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Distribution of *p53* and K-*ras* mutations in human lung cancer tissues

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Studies were performed to examine the mutational pattern of K-*ras* exons 1 and 2 and *p53* exons 5–8 in lung cancer tissues from 27 Chinese patients (10 smokers, 17 non-smokers) using single-stranded conformational polymorphism and DNA sequencing. K-*ras* mutations were found in 13/27 tumors (48%); all mutations were clustered in exon 1 and distributed between codons 9 and 32. The frequency and number of patients with K-*ras* mutations between smokers and non-smokers were not different, except that a high frequency of G → A transitions (11/11) was found in non-smokers. Among cell types, K-*ras* mutations were found in 7/13 (54%) squamous cell carcinoma (SC) and 5/12 (42%) adenocarcinoma (AC) patients. A → T transversions (all six transversions) were present only in SC. In *p53*, 18/27 (67%) tumors contained mutations in exons 7 and 8, frequently at codons 226, 270, 275 and 281. The number of tumors with *p53* mutations in smokers (70%) and in non-smokers (65%) was similar, and the mutation frequency did not differ except for a higher number of G → A (6/7) and T → C (5/6) transitions in non-smokers. Among cell types, the number of tumors with *p53* mutations was 9/13 (69%) in SC and 8/12 (67%) in AC. The A → G (11/16) transitions and A → C (4/4) transversions in *p53* were more frequent in SC than in AC ($P < 0.04$ for A → G; $P < 0.02$ for A → C). The varying mutation patterns in both the K-*ras* and *p53* genes between smokers and non-smokers and among cell types suggest that other than cigarette smoke, environmental and dietary factors may also be involved in the genesis of lung cancer among these patients.

Introduction

Lung cancer is one of the most prevalent cancers in the world. In China, lung cancer is the second most common type of cancer and the incidence is increasing each year (1). Concurrently, environmental air quality is deteriorating and the number of smokers has increased in recent years (2). Cigarette smoking has been shown to be a major source of exposure to a large number of chemical constituents, including initiators, promoters, complete carcinogens and co-carcinogens (3). Mutations in critical genes have been implicated in the development of cancers associated with cigarette smoke exposure (4). In particular, mutational activation of proto-oncogenes and/or inactivation of tumor suppressor genes are associated with carcinogenesis. The most frequent genetic changes found in lung cancer are point mutations in the K-*ras* proto-oncogene and in the *p53* tumor suppressor gene (5). The critical role of these alterations in carcinogenesis has been demonstrated in cell culture by co-transfection of mutated *p53* and activated *ras* proto-oncogenes into primary rat fibroblasts, leading to complete cellular transformation *in vitro* (6–8). Various studies have shown that chemical carcinogens can selectively induce specific base pair changes, such as those found in codons 12, 13 and 61 of K-*ras* and in codons 249 and 273 of the *p53* gene (9–11). Using an animal model, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK*), a carcinogen found in tobacco smoke, induced hamster lung tumors by repeated i.p. injections. Mutations in K-*ras* were found in 50% of the animals at codon 12, but no mutations were detected in the *p53* gene (12). In another study, 77–94% of tumors contained K-*ras* mutations, but only one tumor had a point mutation in the *p53* gene, indicating a distinctive mutation pattern in the K-*ras* gene (13). In molecular genetic studies, K-*ras* mutations, mostly G → T transversions in codon 12, have been found in 30–60% of adenocarcinoma (AC) and in 10% of squamous cell carcinoma (SC) from smokers (14–16). Mutations in the *p53* gene are widely distributed throughout exons 4–9 and the most prevalent mutations are G → T transversions found in tobacco-associated lung cancer (11,17–19). A mixture of highly mutagenic polycyclic aromatic hydrocarbons (PAH) found in tobacco smoke preferentially attack the guanine base. Strauss (20) suggested that the predominant G → T transversion occurs in DNA replication, either by a mispairing of the PAH-adducted guanine with adenine or by a preferential insertion of adenine opposite the non-instructive modified base. Therefore, analysis of patterns of mutations in these two genes may provide clues to the etiology and molecular pathogenesis of lung cancer.

