REPORTS

Clonal Genetic Alterations in the Lungs of Current and Former Smokers

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Background and Purpose: **Genetic damage has been identified at multiple chromosomal sites (i.e., loci) in lung cancer cells. We questioned whether similar damage could be detected in the bronchial epithelial cells of chronic smokers who do not have this disease.** ***Methods:*** **Biopsy specimens from six different bronchial regions were obtained from 54 chronic smokers (40 current smokers and 14 former smokers). The presence of squamous metaplasia and dysplasia (abnormal histologic changes) in the specimens was documented by examination of hematoxylin–eosin-stained sections, and a metaplasia index ([number of biopsy specimens with metaplasia/total number of biopsy specimens] × 100%) was calculated for each subject. Loss of heterozygosity (i.e., loss of DNA sequences from one member of a chromosome pair) involving microsatellite DNA at three specific loci—chromosome 3p14, chromosome 9p21, and chromosome 17p13—was evaluated by means of the polymerase chain reaction. Fisher's exact test and logistic regression analysis were used to assess the data. Reported *P* values are two-sided.** ***Results:*** **Data on microsatellite DNA status at chromosomes 3p14, 9p21, and 17p13 were available for 54, 50, and 44 subjects, respectively. The numbers of individuals who were actually informative (i.e., able to be evaluated for a loss of heterozygosity) at the three loci were 36 (67%), 37 (74%), and 34 (77%), respectively. DNA losses were detected in 27 (75%), 21 (57%), and six (18%) of the informative subjects at chromosomes 3p14, 9p21, and 17p13, respectively. Fifty-one subjects were informative for at least one of the three loci, and 39 (76%) exhibited a loss of heterozygosity. Forty-two subjects were informative for at least two of the loci, and 13 (31%) exhibited losses at a minimum of two loci. Loss of heterozygosity at chromosome 3p14 was more frequent in current smokers (22 [88%] of 25 informative) than in former smokers (five [45%] of 11 informative) (*P* = .01) and in subjects with a metaplasia index greater than or equal to 15% (21 [91%] of 23 informative) than in subjects with a metaplasia index of less than 15% (six [46%] of 13 informative) (*P* = .003). In five informative individuals among nine tested nonsmokers, a loss of heterozygosity was detected in only one subject at chromosome 3p14 (*P* = .03), and no losses were detected at chromosome 9p21 (*P* = .05).** ***Conclusions:*** **Genetic alterations at chromosomal sites containing putative tumor-suppressor genes (i.e., 3p14 and the FHIT gene, 9p21 and the p16 gene [also known as CDKN2], and 17p13 and the p53 gene [also known as TP53]) occur frequently in the histologically normal or minimally altered bronchial epithelium of chronic smokers. [J Natl Cancer Inst 1997;89:857-62]**

It is estimated that 178 100 new cases of lung cancer and 160 400 deaths from this disease (representing the highest cancer-related death rate) will occur in the United States alone in 1997 (*1*). Epidemiologic studies single out cigarette smoking as the number one cause of this devastating illness (*2*). The catastrophic public health problems caused by tobacco have led to tightened federal regulation of cigarette marketing and sales in the United States (*3*).

The major variable in the risk associated with smoking relates to the duration of exposure and dose, since the development of lung cancer requires the accumulation of specific genetic alterations over a long period of time. The risk of developing lung cancer is 15-fold greater in smokers than in nonsmokers (*2*). The relative risk of developing lung cancer decreases from about 15-fold to 1.5- to fourfold in former smokers who have quit for 15 years (*2*). Despite this decreased relative risk, recent studies (*4,5*) in the United States show that more than 50% of new lung cancer cases will occur in former smokers (*4,5*). Morphologic changes caused by cigarette smoking in the bronchial epithelium were first noted four decades ago (*6,7*). However, the underlying genetic basis of tobacco-related lung carcinogenesis has yet to be defined.

