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

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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. Unreated 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.11 The
ROC specimens were divided into two groups according
to DI: intrabronchial wall invasion (25 cases) and
extrabronchial wall invasion (15 cases). The ROC speci-
mens were also divided into another two groups accord-
ing 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 cor-
responding normal tissue were cut from formalin-fixed,
paraffin-embedded blocks. These eight sections then
underwent microdissection according to a technique
described elsewhere.5 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 mark-
ers were as follows: 5'-TCCAGGCTGGGTAACAGG
AG-3' and 5'-ACAGAACTGCCAAACCATCC-3'
for D3S643; 5'-GAGGTGCTAGGGCTCCAG-3'
and 5'-TCCCCTGTGAAGCGTGTG-3'
for TP53. One primer of the
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 electro-
phoresed in 6% polyacrylamide gels including 8 M urea
and 32% formamide, and then were subjected to auto-
radiography. 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.2

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

![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](image)

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<tr>
<th>Table 1. Forty cases of roentgenographically occult bronchogenic squamous cell carcinoma classified by depth of invasion and length of longitudinal extension</th>
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<tbody>
<tr>
<td><strong>Depth of invasion</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>Length of longitudinal excision</td>
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<tr>
<td>10 mm or under</td>
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<td>Over 10 mm</td>
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