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Gene therapy for lung cancer: practice and promise

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Abstract

Gene therapy has emerged as an exciting and promising strategy of cancer therapy. Improved molecular biology techniques and a greater understanding of the mechanisms involved in lung cancer pathogenesis allowed a variety of genes to be validated as molecular targets for gene therapy. A variety of gene therapy strategy have been explored in the pre-clinical research. These include replacement of defective tumor suppressor genes, inactivating oncogenes, introducing suicide genes, immunogenic therapy, and antiangiogenesis-based approach. Clinical trials of gene therapy for lung cancer showed the feasibility of delivering a variety of agent as well as highlighted problems with the delivery of therapeutic constructs. Although some may consider the initial results of these novel therapies to be disappointing, they underscore the complexity of these approaches and the likelihood that these approaches will be effective only when used in a coordinated fashion in the proper clinical context. This review provides an update on our current understanding of lung cancer biology and examines several important issues in cancer gene therapy. In addition, recent results of clinical trials of gene therapy for lung cancer are presented.
Key words: Lung cancer, Gene therapy.

Riassunto

TERAPIA GENETICA PER IL CANCRO DEL POLMONE: ESPERIENZE E FUTURO PREVEDIBILE

La terapia genica appare una stimolante e promettente strategia nella terapia del cancro. Le migliorate tecniche della biologia molecolare ed una maggiore comprensione dei meccanismi implicati nella patogenesi del cancro polmonare hanno permesso ad una molteplicità di geni di essere utilicati come targets molecolari nella terapia genica.
Varie strategie della terapia genica sono state impiegate nella ricerca preclinica. Queste includono il ricambio dei geni soppressori difettosi del tumore, oncogeni inattivi l'introduzione di geni suicidi, terapia immunogenica e l'approccio basato sull'antiangiogenesi.
Esperimenti clinici nella terapia genica del cancro polmonare hanno mostrato la fattibilità della somministrazione di una varietà di agenti, e così pure ha evidenziato i problemi

Introduction

Lung cancer is one of the most prevalent cancers and is the leading cause of cancer deaths in the world (1). Despite advances in chemotherapy, surgery and supportive care, deaths rates for the disease have remained constant for nearly two decades. The poor prognosis associated with lung cancer challenges us to develop novel therapeutic approaches that can prolong life in patients with advanced disease and increase the change of long term survival and cure for patients with local or locally advanced disease.
Gene therapy is an attractive modality for the treatment of lung cancer because lungs are easily accessible via the airway and offer a large surface area for transfection allowing for regional delivery of gene of interest with a reduced risk of systemic side effect (2).
The promise of gene therapy for lung cancer is that, by modifying the genetic code of the tumor cell, it may be killed or forced to undergo to apoptosis. Both laboratory and clinical data suggest that the promise has yet to be kept, but hope remains. Several targets have been identified. The cell cycle control system may be modified to regulate mitosis or force the cell into apoptosis, reintroducing tumor suppressor genes or inactivating dominant oncogenes. Suicide genes can be inserted into the tumor, where they can activate an administered prodrug into an active drug that kills the tumor. Immunogenic therapy uses recombinant DNA constructs to express cytokines and lymphokines, which can retard

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connessi con la formulazione della via di somministrazione dei farmaci.
Mentre alcuni possono ritenere deludenti i risultati iniziali di questa nuova terapia, essi sottovalutano la complessità di queste metodiche e la probabilità che esse saranno efficaci solo quando saranno utilizzate in modo coordinato in un contesto clinico appropriato. Il lavoro costituisce un aggiornamento del nostro modo di considerare la biologia del cancro polmonare ed esamina alcune importanti proposte nella terapia genica del cancro. Inoltre, presenta risultati recenti di esperimenti clinici sulla terapia genica per il cancro polmonare.
Parola chiave: Terapia genetica, cancro del polmone.

tumor growth. Antiangiogenesis gene therapy blocks new vascular growth in the tumor. Ultimately, combinations of these methods will need to be used along with standard therapy, radiation therapy, and chemotherapy to produce their desired effects.

