

The p53 pathway is regulated at the protein level by other tumor suppressor genes and by several oncogenes.[2] For example, *MDM2* normally binds to the N-terminal transactivating domain of p53, prohibiting p53 activation and leading to its rapid degradation. Under normal conditions the half-life of p53 is only 20 min. In the event of genotoxic stress, resulting DNA damage causes phosphorylation of serines on p53, weakening binding to *MDM2* and destabilizing the p53/*MDM2* interaction and prolonging p53 half-life. The resulting increase in p53 DNA binding activity leads to an array of downstream signals that switch other genes on or off. In the normal cell, *MDM2* is inhibited by expression of $p14^{ARF}$, a tumor suppressor gene encoded by the same gene locus as $p16^{INK4a}$ but read in an alternate reading frame.[6] Deletion or mutation of the tumor suppressor gene $p14^{ARF}$, which has been noted in some cancers, results in increased levels of *MDM2* and subsequent inactivation of p53, resulting in inappropriate progression through the cell cycle. The expression of $p14^{ARF}$ is induced by hyperproliferative signals from oncogenes such as *ras* and *Myc*, thus indicating an important role for p53 in protecting the cell from oncogene activation. Importantly, p53 also plays a central role in mediating cell cycle arrest. This function is significant as prolonged tumor stability has often been observed in clinical trials of *p53* gene replacement, suggesting that this effect is predominate in some tumors over apoptosis. p53 is involved in regulating cell cycle checkpoints, and p53 expression can promote cell senescence through its control of cell cycle effectors such as $p21^{Cip1/WAF1}$.

Loss of function in the p53 pathway is the most common alteration identified in human cancer to date. About 50% of common epithelial cancers have *p53* mutations.[7–9] In some cancers, loss of *p53* also appears to be linked to resistance to conventional DNA damaging therapies that require functional cellular apoptosis to accomplish cell death.

Preclinical Studies of *p53* Gene Replacement

The studies described suggest that expressing a wild-type *p53* gene in cancer cells defective in *p53* function could mediate either apoptosis or cell growth arrest. Both results could be a therapeutic benefit in a cancer patient. Our initial studies showed that restoration of functional *p53* suppressed the growth of some, but not all, human lung cancer cell lines.[10] Because of limitations inherent in the use of retroviruses, subsequent studies of *p53* gene replacement in lung cancer made use of an adenoviral vector (Adp53).[11] The first published study of *p53* gene therapy showed suppression of tumor growth in an orthotopic human lung cancer model using a retroviral expression vector.[12]

Adp53 also induced apoptosis in cancer cells with defective *p53* function without significantly affecting normal cells.[13] Adp53 mediated inhibition of tumor growth in a mouse model of human orthotopic lung cancer[14] and induced apoptosis and suppression of proliferation in various other cancer cell lines.[15–18]

Although it was first thought that the inability to transduce every cell in a tumor might limit the effectiveness of gene therapy for cancer, studies[3,19] of three-dimensional cancer cell matrices and subcutaneous xenografts proved that therapeutic genes could penetrate beyond the injection site to nontransduced tumor cells and cause cell death via a "bystander effect." Bystander killing, now known to be an important phenomenon in the success of gene therapy, appears to involve regulation of angiogenesis,[20,21] immune upregulation,[22–24] and secretion of soluble proapoptotic proteins.[25]

Clinical Trials of *p53* Gene Replacement

The first clinical trial protocol for *p53* gene replacement was carried out with a retroviral vector expressing wild-type *p53* under control of a β-actin promoter.[26] The retroviral vector was injected into tumors of nine patients with unresectable non–small cell lung cancer (NSCLC) that had progressed on chemotherapy. Three of the nine patients demonstrated evidence of antitumor activity with no vector-related toxicity, demonstrating for the first time the feasibility and safety of *p53* gene therapy.[27,28]

In a phase I trial of 28 NSCLC patients whose cancers had progressed with conventional treatments, successful

gene transfer using Adp53 was shown in 80% of evaluable patients.[29] Gene expression was detected in 46%, apoptosis was demonstrated in all but one of the patients expressing the gene, and no significant toxicity was observed. Greater than 50% reduction in tumor size was observed in two patients, with one patient remaining free of tumor more than a year after concluding therapy and another having a nearly complete regression of a chemotherapy and radiotherapy resistant upper lobe endobronchial tumor.

Gene Replacement in Combination with Conventional DNA-Damaging Agents in Non–Small Cell Lung Cancer

Many tumors are resistant to chemotherapy and radiation therapy and, therefore, progress after initial treatment. *p53*, often missing or nonfunctional in radiation- and chemotherapy-resistant tumors, is known to play a key role in detecting damage to DNA and either directing repair or inducing apoptosis. Once apoptosis was implicated as a mechanism of cell killing in response to these DNA-damaging agents, it followed that a defect in the normal apoptotic pathway might confer resistance to some tumor cells. Because of Adp53's low toxicity (less than a 5% incidence of serious adverse events) in initial trials, therapeutic strategies combining Adp53 gene replacement and conventional DNA-damaging therapies were logical extensions of earlier studies.[30]

