Alterations in the K-*ras* and *p53* Genes in Rat Lung Tumors

Steven A. Belinsky,[1] Deborah S. Swafford,[1] Gregory L. Finch,[1] Charles E. Mitchell,[1] Gregory Kelly,[1*] Fletcher F. Hahn,[1] Marshall W. Anderson,[2] and Kristen J. Nikula[1]

[1]Inhalation Toxicology Research Institute, Albuquerque, New Mexico; [2]St. Mary's Hospital, Grand Junction, Colorado

Activation of the K-*ras* protooncogene and inactivation of the *p53* tumor suppressor gene are events common to many types of human cancers. Molecular epidemiology studies have associated mutational profiles in these genes with specific exposures. The purpose of this paper is to review investigations that have examined the role of the K-*ras* and *p53* genes in lung tumors induced in the F344 rat by mutagenic and nonmutagenic exposures. Mutation profiles within the K-*ras* and *p53* genes, if present in rat lung tumors, would help to define some of the molecular mechanisms underlying cancer induction by various environmental agents. Pulmonary adenocarcinomas or squamous cell carcinomas were induced by tetranitromethane (TNM), 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK), beryllium metal, plutonium-239, X-ray, diesel exhaust, or carbon black. These agents were chosen because the tumors they produced could arise via different types of DNA damage. Mutation of the K-*ras* gene was determined by approaches that included DNA transfection, direct sequencing, mismatch hybridization, and restriction fragment length polymorphism analysis. The frequency for mutation of the K-*ras* gene was exposure dependent. Only two agents, TNM and plutonium, led to mutation frequencies of > 10%. In both cases, the transition mutations formed could have been derived from deamination of cytosine. The identification of non-*ras* transforming genes in rat lung tumors induced by mutagenic and nonmutagenic exposures such as NNK and beryllium would help define some of the mechanisms underlying cancer induction by different types of DNA damage. Alteration in the *p53* gene was assessed by immunohistochemical analysis for p53 protein and single-strand conformation polymorphism (SSCP) analysis of exons 4 to 9. None of the 93 adenocarinomas examined was immunoreactive toward the anti-p53 antibody CM1. In contrast, 14 of 71 squamous cell carcinomas exhibited nuclear p53 immunoreactivity with no correlation to type of exposure. However, SSCP analysis only detected mutations in 2 of 14 squamous cell tumors that were immunoreactive, suggesting that protein stabilization did not stem from mutations within the *p53* gene. Thus, the *p53* gene does not appear to be involved in the genesis of most rat lung tumors — *Environ Health Perspect* 105(Suppl 4):901–906 (1997)

Key words: K-*ras*, *p53*, rat lung tumors, NNK, beryllium, plutonium, X-ray, diesel exhaust, carbon black

Introduction

Activation of the K-*ras* protooncogene and inactivation of the *p53* tumor suppressor gene are events common to many types of human cancers (*1,2*). Molecular epidemiolgy studies have associated mutational profiles in these genes with specific exposures. For example, lung tumors from cigarette smokers (*3*) or induced in mice by exposure to polycyclic aromatic hydrocarbons (*4*) contain a GGT → TGT transversion mutation in codon 12 of the K-*ras* gene. Further evidence that some carcinogens may produce a specific and recognizable pattern of gene mutations has been demonstrated by mutational hot spots in the *p53* gene in liver and skin tumors associated with exposure to aflatoxin (*5*) and ultraviolet radiation (*6*), respectively. In all these cases, the specific mutation could be ascribed to the type of DNA damage produced by the exposure.

Lung cancer attributable to tobacco use is the leading cause of cancer-related death in the United States (*7*). However, only 16% of habitual smokers develop lung cancer, suggesting that host susceptibility and other environmental factors (e.g., air pollution) contribute to the risk for developing this disease. The best example of a combined exposure increasing lung cancer risk can be seen in underground uranium miners who were highly exposed to radon progeny in conjunction with tobacco use. Their lifetime risk for developing lung cancer is > 50% (*8*). Chronic low-level inhalation of radon in the home has also been estimated to contribute to 10% of all lung cancer deaths in the United States (*8*). The effect of exposure to other types of environmental pollutants (e.g., diesel exhaust, ionizing radiation) may also be an added risk factor for lung cancer (*9*).