Biological considerations in lung cancer

Molecular analysis has demonstrated that lung cancer cells accumulated a number of genetic lesions (predominantly in recessive oncogenes) with perhaps 10 or more such events required for the development of lung cancer. In this section we focus on the hallmark molecular changes in cancer with potential for clinical translation that lead to lung carcinogenesis. Because of space limitations, we focus only the currently understood "major players".

Inactivation of tumor suppressor genes

Tumor suppressor genes play a vital role in normal cell growth control and act by providing antigrowth signals to inhibit the process of tumorigenesis. Inactivation of these genes by deletions, mutations, or aberrant methylation of normally unmethylated CpG islands in the promoter region of many genes results in the loss of tumor suppressor function. Several tumor suppressor genes that are involved in the carcinogenesis of the lung have been identified.
The most studied is the p53 gene encoding a nuclear protein which blocks the progression of cells through the cell cycle in the late G1 phase and trigger apoptosis. Structural alterations of the p53 tumor suppressor protein are observed in approximately 50% of tumors of NSCLC patients, and in some, but not all, studies *p53* mutations are associated with an adverse prognosis (3, 4, 5). Mutations of p53 result in an impaired cellular response to various stresses, including DNA damage, growth factor withdrawal, and oncogenic transformation as well as to genomic instability (6). Moreover, *p53* loss may also abrogate an effective apoptotic response to chemotherapy or radiation treatment (7).
Another tumor suppressor gene that is frequently inactivated in NSCLC is Rb. The *RB* gene is considered the founder of the *RB* family, because two other genes that are structurally and functionally related, namely *p107* and *Rb2/p130* have been identified more recently. A common relevant biological activity shared by the three members of this family is the ability to negatively control the cell cycle. In fact, they negatively modulate the transition between the G_1 and S phases, using mechanisms mostly related to inactivation of transcription factors, such as those of the E2F family, that promote the cell entrance into the S phase. The Rb protein is abnormal in expression level or structure in more than 90% of SCLCs and in 20% to 30% of NSCLC. Several data demonstrated an independent role for the reduction or loss of pRb2/pl30 expression in the formation and/or progression of lung carcinoma and interesting data have recently emerged identifying specific genes that are regulated by pRb2/p 130 (8, 9, 10).
The $p16^{INK4A}$ tumor suppressor gene is the other key component in the Rb/pathway and is often inactivated in many solid tumors. Homozygous deletion or point mutations of $p16^{INK4}$ are not frequently observed among primary lung cancers but are observed among metastatic and advanced NSCLCs (11). An alternative mechanism of $p16^{INK4}$ inactivation is aberrant methylation of the CpG island promoters, and this is common in a number of human cancers. Aberrant methylation of normally unmethylated CpG islands is associated with transcriptional inactivation and loss of expression of tumor suppressor genes in human cancers. Aberrant methylation of the $p16^{INK4}$ gene is observed frequently in NSCLCs; in 36-64% of cell lines (12) and 16-53% of primary tumors (13). Hypermethylation is thought to be the major mechanism through which $p16^{INK4}$ becomes inactivated in primary lung cancers. $P16^{INK4}$ hypermethylation was reported to be frequently detected in premalignant lesions (14). However, it is still unknown whether the methylation status of the $p16^{INK4}$ gene status changes during the progression of lung carcinoma. Some studies have reported that reintroduction of this gene into NSCLC tumor cells lacking it, results in significant tumor suppression growth (15).

Activation of proto-oncogenes

Proto-oncogenes are involved in the regulation of normal cellular growth, development and physiology. In tumors they become activated by genetic mechanism. The Ras gene family code for GTP-binding proteins which plays an essential role in signal transduction pathway (16). Early studies showed that mutations in the Ras oncogene occur frequently in lung cancers, especially in

adenocarcinomas (17). Generally, Ras oncogenes are activated by point mutations at codons 12, 13, or 61. The majority of mutations are G-T transversions, which are associated with cigarette smoking. Several studies suggested that patients whose tumors harbor these mutations have a worse prognosis than tumors without Ras mutations (18).

