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Advances in Brief

Chromosomal Alterations in Lung Adenocarcinoma from Smokers and Nonsmokers[1]

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Abstract

The etiology of lung tumors arising in nonsmokers remains unclear. Although mutations in the *K-ras* and *p53* genes have been reported to be significantly higher in smoking-related lung carcinomas, in the present study we performed a more comprehensive analysis in search of additional genetic changes between lung adenocarcinoma from tobacco- and non-tobacco-exposed patients. We selected a matched cohort of 18 lifetime nonsmoking and 27 smoking patients diagnosed with primary adenocarcinoma of the lung and searched for chromosomal alterations in each tumor by testing normal and tumor tissue with 54 highly polymorphic microsatellite markers located on 28 different chromosomal arms. Allelic losses or gains at chromosomal arms 3p (37 *versus* 6%), 6q (46 *versus* 12%), 9p (65 *versus* 22%), 16p (28 *versus* 0%), 17p (45 *versus* 11%), and 19p (58 *versus* 16%) were present significantly more often in adenocarcinomas from smokers than from nonsmokers. Chromosomal arms showing allelic imbalance in lung tumors from nonsmokers were rare but occurred more often at 19q (22%), 12p (22%), and 9p (22%). The FAL (fractional allelic loss or gain) is defined as the percentage of chromosomal arm losses/gains among the total informative chromosomal arms. Tumors from smokers harbored higher levels of FAL (13 (48%) of 27 showed FAL ≥ 0.3) compared with the lung tumors from the nonsmoker patients (2 (11%) of 18 showed FAL ≥ 0.3; $P = 0.02$; odds ratio, 0.13; 95% confidence interval, 0.01–0.79). Our data demonstrate that widespread chromosomal abnormalities are frequent in lung adenocarcinoma from smokers, whereas these abnormalities are infrequent in such tumors arising in nonsmokers. These observations support the notion that lung cancers in nonsmokers arise through genetic alterations distinct from the common events observed in tumors from smokers.

Introduction

Lung cancer is the most common cause of cancer death in the world, and tobacco has been established as the main etiological agent. The risk of lung cancer increases with the duration of smoking and the number of cigarettes smoked daily. However, ~10% of lung tumors arise in individuals without a history of tobacco use (1). Other carcinogens such as radon and asbestos may contribute to some cases of lung cancer in nonsmokers (2), but the causes of lung cancer in the majority of nonsmokers remain unknown. The most common histological subtypes of non-small cell lung cancer are SqCC[3] and AD. Although AD has been the predominant lung cancer subtype diagnosed in nonsmokers (1), a steady increase in the incidence of AD of the lung among smokers has also been observed. These increases in incidence are though to be related to changes in smoking habits and in cigarette design (3). Specific genetic abnormalities have been identified in lung tumors from smokers, and they differ between the two main histological subtypes. *K-ras* mutations are more common in AD compared with SqCC, whereas *p53* mutations appear to be more frequent in SqCC (4, 5). Moreover, *p53* and *K-ras* gene mutations in lung cancer are predominantly the result of G to T transversions, attesting to the consequences of BaPDE-guanine and other DNA adducts found to be present in the lungs of cigarette smokers (6). Furthermore, higher rates of LOH and allelic gains at many loci have been found in SqCC compared with AD (7, 8).

Most genetic studies in carcinogenesis from nonsmokers have focused on *K-ras* and *p53* gene mutations. Compared with tumors from smokers, lung cancer in nonsmokers shows a very low rate of mutations at these genes (5, 9–13).[4] However, there are few limited reports identifying chromosomal alterations in lung cancers from nonsmokers. To better understand the genetic mechanisms underlying lung tumorigenesis in the absence of tobacco exposure, we compared chromosomal abnormalities in lung ADs from nonsmokers with a matched cohort of smokers using highly polymorphic microsatellite markers.