The F344 rat has been used as a model to evaluate the carcinogenic potential of chronic exposure to environmental chemicals or mixtures. Several exposures have resulted in a significant increase in primary lung tumors. Mutation profiles within the K-*ras* and *p53* genes, if present in rat lung tumors, could help define some of the molecular mechanisms underlying cancer induction by these environmental agents. The establishment of distinct mutational profiles within these genes for a specific environmental agent would also improve risk estimates from exposures in the home and workplace. The purpose of this paper is to review investigations that have examined the role of the K-*ras* and *p53* genes in lung tumors induced in the rat by mutagenic (genotoxic) and nonmutagenic (nongenotoxic) exposures. Lung tumors were induced by tetranitromethane (TNM) (*10*), 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK)(*11*), beryllium metal (*12*), plutonium-239 (*13*), X-ray (*14*), diesel exhaust, or carbon black (*15*). These exposures were chosen because the tumors produced could arise via different types of DNA damage. TNM can stimulate cell proliferation by its irritant

This paper is based on a presentation at the symposium on Mechanisms and Prevention of Environmentally Caused Cancers held 21–25 October 1995 in Santa Fe, New Mexico. Manuscript received at *EHP* 16 April 1996; accepted 11 July 1996.

This research was supported by the Office of Health and Environmental Research and the Assistant Secretary for Defense Programs, U.S. Department of Energy, under contract DE-AC04-76EV01013, in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

Address correspondence to Dr. S.A. Belinsky, Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185. Telephone: (505) 845-1165. Fax: (505) 845-1198. E-mail: sbelinsk@lrri.org

*Present address: Southwest Scientific Resources, Albuquerque, NM 87120.

Abbreviations used: NNK, 4-methylnitrosamino-1-(3-pyridyl)-1-butanone; SSCP, single-strand conformation polymorphism; TNM, tetranitromethane.

properties and cause deamination of DNA (*10*). NNK is a tobacco-specific nitrosamine that is genotoxic to DNA through the generation of alkylating (*11*) and pyridyloxobutylating agents (*16*). Beryllium metal is a nongenotoxic carcinogen that illicits a chronic inflammatory response involving the recruitment of macrophages and neutrophils that can release cytokines and oxygen radicals (*12*). Plutonium-239 and X-ray exposures damage DNA directly and through the production of free radicals (*17*). Diesel exhaust contains 8% particle-associated, extractable, organic compounds by weight in conjunction with the insoluble particles within the carbon matrix, thereby not only producing genotoxic effects on DNA, but also causing chronic inflammation and epithelial proliferation (*15*). Carbon black contains only 0.12% extractable organics and serves as a surrogate for the elemental matrix of diesel exhaust soot (*15*).

Materials and Methods

Exposure and Sampling

The exposures have previously been described in detail (*10–15*). All exposures except NNK were by the inhalation route using respirable aerosols. NNK was administered by sc injection. Lung neoplasms were fixed in 4% buffered paraformaldehyde or in 10% neutral buffered formalin. These samples were embedded in paraffin, cut at 5 µm, stained with hematoxylin and eosin, and examined by light microscopy for histologic diagnosis. Serial sections were also cut for immunohistochemical assays and DNA analysis. If lung tumor specimens were > 0.15 mg, the additional tissue was frozen in liquid nitrogen and stored at –80°C. DNA was isolated from fixed and frozen tissue for the analyses described below using standardized techniques (*12,18*).

Analysis of Mutations in the K-*ras* Gene

The first and second exons of the K-*ras* gene, specifically codons 12, 13, and 61, contain the majority of activating mutations identified in human and murine tumors (*1*). Several methods for detecting mutations within this gene have been used over the past 10 years and have been described previously (*10,12,14,18–22*). These methods include DNA transfection, oligonucleotide mismatch hybridization, direct sequencing of exons 1 and 2 following amplification by the polymerase chain reaction, and *Bst*N1 restriction fragment length polymorphism assay. In all the present studies described, two independent assays were used to confirm the detected mutations.

Analysis of Mutations in the *p53* Gene

Two screening methods were used to detect evidence for alterations in the *p53* gene. First, immunohistochemical analysis of the p53 protein, which relies on the nuclear localization and increased stability of mutant p53 proteins, was used to identify cells with altered expression of the *p53* gene (*12*). Second, to identify the exon containing the possible mutation, exons 4 to 9 of the *p53* gene were screened for mutations by single-strand conformation polymorphism (SSCP) analysis (*18*); > 90% of the mutations in *p53* are localized within these exons (*2*). Finally, mutations were identified by direct sequencing of exon-specific polymerase chain reaction products (*12*).

Results

K-*ras* Mutations in Rat Lung Tumors

The frequency for mutation of the K-*ras* gene was exposure dependent, but was not associated with histologic tumor type. Mutations detected were present at similar frequencies in adenocarcinomas and squamous cell carcinomas. Results are summarized in Table 1. All lung tumors induced by TNM contained a GGT → GAT transition mutation within codon 12 of the K-*ras* gene (*10*). In marked contrast, no activating mutations were present in lung tumors induced by NNK (*19*). Mutation frequencies of 6 and 10% were detected in tumors associated with carbon black and diesel exhaust exposure, respectively. Only 10% of tumors induced by beryllium metal contained a K-*ras* mutation; in both cases a GGT → GTT transversion was observed (*12*). Lung tumors induced by plutonium showed a higher frequency for mutation of the K-*ras* gene than tumors induced by X-ray; 46% of tumors induced by plutonium contained a mutation within this gene, predominantly a GGT → AGT transition in codon 12 (*20*). In contrast, only 3% of X-ray-induced tumors contained an activating mutation (*14*). No mutations were detected in the four sham-exposed lung tumors.

