Diagnostic Usefulness of \textit{p16}/CDKN2A FISH in Distinguishing Between Sarcomatoid Mesothelioma and Fibrous Pleuritis

Di Wu, PhD, \textsuperscript{1} Kenzo Hiroshima, MD, PhD, \textsuperscript{1} Shinji Matsumoto, \textsuperscript{2} Kazuki Nabeshima, MD, PhD, \textsuperscript{2} Toshikazu Yusa, MD, PhD, \textsuperscript{3} Daisuke Ozaki, MD, PhD, \textsuperscript{4} Michio Fujino, MD, PhD, \textsuperscript{5} Hisami Yamakawa, MD, PhD, \textsuperscript{6} Yukio Nakatani, MD, PhD, \textsuperscript{7} Yuji Tada, MD, PhD, \textsuperscript{8} Hideaki Shimada, MD, PhD, \textsuperscript{9} Masatoshi Tagawa, MD, PhD\textsuperscript{10}

Key Words: \textit{p16}; Fluorescence in situ hybridization; Methylation; Mesothelioma; Prognosis; Pleuritis

DOI: 10.1309/AJCPT94JVWIHBKRD

Abstract

The distinction between sarcomatoid mesothelioma and fibrous pleuritis is difficult based on histology, especially when the amount of tumor tissue examined via biopsy is small and immunohistochemical examination is inconclusive. We studied the usefulness of deletion of \textit{p16} with fluorescence in situ hybridization (FISH) and \textit{p16} hypermethylation with polymerase chain reaction for the diagnosis and prognosis of malignant pleural mesothelioma (MPM). We analyzed 50 MPMs, including 22 sarcomatoid mesothelioma cases and 10 fibrous pleuritis cases. We set the cutoff value of homozygous deletion pattern as 14.4\% based on FISH signaling patterns using samples of fibrous pleuritis. The percentage of homozygous deletion pattern was higher than 14.4\% in 55.6\% of the epithelioid mesotheliomas (10/18) and in all of the sarcomatoid mesotheliomas (22/22). Methylation of \textit{p16} was observed in 7 (20.6\%) of 34 informative cases. \textit{p16} FISH analysis can be a reliable test for distinguishing between sarcomatoid mesothelioma and fibrous pleuritis and a prognostic factor for MPM.

Malignant mesothelioma (MM) is a highly aggressive neoplasm with a median survival of 8 to 14 months. In Japan, more than 1,000 new cases are diagnosed each year. The number of deaths between the years 2030 and 2039 is predicted to be 21 times greater than the observed number of deaths between 1990 and 1999, and the number of deaths will peak between 2030 and 2034 in Japan.

Based on the proper clinical and radiologic context, a diagnosis of MM can be rendered when the pathologic findings are typical. However, the pathologic diagnosis of MM can be difficult because of various histopathologic patterns, such as the often deceptively bland appearance of tumor cells and the presence of pseudomesotheliomatous carcinoma. Small surgical biopsy specimens and cytologic specimens also add to the difficulty of proper diagnosis. The International Mesothelioma Panel recommends the application of at least 2 mesothelial and 2 carcinoma markers in addition to a pancytokeratin for the differential diagnosis between epithelioid mesothelioma and metastatic carcinoma.

The absence of clinical and imaging information, pathologic interpretation of benign and malignant mesothelial proliferations can be more difficult, especially when mesothelial proliferations are observed only on a serosal surface or when spindle cells proliferate in the parietal pleura. Differential diagnosis of reactive mesothelial hyperplasia or fibrous pleuritis from malignant pleural mesothelioma (MPM) is crucial to prevent unnecessary invasive and aggressive treatments for MPM in patients with reactive mesothelial hyperplasia or fibrous pleuritis.
Some immunohistochemical markers are useful for differentiating between epithelioid mesothelioma and reactive mesothelial hyperplasia. Epithelial membrane antigen is reported to be useful in the differential diagnosis.\(^4,5\) Strong membranous expression of epithelial membrane antigen was seen in 48 (80%) of 60 MPM cases and in 8 (20%) of 40 reactive mesothelial hyperplastic cases.\(^5\) Glut-1 is expressed in most cases of epithelioid mesothelioma but not in reactive mesothelial hyperplasia.\(^6,7\) Insulin-like growth factor II messenger RNA–binding protein 3 is also expressed in epithelioid mesothelioma but not in reactive mesothelial hyperplasia.\(^8,9\) CD146 was reported to be a sensitive and specific marker for mesotheliomas in cytologic specimens.\(^10\) The combined application of these markers may provide a potential option to differentiate epithelioid mesothelioma from reactive mesothelial hyperplasia.

