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Advances in Brief

Somatic *in Vivo* Alterations of the *JV18-1* Gene at 18q21 in Human Lung Cancers[1]

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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.

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[3] 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 *Eco*RI-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 *Eco*RI 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 [^{32}P]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 *Rsa*I and *Alu*I (Fig. 1). The primer pairs used were: S1 (sense), 5′-AGCTCTAGATGGCTTGCTGCCTTTGGTAAG and AS1 (antisense), 5′-AGCGAATTCACTGGTGTCTCAACTCTCTGA; S2 (sense), 5′-AGCTCTAGAGAAGTATGTGTAAACCCTTAC and AS2 (antisense), 5′-AGCGAATTCCACCTATGTAGTATAAGCGCA; and S3 (sense), 5′-AGCTCTAGAATGACAAGAAGGCATATAGGA and AS3 (antisense), 5′-AGCGAATTCAAGTCTTTTCCATGGGACTTG. The PCR amplification consisted of 35 cycles (94°C for 30 s, 55°C for 45 s, and 72°C for 1 min) 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 *Xba*I-*Eco*RI site of pBluescript SKII(−) (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

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[3] The abbreviations used are: SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; RT, reverse transcription; SSCP, single-strand conformation polymorphism.

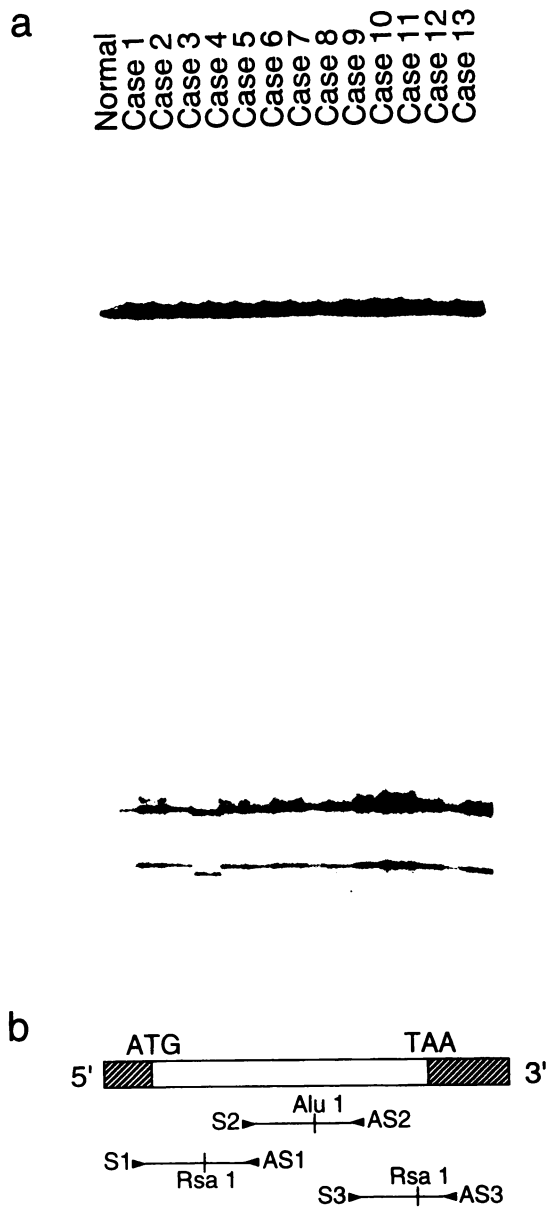

Fig. 1. PCR-SSCP analysis of the *JV18-1* gene in lung cancer specimens. *a*, representative results of PCR-SSCP analysis using S3 and AS3 primers. Abnormal mobility shifts are apparent in cases 3 and 8. *b*, Schematic diagram of the strategy for PCR-SSCP analysis of *JV18-1* cDNAs as well as that of *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.

wild-type alleles were expressed at similar levels in the lung cancer RNA of case 8, consistent with retention of heterozygosity observed on Southern blot analysis.

Sequence analysis of both normal and lung cancer specimens of 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 *Rsa*I 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

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

Histology	No. examined	*JV18-1* mutation	*DPC4* mutation	Allelic loss at 18q21
NSCLC				
Adenocarcinoma	25	1	2[a]	5 (11)[b]
Squamous cell carcinoma	15	0	1[a]	1 (3)
Large cell carcinoma	4	0	0	1 (1)
Adenosquamous carcinoma	1	0	0	0 (1)
SCLC	12	1[c]	0	0 (4)

[a] Previously published in Nagatake *et al.* (10).
[b] Numbers in parentheses, number of informative cases.
[c] Not informative in the allelic loss study.

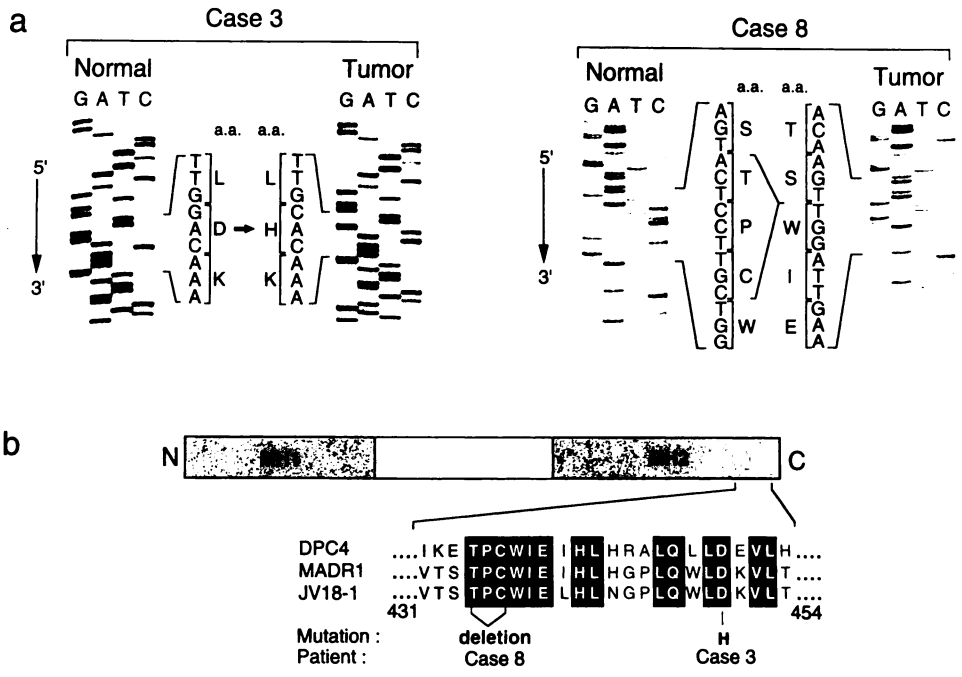

Fig. 2. Sequence analysis of the *JV18-1* gene in lung cancer specimens and the corresponding normal lung samples of cases 3 and 8. *a*, a missense mutation (GAC to CAC) at codon 450 is apparent in lung cancer tissue of case 3, while a 9-bp in-frame deletion is evident in that of case 8. Note that these 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.

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