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Original Articles

Amylase Producing Lung Cancer

Electronmicroscopical and Biochemical Studies

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Summary. A case of the lung cancer associated with marked elevation of serum (7820 IU/l) and of urinary amylase (2225 IU/l) was autopsied. Material was examined histopathologically, electronmicroscopically and biochemically. The pulmonary tumor tissues showed histological pattern of papillary adenocarcinoma. In addition to the small round bodies which were very similar to secretory glanules, many large round bodies were noticed, diffusely distributed in the tumor cell. These large round bodies ranged from 0.2 to 0.7 μm in diameter and showed a lamellar or annular pattern. The soluble phase of the homogenized pulmonary tumor tissues had an amylase level of 11,300 IU/g of protein, which consisted of S-type isoamylase with minor components. Cholesterol, triglyceride and phospholipid were also present at greater concentration in the tumor tissue than the normal pulmonary tissue. The large round bodies appeared too, to be amylase including bodies from the electronmicroscopical and biochemical findings.

Key words: Lung – Lung neoplasms – Amylase – Neoplasm proteins.

Introduction

We have been interested in tumors associated with an elevation of serum amylase and tumor cell amylase production since 1951, when Weiss et al. reported a case of lung cancer associated with elevation of serum amylase. In addition to lung cancer, ovarian and gastric carcinomas with heterogenic amylase production have been reported (Yokosuka et al., 1975; Kameya et al., 1977; Miyawaki et al., 1977). Several cases of amylase producting lung cancer have now been reported but electronmicroscopical and biochemical examination, including cell fraction, has been performed in only a few cases. Recently, we performed an autopsy on a patient who died of lung cancer associated with a marked elevation

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of serum and urinary amylase. We examined this case electronmicroscopically and biochemically and report it here, with some discussion.

Case Report

The patient, a 48-years old man, was admitted complaining of a cough, sputum, shortness of breath and weight loss. These symptoms had begun two months before admission, when he had caught a common cold, and had gradually worsened.

On admission, a physical examination revealed weak breath sounds and bilateral swelling of the lymph nodes of the neck, up to 3 cm in diameter. There were many abnormal shadows scattered diffusely in both lung-field, on chest X-ray and these increased in size markedly during his admission. On laboratory examination, the haematocrit was 31.5% of the total leukocyte count 8,300/mm^3 and the erythrocyte count 459×10^4/mm^3. The total protein in serum was 7.6 g/dl, of which 62.8% was albumin, the total cholesterol level was 235 mg/dl, and the triglyceride level 270 mg/dl. The serum amylase level was 7,820 IU/l(103 IU/g of protein) and 2,225 IU/l in urine.

The other laboratory findings were within normal limits. Histopathological examination of the neck lymph-nodes and cytology of the sputum showed presence of adenocarcinoma.

Macroscopic and Microscopic Findings

The lungs were found to weigh 1,600 g lt. and 1,300 g rt. On cross section, many tumor nodules, up to 3 cm in diameter and grayishwhite in color, were scattered diffusely throughout both lungs.