A *Bst*N1 enrichment assay, which can detect one mutant allele in 10^4 copies of wild-type allele, was required to detect the mutations observed in the beryllium- and X-ray-induced tumors. All other mutations were detected without enriching for the mutant allele. Thus, the activation of the K-*ras* gene in the beryllium- and X ray-induced tumors is both a rare and a late event, possibly stemming from genomic instability during tumor progression.

p53 Mutations in Rat Lung Tumors

All lung tumors, except those induced by TNM (not available for analysis) were examined immunohistochemically for the p53 protein. A sarcoma induced in the nude mouse by the SV40 immortalized rat 2 cell line showed strong immunoreactivity toward the anti-p53 antibody CM1 and was used as a positive control (*12*). Positivity for p53 protein was associated with tumor histology. None of the 93 adenocarcinomas examined (all exposures) were immunoreactive with the p53 antibody. In contrast, 14 of 71 squamous cell carcinomas exhibited nuclear p53 immunostaining as summarized in Table 2. Immunoreactivity was detected in 57 and 50% of squamous cell carcinomas induced by diesel exhaust or carbon black (*18*), albeit with low sample sizes ($n = 7$ and 4), while only 18 and 7% of tumors induced by X-rays (*14*) or plutonium (*21*), respectively, contained aberrant p53 protein. Only 18% of squamous cell tumors

Table 1. Pattern and frequency of K-*ras* gene mutations in rat lung tumors.

Exposure	K-*ras* activation: Frequency	%	Activating mutation
Sham	0/4	0	—
TNM	19/19	100	Codon 12: GGT → GAT
NNK	0/21	0	—
Beryllium	2/24	10	Codon 12: GGT → GTT
Diesel exhaust	2/21	10	Codon 12: GGT → GAT Codon 61: CAA → CAT
Carbon black	1/18	6	Codon 12: GGT → GTT
Plutonium	33/71	46	Codon 12: GGT → AGT Codon 12: GGT → GTT
X-ray	1/35	3	Codon 12: GGT → GAT

Table 2. Frequency for alteration of the *p53* gene in rat squamous cell carcinomas.[a]

Exposure	Immunostaining Frequency	%	SSCP Analysis[b] Frequency	%	Mutation
Sham	1/2	50	ND	–	—
NNK	2/11	18	1/11	9	Exon 5, codon 143: TTG →TCG
Diesel exhaust	4/7	57	1/5	20	Exon 8, codon 272: GTT →GTC
Carbon black	2/4	50	0/4	0	—
X-ray	3/18	18	1/18	6	Exon 9, codon 309: AGC →AAC
Plutonium	2/29	7	2/29	7	Exon 8, codon 280: CGT →CAT Exon 8, codon 283: GAG →AAG

ND, no data. [a]Lung tumors were analyzed for p53 protein with the anti-p53 antibody CM1. SSCP analysis was conducted for exons 4 to 9, and direct sequencing was used to determine the specific mutation. [b]Due to the small size of some neoplasms, not all were included in SSCP analysis.

induced by NNK exhibited nuclear p53 immunostaining. No squamous cell carcinomas were available from the beryllium carcinogenesis study.

The immunostained nuclei in the squamous cell carcinomas were generally distributed throughout the neoplasm in the basilar layers of the neoplastic cords and in the poorly differentiated portions of the neoplasms (Figure 1A, B). The more differentiated polyhedral, keratinizing cells toward the centers of the neoplastic cords showed little to no nuclear reactivity (*14,18,21,22*). Immunoreactivity varied among tumors; it was strong in intensity in multiple foci of some tumors while more focal, intense staining was observed in other tumors. In some tumors staining was diffuse, but weak in intensity (*14,18,21,22*).

Because immunohistochemical analysis of the p53 protein does not detect alterations that result in the loss of protein expression, nonsense mutations, or missense mutations outside of exons 5 to 8, SSCP analysis of exons 4 to 9 was conducted on all tumors. No mutations were detected in any of the adenocarcinomas. In spite of the immunoreactivity observed in the 13 squamous cell carcinomas available for screening, SSCP analysis (Table 2) only detected mutations in one of the NNK-induced tumors (exon 5) and both plutonium-induced tumors (exon 8). Two additional tumors contained mutations: one was a silent mutation within exon 8 from a diesel exhaust-induced tumor, and the other was found in exon 9 from an X-ray-induced tumor. Three of the mutations were G→A transitions, while the fourth mutation was a T→C transition (Table 2, Figure 2).

