Pleomorphic Carcinoma of the Lung Associated with Loss of Heterozygosity of p53 Gene

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ARITA, N., MIKAMI, Y., YOSHIDA, M., KONISHI, I., HORIIKE, N., MIYAUCHI, K., MIYAZAKI, T., NOSE, M. and ONO, M. Pleomorphic Carcinoma of the Lung Associated with Loss of Heterozygosity of p53 Gene. Tohoku J. Exp. Med., 2005, 206 (2), 181-185 — We report a case with pleomorphic carcinoma of the lung in a 70-year-old man. Pleomorphic carcinoma is characterized by a heterogenous composition that includes epithelial and mesenchymal malignancies. In the present case, the tumor was composed of a mixture of unequivocal squamous cell carcinoma and spindle cell components resembling sarcomatous overgrowth. The spindle component did not include a heterologous mesenchymal element characterized by overt differentiation for bone, cartilage, neuron or muscle tissue. To evaluate a state of differentiation of the spindle cell component, we immunohistochemically examined expression of the antigens including vimentin, cytokeratin, sarcomeric actin, α-smooth muscle actin, S-100 protein, CD34, Factor VIII, and CD68. The results showed sole expression of vimentin in the spindle cell component, suggesting an immature state of the mesenchymal lineage. Furthermore, the spindle cell component of this case was genetically characterized by loss of heterozygosity (LOH) at a codon 234 of exon 7 of the p53 gene. This mutation causes an amino-acid replacement (Tyr to Cys), which was previously proven to attenuate p53 function. The present case may suggest a relation between somatic alteration of the p53 gene and histogenesis of pleomorphic carcinoma. —— Pleomorphic; carcinoma; p53 gene; loss of heterozygosity

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In the current World Health Organization (WHO) classification (Travis et al. 1999), pleomorphic carcinoma of the lung is defined as carcinoma with spindle cell and/or giant cell component imparting a close resemblance to sarcoma. The sarcomatous component in pleomorphic carcinoma usually includes atypical spindle cell and/or giant cell components, and lack a heterologous mesenchymal element that was histologically recognized as overt differentiation for bone, cartilage, neuron or muscle tissue. In the case where sarcomatous component in a carcinoma includes...
any heterologous mesenchymal element, it is differentially classified as carcinosarcoma. It remains unclear whether pleomorphic carcinoma and carcinosarcoma share a common genetic mechanism for their histogenesis. In this report, we present a case with pleomorphic carcinoma of the lung associated with somatic alteration of the p53 gene.

**CASE REPORT**

A 70-year-old man with a liver mass and cirrhosis was referred to the Ehime University Hospital for a follow-up examination. The existence of the liver mass was pointed out six years before the admission, but a diagnostic procedure for establishing histopathologic diagnosis was not performed. On the admission, chest x-ray and computed tomography (CT) scanning demonstrated a solitary mass measuring approximately 4.0 x 3.5 cm in size in the left upper lung (Figs. 1A and 1B). Bronchoscopic examination revealed a typical endobronchial growth of this tumor (Fig. 1C). Histopathological examination for the transbronchial biopsy specimen demonstrated a solitary mass measuring approximately 4.0 x 3.5 cm in size in the left upper lung (Figs. 2A and 2B). At the end of the second month after the diagnosis, the patient underwent a radical resection of left upper lobe of the lung. The patients had been smoking 20 cigarettes everyday for 50 years.

The tumor was located in a left upper lobe of the lung, measured 6 x 5 x 3.8 cm in dimension, and adjacent to the left fourth rib as well as parietal pleura. On cut surface, the tumor was well-demarcated from surrounding parenchyma, and solid with scattered necrosis foci. In microscopic examination, the tumor consisted of a unequivocal carcinomatous component and a spindle cell component with a predominance of the latter (Fig. 2C). The carcinomatous component displayed features of well-differentiated squamous cell carcinoma with inflammation and necrotic foci. On the other hand, the spindle cell component consisted of highly atypical cells including bizarre multinucleated tumor giant cells with the increased number of mitoses and atypical mitotic figures. In this component, there was no evidence for overt heterologous mesenchymal differentiation.

In immunohistochemical examination, the spindle cell component was positive for vimentin and negative for cytokeratin, sarcomeric actin, \(\alpha\)-smooth muscle actin, S-100 protein, CD34, Factor VIII, CD68, chromogranin A, thyroid transcription factor (TTF)-1 and \(\alpha\)-fetoprotein (Fig. 3A), whereas the carcinomatous component was

![Fig. 1. Clinical manifestations of the present case. Thoracic x-ray (A) and computed tomography (CT) scanning (B) show a solitary mass measuring approximately 4.0 x 3.5 cm in size in the left upper lung. Bronchoscopic examination (C) reveals its endobronchial growth.](image-url)
solely positive for cytokeratin (Fig. 3B). As summarized in Table 1, the immunohistochemical findings support the absence of heterologous mesenchymal differentiation in this tumor. Both components were positive for p53 expression (data not shown). We considered a case with positive cells exceeding 50% as positive expression of p53.

