Evidence of Cumulative Gene Losses with Progression of Premalignant Epithelial Lesions to Carcinoma of the Bronchus

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Abstract

Human bronchial carcinoma is thought to develop through progressive stages from basal cell hyperplasia to squamous metaplasia, dysplasia, carcinoma in situ, and finally invasive cancer. In this study, we used tissue microdissection to examine loss of heterozygosity of chromosomes 3p21, 5q21, and 9p21 at each stage of the epithelial progression to invasive cancers. Forty-eight premalignant/malignant bronchial sites were biopsied from 13 patients (including 9 subjects without cancer) using fluorescence bronchoscopy. Eighteen sites with moderate/severe dysplasia in 6 patients were subjected to bronchoscopic and molecular follow-up during a 3-month to 2-year period. Seven separate cases of advanced non-small cell bronchial cancers were also analyzed.

From the baseline biopsies, the prevalence of 3p and 9p deletions increased significantly from no deletion in the hyperplasia/metaplasia samples (n = 9) to 37 and 31% of the informative cases, respectively, in the dysplasia samples (n = 29), to 100 and 83%, respectively, for the carcinomas in situ (n = 6), and 100% in the invasive cancers (n = 11). Chromosome 5q deletion was significantly more frequent in invasive cancers (70% of the informative cases) as compared to carcinoma in situ (40%), dysplasia (33%), and hyperplasia/metaplasia samples (11%). The number of chromosome alterations also increased significantly from the lowest to the highest grade lesions, showing evidence of accumulation of genetic damage from one group to another. The molecular follow-up analysis showed that the same genomic alteration can persist in a given dysplastic bronchial area for several months or years, and that the persistence or the regression of the molecular abnormality is well correlated with the evolution of the disease on follow-up.

Our results suggest that molecular analysis of bronchial biopsies obtained by fluorescence bronchoscopy may be a very useful means to study the natural history of preinvasive bronchial lesions and the outcome of interventions, such as chemopreventive treatment.

Introduction

Carcinogenesis of the respiratory epithelium is thought to require multiple steps, such that a sequence of so-called "premalignant" epithelial modifications precedes invasive cancers (1). For squamous cell carcinomas of the bronchus, which represent the majority of cancers arising in the large airways of heavy smokers, a proposed progression model of premalignant lesions to invasive cancer includes the sequential development of basal cell hyperplasia; squamous metaplasia; mild, moderate, and severe dysplasia; and CIS (2). This sequence has been regularly observed in animal models of carcinoma (review in Ref. 3) and is supported by extensive postmortem examination of the bronchial tree from smokers and lung cancer patients (4). As inferred by the results of sputum cytology of individuals at high risk of developing lung cancer, the development of an invasive cancer from a premalignant lesion may take several years (5). However, because of the difficulties in localizing and performing serial sampling of preinvasive lesions of the bronchial tree in vivo, this model has not been yet confirmed in humans.

As hypothesized in other epithelial cancers such as colon cancer (6), each step of the bronchial carcinogenesis may be the result of the accumulation of genetic damages involving several oncogenes and tumor suppressor genes. Increasing information is currently available on the genetic alteration occurring at the final invasive stage of bronchial carcinomas, at both the cytogenetic and molecular levels (reviewed in Refs. 7 and 8). However, very little is known about the genetic abnormalities that may lead to the progression of a low-grade epithelial bronchial lesion to an invasive cancer, especially among individuals who are at risk of developing but do not have invasive cancers. The most frequent molecular alterations described in invasive NSCLCs are deletions of the short arm of chromosomes 3 (9–11) and 9 (12–14), deletion of the q21 band of chromosome 5 (15–18), as well as mutation of the p53 and ras family genes.

Molecular studies of preinvasive bronchial lesions have found a high frequency of 3p deletions in CIS (19) and as early as in epithelial hyperplasia in patients with lung cancer (20). These data suggest that at least some of the molecular abnormalities are very early events. However, to date, the frequency of these genomic abnormalities and their relationship with the histopathological grade of the lesion, their order of alteration (if any), and their evolution with the progression of the disease are not known.