The development of human cancer is a multistep process involving the clonal evolution of abnormal cell populations that gain a selective growth advantage over normal cells by accumulating specific alterations in at least two groups of genes, the protooncogenes and the tumor-suppressor genes (*8,9*). Modern molecular

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See ''Notes'' following ''References.''

technology has made it possible to identify many of these genetic alterations in human tissue, such as the inactivation of both alleles of tumor-suppressor genes in human tumors (*10*) through mutation of one allele and deletion of genetic material containing the other.

Alteration of tumor-suppressor genes and protooncogenes and a loss of heterozygosity (LOH) at multiple chromosomal loci have been identified in lung cancer. Recent studies (*11-13*) show that LOH occurs at chromosomes 3p and 9p in preinvasive lesions of the lungs of patients with non-small-cell lung cancer. The cases in these studies, however, were too few and too heterogeneous to determine if any associations existed between the genetic alterations and smoking or histology. In this study, we assessed a far larger, more homogeneous population of asymptomatic former and current chronic smokers who had bronchial biopsies as part of a prospective study to determine whether genetic alterations at three chromosomal loci that contain putative tumor-suppressor genes could be associated with clinical parameters.

Subjects and Methods

Subjects

We prospectively accrued 54 subjects who were involved in National Cancer Institute-sponsored clinical chemoprevention trials conducted at The University of Texas M. D. Anderson Cancer Center. All subjects were current or former smokers who had smoked at least 15 pack-years (the number of packs per day multiplied by the number of years of smoking). Current smokers included subjects who had quit for a period of less than 1 year. Nine nonsmokers who had undergone lung lobectomies because of metastatic lung tumors of non-lung origin or because of lung hematomas were evaluated as control subjects. This study was approved by the Institutional Review Board, and all participants gave written informed consent.

Sample Collection

All participants in the chemoprevention trials had bronchoscopic examinations at the M. D. Anderson Cancer Center. We performed biopsies at six sites—the carina, right upper lobe, right middle lobe, right lower lobe, left upper lobe, and left lower lobe—before beginning the chemoprevention trials. Blocks of normal lung tissue from the nonsmokers were sectioned and reviewed by an experienced pathologist (B. L. Kemp) to identify normal bronchial epithelium. Each specimen was fixed in 10% buffered formalin, embedded in paraffin, and sliced into 4-μm-thick sections. Four serial sections from each specimen were stained with hematoxylin–eosin for subsequent microdissection.

Histologic Assessment

The biopsy samples were fixed immediately and embedded in paraffin. Ten hematoxylin–eosin-stained histologic sections were examined per site by an experienced pathologist (J. Y. Ro). Histologic changes of squamous metaplasia and dysplasia were carefully documented, and a metaplasia index (the number of biopsy specimens exhibiting metaplasia divided by the total number of biopsy specimens) was calculated for each subject according to the criteria described previously (*14*).

Microdissection and DNA Extraction

For microdissection, the four serial sections were mounted on glass slides and stained with hematoxylin–eosin. The epithelial part of each biopsy section was microdissected by use of a 25½-gauge steel needle. The stroma cells were also microdissected and used as normal control material for each individual. After microdissection, the samples were incubated at 42 °C for 12 hours in 100 μL of a solution containing 50 m*M* Tris–HCl (pH 8.0), 1% sodium dodecyl sulfate, and 0.5 mg/mL proteinase. After adding 10 μL fresh proteinase K (5 mg/mL stock solution), the samples were incubated at 42 °C for another 24 hours. Products of the proteinase K digestion were purified by extraction with phenol–chloroform, and the DNA was precipitated with ethanol, using glycogen (Boehringer-Mannheim Corp., Indianapolis, IN) as the carrier.

