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Advances in Brief

Lung Tumor *KRAS* and *TP53* Mutations in Nonsmokers Reflect Exposure to PAH-Rich Coal Combustion Emissions[1]

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Abstract

We determined the *TP53* and codon 12 *KRAS* mutations in lung tumors from 24 nonsmokers whose tumors were associated with exposure to smoky coal. Among any tumors studied previously, these showed the highest percentage of mutations that (*a*) were G → T transversions at either *KRAS* (86%) or *TP53* (76%), (*b*) clustered at the G-rich codons 153–158 of *TP53* (33%), and (*c*) had 100% of the guanines of the G → T transversions on the nontranscribed strand. This mutation spectrum is consistent with an exposure to polycyclic aromatic hydrocarbons, which are the primary component of the smoky coal emissions. These results show that mutations in the *TP53* and *KRAS* genes can reflect a specific environmental exposure.

Introduction

A recent report (1) has argued that *TP53* mutations in smoking-associated lung tumors are not induced by mutagens in cigarette smoke but are preexisting mutations selected by physiological, nongenotoxic stress. This report has been noted in the medical press (2), and the validity of inferring environmental exposure from tumor gene mutations has been questioned (1, 3). Subsequent analysis by others (4) refutes this position. In this study, we report new data based on an exposure different from cigarette smoke showing that the mutation spectrum in a tumor can, in concert with selection, reflect the exposure linked epidemiologically to that tumor. We demonstrate this by identifying and comparing the *TP53* and *KRAS* mutation spectrum of lung tumors from nonsmokers exposed to polycyclic aromatic hydrocarbon-rich coal emissions to the mutation spectrum of smokers or unexposed nonsmokers.

Materials and Methods

Lung tumors from 24 nonsmoking women from Xuan Wei County, Yunnan Province, China were obtained embedded in paraffin. These women used smoky coal in their homes, which did not have chimneys. The mean age (± SD) of these women at time of surgery was 48.5 ± 8.8 years, ranging from 30 to 63, with a median age of 50. The percentage of p53-positive cells in the tumor and the intensity of staining were determined by the method described previously (5). *KRAS* mutations were determined by denaturing gradient gel electrophoresis by the method described previously (6). *P53* mutations were determined by performing a multiplex PCR for exons 4–9 and then sequencing the amplified exons in both directions by automated sequencing (7). All of the presumptive mutations were confirmed by repeated sequence analysis. χ^2 analyses were performed where appropriate.

Results and Discussion

Almost all (>99%) of the women in Xuan Wei County, Yunnan Province, China are nonsmokers (8), but they have the highest lung cancer mortality rate in China, 25.3/100,000, which is 8× the national average for females. Their cancers are associated with use of smoky coal, a low-sulfur (0.2%) medium-volatile bituminous coal used for cooking and heating in homes without chimneys (8, 9). These emissions contain 81% organic matter, of which 43% is PAH (10).[3] These PAHs account for 61% of the mutagenicity (10), whereas 58% of the mutagenicity of cigarette smoke is in the aromatic amine-rich basic fraction (11). Smoky coal emissions are ~1000× more carcinogenic than cigarette smoke in a murine skin-tumor assay, presumably because of their high concentration of PAHs (12).

Consequently, nonsmokers exposed to these emissions inhale 30× more benzo(*a*)pyrene that do smokers (13), and their urine has high levels of PAH metabolites (14) and 600× more benzo(*a*)pyrene-adducted guanine than does the urine of smokers (13). Smoky coal-associated lung cancer risk is associated with the *GSTM1*-null genotype, consistent with the ability of the GSTM1 enzyme to detoxify PAHs (9). Smoky coal exposure is associated with high levels of TP53 protein accumulation in exfoliated lung cells, suggesting that *TP53* mutation may be a critical feature of smoky coal-associated lung cancer (15). Considering all of the above, nonsmoking women in Xuan Wei with this extreme PAH exposure provided an ideal population to examine whether mutations in tumor genes reflect such an exposure.