Materials and Methods

Patients. Primary lung tumors and matching normal lymphocytes were collected from 45 patients diagnosed with lung AD between 1995 and 1999. Thirty-nine of the patients were diagnosed and treated at the Johns Hopkins University School of Medicine. Two of the nonsmokers were from the Department of Surgery in the Medical College of Wisconsin (Milwaukee, WI) and the other four nonsmokers were diagnosed at the Hospital Universitari Germans Trias I Pujol in Badalona (Barcelona, Spain). Demographic data were collected from patient interviews, review of hospital charts, and review of each institution tumor registry. Subjects were categorized according to smoking status as follows: (*a*) nonsmokers ($n = 18$) were defined as those patients who had smoked <100 cigarettes during their lifetime (14); and (b) smokers ($n = 27$) were current or former smokers who had at least a 10-pack/year(s) history of cigarette smoking (pack-year(s) = number of packs of cigarettes smoked per day multiplied by the number of years of smoking). All of the patients categorized as nonsmokers were carefully reinterviewed to assure their nonsmoking status. Patients with a history of a second primary AD at a different site underwent careful pathological review to exclude lung metastasis from a distant site. Cases in which the possibility of a metastasis could not be excluded were not included in this study. The patients included in the group of smokers were selected from a larger group of smokers with *p53*, *K-ras*, and

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[3] The abbreviations used are: SqCC, squamous cell carcinoma; AD, adenocarcinoma; LOH, loss of heterozygosity; AAH, atypical adenomatous hyperplasia; FAL, fractional allelic loss or gain; OR, odds ratio; CI, confidence interval; BAP, benzo[*a*]pyrene; BPDE, BaP diol epoxide.

[4] S. A. Arhdent, P. A. Deckers, E. A. Alawi, Y. Zhu, M. Sanchez-Cespedes, S. C. Yang, G. B. Haasler, A. A. Balla, M. J. Demeure, J. Jen, and D. Sidransky. Cigarette smoking is strongly associated with mutations of the K-*ras* gene in primary AD of the lung, submitted for publication.

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p16 genetic data available in all of the tumors to closely match the clinical and pathological data characteristic of the nonsmokers. Statistically significant differences in age, tumor type, and pathological stage were not present among the nonsmokers and the smokers.

Microdissection and DNA Extraction. All but four of the tumors (see above) were obtained from fresh-frozen tissue. Representative sections from tissue used for DNA extraction were stained with H&E. Fresh-frozen tissue was meticulously dissected on a cryostat to ensure that the specimen contained at least 75% tumor cells. Approximately 35 12-μm sections were then collected and placed in 1% SDS/proteinase K (0.5 mg/ml) at 58°C for 24 h. The four samples from the Hospital Germans Trias i Pujol were from paraffin-embedded tissue that were deparaffinized with xylene. Digested tissue was then subjected to phenol-chloroform extraction and ethanol precipitation. Normal, control DNA was obtained by veinpuncture, and isolation of lymphocyte DNA was as described previously (15).

Polymorphic DNA Markers and LOH/Allelic Gain Analysis. Fifty-four microsatellite markers located in 28 different chromosomal arms were obtained from Research Genetics (Huntsville, AL). The name and the chromosome location for each of the markers are listed in Table 2. The vast majority of chromosomal arms had at least 90% informativity. Before amplification, 200 ng of one primer from each pair was end labeled with [γ-^{32}P]ATP (Amersham Life Science, Inc., Arlington Heights, IL) and bacteriophage T4 kinase (New England Biolabs, Inc., Beverly, MA) in a total volume of 50 μl. PCR reactions were carried out in a total volume of 10 μl containing 20 ng of genomic DNA, 2 ng of labeled primer, and 60 ng of each unlabeled primer. The PCR buffer included 16.6 mM ammonium sulfate, 67 mM Tris (pH 8.8), 6.7 mM magnesium chloride, 10 mM β-mercaptoethanol, and 1% DMSO, to which 1.5 mM deoxynucleotide triphosphates and 1.0 U of *Taq* DNA polymerase were added (Boehringer Mannheim Biochemicals, Indianapolis, IN). PCR amplifications of each primer set were performed for 35 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 60 s, and extension at 72°C for 60 s. One-third of the PCR product was separated on 8% urea-formamide-polyacrylamide gels and exposed to X-ray film for 4–48 h. Cases were considered informative if two alleles were present in the normal sample. For informative cases, LOH/allelic gains were scored if one allele was decreased by greater than 40% in tumor DNA when compared with the same allele in normal control DNA (16).

