HHS Public Access

Author manuscript

Crit Rev Oncog. Author manuscript; available in PMC 2017 May 24.

Published in final edited form as:

Crit Rev Oncog. 2016 ; 21(1-2): 115–124. doi:10.1615/CritRevOncog.2016016084.

Gene Therapy for Lung Cancer

Humberto Lara-Guerra and **Jack Roth***

Department of Thoracic and Cardiovascular Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Gene therapy was originally conceived to treat monogenic diseases. The replacement of a defective gene with a functional gene can theoretically cure the disease. In cancer, multiple genetic defects are present and the molecular profile changes during the course of the disease, making the replacement of all defective genes impossible. To overcome these difficulties, various gene therapy strategies have been adopted, including immune stimulation, transfer of suicide genes, inhibition of driver oncogenes, replacement of tumor-suppressor genes that could mediate apoptosis or anti-angiogenesis, and transfer of genes that enhance conventional treatments such as radiotherapy and chemotherapy. Some of these strategies have been tested successfully in non-small-cell lung cancer patients and the results of laboratory studies and clinical trials are reviewed herein.

Keywords

lung cancer; gene therapy

I. INTRODUCTION

In 1973, I was completing my first year of general surgery residency at the Johns Hopkins Hospital and contemplating a future career in academia. The chief of surgery, Dr. George Zuidema, suggested that I take time for a research fellowship and recommended a new program in oncology recently established by Dr. Donald Morton at University of California, Los Angeles (UCLA). Intrigued, I read several recent publications from Dr. Morton's group at the National Cancer Institute published prior to his move to UCLA and my interest and excitement escalated. Their studies showed that human immune responses to cancer could be detected and, in mice, that the immune response could eradicate tumors. I interviewed with Dr. Morton and was fortunate to be offered a fellowship. When I arrived at UCLA, I found a clinical department unlike any I had previously encountered. This was a multidisciplinary group with basic scientists, medical oncologists, and surgeons all dedicated to treating cancer in a single administrative unit. This was an exciting time in oncology and the Morton group was leading the way. Clinical trials with novel drugs, immunotherapy, adjuvant therapy, and limb-sparing surgery for sarcoma were initiated. As a fellow, I undertook laboratory research and saw oncology patients in the clinic. Dr. Morton

*Address all correspondence to: Jack A. Roth, M.D., F.A.C.S., Department of Thoracic and Cardiovascular Surgery, The University of Texas M.D. Anderson Cancer Center, T. Boone Pickens Academic Tower (FCT19.5072), 1515 Holcombe Boulevard, Unit 1489, Houston, TX 77030; jroth@mdanderson.org.

stressed the importance of understanding the biology of cancer and pursuing translational research that would benefit patients. The research briefly reviewed here reflects a lifelong application of these concepts that Dr. Morton followed and taught throughout his career.

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer deaths in the United States and in the world. Targeted agents directed against specific genetic abnormalities related to driving the proliferation and survival of NSCLC cells have been developed. However, the prevalence of specific molecular alterations against which clinically approved targeted agents are directed is less than 10% in NSCLC patients.[1] Furthermore, even with an initial response, it is common that patients develop resistance, revealing the complex regulatory networks present in NSCLC cells.

Genetic alterations are usually present in two major classes of genes, oncogenes and tumor-suppressor genes. These genetic alterations regulate malignant growth, including self-sufficiency in growth signals; insensitivity to growth-inhibitory signals; evasion of programmed cell death; limitless replicative potential; induced angiogenesis, invasion, and metastasis; reprogrammed cell metabolism; and evasion of immune destruction.[2] Due to their essential role for the survival of cancer cells, specific targeted agents blocking activated oncogenes are able to induce tumor regression. However, these responses are usually short-lived. The most common genetic alterations present in NSCLC are located in tumor-suppressor genes against which no targeted agents have been developed.[3]

II. RESTORING TUMOR-SUPPRESSOR GENE FUNCTION

The most common gene mutations detected by recent whole-genome sequencing are in tumor-suppressor genes.[3] In most situations, tumor-suppressor genes require both alleles of a gene to be deleted or inactivated to lead to tumor growth. The replacement of just one functional allele may therefore be enough to restore normal growth regulation and to induce tumor apoptosis. The most frequently mutated gene in NSCLC is the tumor-suppressor *p53*.[3–5]

P53 is a transcription factor able to up-regulate apoptotic genes and down-regulate prosurvival genes.[6,7] *P53* functions as a transcriptional gatekeeper, monitoring for DNA damage or oncogene activation, halting the progression of the cell cycle at the G1 stage to facilitate DNA repair or, if changes are irreversible, to induce self-destruction via apoptosis.[6,7]

