Comparison of Mutations in the p53 and K-ras Genes in Lung Carcinomas from Smoking and Nonsmoking Women[1]

Robert Gealy, Lifang Zhang, Jill M. Siegfried, James D. Luketich, and Phouthone Keohavong[2]

Departments of Environmental and Occupational Health [R. G., L. Z., P. K.], Pharmacology [J. M. S.], and Surgery [J. D. L.], University of Pittsburgh, and Lung Cancer Basic Research Program, University of Pittsburgh Cancer Institute [P. K., J. M. S., J. D. L.], Pittsburgh, Pennsylvania 15261

Abstract

Lung cancer incidence is increasing in women with little or no tobacco exposure, and the cause of this trend is not known. One possibility is increased sensitivity to environmental tobacco smoke in women nonsmokers diagnosed with lung cancer. To determine whether mutations associated with tobacco exposure are found in the lung tumors of women who are lifetime nonsmokers or occasional smokers, we compared the p53 and K-ras mutational spectra in lung carcinomas from 23 female nonsmokers, 2 female occasional smokers (<10 pack-years), and 30 female long-term smokers (20–100 pack-years). We also looked for p53 and K-ras mutations in three carcinoid lung tumors, two from female nonsmokers and one from a female occasional smoker. For the *p53* gene, exons 4–8 were examined for mutations; for the K-*ras* gene, exon 1 was examined. No mutations were found in the carcinoid tumors. In lung carcinomas, p53 mutations were identified in six (26.1%) of the cases from lifetime nonsmokers and consisted of five transitions (including three C to T, one G to A, and one T to C) and one T to A transversion. In comparison, p53 mutations were identified in 10 (31.3%) of the 32 lung carcinomas from short-term and long-term smokers and consisted of six transversions (four G to T, one A to T, and one G to C), one A to G transition, one C to T transition, and two deletions of one to four bp. Mutations in the *p53* gene found in nonsmokers also occurred in either different codons or different positions within a codon compared with those seen in long-term smokers. K-ras mutations in codon 12 were identified in two lung carcinomas (8.7%) from lifetime nonsmokers. The K-ras mutations found were a G to T transversion and a G to A transition. Eight (25%) of the 32 lung carcinomas from smokers contained K-ras mutations in codons 12 and 13 (four G to T transversions and four G to A transitions). In addition, six silent mutations that are most likely polymorphisms were found in both smokers and nonsmokers. These results confirm that K-ras mutations are more frequent in smokers than in nonsmokers, but that the same type of mutation in the K-*ras* gene is found in both groups. In contrast, although the frequency of mutation in the *p53* gene was similar in lifetime nonsmokers compared with long-term smokers, the types and spectra of mutation are significantly different. Two of the C to T transitions found in nonsmokers, but none of those found in smokers, occur at the C of a CpG site. These results suggest the mutagen(s) and/or mechanisms of p53 mutations in women nonsmokers are different from those responsible for p53 mutations in women smokers, which are probably largely induced by tobacco mutagens.

Introduction

Lung cancer is the leading cause of cancer mortality for both men and women in the United States (1). In the past decade, the incidence of lung cancer among women has risen, whereas among men it has slightly declined. (1, 2). The link between smoking and lung cancer has been well established in both men and women (3). However, gender-based differences in susceptibility to smoking-related lung cancer have been found, with women showing a higher risk than men of developing lung cancer for a given level of tobacco consumption (4). An increasing number of lung cancer cases have also been documented in nonsmokers, and a disproportionate number of these are adenocarcinomas and occur in women (5). The etiology of lung cancer in nonsmokers is not well understood. Environmental exposures, such as environmental tobacco smoke (6) and exposure to coal fumes (7), as well as hormonal effects (8), have been suggested as potential risk factors for lung cancer in nonsmokers. Individual susceptibility factors may also be important in the development of lung cancer. These factors may include family history of lung cancer, specific xenobiotic metabolic profiles, presence of prior lung disease, or diet (9).