However, distinguishing between sarcomatoid mesothelioma and fibrous pleuritis is difficult, especially when the amount of tumor tissue in the biopsy specimen is small and immunohistochemical examination is inconclusive. Stromal or chest wall fat invasion is the most reliable criterion to diagnose sarcomatoid mesothelioma; however, it cannot be assessed in small biopsy specimens.\(^11\)

The tumor suppressor genes \(p16\) (CDKN2A) and \(p15\) (CDKN2B) map to the 9p21 chromosomal locus and are homozygously deleted in MM and other cancers.\(^12-14\) Point mutation and promoter methylation of both genes have been reported in MM.\(^15,16\) Previous studies have used fluorescence in situ hybridization (FISH) analysis to detect the loss of the \(p16\) gene in mesothelioma\(^15,17-23\) and methylation-specific polymerase chain reaction (PCR) analysis to detect \(p16\) promoter hypermethylation.\(^15,16\) In this study, we evaluate the usefulness of 2 methods, \(p16\) FISH and \(p16\) promoter hypermethylation, in discriminating sarcomatoid mesothelioma from fibrous pleuritis. We also analyze the frequency of homozygous deletion of the \(p16\) gene among histologic subtypes of MPM. Our results confirmed the high prevalence of \(p16\) deletion in MPM and suggest that detection of \(p16\) deletions using FISH analysis could prove useful in the diagnosis of sarcomatoid mesothelioma.

**Materials and Methods**

Fifty MPM cases and 10 fibrous pleuritis cases seen between December 1999 and March 2012 were collected from the archives of the Department of Pathology of Yachiyo Medical Center, Chiba University, Chiba Rosai Hospital, and Chiba East Hospital.\(^1\) Table 1. Paraffin-embedded tissue blocks were available in all cases. Informed consent was obtained from all patients. The diagnosis of MPM was based on the combination of clinical findings, imaging and gross observations at surgery, and routine H&E histology. All cases were reviewed by 2 pathologists (K.H., Y.N.). The diagnosis was confirmed on immunohistochemistry (calretinin, WT-1, D2-40, cytokeratin [CK] 5/6, CAM5.2, CK AE1/AE3, carcinoembryonic antigen, thyroid transcription factor 1, desmin, smooth muscle actin, and S100) using the Bond MAX autoimmunostainer (Leica, Wetzlar, Germany). Representative tissue blocks were selected for FISH analysis. The fibrous pleuritis sample in this study displayed atypical spindle cells. All patients with fibrous pleuritis except 1 had a history of asbestos exposure and pleural effusion. Therefore, we diagnosed these cases as benign asbestos pleural effusion.\(^24\)

**FISH**

FISH was performed on formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections. After paraffin sections were deparaffinized in xylene, dehydrated in ethanol, and air dried, dual-color FISH analysis was performed using a spectrum green–labeled chromosome 9 centromeric probe and a spectrum orange–labeled, locus-specific CDKN2A(p16) probe (Abbott, Abbott Park, IL). Pretreatment steps were performed using a histology FISH accessory kit (DakoCytomation, Tokyo, Japan) as follows: the section was placed in pretreatment solution (MES; 2-ethanesulphonic acid buffer), incubated at 121°C for 1 minute, and digested with pepsin at 37°C for 5 to 11 minutes. The probes were denatured for 5 minutes at 95°C before hybridization. The slides were then hybridized for 48 hours at 37°C and washed in 2 × SSC/0.3% Tween 20 (Sigma, St Louis, MO) at 78°C for 2 minutes, and counterstained with DAPI/antifade (Abbott).