Microsatellite Analysis

Between 400 and 1000 nuclei were dissected from both the epithelium and the stroma of each sample. DNA from at least 150 nuclei was used for each polymerase chain reaction (PCR) amplification. The markers used were D3s1285 (chromosome 3p14), D9s171 (chromosome 9p21), and TP53 (chromosome 17p13) (Research Genetics, Huntsville, AL). For PCR amplification, one of the primers for each marker was end-labeled with $^{32}PO_4$ by use of [γ-^{32}P]adenosine triphosphate (4500 Ci/mmol; ICN Biomedicals, Inc., Costa Mesa, CA) and T4 DNA polynucleotide kinase (New England Biolabs, Inc., Beverly, MA). PCR reactions were carried out in a 12.5-μL mixture containing 3% dimethylsulfoxide, 200 μ*M* deoxynucleoside triphosphates, 1.5 m*M* $MgCl_2$, 0.4 μ*M* unlabeled PCR primers, 0.01 μ*M* [γ-^{32}P]labeled primer, and 0.5 U *Taq* DNA polymerase (Life Technologies Inc., [GIBCO BRL], Gaithersburg, MD). DNA was amplified for 40 cycles at 95 °C for 30 seconds, 56 °C-60 °C for 60 seconds, and 70 °C for 60 seconds in a thermal cycler (Hybaid; Omnigene, Woodbridge, NJ) in 500-μL-capacity plastic tubes, followed by a 5-minute extension at 70 °C. The PCR products were separated in 6% polyacrylamide–urea–formamide gels, and the gels were exposed to x-ray film. LOH was defined on the basis of visual inspection as more than a 50% reduction in the intensity of the autoradiographic signal corresponding to one of the two alleles in comparison with the normal control signals. If any biopsy specimen from an individual showed LOH, that individual was considered to exhibit LOH even if other samples did not display an LOH pattern.

Statistical Analysis

The molecular analyses were performed in the laboratory in a blinded manner with respect to the clinical information of the subjects. Individual characteristics and relevant clinical information, such as smoking status and metaplasia index, were stored separately in the study's biostatistics core. After completion of the molecular analysis, the laboratory data were merged with the clinical data for combined analysis. The LOH analysis was performed on the basis of biopsy site as well as on the basis of the individual. When the individual subject was used as the unit of analysis, LOH in any of the six biopsy sites was considered as LOH in the individual. Fisher's exact test was applied to compare the proportion of LOH between current and former smokers, between subjects with and without metaplasia, between smokers and nonsmokers, and between different chromosome loci. Logistic regression analysis was performed to model the LOH proportion by including smoking status, metaplasia index, site-specific metaplasia status, and other patient characteristics as covariates (*15*). The reported *P* values are two-sided.

Results

Subject Characteristics

There were 33 men and 21 women with a mean age of 55.3 years ± 10.8 years (mean ± standard deviation) and a median pack-year history of smoking of 45 (range, 15-157 pack-years). At the time of bronchial biopsy, 37 subjects were current smokers, three had quit smoking 3, 7, or 10 months prior to biopsy, and the remaining 14 were former smokers who had quit smoking for 12 months or longer (median, 27 months; range, 12-240 months). Overall, current smokers had a higher frequency of metaplasia index greater than or equal to 15% than former smokers (31 [78%] of 40 versus five [36%] of 14; $P = .004$). Of 253 biopsy samples examined, microsatellite information was available for 244 sites from 54 subjects at chromosome 3p14, 221 sites from 50 subjects at chromosome 9p21, and 211 sites from 44 subjects at chromosome 17p13. Sixteen bronchial epithelial areas from nine lifetime nonsmokers, including five men and four women with a mean age of 46.2 years ± 11.6 years, were also obtained.

Frequency of LOH

Of the 54 subjects analyzed for LOH at 3p14, 36 (67%) were informative (i.e., they had two distinct alleles). For LOH analysis at 9p21 and 17p13, 37 (74%) of 50 and 34 (77%) of 44 assessable subjects were informative, respectively. Among the informative individuals, 27 (75%) of 36 exhibited LOH at 3p14, and 21 (57%) of 37 exhibited LOH at 9p21, respec-

tively, in one or more sites analyzed. In contrast, at 17p13, only six (18%) of 34 informative subjects showed LOH (Table 1). The difference in the frequencies of LOH between 17p13 and the other two loci were statistically significant ($P<.001$). Examples of LOH at these loci are shown in Fig. 1. According to biopsy sites, the LOH frequency was 37% (64 of 172 sites), 24% (41 of 168 sites), and 7% (12 of 161 sites) at chromosomes 3p14, 9p21, and 17p13, respectively. Overall, 51 subjects were informative for at least one of the three loci studied, and 39 (76%) of them exhibited LOH for one or more of the loci in 103 (43%) of 238 informative samples analyzed.