Mutations in the *p53* tumor suppressor gene and K-*ras* proto-oncogene are frequent alterations in many human tumors (10, 11). In lung cancer, p53 and K-ras mutations have been well characterized, but most mutations have been reported from smokers. K-ras mutations are found in about 30% of adenocarcinoma of the lung, with 80–90% occurring at codon 12 and consisting predominantly of G to T transversions (12, 13). In comparison, a compilation of p53 mutations in lung cancer shows that these mutations occur in many codons, mostly in the highly evolutionarily conserved regions between exons 4 and 8 (14). They consist predominantly of G to T transversion and, to a lesser extent, G to C transversion, G to A transition, and small bp deletions involving mostly guanine. Although p53 mutations can occur in many codons, there are recognized "hot spots" for

Received 7/27/98; revised 1/4/99; accepted 1/25/99.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by NIH Grant 1-R03-CA71609-01 (to P. K.), by a grant from the University of Pittsburgh Cancer Institute Lung Cancer Basic Research Program (to P. K.), and by NIH Grant R01 CA-50694 (to J. M. S.).
[2] To whom requests for reprints should be addressed, at the Department of Environmental and Occupational Health, 260 Kappa Drive, Pittsburgh, PA 15238. Phone: (412) 967-6526; Fax: (412) 624-1020; E-mail: pho1@vms.cis.pitt.edu.

frequent p53 mutation in lung tumors of smokers that occur in codons 157–158, 179, 245–249, 273, and 282 (14). It has been suggested that such mutations occur in carcinogen-exposed cells during the misreplication of a bulky DNA adduct formed between a carcinogen(s), such as polycyclic aromatic hydrocarbons found in tobacco smoke, and the guanine bases in the *p53* gene (11).

In an attempt to determine the molecular etiology of lung cancer in women nonsmokers, we characterized mutations in the *p53* and K-*ras* genes in primary lung tumors obtained from female lifetime nonsmokers, female occasional smokers, and female long-term smokers. We compare the frequencies, types, and spectra of these mutations among these lung cancer patients.

Materials and Methods

Lung Tumor Tissues. Tissues were fresh-frozen lung tumors collected and stored sequentially between 1988 and 1997 from female patients who underwent lung resection at the University of Pittsburgh Medical Center under an Institutional Review Board-approved protocol. The diagnosis of carcinoma or carcinoid tumor, primary to lung, was confirmed by consulting surgical pathology reports for each patient. Medical records were consulted to determine smoking histories and family history of cancer. A total of 55 specimens of lung carcinoma and three specimens of lung carcinoid were analyzed for p53 and K-ras mutations. Lung carcinomas were obtained from 23 lifetime nonsmokers (12 cases of adenocarcinoma, 2 cases of adenosquamous carcinoma, 4 cases of bronchioloalveolar carcinoma, 4 cases of squamous cell carcinomas, and 1 case of large cell carcinoma), two occasional smokers (one case of squamous cell carcinoma and one case of neuroendocrine carcinoma), and 30 long-term smokers (14 cases of adenocarcinoma, 1 case of adenosquamous carcinoma, 11 cases of squamous cell carcinoma, 3 cases of large cell carcinomas, and 1 case of small cell carcinoma). The 23 nonsmokers, who had no smoking history, and 2 occasional smokers, who had a short smoking history, represented all of the women diagnosed with lung cancer with short or no smoking history who donated tissues for study from 1988–1997. The other 30 carcinomas represented a random sample of women smokers with lung cancer who also donated tissues during the same period as the nonsmokers. Patients, smokers and nonsmokers, had approximately the same age distribution. Three cases of carcinoid lung tumor were also analyzed for mutations; two tumors were from lifetime nonsmokers and one tumor was from an occasional smoker. Occasional smokers were defined as having a total lifetime smoking history of <10 PYs.[3] One PY is defined as one pack of cigarettes/day for 1 year. The two occasional smokers had smoking histories of 2 PYs and 8 PYs, respectively, and had smoked either for a limited period during their lifetime or one or two cigarettes/day for an extended period. The PYs of long-term smokers ranged from 20 to 114, with a mean of 55 PYs (±25.4). The clinical information for lung cancer patients is shown in Table 1. Because carcinoid tumors did not contain mutations in p53 or K-ras, information from carcinoid patients is not included in Table 1 and these cases will not be discussed further.