**Scoring**

Slides were examined and images were obtained using a fluorescence microscope (Axio Imager 2, Carl Zeiss Microscopy, Göttingen, Germany) equipped with filter sets with single exciters for spectrum green, spectrum orange, and DAPI (UV 360 nm). Only individual and well-delineated cells were scored. Overlapping cells were excluded from the analysis. At

---

**Table 1**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients With Mesothelioma</th>
<th>Patients With Fibrous Pleuritis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>44 (88)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>F</td>
<td>6 (12)</td>
<td>1 (10)</td>
</tr>
<tr>
<td><strong>Mean age (range), y</strong></td>
<td>66.2 (44.0-85.0)</td>
<td>69.0 (45.0-86.0)</td>
</tr>
<tr>
<td><strong>MPM subtype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelioid</td>
<td>19 (38)</td>
<td>—</td>
</tr>
<tr>
<td>Biphasic</td>
<td>8 (16)</td>
<td>—</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>23 (46)</td>
<td>—</td>
</tr>
</tbody>
</table>

MPM, malignant pleural mesothelioma.

*Data are presented as number (%) unless otherwise indicated.*
least 100 cells were scored for each case. Homozygous deletion was defined as the absence of both 9p21 signals in the presence of at least 1 chromosome 9 centromere signal. Hemizygous deletion was defined as the presence of only one 9p21 signal in the presence of 2 chromosome 9 centromere signals or when the number of 9p21 signals was less than half the number of chromosome 9 centromere signals.

DNA Extraction

DNA samples were extracted from paraffin-embedded materials. Tissue specimens were accurately removed from two 10-μm serial sections mounted on glass slides by scraping the marked area with a needle under a stereomicroscope. Tumor cells were selectively removed from the sections to minimize contamination by normal tissue specimens. The dissected sections were digested by proteinase K over 2 nights at 37°C.

Methylation-Specific PCR assay

The promoter region methylation status of the p16 gene was evaluated with methylation-specific PCR as previously reported, with slight modification. The DNA sample was modified using bisulfite treatment with the WZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA). Two sets of primers were used to amplify each region of interest. One pair recognized a sequence in which CpG sites were unmethylated (bisulfite modified to UpG), and the other pair recognized a sequence in which CpG sites were methylated (unmodified by bisulfite). Reactions were hot-started at 94°C for 2 minutes. Amplification was carried out in a Takara thermal cycler (Takara, Otsu, Japan) for 40 cycles (94°C/30 sec, 65°C or 60°C/30 sec, 68°C/1 min), followed by an extension at 68°C for 4 minutes. The PCR products were analyzed with electrophoresis in a 4% agarose gel and visualized with ethidium bromide staining.

Statistical Analysis

The differences among categorized groups were compared using the χ² test. The overall survival time for the patients was defined as the time from video-assisted thoracoscopic biopsy until death or the date the patient was last known to be alive. We assessed the correlation between clinicopathologic variables (age, sex, subtype, therapy, p16 deletion, and p16 promoter methylation) and overall survival. Kaplan-Meier curves and survival estimates were calculated, and the Wilcoxon test was used to test for differences between groups. A P value less than .05 was considered statistically significant.

Results

Clinicopathologic Characteristics

The pathologic diagnoses of the 50 patients with mesotheliomas and the 10 patients with fibrous pleuritis are summarized in Table 1. All 50 mesothelioma cases were pleural mesotheliomas. The 5 cases of desmoplastic mesothelioma were classified as sarcomatoid mesothelioma in this study. The majority of specimens were from video-assisted thoracoscopic biopsies (78%), 8 cases were from extrapleural pneumonectomy (EPP) specimens (16%), 2 were from autopsy specimens (4%), and 1 was from resection of a metastatic lymph node (2%).

Cutoff Values for Fibrous Pleuritis

To examine whether FISH analysis of p16 can discriminate mesotheliomas from fibrous pleuritis, we evaluated the frequency of p16 deletion in MPM and in fibrous pleuritis. Cutoff levels were calculated as the mean percentage ± 4 standard deviations (SDs) of cells, with deletion of nuclei in fibrous pleuritis caused by artifactual loss of signals because of nuclear sectioning.

