Chromosomal Duplication Accompanies Allelic Loss in Non-Small Cell Lung Carcinoma

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ABSTRACT

Hemizygous deletion in the short (p) arm of chromosome 3 is a common finding in non-small cell lung carcinoma (NSCLC) and is postulated to be a crucial early change in lung tumorigenesis. Yet one of the most frequent nuclear abnormalities in both NSCLC and premalignant bronchial epithelium is increase in chromosomal copy number. Deletion and duplication have not been assessed in the same tumor set by both molecular and cytogenetic methods to determine whether allelic loss correlates with chromosomal duplication in the same tumor cell populations. It is also not established what biological mechanisms might lead to allelic deletion and chromosomal duplication. We have investigated changes in the copy number of chromosome 3 in touch preparations of 38 NSCLCs (19 adenocarcinomas and 19 squamous cell carcinomas) using FISH, molecular hybridization (FISH) assays. Chromosome 3 centromere probe was matched with a 3p14.2 probe [intron 4 of the fragile histidine triad (FHIT) gene] and a 3p13.1 probe (H.SemalV gene). We then correlated FISH results with results of molecular analyses for allelic losses at loci in the regions to which the FISH probes mapped in 20 of these cases. Although various combinations of FISH abnormalities were sometimes detected within the same specimens, individual cases could be classified according to the predominant FISH pattern, usually with one abnormality present in >60% of tumor cells. Chromosomal duplication, indicated by the presence of more than two centromeric signals, was the most frequent abnormality observed by FISH and was accompanied by loss of specific sequences on 3p in approximately one-half of the specimens in which it was observed. The most frequent abnormality observed by molecular analysis was loss of heterozygosity (LOH) in both of the chromosomal regions tested and was demonstrated in 83% of cases with chromosomal duplication. We conclude that LOH may occur in the presence of chromosomal duplication, suggesting that the duplicated chromosome is homozygous. Our findings imply that LOH occurs before chromosomal duplication during lung carcinogenesis.

INTRODUCTION

Hemizygous deletions in the short (p) arm of chromosome 3 are a nearly constant feature of lung carcinoma and occur in both small cell carcinoma and NSCLC3 (reviewed in Ref. 1). Loss of 3p has been demonstrated in 25–100% of NSCLCs by both karyotypic analysis (2–7) and molecular methods including RFLP (8–15) and LOH at polymorphic microsatellite tandem repeats (16, 17). Recent studies suggest that 3p deletions are early events in lung carcinogenesis, occurring in airway epithelium of smokers before and in the absence of invasive lung carcinoma (18–23).

Despite hemizygous loss of 3p in NSCLC, >80% of these tumors are aneuploid, as determined by flow cytometry (24–31), and FISH studies have revealed elevated chromosome 3 copy numbers (32). These somewhat paradoxical findings suggest that the duplicated chromosome 3 NSCLC may be homozygous. However, the question of whether abnormal chromosome 3 copy number correlates with molecular loss on the short arm of the chromosome in the same tumor set has not been addressed directly.

In the present study, we applied dual-color FISH to investigate deletions in 3p14.2 [intron 4 of the FHIT gene] and 3p21.31 (H. SemalV gene) in NSCLC tumors. We found frequent increases in copy number of chromosome 3, often in association with specific allelic loss. When we compared patterns of hybridization determined by FISH with results of molecular analyses for LOH, we found that LOH may be present, although FISH analysis may indicate chromosomal gain.

MATERIALS AND METHODS

Tumor Imprints. Touch preparations were obtained by imprinting freshly resected lung tumors onto silanized slides. Slides were methanol fixed after drying overnight and were stored at −80°C until use. One Wright-stained slide was examined microscopically for distribution and concentration of tumor cells in relation to stromal cells and for the state of morphological preservation of tumor cells to guide FISH analysis. Imprints with at least 50% well-preserved tumor cells were selected for the hybridization assays. Touch preparations from lung tissue without tumor cells were used as controls for each experiment.

Preparation of Slides for FISH. Touch preparations were brought to room temperature in a dry atmosphere to minimize moisture condensation. Slides were then sequentially digested with RNase A (Sigma Corp.) and pepsin (Sigma Corp.), postfixed in 4% buffered formalin, washed in PBS, dehydrated in graded alcohols, and denatured in 70% formamide at 37°C for 4 min.