Preclinical Studies

The fact that overexpression of *p53* in wild-type *p53* transfected cell lines could induce apoptosis in cancer cells was shown in several studies in vitro.[31–33] Subsequent studies that examined apoptosis in tumor cells treated with radiation or chemotherapeutic agents supported a link between apoptosis induction and functional *p53* expression.[34–39] Preclinical studies of *p53* gene therapy combined with cisplatin in cultured NSCLC cells and in human xenografts in nude mice showed that sequential administration of cisplatin and *p53* gene therapy resulted in enhanced expression of the *p53* gene product,[37,40] and similar studies of Adp53 gene transfer combined with radiotherapy indicated that delivery of Adp53 increased the sensitivity of *p53*-deficient tumor cells to radiation.[15]

Numerous additional studies have generated additional supporting evidence for a critical link between radiation sensitivity and the ability of a cell to induce apoptosis.[41–45] However, the radiosensitivity of some tumor types, for example, epithelioid tumors, does not appear to be correlated with *p53* status.[46–48]

Clinical Trials of Tumor Suppressor Gene Replacement Combined with Chemotherapy

Twenty-four NSCLC patients with tumors previously unresponsive to conventional treatment were enrolled in a phase I trial of *p53* combined with cisplatin.[49] Seventy-five percent of the patients had previously experienced tumor progression on cisplatin- or carboplatin-containing regimens. Up to six monthly courses of intravenous cisplatin, each followed 3 days later with intratumoral injection of Adp53, resulted in 17 patients remaining stable for at least 2 months, two patients achieving partial responses, four patients continuing to exhibit progressive disease, and one patient unevaluable because of progressive disease. Seventy-nine percent of tumor biopsy specimens showed an increase in number of apoptotic cells, 7% demonstrated a decrease in apoptosis, and 14% indicated no change.

A phase II clinical trial evaluated two comparable metastatic lesions in each NSCLC patient enrolled in the study.[50] All patients received chemotherapy, either three cycles of carboplatin plus paclitaxel or three cycles of cisplatin plus vinorelbine, and then Adp53 was injected directly into one lesion. Adp53 treatment resulted in minimal vector-related toxicity and no overall increase in chemotherapy-related adverse events. Detailed statistical analysis of the data indicated that patients receiving carboplatin plus paclitaxel, the combination of drugs providing the greatest benefit on its own, did not realize additional benefit from Adp53 gene transfer; however, patients treated with the less successful cisplatin and vinorelbine regimen experienced significantly greater mean local tumor regression, as measured by size, in the Adp53-injected lesion compared with the control lesion.

Clinical Trials of *p53* Gene Replacement Combined with Radiation Therapy

Preclinical studies suggesting that *p53* gene replacement may increase radiation sensitivity to some tumors[15,41,43–45] led to a phase II clinical trial of *p53* gene transfer combined with radiation therapy.[51] Nineteen patients with localized NSCLC were treated, with a complete response in 1 patient (5%), partial response in 11 patients (58%),

stable disease in 3 patients (16%), and progressive disease in 2 patients (11%), while two patients (11%) were unevaluable because of tumor progression or early death. Three months following completion of therapy, biopsy specimens revealed no viable tumor in 12 patients (63%) and viable tumor in 3 (16%). Tumors of four patients (21%) were not biopsied because of tumor progression, early death, or weakness. The 1-year progression-free survival rate was 45.5%. Among 13 evaluable patients after 1 year, 5 (39%) had a complete response and 3 (23%) had a partial response or disease stabilization. Most treatment failures were caused by metastatic disease, not by local progression.

In this study, pre- and posttreatment biopsies of the tumor were performed for studies of gene expression. Adp53 vector-specific DNA was detected in biopsy specimens from 9 of 12 patients with paired biopsies (day 18 and day 19). The ratio of copies of Adp53 vector DNA to copies of actin DNA was 0.15 or higher in 8 of 9 patients (range, 0.05–3.85), with 4 patients having a ratio >0.5. For 11 patients with adequate samples for both vector DNA and mRNA analysis, 8 showed a postinjection increase in mRNA expression associated with detectable vector DNA. Postinjection increases in *p53* mRNA were detected in 11 of 12 paired biopsies obtained 24 hr after Adp53 injection, with 10 of 11 increasing threefold or greater. Preinjection biopsies that were negative for p53 protein expression by immunohistochemistry were stained for p53 protein expression after Adp53 injection. Staining results confirmed that the p53 protein was expressed in the posttreatment samples in the nuclei of cancer cells. Previous in vitro experiments in human NSCLC cell lines identified four genes (*p21* [*CDKN1A*], *MDM2*, *Fas*, and *Bak*) that showed the greatest increase in mRNA expression after induction of *p53* overexpression with Adp53. Therefore, in the current study, changes in mRNA levels for these four markers were determined at various time points before and during treatment using reverse transcriptase real-time polymerase chain reaction. The study was controlled by obtaining a pretreatment biopsy sample under the same conditions as the posttreatment biopsy sample. The inclusion of a time point during the radiation treatment allowed for a biopsy to be performed immediately before and 24 hr after Adp53 injection, thus allowing determination of the effects of the Adp53 on mRNA expression during treatment. For *p21* (*CDKN1A*) mRNA, increases of statistical significance were noted 24 hr after Adp53 injection and during treatment compared with the pretreatment biopsy. In the case of *MDM2* mRNA, increases were noted during treatment compared with the pretreatment biopsy. Levels of *Fas* mRNA did not show statistically significant changes during treatment. *Bak* mRNA expression increased significantly 24 hr after injection of Adp53, and thus Bak appeared to be the protein most acutely upregulated by Adp53 injection.