The Cancer Imaging Laboratory at the BCCA (Vancouver, Canada) recently developed a simple procedure to increase the sensitivity of detection of preinvasive bronchial lesions during routine bronchoscopy (21). This technique, called LIFE, makes use of the differences in autofluorescence properties of normal and premalignant bronchial epithelia to localize superficial lesions that are invisible under white light bronchoscopy (21, 22). Fluorescence bronchoscopy is currently performed at the BCCA in high-risk subjects to detect and localize preinvasive lesions in the bronchial tree. The same procedure is also used to follow low-grade bronchial lesions. This technique provides a unique opportunity to study the genomic alterations that may occur at each step and during the progression of these lesions.

We report here the results of the genomic evaluation that we performed in a series of preinvasive bronchial lesions sampled during fluorescence bronchoscopy in patients at high risk for lung cancer. The specific goals of this study were to evaluate the frequency of chromosome 3p, 5q, and 9p sequence deletions in preneoplastic bronchial lesions and their relationship with the histopathological grade. Using precise microdissection of the premalignant epithelium...
and DNA amplification of the microdissected material, we show evidence of cumulative genetic damages with the progression of dysplasia to CIS and invasive cancer. We also show that these molecular changes can persist within the premalignant epithelium for several years and, thus, may be used for the follow-up of a given preneoplastic lesion.

Materials and Methods

**Fluorescence Bronchoscopy.** Fluorescence bronchoscopies were performed in high-risk subjects at the BCCA as part of the LIFE investigation trial, using the LIFE system (Xillix Technology Corp., Richmond, British Columbia, Canada). High-risk subjects were smokers or former smokers of more than 45 years of age who had smoked more than 25 pack-years. Written informed consent was obtained from each patient before the investigation. During the procedure, bronchial areas suspicious for dysplasia or cancer under white light or fluorescence examination were biopsied for pathological examination. Bronchial biopsies were systematically reviewed by two pathologists and classified according to the criteria described below. Fluorescence bronchoscopy and biopsy of the same sites were repeated after several months if moderate or severe dysplasia was diagnosed, or after endobronchial treatment of a CIS or a microinvasive lesion.

**Patients.** Thirteen subjects taking part in the LIFE trial, carrying one or more bronchial sites of moderate dysplasia and not previously treated with chemopreventive drugs, were selected for the study of preinvasive lesions. The patients were 12 men and 1 woman, ranging in age from 52 to 71 years. Twelve subjects were heavy smokers (30–120 pack-years), 5 had stopped smoking for 5 years at the time of the first bronchoscopy, and 3 had a history of asbestos exposure. Nine of the 13 subjects did not have evidence of invasive cancer at the time of the first bronchoscopy and during the follow-up period (6 months to 4 years). Two patients were found to have an invasive cancer (one peripheral adenocarcinoma and one squamous cell carcinoma) at the time of the first bronchoscopy, which were successfully resected and did not relapse. One patient had two synchronous microinvasive squamous cell cancers of the left lower lobe and of the right main bronchus. These two lesions were treated by photodynamic therapy with photofrin without recurrence. One patient developed an invasive squamous cell cancer on follow-up 18 months after photodynamic therapy of a CIS. We studied separately 7 cases of proximal advanced invasive tumors (stage IIIa, International Union Against Cancer criteria, 1988) using our microdissection PCR/LOH technique of paraffin-embedded samples. These tumors were used to assess the prevalence of genomic deletions at the latest stages of bronchial carcinogenesis.

**Identification of Preneoplastic Lesions.** The preneoplastic lesions were classified in four groups according to the following histological criteria (5): RCH is an increase in number of otherwise normal appearing basal cells covered with normal ciliated cells. Metaplasia consists of a multilayered epithelium with stratification and cytoplasmic evidence of squamoid differentiation. The dysplasia group was divided into mild dysplasia when the biopsy showed a multilayered epithelium with stratification, with individual cells showing slight increase in nuclear/cytoplasmic ratio and mild hyperchromatism, and moderate or severe dysplasia when the epithelium was irregular in the lower two-thirds. Individual cells show enlargement with a variable increase in nuclear/cytoplasmic ratio always associated with hyperchromatism. The cells are often binucleate with eccentric nuclei, which may show loss of spherical contour. CIS is a multilayered but nonstratified epithelium where superficial cells resemble the basal layer. The epithelium is composed of cells expressing all the nuclear and cytoplasmic features of malignancy, delimited by a microscopically intact basement membrane without invasion of the underlying stroma. Fig. 1 shows an example of one lesion from each group.