Discussion

The results from these investigations indicate that the involvement of the K-*ras* and *p53* genes in the genesis of rat lung tumors is specific to the exposure material and the resulting histologic form of the tumor, respectively. The only two exposures that led to activation of the K-*ras* gene at frequencies > 10% were that of TNM and plutonium. In both cases, the mutations formed could have stemmed from deamination of cytosine. Nitro-containing compounds such as TNM can cause subsequent base mispairing by deaminating a base such as cytosine (*10*).

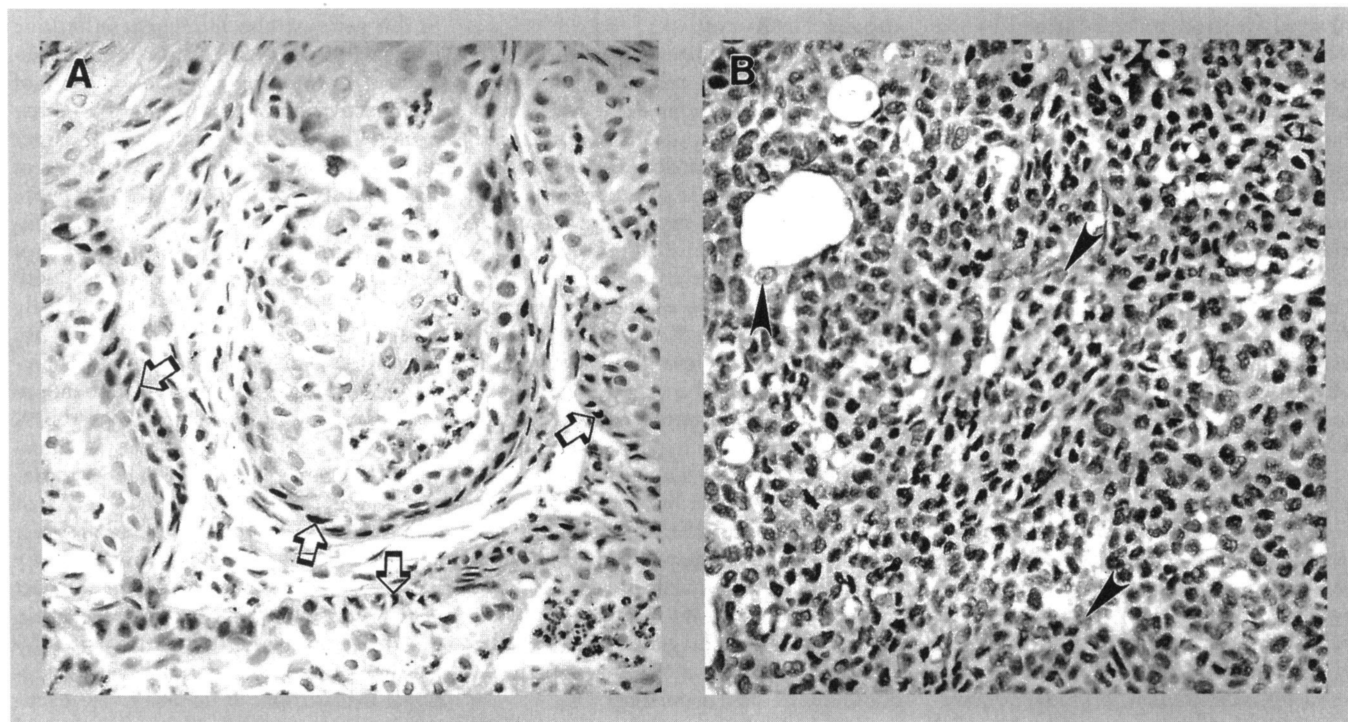

Figure 1. Immunoreactivity of p53 protein in lung squamous cell carcinomas. Magnification is ×300. (*A*) Well-differentiated squamous cell carcinoma. Only the most basal cells of each neoplastic cord exhibit positive nuclear immunoreactivity for *p53* (arrows). (*B*) Poorly differentiated squamous cell carcinoma. Many positively immunostained nuclei are scattered throughout the neoplasm. Arrowheads point to examples of nuclei that are not immunoreactive. Faint hematoxylin counterstain. Reproduced from Belinsky et al. (*22*), with permission.