Nuclear proto-oncogene products, including MYC, encode nuclear DNA-binding proteins that are involved in transcriptional regulation of genes that promote cell division. Abnormal MYC expression was frequently observed in SCLCs but was less common in NSCLCs (19). The MYC proto-oncogenes (MYC, NMYC and LMYC) are activated by gene amplification or transcriptional deregulation, which results in protein overexpression.

Epidermal growth factor receptor (EGFR; erbB-l) is a member of the erb-B family of tyrosine kinase receptor proteins, which also include erb-B2 (HER2/neu), erb-B3, and erb-B4. These receptors play an important role for tumor cell survival and proliferation (20, 21). EGFR overexpression has also been demonstrated in pre-malignant bronchial epithelium, suggesting that EGFR plays an important role in lung carcinogenesis (22). In lung carcinomas, EGFR is more commonly overexpressed than HER2/neu (23). The prognostic association of EGFR overexpression in lung cancer, however, is a controversial issue. Several reports indicated that EGFR was associated with a poor prognosis (24), whereas no prognostic association was reported by other reports (25).

Enhanced tumor angiogenesis

Angiogenesis plays an important role in neoplastic development and progression (26, 27, 28). Increased angiogenesis in lung cancer is associated with poor prognosis and inhibit angiogenesis may inhibit tumor growth and metastasis. VEGF is one of the most important tumor angiogenesis inducers. VEGF and its receptors are frequently expressed in lung carcinomas, and VEGF expression is significantly associated with new vessel formation and with an adverse outcome in NSCLC patients. Another frequent studied marker of angiogenesis is micro vessels density (MVD). MVD is a significant adverse predictor of both disease-free and overall survival in NSCLC patients. In early operable NSCLC, micro vessel count is significantly higher in the invading front of the tumor and in the normal lung adjacent to the tumor as compared with the normal lung distant to the tumor. Currently under clinical investigation are new treatment approaches that affect angiogenesis.

Evading apoptosis

Virtually all human cells are endowed with the capacity to commit suicide using an evolutionarily conserved mechanism that involves activation of caspase-family cell death proteases. Caspase activation culminates in a cell death process known as "apoptosis". The activation of these intracellular proteases is carefully controlled through a delicate balance of anti- and pro-death proteins, serving to precisely regulate cell life. Defects in apoptosis represent a critical step in tumorigenesis and drug resistance. They can result by a defect in the function of pro-apoptotic signals, such as p53 or, alternatively, by a functional excess of anti-apoptotic molecules, such as BCL-2. BCL-2 protein is frequently expressed in SCLC and NSCLC (29, 30). Targeting Bcl-2 may provide a novel therapeutic approach to overcoming chemoresistance in lung cancer.

Gene delivery sistems

Current delivery systems for gene therapy include viral and non-viral. vectors (31, 32). Advantages and disadvantages of these systems are summarized in Table I.

TAB. I – BIOLOGICAL PROPERTIES OF COMMONLY USED GENE DELIVERY SIST

	Retrovirus	*Adenovirus*	*AAV*	*Lipsome*
Type	Viral	Viral	Viral	Non-Viral
Titer	Low	High	Variable	NA
Efficiency	Moderate	High	Moderate	Low
Duration of expression	Stable	Transient	Stable	Transient
Immunogenicity	Low	Moderate	Low	Low
In vitro toxicity	Low	Low	Low	Low
Repeated dosing	Possible	Not possible	Possible	Possible
Clinical trials	Yes	Yes	Yes	Yes
Advantages	Infects hematopoietic and epithelia cells	Does not need proliferating cells	Stable	Easy to produce Safety
Disadvantages	Unstable Low titer Need replicating cells	Immunogenic Temporary Does not infect marrow	Small capacity Low titer Requires helper virus	

