Allelic Loss on 17p13 (TP53) and Allelic Loss on 3p21 in Early Squamous Cell Carcinoma of the Lung

CHI AKI ENDO, MASAMI SATO, SHIGEFUMI FUJIMURA, AKIRA S AkURA D A, HIROKAZU AIKAWA, SATOMI TAKAHASHI, KATSUO USUDA, YASUKI SATO, and MOTOYASU SAGAWA

Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan

Abstract Roentgenographically occult bronchogenic squamous cell carcinomas are early lung cancers that localize in the bronchial wall, and are thought to be a good model to elucidate the carcinogenesis of lung cancer. In the present study, we analyzed the incidence of allelic losses on chromosome regions 3p21 and 17p13 in 40 cases of roentgenographically occult bronchogenic squamous cell carcinomas, using three microsatellite dinucleotide polymorphic markers. We also investigated the relationship between such allelic loss and the clinicopathological findings of those cases. These chromosome regions showed frequent losses. Moreover, the incidence of loss on 17p13 increased gradually along with the advance of the depth of invasion, while the incidence of loss on 3p21 increased along with the advancing length of the longitudinal extension. These results suggested that these chromosome regions play different roles in lung cancer progression, i.e., the 3p21 chromosome region was related to the longitudinal extension of the carcinoma while the 17p13 (p53) region was related to the depth of invasion.

Key words Roentgenographically occult lung cancer · Squamous cell carcinoma · Heterozygosity · Depth of invasion · Longitudinal extension

Introduction

Many investigators have reported frequent allelic losses on the 3p21 and 17p13 allelic regions in advanced lung cancers. A few investigators have also reported losses on these loci in early lung cancers or precancerous lesions of the lung. These allelic losses have been considered to be early events in lung cancer progression. However, the role of such allelic loss in lung cancer progression has yet to be fully elucidated.

Roentgenographically occult bronchogenic squamous cell carcinomas (ROCs) are early lung cancers that are only detected by sputum cytology. In most ROCs, invasion is limited to the bronchial wall. Untreated ROCs develop into advanced lung cancers with radiologically abnormal shadows after several years. Accordingly, ROCs are thought to be a good model for the purpose of elucidating the carcinogenesis of lung cancer.

ROCs are evaluated clinicopathologically based on the depth of invasion (DI) and the length of longitudinal extension (LE) (Fig. 1). Some ROCs have low-grade DI and very long LE (superficial extension type), while others have high-grade DI and very short LE (invasive growth type). DI and LE are considered to represent different biological behaviors in lung carcinogenesis. Allelic losses on 3p21 and 17p13 may be related to DI and/or LE of ROCs. However, there have been no reports concerning the relationship between such biological behaviors and genetic changes in lung carcinogenesis.

In the present study, we analyzed the relationship among the allelic loss on chromosome regions 3p21 and 17p13, DI, and LE in 40 cases of ROC.

Materials and Methods

Forty cases of resected ROC were examined. All cases were male and were classified as pathological stage I. Resected ROC specimens were examined pathologically by serial block sectioning. The bronchial tree from the margin of resection to the ends of the subsubsegmental bronchi was serially cut into blocks perpendicularly to the longitudinal bronchial axis at a thickness of 2 mm. The depth and site of the maximum invasion...
and longitudinal extension of the carcinoma were determined by a histopathological analysis of all blocks. The ROC specimens were divided into two groups according to DI: intrabronchial wall invasion (25 cases) and extrabronchial wall invasion (15 cases). The ROC specimens were also divided into another two groups according to LE: 10 mm or less (17 cases) and over 10 mm (23 cases) (Table 1).

Eight 20-μm-thick sections of the tumors and corresponding normal tissue were cut from formalin-fixed, paraffin-embedded blocks. These eight sections then underwent microdissection according to a technique described elsewhere. DNA was obtained by proteinase K digestion and phenol/chloroform extraction.

The polymorphic DNA markers used in this study were D3S643 and D3S1298 on 3p21, and TP53 on 17p13. These markers were obtained from GenBank (accession numbers D01084, Z16860, and X61505, respectively). The sequences of primers for these markers were as follows: 5′-TCCAGGCTGGGTAACAGGAG-3′ and 5′-ACAGAACTGCCAAACCATCC-3′ for D3S643; 5′-GAGGTGCTAGGGCTCCAG-3′ and 5′-TCCCCTGTGAAGCGTGTG-3′ for D3S1298; 5′-CCCATTCCCCATTCCTA-3′ and 5′-ACTATTCA GCCGAAGGTGAG-3′ for TP53. One primer of each pair was end-labeled with [γ-32P]ATP (10 mCi/ml; DuPont New England Nuclear, Wilmington, DE, USA) by use of T4 polynucleotide kinase (Boehringer-Mannheim, Mannheim, Germany). Polymerase chain reaction (PCR) mixtures in a volume of 15 μl contained 100 ng genomic DNA, 1.5 pmol of each primer, 15 pmol of each dNTP, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 25 mM MgCl2, 0.01% gelatin, and 0.2 units of Taq polymerase (Perkin-Elmer, Wellesley, MA, USA). The PCR conditions were 40 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s. The PCR products were electrophoresed in 6% polyacrylamide gels including 8 M urea and 32% formamide, and then were subjected to autoradiography. When the signal intensity in the tumor tissue was less than 50% of that in the normal tissue based on the findings of a densitometric analysis (Fig. 2), the tumor was regarded to demonstrate allelic loss.

Fig. 1. Schema of roentgenographically occult bronchogenic squamous cell carcinoma (ROC) in the bronchial wall. a, length of longitudinal extension; b, depth of invasion

Fig. 2. Frequency of loss of heterozygosity (LOH) in representative cases of ROCs. Each arrow indicates the position of the deleted allele. N, normal tissue; T, tumor tissue

Table 1. Forty cases of roentgenographically occult bronchogenic squamous cell carcinoma classified by depth of invasion and length of longitudinal extension

<table>
<thead>
<tr>
<th>Depth of invasion</th>
<th>Intrabronchial wall invasion</th>
<th>Extrabronchial wall invasion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of longitudinal excision</td>
<td>10 mm or under</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Over 10 mm</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>15</td>
<td>40</td>
</tr>
</tbody>
</table>