Gene Mutations. The data on gene alterations for *K-ras, p53*, and *p16* genes were collected from our previously published work (13, 17–19) and one report submitted for publication.[4]

Statistical Analyses. Frequencies were summarized in contingency tables, and proportions were compared using Fisher's exact test (2 × 2 tables) or Pearson's χ^2 with exact calculations of *P*s (2× *k* tables). The association between FAL index and clinical, pathological, and genetic features of smokers with lung AD was summarized using odd ratios and exact CIs and *P*s. All of the calculations were performed using statxact-4 software.

Results

We tested 54 microsatellite markers located on 28 different chromosomal arms to search for chromosomal alterations in the lung tumors from our group of smoking ($n = 27$) and nonsmoking ($n = 18$) patients. Representative examples of the results are shown in Fig. 1*A*. Allelic losses or gains were most frequent on chromosomal arms 9p (65%), 19p (58%), 6q (46%), 17p (45%), 8p (41%), 19q (38%), 12p (37%), 3p (37%), 13q (36%), and 5q (30%) in the lung ADs from smokers (chromosomal changes present in more than 30% of the tumor samples; Table 1 and Fig. 1*B*). Strikingly, we observed chromosomal alterations infrequently in nonsmokers, and 12p (22%), 19q (22%) and 9p (22%) were the regions most frequently altered. We compared the lung tumors from smokers and nonsmokers for frequency of LOH/allelic gains at all of the loci evaluated. The frequency of chromosomal alterations was significantly higher in the tumors from smokers compared with nonsmokers on chromosomes 3p ($P = 0.03$), 6q ($P = 0.04$), 9p ($P = 0.007$), 16p ($P = 0.03$), 17p ($P = 0.03$), and 19p ($P = 0.006$). Nine tumors had no chromosomal alterations at any of the markers analyzed; seven (39%) of these tumors were from nonsmokers, and only two (7%) were smokers

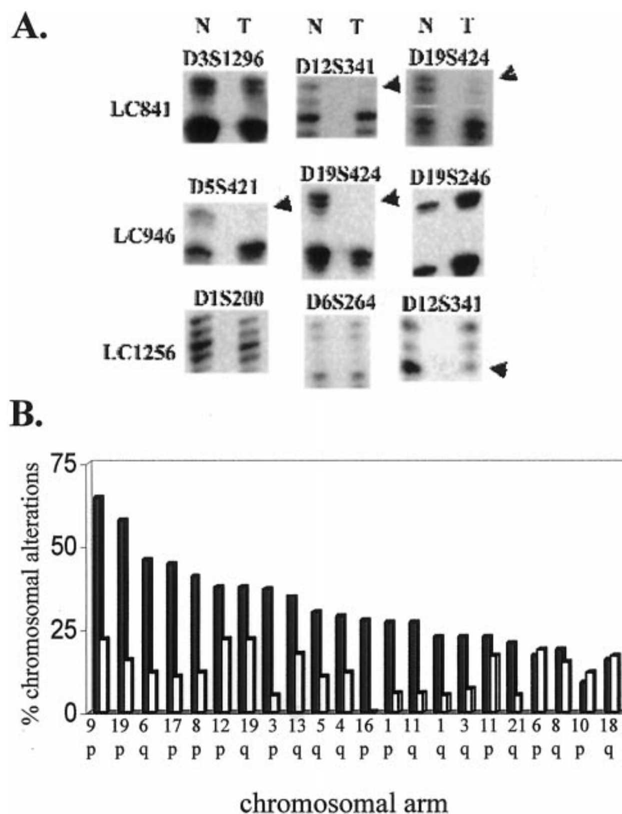

Fig. 1. *A*, representative examples for LOH/allelic gains in non-small cell lung cancer. *On the left*, sample number; *above each autoradiograph*, microsatellite marker; *arrowheads*, the allelic losses or gains; *N*, normal DNA; *T*, tumor DNA. *LC841* and *LC946*, lung tumors from smokers; *LC1256*, tumor from a nonsmoker. *B*, comparison of allelotypes between smokers and nonsmokers. The *bars* indicate the frequency of chromosomal alterations at each chromosomal arm; *black bars*, frequency for the smokers; *white bars*, frequency for the nonsmokers.