Table 1 Clinical profile of female nonsmokers and smokers with lung cancer whose tumors were analyzed for mutations

	Nonsmokers	Smokers
Number of cases	23	32
Mean age ± SE (yrs)	57.8 ± 23.7	61.0 ± 19.5
Histology of tumor		
Adenocarcinoma	12 (52.2%)	14 (43.8%)
Adenosquamous	2 (8.7%)	1 (3.1%)
Bronchioloalveolar	4 (17.4%)	0
Squamous cell	4 (17.4%)	12 (37.5%)
Large cell	1 (4.3%)	3 (9.4%)
Small cell	0	1 (3.1%)
Neuroendocrine	0	1 (3.1%)
Stage at diagnosis		
I	13 (56.5%)	16 (50%)
II	2 (8.7%)	8 (25%)
IIIa or IIIb	3 (13.0%)	7 (21.9%)
IV	4 (17.4%)	0
Unknown	1 (4.3%)	1 (3.1%)
PY smoking		
0	23	0
<10	0	2
10–25	0	1
26–50	0	16
>50	0	13
Environmental tobacco smoke exposure		
Yes	3	n/a
No	1	n/a
Unknown	19	n/a
Cases with K-*ras* mutation	2 (8.7%)	8 (25%)
Cases with p53 mutation	6 (26.1%)	10 (31.3%)
Cases with either mutation	8 (34.8%)	18 (56.3%)

DNA Extraction and Analysis of p53 and K-ras Mutations. For DNA isolation, lung tissues were cut into small pieces, dissociated in a lysis buffer [10 mM Tris (pH 7.4), 0.5% SDS, 150 mM NaCl, and 100 mM EDTA], and digested with RNase A1 (10 μg/ml at 37°C for 2 h) and proteinase K (20 μg/ml at 37°C for at least 4 h), followed by phenol-chloroform extraction and ethanol precipitation. About 0.1 μg of DNA from each sample was used for PCR. The PCR reaction was carried out in a 20-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM of each deoxynucleoside triphosphate (dA, dC, dT, and dG), 0.5 μM each primer, 5 μCi [α-^{32}P]dATP (3000 Ci/mmol), and 1.25 unit of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Foster City, CA). The mixture was incubated for 2 min at 95°C, followed by 35 amplification cycles in a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, CT). To amplify the *p53* gene exons, the following cycle was used: 94°C/30 s, 66°C (55°C for exon 8)/45 s, and 72°C/1.5 min. The following sets of primers were used to amplify individual exons of the *p53* gene:

Exon 4: E4–5′ 5′-TGACTGCTCTTTTCACCCAT-3′
E4–3′ 5′-GGAAGCCAGCCCCTCAGGGC-3′
Exon 5: E5–5′ 5′- AACTCTGTCTCCTTCCTCTT-3′
E5–3′ 5′- GCCCCAGCTGCTCACCATCGCTA-3′
Exon 6: E6–5′ 5′- TCTGATTCCTCACTGATTGC-3′
E6–3′ 5′- CCAGAGACCCCAGTTGCAAA-3′
Exon 7: E7–5′ 5′-GTGCAGGGTGGCAAGTGGCT-3′
E7–3′ 5′-CGTTGGCTCTGACTGTACCAC-3′
Exon 8: E8–5′ 5′-TGCTTCTCTTTTCCTATCCT-3′
E8–3′ 5′-CACCGCTTCTTGTCCTGCTT-3′

The amplified fragments were first checked by PAGE before analysis by SSCP, using the conditions modified from those described previously (15). The SSCP method uses a nondenaturing gel electrophoresis to separate mutant and wild-

[3] The abbreviations used are: PY, pack-year; SSCP, single-strand conformational polymorphism.

type alleles of a DNA fragment on the basis of conformational differences of the respective denatured single-strand forms. Accordingly, by SSCP analysis, each wild type fragment is usually detected in the gel as two major bands representing the two respective single strands of the wild-type allele. The presence of any additional mutant allele in the DNA sample analyzed will be usually detected as two single stranded bands, in addition to the two wild-type bands. We have carried out reconstruction experiments by mixing DNA of wild-type allele with varying fraction of a known *p53* mutant allele and analyzed each mixed DNA sample by using the PCR and SSCP methods. This approach allowed us to detect a *p53* mutant allele present in excess of its corresponding wild type allele at a mutant fraction of as low as 10% (one copy of the mutant allele and nine copies of the wild-type allele; data not shown). This level of sensitivity should be sufficient to detect mutations occurring in tumor tissues. Briefly, aliquots of radiolabeled PCR products were diluted 1:3 in a loading buffer (95% formamide, 10 mM NaOH, and bromphenol blue/xylene cyanole dyes), denatured at 100°C for 2 min, and chilled on ice before loading onto a nondenaturing, 0.5 × MDE gel (FMC BioProducts, Rockland, ME) containing 5% glycerol. Gels were run at eight constant W for 18–24 h at room temperature, dried, and autoradiographed. Because the same exons of the *p53* gene were amplified from each of the tumor samples and analyzed side-by-side by SSCP, mutant bands appearing in the gel can be identified easily and isolated from the gel for further sequencing analysis.

