Somatic in Vivo Alterations of the JV18-1 Gene at 18q21 in Human Lung Cancers¹

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Abstract

The chromosome region 18q21 is frequently deleted in lung cancers. Recent identification of JV18-1 at this locus led us to examine whether or not it might also be altered in lung cancers, as is the case for the closely related DPC4 tumor suppressor gene. A missense somatic mutation and a 9-bp in-frame deletion were detected in the highly conserved region of JV18-1 among 57 lung cancer specimens taken directly from patients. The total alterations in JV18-1 and DPC4, however, are not sufficient to account for all 18q21 deletions in lung cancers. These findings suggest that although JV18-1 and DPC4 may play roles in a limited fraction of lung cancers, another tumor suppressor gene may also exist in this chromosome region.

Introduction

Accumulating evidence indicates that lung cancers carry multiple genetic lesions in tumor suppressor genes (1—6), with frequent allelic losses at the chromosome region 18q21 suggesting the presence of at least one tumor suppressor gene in this location (7, 8). We recently showed that the DPC4 gene, which was initially isolated from 18q21 as a candidate tumor suppressor gene for pancreatic cancers (9), is somatically mutated in a proportion of lung cancers in vivo (10). However, the observed frequency of DPC4 mutations was significantly lower than expected from the numbers of 18q21 deletions in lung cancers, indicating that another tumor suppressor gene might be present in this chromosome region.

Riggins et al. (11) recently reported isolation of a novel gene, termed JV18-1, closely related and forming a MAD-related gene family with the DPC4 gene. Interestingly, it was mapped to the 18q21 region within a short distance (3 Mb) from the DPC4 gene. Moreover, the authors found JV18-1 to be mutated in a fraction of colon cancers which are well known to frequently demonstrate 18q21 deletions (12). These observations led us to examine whether the JV18-1 gene might also be a target for 18q21 deletions in lung cancers.

In the present study, we examined 57 lung cancer specimens, taken directly from patients, for JV18-1 gene alterations along with the molecular status of the DPC4 gene and allelic loss at 18q21. Somatic in vivo JV18-1 mutations were indeed identified, albeit at low frequency, suggesting that the MAD-related genes at 18q21 may play a role in the pathogenesis of lung cancer.

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Materials and Methods

Cell Lines and Tumor Specimens. Tumor samples, along with uninvolved lung tissue where available, were collected from 57 patients diagnosed histologically as having lung cancers (12 cases of SCLC and 45 cases of NSCLC). SCLC tumor samples were obtained at necropsy or biopsy, whereas NSCLC tumors were collected during surgery. All tissues were quickly frozen in liquid nitrogen and stored at −80°C until analyzed.

Southern Blot Analysis. To search for gross alterations in the JV18-1 gene, Southern blot analysis was carried out using EcoRI-digested DNAs as described previously (13). The cDNA probe used in the present study, which covered the entire open reading frame of the JV18-1 gene, was prepared by PCR using the oligonucleotide primers and conditions described below.

Allelic loss at 18q21 was examined by Southern blot analysis using EcoRI polymorphism at the bcl-2 locus at 18q21 and scored when the decrease in signal intensity in densitometric tracings was greater than 50% in tumor specimens as detailed earlier (8).

PCR-SSCP Analysis. PCR using random primed cDNAs was performed with oligonucleotide primers in the presence of [12P]dCTP as described previously (14). PCR products were digested with appropriate restriction enzymes to yield a higher sensitivity due to a smaller size, followed by electrophoretic separation on 6% nondenaturing polyacrylamide gels at 4°C. Restriction enzymes used in this study were RsaI and AluI (Fig. 1). The primer pairs used were: S1 (sense), 5' -AGCTCTAGATGGCTTGCTGCTTTTGTAAG and AS1 (antisense), 5' -AGCGAATTCCTGGTCTCACAATCTCTGTA; S2 (sense), 5' -AGCTCTAGAAGATGTGGTAAACCCTTAC and AS2 (antisense), 5' -AGCGAATTCACCACTATTGATGATAAGCCGA; and S3 (sense), 5' -AGCTCTAGAATGCAAAGA- GAAGGATATAGGA and AS3 (antisense), 5' -AGCGAATTCAGGACTTCTATGCTTTTTCAATGGGACTT.

The PCR amplification consisted of 35 cycles (94°C for 30 s, 55°C for 45 s, and 72°C for 1 mm) after the initial denaturation step (94°C for 2 min).

RT-PCR products of lung cancer specimens showing abnormal PCR-SSCP patterns were digested and cloned into the XbaI-EcoRI site of pBluescript KSII(−) (Stratagene), and the plasmid DNAs prepared from pooled clones were sequenced as described previously (14). RT-PCR products of the corresponding normal lung RNAs were also subjected to PCR-SSCP and sequencing analyses.

