Pseudomesotheliomatous Carcinoma of the Lung

An Immunohistochemical and Ultrastructural Study of Three Cases

Enrico Dessy, MD,* and Giuseppe G. Pietra, MD†

The authors report immunohistochemical and electron microscopic studies on three new cases of pseudomesotheliomatous carcinoma of the lung. Although the distinct clinical and histopathologic features of this peripheral lung cancer were described many years ago, its recognition as a distinct variety of lung carcinoma has not gained wide acceptance. Little is known of its incidence and only few cases have been reported until now. In the current study the authors demonstrate the epithelial nature of this tumor by its positive immunohistochemical reactions for epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), Leu-M1, B 72.3, and surfactant apoprotein. The ultrastructural features and staining of nuclear inclusions with surfactant apoprotein indicate differentiation into type II cells as found in other forms of peripheral lung adenocarcinoma. Despite these morphologic similarities, pseudomesotheliomatous carcinoma is characterized by extensive invasion of the pleura and rapidly fatal course. Because of this biologic behavior it deserves recognition as a distinct variant of peripheral lung carcinoma.
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THE TERM, pseudomesotheliomatous carcinoma, was coined by Harwood *et al.*[1] to identify a distinct variant of peripheral lung cancer characterized by extensive pleural growth and little parenchymal involvement. Because the tumor encases the lung in a thick tumor layer and often involves the parietal pleura, clinically, radiologically and macroscopically the tumor is indistinguishable from malignant pleural mesothelioma.[1-7] Even histopathologically pseudomesothelioma may be confused with malignant pleural mesothelioma because of its tubulopapillary pattern of growth associated with a prominent desmoplastic reaction. Previous reports have emphasized the use of histochemical techniques in the diagnosis of this neoplasm.[1,4,5]

From the *Institute of Pathology and Surgery, Pathologic Anatomy II, University of Cagliari Medical School, Cagliari, Italy; and the †Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

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Address for reprints: Giuseppe G. Pietra, MD, Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical School, Philadelphia, PA 19104.

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In the current report we describe the results of immunohistochemical and ultrastructural observations in three cases of pseudomesotheliomatous carcinoma. We demonstrate immunohistochemical and ultrastructural features suggesting a histogenetic relationship to type II and Clara cell adenocarcinomas. In spite of these morphologic similarities, the biologic behavior of pseudomesotheliomatous carcinoma, characterized by extensive invasion of the pleura and inconspicuous intrapulmonary growth, justifies its recognition as a distinct variant of peripheral lung adenocarcinoma.

Case Reports

Two of the cases reported here were identified from the autopsy files of the Department of Pathology of the Hospital of the University of Pennsylvania (HUP, Philadelphia, PA); the third case was sent for consultation to one of the authors (G.G.P).

Case 1

A 85-year-old man was admitted to HUP with a 3-month history of "cough after swallowing," dyspnea, and pleuritic chest pain. Pertinent findings on admission were diffuse rales, wheezes, and bilateral infiltrates on chest roentgenograms consistent with aspiration pneumonia. His past history was remarkable for 30 pack/year cigarette smoking. There was no known exposure to

asbestos products. Bronchoscopy and bronchial washing of the left lung showed atypical epithelial cells suggestive of bronchioloalveolar carcinoma. The patient had a rapid downhill course and died 2 weeks after admission.

At autopsy the lower lobe of the left lung was encased by a thick layer of gray–white tumor tissue which extended to the lower third of the parietal pleura partially obliterating the pleural space. A tumor nodule, about 4 cm in greatest dimension, was present at the base of the left lower lobe subpleurally, and was associated with dense fibrous adhesions between the base of the lower lobe and the diaphragmatic pleura.

Case 2

A 75-year-old man with unresectable esophageal carcinoma, treated by palliative radiation therapy and substernal gastroesophageal pull-through anastomosis, was admitted to HUP for progressive shortness of breath, anorexia, dysphagia, and weight loss. His past social history was remarkable for 40 pack/year cigarette tobacco use, alcoholism, and probable asbestos exposure. On admission, chest roentgenograms revealed multiple bilateral calcified pleural plaques on the diaphragm and lung apices, and a right lower lobe infiltrate. He developed respiratory alkalosis, leukocytosis, left vocal cord paralysis, and progressive respiratory insufficiency and died 2 days after admission.