Mutations in codons 12 and 13 of the K-*ras* gene were analyzed in each of the above DNA samples by denaturing gradient gel electrophoresis (12).

Statistical Analysis. The results are statistically analyzed using χ^2 or Fisher's Exact test when appropriate.

Results and Discussion

Primary lung carcinomas from 55 women with lung cancer (23 lifetime nonsmokers, 2 occasional smokers, and 30 long-term smokers) were examined for mutations in the *p53* and K-*ras* genes. The clinical information for these patients is shown in Table 1. The mean age was not different among the three groups. The stage distribution was also not significantly different among the three groups; the majority of patients were stage I. The histology of tumors from nonsmokers was predominantly adenocarcinoma (52.2%) or a type of adenocarcinoma (8.7% adenosquamous and 17.4% bronchioloalveolar carcinoma). Thus, 78.3% of tumors from nonsmokers were a type of adenocarcinoma. In contrast, tumors from smokers were evenly divided between adenocarcinoma or adenosquamous cell carcinoma (15 of 32, or 46.9%) and the three types of lung cancer that have the strongest association with smoking [17 of 32 (53.1%) were squamous cell, large cell, or small cell neuroendocrine carcinomas]. Only 21.7% (5 of 23) of cases from nonsmokers fell into these categories. The predominance of adenocarcinoma in nonsmokers was not significantly different from smokers, however, because of the small size of the groups.

Nonsmokers had no lifetime exposure to active tobacco smoke; the two occasional smokers had histories of active smoking of 2 PYs and 8 PYs, respectively; and long-term smokers ranged in PY exposure between 20 and 114. All but one long-term smoker had at least a 30 PY exposure to active tobacco smoke (mean, 55 PYs).

The incidence of K-ras and p53 mutations in the lung carcinomas from lifetime nonsmokers and long-term smokers is summarized in Table 1 and presented in more detail in Table 2. Because carcinoid tumors and those from occasional smokers did not contain mutations, they will not be discussed further. Among the nonsmokers, SSCP analysis showed six mutations (26.1%) in exons 4–8 of the *p53* gene. These mutations included three C to T transitions (patients 89T, 543T, and 829T), one G to A transition (patient 701T), one T to C transition (patient 656T), and one T to A transversion (patient 820T). Thus, the p53 mutations found in women nonsmokers consisted mainly of transitions (five of six), with C to T and G to A accounting for 66.7%. In comparison, p53 mutations were found in 10 of 30 long-term smokers (33.3%), which was not significantly different from the 26.1% incidence in nonsmokers ($P = 0.764$). However, the types of these mutations differed significantly from those found in nonsmokers (Table 2). They consisted of six transversions (60%), including four G to T (patients 142T, 397T, 803T, and 105–87T), one G to C and one A to T (patients 111T and 382T, respectively). In addition, one A to G transition (patient 278T), one C to T transition (patient 789T), and two deletions of one to four bp (759T and 575T), were also found in the smoking group. Therefore, despite the small number of lung tumors obtained from women nonsmokers, our results show a predominance of transversion and deletion in smokers that is significantly different from the predominance of transition found in nonsmokers ($P = 0.035$).

In addition to the different types of p53 mutations, nonsmokers and smokers also differed significantly in the codons where mutations were found ($P = 0.0498$). In long-term smokers, 7 of 10 p53 mutations occurred in codons identified as the hot spot codons 157, 245–249, and 273–274. In nonsmokers, only one mutation occurred in one of the hot spot codons, codon 273. This mutation occurred as a transition in the first base of the codon (CGT to TGT), rather than a transversion in either the first or second base of this codon, which is commonly observed in tumors from smokers (11).

Mutations in the K-*ras* gene at codon 12 were detected in two of the 23 (8.7%) lung carcinomas from women nonsmokers, including a GGT to GAT (302T) and a GGT to GTT (480T). In comparison, eight K-ras mutations (23.3%) were found in tumors from the long-term smokers, and all but one occurred in codon 12 (Table 2). These included four G to T transversions (patients 255T, 346T, 554T, and 823T) and four G to A transitions (patients 766T, 734T, 128–88T, and 851T).