We then carried out a mutation analysis of the p53 gene. Genomic DNA sample was prepared from entire sections of the paraffin-embedded tissue of biopsy specimen. Sequence analysis was performed for polymerase-chain-reaction (PCR) fragment of the exons 5, 6, 7 and 8 of the p53 gene, which encode the DNA binding domain of the p53 protein. These exons are also known to be an open region for somatic mutation associated with oncogenesis and malignancy. The nucleotide sequence of the p53 gene was determined as described previously (Ridanpaa et al. 1995). All experiments were performed in compliance with the institutional guideline of Ehime University School of Medicine.

The nucleotide sequence of the biopsy speci-
men revealed a heterozygous pattern at a codon 234 of exon 7 of the p53 gene (Fig. 4). Of note, this pattern is located in a functional domain of p53 protein, and represents an amino-acid substitution at the codon 234 (Tyr to Cys). To examine whether this pattern was the consequence of a somatic or germline mutation, we analyzed nucleotide sequences in the distinct preparations from spindle cell components, carcinomatous components and non-neoplastic tissues. Each tissue was collected from surgical specimen with a microscope-assisted dissection technique. Although we failed to sequence the p53 gene in carcinomatous and non-neoplastic tissues due to undetermined reasons, the spindle cell component was shown to have LOH pattern at the codon 234 (Fig. 4). Three independent preparations of the spindle cell component demonstrated the same sequence at this codon. The biopsy specimen histologically contained numerous inflammatory cells, so that non-neoplastic cells as well as neoplastic cells must represent the heterozygous pattern proven for biopsy specimen. Given that the spindle cell component, which is the most major component of biopsy specimen, is homozygous, it is reasonable that non-neoplastic cells are heterozygous at this codon. Therefore, we suggest that the LOH in the spindle cell component is a consequence of somatic mutation.

**DISCUSSION**

Tumors currently recognized as pleomorphic carcinoma have been designated as a variety of names, including monophasic carcinosarcoma, sarcomatoid carcinoma, spindle cell carcinoma

**Table 1. Summary of immunohistochemical study**

<table>
<thead>
<tr>
<th>Antibody to (clone name, provider)</th>
<th>Carcinomatous component</th>
<th>Spindle cell component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin (AE1/AE3, DAKO*)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Vimentin (v9, DAKO)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sarcomeric actin (α-Sr-1, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smooth muscle actin (1A4, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S100 protein (ER-PR8, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD34 (NU-4A1, Nichirei†)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Factor VIII (rabbit polyclonal, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD68 (KP-1, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chromogranin A (DAK-A3, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TTF-1 (8G7G3/1, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-fetoprotein (rabbit polyclonal, DAKO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p53 (DO-7, DAKO)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* DakoCytomation, Carpinteria, CA, USA.  † Nichirei, Tokyo, Japan.

Fig. 4. Nucleotide sequences of the exon 7 of p53 gene in endobronchial specimen (Biopsy) and spindle cell component of surgical specimen (Sarcomatoid). An arrow indicates the residue of A-to-G transition.
and carcinoma with metaplastic changes. It remains to be elucidated whether pleomorphic carcinoma represents these differentially termed neoplasms in terms of histogenesis. It is still under debate with pathological importance to distinct pleomorphic carcinoma from carcinosarcoma. Reviewing 33 and 66 examples of pleomorphic carcinoma and carcinosarcoma, respectively, Koss et al. (1999) suggested that there were possible differences in location and a morphological feature of epithelial component between these two. However, there was no significant difference in most clinicopathologic features. To address this debate, it will be important to understand genetic basis for their pathogenesis.

We demonstrated LOH of the p53 gene in the spindle cell component of pleomorphic carcinoma. This mutation was deduced to replace amino acid in a functional domain of p53 protein, suggestive of an influence on expression and a function of p53 protein. Indeed, recent studies have demonstrated that the missense mutation at a codon 234 greatly affects p53 function, and causes accumulation of p53 protein (Smith et al. 1999; Kato et al. 2000; Kato et al. 2003). According to the TP53 mutation database of International Agency for Research on Cancer (IARC), the 138 tumors with somatic mutation at a codon 234 have been registered. Among them, the 83 tumors had A-to-G mutation in the same manner as the present case. The 13 out of the 83 tumors were pulmonary carcinomas, including six squamous cell carcinomas, four adenocarcinomas and three small cell carcinomas. These lines of evidence indicate a role of somatic p53 mutation at this codon in pathogenesis of lung carcinomas.

It is an important question whether the spindle cell component may arise from a preexisting carcinomatous component or from a common epithelial precursor in the early stage. Of note, Przygodzki et al. (1996) demonstrated that pleomorphic carcinoma included significantly fewer p53 point mutations (14%) than adenocarcinoma (27%) and squamous cell carcinoma (43%). They further demonstrated that p53 mutations in pleomorphic carcinoma were more common in exon 7 as shown in this case, whereas those in squamous cell carcinoma and adenocarcinoma were more frequent in exon 8 (Przygodzki et al. 1996). Kawano et al. (1996) analyzed a case with spindle cell carcinoma of the lung, and identified LOH of the p53 gene in sarcomatous component, but not carcinomatous components. Those findings may support the second interpretation as to pathogenic mechanism of the spindle cell component of pleomorphic carcinoma. The present findings support a possibility of the link between p53 mutation and histogenesis of pleomorphic carcinoma.

References