**Microdissection.** Four 7-μm-thick sections were cut from archived, formalin-fixed, paraffin-embedded blocks. One slide was stained with hematoxylin and eosin and coverslipped for diagnosis. Three consecutive cuts, adjacent to the hematoxylin and eosin-stained slide, were used for microdissection according to a technique described elsewhere (11). Using this technique, basement membrane and epithelium are clearly identified and easy to separate, as shown in Fig. 1. After microdissection, both hematoxylin and eosin and microdissected slides were comparatively reviewed by one pathologist (J. L.). This confirmed the histopathological classification of the microdissected areas.

**DNA Extraction.** The microdissected cells were immediately collected in a 0.5-ml Eppendorf tube containing 50 μl of proteinase K buffer [50 mM Tris-HCl (pH 8.8)-0.5 mM EDTA (pH 8)-0.5% Tween 20-500 μg/ml proteinase K] and digested for 24 h at 37°C. The samples were then heated at 100°C for 10 min to inactivate the proteinase K. A 10-μl aliquot was used for each PCR assay without further purification. Normal reference genomic DNA was extracted from blood as described previously.

**Allelic Deletion Analysis.** Primers flanking polymorphic DNA motifs located at chromosomes regions 3p21–22, 5q21, and 9p21–22 were used to evaluate LOH. The chromosome 3p markers used were a 102–124-bp tetranucleotide repeat polymorphism located at the ITIH1 locus (3p21.1; Ref. 11), a 90–98-bp dinucleotide (CA), repeat polymorphism located at the D3S1339 locus (3p21.3; Ref. 23), and a 150-bp dinucleotide (CA), repeat polymorphism located at the D3S1007 locus (3p21.3–22; Ref. 24). Chromosome 5q21 polymorphisms used were the 79/90-bp insertion-deletion polymorphism of the MCC gene exon 10 and the 133/87–46-bp Ralpolymorphism of APC gene exon 11 (25). Chromosome 9 primers were designed from Genebank sequences of known microsatellite dinucleotide (CA), repeat polymorphisms at the IFN-2a gene and the D9S126 locus and amplified 126- and 104-bp products, respectively.

Ten μl of the proteinase K digested sample were subjected to one round of 40 PCR cycles in a total volume of 25 μl. Unpublished primer sequences used in this paper and the exact PCR conditions for each locus are available upon request to the authors (L. T.).

The ITIH1, D3S1007, MCC exon 10, and APC exon 11 (after digestion with Ral) PCR products were fractionated on 10-cm high, 1-mm thick, 8–12% polycrylamide gels (Mini-Protein, Bio-Rad) and were visualized by staining with ethidium bromide. D3S1339, IFN-2α and D9S126 polymorphisms were resolved on 5 M urea-33% formamide denaturing sequencing gels. In these cases, DNA amplification was carried out using one [γ-32P]ATP end-labeled primer.

Normal reference DNA was analyzed from blood lymphocytes, and the genotypy was confirmed from normal submucosal parts of the slides in each case. LOH was scored by visual comparison of the intensity of normal and preneoplastic alleles on gels or on autoradiograms.

**Statistical Analysis.** The significance of differences in the frequency with which gene deletions occurred in different kinds of neoplastic and preneoplastic lesions was estimated using χ² analysis with Yate's correction or Fisher's exact test if required.

**Results**

**Frequency of Deletions in Invasive NSCLCs of the Proximal Bronchi.** We first determined the prevalence of allelic deletions of chromosomes 3p21–22, 5q21, and 9p21–22 sequences in 7 resected NSCLCs of the proximal bronchi (6 large cell carcinomas and 1 squamous cell carcinoma). From these cases, chromosome 3p21–22 deletion was found in 7 of 7 cases, 9p21–22 deletion in 5 of 5 informative cases, and 5q21 deletion in 4 of the 6 informative cases (Table 1). This very high frequency confirmed our previous study showing the high sensitivity of our microdissection/PCR-LOH method as compared to the techniques used previously for LOH detection in invasive tumors (11).