Results and Discussion

We first examined 57 lung cancer specimens for the presence of gross alterations using Southern blot analysis but failed to identify any such somatic abnormalities (data not shown). RT-PCR-SSCP analysis was then performed to search for subtle mutations in the JV18-1 gene, yielding two lung cancer specimens with distinct electrophoretic mobilities (case 3, small cell carcinoma; case 8, adenocarcinoma in Fig. 1). The distinct mobility shifts were present only in lung cancer specimens and not in the corresponding normal lungs, indicating a somatic nature for these changes (data not shown). In contrast to almost exclusive expression of transcripts with the altered mobility shift in case 3, both mutant and
these two cases showed the presence of somatic mutations leading to changes in the predicted JV18-1 gene products (Fig. 2). Case 3 was found to carry a missense mutation (GAC to CAC) in a highly conserved residue at codon 450 within the MAD homology 2 (MH2) region of the JV18-1 protein, producing a nonconservative amino acid substitution (change from aspartic acid to histidine). Case 8 had a 9-bp in-frame deletion encompassing codons 434–436 within the MH2 region. Sequence analysis also revealed that this deletion in case 8 had resulted in the appearance of slower migrating bands in the RT-PCR-SSCP analysis due to removal of the Rsal site used here (Fig. 1).

The present study thus identified two examples of somatic mutations in the JV18-1 gene in lung cancer specimens taken directly from patients, both of which occurred within the highly conserved MH2 region. JV18-1 is a member of the MAD-related gene family, which has been shown to function in the signaling pathway of transforming growth factor β family members (11, 15–18). Since normal bronchial epithelial cells exhibit growth inhibition and induction of differentiation when treated with transforming growth factor β in vitro (19), the present findings suggest that alterations of the JV18-1 gene may play a role in the pathogenesis of a fraction of lung cancers.

We previously reported detection of alterations of the DPC4 gene in a subset of lung cancers (10). In the present cohort, we collectively observed alterations of the MAD-related genes, JV18-1 and DPC4, in 3 (1 JV18-1 and 2 DPC4, 12%) of 25 adenocarcinomas and 1 (DPC4, 7%) of 15 squamous cell carcinomas and 1 (JV18-1, 8%) of 12 small cell carcinomas (Table 1). Neither JV18-1 nor DPC4 mutations were found in four large cell carcinomas and one adenosquamous carcinoma. It is of note that allelic losses at 18q21, where JV18-1 and DPC4 reside, were present in as high as 45% (5/11 informative cases) of the adenocarcinomas (Table 1). Although it is possible that RT-PCR-SSCP analysis using RNAs from microdissected tumor specimens might yield higher mutation frequencies of MAD-related genes, this discrepancy between the frequencies of JV18-1 and DPC4 mutations and that of allelic loss at 18q21 could be reconciled if one assumes that this latter reflects changes in multiple tumor suppressor genes and that there is yet another putative tumor suppressor gene at 18q21 which plays a role in lung carcinogenesis. Alternatively, these MAD-related genes themselves might be inactivated by other molecular mechanisms such as aberrant hypermethylation, leading to transcriptional repression (20). In this regard, it is interesting to note that lung cancers, especially adenocarcinomas, frequently show tumor-specific aberrant hypermethylation at 18q21 (8).

Along with our previous demonstration of DPC4 alterations in lung cancers, the present findings indicate that the biological and biochemical functions of the MAD-related genes, JV18-1 and DPC4, warrant further investigation to gain insights into the molecular pathogenesis of this fatal disease. Future studies

![Diagram](attachment:image.png)

**Fig. 1.** PCR-SSCP analysis of the JV18-1 gene in lung cancer specimens. a, representative results of PCR-SSCP analysis using S3 and AS3 primers. Abnormal mobility shifts are apparent in cases 3 and 8. b, Schematic diagram of the strategy for PCR-SSCP analysis of JV18-1 cDNAs as well as that of JV18-1 mRNA are shown. Open box, coding region; stippled boxes, 5' and 3' untranslated regions. Restriction enzymes used to yield higher sensitivities due to smaller sizes are indicated.

Table 1  Somatic mutations in the MAD-related genes at 18q21 in lung cancers

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. examined</th>
<th>JV18-1 mutation</th>
<th>DPC4 mutation</th>
<th>Allelic loss at 18q21</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>25</td>
<td>1</td>
<td>2$^a$</td>
<td>5 (11)$^b$</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>15</td>
<td>0</td>
<td>1$^a$</td>
<td>1 (3)</td>
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<tr>
<td>Large cell carcinoma</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0 (1)</td>
</tr>
<tr>
<td>SCLC</td>
<td>12</td>
<td>1$^c$</td>
<td>0</td>
<td>0 (4)</td>
</tr>
</tbody>
</table>

$^a$ Previously published in Nagatake et al. (10).
$^b$ Numbers in parentheses, number of informative cases.
$^c$ Not informative in the allelic loss study.
Fig. 2. Sequence analysis of the JV18-1 gene in lung cancer specimens and the corresponding normal lung samples of cases 3 and 8. a, a missense mutation (GAC to CAC) at codon 450 is apparent in lung cancer tissue of case 3, while a 9-bp in-frame deletion is evident in that of case 8. Note that these mutations are not present in the patients' normal lung samples, indicating that they occurred as in vivo somatic mutations in the tumors. b, schematic diagram of the JV18-1 protein and locations of the predicted amino acid alterations resulting from the identified mutations. The amino acid sequence of JV18-1 from amino acids 431—454 of the MH2 domain are aligned with the corresponding regions in MADR1 and DPC4. Conserved sequences are highlighted (black boxes).

may lead to identification of another, yet unidentified, tumor suppressor gene(s) linked with frequent 18q21 deletions in lung cancers.

References


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