DNA Probes and Labeling. Two pairs of cosmids probes were used to analyze chromosomal regions 3p14.2 and 3p21.31. Overlapping cosmids clones c64D1 and c81E9, spanning ~75 kb, were used for 3p14.2. These cosmids map to intron 4 of the FHIT gene and include locus D3S2723 but are proximal to D3S1300 (33). For 3p21.31, overlapping cosmids clones c59H8 and c98A10 were used. These clones map to a region within the H.SemalV gene that is homozygously deleted in small cell lung carcinoma cell lines NCI-H740 and NCI-H450 (34). Cosmid DNAs were isolated by alkaline lysis (35) and individually labeled with biotin-14-dATP (BioNick Labeling System; Life Technologies, Gaithersburg, MD). Similar amounts of the pair of labeled cosmids maps to each region were ethanol precipitated in the presence of salmon sperm and human Cot-1 DNA (Life Technologies). This DNA mixture was dissolved in hybridization buffer (50% formalin, 10% dextran sulfate, 1% w/v Tween 20, and 2× SSC, pH 7.0), denatured at 75°C for 10 min, and incubated at 37°C for 30 min for renaturation of the repetitive sequences. For determination of the chromosome 3 copy number, a specific digoxigenin-labeled α-satellite DNA (D3Z1; Oncor, Gaithersburg, MD) was used. The probe was dissolved in hybridization buffer (50% formamide/2× SSC [1× SSC = 150 mM NaCl, and 15 μM sodium citrate (pH 7.0)] and in 2× SSC for 15 min each,
followed by a wash in 0.3× SSC at 60°C for 5 min. Dual-color detection (biotin-FITC, digoxigenin/rhodamine) was performed at 37°C and consisted of consecutive 30-min incubations with mixtures of: (a) FITC-avidin (2.0 μg/ml; Vector Laboratories, Burlingame, CA) and mouse anti-digoxigenin (0.2 μg/ml; Boehringer Mannheim Laboratories, Indianapolis, IN); (b) biotinylated goat anti-avidin (2.5 μg/ml; Vector Laboratories) and sheep anti-mouse immunoglobulin (20 μg/ml; Boehringer Mannheim); and (c) FITC-avidin (2.0 μg/ml; Vector Laboratories) and mouse anti-digoxigenin rhodamine (20 μg/ml; Boehringer Mannheim Laboratories). Each incubation was followed by three 5-min washes in 0.1 M Tris-HCl (pH 7.5), 0.15 M NaCl, and 0.05% Tween 20 at 37°C. On completion of the detection procedures, slides were washed in PBS, dehydrated in ethanol series, air dried, and counterstained with DAPI solution in DABCO Antifade. Cosmid and α-centromeric probes were tested in the normal lymphoblastoid cell line GM09948 (Coriell Institute Cell Repository) to determine their reliability as FISH probes. Hybridization efficiency was evaluated as the proportion of observed to expected signals (number of analyzed cells × 2) and was estimated to be 93% for the 3p14.2 cosmid probes, 94% for the 3p21.31 cosmid probes, and 97% for D3Z1.

**PCR Protocol.** Tumor imprints used in PCR analyses were made from surgical resection specimens at the same time as the imprints used for FISH analysis. Hematoxylin-stained cells were microdissected from the imprints under 95% glycerin using a dissecting microscope and hand-drawn glass micropipettes attached to a foot-controlled vacuum pump. Microdissected cells were washed in proteinase digestion buffer, and DNA was extracted using a Qiagen QIAamp Tissue kit (Qiagen, Inc., Santa Clarita, CA).

