Lung adenocarcinoma with type II pneumocyte characteristics


ABSTRACT: We report a case of primary lung adenocarcinoma with type II pneumocyte characteristics. Electron microscopic examination demonstrated that the tumour cells had well-developed microvilli and cytoplasmic lamellar inclusion bodies. These ultrastructural features are similar to those seen in type II pneumocytes of normal lung tissue. Western blot analysis, using monoclonal antibody against human surfactant protein A (SP-A), clearly demonstrated that the tumour cells expressed human SP-A, which is a major pulmonary surfactant protein produced by type II pneumocytes. These observations suggest that the tumour was derived from a type II pneumocyte.

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Primary lung adenocarcinomas are heterogeneous with respect to their progenitor cells [1, 2]. The subtype bronchioloalveolar carcinoma is considered to originate from different progenitor cells [3]. With reference to cytological features of tumour cells, SHIMOSATO et al. [3] subclassified primary lung adenocarcinomas into five groups, namely bronchial surface cell, goblet cell, bronchial gland cell, Clara cell and type II pneumocyte. The last type is very rare and needs to be identified ultrastructurally through cytoplasmic lamellar bodies in tumour cells similar to those seen in type II pneumocytes of normal lung tissue [3–6]. We report a case of poorly differentiated adenocarcinoma of the lung, which exhibited type II pneumocyte characteristics ultrastructurally and functionally.

Case report

A 43 year old Japanese man was admitted to Sapporo Medical College Hospital, on November 22, 1989, with a two month history of nonproductive cough. He had no smoking history. Chest examination was unremarkable. No lymph node was palpable, no organ was enlarged and peripheral oedema and clubbing were absent. A chest X-ray disclosed a nodular shadow in the left upper lung field and poorly defined reticular shadows throughout both lungs. Laboratory analyses demonstrated that lactate dehydrogenase (LDH) (518 IU·l⁻¹; normal range 190–450 IU·l⁻¹) and alkaline phosphatase (ALP) (1,042 IU·l⁻¹; normal range 80–230 IU·l⁻¹) were markedly increased, whilst other laboratory data (peripheral blood counts, blood biochemistry, immunoserological examination, blood sugar, stool examination and urinalysis) including tumour markers (carcinoembryonic antigen (CEA), Sialyl Lewis X-1 (SLX), carbohydrate antigen 19-9 (CA19-9), neuron specific enolase (NSE) and α-fetoprotein (AFP) showed values within normal limits. Sputum cytology disclosed malignant cells (adenocarcinoma). Pleural effusion collected from the left pleural cavity was straw-coloured. Cytological examination of this exudate revealed malignant cells. Surfactant protein A (SP-A) content in pleural effusions was measured with SP-A enzyme-linked immunosorbent assay (ELISA) as described previously [7]. High SP-A levels in pleural effusions (>500 ng·ml⁻¹) were found in approximately 40% of primary lung adenocarcinoma. By contrast, other histological types of lung cancers, adenocarcinomas of different primary sites, had low levels of SP-A in their pleural effusions [7]. The SP-A level of the pleural effusion was very high (17,600 ng·ml⁻¹). Transbronchial biopsy from the tumour and peripheral lung tissue in the left anterior segment showed poorly differentiated adenocarcinoma and perivascular lymphatic invasion of malignant cells. The patient was given chemotherapy with cisplatin, 5-fluourouracil and vindesine. No response was obtained. The patient died 13 months after the onset of symptoms.

Histological study

An autopsy specimen was taken from the left lung, fixed with 10% formalin and embedded in paraffin.
After cutting, 5 μ sections were stained with Haematoylin-eosin. As shown in fig. 1, the light microscopic study demonstrated poorly differentiated adenocarcinoma. The tumour cells had abundant, fine vesicular cytoplasm and irregular hyperchromatic nuclei and displayed a glandular pattern of growth with slightly fibrous stroma.

Fig. 1. – Histopathological section of the tumour showing poorly differentiated adenocarcinoma. The tumour cells had a glandular pattern of growth, severe nuclear atypia and fine vesicular cytoplasm. Haematoxylin-eosin staining. Bar = 100 μm.