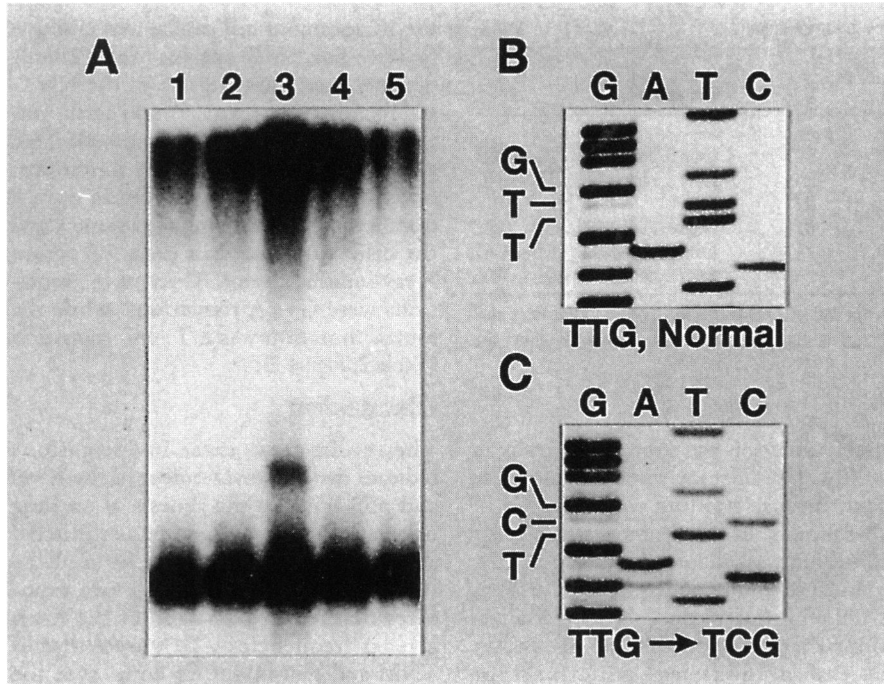

Figure 2. Detection of a mutation in the *p53* gene. (*A*) Exon 5 SSCP analysis of DNA from four lung tumors induced by NNK (lanes 1–4) and normal rat lung (lane 5). A slower migrating band suggestive of a mutation is present in lane 3. (*B*) Wild-type sequence surrounding codon 143. (*C*) TTG→TCG mutation detected by SSCP analysis. Sequencing was done following cloning of PCR products; therefore, the wild-type allele is not present in *C*.

Hydroxyl radicals, hydrogen atoms, or solvated electron species formed by the radiolysis of water and other adjacent molecules resulting from the passage of alpha particles emitted by plutonium can also cause cytosine deamination (*23*). Both transition and transversion mutations were detected in the K-*ras* gene in human adenocarcinomas associated with exposure to radon (*24*). The lack of a specific mutation profile in these human tumors may stem from the fact that most of these uranium miners were also smokers. Adenocarcinomas associated with tobacco use have a higher frequency for transversion than for transition mutations in the K-*ras* gene (*1*).

The difference between alpha particles and X-rays in targeting the K-*ras* gene may stem from the amount of energy deposited. Alpha particles deposit large amounts of energy in small volumes along their paths, while X-rays deposit relatively sparse amounts of energy. Estimation of DNA damage using *p53* expression as a dosimeter indicates that to produce equivalent acute damage requires an absorbed dose of X-rays 10-fold higher than for alpha particles (*25*). Therefore, the energy deposited by plutonium alpha particles would be expected to generate a higher concentration of DNA damage than that deposited by X-rays.

The low frequency of K-*ras* mutations found in the diesel exhaust- and carbon black-induced carcinomas may result from the lack of participation of mutagenic organics in the induction of neoplasia by diesel exhaust. This conclusion is supported by a recent report showing that DNA adducts specific to polycyclic aromatic hydrocarbons are not increased in rats chronically exposed to diesel exhaust (*26*). The fact that K-*ras* mutations are also a rare and late event in rat lung carcinogenesis induced by the nongenotoxic carcinogen, beryllium metal, provides further support for the hypothesis that diesel exhaust acts through a nongenotoxic mechanism. However, the K-*ras* gene in the rat is not always a target for genotoxic carcinogens. For example, despite the dose-related correlation of carcinogen-specific adducts to the induction of rat lung neoplasms by the genotoxic carcinogen NNK (*11*), the expected mutations in the K-*ras* gene were not found in these neoplasms.

The lack of *ras* mutations in rat lung tumors induced by genotoxic exposures is not necessarily an anomaly when compared to human lung carcinogenesis. Activation of the K-*ras* gene is detected in < 40% of human adenocarcinomas but rarely in squamous cell carcinomas. This histologic preference for mutation of the K-*ras* gene was not observed in rat lung tumors, a difference that may stem from the progenitor cells responsible for these two tumor subtypes in rat and human lung cancers (*11,27*). Other oncogenes must also be involved in the development of non-small-cell lung cancers. DNA from cells transformed *in vitro* by X-rays (*28,29*) or *in vivo* by NNK (*19*) could transmit their malignant phenotype to NIH 3T3 cells; however, the oncogenes responsible were not members of the *ras* gene family. Identification of these non-*ras* transforming genes would be invaluable for unraveling some of the mechanisms underlying cancer induction by genotoxic and nongenotoxic exposures.