**Prevalence of Allelic Deletions in Preinvasive Lesions.** A total of 48 separate bronchial sites were sampled from the 13 subjects selected for this study. Including the follow-up material sampled at the same sites over time, there were 80 biopsies. The frequency of allelic deletion in preinvasive lesion was evaluated only from the baseline biopsies sampled before any chemopreventive or endobronchial treatment. This group included 3 cases of RCHs, 6 metaplasias without atypia, 29 dysplasia cases (of which 9 were classified as mild dysplasia and 20 as moderate to severe dysplasia), 6 CISs, and 3 microinvasive and 1 invasive carcinomas.

The microdissection of 45 of these 48 lesions yielded amplified products suitable for analysis at all the informative loci in 3p, 5q, and 9p. Three cases (one CIS and two mild dysplasia samples) failed to amplify using the 9p probes, whereas the same DNA extraction gave...
Table 1. LOH and number of chromosome abnormalities in invasive and intraepithelial bronchial neoplasms

<table>
<thead>
<tr>
<th></th>
<th>No. of cases with LOH at each locus</th>
<th>LOH/no. of chromosome pairs tested</th>
<th>No. of cases with 1, 2, or 3 chromosome abnormalities</th>
<th>At least one of the three abnormalities/informative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RCH/Metaplasia</td>
<td>LOH 3p21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>LOH 5q21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>LOH 9p21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dysplasia</td>
<td></td>
<td>0/9 (9)</td>
<td>1/9 (16%)</td>
<td>0/7 (28%)</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td>3/9 (33%)</td>
<td>1/7 (14%)</td>
<td>1/2 (38%)</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td>7/18 (38%)</td>
<td>2/7 (40%)</td>
<td>5/6 (83%)</td>
</tr>
<tr>
<td>CIS</td>
<td></td>
<td>6/6 (100%)</td>
<td>2/5 (40%)</td>
<td>5/6 (83%)</td>
</tr>
<tr>
<td>Microinvasive</td>
<td></td>
<td>4/4 (100%)</td>
<td>3/4 (75%)</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Stage III NSCLC</td>
<td></td>
<td>7/7 (100%)</td>
<td>4/6 (66%)</td>
<td>5/5 (100%)</td>
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</table>

<sup>a</sup> Metaplasia/RCH versus dysplasia, P < 0.03 (Fisher's exact test); dysplasia versus CIS or higher grade lesion, P < 0.0005 (x², with Yates correction).

<sup>b</sup> Invasive tumors versus CIS/dysplasia, P < 0.05 (Fisher's exact test).

<sup>c</sup> Dysplasia versus CIS or higher grade lesions, P < 0.0002 (x², with Yates correction).

<sup>d</sup> Metaplasia/RCH versus dysplasia, P < 0.0006; dysplasia versus CIS, P < 0.01 (x², with Yates correction).

strong signals at the other loci. Although homozygous deletions may explain these results (12), these 3 cases were considered as noninformative for 9p.

Table 1 shows the allelic deletion frequency at chromosomal regions 3p21–22, 5q21, and 9p21–22 in the 48 baseline biopsies. As can be seen in this table, the same frequency of deletion was found among the invasive/microinvasive squamous cell cancers group and the previously studied stage III NSCLC. High frequency of 3p and 9p deletion (100 and 83%, respectively) and 40% of 5q deletion were also found in the CIS group. Deletions of chromosomes 3p, 5q, and 9p sequences were found in 37, 33, and 31% of the dysplasia samples, respectively. In contrast, LOH was seen in only 1 nondysplastic sample of this series (classified as metaplasia), which represents 1 alteration on 25 chromosome pairs studied in this subgroup. There was a difference in the prevalence of 3p and 9p deletions between CIS or higher grade lesions and the dysplastic lesions (P < 0.002; x², with Yates' correction) and in the prevalence of 3p deletions between dysplasias and lower grade lesions (P < 0.03; unilateral Fisher's exact test). Chromosome 5q21 deletion was found more frequently in invasive carcinomas as compared to CIS and dysplasia lesions (P < 0.05;
Table 2 Histology and molecular follow-up of invasive and preinvasive bronchial lesions