Electron microscopic examination

For the isolation of tumour cells from pretreated pleural effusion (500 ml), we used Ficoll-Hypaque cushion and discontinuous Percoll density gradients as described previously [8]. The isolated tumour cells and the specimen obtained at autopsy were fixed with 4% paraformaldehyde and 2% glutaraldehyde solution, buffered with 0.1 M cacodylate buffer, pH 7.4 for electron microscopic examination. Since autopsy was performed next morning (12 h after death), electron microscopic findings in the autopsy material were not demonstrable because of degeneration of ultrastructure. Therefore, the electron microscopic photograph shows the tumour cells from the pleural effusion (fig. 2).

The ultrastructural study demonstrated that the tumour cells had well-developed microvilli (100%) and cytoplasmic lamellar inclusion bodies (74%; 148 out of 200 tumour cells), and these ultrastructural features are similar to those seen in type II pneumocytes of normal lung tissue although lamellar bodies of the tumour cells were more immature than those seen in type II pneumocytes. The tumour cells without cytoplasmic lamellar inclusion bodies were considered to be a variant form of type II pneumocyte carcinoma cells because they were similar to type II pneumocyte carcinoma cells in all other respects.

Western blot analysis

Western blot analysis with monoclonal antibody against human SP-A PE10, was performed according to the method of TOWNIN et al. [9], using tumour cells isolated from the pre-treated pleural effusion. As depicted in figure 3, the tumour cells showed two bands at the molecular mass of 34–37 kDa and 60–70 kDa (lane a) under reducing condition, whereas they failed to react with control mouse serum (lane b). These bands exhibited the same property of human SP-A with respect to molecular mass, because the 60–70 kDa proteins were reported to be a dimer of 34–37 kDa SP-A monomer [10, 11].

Fig. 2. – Electron microscopic examination of the tumour cells isolated from pretreated pleural effusion. One out of two tumour cells had lamellar inclusion bodies (arrow heads) that are typical ultrastructural feature of type II pneumocyte type carcinoma and all the tumour cells had well-developed microvilli. Bar = 5 μm.

Fig. 3. – Western blot analysis of the tumour cells performed using monoclonal antibody against human SP-A, PE10 (lane a) and control mouse serum (lane b) under reducing condition. The tumour cells showed 34–37 and 60–70 kDa bands which are the same as human SP-A (monomer and dimer) with respect to molecular mass.
Discussion

SP-A is the predominant phospholipid-associated glycoprotein in pulmonary surfactant, which is produced from type II pneumocytes in normal lung tissue [12, 13]. SP-A is thought to play a crucial role in reducing the surface tension of the alveolar interface [11]. We recently observed [7] that when SP-A content was measured with SP-A ELISA system, using two monoclonal antibodies, a very small number of primary lung adenocarcinomas, including this case, exhibited extremely high SP-A levels (>10,000 ng·ml⁻¹) in malignant pleural effusion. Therefore, we performed the electron microscopic study and Western blot analysis of SP-A to clarify whether or not this lung adenocarcinoma was type II pneumocyte type carcinoma. Western blot analysis showed the expression of SP-A in the tumour cells indicating the tumour cells of this case synthesised SP-A.

By the cytological subclassification primary lung adenocarcinomas are divided into five groups (bronchial surface cell, goblet cell, bronchial gland cell, Clara cell and type II pneumocyte). All of the five cell types are seen in peripheral adenocarcinomas. Clara cell type is the most frequently seen, followed by bronchial surface cell (mucoblast) type. Type II pneumocyte is extremely rare [3]. Type II pneumocyte type carcinoma is reported to have specific light microscopic features. The tumour cells are cuboidal, arranged in bronchioloalveolar or papillary patterns, and uniform in appearance with a dome-shaped free cell border and finely vesicular cytoplasm [3]. In this case, the tumour cells had no typical features of type II pneumocyte type carcinoma except fine vesicular cytoplasm. However, the electron microscopic examination demonstrated that the tumour cells had lamellar inclusion bodies similar to those seen in type II pneumocytes, although the lamellar bodies of the tumour cells were more immature than those seen in type II pneumocytes. It is, as yet, unclear whether type II pneumocyte type carcinomas possess the function of pulmonary surfactant secretion similar to type II pneumocytes of normal lung tissue, although they conserve the morphological structures of type II pneumocytes and may produce SP-A. Fortunately, we were able to establish a tumour cell line originating from the tumour cells of the malignant pleural effusion of this case. The tumour cell line is now under investigation with respect to the functional potential of pulmonary surfactant secretion.

References