Our results confirm that K-ras mutations are infrequent in lung tumors from nonsmokers compared with smokers. However, in both groups they occur most often in adenocarcinoma, compared with squamous cell or large cell carcinoma, in agreement with previous studies (12, 13). In both smokers and nonsmokers, the K-ras mutations occur predominantly in the same codon 12, and have the same molecular signature (GGT to GAT or GTT). Although we recognize the number of mutations observed in nonsmokers is limited, our results suggest that K-ras mutations in nonsmokers arise by the same mechanism as in smokers and points to environmental tobacco exposure as a possible etiological agent.

In contrast, p53 mutations occur frequently in nonsmokers but, in this study, there was no statistical difference in the incidence between smokers and nonsmokers. p53 mutations in nonsmokers were also found predominantly in some types of adenocarcinoma (five of six, or 83.3%). In contrast, only 3 of 10 (30%) p53 mutations in smokers were detected in adenocarcinoma, but this difference was not significant. Of all types of adenocarcinoma, 5 of 18 (27.8%) contained p53 mutations in nonsmokers, compared with 3 of 15 (20%) in smokers who contained these mutations. For squamous cell carcinoma, 5 of 11 (45.5%) contained p53 mutations in smokers, compared

Table 2 *p53* and K-*ras* mutations in lung tumor tissues from 30 female smokers and 23 female nonsmokers

Patient no.	Codon (exon)	Base change			Amino acid change	Histology	Age	PYs
Smokers (n = 30)								
p53								
759T	293–294 (8)	GGGGAG	GGGAG	Deletion	Frameshift	SC[a]	50	80
382T	279 (8)	CAT	CTT	A to T	His-Leu	SC	56	80
111T	245 (7)	GGC	GCC	G to C	Gly-Ala	SC	77	60
142T	157 (5)	GTC	TTC	G to T	Val-Phe	SC	77	100
397T	249 (7)	AGG	ATG	G to T	Arg-Met	AD	76	50
278T	234 (7)	TAC	TGC	A to G	Tyr-Cys	SC	62	40
575T	247–248 (7)	CCGG		Deletion	Frameshift	AD	63	36
803T	157 (5)	GTC	TTC	G to T	Val-Phe	SC	73	30
789T	194 (6)	CTT	TTT	C to T	Leu-Phe	AD	69	60
105-87T	274 (8)	GTT	TTT	G to T	Val-Phe	LC	53	40
554T	213 (6)	CGA	CGG	A to G	None (Silent)	AD	38	30
K-*ras*								
766T	12 (1)	GGT	GAT	G to A	Gly-Asp	AD	49	50
734T	13 (1)	GGC	TAC	G to A	Gly-Asp	AD	51	50
128-88T	12 (1)	GGT	AGT	G to A	Gly-Ser	SC	53	60
255T	12 (1)	GGT	GTT	G to T	Gly-Val	AD	63	45
346T	12 (1)	GGT	TGT	G to T	Gly-Cys	AD	69	75
851T	12 (1)	GGT	GAT	G to A	Gly-Asp	LC	62	40
823T	12 (1)	GGT	TGT	G to T	Gly-Cys	AD	51	45
554T	12 (1)	GGT	GTT	G to T	Gly-Val	AD	38	30
105-87T	3 (1)	GAA	GAG	A to G	None (Silent)	LC	53	40
	8 (1)	GTA	GTG	A to G	None (silent)			
789T	8 (1)	GTA	GTG	A to G	None (Silent)	AD	69	60
Nonsmokers (n = 23)								
p53								
89T	165 (5)	CAG	TAG	C to T	Gln-Stop	ADSC	72	0
656T	243 (7)	ATG	ACG	T to C	Met-Thr	AD	70	0
829T	175 (5)	CGC	TGC	C to T	Arg-Cys	BA	46	0
543T	273 (8)	CGT	TGT	C to T	Arg-Cys	BA	46	0
701T	146 (5)	TGG	TGA	G to A	Trp-Stop	LC	80	0
820T	127 (5)	TCC	ACC	T to A	Ser-Thr	AD	71	0
452T	36 (4)	CCG	CCA	G to A	None (silent)	BR-AL	77	0
307T	213 (6)	CGA	CGA	A to G	None (silent)	SC	65	0
K-*ras*								
302T	12 (1)	GGT	GAT	G to A	Gly-Asp	AD	41	0
480T	12 (1)	GGT	GTT	G to T	Gly-Val	BA	60	0