Because conventional chemotherapy and radiotherapy act by inducing DNA damage, it is expected that cancer cells with mutated or absent *P53* protein might show resistance to therapy. However, because neoplastic cells have multiple somatic mutations, it was not intuitively obvious that the single correction of *P53* protein by the addition of a wild-type *p53* into the cancer cell genome would be sufficient to induce apoptosis. Nevertheless, experiments in different types of neoplastic cells have shown that the single correction of defective/absent *p53* with wild-type *p53* induces apoptosis.[8,9] In NSCLC, the observation that reintroduction of wild-type *p53* in NSCLC cell lines carrying mutated or deleted *p53* is able to trigger apoptosis provided the justification of exploring its therapeutic use.[8] Some

p53 mutations may impart oncogenic potential to *p53* and are considered gain-of-function mutations. However, in most cases, overexpression of wt*p53* protein will overcome this and trigger apoptosis.

III. PRECLINICAL STUDIES OF *P53* GENE REPLACEMENT

The first of *p53* gene replacement used a retroviral vector and showed that restoration of functional *p53* suppressed the growth of some, but not all, human NSCLC cell lines *in vitro*.[10–12] With this retroviral vector, intratumoral *p53* gene therapy showed suppression of tumor growth in an orthotropic human NSCLC model with absent or mutant *p53*.[13] This was the first study demonstrating that the restoration of a single tumor-suppressor gene could induce tumor regression *in vivo*. Subsequent studies, including clinical trials, have used an adenoviral vector (Ad-*p53*) of a serotype 5 replication-defective vector with a deleted E1 region.[14] Ad-*p53* was able to induce apoptosis and to suppress proliferation in NSCLC cell lines, to increase sensitivity to chemotherapeutic agents regardless of cellular *p53* status, and to inhibit tumor growth in NSCLC orthotropic mouse models.[15–17] In addition, killing of non-transduced tumor cells through a bystander effect initiated by Ad-*p53*-transduced tumor cells has been reported, potentially induced via regulation of proapoptotic signals, angiogenesis, and immune response.[18–22]

IV. CLINICAL TRIALS OF *P53* GENE REPLACEMENT

The first clinical trial implementing *p53* gene replacement utilized an intratumoral therapy of a replication-defective retroviral vector expressing wild-type *p53* driven by a beta-actin promotor.[23] The therapeutic agent was provided via intratumoral injection in nine unresectable NSCLC patients progressing after conventional therapy. Three of the nine patients showed evidence of tumor regression with no vector-related toxicity, demonstrating the feasibility and safety of *p53* gene therapy. A dose-escalation trial in 28 NSCLC patients who had not responded to conventional therapy showed successful gene transfer via intratumoral injection in 80% of evaluable patients, with expression of *p53* detected in 46%.[24] With no significant toxicities detected, apoptosis was observed in all but one of the patients expressing *p53*. More than 50% tumor reduction was observed in two patients, with one experiencing a disease-free survival of more than a year and another experiencing a nearly complete response (CR).

In a subset analysis of patients with tumors causing airway obstruction, the degree of toxicity and antitumor activity of the delivery of Ad-*p53* gene therapy via bronchoscopy were assessed.[25] Twelve patients (median age, 60 years) with advanced endobronchial NSCLC were evaluated. The median tumor area was 5 cm x 3.2 cm. All patient tumors contained a *p53* gene mutation. Ad-*p53* was administered via bronchoscopic intratumoral injection once every 28 days. Toxicity attributed to the Ad-*p53* vector was minimal. Six of the 12 patients had significant improvement in airway obstruction, with three patients meeting the criteria for partial response (PR). In addition, a phase I trial evaluated the safety of Ad-*p53* gene transfer via bronchoalveolar lavage.[26] In 23 assessable patients with bronchioloalveolar carcinoma, 16 experienced stabilization of their disease, but limited distribution of vector was observed.

V. *P53* GENE REPLACEMENT IN COMBINATION WITH CHEMOTHERAPY

In vitro and xenograft NSCLC models have shown that sequential therapy of cisplatin and *p53* gene therapy enhances *p53* expression.[27,28] In a phase I study of intratumoral delivery of Ad5CMV-*p53*, 15 patients with histologically confirmed NSCLC and *p53* mutations were enrolled.[29] Nine patients received escalating dose levels of Ad5CMV-*p53* as monotherapy once every 4 weeks. Six patients were treated on a 28-day schedule with Ad5CMV-*p53* in combination with intravenous administration of cisplatin (80 mg/m^2). There were no dose-limiting toxicities. The most common treatment-related toxicity was transient fever. Specific *p53* transgene expression was detected in tumor tissues despite the presence of neutralizing anti-adenovirus antibody. Thirteen of 15 patients were evaluated for efficacy. One patient had a PR, 10 had stable disease with three lasting at least 9 months, and two patients had progressive disease.