Among the 24 women studied here, 54% had bronchioloalveolar adenocarcinoma and 46% had acinar adenocarcinoma (Table 1). Of the tumors, 29% were mutant at codon 12 of *KRAS*, with 86% of the mutations being G → T transversions. All of the 24 tumors stained positive for TP53 protein accumulation, and mutation analysis showed that 71% of the tumors contained base substitutions, with 17% having two *TP53* mutations, 21% having mutations in both *KRAS* and *TP53*, and only 21% having no detected mutations at either locus (Table 1).

The differing chemical compositions of smoky coal combustion emissions and cigarette smoke were reflected in their respective mutation spectra (Table 2). Of the *KRAS* mutations, 86% were G → T transversions in smoky coal-associated tumors, whereas only 66% were among smokers. Of the *TP53* mutations, 76% in smoky

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; NTS, nontranscribed strand.

Table 1 *Characteristics of lung tumors*

	Type[a]	*KRAS*[b]	TP53 staining[c]	*TP53* mutation[d]
1	BA	TGT	70/++	
2	BA	TGT	80/++	
3	BA	TGT	70/++	E5/154/GGC → GTC
4	BA	TGT	80/++	E5/154/GGC → GTC
5	AD		50/++	E5/158/CGC → CTC
6	AD		75/++	E5/175/CGC → CTC
				E8/280/AGA → ATA
7	AD		75/++	E8/273/CGT → CAT
8	BA		75/++	
9	BA		75/++	E7/249/AGG → ATG
10	AD	TGT	90/++	E8/272/GTG → TTG
11	BA		90/++	E5/156/CGC → CCC
12	AD		70/+	E7/249/AGG → ATG
13	AD		90/+++	E7/249/AGG → ATG
				E8/273/CGT → CTT
14	BA		90/++	E8/272/GTG → TTG
15	AD		50/++	E5/154/GGC → GTC
16	AD	GAT	50/++	E5/153/CCC → CCT
17	BA		75/++	E5/158/CGC → CTC
18	BA		50/++	E5/175/CGC → CTC
				E8/280/AGA → ATA
19	BA		50/++	
20	BA		10/+	E8/273/CGT → CTT
21	AD		50/+	
22	AD		10/++	
23	BA	GTT	20/+	E5/126/TAC → TGC
				E7/260/TCC → CCC
24	AD		50/++	

[a] BA, bronchioloalveolar adenocarcinoma; AD, acinar adenocarcinoma.
[b] Codon 12 wild-type sequence is GGT.
[c] Percentage of positive cells in the tumor/intensity of staining.
[d] Exon/codon/mutation. All were heterozygous except for the mutation in patient 16, which was homozygous. *TP53* exons 4–9 were amplified by a multiplex PCR; the products were sequenced by cycle-sequencing, and the sequence was resolved on an automated sequencer.

coal-associated tumors were G → T transversions, compared with only 29% or 11% among smokers or nonsmokers, respectively. χ^2 analysis for G → T transversions in smoky coal *versus* smokers, *versus* Asians, and *versus* nonsmokers gave *P*s of 0.09, 0.04, and 0.0003, respectively. The *TP53* database contained an insufficient number of Chinese female cigarette smokers to permit a comparison with this subgroup. Although exposure to smoky coal emissions is associated more with adenocarcinomas, and smoking is associated more with squamous-cell carcinomas (5), the high frequency of G → T transversions among smoky coal-exposed individuals was not attributable to tumor type because the frequency of such transversions in adenocarcinomas in the IARC *TP53* Mutation Database is only 29% (4). Furthermore, 33% of the mutations in the *TP53* gene from smoky coal-associated tumors cluster within the GC-rich region of codons 153–158, whereas only ~9% of the mutations in smokers or nonsmokers (and only 3% in nonsmoking-related tumors) are in this region. This site specificity and high frequency of G → T transversions are consistent with high PAH exposure and the fact that most PAHs produce DNA damage primarily at guanines (17).