To examine whether LOH involved specific alleles as reported previously (*11*,*12*), we analyzed the patterns of allelic loss. Of the 27 individuals who exhibited LOH at the 3p14 locus, 20 (74%) had LOH in two or more biopsy sites. This LOH involved different alleles in eight subjects and the same allele in 12 subjects. Similarly, of the 11 subjects who demonstrated LOH at the 9p21 locus in two or more biopsy sites, five exhibited the loss of different alleles and six exhibited the loss of the same allele. These results suggest the presence of multiple clonal abnormalities in these subjects.

Among the 54 total individuals studied, 42 (78%) displayed informative patterns for at least two of the chromosome loci. Thirteen (31%) of the 42 subjects had LOH in at least two loci. In 12 biopsies from these individuals, LOH was detected at both 3p14 and 9p21 in six biopsy specimens, at both 3p14 and 17p13 in four biopsy specimens, and at all three markers in two biopsy specimens. Interestingly, among six biopsy specimens that showed LOH at 17p13 and were informative at 3p14 and/or 9p21, four exhibited LOH at 3p14 and the other two exhibited LOH at both 3p14 and 9p21. These data indicate that multiple genetic alterations are present in the lungs of some smokers and suggest that LOH at 17p13 occurs later than LOH at 3p14 and 9p21.

Smoking Status and LOH

We examined the association between LOH and smoking status (Table 2). Current smokers tended to have a higher frequency of overall LOH (defined as LOH at any of the three loci tested) than former smokers (31 [82%] of 38 versus eight [62%] of 13), but the difference did not reach statistical significance ($P = .25$). Despite smoking cessation, however, almost two thirds of the former smokers (i.e., 62%) harbored clonal genetic alterations in their lungs. When the LOH at a single locus was associated with smoking status, current smokers had a significantly higher frequency of LOH at 3p14 than former smokers (22 [88%] of 25 versus five [45%] of 11; $P = .01$). However, the frequencies of LOH at 9p21 and 17p13 were similar among current and former smokers. These results suggest that the genetic loci differ in their sensitivity to the mutagenic effects of cigarette smoking. Alternatively, 3p14 alterations may be sublethal, or cells with LOH at 9p21 or 17p13 may have a growth advantage, resulting in clonal expansion. In contrast to smokers, among five informative cases from the nine nonsmokers, only one (20%) exhibited LOH at 3p14 ($P = .03$) and none had LOH at 9p21 ($P = .05$). Similarly, among 11 informative sites, LOH was observed in only one (9%) site at 3p14 ($P = .07$) and none of the sites at 9p21 ($P = .10$). These data indicate that cigarette smoking may be one of the causes of frequent LOH observed in this study. When LOH frequencies at the three loci in individuals who smoked 30 or more pack-years were compared with the frequencies in individuals who smoked fewer than 30 pack-years, the loss of 17p13 seemed more frequent in those with more extensive smoking histories, although no statistical significance was found in a two-sided Fisher's exact test ($P = .15$) (Table 2). However, a marginally significant difference was reached when the data were analyzed in a chi-squared test ($P = .06$). The difference may become statistically significant when more samples are analyzed in our ongoing clinical trial.

Metaplasia Index and LOH

To test whether tissue sections exhibiting squamous metaplasia harbor more genetic alterations, we associated LOH with the metaplasia index. Among the three loci studied, only LOH at 3p14 showed a strong association with metaplasia status (Table 2). Among the informative subjects with a metaplasia index greater than or equal to 15%, 21 (91%) of 23 had LOH at 3p14 compared with six (46%) of 13 who had a metaplasia index of less than 15% ($P = .003$). In contrast, there was no significant association between metaplasia status and LOH frequency for 9p21 and 17p13.