Two PCR primer sets mapping to within 40 kb of the 3p14.2 and 3p21.31 regions recognized by the FISH cosmids probes were used to assess LOH in the short arm of chromosome 3. Primer sets for the 3p14.2 region included D3S1300 and D3S1481, whereas primer sets for the 3p21.31 region included D3S2968 and D3S1573. Peripheral blood DNAs were washed in proteinase digestion buffer, and DNA was extracted using a Qiagen QIAamp Tissue kit (Qiagen, Inc., Santa Clarita, CA). The second-round reaction mixture contained a 5' biotinylated forward primer (Molecular Resources, Inc.). Two amplification protocols were used. In both protocols, concentrations of template DNA were adjusted to ~200 pg in a final volume of 10 μl. For efficient primers, first round of thermal cycling began at 94°C for 10 min, followed by 10 amplification rounds consisting of denaturation at 94°C for 30 s, annealing at 72°C for 30 s, extension at 72°C for 75 s, and finishing with 8 min at 72°C. This first round was followed by a second in which 3 μl of reaction mix were added to additional primers, Taq polymerase, and other reagents, and a second amplification round commenced. This second round was begun at 94°C for 10 min, followed by 20 touchdown cycles of 94°C for 30 s, annealing at 82°C (10°C above ideal annealing temperature) for 30 s with a 0.5°C drop in temperature per cycle to finish at the optimal annealing temperature, and extension at 72°C for 75 s. During the touchdown cycles, only sequences that bind primers at high stringency are amplified, resulting in high levels of specific product and low non-specific background amplification. The touchdown cycles were followed by 10 cycles of denaturation at 94°C for 30 s, annealing at the optimal temperature for each primer set (50–55°C) for 30 s, and extension at 72°C for 75 s. The program finished with 8 min at 72°C. For less efficient primers, the first round consisted of 15 cycles rather than 10, and the second round consisted of 35 cycles (20 touchdown cycles plus 15 extension cycles). PCR products were electrophoresed on a denaturing sequencing gel (7% acrylamide, 7 M urea, and 35% formamide). Polycrylamide gels were blotted on an Immobilon S nylon membrane (Millipore Corp., Bedford, MA) incorporating streptavidin, biotinylated alkaline phosphatase, and 1,2-dioxetane substrate. Bands were visualized by exposure of membranes to X-ray film (Fuji RX; Fuji Medical Systems, Stamford, CT) in an X-ray cassette for 2–10 min.

The PCR protocol was designed to optimize signal from a small quantity of DNA. Preliminary experiments indicated that the primers used in this study were of variable efficiency in generating PCR product from a standard quantity of DNA. To avoid the possibility of artificially induced allelic imbalance, serial dilutions of template DNA ranging from 20 pg to 200 ng were amplified, and the number of cycles per round required to produce uniform PCR bands at 200 pg (~25 diploid cell equivalents) was determined. Loss was defined as a reduction of band density of 50% relative to both the retained allele and normal blood alleles. A water blank control was included in all standardization tests, and a consistently negative result in this control was a requirement for acceptance of a particular protocol for testing of clinical material.

**RESULTS**

**FISH.** Imprints from a total of 38 NSCLCs (19 adenocarcinomas and 19 squamous carcinomas) were analyzed by FISH. In 30 cases, probes for both 3p14.2 and 3p21.31 regions were successfully applied to a sufficient number of cells. In the remaining eight cases, sufficient tumor cells were available for analysis of only one of the two regions. A total of 31 tumors were analyzed with the 3p14.2 probes and 37 with the 3p21.31 probes. Imprints from nine normal lung specimens provided a measure of background variation inherent in the FISH procedures (data not shown). The mean percentage of aneuploid cells in normal lung imprints was 9.1%, with a SD of 2.5% and SE of 0.7%. Fifteen % (mean percentage of aneuploid cells in normal lung + 2SD) was therefore set as the upper limit of normal.

Thirty-six of the 38 tumors examined (95%) exhibited a 3p abnormality in >15% of cells, and in 33 tumors (89% of adenocarcinomas and 84% of squamous carcinomas), >60% of tumor cells were abnormal. Six patterns of gain or loss could be recognized by relating the number of specific allelic signals to the number of centromeric signals. The designations of the various patterns are indicated in Table 1. The presence of only one centromeric signal indicated monosomy. Monosomy could potentially have existed in association with specific allelic loss, which would have indicated homozygous loss. In fact, monosomy with specific loss was not observed as a dominant pattern in this series. Two centromeric signals indicated disomy, which was occasionally associated with specific allelic loss. Finally, a dominant pattern of more than two centromeric signals was interpreted as an indication of chromosomal duplication, which may or may not be accompanied by specific allelic loss.

Examples of chromosomal duplication are depicted in Fig. 1. The incidences of the various patterns of loss and duplication are graphically represented in Fig. 2 and 3. As indicated in these graphs,

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<table>
<thead>
<tr>
<th>Allelic signal</th>
<th>Monosomy/ Homozygous detection</th>
<th>Specific loss</th>
<th>Duplication/ Specific loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; centromere</td>
<td>Monosomy</td>
<td>Specific loss</td>
<td>Duplication</td>
</tr>
<tr>
<td>= centromere</td>
<td>Monosomy</td>
<td>Disomy</td>
<td>Duplication</td>
</tr>
</tbody>
</table>
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Table 1 Two-color FISH in NSCLC patterns of chromosomal loss
Fig. 1. Chromosome 3 abnormalities detected by α-satellite centromere-specific probe (red) and cosmid probes mapped to 3p21.3 or 3p14.2 (green) including: A, specific 3p21 loss (one green signal and two red signals) in an adenocarcinoma; B, duplication with specific loss (four green signals and eight red signals) of 3p21.3 in a squamous carcinoma; C, duplication with specific loss (four green signals and seven red signals) of 3p14.2 in an adenocarcinoma; and D, duplication without 3p14.2 loss (four green signals and four red signals) in a squamous carcinoma.