Retrovirus are RNA viruses that are able to integrate DNA within the host cell genome (33, 34, 35). The virus enters the cell using envelope glycoproteins to bind to specific receptors on the cell surface. Viral RNA is then reverse transcribed to DNA by the virally encoded reverse transcriptase. The viral DNA is transported to the nucleus where it integrates in the host chromosome and direct transcription of the provirus. Viral transcripts are translated by the infected cell to form viral structural proteins. Some of the un-spliced viral transcripts are packaged into the newly formed viral particles and are released by budding. Their advantages include the ability to stably integrate into the host genome thereby theoretically ensuring a prolonged and stable expression of the therapeutic gene and the absence of viral protein expression. Nevertheless, they have the disadvantage of limited carrying capacity of roughly 8-12 kb, are reproduced at low titers and can transduce only the solid tumor cells that are actively dividing.

Adenoviruses have a 36 kb chromosome which is divided into "early gene regions" which are expressed prior to viral DNA replication and "late genes" which encode viral structural proteins (36, 37, 38). E1A, the first early gene, encodes two proteins via alternative splicing that suppress or activate transcription of viral and cellular genes and regulate the cell cycle. Therefore, in gene therapy studies, the majority of adenoviral vectors developed have a deletion of this gene. This allows the virus to be infective but not replicative. Adenoviral vectors are tropic for respiratory epithelium and transduce pulmonary cells efficiently. They have the further advantages of being able to transduce non-dividing cells in culture and in vivo and can be produced at high titers. The major disadvantage is their ability to trigger non-specific infiammatory and specific anti-viral immune responses. These problems may be ameliorated by co-administration of immunosuppressant agents, re-administering adenoviruses of alternative serotype o using "third generation", fully deleted adenoviruses. Moreover, adenoviral DNA remains episomal in the host cells nucleus and the virus does not integrate into cellular DNA, making the infection temporary.

Another viral vector that has been used is the adeno-associated virus (AAV). AAV is a member of the parvovirus family and is a single stranded DNA virus that requires a helper virus, such as adenovirus, for replication (39, 40, 41). The AAV integrates at a site-specific area on chromosome 19, and then remains dormant until infection with a helper virus (usually an adenovirus) allows its replication. Major advantages are that AAV can integrate stably into the host cell genome is able to infect a broad host range of cell types and it is not implicated in any human disease. Additionally, AAV are able to raise long-lasting gene expression *in vivo*, even after a single virus injection. Several authors reported persistence of expression of foreign genes transduced with rAAV from 180 days up to 18 months. Despite this advantages, the AAV has not been used clinically to date because it has a small capacity to hold DNA and it has not been produced in high titers. Like all vectors that requires promoters that utilize host cell transcriptional machinery, gene expression of AAV is limited by the ability of a particular promoter to function in a given cell type HSV has also been investigated as a potential target for gene delivery (42). A primary advantage of HSV is the large mount of gene expression that is possible because the vector is very large. Moreover, it infects a wide range of cell types with prolonged expression and high titers. Unfortunately, virus cytotoxicity and difficulty to manipulate its large genome, limited its clinical use.

Non-viral methods of gene delivery have employed primarily liposomes. Liposomes are cationic lipids complexed to DNA (43, 44). The overall positive surface charge of the cationic liposome interact with the negative charge of the DNA back-bone forming a stable complex which is internalized into cells for its electrical charge properties. Cationic lipid formulations have already been used to delivery genes to the lung in vivo (45). The advantages of liposomes include no DNA size constraints, easy bulk preparation and low immune response.

