Advances in Brief

Prognostic Significance of p53 Mutations and 3p Deletions in Primary Resected Non-Small Cell Lung Cancer

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Abstract

We evaluated the prognostic significance of p53 mutations and an allelic loss of chromosome 3p in 71 patients with non-small cell lung cancer who underwent potentially curative resection. p53 mutations were detected in 38 cases (49%), while 3p deletions were observed in 34 of 70 informative cases (49%). The presence of the p53 mutation was associated with a shortened survival in all patients (P = 0.014 by log rank test), including those in early stages of the disease (stage I or II, n = 48) (P = 0.016 by log rank test). Multivariate analysis by the Cox proportional hazards model also revealed that p53 mutation was an independent yet unfavorable prognostic factor (P = 0.013). Patients with 3p deletion tended to have a poorer prognosis, but not to a statistically significant extent.

Introduction

Lung cancer is soon expected to become the leading cause of cancer deaths in Japan inasmuch as it currently claims more than 36,000 lives annually (1). Of patients with NSCLC3 who undergo potentially curative resection, the long-term survival rate remains unsatisfactory even in patients undergoing "potentially curative resection" (2). Our understanding of the molecular pathogenesis of this fatal disease may have reached a certain point at which a basis can now be established for designing novel strategies to better diagnose and treat lung cancer patients. Among the genetic abnormalities identified in NSCLC thus far, mutations in the p53 gene (3-5) and an allelic deletion of chromosome 3p (6-10) appear to be the most frequent targets. The p53 gene is now thought to regulate transcription through sequence-specific DNA binding, while mutations in the p53 gene abrogate this trans-activating activity, resulting in uncontrolled proliferation of the affected cells (11). We previously showed that nearly one-half of all primary resected NSCLC specimens carried mutations in the p53 gene (5) and that wild type p53 could indeed function as a potent in vitro and in vivo growth suppressor in human lung cancer (12). In addition to the p53 gene on chromosome 17p, a frequent occurrence of 3p deletion in lung cancer (100% in small cell lung cancer and 50% or more in NSCLC) strongly suggests the presence of a tumor suppressor gene(s) in this chromosomal region (6-10). 3p deletion has also been identified in other human malignancies such as renal cancer, suggesting a significant role of the putative tumor suppressor gene(s) on 3p in the development of human cancers (13).

In this study, we investigated the prognostic significance of p53 mutations and 3p deletions in 71 patients with NSCLC who could undergo complete resection by radical surgery. We report here that the presence of the p53 mutation is associated with shortened survival and may define a certain subset of NSCLC patients as suitable candidates for investigational therapeutic approaches.

Materials and Methods

Patients and Tumor Samples. Tumor samples along with uninvolved lung tissue were obtained from 71 patients with NSCLC (33 squamous cell carcinomas, 34 adenocarcinomas, and 4 adenosquamous carcinomas) who had undergone a potentially curative resection at the Aichi Cancer Center and the National Chubu Hospital, from 1982 to 1991. All tissues were quickly frozen in liquid nitrogen and stored at −80°C until analysis.

Detection of p53 Mutations. PCR-SSCP analysis was performed to detect p53 mutations in the region between exons 5 and 8 as described by Orita et al. (14) with slight modification. Genomic DNAs (100 ng) prepared from frozen samples as described previously (3) were subjected to amplification at each exon including the exon-intron boundary. The primers pairs used in this study were

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer Set</th>
<th>Sequences</th>
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<tbody>
<tr>
<td>Exon 5</td>
<td>e5s, 5′-AGCAAGCTTGACATTTCAACTCTGCTCCTT</td>
<td>e5as, 5′-AGCGGATCCACCGACCGTGCCTCCTCCA</td>
</tr>
<tr>
<td>Exon 6</td>
<td>e6s, 5′-AGCAAGCTTAGGCTCCTGATTCCTCACTG</td>
<td>e6as, 5′-AGCGGATCCCCACAGACCCAGTGCACAC</td>
</tr>
<tr>
<td>Exon 7</td>
<td>e7s, 5′-AGCAAGCTTAGGCTCCTGATTCCTCACTG</td>
<td>e7as, 5′-AGCGGATCCCCACAGACCCAGTGCACAC</td>
</tr>
<tr>
<td>Exon 8</td>
<td>e8s, 5′-AGCAAGCTTAGGCTCCTGATTCCTCACTG</td>
<td>e8as, 5′-AGCGGATCCCGACAGACCCAGTGCACAC</td>
</tr>
</tbody>
</table>

For exons 5, 7, and 8, the PCR products labeled with [32P]dCTP were electrophoretically separated by 6% nondenaturing polyacrylamide gels at 40 W for 3.5 h at 5°C. Genomic DNAs extracted from tumor samples with abnormal electrophoretic mobilities were vectored with 100,000 cpm [32P]dCTP electrophoresed in 6% nondenaturing polyacrylamide gel. With the aid of primers described by Orita et al. (14), the PCR products were subcloned into the pBluescript vector (Stratagene). The resulting plasmids were sequenced to determine the nature of the mutation.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; RFLP, restriction fragment length polymorphism.
Allelic loss was scored when densitometric tracing indicated at least a 50% decrease in intensity.

**Clinical Data and Statistical Analysis.** The study was unblinded following molecular genetic analysis, and statistical analysis was performed to examine the prognostic significance of p53 mutations and 3p deletions in NSCLC. The new international staging system for lung cancer was used for postoperative pathological staging. The Kaplan-Meier method was used to estimate survival possibility as a function of time, and survival differences were analyzed by the log rank test (15). Cox regression analysis of factors potentially related to survival was performed to identify which independent factors would jointly have a significant influence on survival (15).