Recently the first randomized clinical trial of *p53* gene therapy was reported. Ninety patients with squamous cell carcinoma of the head and neck were randomly allocated to receive intratumoral injection of Adp53 (10^{12} VP/dose/week for a total of 8 weeks) in combination with radiation therapy (70 GY/8 weeks) or radiation therapy alone. Complete remission was seen in 64.7% of patients receiving Adp53 combined with radiation therapy compared with 20% of patients receiving radiation therapy alone, which was highly significant statistically.[52]

Systemic Gene Therapy

Local control of cancers is important, but most patients with lung cancer die from systemic metastases. Thus gene delivery to distant sites of cancer is essential if cancer gene therapy is going to have an impact on survival. Recently, nanoscale synthetic particles that can encapsulate plasmid DNA and deliver it to cells after intravenous injections have been developed. This has been studied in mouse xenograft models of disseminated human lung cancer with delivery of *p53* and other tumor suppressor genes. Multiple *3p21.3* genes show different degrees of tumor suppressor function in various human cancers in vitro and in preclinical animal models. One of the tumor suppressor genes at this locus is *FUS1* which is not expressed in most lung cancers. When wild-type *FUS1* is expressed in a lung cancer cell, apoptosis occurs. To translate these findings to clinical applications for molecular cancer therapy, we recently developed a systemic treatment strategy by using a novel *FUS1*-expressing plasmid vector complexed with DOTAP: cholesterol (DOTAP:Chol) liposome, termed FUS1 nanoparticle, for treating lung cancer and lung metastases.[53,54] In a preclinical trial, we showed that intratumoral injection of FUS1 nanoparticles into subcutaneous NSCLC H1299 and A549 lung tumor xenografts resulted in significant inhibition of tumor growth. Intravenous injections of FUS1 nanoparticles into mice bearing experimental A549 lung metastasis caused a decrease in the number of metastatic tumor nodules. Treating lung tumor–bearing animals with DOTAP:Chol–FUS1 complexes resulted in prolonged survival (median survival time, 80 days) compared with control animals. These results demonstrate that the *FUS1* gene is a promising therapeutic agent for treatment of primary and disseminated human lung cancer.[53,54] Based on these studies, a phase I clinical trial with *FUS1*-mediated molecular therapy by systemic administration of FUS1 nanoparticles is now underway in stage IV lung cancer patients at the University of Texas M. D. Anderson Cancer Center in Houston, Texas.

Conclusion

Current cancer treatment, including radiation and chemotherapy, controls less than 50% of lung cancers, with an overall 5-year survival rate of approximately 15%. Combining existing treatments has reached a plateau of efficacy, and the addition of conventional cytoxic agents is limited because of toxicity. The clinical trials summarized in this chapter clearly demonstrate that, contrary to initial predictions that single gene therapy would not be effective for cancer because of multiple genetic lesions, gene replacement therapy targeted to a tumor suppressor gene can cause cancer regression by activation of multiple apoptotic and growth inhibitory pathways with minimal toxicity.

Gene expression has been documented and occurs even in the presence of an antiadenovirus immune response, clinical trials have demonstrated that direct intratumor injection can cause tumor regression or prolonged stabilization of local disease, and the low toxicity associated with gene transfer indicates that tumor suppressor gene replacement can be readily combined with existing and future treatments. Studies combining transfer of tumor suppressor genes in combination with conventional DNA-damaging treatments indicate that correction of a defect in apoptosis induction can restore sensitivity to radiation and chemotherapy in some resistant tumors, and indications that sensitivity to killing might be enhanced in already sensitive tumors may eventually lead to reduced toxicity from chemotherapy and radiation therapy from reduced doses. The most recent data from the laboratory showing damage to tumor suppressor genes in normal tissue and premalignant lesions even suggests that this treatment strategy may someday be useful in early intervention and even prevention of cancer. Preclinical studies have shown that systemic delivery can treat disseminated metastases. The ready availability of gene libraries, the ability to administer the genes without the extensive reformulation required of small molecules, and their specificity makes this an attractive therapeutic approach. Despite the obvious promise evident in the results of these studies though, it is critical to recognize that there are still gaps in knowledge and technology to address. The major issues for the future development of gene therapy include the following:

1. Developing more efficient and less toxic gene delivery vectors for systemic gene delivery
2. Identifying the optimal genes for various tumor types
3. Optimizing combination therapy
4. Monitoring gene uptake and expression by cancer cells
5. Overcoming resistance pathways

However, given the rapid progress in the field, it is likely that many of these technological problems will be solved in the near future.

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