<table>
<thead>
<tr>
<th>Patient 10</th>
<th>Date of biopsy</th>
<th>Treatment</th>
<th>Bronchial site</th>
<th>Date of biopsy</th>
<th>Treatment</th>
<th>Bronchial site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>September 1990</td>
<td></td>
<td>RB8 RML</td>
<td>September 1992</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>13 cis-retinoic acid (3 months)</td>
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<tr>
<td>Patient 15</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
</tr>
<tr>
<td></td>
<td>June 1991</td>
<td>Left upper lobectomy</td>
<td>LUL stump RML RB6</td>
<td>September 1991</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>August 1992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 18</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
</tr>
<tr>
<td></td>
<td>August 1992</td>
<td>PDT right lower lobe</td>
<td>RB2 RBLB</td>
<td>September 1993</td>
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<tr>
<td>Patient 31</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
</tr>
<tr>
<td></td>
<td>February 1992</td>
<td>None</td>
<td>RBLB</td>
<td>April 1993</td>
<td></td>
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<tr>
<td>Patient 40</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
</tr>
<tr>
<td></td>
<td>June 1992</td>
<td>None</td>
<td>LB1 + 2 LB3 RB7</td>
<td>May 1993</td>
<td></td>
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<td></td>
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<tr>
<td>Patient 78</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
<td>Date of biopsy</td>
<td>Treatment</td>
<td>Bronchial site</td>
</tr>
<tr>
<td></td>
<td>February 1993</td>
<td>PDT, L8 + RMB</td>
<td>LB6 RMB LUL LB3</td>
<td>October 1993</td>
<td>Retinol</td>
<td></td>
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<td>March 1994</td>
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</table>

- Histology: Mod dysp, moderate dysplasia; RCH, reserve cell hyperplasia; CIS, carcinoma in situ. Bronchial sites: RB/LB, right bronchus/left bronchus; RML, right middle lobe; LUL, left upper lobe; RMB, right main bronchus. Molecular analysis: N, not deleted; F, failed to amplify; NI, not informative; NA, not analyzed. PDT, photodynamic therapy.

Fisher’s exact test. Table 1 shows that the frequency of chromosomal alterations significantly increases from the RCH/metaplasia group (1 of 25 chromosome pairs studied) to the dysplasia group (24 of 70), to the CIS group (11 of 15), indicating a progressive accumulation of genetic damages at these three critical chromosomal regions with the progression of the epithelium from one stage to the other.

As shown in Table 1, at least one of these genomic alterations was found in 12% of the metaplasia/RCH samples (1 of 9), 55% of the dysplasia samples (16 of 29), and 100% of the CIS (6 of 6) and the more advanced tumors (11 of 11). No lesion from the RCH/metaplasia group had more than one of the three somatic genetic alteration tested. In contrast, multiple chromosomal abnormalities were frequently seen in more advanced lesions. Nine of the 16 deleted dysplasia cases, 4 of the 6 CIS, and all of the microinvasive or invasive cancers carried 2 or 3 abnormalities.

Molecular Follow-up Analysis. Table 2 shows the results of the molecular follow-up from 18 bronchial sites in 6 patients. These samples included the follow-up of 2 microinvasive cancers, 3 CIS, 8 dysplasia (including 5 moderate dysplasias), and 1 metaplasia samples. Three additional lesions (one CIS and two mild dysplasias), arising in non-previously biopsied bronchi, were diagnosed during the follow-up period. Examples of molecular follow-up data are presented in Fig. 2. With respect to the treatment of each lesion, the regression of the molecular abnormality usually predicted the regression of a CIS or of a dysplastic lesion to a lower grade lesion (case 10, RB8-RML; case 15, RML; case 31, RB8; case 78, LB6-RMB). Conversely, the persistence of the genetic alteration or the appearance of additional genomic damages with time at the same bronchial site was seen in 6 cases during a 3-month to 2-year period (Table 2 and Fig. 2). These alterations were associated with the persistence of the dysplastic epithelium on the following bronchial exploration (case 31, RB6; case 40, LB1 + 2-LB3-RB7; case 78, LB3) or with the progression of the lesion (case 18, RB8).