[a] SC, squamous cell; AD, adenocarcinoma; LC, large cell; ADSC, adenosquamous; BA, bronchiolo-alveolar.

with 0 of 4 in nonsmokers. Our results demonstrate that p53 mutations found in women nonsmokers contain a higher number of transitions (83.3%) than in women smokers (20%; P = 0.035). In women smokers, p53 mutations consisted mainly of transversions (60%) and deletions (20%). The type of p53 mutation, the mutated codon, and the base within the codon that is altered all differ between women smokers and nonsmokers. In addition, the histology of the tumors containing a p53 mutation are predominantly adenocarcinoma in nonsmokers and squamous cell carcinoma in smokers.

In addition to mutations that changed the amino acid produced at specific codons, we also observed silent mutations that did not change the protein coding sequence (Table 2). These mutations were observed in codons 8 (patient 789T) and codons 3 and 8 (patient 105–87T) of the K-*ras* gene and in codons 36 (patient 452T) and 213 (patients 307T and 554T) of the *p53* gene. Although the mutations found in codons 36 and 213 of the *p53* gene had been identified previously in lung and other tumors (16), those found in codons 3 and 8 of the K-*ras* gene have not been reported in the literature. Nevertheless, these silent mutations represent presumably polymorphisms. However, in one study that compared the distribution of codon 213 mutation in various areas of papillary thyroid carcinomas, this mutation was detected in only tumor cells but not in nontumor cells, suggesting that it arises during the evolution of the tumor (17). In the present study, both patients with silent K-ras mutations (patients 105–87T and 789T) also harbored each a missense p53 mutation, whereas one of the patients with a silent p53 mutation (patient 554T) also harbored a missense K-ras mutation (Table 2). Therefore, we cannot rule out the possibility that some of the silent mutations may have occurred during tumor progression rather than representing polymorphisms. We cannot, however, investigate further this possibility due to a lack of normal tissues or lymphocytes from the patients who harbored these silent mutations.

There has been extensive study of p53 mutations in lung cancer. However, these mutations are almost exclusively from smokers (14). A question that arises from our data is whether the preponderance of adenocarcinoma in nonsmokers is a factor in the type of p53 mutation observed. There is no convincing evidence that there is a relationship between type of p53 mutation and histology of lung tumors. A study by Husgafvel-Pursiainen *et al.* (18) in male and female lung cancer patients who smoke showed that G to T transversions are more common in adenocarcinoma (42%) than in squamous cell carcinoma (29%), whereas Kure *et al.* (19) found a similar frequency in the two histologies: 34% G to T transversions in adenocarcinoma and 31% in squamous cell carcinoma. A recent report by

Hernandez-Bousssard and Hainaut (14) showed a slightly more frequent rate of G to T transversion in squamous cell (33%) compared with adenocarcinoma (27%). These data together argue that the rate of G to T transversion is approximately the same in the two histological types of lung cancer and suggest that, if histology were the determining factor in type of p53 mutations seen in nonsmokers, we should have seen a larger proportion of G to T transversions in that population. Instead, we observed transitions. The fact that we observed no G to T transversions in nonsmokers and that the mutations did not occur in the tobacco hot spots, suggests a different mechanism of mutation formation in nonsmokers compared with smokers.

With regard to p53 mutations in nonsmokers, Husgafvel-Pursiainen and Kannio (20) showed a mutation frequency of 28%, which is in close agreement with our present mutation frequency of 26% in nonsmokers. However, the types of p53 mutations in these patients were not determined by sequencing. Another study by Takeshima *et al.*, (21) compared p53 mutations in 17 Japanese women nonsmokers and 77 smokers, who were mostly Japanese men. The histological types of lung cancer from the 17 nonsmokers included 10 adenocarcinomas, 4 squamous cell carcinomas, 2 small cell carcinomas, and 1 large cell carcinoma, which are very similar to those observed among the women nonsmokers with lung cancer in our study. Eight p53 mutations were identified, including four C to T and one G to A transitions, and three transversions, including one G to T, one G to C and one T to A. The four C to T transitions were found in three squamous cell carcinomas and one of the small cell carcinomas, whereas the G to A transition was found in one of the small cell carcinomas. All three transversions were found in adenocarcinomas. Our results are in agreement with this study (21), suggesting the predominance of transitions in lung tumors of women nonsmokers. Furthermore, the fact that these transitions were detected in both adenocarcinoma, by us, and squamous cell carcinoma, by Takeshima *et al.* (21), suggests that the predominance of transitions observed in lung tumors of women nonsmokers is not a function of histology.

