A Frequent Deletion of Chromosome 5q21 in Advanced Small Cell and Non-Small Cell Carcinoma of the Lung

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ABSTRACT

We have examined the deletion of the long arm of chromosome 5 (5q) in 59 cases of advanced lung cancer [39 cases of small cell lung cancer (SCLC) and 20 cases of non-SCLC] using 12 restriction fragment length polymorphism markers on 5q. Of 59 lung cancer cases, 48 (81%) exhibited deletion at any portion of the 5q locus (loci). Such a high frequency of 5q deletion has not been reported in surgically resectable non-SCLC. One SCLC case showed a 5q deletion only in metastatic sites but not in the primary cancer. These data suggest that the inactivation of putative tumor-suppressor genes(s) on 5q may be a late event in the progression of lung cancer. There was no significant difference in frequency of 5q deletion between SCLC and non-SCLC. Compared to non-SCLC, however, SCLC usually showed widespread deletion on 5q. While the most frequent target region was estimated to be about 3-5 megabases at 5q21 around the adenomatous polyposis coli (APC) gene locus, some cases showed more telomeric deletion (5q33-35), suggesting that there are at least two different tumor-suppressor genes on 5q associated with the progression of lung cancer.

INTRODUCTION

Tumorigenesis is a multistep process of genetic abnormalities involving the functional inactivation of tumor-suppressor genes as well as the activation of oncogenes (1). There has been increasing evidence for the functional inactivation of tumor-suppressor genes in many neoplasms (2-5). Homozygous functional inactivation of a tumor-suppressor gene is often accomplished by mutations in one copy of the gene combined with physical loss of the chromosomal region harboring the remaining allele of the gene. Comparison of constitutional and tumor genotypes by RFLP analysis has revealed that allelic deletions occur on specific chromosomes in a variety of human malignancies. In lung cancer, common targets of the allelic loss are 3p (6-9), 11p (10), 13q (9), and 17p (9). Furthermore, mutations in the remaining allele including the Rb gene on 13q and the p53 gene on 17p have frequently been detected in lung cancer (11, 12). These genetic abnormalities seem to be involved in the development of lung cancer, because these findings could be detected even at the early stage of the malignancy.

Based on the differences in prognosis, resectability, and chemoradiosensitivity, lung cancer is divided grossly into SCLC and non-SCLC. SCLC is a highly malignant neoplasm, and its rapid growth rate and high metastasizing ability preclude surgical cure, while non-SCLC is a disease entity of surgery because of its lower grade malignant phenotypes than those of SCLC. The chromosomal abnormalities described above are seen almost equally in both SCLC and non-SCLC. SCLC is a highly malignant neoplasm, and its rapid growth rate and high metastasizing ability preclude surgical cure, while non-SCLC is a disease entity of surgery because of its lower grade malignant phenotypes than those of SCLC. The chromosomal abnormalities described above are seen almost equally in both SCLC and non-SCLC. SCLC has been shown in masses obtained at early but not advanced stages.

To investigate these possibilities, this study was undertaken using tumor specimens of SCLC and non-SCLC at advanced stages. In this study, we detected 48 of 59 (81%) advanced lung cancer cases [34 of 39 (87%) SCLC cases, 14 of 20 (70%) non-SCLC cases] in which a deletion at one or more loci on 5q was found. There was no significant difference in the frequency of 5q loss between SCLC and non-SCLC. However, SCLC usually has widespread 5q deletion compared to non-SCLC. The common region of deletion lies 3-5 megabases at 5q21 around the APC locus. In addition to frequent loss of this region, 5q33-35 was considered to be another target in some cases.

MATERIALS AND METHODS

Samples. Primary tumors and corresponding normal tissues were obtained at autopsy and stored at -80°C until DNA extraction. Tissues from metastatic sites were obtained from an SCLC case (case 6) at the same time. All cases were categorized as stage IIIb or IV, according to the criteria of the Japan Lung Cancer Society (17).

DNA Extraction and Southern Blotting. Minced frozen tissues were transferred into lysis buffer. After treatment with proteinase K, DNAs were digested with appropriate restriction enzymes (Bethesda Research Laboratories, Inc., Bethesda, MD) and separated by O.H% agarose gel electrophoresis. Southern blot analysis was carried out according to methods described previously (18). Allelic loss was considered when the signal intensity in tumor tissue was <50% of that in normal tissue by densitometric analysis.