Discussion

Loss of tumor suppressor gene function plays a major role in the development of many human cancers (26). Previous studies have demonstrated that recurrent hemizygous or homozygous allelic deletions in tumors are indicative of the inactivation of such genes. Progressive loss of tumor suppressor gene functions releases the cells from control growth contributing to the malignant phenotype and are believed to be early events in tumorigenesis. The present study provides information on the frequency of gene losses occurring on chromosomes 3p21–22, 5q21, and 9p21 at the invasive and preinvasive stages of bronchial carcinogenesis, as well as the temporal relationship of these losses during progression from early preneoplastic lesions to CIS and invasive cancers.

Because of the high sensitivity of the microdissection/PCR method used for LOH assessment, the prevalence of 3p21–22, 5q21, and 9p21 losses is likely underestimated. However, the microdissection/PCR method is considered to be the gold standard for the detection of LOH. Therefore, the results of this study are in line with previous findings, suggesting that the loss of tumor suppressor gene function plays a major role in the development of bronchial carcinogenesis.
deletion found in this series is likely to represent the true frequency of alterations occurring in invasive NSCLCs of the proximal bronchi. This underlines the crucial importance of these specific genomic alterations in bronchial tumorigenesis.

Chromosome 3p alterations have been found in all histological subtypes of lung cancers, and previous study has shown that the incidence of 3p deletion is particularly high in small cell cancers (10), but also in other cell types (9, 11). Using the same microdissection PCR/LOH technique, we found, in a separate study, 85% of 3p21–22 deletion in squamous cell and large cell undifferentiated carcinomas in a series of 86 NSCLCs (11), a result consistent with the frequency of 3p deletion found in the present study.

The reported frequency of 9p deletion in invasive NSCLC is also very high (up to 90%) in most cytogenetic (12) and molecular genetic (13, 14) studies. Further evidence implicating chromosome 9p in lung carcinogenesis has been provided by data showing frequent homozygous 9p21 deletions in both small cell and non-small cell bronchial cancers (13, 14) in the region including the *NF-2* and *D9S126* loci. Two tumor suppressor genes, *p16 (MST1)* and *p15 (MST2)*, members of the cyclin-dependent kinase inhibitor family, have been isolated from this region (27) which may be especially involved in non-small cell carcinogenesis (28).

The *MCC* (mutated in colon cancer) and *APC* genes (adenomatous polyposis coli) studied in our series are two known tumor suppressor genes for colon cancers localized in 5q21. LOH of these two gene loci has been frequently detected in NSCLC, where it seems to be more frequently found in squamous cell tumors (16–18). The frequency of 5q21 deletion in NSCLC series varies from 25 to 30% (15, 18) and up to 70% (16, 17). Besides differences in tumor stages between these series, such differences may be related to the purity grades of the examined tumor tissues. To date, the only other study using microdissection to assess 5q21 loss frequency found LOH at the APC locus in 5 of 7 informative squamous cell cancer (17), a result similar to our findings in invasive and microinvasive tumors (7 of 9 informative cases).

Besides the confirmation of consistent 3p deletion, our study also demonstrates 9p21 and 5q21 deletion in 80 and 40% of the bronchial CIS, respectively. The concomitant 3p and 9p21 deletion found in 4 of 5 doubly informative CIS cases of this series is a striking observation, the prognostic importance of which has to be explored further. In contrast, 5q21 deletion was found more frequently in invasive carcinoma, as compared to CIS and lower grade lesions, appearing to be a later event than the 3p and 9p alterations. Recently, the APC protein has been found to be associated with catenins, molecules known to be involved in cell adhesion (29), suggesting that the alteration of the APC gene may influence invasion and metastatic capabilities of the tumor. It is therefore possible that bronchial CISs with 3p, 9p, and 5q deletions behave more aggressively than the...
non-Sq-deleted ones. It was of interest that the only CIS of our series displaying all three alterations progressed into an invasive cancer after 1 year follow-up, despite endobronchial photodynamic therapy. If confirmed in future longitudinal studies of bronchial CIS, this observation may be of great prognostic and therapeutic importance.