Another phase I trial of NSCLC patients resistant to cisplatin-based therapy tested the addition of Ad-*p53* to these regimens.[30] Intratumoral Ad-*p53* gene therapy injection in sequence after six monthly courses of cisplatin resulted in two of 24 patients achieving PR and 17 achieving stable disease for at least 2 months with a safe toxicity profile. Seventy-nine percent of tumor biopsies showed an increase in the number of apoptotic cells.

VI. *P53* GENE THERAPY IN COMBINATION WITH RADIATION THERAPY

Ad-*p53* gene transfer increases the sensitivity of *p53*-deficient tumor cells to radiation.[31]

The combination of three intratumoral injections of Ad-*p53* with concomitant radiotherapy (60 Gy over 6 weeks) showed 5% and 58% of patients with a CR or PR, respectively.[32] In biopsies obtained 3 months after therapy, no viable tumor was observed in 63% of cases. Among 13 evaluable patients after 1 year, 39% had a CR and 23% had a PR or disease stabilization. The 1-year progression-free survival rate was 45.5%. Most treatment failures were caused by metastatic disease without local progression.

In that study, paired biopsies (days 18 and 19) before and after Ad-*p53* gene therapy were obtained. Ad-*p53* vector-specific DNA was detected in nine of 12 patients. From 11 patients with adequate samples for both vector DNA and mRNA analysis, eight showed an increase in mRNA expression associated with detectable vector DNA. Post-injection increases in *p53* mRNA were detected in 11 of 12 paired biopsies, with 10 of 11 increasing threefold or more. Before Ad-*p53* gene therapy, biopsies were negative for *p53* expression by immunohistochemistry. After Ad-*p53* injection, staining confirmed nuclear *p53* expression in the posttreatment biopsies. The expression of genes that are targets of *p53* was also modified, with *p21* (*CDKN1A*), *MDM2*, and *BAK* showing an up-regulation 24 hours after Ad-*p53* injection.

The most frequently reported adverse events related to Ad-*p53* treatment were fever and chills, asthenia, injection site pain, nausea, and vomiting. The vast majority of these events were mild to moderate. To date, no maximum tolerated dose for Ad-*p53* injection has been established. Beginning in 1998, a similar adenovirus-*p53*-expressing vector was tested in China in clinical trials under the name Gendicine. A multicenter, randomized clinical trial

was conducted in which Ad-*p53* was administered to 135 patients with head and neck squamous cell carcinoma.[33] One group received gene therapy in combination with radiotherapy (GTRT) and the other group received radiotherapy alone (RT). In the GTRT group, the CR rate determined by computed tomography was 64% with 29% PR. The response rate in the RT group was 19% of the patients showing CR and 60% PR. There was a significant difference between the two groups in both the CR rate and the PR rate. This study served as the basis for the approval of Ad-*p53* (Gendicine) by the Chinese Food and Drug Administration and Gendicine thus became the first gene therapy agent approved for human use.

VII. SYSTEMIC GENE THERAPY

Most NSCLC patients die from systemic metastases. Nanovesicles capable of encapsulating plasmid DNA allow the delivery of gene therapeutic agents intravenously to distant metastases.[34,35] Allele losses and genetic alterations on the short arm of chromosome 3 (3p25, 3p21–22, 3p14, and 3p12–13) are among the most frequent and earliest genomic abnormalities involved in lung cancer and many other human cancers.[36–38] Multiple overlapping homozygous deletions have also been found in the 3p21.3 region spanning a 120 kb genomic locus in human lung cancer cell lines.[39,40] In addition, chromosomal abnormalities in the 3p21.3 region have been detected frequently in smoke-damaged respiratory epithelium and preneoplasic lesions.[39,41,42] These findings suggest that one or more putative tumor-suppressor genes are present in the 3p21.3 region and that they function as “gatekeepers” in the molecular pathogenesis of lung cancer.[39,41,43] When re-expressed in NSCLC cell lines, 3p21.3 genes show various degrees of tumor suppression *in vitro* and xenograft models with the Tumor Suppressor Candidate 2 gene (*TUSC2*) showing the greatest suppressor activity. [42,44] The TUSC2 protein is absent in the majority of NSCLC cell lines.[38,44–46] Loss or reduction of TUSC2 expression was associated with significantly worse overall survival. Premalignant lesions also expressed significantly lower levels of TUSC2 compared with normal and hyperplastic bronchial epithelia. Expression of *TUSC2* showed the most potent pro-apoptotic activity in human NSCLC cells among the candidate 3p21.3 tumor-suppressor genes.[44] The apoptotic protease-activating factor 1 (Apaf-1) was identified as a potential cellular target of TUSC2 protein by its direct protein–protein interaction. [47] Apaf-1 plays an important role in the mitochondria- dependent apoptotic pathway, but a relatively high level of endogenous Apaf-1 protein was detected in NSCLC cells.[48–50] These Apaf-1 proteins appeared to be functionally inactive, as indicated by their lack of intrinsic ATPase activity. Forced expression of *TUSC2* in *TUSC2*-deficient tumor cells can trigger cytochrome c release from the mitochondria into the cytosol and cause TUSC2 binding to Apaf-1, thus recruiting it to critical cellular locations, activating Apaf-1, initiating Apaf-1-mediated caspase activation, and inducing apoptosis.[51]