TAB. II – CLINICAL TRIAS OF GENE THERAPY IN LUNG CANCER

Author (Rej)	*Gene targeted*	*Mechanism*	*Delivery sistem*
Roth (50)	p. 53	Oncosuppressor gene	Retrovirus
Schuler (51)	p. 53	Oncosuppressor gene	Adenovirus
Swisher (52)	p. 53	Oncosuppressor gene	Adenovirus
Kubba (53)	p. 53	Oncosuppressor gene	Adenovirus
Nemunaitis (58)	p. 53	Oncosuppressor gene	Adenovirus
Schuler (59)	p. 53	Oncosuppressor gene	Adenovirus
Swisher (60)	p. 53	Oncosuppressor gene	Adenovirus
Nemunaitis (65)	p. 53	Oncosuppressor gene	ONYX
Robinson (81)	IL-2	Immunogenic therapy	Adenovirus
Escudier (82)	IL-2	Immunogenic therapy	Vaccinia Virus

Clinical trials of gene therapy for NSCLC

Studies done to date have all been early, mostly phase I trials evaluating the safety and feasibility of delivering genes to patients with advanced or recurrent NSCLC. Published studies have looked at the effectiveness of: 1) reintroduction of tumor suppressor genes 2) inhibition or downregulation of dominant oncogenes 3) suicide gene therapy 4) inhibition of angiogenesis 5) enhancing immune response. Table II summarizes these trials.

Reintroduction of tumor suppressor gene

Antitumor activity after p53 gene transfer in NSCLC has been demonstrated in preclinical studies in vitro ad in vivo. Fujiwara et al showed that introduction of wt-p53 in an orthotopic lung cancer model is able to suppress tumor growth of lung cancer cells (46). Adenoviral and retroviral vectors were both used to successfully deliver p53 to human lung cancers in athymic mouse models (47). A recent study showed that introduction of wt-p53 into NSCLC cell line is able to suppress tumor growth by inducing apoptosis (48). Further investigations present evidence of an anti-angiogenic effect of p53 replacement (49). After infection with the p. 53-adenoviral vector, a reduced expression of VEGF was reported as well in transduced as in non-transduced human lung cancer cells.

On the basis of these results, clinical trials of p53 replacement were performed in patients with unresectable lung cancer.

The first study was performed by Roth et al at the M.D. Anderson Cancer Center (50). The wt-p. 53 was administered to the tumors by direct intratumoral injection of a retrovirus vector carrying a wt-p. 53 cDNA driven by the B-actin promoter. The tumors were biopsied before and after wt-p. 53 therapy. In six of seven tumor biopsied increased TUNEL staining was observed in post-treatment biopsies compared to the pre-treatment specimens. Interestingly, the percentage of cells in the TUNEL assay exceeded the percentage of cells containing vector DNA in more specimens, indicating the presence of a bystander effect. Nine patients entered the protocol. Eight of the nine patients completed the protocol, and all eight showed evidence of gene transfer. Three of the seven who were assessable showed evidence of local tumor regression in treated lesions, whereas other untreated lesions continued to progress. No toxic effects directly attributable to the vectors were observed in those seven assessable patients; however the low transduction efficiency of the retroviral vector was limiting. A11 subsequent trials have utilized adenoviral vectors for gene transfer since such vectors are relatively easy to manufacture at large scale, can be produced at higher viral titers, and have the ability to transduce both dividing and non-dividing cells.

A phase I clinical trial of an adenoviral vector expressing wild-type p53 was carried out (51). Vector DNA was detected in 80% of the assessable patients, indicating successful gene transfer. Vector-specific p53 mRNA, as indicator of gene expression, was detected in 46% of patients. Measurement of apoptosis revealed tumor cells undergoing apoptosis in all but one of the group of patients expressing the gene. Vector-related toxicity was minimal, despite up to six injections per patient repeated at monthly intervals. Of the 25 assessable patients, two (8%) exhibited partial responses, 16 (64%) exhibited disease stabilization ranging from 2 to 24 months, and the remaining seven (28%) exhibited disease progression. Similar results in terms of gene transfer and toxicity were reported in another study by Swisher et al (52).