($P = 0.02$; Fisher's Exact Test). No apparent significant differences were found among the nonsmoker lung ADs based on country origin (Spain *versus* United States).

The frequency of LOH and allelic gains identified by microsatellite analysis is a good indicator of chromosomal instability. Aneuploidy attributable to the gain or loss of entire chromosomes can be detected as LOH/allelic imbalance in every informative marker for the same chromosome, whereas partial chromosomal losses or gains are represented as a loss of only a portion of the informative markers of a single chromosome. FAL (defined as the number of chromosomal arms showing LOH and/or allelic gains at least at one of the markers divided by the number of informative chromosomal arms) was calculated for each tumor. The median FAL in the group of smokers was 0.28, whereas in the nonsmokers the FAL was 0.11 (Fig. 2). Because 0.3 is just above the calculated 0.28 median of FAL in smokers, we used it as a cutoff to define patients with high and low number of chromosomal abnormalities. Tumors from 13 (48%) of 21 smokers harbored higher FAL levels (FAL $\geq$ 0.30) compared with only 2 (11%) of 18 lung tumors from nonsmokers ($P = 0.01$; OR, 0.13; CI, 0.01–0.79). As expected**,** in those tobacco-related tumors with high FAL, we observed a greater frequency of LOH and allelic gains in almost every chromosomal arm compared with the low FAL tumors (changes in 3p, 3q, 8p, 9p, 13q, 17q, 18q, and 19p were statistically significant). Chromosomes 6q, 4q and 12p showed a very similar frequency of abnormalities in the two groups of smokers (Table 2).

We also compared several clinicopathological and genetic features of the primary tumors from smokers with the FAL data (Table 3). The distribution of patients according to the FAL index was very similar

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Table 1 *Microsatellite markers used in the chromosomal instability assessment and frequency of LOH or allelic gains (AGs) in smoking- and nonsmoking-related lung tumors*

