

Molecular targets and gene therapy of lung cancer

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Lung cancer is of great interest in human pathology because its apparent aggressiveness cannot be stopped by applied treatment procedures. The lack of highly specific screening tests prevents an early diagnosis of the disease. Insidious beginning and diverse and unclear clinical picture are responsible for the fact that most cases are diagnosed at advanced stages. An increasing number of patients and a short length of survival are additional factors that make this disease an imperative in the clinical practice, while vague and mutually dependent etiological factors represent a challenge in laboratory studies of the pathogenesis. The objective of this review is to describe some of the potential molecular targets available for manipulation in lung cancer; vector currently used by thoracic investigators to deliver therapy, and illustrated the experience with clinical trials of gene therapy in lung cancer. While gene therapy offers new hopes for lung cancer treatment, it is the need to develop valid clinical protocols of randomized trials before safety using to various lung cancer patient populations.

Key Words: Lung Neoplasms; Gene Therapy; Clinical Trials as Topic; Clinical Protocols

INTRODUCTION

Beginning in the late 1960s, published reports theorized that genes of interest could be transferred into human cells using viral DNA shuttles to treat monogenic deficiency disease states (1,2). Over two decades later, in September 1990, Blaese et al. launched the first approved gene therapy clinical trial in an attempt to study the rare adenosine deaminase deficiency (3). In the 12 years that have passed since that inaugural patient was enrolled, a gene therapy revolution has transpired, with hundreds of clinical trials completed for a variety of conditions, including more genetically-complex disorders such as cancer.

Gene therapy, which can be defined as the introduction of new genetic material into cells for therapeutic intent, is a tool that may aid in the development of novel cancer therapies based on important basic scientific advances in the understanding of the immune system and the molecular biology of cancer. Two requirements exist for successful genetic manipulation of a eukaryotic cell. First, a method must exist to provide successful gene insertion into the correct cell type with adequate efficiency for a particular therapeutic purpose. Some therapeutic approaches may require permanent gene transfer, whereas for others, transient activity may suffice. Second, the inserted gene must be adequately expressed by that cell.

Indeed, great strides have been made but the race toward more promising results will no doubt take time. The price of developing such molecular-based therapies needs to be justified by a number of factors, including the population of individuals affected and the subsequent burden to society, along with the lack of currently available efficacious treatments.

Lung cancer is the leading cause of cancer death in both men and women worldwide. An estimated 215,020 new cases in the USA (114,690 in men and 100,330 in women) of lung and bronchus cancer will be diagnosed in 2008 and 161,840 deaths (90,810 in men, 71,030 in women) are estimated to occur due to this disease (4). Only 15% of all lung cancer patients are alive 5 years or more after diagnosis. The relative 5-year survival rate of 15% is poor in comparison to other cancer including breast (86%), colon (62%), and prostate (97%) (5). In addition, this number has not changed significantly over the past 30 years. The lack of effective screening procedures means the majority of patients present in later stages in which tumors are often resistant to even multimodal therapy. Accordingly, it is not surprising to see that the molecular characterization and subsequent application of gene transfer meth-

ods in lung cancer has played a large role in the development of gene therapy over the last decade.

The objective of this review is to discuss some of the potential molecular targets available for manipulation in lung cancer, describes vector currently used by thoracic investigators to deliver therapy, and illustrated the experience with clinical trials of gene therapy in lung cancer.

MOLECULAR TARGETS IN LUNG CANCER

During the progression from normal to malignant cell transformation in lung cancer, a myriad of potential causative and molecular factors have been elucidated. Chromosomal aberrations are seen at an early stage. Lung cancer cells display chromosomal instability- that is, numeric abnormalities (aneuploidy) of chromosomes, as well as structural cytogenetic abnormalities (6). Deletions of both chromosome 3 and 13 have been documented in lung tumor specimens (7,8). Although the specific genes associated with the chromosomal changes are being rapidly identified, the underlying mechanisms of this chromosomal instability are not yet known. Alterations in microsatellite polymorphic repeat sequences are another type of instability, found in 35% of small-cell lung cancer (SCLC) and 22% of non-small cell lung cancer (NSCLC) (9). However , the DNA repair genes affected in lung cancer that give these changes are still unknown. These may be followed by alterations in cell cycle regulation and growth factor signaling that assists the tumor as it continues to grow. The cancer also develops mechanisms to fight nature's methods of surveillance and programmed death, leading to future local infiltration and eventually systemic spread. With both hereditary components and various environmental carcinogens (most common tobacco exposure) exercising roles in this progression, there are no doubt a number of events that can occur.