Because current smokers had a higher frequency of metaplasia index greater than or equal to 15% than former smokers and because smoking cessation is known to induce a reversal of squamous metaplasia (*14*), we examined site-specific data to determine whether the association between LOH at 3p14 and smoking status was dependent on the presence of squamous metaplasia. We first examined the association between site-specific LOH at 3p14 and histology, and then we performed a logistic regression analysis to delineate the relationship between LOH at 3p14 and smoking status and between LOH at 3p14 and squamous metaplasia status (Table 3). The outcome variable was LOH at 3p14, and the covariates were smoking status (current versus former), site-specific metaplasia status (present versus absent), and metaplasia index ($\geq$15% versus <15%). In the univariate analysis, site-specific metaplasia status, metaplasia index, and smoking status were positively associated with LOH at 3p14 ($P = .04$, $P = .007$, and $P = .09$, respectively). In the multivariate analysis, neither smoking status nor site-specific metaplasia status was associated with LOH at this locus. Metaplasia index, however, remained significantly associated with LOH at 3p14. These results indicate that the low frequency of LOH at 3p14 in former smokers is linked to the reversal of squamous metaplasia following smoking cessation. However, the lack

Table 1. Frequency of loss of heterozygosity (LOH) in the bronchial epithelium of smokers

	Chromosome locus			
Analysis based on	3p14	9p21	17p13	Any*
Subjects	27/36 (75)†	21/37 (57)	6/34 (18)	39/51 (76)
Biopsy sites	64/172 (37)	41/168 (24)	12/161 (7)	103/238 (43)

*LOH at one or more of the three indicated loci.
†Number with LOH/total number informative (%).