Chrom.	Marker	Smokers LOH-AG/I[a] (%)	Nonsmokers LOH-AG/I (%)	*P*
1p13	D1S252	4/22 (18)	1/13 (8)	
1p35-34	D1S200	4/23 (17)	1/18 (6)	
1p		**6/26 (23)**	**1/18 (6)**	**0.2**
1q24-31	D1S158	5/25 (20)	1/17 (6)	
1q32-44	D1S103	6/19 (32)	1 /9 (11)	
1q		**7/26 (27)**	**1/18 (6)**	**0.1**
2q32	D2S202	1/23 (4)	1/13 (8)	
2q33-37	D2S143	3/24 (12.5)	1/18 (6)	
2q		**3/27 (11)**	**1/18 (6)**	**0.6**
3p12	D3S1284	5/18 (28)	1/14 (7)	
3p14	D3S1296	7/19 (37)	1/10 (10)	
3p14	D3S1300	5/14 (36)	1/10 (10)	
3p21	D3S1578	8/25 (32)	1/16 (6)	
3p21	D3S1067	7/19 (37)	0/14 (0)	
3p		**10/27 (37)**	**1/18 (5)**	**0.03**
3q25	D3S1268	**5/21 (24)**	**1/15 (7)**	**0.4**
4p15	D4S1608	2/11 (18)	1 /9 (11)	
4p16-11	D4S230	2/20 (10)	0/13 (0)	
4p		**4/23 (17)**	**1/16 (6)**	**0.6**
4q33	D4S171	7/17 (41)	1/13 (8)	
4q35	D4S426	3/20 (15)	1 /9 (11)	
4q		**7/24 (29)**	**2/16 (12.5)**	**0.4**
5p13	D5S455	**2/17 (12)**	**3/18 (17)**	**1.0**
5q21	D5S421	8/25 (32)	0/13 (0)	
5q21	D5S346	5/19 (26)	1/14 (7)	
5q34-35	D5S429	3/23 (13)	1/14 (7)	
5q		**8/27 (30)**	**2/18 (11)**	**0.2**
6p24-25	D6S470	1/19 (5)	2/12 (17)	
6p21-22	D6S105	3/16 (19)	1/12 (8)	
6p		**4/23 (17)**	**3/16 (19)**	**1.0**
6q25	D6S255	7/21 (33)	1/13 (8)	
6q25-27	D6S264	9/18 (50)	2/12 (17)	
6q		**11/24 (46)**	**2/17 (12)**	**0.04**
8p21-22	D8S549	8/16 (50)	1 /8 (12.5)	
8p22	D8S261	4/18 (22)	2/16 (12.5)	
8p		**9/22 (41)**	**2/16 (12.5)**	**0.08**
8q11-12	D8S285	**4/21 (19)**	**2/13 (15)**	**1.0**
9p21	D9S171	15/24 (62.5)	4/18 (22)	
9p21	IFNA	12/21 (57)	3/17 (18)	
9p		**17/26 (65)**	**4/18 (22)**	**0.007**
10q22-24	D10S185	2/22 (9)	2/11 (18)	
10q23-25	D10S254	0 /4 (0)	1/12 (8)	
10q		**2/23 (9)**	**2/17 (12)**	**1.0**
11p12-13	D11S905	4/14 (29)	3/11 (27)	
11p15.5	D11S861	5/19 (26)	2/18 (11)	
11p		**5/22 (23)**	**3/18 (17)**	**0.7**
11q23-24	D11S934	4/23 (17)	1/12 (8)	
11q23-25	D11S912	4/22 (18)	1/15 (7)	
11q		**7/26 (27)**	**1/17 (6)**	**0.1**
12p12-13	D12S77	7/23 (30)	2/11 (18)	
12pter	D12S341	8/20 (40)	3/17 (18)	
12p		**10/27 (37)**	**4/18 (22)**	**0.3**
13q14.3	D13S135	9/20 (45)	3/17 (18)	
13q34	D13S1315	3/18 (17)	1 /7 (14)	
13q		**9/25 (36)**	**3/17 (18)**	**0.3**
16p12.3	D16S403	6/21 (29)	0 /4 (0)	
16p12.3	D16S412	4/16 (25)	0 /8 (0)	
16p		**7/25 (28)**	**0/16 (0)**	**0.03**
17p13.1	TP53	**9/20 (45)**	**2/18 (11)**	**0.03**
17qter	D17S579	3/18 (17)	1/17 (6)	
17qter	D17S789	2/19 (11)	0/16 (0)	
17q		**4/26 (15)**	**1/17 (6)**	**0.6**
18q21	D18S51	3/21 (14)	3/14 (21)	
18q23	D18S70	2/23 (9)	1/17 (6)	
18q		**4/25 (16)**	**3/18 (17)**	**1.0**
19p13	D19S424	15/25 (60)	3/18 (17)	
19p13	D19S411	9/18 (50)	0/12 (0)	
19p		**15/26 (58)**	**3/19 (16)**	**0.006**
19q13.3	**D19S246**	**10/26 (38)**	**4/18 (22)**	**0.3**
21q21	D21S11	5/22 (23)	0/15 (0)	
21q21	D21S417	4/21 (19)	1/18 (6)	
21q		**5/24 (21)**	**1/18 (6)**	**0.2**
22q13	D22S282	1/27 (4)	1/11 (9)	
22q13	D22S928	4/20 (20)	1/12 (8)	
22q		**5/27 (19)**	**1/16 (6)**	**0.4**
Xq21-22	DXS990	**0/10 (0)**	**0/11 (0)**	

[a] I, number of patients that were informative for every marker tested.