Tumor suppressor genes

p53 maintains genomic integrity in the face of cellular stress from DNA damage (for example, caused by gamma and ultraviolet irradiation, carcinogens, and chemotherapy). It functions as a transcription factor to activate the expression of genes that control cell-cycle checkpoint, apoptosis (BAX), DNA repair (GDD45), and angiogenesis (thrombospondin). The p53 gene, located at chromosome 17p13, is the most frequently mutated tumor suppressor gene (TSG) in human malignancies, and mutations affect approximately 90% of SCLC and 50% of NSCLC. In NSCLC, p53 alterations occurred more fre-

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Abbreviations

DNA- deoxyribonucleic acid, RNA-ribonucleic acid, mRNA- messenger ribonucleic acid, siRNA- vector-based small interfering ribonucleic acid, the USA- the United States of America, SCLC- small-cell lung cancer, NSCLC- non-small cell lung cancer, TSG- tumor suppressor gene, G→T- guanine → thymine, FHT- fragil histidine triad, VEGF- vascular endothelial growth factor, bFGF- basic fibroblast growth factor, PDGF- platelet derived growth factor, IL-8- interleukin-8, EGFR- epidermal growth factor receptor, KD- kiloDalton, TNF- α - tumor necrosis factor - α , NF- κ B- nuclear factor of kappa light chain gene enhancer in B cells, NF- κ BNIK- nuclear factor of kappa light chain gene enhancer in B cells - inducing kinase, I κ B-IKK- inhibitor of kappa B kinase, NIK- inducing kinase, IKK- inhibitor of kappa α kinase, IKK- IkappaB kinase, VSV-G- vesicular stomatitis virus, CAR- Coxackie adenovirus receptor, CT- Computed Tomography, Ad-53- Adenoviral-p53, BAL- bronchial lavage, BAC- bronchoalveolar lung cancer, vp- viral particles, HSVtk- herpes simplex virus thymidine kinase, GCV- ganciclovir, UCMS- umbilical cord matrix stem

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quently in squamous cell (51%) and large cell (54%) carcinomas than adenocarcinomas (39%) (10-12). *p53* mutations correlate with cigarette smoking, and most are G→T transversions. The observation that *p53* mutations occur in over 50% of lung cancers and the confirmations of its role as an anti-oncogene made this an attractive target for initial preclinical studies (13-15). The fragile histidine triad (*FHIT*) gene is found at chromosome 3p14.2, and is absent in many lung cancers, including the squamous cell type (87%) and adenocarcinoma (57%). The loss of *FHIT* protein is strongly associated with smoking and occurs in some preneoplastic lesions. Functionally transfection of wild-type *FHIT* into lung cancer cells, induces apoptosis and suppresses tumorigenicity (16). Associated with decreased survival in NSCLC when absent (17), the gene has been noted to inhibit cell growth *in vitro* and eliminate tumorigenicity *in vivo* (18). Another TSG known as melanoma differentiation associated gene (fragile histidine triad) was initially discovered by subtraction hybridization in melanoma cell lines and has effective apoptosis-inducing effects in NSCLC (19,20).

Angiogenesis and growth factors

Cancer metastasis is a sequential and selective process that consists of a series of interlinked, but independent steps, including angiogenesis, motility, invasion, adhesion, extravasation, and proliferation. Tumor angiogenesis is the process whereby a tumor creates new blood vessels to assist in growth and the development of metastasis. Tumor angiogenesis is necessary for tumor mass to grow beyond a few millimeters in size and is regulated by the balance of inducers and inhibitors that are released by tumor cells and host cells. This discovery by Folkman in the 1970s, has led to intensive investigation into methods to inhibit tumor neovessel formation. This increase in microvasculature is a predictor of the probability of metastatic disease and prognosis in NSCLC (21).