Results summarized in this review indicate that the *p53* gene may be involved in the development of some rat squamous cell carcinomas, while alterations in this gene have not been detected in adenocarcinomas. Consistent with these findings, several recent reports indicate that alterations in *p53* are infrequently found in primary rat neoplasms regardless of tissue or etiology (*30–37*), with the exception of squamous cell carcinomas (*38,39*). Mutations in this gene are also infrequent in murine lung tumors (*40–42*). While immunohistochemical staining revealed evidence of p53 inactivation in 14 squamous cell carcinomas, both SSCP analysis and direct sequencing detected mutations in only 3 of 13 immunoreactive samples that were examined. Given the extent and distribution of immunoreactivity in the majority of these tumors and the sensitivity of SSCP (*43*) and direct sequencing, it is unlikely that mutant alleles present would not have been detected. The p53 protein immunoreactivity in these neoplasms may be due to mutations outside exons 4 through 9, although this is unlikely because such mutations have rarely been associated with protein stabilization and occur in < 5% of human neoplasms with *p53* mutations (*44*).

Alternatively, p53 immunoreactivity may be due either to stabilization by other gene products or the disruption of protein degradation. Stabilization of p53 by another protein may correlate with functional inactivation, analogous to its inactivation and extended half-life when bound by the SV40 large-T antigen. Precedent for this finding exists in several other reports in which enhanced detection of p53 by immunohistochemistry occurs in

the absence of mutations within the conserved region (*33,44,45*). One known method of inactivating wild-type *p53* is the overexpression of the *MDM2* gene product, which may stabilize the p53 protein to detectable levels. Immunohistochemical staining of lung tumors induced by diesel exhaust, carbon black, or X-rays detected overexpression of *MDM2* in only one squamous cell carcinomas induced by X-ray exposure (*14,18*). This tumor also stained positive for p53. The *MDM2* gene was not amplified in the immunoreactive X-ray-induced tumor, suggesting that the mechanism underlying this overexpression could stem from enhanced translation as described by Landers et al. (*46*).

It is clear that while mutation of the *p53* tumor suppressor occurs in 37 and 65% of human pulmonary adenocarcinomas and squamous cell carcinomas, respectively, many cancers develop independently of this tumor suppressor gene. The recent identification of the $p16^{INK4a}$ gene at chromosome 9p21 (*47*) and other genes being identified at chromosomes 3p14, 3p21, and 3p25 (*48*) should provide additional candidate genes for analysis in rat lung tumors.