<table>
<thead>
<tr>
<th>Deletion Pattern</th>
<th>Frequency of p16/CDKN2A Deletion Pattern With FISH in Fibrous Pleuritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous</td>
<td>0.7-4.0</td>
</tr>
<tr>
<td>Homozygous/hemizygous</td>
<td>7.0-25.0</td>
</tr>
<tr>
<td>Homozygous/</td>
<td>3.9 ± 2.6</td>
</tr>
<tr>
<td>hemizygous</td>
<td>17.2 ± 6.3</td>
</tr>
<tr>
<td>Cutoff Value</td>
<td>&gt;14.4</td>
</tr>
<tr>
<td>for % Nuclei</td>
<td>&gt;42.3</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

Table 2

FISH for p16/CDKN2A Deletion in Cases With MPM

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Homozygous Deletion</th>
<th>Homozygous/ Hemizygous Deletion</th>
<th>Normal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioid</td>
<td>10 (65.6)</td>
<td>12 (66.7)</td>
<td>6 (33.3)</td>
<td>18</td>
</tr>
<tr>
<td>Biphasic</td>
<td>7 (87.5)</td>
<td>7 (87.5)</td>
<td>1 (12.5)</td>
<td>8</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>22 (100.0)</td>
<td>22 (100.0)</td>
<td>0 (0)</td>
<td>22</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization; MPM, malignant pleural mesothelioma.

* Data are given as number (%) unless otherwise indicated.
Homzygous deletions of p16 were seen in 39 (81.3%) of 48 MPM cases. In epithelioid mesothelioma, the percentage of homozygous deletion pattern was higher than the cutoff value in 55.6% (10/18) of the cases. In biphasic mesothelioma, the percentage of homozygous deletion pattern was higher than the cutoff value in 87.5% (7/8) of the cases. In all of the sarcomatoid mesothelioma cases, the percentage of homozygous deletion pattern was higher than the cutoff value (Figure 1A).

We analyzed the homozygous deletion pattern and the hemizygous deletion pattern together. The mean percentage + 4 SDs for fibrous pleuritis was 42.3%. When we applied this cutoff value, 66.7% (12/18) of epithelioid mesothelioma could be diagnosed with p16 FISH analysis (Figure 1B).

**Frequency of Methylation in MPM and Fibrous Pleuritis**

We performed methylation-specific PCR for the p16 promoter in 35 mesothelioma and 7 fibrous pleuritis cases. Methylation-specific PCR was not successful in 1 mesothelioma case because the amount of extracted DNA was low. Of the remaining mesothelioma cases, the frequency of promoter methylation was 20.6% (7/34). The frequency of promoter methylation in fibrous pleuritis cases was 42.9% (3/7). No significant difference was observed in the frequency of promoter methylation between MPM and fibrous pleuritis. We investigated the relationship between the homozygous deletion and promoter methylation of the p16 gene in MPM. Twenty-two (81.5%) of the 27 mesothelioma cases with homozygous deletion were without methylation; however, 5 (18.5%) of the 27 mesothelioma cases with...
homogeneous deletion did show methylation. Only 2 of 6 mesotheliomas without homogeneous deletion that were analyzed showed promoter methylation. Therefore, no association was found between $p16$ homogeneous deletion and $p16$ promoter methylation.

### Prognostic Analysis of $p16$ Deleted vs Nondeleted MPM

In this study, we analyzed the prognosis of 43 patients with MPM who had been treated during the period between December 1999 and March 2012. Table 4 summarizes the predictors of prognosis in MPM. Patients with sarcomatoid MPM showed the worst survival, whereas patients with epithelioid MPM showed better survival (Table 4). The median survival times for patients with respect to histologic subtypes of MPM are as follows: 22 months for epithelioid mesothelioma, 12 months for biphasic mesothelioma, and 5 months for sarcomatoid mesothelioma ($P = .0015$). $p16$ homogeneous deletion is associated with shorter survival (Table 4). For patients with $p16$ homozygous deletion, the median survival time was 7 months. In contrast, patients without $p16$ homozygous deletion showed a longer survival time of 22 months ($P = .0363$). Patients without $p16$ methylation had longer survival times than patients with $p16$ methylation ($P = .0442$).

### Discussion

Loss of the $p16$ gene is one of the most frequent genetic alterations in MPM. Illei et al. found homogeneous deletion of $p16$ in 70 (74%) of 95 MPM cases, including 49 (69%) of 71 epithelioid, 16 (84%) of 19 biphasic, and 5 (100%) of 5 sarcomatoid mesotheliomas. In a recent study of MPM, Chiosea et al. detected homogeneous 9p21 deletion with FISH analysis in 35 (67%) of 52 cases of MPM. Our study showed similar results, with homogeneous deletion of $p16$ seen in 81.3% (39/48) of Japanese MPM cases. Furthermore, homogeneous deletion was observed in 55.6% (10/18) of epithelioid mesotheliomas, 100% (22/22) of sarcomatoid mesotheliomas, and 87.5% (7/8) of biphasic mesotheliomas. These results indicate that $p16$ FISH analysis in MPM is a useful test that may guide diagnosis and treatment decisions for patients.