In this study we examined the genetic alterations using single-stranded conformational polymorphism (SSCP) and automated DNA sequencing techniques to analyze point mutations in the K-*ras* and *p53* genes from lung cancer tissues. The association between exposure to cigarette smoke and mutations found in the K-*ras* and *p53* genes was assessed by comparing the mutational patterns between smokers and non-smokers among different tumor types.

Materials and methods

Lung cancer tissues

Fresh tumor samples were obtained from 27 lung cancer patients who, for other purposes, had undergone lobectomy or pneumonectomy in the hospitals of Guangzhou (Guangdong, People's Republic of China) during 1989–1991. The histological tumor types were determined according to WHO

***Abbreviations:** NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; AC, adenocarcinoma; SC, squamous cell carcinoma; PAH, polycyclic aromatic hydrocarbons; SSCP, single-stranded conformational polymorphism; SCC, small cell carcinoma; 4-ABP, 4-aminobiphenyl; CSC, cigarette smoke condensate.

classifications. The patient's smoking history was obtained from personal interviews and medical records. Smokers were classified as patients who in their lifetime smoked at least 1 cigarette/day continuously for 6 months.

DNA preparation

High molecular weight DNA was isolated and extracted from homogenized tumor tissues according to a standard protocol (21). The tissues were digested overnight at 37°C with proteinase K. DNA was isolated by phenol/chloroform extraction and ethanol precipitation, purified by RNase digestion and then resuspended in 1 ml TE buffer (10 mM Tris, 5 mM EDTA). The concentration and purity of DNA were determined by measuring the OD at 260 and 280 nm wavelength using a spectrophotometer.

Polymerase chain reaction

Oligonucleotide primers used for PCR applications were synthesized using a PS-250 DNA synthesizer (Cruachem, Herndon, VA). The sequences of the primers used were based on published information (22,23), as follows: *p53* exon 5, 5′ primer, 5′-TTCCTCTTCCTGCAGTAGTC-3′, 3′ primer, 5′-CTGGGGACCCTGGGCAA-3′; *p53* exon 6, 5′ primer, 5′-GAGACGACAG-GGCTGGGT-3′, 3′ primer, 5′-CCACTGACAACCACCCTT-3′; exon 7, 5′ primer, 5′-TGGCTCTGACTGTACCACCA-3′, 3′ primer, 5′-CAAGTGGCT-CCTGACCTGGA-3′; exon 8, 5′ primer, 5′-ATCCTGAGTAGTGGTAATCT-3′, 3′ primer, 5′-TACCTCGCTTAGTGCTCCCT-3′; K-*ras* codons 12 and 13, 5′ primer, 5′-ATGACTGAATATAAACTTGT-3′, 3′ primer, 5′-CTCTATTGT-TGGATCATATT-3′; codon 61, 5′ primer, 5′-GCAAGTAGTAATTGATGGAG-3′, 3′ primer, 5′-AGAAAGCCCTCCCCAGTCCT-3′. Specific DNA amplification of the *p53* and K-*ras* genes was performed as described by Saiki *et al*. (24). PCR conditions were modified as follows. The reaction mixture contained 100 ng chromosomal DNA, 30 pmol each primer, 0.1 mM deoxynucleotide triphosphates, 0.5 U Taq polymerase and reaction buffer (10 mM Tris−HCl, pH 8.3, 1.5 mM MgCl, 50 mM KCl, 0.001% w/v gelatin) in a total volume of 100 µl. The mixture was incubated for 2 min at 95°C in a thermal DNA cycler (Perkin Elmer Cetus, Norwalk, CT) to denature the DNA, then 40 amplification cycles were carried out as follows: 1 min denaturing at 95°C, 1 min annealing at 56–58°C and 2 min DNA extension at 72°C. The PCR products were checked in a 1% agarose gel using GelMarker-I (Research Genetics, Huntsville, AL) as a standard. PCR conditions for SSCP were the same as described above, except that in a total volume of 10 µl, 10 ng chromosomal DNA were incubated in a solution of reaction buffer, 3 pmol each primer, 0.01 mM deoxynucleotide triphosphate, 2.5 µCi [α-^{32}P]dATP (3000 Ci/mmol) and 0.1 U Taq polymerase.