**Results and Discussion**

**Detection of p53 Mutations in NSCLC Specimens.** We examined exons 5 through 8 in the p53 gene by the PCR-SSCP method, since previous studies have shown that most of the mutations occurring in NSCLC are found in this region (4, 5). Using 18 tumor samples identified by sequencing to have p53 mutations (5, 16), we first determined the optimal conditions for PCR-SSCP analysis so that all mutations could be detected.

We searched for p53 mutations in genomic DNAs extracted from 71 paired tumor and normal lung specimens which had been surgically removed during potentially curative resection (Table 1). p53 mutations were found in 35 of these 71 NSCLC tumors (49%), but in none of the corresponding normal lung tissues, indicating that these mutations were somatically acquired events. No significant differences in the frequency of p53 mutations were observed with respect to the clinicopathological status of the disease including tumor size, nodal involvement, histology, and stage (Table 1). These findings generally agree with our previous report (5).

**Detection of 3p Deletions in NSCLC Specimens.** Seventy of 71 cases were informative at 1 or more of the 5 loci tested, and allelic loss was observed in 34 of these 70 informative cases (49%). The prevalence of 3p deletion was slightly lower than that observed in our previous report (79%) (9) probably due to the more strict criteria applied in the present study for determining partial loss of heterozygosity. We found no significant links between the presence of 3p deletion and the clinicopathological status of the disease (Table 1).

**Prognostic Significance of p53 Mutations and 3p Deletions.** Recent molecular biological studies have demonstrated that alterations in the tumor suppressor genes play an important role in the pathogenesis of lung cancer and that the p53 gene along with a putative tumor suppressor gene(s) on 3p appears to be frequently involved in this process. The primary aim of this investigation was to determine whether the presence of these genetic alterations could serve to identify a group of patients who would experience a distinct outcome after complete resection of the NSCLC tumors.

As shown in Fig. 1A, the Kaplan-Meier survival curve demonstrated that when all patients were considered, those with p53 mutations survived for a shorter period of time (P = 0.014 by the log rank test). A significant association was also observed between the presence of p53 mutation and shortened survival among patients in stages I or II of the disease, a subset of patients who should have actually had the best hope for cure (P = 0.016 by the log-rank test) (Fig. 1B). Cox proportional hazards regression analysis revealed that the disease stage and p53 mutation were significant prognostic factors and that the risk ratio of death was 3.499 among patients with the p53 mutation versus those without the p53 mutation (P = 0.013) (Table 2).

This study is the first to report that the presence of mutations in the p53 gene is an unfavorable prognostic factor for patients with NSCLC undergoing complete resection. Using in vitro-cultured cell lines of NSCLC histology, however, Mitsudomi et al. (17) found no association between the p53 mutation and a poor prognosis. This discrepancy may be explained by the fact that cell line establishment itself is known as an independent, unfavorable prognostic factor in NSCLC (2) and that p53 gene mutations seem to aid in the establishment of human lung cancer cell lines (16). We note that a significant association between accumulation of p53 protein and a poor prognosis in human NSCLC was recently reported by Quinlan et al. (18) using p53-specific monoclonal antibody. An association between the p53 alteration and a poor prognosis after surgical resection, similar to the
results in human NSCLC, has been also reported for gastric cancer cases subjected to immunohistological analysis using an anti-p53 antibody (19), although other types of human cancer including colon and breast cancer were found to have no such association (20, 21). This may be due to differences in the timing and role of p53 mutations in the pathogenesis of each cancer in which distinct but partly overlapping sets of genetic alterations may be necessary.

In contrast to the significant effect of the p53 mutation, the influence of 3p deletion on the survival of NSCLC patients was statistically insignificant, although patients with the 3p deletion tended to have a poorer prognosis \((P = 0.084\), all patients; \(P = 0.068\), those with stage I or II disease by the log rank test) (Fig. 2). Multivariate analysis using the Cox proportional hazards model, however, showed no association between the presence of 3p deletion and shortened survival. Since there may be three putative tumor suppressor genes on 3p as previously reported (9), isolation of as yet unidentified genes may reveal a close relationship between mutations and prognosis which may be currently obscured by the intrinsic limitation of RFLP analysis.

It should be noted that 23 (66%) of 35 cases with p53 mutations showed 3p deletions in contrast to less frequent occurrence of 3p deletions in tumors without p53 mutations (11 of 35, 31%). In fact, there was weak but significant association between the presence of p53 mutation and 3p deletion \((x^2 = 8.24, P = 0.004\), by \(x^2\) test for independence). Emerging evidence suggests that wild type p53 protein may play a key role to maintain the integrity of the genome, while p53 mutations may result in genetic damages such as mutation and aneuploidy (22). Association between the presence of p53 mutation and 3p deletion suggests a possibility that genetic instability leading to an inactivation of a putative tumor suppressor gene(s) on 3p may be induced by p53 mutations or vice versa.

**Pilot studies reported thus far (Ref. 18; this study) suggest that NSCLC patients with p53 mutations may be consequently defined as candidates for new investigational adjuvant therapies, although additional definitive/confirmatory studies with much larger cohorts are needed before initiating such prospective clinical trials. In the future, identification of other genetic lesions such as the putative tumor suppressor gene(s) on 3p may also provide an adjunctive means toward identifying a subset of NSCLC patients with a very poor prognostic outcome.**

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**References**

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