At autopsy, the major findings consisted in squamous cell carcinoma of the proximal esophagus with invasion and compression of the trachea. Additional findings were severe centrilobular emphysema, and multiple calcified plaques bilaterally on the diaphragmatic and parietal pleura. Over the lateral aspect of the right lower lobe, the visceral pleura was thickened by a 3-cm tumor mass which extended into the subpleural connective tissue to a depth of 5 mm. The tumor did not invade the underlying lung tissue. The macroscopic impression at autopsy was that of a malignant pleural mesothelioma arising within a pleural plaque. Microscopic examination revealed a tubular, mucin-secreting carcinoma associated with an intense desmoplastic reaction (Fig. 1). The tumor cells were widely disseminated in the lymphatics of the right visceral pleura.

Case 3

A 70-year-old woman was admitted to an outside hospital for palliative right pleura stripping for a malignant mesothelioma involving her right visceral and parietal pleurae. The diagnosis of mesothelioma had been established 2 months earlier during a hospitalization for recurrent right pleura effusion. There was history of possible household exposure to asbestos. At the time of the first hospitalization focal pleural based densities involving the anterior and posterior aspects of the right hemithorax had been seen on chest roentgenograms and computed axial tomography (CAT) scan of the chest. However, no intrapulmonary masses had been detected. Bronchoscopy and bronchial biopsy were negative for malignancy. A needle biopsy of the right pleura revealed a tubulopapillary malignant epithelial tumor negative for epithelial mucin. At thoracotomy, the right pleural space contained 1400 ml of bloody fluid, and the pleura in the lower 50% of the chest and the costophrenic angle was thickened with tumor nodules. The tumor involved predominantly the parietal pleura but extended also the visceral pleura and the fissure between the middle and lower lobes. Metastatic tumor was present in the mediastinum. After pleural stripping the patient was treated with six courses of platin and mitomycin C. She did well for 19 months when reoccurrence and metastatic spread of the tumor were noticed. She received further chemotherapy with cytoxan, doxorubicin, and 5-fluorouracil (5-FU), but eventually died of widespread metastases 25 months after the original diagnosis. No autopsy was performed.

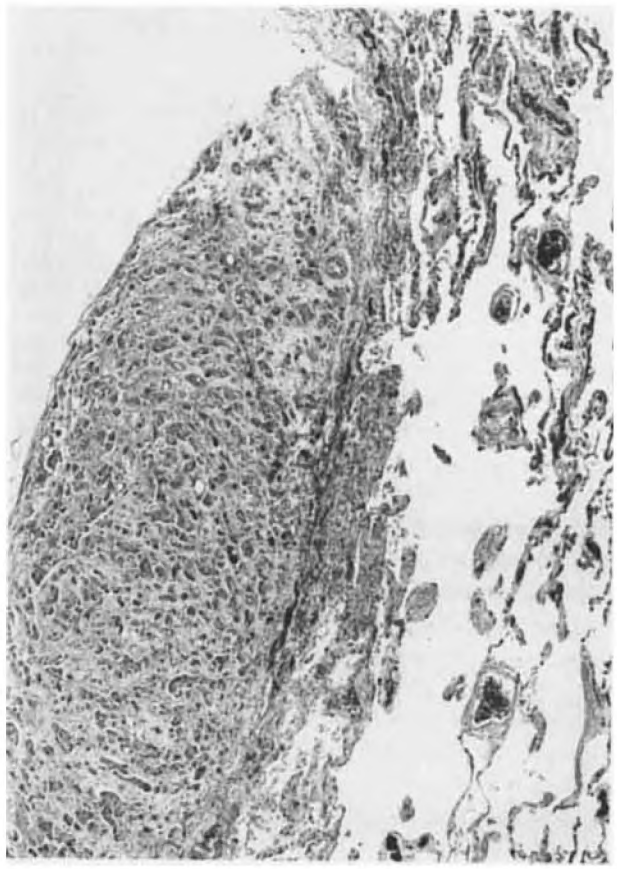

FIG. 1. Patient 2. Histologic appearance of pseudomesotheliomatous carcinoma. Low magnification showing the superficial spread of the neoplasm (H & E, ×40).