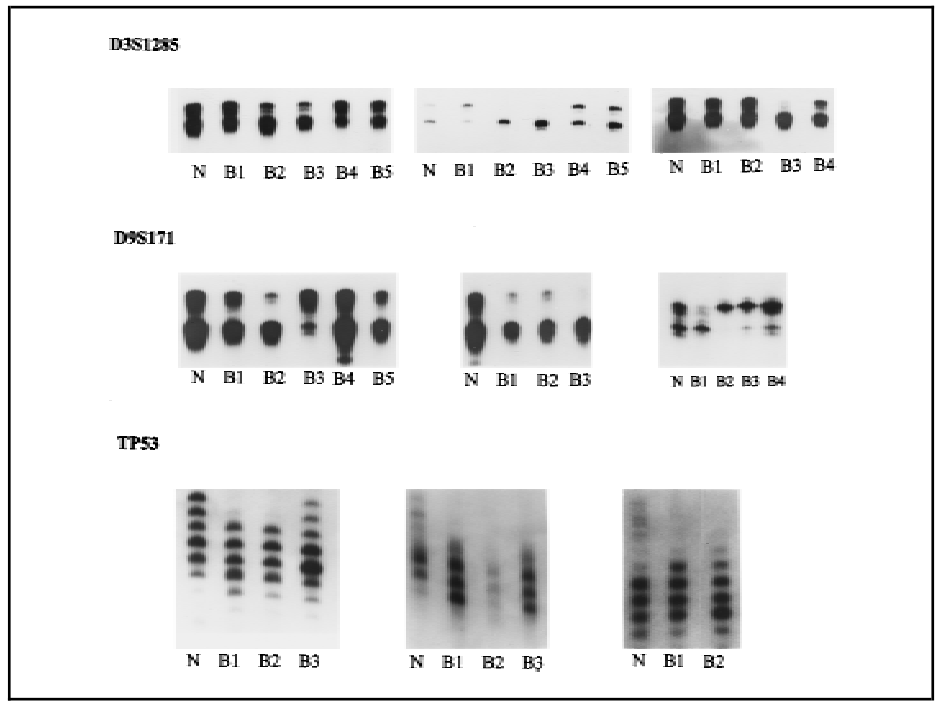

Fig. 1. Loss of heterozygosity (LOH) observed at microsatellite markers. Examples are shown for nine subjects, including three assessed at D3S1285 (chromosome 3p14), three assessed at D9S171 (chromosome 9p21), and three assessed at TP53 (chromosome 17p13). The analysis was performed by use of specific primer pairs, with one primer in each pair end labeled with $^{32}PO_4$, and the polymerase chain reaction. The amplification products were resolved in 6% polyacrylamide–urea–formamide gels, which were subsequently exposed to x-ray film. N = normal DNA from stromal cells, and B followed by a number = DNA from bronchial epithelial cells from individual biopsy sites (maximum of six sites examined per subject). LOH (defined by more than a 50% reduction in radioactive signal intensity) at chromosome 3p14 (D3S1285) appears in B2, B3, and B4 of subject 1; in B1, B2, and B3 of subject 2; and in B3 of subject 3. LOH at chromosome 9p21 (D9S171) appears in B2 and B3 of subject 4; in B1, B2, and B3 of subject 5; and in B1, B2, B3, and B4 of subject 6. LOH at chromosome 17p13 (TP53) appears in B1, B2, and B3 of subject 7; in B1, B2, and B3 of subject 8; and in B1 and B2 of subject 9.

of a direct association between LOH at 3p14 and squamous metaplasia suggests that the area of squamous metaplasia itself may not necessarily harbor the genetic alterations. Nevertheless, metaplastic changes occurring in the lungs appear to indicate a field effect of smoking, i.e., the more the metaplasia in the field, the greater the frequency of LOH at 3p14.

Discussion

To our knowledge, this large prospective study (54 subjects and 253 biopsy specimens) is the first molecular study of the genetic effects of tobacco smoke on the histologically normal or minimally altered lung tissue of healthy chronic smokers. A high frequency of LOH occurred at one or more of the study's three chromosomal loci (3p14, 9p21, and 17p13). The well-described p53 tumor-suppressor gene (also known as TP53) resides at 17p13 (*15*). The other two loci are also potentially important tumor-suppressor loci because they contain the FHIT[1] (3p14) and the p16 (also known as CDKN2) (9p21) genes (*16-18*). The highest rate of LOH occurred at 3p14 (75%), followed by 9p21 (57%) and 17p13 (18%). In the lifetime nonsmokers, LOH was observed at lower rates (20% at 3p14 and none at 9p21). However, the sample size of the nonsmokers was small because of the extreme difficulty in obtaining bronchial epithelial specimens from nonsmokers, and the mean age of the nonsmokers was slightly younger (46.2 years versus 55.3 years). Nonetheless, the differences in the numbers of cases with LOH at 3p14 and 9p21 between the smokers and the nonsmokers were statistically significant ($P = .03$ and $P = .05$, respectively). These data indicate that clonal genetic alterations are common events in the lung tissue of chronic smokers. Since the overall number of samples obtained from the nonsmokers was small and since the samples were collected from more distal sites in the airway, our data need further validation with a larger number of comparable proximal control samples.

The overall frequency of LOH was lower in former smokers (62%) than in current smokers (82%). This finding is consistent with recent molecular data showing that smoking cessation is associated with a decline in tobacco-related DNA adducts (*19*). Although the trend in our data suggests that smoking cessation may reverse the clonal expansion of abnormal cells, the 62% LOH rate in former smokers indicates that a high rate of

Table 2. Association of loss of heterozygosity (LOH) with smoking status and metaplasia index*

	Chromosome locus			
	3p14	9p21	17p13	Any†
Current smokers	22/25 (88%)‡	16/27 (59%)	5/29 (17%)	31/38 (82%)
Former smokers	5/11 (45%)	5/10 (50%)	1/5 (20%)	8/13 (62%)
P values§	.01	.72	1.00	.25
Smoked <30 pack-years‖	7/8 (88%)	6/10 (60%)	0/11 (0%)	10/13 (77%)
Smoked ⩾30 pack-years	20/28 (71%)	15/27 (56%)	6/23 (26%)	29/38 (76%)
P values§	.65	1.00	.15	1.00
Metaplasia index <15%	6/13 (46%)	9/13 (69%)	2/10 (20%)	12/18 (67%)
Metaplasia index ⩾15%	21/23 (91%)	12/24 (50%)	4/24 (17%)	27/33 (82%)
P values§	.003	.32	1.00	.30

*Smoking status categorized as current (current smokers and those who had quit <1 year) or former. Metaplasia index = number of biopsy specimens exhibiting metaplasia divided by total number of biopsies, with the quotient multiplied by 100%; calculated for each subject (*see* the ''Subjects and Methods'' section for details).

†LOH at one or more of the three indicated loci.