Single-stranded conformational polymorphism analysis

SSCP was performed essentially as described by Orita *et al*. (25). PCR amplification products were diluted 1:1 in sequence loading buffer (Sigma, St Louis, MO), denatured at 95°C for 5 min and kept at 0°C. Aliquots of 4 µl of each PCR product were denatured and rapidly loaded into a non-denaturing MDE gel (GAD Baker, Phillipsburg, NJ). Electrophoresis was carried out at room temperature using 1 × Tris−borate−EDTA buffer at 8 W constant power for 8–24 h. Following electrophoresis, the gel was subjected to drying and autoradiographed using an intensifying screen and Dupont Reflectionfilm^{TM} at −80°C for 16–72 h.

DNA sequencing

PCR amplification products were cloned directly using the TA cloning method (26). Phagemid DNA was purified and gels prepared and electrophoresed according to the protocols described in the ABI 373A sequencer manual (Foster City, CA). Sequencing of cloned DNA was performed in both directions using the fluorescent-labeled dideoxynucleotide triphosphate terminator method with the Taq dideoxy terminator cycle sequencing kit (Applied Biosystems Inc.). Sequencing conditions were according to the manufacturer's instructions.

Statistical analysis

The χ^2 test was employed for data analyses. When expected values in the χ^2 test were lower than 5, Fisher's exact test was used.

Results

Information related to age, sex, smoking status and cancer types of the patients involved in the study is listed in Table I. Most of the patients were male (20/27) with an average age of 55.2 ± 11.29 years. The distribution of lung cancers based on the histological classifications was: 13 SC, 12 AC, 1 adenosquamous carcinoma and 1 small cell lung cancer (SCC). Control DNA was obtained from the normal lung tissue of a human embryo. Eight patients (62%) in the SC group and two patients (16%) in the AC group were current smokers.

Table I. Histological subtypes and smoking history of lung cancer patients studied for the presence of K-*ras* and *p53* gene mutations[a]

Patient ID no.	Sex	Age	Smoking
Squamous cell carcinoma			
1	F	42	NS
3	M	49	NS
5	M	55	SM
6	M	65	SM
8	M	65	SM
11	M	51	SM
12	M	55	SM
13	M	61	SM
16	M	62	SM
17	M	55	SM
19	M	66	NS
23	F	52	NS
26	M	62	NS
Adenosquamous cell carcinoma			
24	M	68	NS
Adenocarcinoma			
2	M	67	NS
4	M	40	SM
7	M	52	NS
9	M	49	NS
10	M	24	NS
14	M	52	SM
15	F	51	NS
20	F	58	NS
21	M	54	NS
22	M	58	NS
25	F	76	NS
27	F	58	NS
Small cell lung cancer			
18	F	36	NS

[a]ID no., identification number; F, female; M, male; NS, non-smoker; SM, smoker.

Point mutations in the K-*ras* proto-oncogene and/or *p53* tumor suppressor gene were first screened by PCR-SSCP. In K-*ras* exon 1 (including codons 12 and 13) and in *p53* exons 7 and 8, a shift of DNA fragments was observed in some cancer tissues, indicating the presence of mutations in these samples. However, this phenomenon was not observed in exon 2 of K-*ras* (including codon 61) and in *p53* exons 5 and 6. Cancer tissue DNAs harboring mutations identified by SSCP were cloned in a TA vector and sequenced to determine the exact nature of the mutations. All the mutations were confirmed by repeated experiments and sequencing from both directions. The K-*ras* and *p53* mutations observed in the cancer tissues are listed in Tables II and III respectively. As shown in Table II, tumors from 13 patients (48%) contained K-*ras* mutations. Based on the tumor types, K-*ras* mutations were found in 7/13 (54%) SC, 5/12 (42%) AC and in the single SCC. The pattern of K-*ras* mutations found in exon 1 is presented in Figure 1a. Most of the K-*ras* mutations contained a single base pair change, except codons 31 and 32 of SC and codon 15 of SCC, which contained two changes per codon. Within the codons, 15 and 16 were the most frequently mutated. The maximum number of mutations at any specific codon in K-*ras* was less than five. The number of tumors with mutations and mutation frequencies of the K-*ras* gene did not differ between smokers and non-smokers, except that a higher number of G → A transitions was observed in non-smokers (11/11) than in smokers (0/11) ($P < 0.01$) (Table IV). Among

Table II. K-*ras* gene mutations in lung cancer tissues from 13 squamous cell carcinoma, 12 adenocarcinoma and one small cell carcinoma