VIII. PRECLINICAL STUDIES OF *TUSC2* GENE REPLACEMENT

A *TUSC2*-expressing plasmid vector was packaged in N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTAP):cholesterol (DOTAP:chol) nanovesicles to create a gene delivery agent that can be delivered intravenously. The nanovesicles are taken up selectively by both cultured and fresh tumor cells with 10-fold more than normal cells,

thus imparting a passive targeting property without the need to attach ligands.[52] Intratumoral *TUSC2* nanovesicle delivery in subcutaneous xenografts inhibits tumor growth. Intravenous delivery in an NSCLC metastasis mouse model significantly decreased the number of metastatic nodules and prolonged survival.[35] Analysis of TUSC2 expression showed distribution of TUSC2 throughout the tumor in a high percentage of the tumor cells.

IX. CLINICAL TRIAL OF *TUSC2* GENE THERAPY

A phase I clinical trial using *TUSC2* nanovesicles was performed in stage IV NSCLC patients.[53] Patients with recurrent or metastatic NSCLC previously treated with platinum-based chemotherapy were treated with escalating doses of intravenous DOTAP:chol nanovesicles encapsulating a *TUSC2* expression plasmid (DOTAP:chol-*TUSC2*) every 3 weeks for a maximum of six doses. Thirty-one patients were treated at six dose levels ranging from 0.01 to 0.09 milligrams per kilogram. Seventy-percent had received two or more prior chemotherapy regimens. The only dose-limiting toxicities were two episodes of transient grade 3 hypophosphatemia, resulting in an MTD of 0.06 mg/kg. Twenty-three patients received two or more doses. Five patients achieved stable disease (range, 2.6–10.8 months, 95% confidence interval [CI] 2.0–7.6) and all other patients progressed. Two patients had reductions in primary tumor size of 14% and 26%. One patient with stable disease had evidence of a durable metabolic response on positron emission tomography imaging and received 12 cycles of therapy. This patient remains alive on subsequent therapy 14 months after the final treatment with DOTAP:chol-*TUSC2*. Median survival for all patients was 8.3 months (95% CI 6.0–10.5 months).

Pretreatment and 24 hour posttreatment tumor biopsies were obtained from seven patients by percutaneous computed tomographic guidance from a central tumor location. RT-PCR analysis detected high levels of *TUSC2* plasmid expression in six of seven posttreatment tumor specimens, but not in pretreatment specimens or negative controls. Proximity ligation assay performed on paired biopsies from three patients demonstrated no TUSC2 protein staining in pretreatment tissues compared with intense TUSC2 protein staining in posttreatment tissues. RT-PCR gene expression profiling analysis of apoptotic pathway genes in one paired specimen with high posttreatment levels of TUSC2 mRNA and protein showed significant up-regulation and down-regulation of genes involved in both the intrinsic and extrinsic apoptotic pathways. Antibodies to single- and double-stranded DNA were not detected 14 months after completion of 12 cycles of therapy in one patient. We concluded that DOTAP:chol-*TUSC2* nanovesicles can be safely administrated intravenously in lung cancer patients with demonstrable gene delivery to tumors with protein expression and evidence of antitumor activity.

X. FUTURE DIRECTIONS

TUSC2 interaction down-regulates different tyrosine kinases.[51] Forced TUSC2 expression in NSCLC cell lines lacking activated c-Abl kinase leads to decreased levels of c-Abl's tyrosine kinase activity, diminishing the size and number of colonies in soft agar. Furthermore, re-expression of wild-type TUSC2 into wild-type EGFR, gefitinib-resistant NSCLC cell lines sensitized their response to gefitinib by enhancing growth inhibition and

synergistically inducing apoptosis *in vitro* and in an orthotropic mouse model at concentrations of gefitinib similar to steady-state serum concentrations achievable with oral dosing. *TUSC2* nanovesicle treatment alone or with gefitinib inactivated EGFR and Akt. Finally, a combination of *TUSC2* nanovesicles and erlotinib induced higher levels of cell growth inhibition, apoptosis induction, and inactivation of EGFR and Akt than those observed with erlotinib alone. *TUSC2* nanovesicle and erlotinib treatment showed cooperative growth inhibition in different colony formation assays, including in cell lines resistant to erlotinib. Cooperative antitumor interaction of *TUSC2* nanovesicles and erlotinib treatment were confirmed *in vivo* using a lung colony formation metastatic model, with the greatest reduction in colonies occurring with the *TUSC2* nanovesicle and erlotinib combination. This new evidence supporting a cooperative effect of TUSC2 re-expression in combination with erlotinib supports a phase I/II trial assessing the combination of *TUSC2*-nanovesicles with erlotinib in stage IV or recurrent NSCLC patients with absence of EGFR TK-activating mutations or showing progression after erlotinib treatment.[54]