Previous studies on $p16$ FISH analysis used a cutoff value of 20%. It is reported that no reactive pleural mesothelial proliferations showed $p16$ deletion. We believe that spindle cells in fibrous pleuritis do not harbor deletion of the $p16$ gene; therefore, we established an alternative cutoff value for $p16$ deletion based on FISH signaling patterns obtained from spindle cells observed in fibrous pleuritis. In a standard data distribution, 99.994% of the data values are within 4 SDs. Therefore, in this study we established the cutoff value for homogeneous deletion as 14.4%. Chung et al. proposed a cutoff value for the diagnosis of mesothelioma as 10% for homogeneous deletion and 44% for hemizygous deletion based on FISH signaling patterns obtained from benign controls. Because of the variability in FISH analysis and scoring methods from one institute to another, each institute should develop its own applicable cutoff value.
Iliei et al.\textsuperscript{17} demonstrated that homozygous \textit{p16} deletion detected with FISH is a very powerful technique for confirming the diagnosis of MPM on reactive mesothelial cells with cytology. Differentiation of sarcomatoid mesothelioma and fibrous pleuritis via histological and immunohistochemical staining is difficult, especially in small biopsy specimens. However, few sarcomatoid mesothelioma cases have been analyzed with FISH to date. To our knowledge, the number of sarcomatoid mesothelioma cases in each report did not exceed 5 and the results are contradictory.\textsuperscript{18-21,23} We investigated 22 cases of sarcomatoid mesothelioma with FISH analysis and found that all sarcomatoid mesotheliomas showed a homozygous deletion pattern in more than 14.4% of tumor cells, and all of the fibrous pleuritis showed a homozygous deletion pattern in less than 14.4% of spindle cells. Both sensitivity and specificity of \textit{p16} FISH analysis using a cutoff value of 14.4% for the diagnosis of mesothelioma are 100%.

Most studies of FISH analysis reported that homozygous deletion was observed frequently in MPM\textsuperscript{17,18,21,22,26,27}; more than 90% of analyzed MPM cells showed a homozygous deletion pattern.\textsuperscript{22,25} Hemizygous deletion was not identified in any of the analyzed cases of MPM in some reports.\textsuperscript{22,25} However, Chung et al.\textsuperscript{23} reported that the mean percentage of nuclei with a homozygous deletion pattern was 61% (range, 1%-87%), with 3 cases showing less than 30%; hemizygous deletion occurred in 10 (18%) of 54 MPM cases. They reported that hemizygous deletion is sufficient for the diagnosis of MPM.\textsuperscript{23} Frequency of \textit{p16} gene homozygous deletion in epithelioid mesothelioma was 55.6% (10/18) in our study. When we combined the homozygous and hemizygous patterns, the cutoff value for mesothelioma was 42.3%. Two of the 6 epithelioid mesothelioma cases without homozygous deletion had a higher value than our established cutoff value. Sensitivity of \textit{p16} deletion for the diagnosis of epithelioid mesothelioma increased to 66.7% (12/18) when we combined both homozygous and hemizygous patterns. The \textit{p16} gene is a known tumor suppressor, and loss of both alleles is required for malignant transformation.\textsuperscript{12} Therefore, homozygous deletion fully explains the association between the \textit{p16} gene deletion pattern and a diagnosis of MPM observed in our study. Alternatively, hemizygous deletion would be the first hit of the Knudson 2-hit hypothesis that would require a second hit, such as promoter hypermethylation, to eliminate the second gene copy.\textsuperscript{30}