REFERENCES

1. Rodenhuis S. *Ras* oncogenes and human lung cancer. In: Lung Cancer Principles and Practice (Pass H, Mitchell JB, Johnson DH, Turrisi AT, eds). New York:Lippincott-Raven, 1996;73–82.
2. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 54:4855–4878 (1994).
3. Hollstein M, Sidransky D, Vogelstein B, Harris CC. *p53* Mutations in human cancers. Science 253:49–53 (1991)
4. You M, Candrian U, Maronpot RR, Stoner GD, Anderson MW. Activation of the Ki-*ras* protooncogene in spontaneously occurring and chemically induced lung tumors of the strain A mouse. Proc Natl Acad Sci USA 86:3070–3074 (1989).
5. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the *p53* gene in human hepatocellular carcinomas. Nature 350:427–428 (1991).
6. Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Pontén J. A role for sunlight in skin cancer: UV-induced *p53* mutations in squamous cell carcinoma. Proc Natl Acad Sci USA 88:10124–10128 (1991).
7. Boring CC, Squires TS, Tong T. Cancer statistics. J Clin Cancer 86:7–26 (1993).
8. Lubin JH, Boice JD, Edling C, Hornung RW, Howe GR, Kunz E, Kusiak RA, Morrison HI, Radford EP, Samet JM et al. Lung cancer in radon-exposed miners and estimation of risk from indoor exposure. J Natl Cancer Inst 87:817–827 (1995).
9. Cohen AJ, Pope CA. Lung cancer and air pollution. Environ Health Perspect 103(Suppl 8):219–224 (1995).
10. Stowers SJ, Glover PL, Reynolds SH, Boone LR, Maronpot RR, Anderson, MW. Activation of the K-*ras* protooncogene in lung tumors from rats and mice chronically exposed to tetranitromethane. Cancer Res 47:3212–3219 (1987).
11. Belinsky SA, Foley JF, White CM, Anderson MW, Maronpot RR. Dose–response relationship between O^6-methylguanine formation in Clara cells and induction of pulmonary neoplasia in the rat by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res 50:3772–3780 (1990).
12. Nickell-Brady C, Hahn FF, Finch GL, Belinsky SA. Analysis of K-*ras p53* and c-*raf*-1 mutations in beryllium-induced rat lung tumors. Carcinogenesis 15(2):257–262 (1994).
13. Herbert RA, Gillett NA, Rebar AH, Lundgren DL, Hoover MD, Chang IY, Carlton WW, Hahn FF. Sequential analysis of the pathogenesis of plutonium-induced pulmonary neoplasms in the rat: morphology, morphometry, and cytokinetics. Radiat Res 134:29–42 (1993).
14. Belinsky SA, Middleton SK, Picksley SM, Hahn FF, Nikula KJ. Analysis of the K-*ras* and *p53* pathways in X-ray-induced lung tumors in the rat. Radiat Res, 145:449–456 (1996).
15. Nikula KJ, Snipes MB, Barr EB, Griffith WC, Henderson RF, Mauderly JL. Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon black in F344 rats. Fundam Appl Toxicol 25:80–94 (1995).
16. Murphy SE, Palomino A, Hecht SS, Hoffmann D. Dose–response study of DNA and hemoglobin adduct formation by 4-methylnitrosamino-1-(3-pyridyl)-1-butanone in F344 rats. Cancer Res 50:5446–5452 (1990).
17. Bremmer LH. Ionizing radiation-induced mutagenesis. Br J Cancer 57:6–18 (1988).
18. Swafford DS, Nikula KJ, Mitchell CE, Belinsky, SA. Low frequency of alterations in *p53*, K-*ras*, and *mdm2* in rat lung neoplasms induced by diesel exhaust or carbon black. Carcinogenesis 16:1215–1221 (1995).
19. Belinsky SA, Devereux TR, White CM, Foley JF, Maronpot RR, Anderson MW. Role of Clara cells and type II cells in the development of pulmonary tumors in rats and mice following exposure to a tobacco-specific nitrosamine. Exp Lung Res 17:263–278 (1991).
20. Stegelmeier BL, Gillett NA, Rebar AH, Kelly G. The molecular progression of plutonium-239-induced rat lung carcinogenesis. Mol Carcinog 4:43–51 (1991).
21. Kelly G, Stegelmeier BL, Hahn FF. *p53* Alterations in plutonium-induced F344 rat lung tumors. Radiat Res 142:263–269 (1995).
22. Belinsky S, Mitchell CE, Nikula KJ, Swafford DS. Pulmonary toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats: examination of possible target genes. Health Effects Research Institute Report 68. Cambridge, MA:Health Effects Reasearch Institute, 1995.
23. Growovsky AJ, de Boer JG, de Jong PJ, Drobetsky EA, Glickman BW. Base substitutions, frameshifts, and small deletions constitute ionizing radiation-induced point mutations in mammalian cells. Proc Natl Acad Sci USA 85:185–188 (1988).
24. McDonald JW, Taylor JA, Watson MA, Saccomanno G, Devereux TR. *p53* and K-*ras* in radon-associated lung adenocarcinoma. Cancer Epidemiol Biomarkers Prev 4:791–793 (1995).
25. Hickman AW, Jaramillo RJ, Lechner JF, Johnson NF. α-Particle-induced p53 protein expression in a rat lung epithelial cell strain. Cancer Res 54:5797–5800 (1994).
26. Gallagher J, Heinrich U, George M, Hendee L, Phillips DH, Lewtas J. Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. Carcinogenesis 15(7):1291–1299 (1994).
27. Gazdar, AF, Linnoila, RI. The pathology of lung cancer: changing concepts and newer diagnostic techniques. Semin Oncol 15:215–225 (1988).
28. Leuthauser SWC, Thomas JE, Guernsey DL. Oncogenes in X-ray-transformed C3H10T1/2 mouse cells and in X-ray-induced mouse fibrosarcoma (RIF-1) cells. Int J Radiat Biol 62:45–51 (1992).
29. Borek C, Ong A, Mason H. Distinctive transforming genes in X-ray-transformed mammalian cells. Proc Natl Acad Sci USA 84:794–798 (1987).
30. Asamoto M, Mann AM, Cohen SM. *p53* Mutation is infrequent and might not give a growth advantage in rat bladder carcinogenesis *in vivo*. Carcinogenesis 15(3):455–458 (1994).