Materials and Methods

Histochemical and Immunohistochemical Study

Histologic, histochemical, and immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tissues.

Epithelial mucin was demonstrated by staining 5-μm sections with Meyer's mucicarmine or with periodic acid-Schiff (PAS) before and after diastase digestion (D-PAS). Intracellular hyaluronic acid was demonstrated with colloidal iron before and after digestion with *Clostridium perfringens* hyaluronidase (Sigma Chemical Co., St. Louis, MO).

The avidin–biotin complex immunoperoxidase technique was used for immunohistochemical studies.[8] After deparaffinization, with two changes in xylene, 5-μm sections were hydrated using graded ethanol solutions to distilled water and incubated in 0.3% (v/v) hydrogen peroxide (H_2O_2) in absolute methanol for 45 minutes to quench endogenous peroxidase activity. The sections were subsequently incubated in 20% (v/v) normal goat serum (30 minutes), followed by 1-hour incubation at room temperature with primary antiserum, 45 minutes with secondary biotinylated antibody, and 45 minutes with avidin–biotin complex reagent. After each step the sections were washed repeatedly with phosphate-buffered saline (PBS, pH 7.4), and then exposed to the Chromagen reaction solution (0.035% [w/v] diaminobenzidine (Sigma) in 10 ml TRIS buffer, filtered, and brought to 0.03% [w/v] H2O2) for 5 minutes. After being washed in tap water the sections were counterstained in hematoxylin, dehydrated, cleared, and coverslips were applied.

The sources and dilution of antibodies tested are shown in Table 1. Both negative and substitution serum controls were used. Positive tissue controls were inserted in each test.

Sections were evaluated for immunoreactivity by the two authors independently. The intensity of staining of individual cells was graded on a scale of 1+ to 3+; the percentage of positive cells was recorded.

Electron Microscopic Analysis

Patients 2 and 3 were evaluated by electron microscopic study after fixation of fresh tissue in 1.5% (w/v) paraformaldehyde-1.5% (w/v) glutaraldehyde in 0.1 mol/l sodium (Na)-cacodylate buffer. In Patient 1, electron microscopic studies were performed on representative sections cut from paraffin blocks and postfixed in aldehydes.[8] Samples were postfixed for 10 minutes in 1% (w/v) osmium tetroxide (OsO_4) in cacodylate buffer, dehydrated in ascending grades of ethanol, and embedded in Poly-bed 812 (Polysciences Inc., Warrington, PA). One micron-thick sections were stained with alkaline toluidine blue for survey; selected areas were further sectioned at 60 nm for electron microscopic study after staining with lead citrate and uranyl acetate.

TABLE 1. Source and Dilution of Antibodies

Reagent	Source	Dilution
Anticarcinoembryonic antigen	Dako, Santa Fe, CA	1:1250
Anti-B 72.3	National Cancer Institute, Bethesda, MD	1:5000
Anti-leu M1	Becton-Dickinson, Sunnyvale, CA	1:50
Antiepithelial membrane antigen	Dako	1:50
Polyclonal antisurfactant apoproteins	Dr. G. Singh, Department of Pathology, University of Pittsburgh, PA	1:1000
Monoclonal PE-10	Dr. T. Akino, Department of Biochemistry, Sapporo Medical College, Sapporo, Japan	1:400

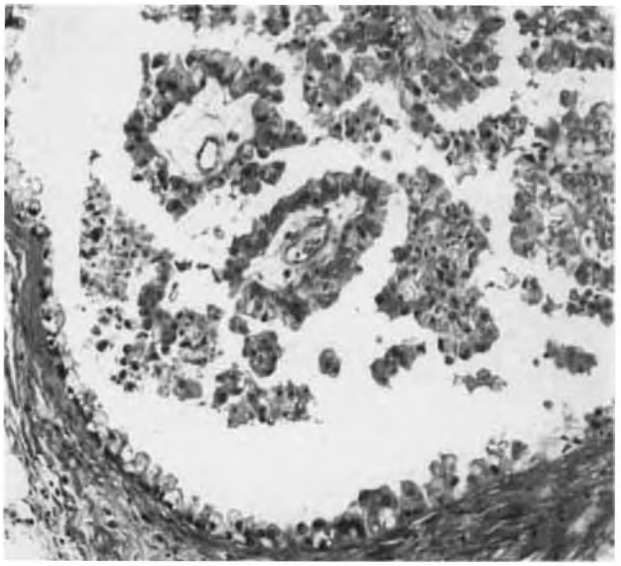

FIG. 2. Patient 3. The tumor has a predominant papillary pattern (H & E, ×100).