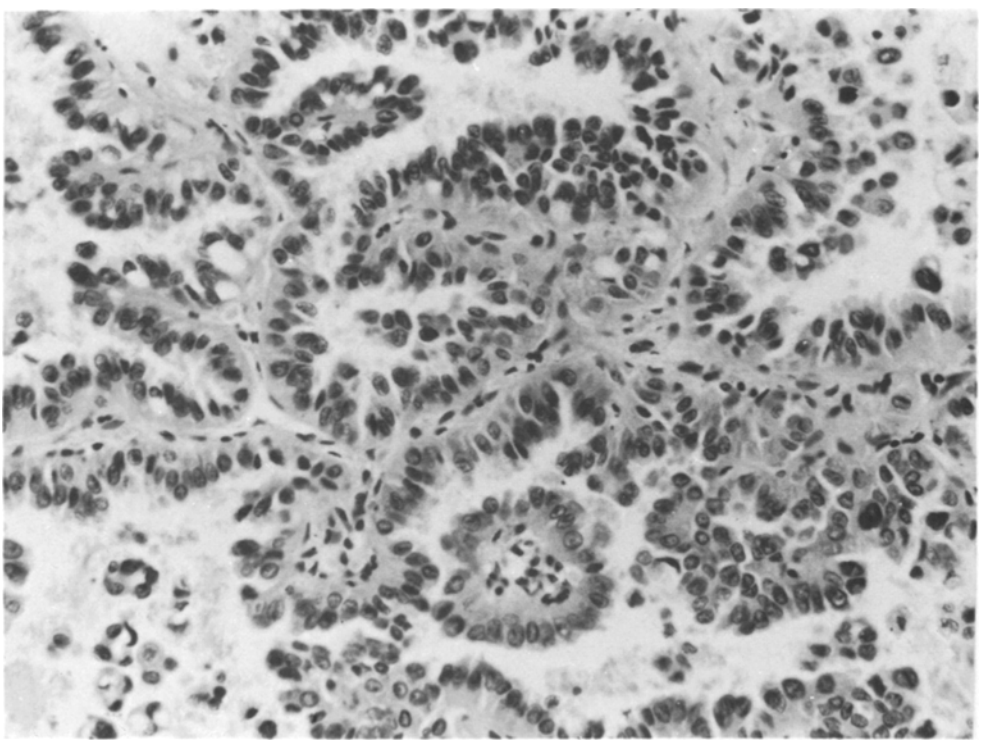

Fig. 1. Histological findings of the lung tumor: atypical epithelial cells with a thin fibrous stroma, forming a papillary pattern. Hematoxylin-eosin. ×200

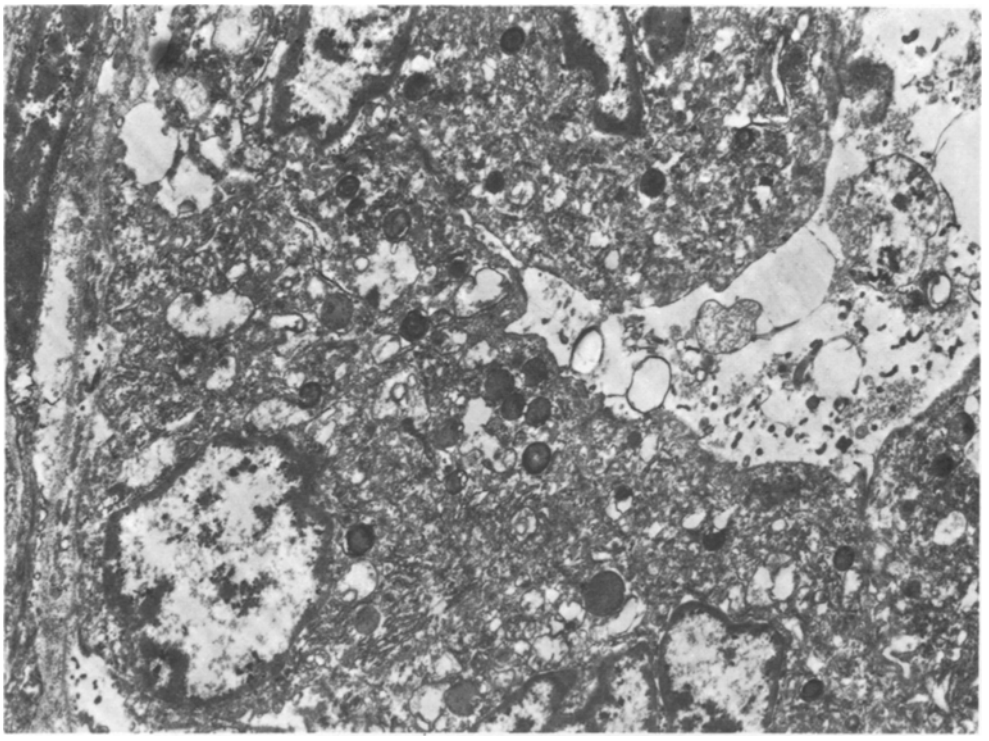

Fig. 2. Electronmicroscopical findings of the lung tumor: Tumor cells are arranged on a basal membrane and have numerous electron dense bodies at the apical region. ×4,000

The largest tumor nodule was noticed at the subpleural region of rt. middle lobe. Almost all of the bronchial lumena were filled with a dense mucinous substance. Histopathologically, atypical columnar epithelial cells, having hyperchromatic and large pleomorphic nuclei, are proliferating markedly and invasively, forming papillary patterns with thin fibrous connective stroma (Fig. 1). In some parts these atypical cells were proliferating along the alveolar wall and showed a structure similar to alveolar cell carcinoma.