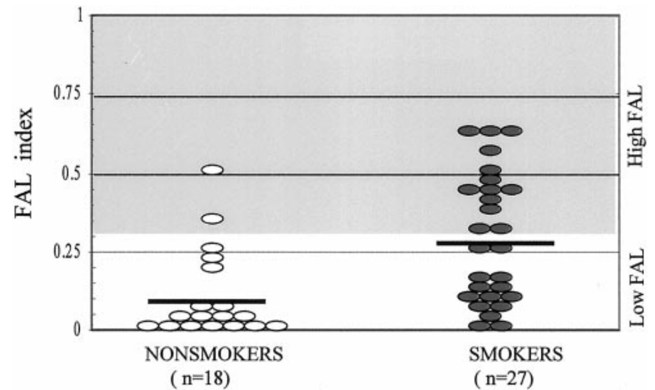

Fig. 2. FAL distribution for nonsmoking- and smoking-derived lung cancer. *Bars*, the mean for each group of subjects (the mean FAL is 0.11 for nonsmokers and 0.28 for smokers). The smokers were also subdivided into two groups: *High FAL*, tumors with FAL higher than 0.3; *Low FAL*, tumors with FAL lower than 0.3.

Table 2 *Comparison between the frequency of LOH and allelic imbalances in the two groups of lung adenocarcinomas from smokers*

Chromosomal arm	High FAL (n = 13)	Low FAL (n = 14)	*P*
9p	11/12 (92%)	6/14 (43%)	0.01
19p	10/12 (83%)	5/14 (36%)	0.02
17p	8/11 (73%)	1/10 (10%)	0.008
13q	8/12 (67%)	1/14 (7 %)	0.003
8p	7/11 (64%)	2/12 (17%)	0.04
3p	8/13 (62%)	2/14 (14%)	0.02
19q	7/12 (58%)	3/14 (21%)	0.1
6q	6/12 (50%)	5/12 (42%)	1.0
12p	6/13 (46%)	4/14 (29%)	0.4
5q	6/13 (46%)	2/14 (14%)	0.1
16p	5/11 (45%)	2/13 (15%)	0.2
1p	5/12 (42%)	1/14 (7 %)	0.07
3q	5/12 (42%)	0/11 (0 %)	0.04
11p	4/10 (40%)	1/13 (8 %)	0.1
18q	4/11 (36%)	0/14 (0 %)	0.03
6p	4/12 (33%)	0/12 (0 %)	0.09
4q	4/13 (31%)	3/11 (27%)	1.0

between the different AD subtypes (bronchoalveolar *versus* non-bronchoalveolar), sex and age of the patients, and the different stages of the disease. Moreover, there was no correlation between the quantity of cigarettes smoked during the lifetime and the FAL index. Interestingly, *p53* gene mutations were detected more frequently in tumors with high FAL [7 (54%) of 13] compared with tumors with low FAL [3 (21%) of 14; P = 0.2; OR, 4.3; 95% CI, 0.63–33.8). However, *K-ras* gene mutations were more common in tumors with a low FAL [8 (57%) of 14] compared with those with a high FAL [4 (31%) of 13; P = 0.3; OR, 0.33; 95% CI, 0.05–2.1]. Finally, *p16* gene inactivation was distributed similarly between both groups of lung tumors.

From these observations, we suggest a preliminary division of lung ADs into three main groups: Group Ia, tobacco-associated lung AD with a high rate of chromosomal abnormalities (high FAL). This group includes about 50% of lung ADs in patients with a history of tobacco exposure and displays frequent *p53* gene alterations; Group Ib, tobacco-associated lung AD with a low rate of chromosomal alterations (low FAL), representing the other half of the lung ADs arising in smokers. These tumors show a high frequency of *K-ras* mutations; Group II, non-tobacco associated lung ADs with a very low level of chromosomal abnormalities and rare *p53* or *K-ras* mutations. All three of the groups harbor frequent *p16* gene inactivation (Fig. 3).