Results

Histopathologic Findings

In every case the predominant histopathologic feature was that of a tubulopapillary adenocarcinoma (Fig. 2). The tumor cells were polygonal to columnar, with abundant cytoplasm, irregular hyperchromatic nuclei, often containing one to two prominent nucleoli. Numerous psammoma bodies were present in Patients 1 and 3. Desmoplastic reaction was marked in Patients 1 and 2. In Patient 1, the glandular areas gradually blended into solid aggregates of malignant polyhedral and spindle cells, mimicking the biphasic epithelial and sarcomatous patterns of malignant mesothelioma (Fig. 3).

Epithelial mucin was demonstrated in a high percentage of the tumor cells with either D-PAS (Table 2) or colloidal iron. Mucicarmine gave less consistent results.

Immunohistochemical Results

The results of the immunohistochemical reactions are summarized in Table 2. In every case, the cytoplasm of the tumor cells was decorated with anti-CEA, EMA, Leu-M1, and B 72.3. The intensity and frequency of decoration was, however, variable. In all three cases, there was finely granular decoration of the tumor cell cytoplasms with

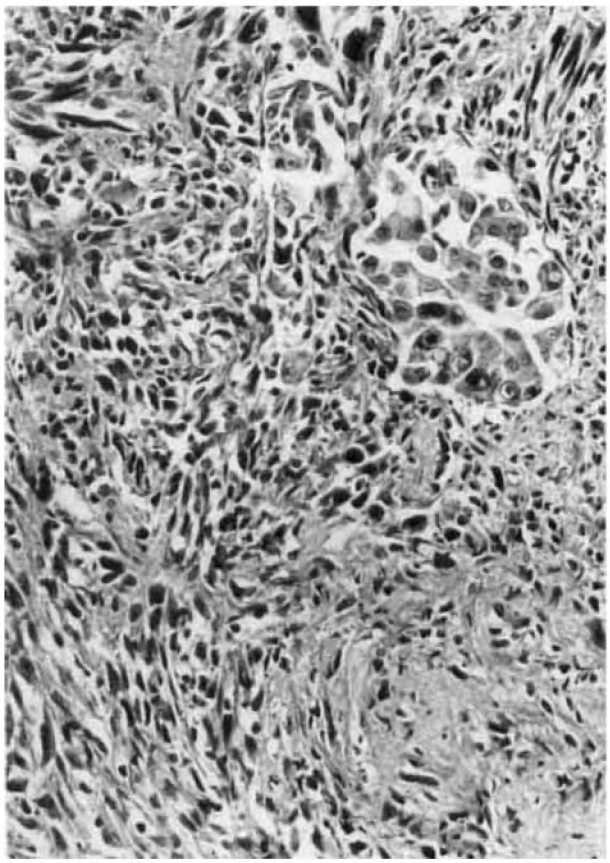

FIG. 3. Patient 1. The tumor is composed of nests of epithelioid and spindle cells, mimicking the biphasic features of mesothelioma (H & E, ×100).