Small metastatic nodules, up to 0.3 cm in diameter, were observed in the liver, similar masses up to 1.5 cm were seen in both adrenal glands and a nodule, measuring 0.8 cm, was found in the pancreas. Histologically, metastatic lesions are observed in the heart and prostate. Pulmonary hilar, paratracheal and bilateral neck lymph nodes were invated. The salivary glands were normal.

Electron Microscopical Findings

The tumor cells are arranged on a basal membrane and joined by complex cellular interdigitations. Some microvilli are observed on the free cell margin. Almost all of the tumor cells have round to oval nuclei with deep notches. The mitochondria are relatively swollen and have increased in number. The rough surface endoplasmic reticulum and the Golgi's apparatus are moderately developed (Fig. 2).

The most conspicuous findings in the tumor cells are the presence of numerous electron dense bodies, which are scattered diffuse and surrounded by

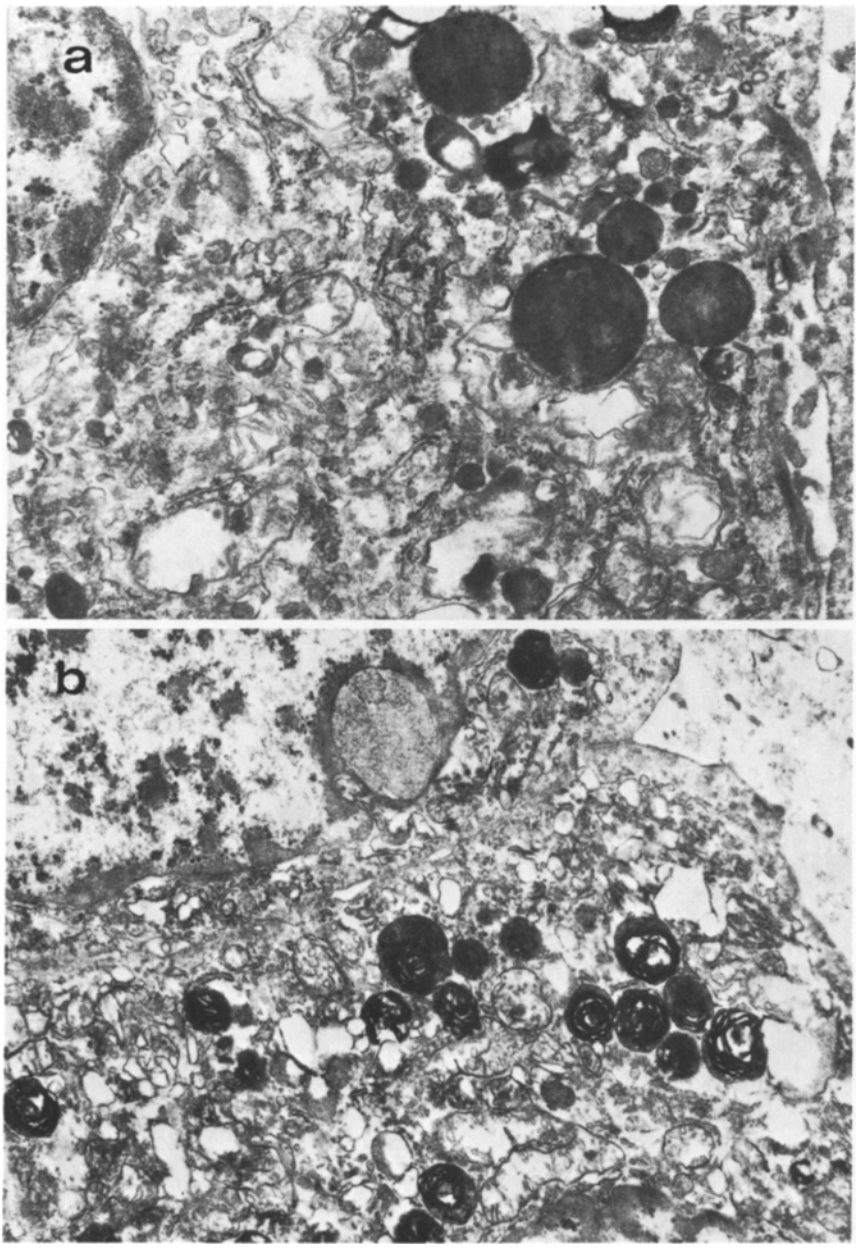

Fig. 3a and b. Electron dense bodies in the tumor cells. **a** Small round bodies surrounded by single limiting membrane and having fine granular matrix. ×12,000. **b** Large round bodies showing lamellar pattern. ×8,000

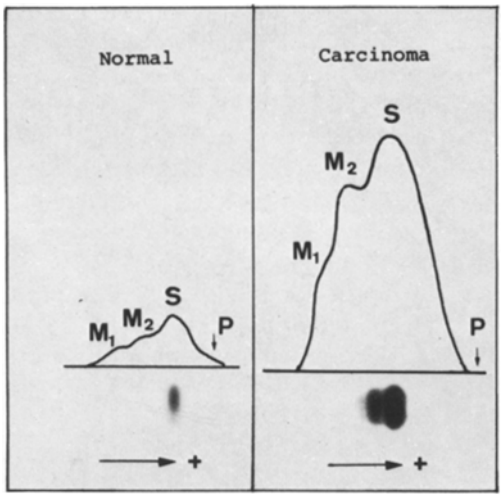

Fig. 4. Electrophoretic and densiometric pattern of amylase in normal and carcinomal lung extracts (C-5 and S-5). *P* pancreas type; *S* salivary type; *M1* and *M2* minor components

a single limiting membrane. These bodies vary in size, ranging from 0.2 to 0.7 μm in diameter, and show two patterns as follows:

I. Small Round Bodies. In which the matrix is finely granular and very similar to secretory granules such as zymogen granules in acinar cells of pancreas or salivary glands. These bodies are not numerous (Fig. 3a).