In a subsequent study a novel treatment concept was carried out by Kubba (53). In this study patients with bronchiolo-alveolar carcinoma were treated with Ad-p53 administered by bronchial lavage. Bronchiolo-alveolar carcinoma is an uncommon subtype of NSCLC in which tumor cells tend to spread aerogenously in thin layers along distal airways, potentially allowing for disseminated gene transfer by vector administration via the airways. A pilot dose escalation study was performed in which two treatments at the same dose level were administered 2 weeks apart to a single involved lobe followed by additional treatments to all involved lobes as tolerated. 24 patients were treated and individual patients received up to 14 cycles of therapy. One patient demonstrated a partial response in the lung and an additional patient demonstrated tumor regression at metastatic sites in the liver and brain raising the possibility of a systemic immunologic bystander effect. A future study combining airway delivery of Ad-p. 53 with chemotherapy in bronchiolo-alveolar carcinoma is planned. Alternative approach to enhance aerosolized gene delivery of p53 and other genes are also being investigated including aerosolization of adenoviral vectors incorporated into calcium phosphate precipitates and formulation with cationic polymers such as polyethyleneamine (PEI).

Preclinical studies showed that synergistic growth inhibition could be achieving by combining p53 gene therapy with cisplatin and irradiation (54, 55, 56, 57).

On the basis of results of preclinical studies, Nemunaitis et al initiated a phase I trial of p53 gene transfer in sequence with cisplatin in 24 NSCLC patients with nonfunctional P53 genes (58). Intravenous cisplatin was administered and 3 days later p53 was delivered directly in the tumor. Up to a total of 6 monthly courses were carried out. Seventeen patients remained stable for at least two months, two achieved partial responses, and five continued a progressive disease. When tumor biopsies were analyzed for apoptosis, 14% demonstrated no change, 7% showed a decrease in apoptosis, and 79% demonstrated an increased number of apoptotic cells. Notably, 75% of the patients entered onto the trial experienced tumor progression while being treated with cisplatin or carboplatin-containing regimens.

In a subsequent study, Schuller et al failed to demon-

strate an additional benefit from intratumoral adenoviral p53 gene therapy in patients receiving an effective first-line chemotherapy for advanced NSCLC (59). No difference between the response rate of lesions treated with *p53* gene therapy in addition to chemotherapy (52% objective responses) and lesions treated with chemotherapy alone (48% objective responses) has been detected.

A phase II study combining Ad-p53 with radiation therapy was carried out by Swisher et al (60). This trial was designated for patients with loco-regionally advanced non metastatic NSCLC who could not tolerate chemo-radiation because of age or co-morbidities.

Additionally, patients with localized disease who were unable to tolerate surgical resection because of poor pulmonary function were also eligible. Patients were treated with three intratumoral injections of Ad-p53 on days 1, 18 and 32 in combination with 60 Gy of radiation. Ad-p53 doses were injected directly into the primary tumor using bronchoscopy or CT guidance. Post treatment biopsies demonstrated pathologic complete response in 8 of 11 patients who underwent biopsies, suggesting a high pathologic control rate at the primary tumor. As a matter of fact, historical data demonstrating only a 20% pathologic complete response rate with radiation alone. The 1-year progression free survival was 45.5%, with most failures occurring because of metastatic progression rather then local failure. Other than injection-related pneumothoraces in 13 patients, treatment was well tolerated. All patients with pneumothorax cases were managed as outpatients by observation (eight patients) and use of a percutaneous pleural catheter (five patients). No treatment-related mortality was observed and most patients were successfully treated as outpatients. This study shows that Ad-p53 can be administered in conjunction to radiation therapy in an outpatient setting in patients with locally advanced NSCLC with low toxicity. The high negative pathologic control rate is encouraging but the continued metastatic failure emphasizes the need to combine Ad-p53 with chemotherapy agents to try to address the distant disease. Phase III trials will be required to determine whether the potentially improved local control rate obtained with Ad-p53 can translate into improved overall survival. These trials will require randomization with Ad-p53 administered in combination with chemo-radiation regimens.