Codon[a]	Base change	Amino acid change	Patient ID no.[b]		
			SC[c]	AC[c]	SCC[c]
9	GTT → GTC	Val → Val	3		
12	GGT → TGT	Gly → Cys		4	
13	GGC → GTC	Gly → Val	3		
13	GGC → AGC	Gly → Ser		7	
14	GTA → ATA	Val → Ile	20		
15	GGC → ACC	Gly → Thr			18
15	GGC → AGC	Gly → Ser	19,20		
15	GGC → AGC	Gly → Ser		15,21	
16	AAG → AAA	Lys → Lys			18
16	AAG → AAA	Lys → Lys	19,20	7	
16	AAG → ATG	Lys → Met	3		
18	GCC → GTC	Gly → Val			18
25	ACA → TCA	Thr → Ser	8		
25	ACA → GCA	Thr → Ala		4	
26	AAT → TAT	Asn → Tyr	11		
27	CAT → CGT	His → Arg		2	
30	GAC → GTC	Asp → Val	23		
30	GAC → GCG	Asp → Ala	16		
31	GAA → TAT	Glu → Tyr	16		
32	TAT → ATA	Tyr → Ile	16		

[a]Codons 9–32 belong to exon 1 of the K-*ras* gene.
[b]Patient ID no. is as assigned in Table I.
[c]SC, squamous cell carcinoma; AC, adenocarcinoma; SCC, small cell carcinoma.

tumor types, SC contained a higher number (but not statistically significant) of K-*ras* mutations (66%) commpared with AC (21%). The SC also contained a higher number of A → T (6/6) transversions compared with AC (0/6) ($P < 0.01$). Different types of substitution mutations observed in exon 1 of the K-*ras* gene are summarized in Table IV.

Tumors from 18 patients (67%) contained *p53* mutations. The number of mutations and specific base changes at different codons in exons 7 and 8 are listed in Table III. Based on the tumor type, *p53* mutations were found in 9/13 (69%) SC, 8/12 (67%) AC and in the single SCC. Most of the cancer tissues were found to carry multiple mutations in the *p53* gene. Approximately 20% of the total mutations were a single base pair change in the third position of the codons which did not alter the amino acid (silent mutations) and most of them were Gly → Gly. Two mutations per codon were observed in two tissues: a GG → TT mutation at codon 248 in an AC and an AA → GG at codon 292 in a SC. The mutation pattern of *p53* is illustrated in Figure 1b and c. Codons 226, 270, 275 and 281 were the most frequently mutated sites. More than two mutations in codons 226, 245, 266, 281, 282, 298, 299 and 305 were at the third base of the codon, whereas codons 250, 270 and 275 contained first base mutations. Codons 247, 269, 281, 286, 290 and 292 contained more than one mutation at the second base. The maximum number of base pair substitutions at any specific codon were ⩽10 for each mutation type.

p53 gene mutational patterns for both smokers and non-smokers and for tumor types are summarized in Table IV. The number of tumors with *p53* mutations in smokers (70%) was similar to that of the non-smokers (65%) (Table III), and the mutation frequencies were not different between the two groups (Table IV). The most frequent base changes in the *p53* gene were G → T (29/110), A → G (16/110), C → G (12/110) and C → T (11/110). However, the mutational pattern did not differ between smokers and non-smokers and the distributions of transitions and transversions were similar in both groups. The mutational pattern of SC showed an increased number of A → G transitions (11/16) ($P < 0.04$) and A → C (4/4) transversions ($P < 0.02$) in the *p53* gene.

Discussion

Accumulated mutations in genes regulating cellular growth are associated with carcinogenesis. The causes of DNA damage include both endogenous factors which increase infidelity of DNA replication and exogenous factors such as chemical mutagens and radiation exposure. Various genotoxic compounds have been shown to selectively induce alterations in specific base pairs in genes that are related to cancer. The molecular basis for these changes is not yet fully understood, although several hypotheses have been suggested (20,27).