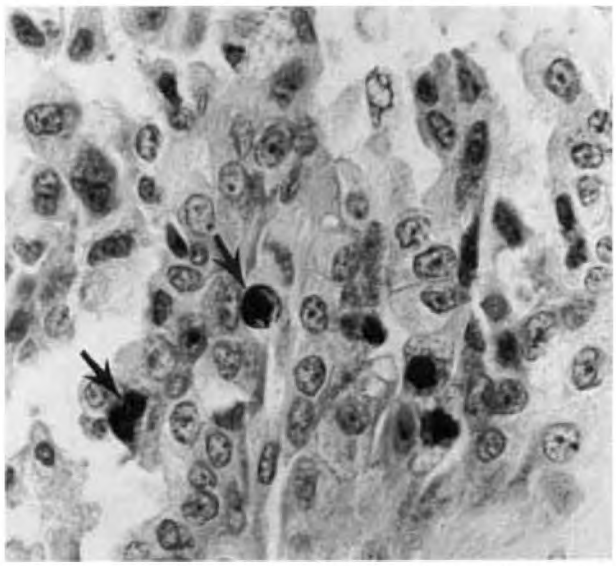

FIG. 4. Patient 3. Immunohistochemical staining with surfactant-apoprotein antibodies showing strong decoration of nuclear inclusions (arrows) (monoclonal PE-10, ×250).

both polyclonal[9] and monoclonal antisurfactant apoprotein antibodies[10] (Figs. 3 and 4). In Patients 1 and 3 there was also strong decoration of intranuclear inclusions[11] (Fig. 4). In Patient 1 decoration of the tumor spindle cells was more variable and only few cells were decorated.

Electron Microscopic Findings

The cytologic details were best preserved in Patient 3. In this case, the tumor cells lined papillary formations or microglandular structures. The tumor cells were interconnected, near their apical surface, by short desmosomes (Fig. 5). They had abundant cytoplasm containing numerous mitochondria, electron-lucent vacuoles, and lamellar bodies of variable size (Figs. 5 and 6). The smooth and rough endoplasmic reticulum were not prominent and small bundles of cytofilaments were irregularly dispersed within the cytoplasm. The luminal surface of the tumor cells had a variable complement of microvilli which varied in size and shape, ranging from short, stubby, often branched forms to long, thin structures, with a length/width ratio of about 10:1 (Fig. 6). In Patients 1 and 2, postmortem autolytic changes and paraffin embedding caused marked swelling and electron lucency of the matrix of the mitochondria and endoplasmic reticulum. However, the remaining cytoplasmic features were sufficiently preserved to identify long, thin microvilli, lamellar bodies, and intracellular microacini.

TABLE 2. Histochemical and Immunohistochemical Features of Pseudomesotheliomatous Carcinoma

Patient no.	Age (yr)	Sex	D-PAS	CEA	EMA	Leu-M1	B72.3	Surfactant apoprotein†	
								Monoclonal	Polyclonal
1	85	M	++ (70)	++ (20)	++ (80)	+++ (80)	+++ (60)	++ (100)	++ (80)
2	75	M	++ (20)	++ (10)	+++ (100)	++ (30)	++ (50)	++ (40)	++ (30)
3	70	W	++ (30)	++ (60)	nd	+++ (80)	++ (50)	++ (60)	++ (30)

D-PAS: periodic acid-Schift after diastase digestion; CEA: carcinoembryonic antigen; EMA: epithelial membrane antigen.

* For each case the intensity of reaction is graded from + to +++, with the percentage of stained cells in brackets.

† Positive nuclear inclusions in Patients 1 and 3.