Discussion

We have characterized the chromosomal abnormalities present in lung AD by microsatellite analysis in smokers and nonsmokers.

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Table 3 *Correlation between the FAL index and the different clinical, pathological, and genetic characteristics in smokers with AD of the lung*

Characteristics	FAL <0.3	FAL ≥0.3	*P*	OR	95% exact CI
Age					
<55 yr	2	1	1	0.5	0.008–11.1
≥55 yr	12	12			
Pack/yr[a]					
≤25	3	3	1	1	0.10–9.6
>25	9	9			
Sex					
Female	9	7	0.9	0.65	0.11–0.39
Male	5	6			
Stage					
I–II	14	12	0.04	0	0–0.93
III–IV	0	2			
Histology					
AD	10	11	0.7	2.2	0.24–28.6
bAD[b]	4	2			
p53 status					
Mutant	3	7	0.2	4.3	0.63–33.8
Wild type	11	6			
K-ras status					
Mutant	8	4	0.3	0.33	0.05–2.1
Wild type	6	9			
p16 status					
Altered	6	6	1	1.1	0.19–6.7
Wild type	8	7			

[a] Pack-year not available from three patients.
[b] bAD, bronchoalveolar AD.

Previous reports generating lung cancer allelotypes have mostly compared the two main histological types, AD and SqCC (7, 20). The most frequently altered loci identified in the present study are in general agreement with previously published studies on AD (7, 20, 21). LOH on chromosomes 9p and 17p likely target the *p16* and *p53* genes, respectively. However, putative genes targeted by other chromosomal alterations remain to be identified. The high frequency of alterations at chromosome 19p is of special interest. We found chromosome 19 abnormalities in over one-half of the lung ADs from smoking patients. Recently, Virmani *et al.* (21) observed that 86% of lung tumors showed LOH at chromosome 19p using lung cancer cell lines from primary tumors. Our observations in primary tumors further point to the existence of an important tumor suppressor gene located on chromosome 19p.

The comparison of allelotypes between the tobacco- and non-tobacco-related tumors revealed that lung ADs from nonsmokers harbored few chromosome abnormalities. No areas of LOH or allelic gains at any chromosomal arm were present in more than 25% of the tumors. Although other investigations have previously shown that LOH at 3p and 9p may be less common in lung tumors from nonsmokers (7, 22, 23), our work is the first to look at almost every chromosomal arm in lung ADs from nonsmokers. The analysis of individual chromosomal abnormalities demonstrated that, in nonsmokers, the frequency of chromosomal instability per tumor is significantly lower compared with tobacco-related tumors. Because the distribution of the tumor stage was similar between the two group of patients, the high frequency of chromosomal instability in lung ADs from smokers cannot be attributed simply to tumor progression. Other genetic alterations such as *K-ras* and *p53* mutations have also been reported as rare events in non-smoking-related lung tumors (5, 9–12).

We have previously observed that *p16* gene alterations are independent of the smoking history of the patient (19). However, although tobacco-related tumors show a high frequency of homozygous deletions and LOH/point mutations, promoter hypermethylation is the main mechanism of *p16* inactivation in lung ADs from nonsmokers (19). With the exception of *p16* alterations no other common genetic abnormalities were detected in lung cancer from nonsmokers. Point mutations in these tumors in oncogenes and tumor suppressor genes still not identified, and small and very localized deletions may be responsible for tumor development in most of the nonsmoking lung cancer patients. It is tempting to speculate that distinct endogenous mechanisms of gene activation or inactivation lead to the development of lung cancer in nonsmoking patients.