II. Large Round Bodies. In which the matrix varies in electron density and in which lamellar or annular patterns are noted in addition to the fine granular pattern. These bodies are very similar to cytosomes in large alveolar cells (Fig. 3b).

These two types of dense bodies are both scattered in the same tumor cells.

Biochemical Analysis

A homogenate of the pulmonary tumor (S-1) and of the normal pulmonary tissues (C-1) was prepared. Each homogenate was separated by centrifugation of 600 G/10 min into the recipitate (S-2 and C-2) containing the nuclear elements. Centrifugation at 10,000 G/10 min produced the precipitate (S-3 and C-3) containing the mitochondria. Ultra-centrifugation at 105,000 G/60 min produced a precipitate (S-4 and C-4) containing the microsomes and the supernate (S-5 and C-5) containing the soluble phase. Amylase activity was measured using a chromogenic substrate method with blue starch polymer and a photoelectric colorimeter. The total protain was measured by Biuret's method. The fractionated specimen S-1 showed an amylase level of 4,500 IU/g of protein, S-2, 1,000 IU/g of protein, S-3, 1,100 IU/g of protein, S-4, 4,300 IU/g of protein and

S-5, 11,300 IU/g of protein. The fractionated specimen C-1 showed a level of 150 IU/g of protein, C-2, C-3 and C-4, only few units and C-5, 360 IU/g of protein. S-4 included cholesterol level of 0.9 mg/g of wet tissue (gwt), a triglyceride level of 1.14 mg/gwt and a phospholid level of 1.1 mg/gwt. S-5 had a cholesterol level of 0.9 mg/gwt, a triglyceride level of 6.4 mg/gwt and a phospholipid level of 4.5 mg/gwt. The control specimen C-4 had a cholesterol level of 0.14 mg/gwt, a triglyceride level of 0.14 mg/gwt and a phospholipid level of 0.39 mg/gwt. In C-5 the cholesterol level was 0.5 mg/gwt, the triglyceride level, 1.9 mg/gwt and the phospholipid level, 1.4 mg/gwt.