RFLP Markers. RFLP markers used in this study are listed in Table 1. LMS8 was a gift from Dr. Arcine Jeffreys. L45 was purchased from Collaborative Research, Inc. (Bedford, MA) through the Health Science Research Institute (Yokohama, Japan). PHF12-65 and p1227 were provided by the Japanese Cancer Research Resource Bank. New polymorphisms of two markers are described in Table 2. These markers have been mapped by fluorescence in situ hybridization (19).

RESULTS

Deletion Map of 5q in Advanced Lung Cancer. From 59 advanced lung cancer specimens (39 SCLC and 20 non-SCLC), the deletion map shown in Fig. 1 was created using 12 RFLP markers on 5q. All markers used in this study (Table 1) have been published elsewhere and mapped to 5q. The orders of these markers were estimated using data from the references listed in Table 1. New polymorphisms of two markers, cC15-7 and cC15-22, have been mapped by fluorescence in situ hybridization to 5q13 and 5q23.
Table 1. RFLP markers on 5q used in this study

<table>
<thead>
<tr>
<th>Markers</th>
<th>Locus</th>
<th>Chromosomal location</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cCI5-7</td>
<td>DSS37</td>
<td>q13</td>
<td>19</td>
</tr>
<tr>
<td>p227</td>
<td>DSS85</td>
<td>q21</td>
<td>13, 30</td>
</tr>
<tr>
<td>KK5.33</td>
<td>DSS141</td>
<td>q21</td>
<td>13, 31, 32</td>
</tr>
<tr>
<td>L5.71</td>
<td>DSS141</td>
<td>q21</td>
<td>4, 5, 33</td>
</tr>
<tr>
<td>EF5.44</td>
<td>DSS141</td>
<td>q21</td>
<td>4, 33</td>
</tr>
<tr>
<td>YN5.48</td>
<td>DSS141</td>
<td>q21</td>
<td>4, 13, 32, 33</td>
</tr>
<tr>
<td>L5.53</td>
<td>DSS141</td>
<td>q21</td>
<td>33</td>
</tr>
<tr>
<td>MC5.61</td>
<td>DSS64</td>
<td>q21</td>
<td>30, 31, 32</td>
</tr>
<tr>
<td>cCI5-222</td>
<td>DSS64</td>
<td>q21</td>
<td>30, 31</td>
</tr>
<tr>
<td>L4.5</td>
<td>DSS61</td>
<td>q33-34</td>
<td>30, 31</td>
</tr>
<tr>
<td>pH12-65</td>
<td>DSS2</td>
<td>q34</td>
<td>30, 31, 34</td>
</tr>
<tr>
<td>LMS8</td>
<td>DSS43</td>
<td>q35</td>
<td>30, 31, 34</td>
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Table 2. New polymorphisms of two 5q markers

<table>
<thead>
<tr>
<th>Physical location</th>
<th>Enzyme-detected polymorphism</th>
<th>Allele size (kilobases)</th>
<th>Allele frequency</th>
<th>% Frequency of heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>cCI5-7 5q13.2-13.3</td>
<td>Pvu II</td>
<td>A1</td>
<td>9.0</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>7.5</td>
<td>0.34</td>
</tr>
<tr>
<td>cCI5-222 5q23.1-23.2</td>
<td>Msp I</td>
<td>A1</td>
<td>8.5</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>6.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Determined by fluorescence in situ hybridization (19).
* Calculated among 128 chromosomes from 64 unrelated individuals.
* Also polymorphic with Hind III, not polymorphic with Taq I, Msp I, Bam HI, and Pst I.
* Not polymorphic with Pvu II, Hind III, Taq I, Bam HI, and Pst I.

respectively (19), are described in Table 2. Allelic loss detecting these two markers is shown in Fig. 2. All cases were informative for at least one locus on 5q. A deletion at any 5q locus (loci) was present in 81% of all cases. Among SCLC and non-SCLC, there was no significant difference in frequency of deletion at 5q. However, the percentage of deleted loci in all informative loci on SCLC was significantly higher than that on non-SCLC (Table 3).

Common Region of Deletion on 5q. Picking up the cases showing partial and interstitial deletion, we estimated the common region of deletion on 5q in advanced lung cancer (Fig. 3). Cases 49 and 31 showed a deletion at the telomeric to L5.71, whereas cases 41 and 34 did it at the centromeric to YN5.48. These data suggest that a common region of deletion lies between markers L5.71 and YN5.48 at 5q21. Cases 23, 24, 27, and 29 showed a distal deletion at telomeric to YN5.48. Among these cases, the common region of deletion was estimated to be between L4.5 and LMS8, q33-35. Case 62 showed an unusual deletion at centromeric to cCI5-7 (Fig. 1).