Our results suggest that the origin(s) and/or mechanisms of mutation in the *p53* gene in women nonsmokers who develop lung cancer are different from those in women smokers. We acknowledge that these results are obtained from a small number of women nonsmokers and need to be confirmed by other studies. Although the number of lung cancers is increasing in nonsmokers, particularly in women nonsmokers, nonsmokers still represent a very small proportion of total lung cancer cases. The predominance of transversion and deletion involving guanine residues in women smokers, and the occurrence of many of these mutations at codons 157, 245, and 247–249, known to be hot spots for mutations in lung tumors in smokers, is consistent with an induction by carcinogens (such as polycyclic aromatic hydrocarbons in tobacco smoke) as has been documented previously (11). In contrast, the predominance of C to T and G to A transitions found in nonsmokers may be produced by a different mutagen(s) than in smokers. For instance, two of the three C to T transitions (codons 175 and 273) observed in nonsmokers by us and one of four other C to T transitions found by Takeshima *et al.* (21) occurred at the C in a CpG site. It has been suggested that the C at a CpG site is prone to methylation *in vivo*. The resulting 5-methyl C is, in turn, preferentially involved in spontaneous mutation. Deamination of the 5-methyl C results in a C to T transition (22). In addition, some p53 mutations found in lung tumors of women nonsmokers, particularly those that occur at a non-CpG site, may be spontaneously induced by errors in DNA replication. Both G to A and A to G transitions have been shown to be produced by some polymerases during DNA replication *in vitro* (23). There may be other origins that can explain the formation of the transitions observed in women nonsmokers, including the induction by endogenously formed compounds (24), as well as the possible carcinogenic effect of estrogens, because the hormonal status of patients has been suggested to be a risk factor in lung cancer in women (8).

In summary, this study shows that the predominant types and spectra of p53 mutation in lung tumors are different between women smokers and nonsmokers, suggesting different origins and/or mechanisms of formation of mutation in these two groups. Host susceptibility factors such as metabolic enzymes responsible for activation and detoxification of specific chemicals or classes or chemicals, or the capacity for DNA repair in different individuals, may be important in the induction of mutations in nonsmokers. It is also possible that p53 mutations in nonsmokers are induced by a different exposure than tobacco smoke. Comparison of mutational spectra in male smokers and nonsmokers may also provide insight into gender-specific differences in lung cancer etiology.

References

1. Boring, C. C., Squires, T. S., and Tong, T. Cancer Statistics. CA Cancer J. Clin., *43:* 7–26, 1993.

2. United States Department of Health and Human Services. Strategies to control tobacco use in the United States. Tobacco Control Monogr., *1:* 1–307, 1991.

3. United States Surgeon General. Reducing the health consequences of smoking. Twenty-five years of progress. USPHS Pub. No. (CDC) 89–8411. Rockville, MD: Department of Health and Human Services, 1989.

4. Harris, R. E., Zang, E. R., Anderson, J. I., and Wynder, E. L. Race and sex differences in lung cancer risk associated with cigarette smoking. Int. J. Epidemiol., *22:* 592–599, 1993.

5. Kabat, G. C. Aspects of the epidemiology of lung cancer in smokers and nonsmokers in the United States. Lung Cancer, *15:* 1–20, 1996.

6. Fontham, E. T. H., Correa, P., Wu-Williams, A., Reynolds, P., Greenburg, R. S., Buffler, P. A., Chen, V. W., Boyd, P., Alterman, T., Austin, D. F., Liff, J., and Greenburg, S. D. Lung cancer in nonsmoking women: a multicenter case-control study. Cancer Epidemiol. Biomark. Prev., *1:* 35–43, 1991.