Electrophoresis on cellulose acetate membrane showed that S-5 included salivary isoamylase (S-type isoamylase: 51%) and minor components (M1: 17% and M2: 31%) and C-5, S-type isoamylase (55%) and minor components (M1: 17% and M2: 23%) (Fig. 4).

Discussion

In this case, we found that the tumor origined from the peripheral region of the middle lobe of rt. lung, since tumor nodules were spreaded dominantly in lungs and the largest tumor nodule was observed at the middle lobe of rt. lung, in addition to histological and electromicroscopical findings.

Amylase producing lung cancer has attracted our interest since 1951, when Weiss et al. reported a case of "Elevated serum amylase associated with bronchogenic carcinoma". Our reference indicate since then, 22 cases of lung cancer associated with hyperamylasemia have been reported (Weiss et al., 1951; Gasser et al., 1959; Harada et al., 1971; Abe et al., 1973; Amman et al., 1973; Okada et al., 1973; Handular et al., 1975; Gomi et al., 1976; Sirsat et al., 1976; Sudo et al., 1976; Oshima et al., 1977; Otsuki et al., 1977; Yokoyama et al., 1977; Feood et al., 1978; Mitsuzawa et al., 1979 Morohoshi et al., 1980) 17 out of the 20 cases which have been examined histologically show adenocarcinoma, including papillary adenocarcinoma and alveolar carcinoma. The other three cases show undifferentiated carcinoma. Isoamylase has been examined in 18 out of the 22 cases and in all of these there is an elevation of S-type isoamylase. In addition to S-type isoamylase, Hadrada et al., Gomi et al., Otsuki et al., Yokoyama et al. and Mitsuzawa et al. found unusual S-type isoamylase which were sited at positive points from the migration point of normal S-type isoamylase in electrophoresis. We found minor components (M1 and M2) sited at positive points from the migration points of normal S-type isoamylase, which seemed to come of the alterative change of S-type isoamylase.

It is interesting that most of the amylase-producing lung cancers show histological pattern of adenocarcinoma. As amylase is an enzyme produced by exocrine cells and normal pulmonary tissues produce minimal amount of amylase (Ende, 1969), it is reasonable to suppose that adenocarcinoma, which originates from bronchioloalveolar epithelium may be maintaining its secretory function and producing amylase. Lung cancer associated with the ectopic production of hormones such as ACTH, ADH, MSH and others is usually an undifferenciated carcinoma (Ioachim, 1978) and considered often to be of neural crest origin.

Electronmicroscopical examination was performed by Amman et al., Gomi et al. and Yokoyama et al. They found electron dense granules similar to zymogen granules in the apical region of the tumor cells. In our case, it is noteworthy that only a few typical secretory-like granules were recognized, despite the amount of amylase in serum and in the tumor tissues. A great many large round bodies with a laminated or an anular pattern were noticed. We suspect therefore, that in addition to the small round bodies, the large round bodies may include amylase. This is supported by the results of cell fraction: the supernatants containing the microsomes and the soluble phase were found to contain the highest amylase level. Yokoyama et al. also found electron dense bodies similar to our large round bodies and suggested that they were young zymogen granules. However, the large round bodies seem to us to resemble cytosome-like bodies rather than secretory type granules. Because the laminated substance contained in cytosomes in normal large alveolar cells is considered to be alveolar surfactants (including lecithin, Yamamoto, 1972), these large round bodies would have to be complex proteins including amylase. This view is supported by the biochemical results, since phospholipids, cholesterol and triglycerides were found in high concentration in the supernatant showing the highest amylase level. In this case, therefore, we speculate that in addition to the exocrine secretory-system, the alveolar surfactant producing-system may play a part in ectopic amylase production.

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