A different p53 gene replacement approach is use of gene-modified adenovirus against p53 mutant tumors (61, 62, 63, 64). The ONYX vector system is the most widely studied of the conditionally replication-competent vectors for cancer gene therapy. This vector system is designed on the basis of the proposition that cells with w-t functional p53 inhibits the replication of the adenoviral vector because intracellular p53 was inactivated by E1B, a 55-Kd protein. ONYX-015 is an adenovirus mutant that lacks the E1b gene. As a result it cannot neutralize p53. Therefore it should only grow in cells that lack p53, and should replicate selectively in cancer cells. Antitumoral efficacy was documented following intratumoral or intravenous administration of ONYX-015 to nude mouse-human tumor xenografts. Moreover, efficacy of ONYX-015 plus chemotherapy (cisplatin, 5fluorouracil) was significantly greater than with either agent alone. A pilot study of e.v. administration of ONYX-015 in patients with cancer metastatic to the lung has been performed and included 2 patients with NSCLC (65). In one of these patients intratumoral viral replication without associated replication in surrounding normal lung was documented on a post-treatment biopsy and increasing viral genome copy number was detected in plasma for at least 7 days consistent with possible in vivo viral replication. Nevertheless, all patients developed anti-adenoviral antibodies and no tumor responses were seen.

Antisense therapy

Antisense therapy is a technique designed to ablate expression of dominant oncogenes (66). Inhibition of oncogenic function can be attempted at different levels. First, transcription of the oncogene can be inhibited using triplex-forming oligonucleotides or other sequences that bind transcriptional start sites in the genomic DNA. Second, translation of the oncogene messenger RNA can be blocked using specific antisense sequences, which function by promoting degradation of the complementary message. These molecules are currently undergoing clinical tests. Approximately 30% of lung adenocarcinomas express mutant k-ras alleles. Because mutated k-ras appears to contribute to the malignant phenotype, it was hypothesized that an approach targeting the k-ras may have therapeutical efficacy. In NSCLC, inhibition of c-myc and k-ras expression by the antisense technique has also been shown to inhibit cell proliferation in vitro (67, 68). Clinical trials using antisense k-ras delivered by retroviral vectors have been approved for patients with NSCLC (69). Third, protein product of oncogene can be blocked by vectors encoding oncoprotein neutralizing antibody fragments (70, 71). Cochet et al showed that the introduction of an anti-ras single-chain antibody fragment could mediate apoptosis in the ras transformed lung carcinoma cell line H460. Alternatively, the overexpressed protein can be functionally disabled by preventing its trans-membrane expression (72). The feasibility of this approach was demonstrated by using a vector encoding an intracellular single-chain antibody fragment directed against HER-27neu that bound it intracellularly and abrogated its growth-promoting properties.

Suicide gene therapy

Suicide gene therapy is used to transduce cancer cells with a gene construct that is able to convert a pro-drug

into active drug which is toxic for target cells. One such suicide gene is the herpes simplex thymidine kinase (HSV-tk) gene. This gene encodes for an enzyme that converts the normally nontoxic nucleoside analogue, gancyclovir, to its activated triphosphate form, a toxic compound that leads to cell death. The process requires a bystander effect to kilI cells not infected with vector (73). Bystander effect seems to occur for phosphorylated GCV transfer from transduced to un-transduced cells by intercellular bridges, gap junctions, or by uptake of small vesicles containing activated GCV released by apoptosis. Preclinal studies demonstrated the value of HSV-tk plus GCV in an immunocompetent orthotopic lung cancer model. Fukunaga et al reported a prolonged survival of mice inoculated with AdHSVt transfected tumor cells following treatment with gancyclovir compared with control (74, 75). Clinical trials of suicide gene therapy in lung cancer patients have not been performed to date. However, interesting results have been reported in mesothelioma patients. Two clinical trials utilizing an adenoviral vector to deliver the HSV-tk gene to patients with mesothelioma have been reported (76, 77). Gene transfer was confirmed in more than half of the patients and several partial tumor regressions were noted.