Finally, the Cancer Genome Atlas Network has reported the first NSCLC comprehensive characterization.[55] This comprehensive genomic sequence of lung squamous cell carcinomas confirmed the importance of *p53* and also identified novel or previously underestimated tumor-suppressor genes such as *CDKN2A*, which was found to be inactivated in 72% of cases, resulting in different patterns of expression of its encoded proteins ARF and INK4A. *RB1* was also found to be frequently mutated. These studies can guide the development of future gene therapies against NSCLC.

XI. CONCLUSIONS

Contrary to initial beliefs, the body of work on *p53* has demonstrated that gene replacement targeting a single tumor-suppressor gene can cause cancer regression. In addition, DOTAP:chol-*TUSC2* confirmed that gene therapy could be administrated systemically. With both gene therapeutic options showing clinical response with a safe toxicity profile, combinations with other NSCLC therapies, including targeted agents, deserve to be explored. With other promising gene therapeutic options currently in laboratory development in addition to new potential targets discovered by genomic sequencing, the matching of a targeted gene therapy with specific molecular profiles or therapeutic regimens has a promising future.

Acknowledgments

This work was supported in part by the National Institutes of Health/National Cancer Institute through The University of Texas M.D. Anderson Cancer Center's Cancer Center Support Grant CA-016672; Lung Program and Shared Core Facilities Specialized Program of Research Excellence (SPORE) Grant CA-070907 (J. Minna and J.A Roth); R01 Grant CA-116322 (L. Ji); and Tobacco Settlement Funds as appropriated by the Texas Legislature and by a sponsored research agreement from Genprex, Inc. (R. Mehran). H.L.G. was supported by the Cancer Prevention Research Institute of Texas (Institutional Training Grants RP101502 and RP140106).

ABBREVIATIONS

NSCLC non-small-cell lung cancer

References

1. Pal SK, Figlin RA, Reckamp K. Targeted therapies for non-small cell lung cancer: an evolving landscape. Mol Cancer Ther. 2010; 9(7):1931–44. [PubMed: 20571071]
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646–74. [PubMed: 21376230]
3. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012; 489(7417): 519–25. [PubMed: 22960745]
4. Isobe T, Hiyama K, Yoshida Y, Fujiwara Y, Yamakido M. Prognostic significance of *p53* and ras gene abnormalities in lung adenocarcinoma patients with stage I disease after curative resection. Jpn J Cancer Res. 1994; 85:1240–6. [PubMed: 7852188]
5. Quinlan DC, Davidson AG, Summers CL, Warden HE, Doshi HM. Accumulation of *p53* protein correlates with a poor prognosis in human lung cancer. Cancer Res. 1992; 52:4828–31. [PubMed: 1324796]
6. Malkin D. The role of *p53* in human cancer. J Neurooncol. 2001; 51(3):231–43. [PubMed: 11407595]
7. Burns T, El-Deiry W. The *p53* pathway and apoptosis. J Cell Physiol. 1999; 181:231–9. [PubMed: 10497302]
8. Fujiwara T, Grimm EA, Mukhopadhyay T, Cai DW, Owen-Schaub LB, Roth JA. A retroviral wild-type *p53* expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis. Cancer Res. 1993; 53(18):4129–33. [PubMed: 8395976]
9. Baker S, Markowitz S, Fearon E, Willson J, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type *p53*. Science. 1990; 249:912–5. [PubMed: 2144057]
10. Raycroft L, Wu H, Lozano G. Transcriptional activation by wild-type but not transforming mutants of the *p53* anti-oncogene. Science. 1990; 249:1049–51. [PubMed: 2144364]
11. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science. 1998; 281(5381): 1322–6. [PubMed: 9735050]
12. Cai DW, Mukhopadhyay T, Roth JA. Suppression of lung cancer cell growth by ribozyme-mediated modification of *p53* pre-mRNA. Cancer Gene Ther. 1995; 2(3):199–205. [PubMed: 8528963]
13. Fujiwara T, Cai DW, Georges RN, Mukhopadhyay T, Grimm EA, Roth JA. Therapeutic effect of a retroviral wild-type *p53* expression vector in an orthotopic lung cancer model (Commentary). J Natl Cancer Inst. 1994; 86(19):1437–8. [PubMed: 8089857]
14. Zhang WW, Fang X, Mazur W, French BA, Georges RN, Roth JA. High-efficiency gene transfer and high-level expression of wild-type *p53* in human lung cancer cells mediated by recombinant adenovirus. Cancer Gene Ther. 1994; 1(1):5–13. [PubMed: 7621238]
15. Georges RN, Mukhopadhyay T, Zhang Y, Yen N, Roth JA. Prevention of orthotopic human lung cancer growth by intratracheal instillation of a retroviral antisense K-ras construct. Cancer Res. 1993; 53(8):1743–6. [PubMed: 8467490]
16. Bouvet M, Fang B, Ekmekcioglu S, Ji L, Bucana CD, Hamada K, Grimm EA, Roth JA. Suppression of the immune response to an adenovirus vector and enhancement of intratumoral transgene expression by low-dose etoposide. Gene Ther. 1998; 5:189–95. [PubMed: 9578838]
17. Weill D, Mack M, Roth J, Swisher S, Proksch S, Merritt J, Nemunaitis J. Adenoviral-mediated *p53* gene transfer to non-small cell lung cancer through endobronchial injection. Chest. 2000; 118(4): 966–70. [PubMed: 11035664]
18. Miyashita T, Reed JC. Tumor suppressor *p53* is a direct transcriptional activator of human bax gene. Cell. 1995; 80(2):293–9. [PubMed: 7834749]
19. Carroll JL, Nielsen LL, Pruett SB, Mathis JM. The role of natural killer cells in adenovirus-mediated *p53* gene therapy. Mol Cancer Ther. 2001; 1(1):49–60. [PubMed: 12467238]
20. Molinier-Frenkel V, Le Boulaire C, Le Gal FA, Gahery-Segard H, Tursz T, Guillet JG, Farace F. Longitudinal follow-up of cellular and humoral immunity induced by recombinant adenovirus-mediated gene therapy in cancer patients. Human Gene Ther. 2000; 11(13):1911–20. [PubMed: 10986563]