These angiogenic factors are mainly proteins, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), placental growth factor, pleiotropin, transforming growth factor- β , E-cadherin and others.

One gene therapy strategy, therefore, has been to inhibit the production or function of proangiogenic cytokines. A second strategy involves the delivery of genes that encode inhibitors of angiogenesis.

VEGF is a proangiogenic cytokine that can bind to two high-affinity receptors (VEGFR-1 or Flt-1, and VEGFR-2 or KDR) expressed on vascular endothelial cells. Angiogenesis is required for tumor progression and metastasis and can be induced by various factors, including the vascular endothelial growth factor-C (VEGF) family of polypeptide growth factors, which is comprised of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. Among the VEGF family, VEGF-C has recently been found to induce not only angiogenesis, but also lymphangiogenesis, via VEGF receptor-3 (VEGFR-3) and VEGF receptor-2 (VEGFR-2). Expression of VEGFR-3 is highly restricted to lymphatic endothelial cells and stimulated by only VEGF-C and VEGF-D.

bFGF and angiogenic CXC chemokines, such as interleukin-8 (IL-8), have all been implicated in lung cancer. Reduced E-cadherin expression, which can be occur by promoter hypermethylation, was associated with tumor dedifferentiation, increased lymph node metastasis, and poor survival in NSCLC patients. Reduced α 3 integrin expression correlated with a poor prognosis

of patients with lung adenocarcinoma. Specific CD44 isoforms may also be associated with lung cancer metastasis.

A correlation between upregulated VEGF and progression of disease in NSCLC has been shown (22,23). A number of agents attacking either the protein or the receptor of VEGF have been developed, including bevacizumab (Avastin®), which is a human monoclonal antibody against VEGF that has shown increased time-to-progression in NSCLC when combined with chemotherapy in phase II trials (24). Originally identified as a promoter of fluid permeability in ascites (25), the use of similar therapy to reduce malignant effusions in mesothelioma and NSCLC is currently under study (26).

Many growth factor/receptor system are expressed by either the lung tumor or adjacent normal cells, thus providing autocrine or paracrine growth stimulatory loops. Overexpression of epidermal growth factor receptor (EGFR) is observed in approximately 70% of NSCLC and may be a prognostic factor for poor survival. Coexpression of EGFRs and their ligands, especially transforming growth factor- α , by lung cancer cells indicates the presence of an autocrine (self-stimulatory) growth factor loop.

Endostatin is a 20-kD cryptic fragment of collagen XVIII and is the first endogenous angiogenesis inhibitor to be discovered as a cryptic fragment of a basement membrane protein. Endostatin inhibited tumor necrosis factor (TNF)- α -induced angiogenic signaling by down-regulating the TNF- α receptor. In addition, NF κ B (nuclear factor of kappa light chain gene enhancer in B cells) was down-regulated by endostatin. Both, nuclear factor NF κ B -inducing kinase (NF κ BNIK) and inhibitor of kappaB kinase (Ik κ BKK) have been implicated as essential components for activation NF κ B in response to many external stimuli. However, the exact roles of inducing kinase (NIK) and inhibitor of kappa α kinase (IKK α) in cytokine signaling still remain controversial. Cells lacking functional NF κ B die after ligation of some TNF receptor family members through failure to express NF κ B-dependent anti-apoptotic genes. NF κ B activation requires the IkappaB kinase (IKK) complex containing two catalytic subunits named IKK α and IKK β that regulate distinct NF κ B pathways. IKK β is critical for classical signaling that induces pro-inflammatory and anti-apoptotic gene profiles, whereas IKK α regulates the non-canonical pathway involved in lymphoid organogenesis and B-cell development. These results argue that endostatin may function as cryptic tumor suppressor proteins, offering an additional line of defense against tumor progression by blocking angiogenesis with endostatin gene therapy (27). *ERBB2* (*HER2/neu*) is highly expressed in more than one-third of NSCLC, especially adenocarcinomas, although gene amplification as seen in breast cancer is not usually the underlying mechanism in lung cancer. A meta-analysis suggested that overexpression of *ERBB2* is a factor of poor prognosis for survival in NSCLC (28,29). Trastuzumab (Herceptin®), a recombinant humanized monoclonal antibody that recognizes *HER2* and thus blocks its activity, is being tested for efficacy in NSCLC as a single agent or in combination with chemotherapy (30).