7. Mumford, J. L., He, X. Z., Chapman, R. S., Cao, S. R., Harris, D. B., Li, X. M., Xian, Y. L., Jiang, W. Z., Xu, C. W., Chuang, J. C., Wilson, W. E., and Cooke, M. Lung cancer and indoor air pollution in Xuan Wei, China. Science (Washington DC), *235:* 217–220, 1987.

8. Taioli, E., and Wynder, E. L. Endocrine factors and adenocarcinoma of the lung in women. J. Natl. Cancer Inst., *86:* 869–870, 1994.

9. Ernster, V. L. The epidemiology of lung cancer in women. Ann. Epidemiol., *4:* 102–110, 1994.

10. Rodenhuis, S., and Slebos, R. J. C. Clinical significance of *ras* oncogene activation in human lung cancer. Cancer Res., *52* (Suppl.)*:* 2665s–2669s, 1992.

11. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.

12. Keohavong, P., DeMichelle, M. A. A., Melacrinos, A. C., Landreneau, R. J., Weyant, R. J., and Siegfried, J. M. Detection of K-ras mutations in lung carcinomas: relationship to prognosis. Clin. Cancer Res., *2:* 441–418, 1996.

13. Siegfried, J. M., Gillespie, A. T., Mera, R., Casey, T. J., Keohavong, P., Testa, J. R., and Hunt, J. D. Prognostic value of specific K-ras mutations in lung adenocarcinomas. Cancer Epidemiol. Biomark. Prev., *6:* 841–847, 1997.

14. Hernandez-Boussard, T. M., and Hainaut, P. A specific spectrum of p53 mutations in lung cancer from smokers: review of mutations compiled in the IARC p53 database. Environ. Health Perspect., *106:* 385–391, 1998.

15. Orita, M., Iwahana, H., Kanazava, H., Hayashi, K., and Sekiya, T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformational polymorphisms. Proc. Natl. Acad. Sci. USA, *86:* 2766–2770, 1989.

16. Hainaut, P., Hernandez, T., Robinson, A., Rodriguez-Tome, P., Flores, T., Hollstein, M. Harris, C. C., and Montesano, R. IARC database of p53 mutations in human tumors and cell lines: updated compilation, revised formats and new visualization tool. Nucleic Acids Res., *26:* 205–213, 1998.

17. Smida, J., Zitzelsberger, H., Kellerer, A. M., Lehmann, L., Minkus, G., Negele, T., Spelberg, F., Hieber, L., Demidchik, E. P., Longfelder, E., and

Bauchinger, M. p53 mutations in childhood thyroid tumors from Belarus and in thyroid tumors without radiation history. Int. J. Cancer, *73:* 802–807, 1997.

18. Husgafvel-Pursiainen, K., Ridanpaa, M., Anttila, S., and Vainio, H. *p53* and *ras* gene mutations in lung cancer: implications for smoking and occupational exposure. J. Occup. Environ. Med., *37:* 69–76, 1995.

19. Kure, E. H., Ryberg, D., Hewer, A., Phillips, D. H., Skaug, V., Baera, R., and Haugen, A. p53 mutations in lung tumors: relationship to gender and lung DNA adducts levels. Carcinogenesis (Lond.), *17:* 2201–2205, 1996.

20. Husgafvel-Pursiainen, K., and Kannio, A. Cigarette smoking and p53 mutations in lung cancer and bladder cancer. Environ. Health Perspect., *104* (Suppl. 3)*:* 553–556, 1996.

21. Takeshima, Y., Seyama, T., Bennett, W. P., Akiyama, M., Tokuoka, S., Inai, K., Mabuchi, K., Land, C. E., and Harris, C. C. p53 mutations in lung cancers from non-smoking atomic bomb survivors. Lancet, *342:* 1520–1521, 1993.

22. Rideout, W. M., Coetzee, G. A., Olumi, A. F., and Jones, P. A. 5-methylcytosine as an endogenous mutagen in the human LDL receptor and *p53* gene. Science (Washington DC), *249:* 1288–1290, 1990.

23. Keohavong, P., and Thilly, W. G. Fidelity of DNA polymerases in DNA amplification. Proc. Natl. Acad. Sci. USA, *86:* 9253–9257, 1989.

24. Bartsch, H., Ohshima, H., Shuker, D. E. G., Pignatelli, B., and Calmels, S. Exposure of humans to endogenous N-nitoso compounds: implications in cancer etiology. Mutat. Res., *238:* 255–267, 1990.