21. Yen N, Ioannides CG, Xu K, Swisher SG, Lawrence DD, Kemp BL, el Naggar AK, Cristiano RJ, Fang B, Glisson BS, Hong WK, Khuri FR, Kurie JM, Lee JJ, Lee JS, Merritt JA, Mukhopadhyay T, Nesbitt JC, Nguyen D, Perez-Soler R, Pisters KMW, Putnam JB Jr, Schrump DS, Shin DM, Walsh GL, Roth JA. Cellular and humoral immune responses to adenovirus and *p53* protein antigens in patients following intratumor injection of an adenovirus vector expressing wild-type *p53* (Ad-*p53*). Cancer Gene Ther. 2000; 7(4):530–6. [PubMed: 10811470]
22. Owen-Schaub LB, Zhang W, Cusack JC, Angelo LS, Santee SM, Fujiwara T, Roth JA, Deisseroth AB, Zhang WW, Kruzel E, Radinsky R. Wild-type human *p53* and a temperature-sensitive mutant induce Fas/APO-1 expression. Mol Cell Biol. 1995; 15(6):3032–40. [PubMed: 7539102]
23. Roth JA, Nguyen D, Lawrence DD, Kemp BL, Carrasco CH, Ferson DZ, Hong WK, Komaki R, Lee JJ, Nesbitt JC, Pisters KMW, Putnam JB Jr, Schea R, Shin DM, Walsh GL, Dolormente MM, Han CI, Martin FD, Yen N, Xu K, Stephens LC, McDonnell TJ, Mukhopadhyay T, Cai D. Retrovirus-mediated wild-type *p53* gene transfer to tumors of patients with lung cancer. Nat Med. 1996; 2(9):985–91. [PubMed: 8782455]
24. Swisher SG, Roth JA, Nemunaitis J, Lawrence DD, Kemp BL, Carrasco CH, Connors DG, el Naggar AK, Fossella F, Glisson BS, Hong WK, Khuri FR, Kurie JM, Lee JJ, Lee JS, Mack M, Merritt JA, Nguyen DM, Nesbitt JC, Perez-Soler R, Pisters KMW, Putnam JB Jr, Richli WR, Savin M, Schrump DS, Shin DM, Shulkin A, Walsh GL, Wait J, Weill D, Waugh MKA. Adenovirus-mediated *p53* gene transfer in advanced non-small cell lung cancer. J Natl Cancer Inst. 1999; 91(9):763–71. [PubMed: 10328106]
25. Saeki T, Mhashilkar A, Swanson X, Zou-Yang H, Sieger K, Kawabe S, Branch CD, Zumstein L, Meyn RE, Roth JA, Chada S, Ramesh R. Inhibition of human lung cancer growth following adenovirus-mediated mda-7 gene expression *in vivo*. Oncogene. 2002; 21:4558–66. [PubMed: 12085234]
26. Keedy V, Wang W, Schiller J, Chada S, Slovis B, Coffee K, Worrell J, Thet LA, Johnson DH, Carbone DP. Phase I study of adenovirus *p53* administered by bronchoalveolar lavage in patients with bronchioloalveolar cell lung carcinoma: ECOG 6597. J Clin Oncol. 2008; 26(25):4166–71. [PubMed: 18757331]
27. Fujiwara T, Grimm EA, Mukhopadhyay T, Zhang WW, Owen-Schaub LB, Roth JA. Induction of chemosensitivity in human lung cancer cells *in vivo* by adenoviral-mediated transfer of the wild-type *p53* gene. Cancer Res. 1994; 54(9):2287–91. [PubMed: 8162565]
28. Nguyen DM, Spitz FR, Yen N, Cristiano RJ, Roth JA. Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated *p53* gene transfer. J Thorac Cardiovasc Surg. 1996; 112(5):1372–6. discussion 6–7. [PubMed: 8911337]
29. Fujiwara T, Tanaka N, Kanazawa S, Ohtani S, Saijo Y, Nukiwa T, Yoshimura K, Sato T, Eto Y, Chada S, Nakamura H, Kato H. Multicenter phase I study of repeated intratumoral delivery of adenoviral *p53* in patients with advanced non-small-cell lung cancer. J Clin Oncol. 2006; 24(11): 1689–99. [PubMed: 16505415]
30. Schuler M, Herrmann R, De Greve JL, Stewart AK, Gatzemeier U, Stewart DJ, Laufman L, Gralla R, Kuball J, Buhl R, Heussel CP, Kommoss F, Perruchoud AP, Shepherd FA, Fritz MA, Horowitz JA, Huber C, Rochlitz C. Adenovirus-mediated wild-type *p53* gene transfer in patients receiving chemotherapy for advanced non-small-cell lung cancer: results of a multicenter phase II study. J Clin Oncol. 2001; 19(6):1750–8. [PubMed: 11251006]
31. Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, Roth JA. Adenoviral-mediated wild-type *p53* gene expression sensitizes colorectal cancer cells to ionizing radiation. Clin Cancer Res. 1996; 2(10):1665–71. [PubMed: 9816114]
32. Swisher SG, Roth JA, Komaki R, Gu J, Lee JJ, Hicks M, Ro JY, Hong WK, Merritt JA, Ahrar K, Atkinson NE, Correa AM, Dolormente M, Dreiling L, El-Naggar AK, Fossella F, Francisco R, Glisson B, Grammer S, Herbst R, Huaringa A, Kemp B, Khuri FR, Kurie JM, Liao Z, McDonnell TJ, Morice R, Morello F, Munden R, Papadimitrakopoulou V, Pisters KM, Putnam JB Jr, Sarabia AJ, Shelton T, Stevens C, Shin DM, Smythe WR, Vaporciyan AA, Walsh GL, Yin M. Induction of *p53*-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral *p53* (INGN 201) and radiation therapy. Clin Cancer Res. 2003; 9(1):93–101. [PubMed: 12538456]