Oncogenic mutations

Normal genes altered by mutations, amplification, or chromosomal rearrangement can converted into oncogenes that are involved in carcinogenesis. Genes that normally conduct signals or play a role in the proliferation of cells can suddenly become overactive.

Proto-oncogene is assumed to be functioning in mitogenic processes in the following way: growth factors initiate transmission of signals for growth

through a cell by binding for its receptor. The signal is then transmitted by tyrosine kinases at the inner side of the cell membrane produced by *SRC*, *ABL*, *FES* genes. Increased levels of *SRC* expression have been found in a range of cancers, especially breast, colorectal, prostate and lung. Preliminary preclinical data and pharmacodynamic data suggest that *SRC* inhibition is a viable therapeutic option in the treatment of advanced NSCLC (31). RAS proteins, similar to G proteins that are at the cytoplasmic side of the membrane, function as GTP phosphatases and transmit those signals to the serine-threonine kinase in the cytoplasm, produced by *MYC* and *MOS* genes. Finally, the signal ends in the nucleus where it induces nuclear proto-oncogene – *FOS*, *JUN*, *MYB*, whose proteins bind directly or in a complex with other proteins to specific regulatory sequences of the target genes thus changing the transcription and finally bringing to DNA synthesis. Kinases family is signaling enzymes that have long been recognized to regulate critical cellular processes such as proliferation, survival, migration, and metastasis.

RAS genes belong to the group of ubiquitous, eukaryotic genes that play an important role in the cell proliferation and differentiation. The group of *RAS* genes consists of *H*-, *K*- and *N-RAS* genes that code almost identical 21 kD proteins designated as p21. The *RAS* gene family can be activated by point mutations at codons 12,13, or 61, and one member of this family is mutated in approximately 20% to 30% of NSCLC (particularly adenocarcinomas), but hardly ever in SCLC. The *K-RAS* genes are a set of oncogenes mutated in one-third of lung adenocarcinomas and associated with very poor prognosis (32). Characteristically, approximately 70% of *K-RAS* mutations are G→T transversions, with the substitution of the normal glycine by either cystine or valine. Similar G→T transversions also affect the *p53* gene in lung cancer (33). Efforts to combat the increase in signal transduction present after mutation is identified include: antisense sequences (either synthesized as oligonucleotides or carried in vectors) that bind to complimentary *K-RAS* mRNA inducing enzymatic degradation (34,35) and vectors containing ribozymes that specifically target destruction of known *K-RAS* mutant sequences (36).

Apoptosis mediators

The *BCL-2* family represents a set of genes that work downstream of *p53* in the apoptotic cascade. The identification of specific genes involved in either promoting (*BAX*, *BAK*) or antagonizing apoptosis (*BCL-2*, *BCL-X1*) has led to another area for gene manipulation in lung cancer (37). Abnormalities in the expression of all of these genes are present in different degrees depending on lung cancer type. In certain NSCLC, the *BCL-2* protein is overexpressed; however, this is more common in those with neuroendocrine features and in SCLC (38). Because these genes work in the late stages of apoptotic cascade, the potential to bypass earlier conflicting genetic alterations is present, provided overall toxicity can be limited.