Immunogenic therapy

Immunogenic therapy is used to transduce cancer cells with cytokine genes that are able to enhance their immunogenicity. The cytokine is produced in high concentrations in the vicinity of the tumor, thereby altering the local immunologic environment of the turnor cell so as to either enhance presentation of tumor specific antigens to APC or to enhance the activation of tumor-specific lymphocytes. Many cytokine genes have been introduced into tumor cells with varying effects on both tumorigenicity and immunogenicity. It has not yet been determined which cytokines are optimal for lung cancer but are of particular interest IL-7, IL-12, GMCSF (78, 79, 80). Unfortunately, clinical trials showed discouraging results in lung cancer. Nine patients with NSCLC were treated with intratumoral injection of an adenoviral vector expressing IL-2 without evidence of activity (81). One study of intratumoral administration of a vaccinia virus expressing IL-2 in patients with chest wall masses associated with pleural mesothelioma has been conducted. Transient tumor associated expression of IL-2 was detected but immune responses were minimal and no tumor regressions were noted. Neutralizing anti-vaccinia antibody responses were detected in all patients (82). An altemative approach is to use modified lung cancer cells to delivery a variety of antigens but to increase the immune response through concomitant administration of cytokines, such as GMCSF. In practice, GMCSF is introduced into autologous tumor cells. Cells are then re-inoculated into the subcutaneous tissues of the patient. The GMCSF gene produces local GMCSF which increases the immune response to the autologous tumor. This approach has been evaluated in a phase I trial in patients with early and advanced NSCLCs (83). Results of this study are discouraging because objective responses have been observed only in a minority of advanced-stage patients and prolonged remission free duration has been observed only in some early-stage patients immunized after tumor resection. Moreover, this approach is complex because it requires tumor resection/biopsy with tumor processing before it can be undertaken.

Anti-angiogenesis gene therapy

Delivering genes with anti-angiogenic properties directly to the tumor vasculature is an emerging and promising therapeutic strategy since Judah Folkman has popularized the concept that angiogenesis is essential to tumor growth and represents an exciting and complex molecular target (84). Sauter et al published the results of animal studies using adenoviral vectors carrying a endostatin transcription unit (85). These studies showed that the intravenous injection of adenoviral vectors produces constant levels of endostatin and is associated with a reduction of the growth of the Lewis lung cancer cells lines in nude mice. Gene therapy techniques also have been used to directly inhibit VEGF activity. VEGF plays an important role in the pathogenesis of lung cancer as demonstrated by several studies reporting a correlation between VEGF levels and survival (86). For example antisense oligonucleotides targeting VEGF are able to decrease tumor growth in lung cancer. Alternatively, VEGF activity can be blocked by interfering with the transcription factors that mediate its effect (87). These approaches has not yet to be expressed in clinical programs.

Conclusions

Gene therapy is perhaps the most prominent ed exciting new approach to the treatment of cancer. Clinical evaluation of gene therapy in lung cancer has just began. Lesson learned from these studies is very important. First, gene therapy has an excellent profile of safety and does not appear to enhance the toxicity of chemotherapy or radiation. So that, if toxicity can be controlled, combinations of gene therapy modalities might work, especially in concert with conventional multimodality modes of therapy including surgery, radiation therapy and chemotherapy. Second, response rate reported are acceptable but they are localized in the site of injection of vector. Use of gene therapy as loco regional treatment could be another important area of research in lung cancer because, despite improvements in radiation and chemo-

radiation, loco regional control remains poor in his disease. Third, a number of biologic problems must be solved: designing gene delivery vehicles that carry out efficient delivery of exogenous DNA into cells; obtaining proper qualitative tissue-specific expression of transferred, therapeutic genes; obtaining proper quantitative expression of transferred genes; obtaining the proper temporal expression of transferred genes; avoiding immunological responses to foreign gene products. Each of these problems remains today a challenge.

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