Fig. 2. Predominant patterns of chromosome 3/3p21.31 anomaly detected by FISH in 31 NSCLCs. The most frequent patterns of loss are duplication and duplication with specific loss.

duplication of the chromosome with or without specific loss was the most common abnormality found in this series of tumors, occurring in 27 of 38 cases in at least one chromosomal region. Specific loss in disomic tumor cells was the dominant pattern in 5 of 38 cases, whereas disomy without detectable loss was found in two cases. Finally, monosomy was the dominant pattern in four cases, although it was found together with other dominant abnormalities in three additional cases.

A high degree of concordance was found between results for the separate chromosomal regions. For all 30 cases in which specimens were adequate for evaluation of both chromosomal regions, there was 100% concordance between dominant FISH patterns for 3p14.2 and 3p21.31 (Table 2). Intratumoral heterogeneity in which two distinct abnormal cell populations were present at frequencies higher than 15% was observed in eight specimens (21% of the adenocarcinomas and 21% of the squamous carcinomas). Secondary FISH patterns were also concordant in all cases. The two FISH patterns of each heterogeneous tumor are listed in Table 3. Specimens with a population of tumor cells bearing homozygous losses listed in the table were both adenocarcinomas, and both deletions were detected in the 3p21.31 region. In these two specimens, a FISH assay was also performed with a probe mapped distal to 3p31.3 (PAC197c13), and only hemizygous deletion was detected at this region.

There were few differences between the two different types of NSCLC, adenocarcinoma and squamous carcinoma, evaluated in this study. Fig. 4 compares the patterns of loss in adenocarcinoma and squamous carcinoma. Duplication was slightly more common in adenocarcinoma than in squamous carcinoma, but the numbers of cases...
Table 2 Concordance of FISH results: 3pl4.2 versus 3pl21.31

<table>
<thead>
<tr>
<th>3p21.31</th>
<th>Disomy</th>
<th>Duplication</th>
<th>Specific loss</th>
<th>Monosomy</th>
<th>Specific loss</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>3pl4.2</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Duplication</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Specific loss</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Monosomy</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
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</tr>
<tr>
<td>Specific loss</td>
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<td>0</td>
<td>5</td>
<td>5</td>
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</tr>
<tr>
<td>Totals</td>
<td>1</td>
<td>8</td>
<td>13</td>
<td>3</td>
<td>5</td>
<td>30</td>
</tr>
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</table>

Table 3 Tumors with mixtures of abnormal cells: Dominant and secondary FISH patterns

<table>
<thead>
<tr>
<th>Case</th>
<th>Dominant pattern (% tumor cells)</th>
<th>Secondary pattern (% tumor cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Duplication with specific loss (60%)</td>
<td>Monosomy (40%)</td>
</tr>
<tr>
<td>2</td>
<td>Specific loss, homozygous (3p21.31 only, 50%)</td>
<td>Specific loss, hemizygous (40%)</td>
</tr>
<tr>
<td>3</td>
<td>Specific loss, hemizygous (50%)</td>
<td>Specific loss, homozygous (3p21.31 only, 40%)</td>
</tr>
<tr>
<td>4</td>
<td>Duplication (70%)</td>
<td>Duplication with specific loss (30%)</td>
</tr>
<tr>
<td>5</td>
<td>Duplication (50%)</td>
<td>Duplication with specific loss (40%)</td>
</tr>
<tr>
<td>6</td>
<td>Specific loss, hemizygous (60%)</td>
<td>Duplication (30%)</td>
</tr>
<tr>
<td>7</td>
<td>Duplication (70%)</td>
<td>Monosomy (20%)</td>
</tr>
<tr>
<td>8</td>
<td>Duplication with specific loss (70%)</td>
<td>Monosomy (20%)</td>
</tr>
</tbody>
</table>

DISCUSSION

This study documents the frequent association of chromosome 3 duplication with LOH at specific polymorphic sites on chromosome 3p. The most frequently observed FISH alteration in this series of tumors was an increase in chromosome 3 copy number. This abnormality occurred in 71% of all lung cancers and was found in a high percentage of both adenocarcinomas and squamous carcinomas. Specific allelic loss was detectable by FISH in 13 of the 27 specimens in which chromosomal duplication was observed. In only a small minority of tumors (5 of 38) did specific loss occur without chromosome duplication. In a large minority of cases (14 of 38), duplication occurred without specific loss. Disomy without specific loss was uncommon (two cases). Microsatellite analysis determined that LOH was present in 83% (15 of 18 cases) of informative tumors in which chromosomal gain was observed. In most cases of LOH, no normal allelic band was found in electrophoretic gels, suggesting that allelic loss was complete.