GENE THERAPY VECTOROLGY IN LUNG CANCER

Delivery of therapeutic gene constructs to tumors in the lung present many challenges, but also presents interesting avenues for research. The anatomy and unique physiology of the lung as a collapsible air-containing structure surrounded by the largest blood vessels in the body provide for an increased degree of difficulty when considering invasive delivery procedures, compared to other organs such as the liver, bowel, or even brain. This being said, central tumors can often be reached by flexible bronchoscopy, and peripheral tumors by established transthoracic radiologic-guided approaches. There is also the

possibility of utilizing inhaled delivery systems (39) or even bronchial lavage (40). Finally, investigators have demonstrated the fact that using the bronchial circulation may be a better way to deliver conventional chemotherapy to lung tumors, and this may be an area worthy of pursuit for application of gene therapy as well (41). However, all approaches, as for all other anatomic areas of the human body, are limited in effectiveness by shortcomings in gene therapy vector delivery, persistence, and targeting.

With the completion and refinement of the Human Genome Project (42) and subsequent new developments in proteomics, one can only anticipate that the discovery of even more targets is on the horizon for diseases such as lung cancer. From the beginning, the major problem facing scientists has been not so much which molecular aberrations to pursue, but how to deliver the specific genes or therapies of interest. The ideal vector would possess a number of characteristics, including specificity to tumor cells over normal cells, growth in both dividing and non-dividing cells (i.e., broad tropism), resistance to cytotoxic and humoral-mediated destruction, extended transgene expression, and low inflammatory and toxic response. Currently, viral based vectors are most commonly used, but they incorporate only some of the above characteristics. The major viral vectors used in lung cancer can be furthered divided into those that integrate into host chromatin (oncoretrovirus) and those that remain in the cell nucleus as episomal forms (adenovirus).

Retrovirus

Retroviruses are RNA viruses that are capable of stably integrating DNA within the host cell genome. The replication cycle of a retrovirus begins with viral attachment to a cell by a specific receptor. The virus enters the cell and the viral RNA is reverse transcribed to DNA by the virally encoded reverse transcriptase. The viral DNA is then transported to the nucleus, where it integrates into the host cell genome. The integrated viral DNA, termed the provirus, is transcribed, and then both spliced and unspliced transcripts are translated to form the viral proteins. Some of the unspliced transcripts are packaged, via a packaging signal sequence (ψ), into viral capsids. The mature viruses then bud from the host cell membrane.

Retroviral vectors for gene transfer have been constructed by substituting the gene of interest in place of the viral protein coding regions, which, thus makes these vectors replication incompetent. These vectors are packaged into retroviral particles using helper, or packaging, cell lines that contain the structural viral protein genes in trans (i.e., from another site in the packaging cell genome). Because the retroviral vector contains the ψ sequences, it is packaged into the mature virus and is capable of infecting target cells but incapable of replication due to the absence of the retroviral protein coding regions. The viral structural genes provided in trans are not packaged due to the absence of the ψ sequences.

Single-stranded RNA retrovirus was the first viral vector used in preclinical and clinical studies. The *GAG*, *POL*, and *ENV* genes required for replication of the virus are replaced with a therapeutic construct. Initially, its main advantage was the ability of this vector to integrate into the host genome and lead to long-term expression. Inherent with this property came what was one thought to be a negligible risk for promoting new cancer through insertional mutagenesis, but is now considered a real concern after recent reports of the development of leukemia in patients treated with gene replacement (43).

VEGF-C and VEGF-A are involved in lymphangiogenesis and angiogenesis. To inhibit metastasis, combination therapy with vector-based small interfering RNA (siRNA) against VEGF-C and/or VEGF-A was conducted on murine metastatic mammary cancer. Shibata et al. (44) suggest that specific silencing of the VEGF-C or VEGF-A gene alone can inhibit lymph node metastasis. However, combination siRNA therapy targeting both VEGF-C and VEGF-A inhibits both lymph node and lung metastasis, rendering this combined therapy more beneficial than either alone. The observed anti-metastatic activity of siRNA-expressing vectors targeting VEGF-C or VEGF-A may be of high clinical significance in the treatment of metastatic breast cancer. This data offer a new highlight for lung cancer gene therapy. For accomplishing to prevent adverse side effects, is to more accurately target tumor cells. That method known as pseudotyping consists from this: the viral envelope of the retrovirus is altered by substituting receptor binding proteins from an alternative virus such as vesicular stomatitis virus (VSV-G), leading to an increased range of cells available as hosts, and allowing more efficient amplification during production (45,46). Clinical use of retroviruses for lung cancer gene therapy treatment is limited by the difficulties in effective supernatant *in vivo* delivery, the inability to remove and perfuse (or perfuse *in situ*) the lung for long periods of time, or to reliably transfer some kind of native "packaging cells" to the lung.