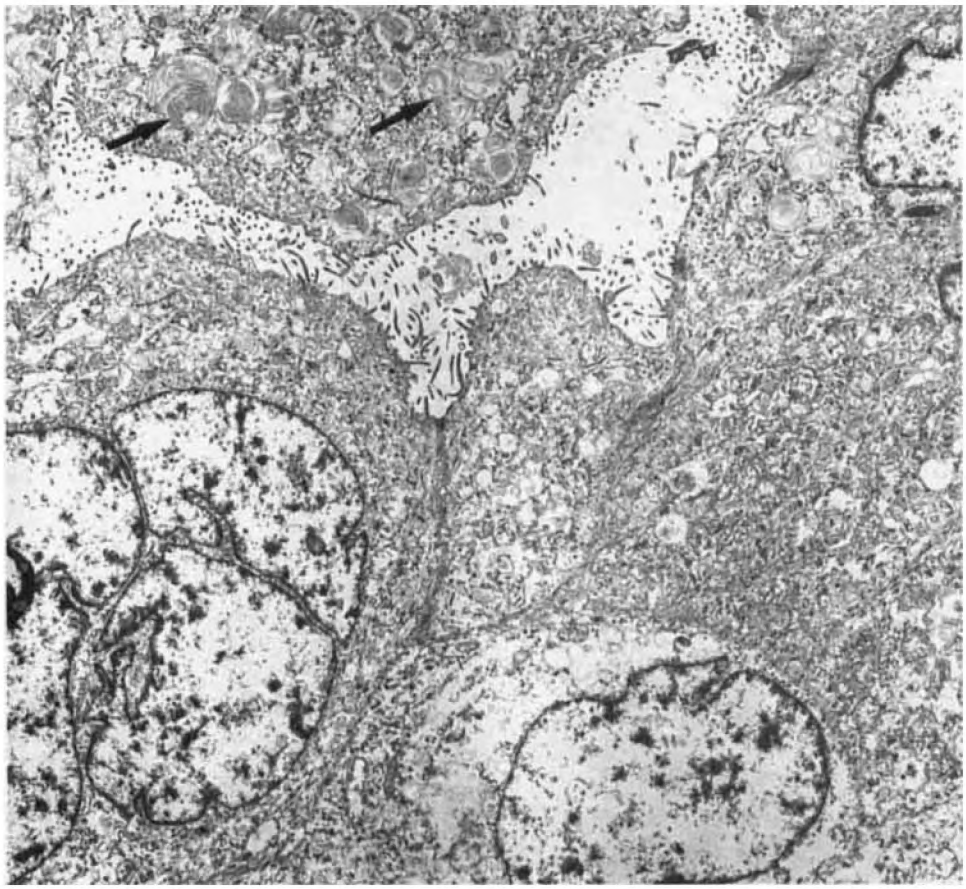

FIG. 5. Patient 3. Ultrastructural aspect of the tumor cells showing acinar arrangement. The cells contain lamellar bodies (arrows) and slender microvilli and are interconnected by small desmosomes (original magnification ×4000).

Discussion

Harwood *et al.*,[1] in reporting six cases of pseudomesotheliomatous carcinoma of the lung, made a convincing case for considering this entity as a distinct variant of lung cancer. The tumor is clinically, radiologically, and macroscopically indistinguishable from malignant pleural mesothelioma, and shares with the latter a rapidly fatal course. In contrast to mesothelioma, there was no apparent causal association with exposure to asbestos or other environmental carcinogens.

However, their observation has not gained wide acceptance. The entity of pseudomesotheliomatous carcinoma is briefly mentioned in current pulmonary pathology textbooks,[12,13] but it has not been included in the revised classifications of lung tumors by the World Health Organization (WHO)[14] or Lung Cancer Study Group.[15] Based on our experience of other cases referred for second opinion and presumed to be pleural mesotheliomas, the tumor is more common than the fifteen cases reported in the literature would lead one to believe. With the increasing reliance on CAT scans and noninvasive diagnostic procedures, and the general decline in the number of autopsies performed on patients with cancer, it is likely that pseudomesothelioma will continue to be confused with malignant pleural mesothelioma.

The correct diagnosis of pseudomesotheliomatous carcinoma of the lung is based on the recognition of distinct histopathologic features which allow its differentiation from the epithelial variant of malignant mesothelioma.

A variety of histochemical and immunohistochemical methods have been proposed to help in the differential diagnosis between pleural malignant mesothelioma and pleural metastases of adenocarcinoma.[7,16–19] We have found, as have others,[7] that immunohistochemical reactions, particularly the positive reaction for CEA, Leu-M1 and B 72.3, are useful in distinguishing pseudomesotheliomatous carcinoma from mesothelioma. The results of the current study suggest that the immunohistochemical demonstration of surfactant apoprotein in tumor cells[20] invading the pleura, may be of further help in differentiating metastases of lung carcinoma from metastases of extrapulmonary malignancies. Others have emphasized the diagnostic importance of electron microscopic study[21,22] and the length:width ratio of microvilli in the diagnosis of malignant mesothelioma.[7,22,23] We have found that the length:width ratio of microvilli, by itself, is not a reliable diagnostic feature to distinguish meso-