LOH was not predictive of FISH results because specific allelic loss could be detected by FISH in only 56% of specimens exhibiting LOH by microsatellite analysis. This finding is consistent with the idea that evaluating each category were small, and their biological and statistical significances were unclear.

Molecular Results. At least one of the four polymorphic markers tested in this series was informative in 19 of the 20 tumors. Fig. 5 shows representative results for a squamous carcinoma and an adenocarcinoma. There was complete agreement of testing results among the various markers within chromosomal regions and nearly complete agreement between regions (Table 4). Because of this concordance, all markers could be considered together in comparing molecular to FISH results. As indicated in Table 5, LOH was detected in at least one marker in 16 of the 19 cases. Duplication or duplication with specific loss was the predominant FISH result occurring in 15 of these 16 cases. LOH was detected in all but three of the cases examined, and all informative sites tested in these three cases were heterozygous.

Fig. 4. Comparison of chromosome 3p21.31 anomalies detected by FISH in adenocarcinoma and squamous cell carcinoma. Slightly more cases of duplication were observed in adenocarcinomas than in squamous carcinomas, and monosomy was slightly more frequent in squamous carcinoma. Otherwise, FISH patterns were similar for the two tumor types.

Fig. 5. Patterns of LOH on chromosome 3p at 3 informative sites are shown for an adenocarcinoma on the right and a squamous carcinoma on the left. T, tumor lanes; N, lanes containing amplification products of nonneoplastic lung cells (including inflammatory cells). Single alleles (arrows) are lost from tumor DNA at all informative loci.

Table 4 Loss of heterozygosity in NSCLC

<table>
<thead>
<tr>
<th>p14.2</th>
<th>Loss</th>
<th>No loss</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss</td>
<td>11</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>No loss</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>11</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 5 Molecular analysis versus FISH NSCLC

<table>
<thead>
<tr>
<th>Molecular results (LOH)</th>
<th>FISH results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplication</td>
<td>Duplication + Specific loss</td>
</tr>
<tr>
<td>Loss</td>
<td>7</td>
</tr>
<tr>
<td>Retention</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>9</td>
</tr>
</tbody>
</table>
(discussed below) that chromosome 3 may have been reduced to homozygosity early in lung carcinogenesis, and the homozygous chromosome may have been duplicated, resulting in two or more copies of the same homologue, the homozygosity of which would be undetectable by FISH. An alternative explanation is that the region of loss detected by PCR was too small to be detected by FISH. This seems unlikely because there was a high degree of correlation of PCR results within and between chromosomal regions, suggesting that loss involved a large part of the chromosome.

Few previous studies have directly and systematically addressed chromosome 3 copy numbers in NSCLC, and only one of these studies has evaluated interphase tumor cells by FISH (32). Most of these previous studies have found that virtually all NSCLC are aneuploid and that their karyotypic profiles are complex (6, 36, 37). Increase in chromosome 3 copy number is frequent and is proportional to the high degree aneuploidy that exists in these tumors. In the single FISH study that is thus far reported, mean chromosome 3 copy number ranged from 2.5 to 4.7 and was associated with comparably increased numbers of chromosomes 8, 11, 12, 17, and 18 (32). The present study thus is consistent with the high degree of aneuploidy observed previously in NSCLC.

Structural losses in the short arm of chromosome 3 are commonly found in NSCLC. Since the original descriptions of hemizygous 3p deletion in small cell carcinoma by Whang-Peng et al. (38, 39), several cytogenetic studies have documented frequent loss at 3p in NSCLC as well (6, 36, 37). Only a single analysis has used FISH (17), and this primarily to confirm the presence of homozygous loss in a small number of NSCLCs.