Adenovirus

The double stranded DNA adenovirus is currently the most widely used vector in gene therapy and is known for its high transfer efficiency in multiple tissues, especially lung. First generation vectors were created by deleting early viral genome sequences, which are necessary for replication, and replacing them with cassettes containing the desired recombinant DNA. Advantages include large packaging ability, broad tropism, and ease of creation. Clinical trials have demonstrated low serious toxic effects when high viral load is delivered locally. The main problems encountered by the vector and the immunologic response generated by the vector and the transient nature of transgene expression. An evolution in the production of the virus has continued since its initial use; however, newer vectors containing greater viral genome deletions (often referred to as "gutted" vectors) are ultimately decreasing the cytotoxic response (47). Besides pseudotyping, other transductional targeting approaches have attempted to link vector capsids to antibodies that direct specific receptor binding and evade neutralizing antibodies (48), or by upregulating receptors involved in adenoviral transduction such as the Coxsackie adenovirus receptor (CAR) (49).

CLINICAL TRIALS OF GENE THERAPY IN LUNG CANCER

Preclinical studies and initial clinical trials

The product of the *p53* gene is capable of binding damaged DNA for repair or induction of apoptosis. In lung cancer, there is the overwhelming link between tobacco exposure and acquired *p53* mutation (50).

Preclinical studies using both retroviral and adenoviral delivery confirmed the therapeutic effect of *p53* (51,52) and eventually paved the way for the initiation of clinical trials. These studies also hypothesized that single intratumoral injections given at specific intervals could be effective, due to factors such as the *bystander effect* (where neighboring cells not transduced are destroyed by various local mechanisms) (53). In 1996, Roth et al. (54) published results of the first clinical trial for *p53* replacement in NSCLC. A retroviral vector containing wild-type *p53* was injected into the tumors of nine advanced stage patients, using either bronchoscopic or

CT (computed tomography) guidance. Evidence of *p53* expression, apoptosis, and tumor regression was described in some patients. This study proved that gene therapy could be administered safely with no serious grade toxicities. Building on the success of the above delivery method, Keddy et al. (55) used Adenoviral-*p53* (Ad-53) with bronchial lavage (BAL) to treat patients with bronchoalveolar lung cancer (BAC). This diffuse form of lung cancer normally grows along the alveoli making direct treatment approaches difficult. Chemotherapy has been largely ineffective in its treatment. Patients were given two cycles of BAL with escalating doses. Twenty-five patients were treated viral particles (vp) at doses between $2 \times 10(9)$ and $2 \times 10(12)$ vp. At $2 \times 10(12)$ vp, one patient experienced grade 4 pulmonary toxicity, and one patient died 25 days after his second cycle. The most frequent toxicities included low-grade fever, hypoxia, and dyspnea. Of the 23 assessable patients, 16 had stable disease as their best response. Subjective improvement in breathing was noted in eight patients. Ad-*p53* can be administered safely by BAL at $5 \times 10(11)$ vp with repeated dosing. Stabilization of disease and symptomatic improvement may warrant further studies of Ad-*p53* or other adenoviruses administered by BAL in patients with BAC. Symptomatic response encourages further experimentation with this form of treatment and phase II trials in conjunction with chemotherapy (55).

Prodrug gene therapeutics

The concept of prodrug therapy in cancer was first proposed in 1986, because of systemic chemotherapy's general toxicity, which is due to its lack of specificity for malignant cells (56). The basic principle relies on the delivery of an enzyme-producing gene into the cell, which then converts a nontoxic drug into a cytotoxic substance. Because this form of therapy does not target specific genetic or tissue abnormalities, it was first applied to cancers that are generally restricted to specific anatomic spaces or cavities, including malignant mesothelioma (57).