Our study showed no correlation between the deletion of \textit{p16} as determined with FISH and methylation status of the \textit{p16} promoter. Four of 6 mesotheliomas without \textit{p16} homozygous deletion showed no promoter methylation, whereas 5 of 27 mesotheliomas with \textit{p16} homozygous deletion also showed promoter methylation. If \textit{p16} is deleted in tumor cells, the corresponding promoter region would also be deleted and methylation of \textit{p16} would not be observed. In some cases of mesothelioma that showed a homozygous deletion pattern of \textit{p16}, the tumor cells also showed a normal \textit{p16} pattern on FISH analysis, and therefore displayed promoter hypermethylation because the promoter was not deleted in these tumor cells. Alternatively, these tumor cell samples may have been contaminated by non–tumor cells that, when analyzed with methylation-specific PCR, showed promoter methylation. Our results showed that the hypermethylation of the \textit{p16} gene was observed in 3 (42.9%) of 7 fibrous pleuritis cases. Studies have reported aberrantly methylated \textit{p15} alleles in the lymphocytes of healthy donors,\textsuperscript{31} \textit{p16} hypermethylation in normal breast tissue,\textsuperscript{32} and \textit{p15} hypermethylation and \textit{p16} hypermethylation in normal colon mucosa.\textsuperscript{33} However, further study is needed to evaluate the relationship between methylation status of the \textit{p16} gene and tumorigenesis.

It is reported that the most important predictors of poor prognosis in MPM are poor performance status, gender, low hemoglobin concentration, high platelet count, and high WBC count.\textsuperscript{34-36} Our study showed that younger patients and patients treated with EPP have a better survival. Biphasic or sarcomatous subtypes are reported to be associated with poor survival.\textsuperscript{34-38} Our study results showed that patients with epithelioid mesothelioma had longer survival than those with nonepithelioid mesothelioma. Homozygous deletion of \textit{p16} is reported to be associated with a worse prognosis.\textsuperscript{23,29} Dacic et al.\textsuperscript{39} demonstrated that loss of \textit{p16}, as detected with FISH in 21 (60%) of 35 MPM cases with epithelioid subtype, was more frequently associated with a short survival. The estimated median survival time was 12 months for subjects positive for \textit{p16} deletion. Loss of \textit{p16} immunoreactivity in mesotheliomas is also reported to be a negative prognostic factor in pleural or peritoneal mesotheliomas.\textsuperscript{29,39} However, these studies analyzed mainly patients with epithelioid mesothelioma. Our study included not only epithelioid mesotheliomas but also biphasic and sarcomatoid mesotheliomas. Using Wilcoxon analysis, we confirmed that histology is a strong factor influencing overall survival, and \textit{p16} deletion is a prognostic predictor for patients with MPM.

In summary, our data demonstrated that FISH analysis using a commercially available dual-color FISH probe can be reliably performed on archival paraffin-embedded tissue that has been kept at room temperature for a long duration. However, signal intensity did decrease 8 years after biopsy. We also demonstrated that \textit{p16} deletion detected with FISH analysis is useful for differentiating sarcomatoid mesothelioma from fibrous pleuritis. Analysis of methylation status of the \textit{p16} gene may not be useful for the diagnosis of mesothelioma. Homozygous deletion of \textit{p16} detected with FISH analysis and \textit{p16} promoter methylation evaluated with methylation-specific PCR may predict the prognosis of patients with MPM.
of Pathology, Fukuoka University, Fukuoka, Japan; 3Departments of Thoracic Surgery and 4Pathology, Chiba Rosai Hospital, Ichihara, Japan; 5Department of Thoracic Surgery, Chiba Medical Center, Chiba, Japan; 6Department of Thoracic Surgery, Yarita Hospital, Ichihara, Japan; 7Departments of Diagnostic Pathology and 8Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan; 9Department of Surgery, School of Medicine, Toho University, Tokyo, Japan; and 10Division of Pathology and Cell Therapy, Chiba Cancer Center Research Institute, Chiba, Japan.

This study was partially supported by Grants-in-Aid from the Ministry of the Environment of Japan and a Grant-in-Aid from the Nichias Corporation, Tokyo, Japan.

Address reprint requests to Dr Hiroshima: Dept of Pathology, Tokyo Women’s Medical University Yachiyo Medical Center, 477-96 Owada-Shinden, Yachiyo, Chiba 276-8524, Japan.

References


© American Society for Clinical Pathology

DOI: 10.1309/AJCPT94JVWIHBKRD

Downloaded from https://academic.oup.com/ajcp/article-abstract/139/1/39/1766082 by guest on 30 December 2017