33. Peng Z. Current status of gendicine in China: recombinant human Ad-*p53* agent for treatment of cancers. Human Gene Ther. 2005; 16(9):1016–27. [PubMed: 16149900]
34. Uno F, Sasaki J, Nishizaki M, Carboni GL, Xu K, Gao B, Kondo M, Atkinson EA, Minna JD, Roth JA, Ji L. Myristoylation of the fus1 protein is required for tumor suppression in human lung cancer cells. Cancer Res. 2004; 64(9):2969–76. [PubMed: 15126327]
35. Ito I, Ji L, Tanaka F, Saito Y, Gopalan B, Branch CD, Xu K, Atkinson EN, Bekele BN, Stephens LC, Minna JD, Roth JA, Ramesh R. Liposomal vector mediated delivery of the 3p FUS1 gene demonstrates potent antitumor activity against human lung cancer *in vivo*. Cancer Gene Ther. 2004; 11:733–9. [PubMed: 15486560]
36. Sekido Y, Fong KM, Minna JD. Molecular genetics of lung cancer. [Review] [70 refs]. Annu Rev Med. 2003; 54:73–87. [PubMed: 12471176]
37. Zabarovsky ER, Lerman MI, Minna JD. Tumor suppressor genes on chromosome 3p involved in the pathogenesis of lung and other cancers. Oncogene. 2002; 21(45):6915–35. [PubMed: 12362274]
38. Wistuba II, Behrens C, Virmani AK, Mele G, Milchgrub S, Girard L, Fondon JW III, Garner HR, McKay B, Latif F, Lerman MI, Lam S, Gazdar AF, Minna JD. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. Cancer Res. 2000; 60(7):1949–60. [PubMed: 10766185]
39. Daly MC, Xiang RH, Buchhagen D, Hensel CH, Garcia DK, Killary AM, Minna JD, Naylor SL. A homozygous deletion on chromosome 3 in a small cell lung cancer cell line correlates with a region of tumor suppressor activity. Oncogene. 1993; 8(7):1721–9. [PubMed: 8390035]
40. Zochbauer-Muller S, Gazdar AF, Minna JD. Molecular pathogenesis of lung cancer. Annu Rev Physiol. 2002; 64:681–708. [PubMed: 11826285]
41. Wistuba II, Lam S, Behrens C, Virmani AK, Fong KM, LeRiche J, Samet JM, Srivastava S, Minna JD, Gazdar AF. Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst. 1997; 89(18):1366–73. [PubMed: 9308707]
42. Ji L, Minna JD, Roth JA. 3p21.3 tumor suppressor cluster: prospects for translational applications. Future Oncol. 2005; 1(1):79–92. [PubMed: 16555978]
43. Kondo M, Ji L, Kamibayashi C, Tomizawa Y, Randle D, Sekido Y, Yokota J, Kashuba V, Zabarovsky E, Kuzmin I, Lerman M, Roth J, Minna JD. Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells. Oncogene. 2001; 20(43):6258–62. [PubMed: 11593436]
44. Ji L, Nishizaki M, Gao B, Burbee D, Kondo M, Kamibayashi C, Xu K, Yen N, Atkinson EN, Fang B, Lerman MI, Roth JA, Minna JD. Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities *in vitro* and *in vivo*. Cancer Res. 2002; 62(9):2715–20. [PubMed: 11980673]
45. Thu KL, Vucic EA, Chari R, Zhang W, Lockwood WW, English JC, Fu R, Wang P, Feng Z, MacAulay CE, Gazdar AF, Lam S, Lam WL. Lung adenocarcinoma of never smokers and smokers harbor differential regions of genetic alteration and exhibit different levels of genomic instability. PLoS One. 2012; 7(3):e33003. [PubMed: 22412972]
46. Prudkin L, Behrens C, Liu DD, Zhou X, Ozburn NC, Bekele BN, Minna JD, Moran C, Roth JA, Ji L, Wistuba IW. Loss and reduction of FUS1 protein expression is a frequent phenomenon in the pathogenesis of lung cancer. Clin Cancer Res. 2008; 14(1):41–7. [PubMed: 18172250]
47. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW, Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. Mol Cell. 2002; 9(2):423–32. [PubMed: 11864614]
48. Riedl SJ, Li W, Chao Y, Schwarzenbacher R, Shi Y, Riedl SJ, Li W, Chao Y, Schwarzenbacher R, Shi Y. Structure of the apoptotic protease-activating factor 1 bound to ADP. Nature. 2005; 434(7035):926–33. [PubMed: 15829969]
49. Riedl SJ, Salvesen GS, Riedl SJ, Salvesen GS. The apoptosome: signalling platform of cell death. Nat Rev Mol Cell Bio. 2007; 8(5):405–13. [PubMed: 17377525]