Smoky coal-associated *TP53* mutations also exhibit an extreme strand bias, such that 100% of the guanines of the G → T transversions are on the NTS (Table 2). This suggests the presence of exogenous damage on the NTS that is not repaired by transcription-coupled repair. On the other hand, C → T transitions at CpG sites are considered to result from an endogenous mutational mechanism, and their frequency is only ~10% in tumors from smoky coal-exposed subjects and smokers (Table 2). Thus, most mutations in smoky coal-exposed individuals and smokers appear to result from unrepaired, exogenous damage on the NTS rather than resulting from DNA damage that is endogenous in origin.

The three mutational hot spots in the tumors associated with exposure to smoky coal emissions coincide with a hot spot for PAH adducts (codon 154), a hot spot for cigarette smoke-associated mutations (codon 249), and a hot spot for both events (codon 273; Table 3). Although bearing some similarity to the cigarette smoke-associated tumor spectrum (codons 249 and 273), the smoky coal mutation spectrum is also distinctly different, having a cluster of mutations at codon 154, which is not a hot spot for cigarette smoke-associated mutations.

Along with studies on environmental tobacco smoke (18, 19) and radon (20), this study on smoky coal presents a unique mutation

Table 3 *Hot spots in TP53 of PAH adducts and mutations associated with exposure to cigarette smoke or smoky coal emissions*

		Hot spots[a]		
Exon	Codon	PAH adducts	Cigarette mutations	Smoky coal mutations
5	154	+		+
	156	+		
	157	+	+	
	158	+	+	
	159	+		
7	237	+		
	245	+	+	
	248	+	+	
	249		+	+
8	273	+	+	+

[a] References for PAH adducts and cigarette smoke mutations are 17 and 4, respectively. The smoky coal mutation spectrum had four codons with one mutation, four codons with two mutations, and three codons with three mutations. We identified hot spots as those three codons that each contained three mutations.

Table 2 *Mutational characteristics of lung tumors*

	KRAS codon 12 (%)[a]			*TP53* (%)[b]					
Type	No. of subjects	Positive	G → T	No. of mutations	G → T	G → T on NTS	C → T at CpG	C → T at CpG on NTS	Codons 153–158
Lung									
All smokers	995	238 (23.9)	(65.7)[c,d]	343	101 (29.1)[d]	85 (85)[e]	39 (11.3)[d]	17 (43.5)[f]	32 (9.3)[d]
All nonsmokers	122	8 (6.6)	[g]	92	10 (10.9)	7 (70)	20 (21.7)	12 (60)	8 (9.4)
Smoky coal	24	7 (29.2)	6 (85.7)	21	16 (76.2)	16 (100)	2 (9.5)	1 (50)	7 (33.3)
Asians (S and NS)[h]	747	92 (12.3)	41 (44.6)	513	129 (25.1)	111 (86)	69 (13.4)	37 (53.6)	41 (8)
NTR[i]	[g]	[g]	[g]	2941	266 (9)	198 (74.4)	915 (31.1)	423 (46.2)	82 (2.8)

[a] Data other than smoky coal from Refs. 6 and 16.
[b] Data other than smoky coal from IARC *TP53* Mutation Database, DataR4 version (April 2000), available at http://www.iarc.fr/TP53. Data from cell lines, metastases, and individuals exposed to any carcinogen other than tobacco smoke were excluded. The studies by Gao *et al.* (21) and Fujimoto *et al.* (22) were also excluded.
[c] Based on 65 of 99 because the type of mutation (*e.g.*, G → T) was not always stated in the literature.
[d] Percentage of all of the mutations.
[e] Percentage of G → T transversions on the NTS.
[f] Percentage of all of the C → T transitions at CpG sites.
[g] Insufficient data to permit a calculation.
[h] Asians from Taiwan, Japan, and Hong Kong; smokers and nonsmokers.
[i] NTR, non-tobacco-related tumors (breast, brain, colon).

spectrum in lung tumors from nonsmokers whose cancers are linked epidemiologically to a well-characterized exposure. As such, our results and other analyses (4) support the view that mutation spectra in tumor genes reflect, in concert with selection, the primary DNA damage induced by mutagenic exposures linked epidemiologically to those tumors.

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