31. Weghorst CM, Dragnev KH, Buzard GS, Thorne KL, Vandeborne GF, Vincent KA, Rice JM. Low incidence of point mutations detected in the *p53* tumor suppressor gene from chemically induced rat renal mesenchymal tumors. Cancer Res 54:215–219 (1994).
32. Jones RF, Matuszyk J, Debiec-Rychter M, Wang CY. Mutation and altered expression of *p53* genes in experimental rat bladder tumor cells. Mol Carcinog 9:95–104 (1994).
33. Asamoto M, Mann AM, Macatee TL, Cohen SM. Mutations and expression of the *p53* gene in rat bladder carcinomas and cell lines. Mol Carcinog 9:236–244 (1994).
34. Ushijima T, Kakiuchi H, Makino H, Hasegawa R, Ishizaka Y, Hirai H, Yazaki Y, Ito N, Sugimura T, Nagao M. Infrequent mutation of Ha-*ras* and *p53* in rat mammary carcinomas induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Mol Carcinog 10:38–44 (1994).
35. Tokusashi Y, Fukuda I, Ogawa K. Absence of *p53* mutations and various frequencies of Ki-*ras* exon 1 mutations in rat hepatic tumors induced by different carcinogens. Mol Carcinog 10:45–51 (1994).
36. Fukuda I, Ogawa K. Detection of *p53* gene mutations in rat hepatocellular carcinoma cell lines by denaturing gradient gel electrophoresis. Mol Carcinog 7:257–262 (1993).
37. Smith ML, Yeleswarapu L, Lombardi B, Shinozuka H. Lack of mutations of the *p53* tumor suppressor gene in hepatocellular carcinomas induced in rats by a peroxisome proliferator. Mol Carcinog 7:89–93 (1993).
38. Recio L, Sisk S, Pluta L, Bermudez E, Gross EA, Chen Z, Morgan K, Walker C. *p53* Mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. Cancer Res 52:6113–6116 (1992).
39. Makino H, Ishizaka Y, Tsujimoto A, Nakamura T, Onda M, Sugimura T, Nagao M. Rat *p53* gene mutations in primary zymbal gland tumors induced by 2-amino-3-methylimidazo[4,5-f]quinoline, a food mutagen. Proc Natl Acad Sci USA 89:4850–4854 (1992).
40. Goodrow TL, Storer RD, Leander KR, Prahalada SR, van Zwieten MJ, Bradley MO. Murine p53 intron sequences 5–8 and their use in polymerase chain reaction/direct sequencing analysis of *p53* mutations in CD-1 mouse liver and lung tumors. Mol Carcinogen 5: 9–15 (1992).
41. Hegi ME, Soderkvist P, Foley JF, Schoonhoven R, Swenberg JA, Kari F, Maronpot R, Anderson MW, Wiseman RW. Characterization of *p53* mutations in methylene chloride-induced lung tumors from B6C3F1 mice. Carcinogenesis 14:803–810 (1993).
42. Chen B, Liu L, Castonguay A, Maronpot RR, Anderson MW, You M. Dose-dependent *ras* mutation spectra in *N*-nitrosodiethylamine induced mouse liver tumors and 4-(methylnitroamino)-1-(3-pyridyl)-1-butanone induced mouse lung tumors. Carcinogenesis 14:1603–1608 (1993).
43. Suzuki Y, Orita M, Shiraishi M, Hayashi K, Sekiya T. Detection of *ras* gene mutations in human lung cancers by single-strand conformation polymorphism analysis of polymerase chain reaction products. Oncogene 5:1037–1043 (1990).
44. Bodner SM, Minna JD, Jensen SM, D'Amico D, Carbone D, Mitsudomi T, Fedorko J, Buchhagen DL, Nau MM, Gazdar AF et al. Expression of mutant p53 proteins in lung cancer correlates with the class of *p53* mutation. Oncogene 7:743–749 (1992).
45. Lehman TA, Bennett WP, Metcalf RA, Welsh JA, Ecker J, Modali RV, Ullrich S, Romane JW, Appella E, Testa JR et al. *p53* Mutations, *ras* mutations, and *p53*-heat shock 70 protein complexes in human lung carcinoma cell lines. Cancer Res 51:4090–4096 (1991).
46. Landers JE, Haines DS, Strauss JF, George DL. Enhanced translation: a novel mechanism of *mdm2* oncogene overexpression identified in human tumor cells. Oncogene 9:2745–2750 (1994).
47. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian V, Stockert E, Day RS, Johnson BE, Skolnick MH. A cell cycle regulator potentially involved in genesis of many tumor types. Science 264:436–440 (1994).
48. Hosoe S, Shigedo Y, Ueno K, Tachibana I, Osaki T, Tanio Y, Kawase I, Yamakawa K, Nakamury Y, Kishimoto T. Detailed deletion mapping of the short arm of chromosome 3 in small cell and non-small cell carcinoma of the lung. Lung Cancer 10:297–305 (1994).