To date, the relationship between dysplastic lesions and bronchial cancers appears more complex. The reversibility of bronchial cell atypia, that may occur spontaneously or after smoking cessation, has been well documented both in animal models and in humans (3, 30), and confirmed by the follow-up data presented here. On the other hand, the probability that a dysplastic lesion will progress to invasive cancer is still unknown. However, emerging evidence, including the high frequency of genomic alterations found in the dysplastic samples of the present study, indicates that the same genetic mechanisms contribute to the emergence of both dysplasias and invasive cancers of the bronchus. Further proof of the relationship between genomic damages and the occurrence of dysplasia is shown by our findings that the same genomic alteration can persist in a given dysplastic bronchial area for several months or years, and that the persistence or the regression of the molecular abnormality is well correlated with the evolution of the disease on follow-up. Our observation of cumulative gene losses with the progression from one preinvasive stage to another also lends strong support to the multistep carcinogenesis theory of the bronchial epithelium. However, longitudinal studies in a larger population with a longer follow-up period than the present study are needed to determine whether dysplastic lesions with 3p, 5q, or 9p alterations are the ones that would progress to invasive cancer. Other nonrandom chromosomal gain or losses involving chromosomes 1, 7, 11, and 17 have been recorded by cytogenetic and molecular analysis in lung cancer (7) that may also contribute to the carcinogenesis of the bronchial epithelium.

In marked contrast to the dysplastic samples, LOH was very rarely observed in the RCH/metaplasia cases. This may indicate that the first preneoplastic modifications observed in the bronchial epithelium are related to epigenetic changes more than true genetic alterations. Such changes leading to an hyperproliferative state have been shown in tracheal epithelial cell culture after carcinogen exposure and may facilitate the occurrence of further genetic damages (31). Alternatively, although tissue microdissection allows the study of histologically defined cell types, we cannot exclude that the low LOH frequency in metaplasia and RCH samples is linked to the polyclonal nature of these proliferations, which can have masked premalignant cell-specific LOH.

To our knowledge, only one other series of low-grade preneoplastic lesions has been assessed for LOH, but this study assessed only lung cancer patients, and mostly bronchiolar or alveolar preneoplastic lesions. In this series, 3p deletions were found in 11 of 20 hyperplasia/metaplasia cases (20). This discrepancy may be due to the fact that most of our subjects (10 of 13) were noncancerous smokers or former smokers, suggesting differences in metaplasia/hyperplasia lesions between cancer and noncancer patients. Because 9 of 10 chromosome 3p-deleted hyperplasia cases were sampled in bronchioles in the previous study (20), this may also indicate differences in tumorigenesis between bronchial and bronchiolar epithelia.

In summary, our data are consistent with the simple serial model of bronchial carcinogenesis predicted by the studies of Auerbach et al. (4) and Saccomanno et al. (5) 30 years ago, where bronchial cancers arise from the progressive development of preneoplastic lesions of increasing severity. At the molecular level, our data also suggest that 3p and 9p deletions are earlier genetic events than 5q deletions. However, the identical frequency (30%) of 3p, 5q, and 9p losses in dysplasia samples indicates that none of these three alterations is restricted to a particular stage of bronchial carcinogenesis. Such findings have also been described in colonic adenomas (6), suggesting that the accumulation of specific molecular changes, or their association, is more important than their order of occurrence with respect to one another.

Further molecular studies are needed to better characterize the genetic alterations that define the malignancy of the bronchial epithelium, as well as to identify critical factors, or association of factors, that would be predictive of the progression of preneoplastic lesions to invasive cancers. In conjunction with very sensitive methods of endobronchial detection of the preneoplastic foci, such studies may help to develop a more effective strategy for the management of this highly fatal disease.

Note Added in Proof

After submission of the present paper to Cancer Research, Kishimoto et al. (32) published the results of the 9p21 LOH assessment in their series of preneoplastic lesions accompanying lung cancers previously studied for 3p deletions (20). This study, which mostly assessed bronchiolar and alveolar lesions, also showed a very high frequency of 9p21 deletions in preinvasive lesions beginning at the stage of hyperplasia.

References


Evidence of Cumulative Gene Losses with Progression of Premalignant Epithelial Lesions to Carcinoma of the Bronchus

Luc Thiberville, Peter Payne, Jürgen Vielkinds, et al.