In considering possible mechanisms by which LOH could occur in the presence of increased chromosome 3 copy number, it seems probable that LOH occurred first. If duplication were to have occurred first, allelic loss would have had to affect multiple duplicate chromosomes, which seems unlikely. More probably, LOH occurred before duplication, resulting in multiple copies of the same homologous chromosome. Two possible explanations for the patterns of loss and duplication are illustrated in the accompanying diagram (Fig. 6). In one, replication of only one chromosome or chromosomal region occurs. Monosomy permits expression of a mutant cellular phenotype and may result in a deleterious dosage effect on other genes, in particular the housekeeping genes. The duplication of the remaining homologue may be important to maintain homeostasis for these genes. Classical cytogenetics and FISH techniques cannot differentiate cells displaying normal disomic status from cells displaying two copies of the same homologue after a chromosome loss and endoreduplication. In contrast, molecular genetic analysis can allow for the detection of allelic loss. Additional series of chromosome losses and endoreduplications will generate near-triploid and near-tetraploid cells, always maintaining allelic loss. The second possibility is that specific loss occurs early and that the abnormal chromosome pair replicate abnormally, resulting in duplication with specific loss in cell progeny.

This hypothesis of chromosome loss and reduplication in epithelial tumorigenesis is supported by studies of tumors from several other organs besides lung. Dutrillaux (40) has postulated that in colorectal, breast, and NSCLCs, the first clonally abnormal cells are hypodiploid, with loss of entire chromosomes or chromosomal arms. These hypodiploid cells are proposed to undergo progressive endoreduplication, leading to the formation of hypotetraploid subclones. The coexistence of hypodiploid and hypotetraploid clones has been confirmed in colorectal tumor cells by Muleris et al. (41, 42) and in a lung squamous carcinoma cell line by Drouin et al. (43). Severe hypodiploidy or near-haploidy in epithelial tumors could be more frequent than reported by cytogenetic analysis, because tumor cells are mostly karyotyped at a late stage in tumor progression, when the haploid cells have been replaced by the hyperdiploid or polyploid cells.

Our findings are also consistent with current understanding of lung carcinogenesis in which inactivation of tumor suppressor genes is recognized as a major step. Gene inactivation mechanisms include physical deletion, point mutation, or methylation. In lung tumors, putative suppressor genes including p16/MTSI and p15/MTS2 are preferentially lost by homozygous deletion on chromosome 9 (44–47). Conversely, p53 in chromosome 17p, Rbl in chromosome 13q, and APC in chromosome 5q are frequently inactivated by a combination of allelic deletion and usually point mutations in the remaining allele (48–50).

The high frequency of hemizygous genetic loss at 3p has suggested that this region may harbor one or more tumor suppressor genes that may be inactivated by mutation or homozygous loss in the morphologically intact homologous chromosome. Regions suspected of harboring tumor suppressor genes include the two regions tested in this study, 3p14.2 and 3p21.31. In lung, LOH has been consistently demonstrated at 3p14.2 in both invasive carcinomas and preneoplastic lesions (22, 51–53), and homozygous deletions have been found in lung carcinoma cell lines (22, 51). 3p14.2 also has been found to harbor the most common inducible fragile site in the human genome, FRA3B (54), suggesting that genomic instability may explain the high frequency of deletions in 3p14. A possible tumor suppressor gene located at 3p14.2 is the FHIT gene, which codes for a protein with hydrolase activity. This gene was first proposed as a tumor suppressor candidate in digestive tract tumors by Ohta et al. (55). In lung tumors, Sozzi et al. (56) have described aberrant FHIT transcripts in both NSCLC and small cell lung carcinoma, and transfer of the wild-type FHIT gene into lung tumor cells is reported to suppress tumor growth (57). However, transfer of hydrolase-inactive mutant FHIT gene also suppresses tumor growth (57), and the suppressive effect of FHIT gene transfer on tumor cell proliferation, tumor cell colony formation, and in vivo tumor formation have not been confirmed (58), leaving the role of the FHIT gene in lung carcinogenesis to yet be clearly established.

A second region of consistent loss in lung cancer and preneoplastic
epithelium is 3p21.31. Homozygous loss at 3p21.31 has been reported in three small cell lung carcinoma cell lines (34, 59–61). This is a highly gene-rich region (62), and genes mapped to this region include GA1TI (63) and GA112 (64) coding for GTP-binding, retinal-specific, α-transducing proteins; 3 pk (65) coding for a mitogen-activated protein kinase-activated protein kinase kinase; HsEna IV and two semaphorin genes, (34, 66) and HsEna V (67). To date, no tumor-associated mutations have been found in these genes, and their roles in lung carcinogenesis also remain to be determined.

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