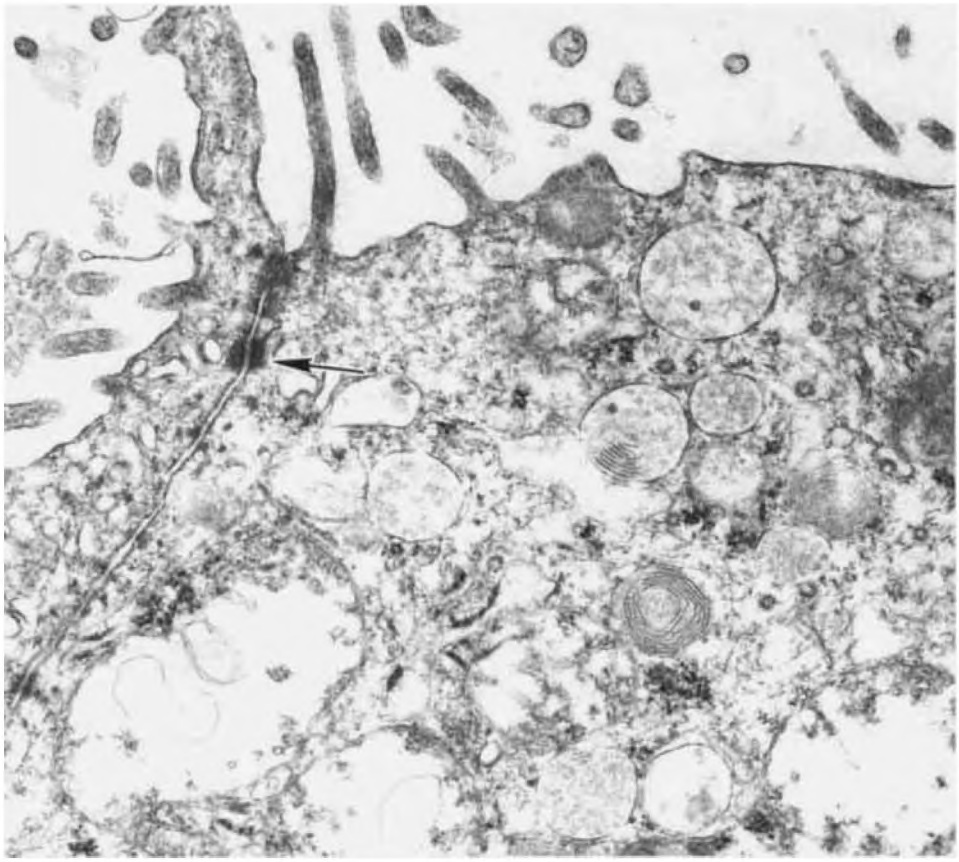

FIG. 6. Patient 3. Higher magnification shows slender microvilli and small desmosomes (arrows) and lamellar bodies (original magnification ×30,000).

thelioma from pseudomesothelioma, since the latter may also show long microvilli.

The immunohistochemical demonstration of surfactant apoprotein in nuclear inclusions and the ultrastructural features of lamellar bodies suggest a derivation of this tumor from type II cells or Clara cells. Because of its marked desmoplastic reaction, a relation to the so-called "scar carcinoma" has been proposed.[4] In recent years it has become apparent that assumptions as to the histogenesis of a lung neoplasm should not be made solely on the basis of morphologic features. The mere fact that a neoplasm shows features of type II cells or Clara cells does not warrant the conclusion that these cells have dedifferentiated to become neoplastic. During the development of cancer there may be activation of repressed genes that regulate differentiation, leading to the transformation of columnar epithelial cells into highly specialized cells, like type II and Clara cells.

In spite to its similarity to bronchioloalveolar and other peripheral lung papillary carcinomas composed of type II pneumocytes and Clara cells, pseudomesotheliomatous carcinoma has a markedly dissimilar biologic behavior. Because of the extensive pleural involvement, its prognosis is poor. In the reported cases, the average survival, from onset of symptoms, was 4.2 months (range, 0.8 to 9 months). In our own material, the first patient died a few months after the onset of symptoms; in the second patient the tumor was discovered at autopsy. The third patient died of widespread metastases in approximately 2 years after diagnosis. Further studies are needed to determine whether the aggressive behavior is due to intrinsic invasive properties of the tumor cells or to the unique location of the tumor in close proximity to the vast pleura lymphatic network.

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