The proto-oncogenes of the *ras* family have provided the link between the action of carcinogens and the activation of proto-oncogenes, in which point mutations take place in the critical region required for intrinsic GTP hydolysis. Although the role of tobacco smoke exposure in the induction of lung neoplasms in humans has been convincingly demonstrated, the specific components responsible for the mutations have not been identified. Exposure to mixtures of potentially mutagenic chemicals, however, surprisingly induces only one of a series of possible *ras* mutations, i.e. a point mutation in codon 12 of K-*ras*, mostly a G → T transversion (28,29). However, in addition to codon 12, K-*ras* mutations at codons 13 and 61 have also been reported in some lung tumor tissues (30). This may indicate that a specific component of tobacco smoke is responsible for the mutations in codons 12, 13 and/or 61 of the K-*ras* proto-oncogene which are critical for carcinogenesis. Within codon 12 of K-*ras*, G → T transversion in position 1 is the most frequent mutation in lung AC (29). Interestingly, the results of this study show that all the mutations observed in K-*ras* were distributed between codons 9 and 32 of exon 1 and were most frequently in codon 15, principally a G → A transition. Even though the exact mechanism for this specificity is not known, it has been shown that mutations in this region of the gene are critical for the activity of the ras oncoprotein. The higher frequency of mutations in codon 15 observed in this study may be due to the exposure to different mutagenic agents or differences in the ethnic background (18). As reported elsewhere, many codons of exon 1 (Figure 1a) other than codon 12 may also be important in oncogenesis (31). In addition to codon 12, mutations observed in other codons in this study could be attributable to differences in the screening of mutations. Most of the previous studies were designed to detect K-*ras* mutations in codons 12, 13 and 61 only, since these codons are most frequently mutated in many tumor tissues. However, screening of the complete sequence for mutations is important for analysis of mutation pattern. Therefore, in this study we used PCR-SSCP to detect point mutations in the complete exon and all samples which showed a shift of DNA fragments were cloned and sequenced to determine the exact nature of the mutations. Thus the possibility of detecting all mutations in a gene is enhanced. However, further studies are required to validate their role, since the number of tumor samples studied is small and geographic and ethnic diversity have not been fully investigated.

Tobacco smoke contains a large number of carcinogens and tumor promoters (14,18,32). In lung cancer patients it has

Table III. Mutations in exons 7 and 8 of the *p53* gene in lung cancer tissues from 13 squamous cell carcinoma, 12 adenocarcinoma and one small cell carcinoma

Exon	Codon	Base change	Amino acid change	Patient ID no.[a]	
				SC[b]	AC[b]
7	226	GGC → GGT	Gly → Gly	3,5,8,11,12	2,7,10
7	229	TGT → TGC	Cys → Tyr	8	20
7	235	AAC → ACC	Asn → Thr		20
7	239	AAC → TAC	Asn → Tyr		20
7	240	AGT → AGC	Ser → Ser	8	
7	241	TCC → CCC	Ser → Pro	12	
7	242	TGC → TGG	Cys → Trp		20
7	245	GGC → GGG	Gly → Gly	5,11,12	4,7,9
7	246	ATG → ATT	Met → Ile		20
7	247	AAC → ACC	Asn → Thr	13	20
7	247	AAC → AGC	Asn → Thr		2
7	248	CGG → CTT	Arg → Leu	19	14
7	250	CCC → GCC	Pro → Ala	3,12,13	7,25
7	253	ACC → ATC	Thr → Met		20
7	259	GAC → GAA	Asp → Glu		20
7	263	AAT → GAT	Asn → Asp	13	
8	266	GGA → GGG	Gly → Gly	1,8	4,7
8	269	AGC → AAC	Ser → Asp	3	9
8	270	TTT → ATT	Phe → Ile	1,8,12	4,7,9,10,14
8	275	GCC → TCC	Ala → Ser	1,8,12,13,23	4,7,9,10,14
8	276	TGT → AGT	Cys → Ser		10
8	277	CCC → TCC	His → Ser	8	7
8	280	AGA → GGA	Arg → Gly	19	
8	281	GAC → GAA	Asp → Glu	1,11,12,13	7,14
8	281	GAC → GAA	Asp → Glu	18 (SCC)	
8	281	GAC → GGC	Asp → Gly	8	9
8	282	CGG → CGT	Arg → Arg		20
8	282	CGG → CGA	Arg → Arg	13	4,9
8	283	AAT → GAT	Asn → Asp	8	
8	286	GAA → GGA	Glu → Gly	13	7
8	289	CTC → CCC	Leu → Pro	3	7
8	290	CGC → CAC	Arg → His		20
8	290	CGC → CCC	Arg → His		25
8	290	CGC → CTC	Arg → Leu		4,7,9
8	291	AAG → CAG	Lys → Glu	13	
8	292	AAA → GGA	Lys → Gly		20
8	292	AAA → AGA	Lys → Arg	3	
8	292	AAA → AAG	Lys → Lys	8	
8	293	GGG → GGT	Gly → Gly		20
8	294	GAG → AAG	Glu → Lys	8	
8	297	GAG → GAT	Glu → Asp		20
8	298	GAG → GAT	Glu → Asp	3,5,8	
8	298	GAG → CAG	Glu → Glu		20
8	299	CTG → CTC	Leu → Leu	1,8	4,7
8	302	GGG → GCG	Gly → Ala	13	
8	305	AAG → AAT	Lys → Asn	1,11,13	7,9