50. Deng W, Kawashima H, Wu G, Jayachandran G, Xu K, Minna JD, Roth JA, Ji L. Synergistic tumor suppression by coexpression of FUS1 and *p53* is associated with down-regulation of murine double minute-2 and activation of the apoptotic protease-activating factor 1-dependent apoptotic pathway in human non-small cell lung cancer cells. Cancer Res. 2007; 67:709–17. [PubMed: 17234782]

51. Ji L, Roth JA. Tumor suppressor FUS1 signaling pathway. J Thorac Oncol. 2008; 3(4):327–30. [PubMed: 18379348]

52. Ramesh R, Saeki T, Templeton NS, Ji L, Stephens LC, Ito I, Wilson DR, Wu Z, Branch CD, Minna JD, Roth JA. Successful treatment of primary and disseminated human lung cancers by systemic delivery of tumor suppressor genes using an improved liposome vector. Mol Ther. 2001; 3(3):337–50. [PubMed: 11273776]

53. Lu C, Stewart DJ, Lee JJ, Ji L, Ramesh R, Jayachandran G, Nunez MI, Wistuba II, Erasmus JJ, Hicks ME, Grimm EA, Reuben JM, Baladandayuthapani V, Templeton NS, McMannis JD, Roth JA. Phase I clinical trial of systemically administered TUSC2(FUS1)-nanoparticles mediating functional gene transfer in humans. PLoS One. 2012; 7(4):e34833. [PubMed: 22558101]

54. Genprex, Inc. FUS1-nanoparticles and erlotinib in stage IV lung cancer. Available from: http://clinicaltrials.gov/show/NCT01455389

55. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012; 489:519–25. [PubMed: 22960745]