After a number of successful preclinical studies (58,59), clinical trials evaluating the efficacy of adenoviral-mediated herpes simplex virus thymidine kinase (HSVtk)/ganciclovir (GCV) prodrug therapy were completed at the University of Pennsylvania by Sterman et al. (60). A total of 21 patients with untreated disease were treated with adenoviral vector administered through thoracostomy tubes, followed by 2 weeks of systemic GCV chemotherapy. Toxicity was minimal and 11 of 20 patients exhibited gene transfer. Complete tumor regression associated with survival after 4.5 years was observed in two subjects (61), but the majority of patients demonstrated poor response. Improved results with this technology are likely still dependent on the development of more efficient delivery strategies. Current studies to increase killing competence by improving the HSVth enzyme through the use of alternative mutant constructs also shows some promise for the application of this system in mesothelioma, and similar studies have been completed for lung cancer (61,62).

Clinical trials in conjunction with chemotherapy and radiation

Early studies attempting to define the role of *p53* demonstrated the importance of *p53* activation in mediating chemotherapeutic cytotoxicity (63) and the prediction of resistance in cancer with aberrant *p53* expression (64). Additional preclinical studies with wild-type *p53* adenoviral transfer supported the induction of chemosensitivity when the gene activity was restored (65). Shuler et al. (66) continued the investigation of combining Ad-*p53* (SCH 58500) with chemotherapy in a phase II trial. A total of 25 patients with

metastatic NSCLC were enrolled and received one of two chemotherapy regimens, combined with intratumoral Ad-p53. Only patients with two lesions of comparable size in the same organ were enrolled, one for injection and one for observation. Once again the combination of all agents produced minimal toxicity. Some tumor regression was seen in the injected specimens but overall response rate was not significantly different between the two groups. The benefit of this study was seen in the lack of cumulative toxicity with multiple regimens and Ad-p53, however randomized trials are necessary before the therapeutic benefit of this combination can be accurately determined.

The association between p53 and apoptosis, together with the demonstration of radiation-induced apoptosis, provides a link for possible exploitation. To assess if p53 restoration radiosensitizes patients, a phase II trial was recently completed by Swisher et al. (67). The combination was well tolerated by patients and evidence of tumor regression was provided. Upregulation of the proapoptotic gene BAK was seen in tumor specimens and proposed as one possible mechanism for the increased cell-killing seen with this combination. The proven safety of adenoviral p53 transfer and specific cases of regression seen in phase I and II trials will no doubt encourage continued evolution of p53 replacement therapy. A number of clinical trials are needed to assess various lung cancer patient populations and combine different treatment protocols.

CONCLUSION

In the past two decades, hope for a lung cancer cure has remained unfulfilled due to multiple factors: delayed diagnosis from lack of effective screening methods, poor local and regional primary tumor control, the lack of effective systemic therapy to target distant metastatic disease, and morbidity/mortality from aggressive combined modality therapy.

Recent research advances in cancer and molecular biology have furthered our understanding of the etiology and natural history of lung cancer. Through translation research, a growing understanding of the molecular changes that underlie cancer progression has contributed to the development of novel molecular approaches for identifying new therapeutic targets. Much preclinical research has been applied to clinical studies. This progress made in translation research of lung cancer defining gene therapy as the foundation for future improvements in lung cancer treatment. While gene therapy offers new hopes for lung cancer treatment, it is the need to develop valid clinical protocols of randomized trials before safety using to various lung cancer patient populations.

Umbilical cord matrix stem (UCMS) cells offer new horizon for lung cancer gene therapy research. Stem cells are defined by two main characteristics: self-renewal capacity and commitment to multi-lineage differentiation. The cells have a great therapeutic potential in repopulating damaged tissues as well as being genetically manipulated and used in cell-based gene therapy. Umbilical cord vein is a readily available and inexpensive source of stem cells that are capable of generating various cell types. UCMS cells can potentially be used for targeted safety delivery of lung cancer therapeutics. Progress in gene therapy is dependent on the techniques of gene transfer (68).

Conflict of interest

We declare no conflicts of interest.

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