Progression of Lung Cancer and 5q Loss. To investigate correlations between tumor progression and 5q loss, the deletion pattern was compared between the primary tumor and its metastatic focus. In one SCLC case (case 6), a tumor cell line was established from the primary tumor specimen obtained by the bronchofiberscope, as described previously (20). The primary tumor and a metastatic mass in the adrenal gland were obtained at autopsy. The metastatic tumor and cell line showed loss of one allele, whereas the primary tumor showed retention of heterozygosity (Fig. 4). In this case, all informative 5q loci except LMS8 were deleted in the metastatic tumor (Fig. 1) and tumor cell line.

DISCUSSION

We report here that alleles at loci on chromosome 5q were frequently lost in advanced lung cancer. It has been reported that only 20–30% of surgically resected non-SCLC cases have 5q losses (13, 14). Other than these resectable non-SCLC, two reports have indi-
Table 3  Comparison of 5q allelic loss between SCLC and non-SCLC

<table>
<thead>
<tr>
<th></th>
<th>SCLC</th>
<th>Non-SCLC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases showing allelic loss/total informative cases</td>
<td>34/39 (87%)</td>
<td>14/20 (70%)</td>
<td>48/59 (81%)</td>
</tr>
<tr>
<td>Total deleted loci/total informative loci</td>
<td>91/156 (58%)</td>
<td>28/80 (35%)</td>
<td>119/236 (50%)</td>
</tr>
</tbody>
</table>

* Not significant.

Fig. 4. Southern blot demonstrating normal (N), primary (Pr), adrenal metastasis (M), and cell line (C) established from primary tumor from an SCLC patient (case 6). Each DNA was digested with MspI and hybridized to RFLP marker, D5S81.

The most common region of overlap of deleted segments lies at 5q21 between markers L5.71 and YN5.48. The physical distance between these markers was estimated to be several megabases (4, 22).

Two candidate tumor-suppressor genes (MCC and APC) at this region have been cloned and shown to be somatically altered in some colorectal tumors (3-5). Therefore, these genes should be considered the best candidates for tumor-suppressor genes on 5q21 involved in lung cancer (16). However, alteration of these genes in lung cancer has not been reported so far. In addition to reports concerning 5q deletion in colorectal cancer, there have been some reports about 5q deletion in carcinomas of esophagus (23), stomach (24), pancreas (25) lung (13-16), kidney (21), and liver (26). Among these neoplasms with 5q loss, Horii et al. (24, 25) reported that only carcinomas in the digestive system, such as colon, stomach, and pancreas, had the alteration of the APC gene but carcinomas of lung and kidney did not. Moreover, D’Amico et al. (16) also reported that some exons in the MCC/APC genes and highly conserved tyrosine kinase domain of the FER gene, which is located close to the MCC/APC gene loci, have no identified abnormalities. Taken together, these results suggest that there may be another tumor-suppressor gene at 5q21, located close to the APC gene. It seems to be involved in the development and/or progression of several types of human cancer.

Some cases showed telomeric deletion at 5q33-35, with the remaining heterozygosity at 5q21 loci. This suggests that at least two kinds of tumor-suppressor genes on 5q may be involved in the progression of lung cancer.
It is known that 5q deletion also occurs in several malignancies of the hematopoietic system. Interferon-regulating factor-1 (IRF-1) on 5q31 is thought to be a candidate tumor-suppressor gene, because inactivating rearrangements of one IRF-1 allele, accompanied by deletion of the second allele, were identified in one case of acute leukemia (27). More recently, Le Beau et al. (28) demonstrated a small but common deletion at 5q31 in malignant myeloid diseases. They reported that the target region was estimated to be 2.8 megabases and did not contain the IRF gene locus. However, these regions are not consistent with the target involved in lung cancer described in this study. Thus, it is possible that different tumor-suppressor genes on 5q may be involved in the pathogenesis of hematopoietic malignancies and solid tumors.

Some cDNA segments, which are homozygously deleted in lung cancer cell lines, have been isolated by a genomic subtraction hybridization technique (29). One of these was mapped to chromosome 5. Since detailed location of this cDNA clone has not been reported, it is unknown whether the cDNA is located within the commonly deleted region reported here. Taken together, these results will be useful to isolate putative tumor-suppressor gene(s) and will provide an important clue for understanding the mechanism(s) of the progression of human cancers.

ACKNOWLEDGMENTS


REFERENCES


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