‡Number with LOH/total number informative (%).

§*P* values based on two-sided Fisher's exact tests.

‖Pack-years = the number of packs per day multiplied by the number of years of smoking.

Table 3. Logistic regression model for site-specific loss of heterozygosity at chromosome 3p14

Model	*P*
Univariate model	
Squamous metaplasia (present versus absent)	.04
Metaplasia index (≥15% versus <15%)*	.007
Smoking status (former versus current)†	.09
Multivariate model	
Squamous metaplasia (present versus absent)	.10
Smoking status (former versus current)	.23
Metaplasia index (≥15% versus <15%)	.03
Smoking status (former versus current)	.61

*Metaplasia index = number of biopsies exhibiting metaplasia divided by total number of biopsies, with the quotient multiplied by 100%; calculated for each subject (*see* the ''Subjects and Methods'' section for details).

†Current smokers include individuals who had quit for a period of less than 1 year.

clonal genetic damage persists even after smoking cessation.

LOH at 3p14 was significantly lower in former smokers; therefore, it was the most sensitive marker of smoking status. This finding, coupled with other recent molecular data (*20*,*21*), suggests that the known fragile locus 3p14 (or possibly the FHIT gene within it) is a major target of carcinogens in cigarette smoke. It may be, therefore, that LOH at 3p14 is a frequently detected clonal event that may not be critically involved in the multistep carcinogenic process. Since LOH at 9p21 and LOH at 17p13 were not significantly associated with smoking status, these relatively stable clonal alterations may mark the enduring process of multistep carcinogenesis and may thus identify former smokers who are at the highest risk of lung cancer.

Mutations in the p53 tumor-suppressor gene are the most common genetic alterations identified in human cancer, and they provide a fingerprint of the effects of carcinogen exposure (*22*-*24*). LOH at the p53 locus and overexpression of (mutant) p53 protein have previously been shown in the dysplastic bronchial epithelium of patients without lung cancer (*23*). These observations suggest that the inactivation of p53 function may play a role in early tumorigenesis in the lung (*23*). Our finding that LOH at 17p13 may be associated with exposure to tobacco smoke is supported by major recent findings involving this locus (*24*,*25*). Mutations in p53 are strongly associated with cigarette smoking in patients with lung cancer and squamous cell carcinoma of the head and neck (*24*,*26*). The direct molecular etiologic link between tobacco smoke and lung cancer was established by the finding that benzo[*a*]pyrene-diol-epoxide adducts of tobacco smoke are distributed along the exons of the p53 gene (*25*).

Smoking and smoking-cessation efforts are enormous national public health issues, largely because of the devastating morbidity and mortality caused by lung cancer. Epidemiologic data establish that the lung cancer risk of smokers who quit declines over time to significantly lower levels (*2*). Our findings provide molecular insight into these data—an overall trend of lower LOH frequency and significantly lower LOH frequency at 3p14 are detected in former smokers compared with current smokers. Epidemiologic data also temper the good news for chronic smokers who quit—their risk of lung cancer never reverts to that of never smokers (*27*,*28*), and more than 50% of all patients with lung cancer are former smokers (*4*,*5*). These data also receive molecular underpinnings from our findings—the overall LOH frequency was 62% in our former smokers who had quit for at least 12 months and for an average of 27 months.

National smoking cessation and prevention efforts, especially among children and other high-risk groups, are beginning and will continue to have a large, positive impact on smoking-related diseases. More and more smokers will quit, and, therefore, the percentage of individuals with lung cancer who are former smokers is bound to grow. Future efforts to control lung cancer should include the development of chemoprevention approaches for former smokers. The development of specific clonal genetic markers for identifying individuals at highest risk should be included among those efforts.

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Notes

[1]FHIT = fragile histidine triad. The FHIT gene encodes a dinucleoside 5′,5‴-P1,P3-triphosphate hydrolase (*29*). One substrate for this enzyme, 5′,5‴-P1,P4-tetraphosphate (Ap4A), has been reported to promote DNA synthesis (*30-33*). Loss of FHIT function may lead to Ap4A accumulation and unregulated DNA synthesis.

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