[a]Patient ID no. is as assigned in Table I.
[b]SC, squamous cell carcinoma; AC, adenocarcinoma; SCC, small cell carcinoma.

been demonstrated that, when compared with non-smokers, smokers have higher frequencies of K-*ras* gene mutations (33). However, in our studies the K-*ras* mutations were not different between smokers and non-smokers. The only difference we observed was higher frequencies of G → A transitions in non-smokers (11/11) compared with smokers (0/11) ($P < 0.01$). The smoke-related factors, if any, involved in the specificity of A → T transversions are not clear. The carcinogen 4-aminobiphenyl (4-ABP) found in cigarette smoke predominantly complexes with guanine forming *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl. It has been shown that 4-ABP is weakly mutagenic and induces primarily G → T and A → T transversions in DNA (34,35). The modified base, O^6-methylguanine, an adduct derived from treatment with methylating agents, is read as an adenine base by DNA polymerases, thus leading to frequent generation of G → A transitions (36). The very high number of G → A transitions in non-smokers, compared with smokers, observed in our study suggests the presence of similar types of chemical compounds in the environment. However, the agent(s) responsible for the high frequency of G → A transitions (62%) in non-smokers is not known.

Consistent with previous reports (37–39), the mutation frequency of K-*ras* was higher in SC (66%) compared with AC (21%) ($P < 0.05$). This suggests that mutations either occur most frequently in the proximal portion of the lung or airway epithelial cells that are involved in the morphopathogenesis of SC. Mutation frequencies between smokers and non-smokers within the tumor types were similar. This could be due to the small number of tumor samples studied. The only difference observed between the cell types for base changes

Table IV. Spectra of base changes in exon 1 of K-*ras* and exons 7 and 8 of *p53* in human lung cancer tumors[a]

K-*ras*							*p53*						
Mutation		Smoking		Cancer type			Mutation		Smoking		Cancer type		
Type	*n*	SM	NS	SC	AC	SCC	Type	*n*	SM	NS	SC	AC	SCC
G → T	3	2	1	2	1	0	G → T	29	12	17	15	14	
G → A	11	0	11	5	4	2	G → A	7	1	6	5	2	
G → C	1	0	1	0	0	1	G → C	7	3	4	4	3	
C → T	1	0	1	0	0	1	C → T	11	5	6	6	5	
C → A	0	0	0	0	0	0	C → A	8	4	4	4	3	1
C → G	1	1	0	1	0	0	C → G	12	6	6	7	5	
A → G	2	1	1	1	1	0	A → G	16	5	11	11	5	
A → C	1	1	0	1	0	0	A → C	4	1	3	4	0	
A → T	6	4	2	6	0	0	A → T	1	0	1	1	0	
T → C	1	0	1	1	0	0	T → C	6	1	5	4	2	
T → A	2	2	0	2	0	0	T → A	9	4	5	3	6	
T → G	0	0	0	0	0	0	T → G	0	0	0	0	0	
Total	29	11	18	19	6	4		110	42	68	64	45	1

[a]SM, smokers; NS, non-smokers; SC, squamous cell carcinoma; AC, adenocarcinoma; SCC, small cell carcinoma.

