An Atypical Carcinoid Tumor of the Lung With Mutations in the p53 Gene and the Retinoblastoma Gene*

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Molecular analysis of a metastatic lesion of an atypical carcinoid tumor of the lung obtained from a 77-year-old man at autopsy revealed a point mutation in the p53 gene and a deletion in the retinoblastoma (Rb) mRNA. This case suggests that both these antioncogenes may be involved in the progression of atypical carcinoid tumor.

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In the carcinogenesis and progression of lung cancer, various genes have been reported to be involved. Among the candidates, there are oncogenes, such as the myc and ras families, and antioncogenes, such as the p53 and the retinoblastoma (Rb) genes. The deletion of the Rb gene and the mutation of the p53 gene are frequently detected in small-cell lung cancer (SCLC).\(^2\) Barbareschi et al reported that in neuroendocrine lung neoplasms, the p53 mutation was seen only in SCLCs and not in atypical carcinoids, while the Rb deletion seemed quite specific for SCLCs and most atypical carcinoids. They also investigated the proliferation (PCNA and Ki67) and neuroendocrine differentiation markers of typical carcinoid, atypical carcinoid, and SCLC, concluding that the decrease in neuroendocrine features during the progression from typical carcinoid to SCLC is paralleled by an altered expression of tumor suppressor gene products, ie, the p53 and the Rb proteins.

We have investigated the abnormalities in the p53 gene and the Rb gene in lung cancer specimens obtained by biopsy, operation, or autopsy. One of the autopsy cases was diagnosed as atypical carcinoid and one metastatic lesion on the pericardium was rendered to molecular analysis. Using polymer chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique, mutations both in the p53 gene and the Rb gene were detected.

CASE REPORT

A 75-year-old man was admitted to Hiroshima (Japan) University Hospital with a 6-month history of weight loss and shortness of breath. He was a heavy smoker (2 packs a day for 40 years). The chest radiograph and the computed tomographic scan revealed a 5×4-cm-diameter tumor over the left hilum with irregular margins and spicula formation. The serum level of tumor markers were as follows: carcinoembryonic antigen (CEA), 50.5 ng/ml; and neuron-specific enolase (NSE), 7.8 ng/ml. Although transbronchial fiberoptic and cytologic study of sputum failed to detect malignant cells, we diagnosed the lesion as primary lung cancer stage IIIb (T4N2M0) with invasions into the aorta and pulmonary artery. Following a 65-Gy course of radiotherapy, the tumor shadow diminished to 4.5×3 cm and the serum CEA level decreased to 24.4 ng/ml. After an asymptomatic period of one year without further anticancer therapy, he developed a pneumonia. The chest radiographs exhibited a rapid dissemination of small tumor shadows bilaterally, and the patient died one month later of respiratory failure. The serum levels of tumor markers were as follows: CEA, 80.0 ng/ml; and NSE, 5.3 ng/ml. Autopsy disclosed disseminated metastatic lesions also on the pericardium and pleura. Both the primary and metastatic lesions were histologically diagnosed as atypical carcinoid by hematoxylin-

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**Figure 1.** PCR-DGGE analysis of the 310bp fragments of p53 exon 5 (A) and the 1099bp fragments of Rb exons 20-26 (B). Each PCR product in (B) was cleaved into three fragments with restriction enzyme Tsp1 prior to DGGE. 100 percent denaturant = 40 percent (V/V) Formamide, 7M Urea. Lanes 8 and 13: PCR products from the metastatic lesion of this case. N = normal homoduplex; V = variant homoduplex; N/V = heteroduplex of normal and variant strands. Lanes 1-7 and 9-12 = PCR products from other cases of lung cancer.
obtained the sequence by CATGAAATCTACC-3')and reverse primer TGGCATTTACACAAGAT-3') to amplify exons 20-26 of the Rb gene. From the genomic DNA, exon 5 of the p53 gene was amplified using the primers reported by Hsu et al. The PCR products were subjected to DGGE following the method of Takahashi et al and exhibited abnormal bands indicating the existence of mutations (Fig 1). Loss of the normal allele of the p53 gene was also indicated by the disappearance of the normal homoduplex band. Direct sequencing of the PCR products revealed a TGG (Cys) to TTC (Phe) mutation in codon 135 of the p53 gene and a 31 bp deletion, corresponding to exon 24 in its entirety, of the Rb mRNA (Fig 2).

**DISCUSSION**

Although D’Amico et al. reported that the p53 gene mutation in SCLC was not associated with tumor response to therapy or to patient survival, the incidence of the p53 gene mutations in established lung cancer cell lines is higher than in nonimmortalized lung neoplasms. The implication, therefore, is that a p53 mutation may confer a growth advantage to cancer cells. In this case, during the first year from diagnosis, the progression of the tumor was very slow. After one year of a symptom-free period, the tumor dispersed very rapidly, and the patient died one month later. Unfortunately, we could not obtain a tissue sample before the clinical turning point; however, we speculate that the mutation of p53 gene, loss of the normal allele of p53 gene, and/ or the deletion of Rb mRNA might have promoted the rapid progression and dissemination of the tumor in this case. Also, the mutation of p53 gene seems not to be specific for SCLC in neuroendocrine lung neoplasms, but to be involved also in the progression of atypical carcinoid tumor. It has been reported that most of the naturally occurring Rb mutations contain deletions in either one or both of the binding sites of Rb to adenovirus E1A or SV40 large T antigen.* This case reveals that a deletion of exon 24, outside of those binding sites, may also depress the function of Rb as an antionogene.

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**Fatal Haemophilus influenzae Septicemia Following Bronchoscopy in a Splenectomized Patient**

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We describe a 46-year-old splenectomized patient who died of Haemophilus influenzae septicemia 16 h following bronchoscopy. Although rare, postsplenectomy overwhelming sepsis is always a danger in splenectomized patients under-

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