Tobacco contains several carcinogens such as BaP and some *N*-nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; Ref. 24). The activated form of BaP is the BPDE that originates as a consequence of the metabolizing of BaP inside the cells and that can form stable covalent DNA adducts and induce DNA single-strand breaks (25). Selective BDPE adduct formation at codons 157, 248, and 273 of the *p53* gene have been reported as one the causes for *p53* gene mutations in lung cancer (6). Moreover, *in vitro* studies revealed that cultured lymphocytes treated with BDPE accumulate chromosomal aberrations (25), and a recent study showed that the efficiency of the repair of BDPE adducts is dependent on *p53* wild-type expression (26). Therefore, it is possible that BPDE-induced *p53* mutations prevent the efficient repair of the DNA adducts generated by BDPE and other tobacco carcinogens in smoking-related lung cancer. Such a genetic environment would lead to widespread chromosomal abnormalities that alter other important oncogenes and tumor suppressor genes, thus allowing further tumor progression. In agreement with this hypothesis is the fact that *p53* mutations are considered an early alteration in lung tumors because they can be detected in preneoplastic lesions of the bronchus (27). Allelic losses at chromosomal arms 3p and 9p have been detected in preneoplastic lesions and even in normal bronchial epithelium (28), sometimes preceding the presence of *p53* gene mutations (29). However, larger 3p deletions or LOH at other loci are more frequent in invasive carcinoma than in dysplasia or metaplastic lesions (27–29).

Finally, we observed two distinct groups among the tobacco-related lung ADs, those with a high level (high FAL) and those with a low level (low FAL) of chromosomal abnormalities. Interestingly, *p53* gene mutations clustered in the tumors with a high frequency of chromosomal alterations, whereas *K-ras* mutations were more frequent in the tumors with low FAL. In lung AD, AAHs have been suggested to be the tumor precursor lesions (30). Genetic screening of low and high grade AAH lesions revealed the presence especially of *K-ras* mutations but no *p53* mutations (30, 31). Moreover, *p16* promoter hypermethylation has been shown to constitute an early event in lung AD progression (32). These observations suggest that AAH

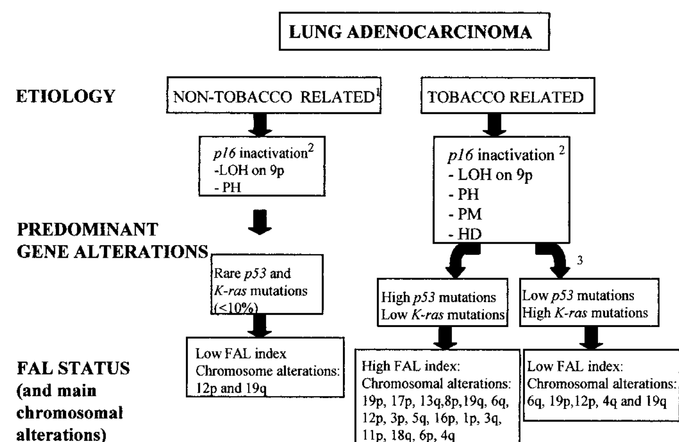

Fig. 3. Lung AD classification based on smoking history and genetic profile. *1*, ~20% of lung AD arising in nonsmokers (3); *2*, *PH*, promoter hypermethylation; *PM*, point mutation; *HD*, homozygous deletion; *3*, a proposed precursor lesion AAH, identified in the lungs of smokers, harbors frequent *K-ras* mutations.

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lesions may be precursors to group Ib lung ADs. Because tobacco targets both the *p53* and *K-ras* genes for mutation, the first tobacco-induced mutation may dictate the pathway for progression in smoking-associated tumors.

Chromosomal instability has been studied in colorectal tumors, and two independent genetic pathways have been suggested for colorectal carcinogenesis (33). One pathway includes a minor group of colorectal tumors showing general microsatellite instability, nearly diploid cells, and the absence of polypoid lesions often associated with the hereditary nonpolyposis colorectal cancer syndrome. The other pathway is associated with widespread aneuploidy and is though to arise from polypoid adenomas with a high frequency of *K-ras* mutations (34). Our observations suggest that ADs of the lung may also progress through two distinct genetic pathways in smokers.

We now present molecular evidence that lung AD also represents a heterogeneous group of genetically different entities. Additional studies will determinate whether these molecular subclassifications are associated with certain clinical and pathological features and specific responses to various therapies. A complete characterization of the genetic alterations present in these different categories will allow us to more fully understand lung cancer development in smokers and nonsmokers.

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