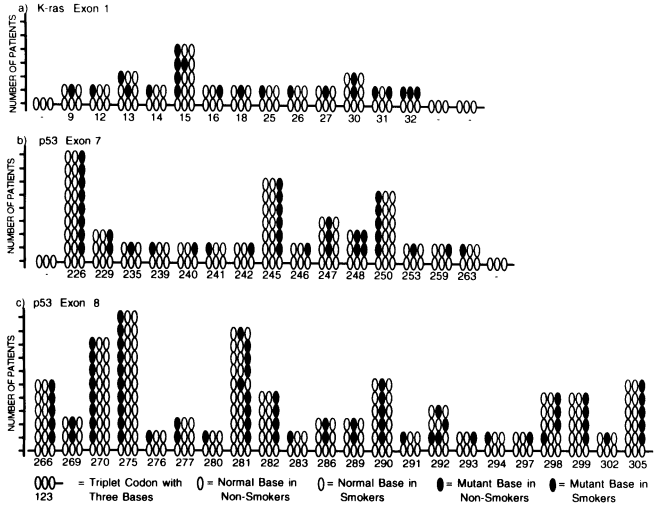

Fig. 1. The patterns of mutations in (**a**) exon 1 of the K-*ras* and (**b** and **c**) exons 7 and 8 of the *p53* genes from 27 lung cancer tissues. Each triplet codon which is located above the normal codon represents the mutated codon of one individual.

in K-*ras* was a higher frequency of A → T transversions in SC (all six of the A → T transversions).

Mutational analyses in exons 7 and 8 of the *p53* gene revealed that 18/27 tumors contained mutations. A large number of mutations were in the sequences determining the highly conserved domains of the protein reported to be hotspots for mutations (40). Recently many studies have reported preferential mutations in the *p53* gene with specific carcinogenic agents. G → T transversions at codon 249 have been demonstrated in hepatocellular carcinomas in geographic regions where aflatoxin is a known risk factor (41). Lung cancers contain a high percentage of G → T transversions, a mutational type known to be induced by activated benzo[*a*]pyrene metabolites, a constituent of cigarette smoke (42). In agreement with the above reports, our results also show a higher percentage of G → T (26% of total mutations) transversions and the presence of hypermutable codons. In addition to the G → T transversions, A → G (15%), C → G (11%) and C → T (10%) mutations were also observed in the *p53* gene. Interestingly, 30% of the mutations were silent mutations, and the majority of these were Gly → Gly (20%) concentrated in codons 226, 245, 266, 286 and 293. Even though silent mutations may not contribute to the process of mutagenesis, their frequency can be included for the analyses of mutation pattern.

Tobacco-associated *p53* mutations in lung cancer are widely distributed throughout exons 4–9, predominantly G → T transversions in smokers (19). In the *Salmonella* mutagenicity assay, cigarette smoke condensate (CSC) induced 80% G → T transversions at the base substitution allele (*his*G46) in strain TA100, suggesting the presence of a specific compound in cigarette smoke (43). As reported elsewhere (19,42), G → T transversion is the most commonly found mutation in the *p53* gene. In our study also, the predominant type of mutation found in *p53* was G → T transversions in both smokers and non-smokers compared with other types of base changes. Other types of base mutations appeared to be unaffected by smoking status.

The mutational pattern among tumor types for the *p53* gene showed no differences between the patients. Mutation frequencies between smokers and non-smokers within the tumor types were also similar. Cell type differences in *p53* mutations were limited to a higher number of A → G transitions (11/16) ($P < 0.04$) and A → C transversions (4/4) ($P < 0.02$). These results suggest that alterations in the *p53* gene induced by environmental mutagens may also lead to SC.

There is no conclusive evidence that the relative risk linking smoking to lung cancer is different for different histological types (44). However, some early reports (37) and studies in China (38) suggest that the relative risk is slightly lower for AC. From the perspective of pulmonary anatomy, it is possible that airborne mutagens can exert a carcinogenic effect upon different parts of the bronchial tree, usually causing SC or SCC if the proximal bronchial areas are affected and possibly AC if the distal parts are involved (39). Elevated mutational patterns observed in our studies for both the K-*ras* and *p53* genes in SC when compared with AC may reflect the higher probability of causing mutations in airway epithelial cells that produce SC. The general consensus is that mutations in tumor suppressor genes prime for the accumulation of mutations in oncogenes. Our results show that 83% of tumors carrying

mutations in the K-*ras* gene also contained *p53* mutations. It has also been demonstrated by others that mutations in both *p53* and K-*ras* are common events which often occur independently (33).

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