Ultrastructural Comparison of "Alveolar" and "Solid" Areas in Bronchioloalveolar Carcinoma

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Abstract. The purpose of this study was to compare the ultrastructural features of bronchioloalveolar carcinomas, contrasting the well-differentiated alveolar component and the poorly-differentiated solid component in the same tumor. We studied 7 cases of non-mucinous bronchioloalveolar carcinomas by electron microscopy. Two of these cases showed lamellar bodies in both the alveolar and solid components and the remaining 5 cases revealed Clara cell granules in both components. We conclude that the neoplastic cells in bronchioloalveolar carcinoma retain their ultrastructural phenotypes after becoming invasive carcinoma with loss of alveolar differentiation. *(received 6 February 2002; accepted 15 March 2002)*

Keywords: bronchioloalveolar carcinoma, electron microscopy, lamellar bodies, Clara cell granules

Introduction

Bronchioloalveolar carcinoma (BAC) is a distinct type of pulmonary adenocarcinoma. BAC, a neoplasm of unknown etiology, tends to be multifocal and occurs disproportionately in females and non-smokers [1-3]. Survival of patients with stage I (T1N0M0) BAC is >90% at 5 yr after surgical excision and without adjuvant therapy [4]. Since BAC is such a treatable disease, pathologists must observe the utmost care in classifying lung tumors. Ultrastructural studies of BAC point to a type II pneumocyte or Clara cell origin, but the precursor cell is still unknown [5-8]. This comparative study was performed to characterize the ultrastructural features of BAC at different stages of differentiation, with attention to the definition of BAC in the World Health Organization (WHO) classification of lung tumors [9].

Materials and Methods

We selected 7 recent cases of non-mucinous BAC for ultrastructural examination. Six of the 7 patients were female. Their mean age was 74 yr. The tumors were all located peripherally and ranged from 1 to 2.5 cm in diameter (average 1.4 cm) (Table 1). The specimens were all taken from surgically resected lobes of lung.

The lung tissue was prepared for electron microscopic examination by standard methods. Sections (1 μm) of toluidine blue-stained tissue were examined by light microscopy and two foci were selected for examination in each case, one from the alveolar component and the other from the solid component. Electron microscopy was performed with a JEOL JEM 100 CXII transmission electron microscope.

Results

The selected BACs showed two distinct growth patterns by light microscopy. One was the well-differentiated, alveolar, or so-called "typical bronchioloalveolar" pattern, and the other was the poorly-differentiated, solid growth pattern, without recognizable features of BAC (Fig. 1). The alveolar component showed a linear arrangement of hobnail cuboidal to columnar cells, resting on the alveolar and bronchiolar basement membranes. The solid component was composed of solid nests and

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Table 1. Summary of ultrastructural findings in 7 cases of bronchioloalveolar carcinoma (BAC).

Case #	Age (yr)	Sex	Site	Diameter (cm)	EM (A)	EM (solid)
1	90	Female	LLL	1.0	LB	LB
2	79	Male	LUL	1.6	CCG	CCG
3	55	Female	RLL	2.3	CCG	CCG
4	77	Female	LUL	1.0	LB	LB
5	59	Female	RUL	2.5	CCG	CCG
6	90	Female	LUL	1.0	CCG	CCG
7	74	Female	RLL	1.6	CCG	CCG

LLL: left lower lobe; LUL: left upper lobe; RLL: right lower lobe; RUL: right upper lobe; EM: electron microscopy; A: alveolar pattern; LB: lamellar bodies; CCG: Clara cell granules

conglomerates of neoplastic cells with features suggestive of adenocarcinoma embedded in a fibrotic stroma.

Electron microscopy revealed either lamellar bodies or Clara cell granules in the cytoplasm of neoplastic cells (Figs. 2 & 3). In 2 of the 7 cases (cases #1 and #4), the neoplastic cells showed cytoplasmic lamellar bodies in both the alveolar and solid components indicative of type II pneumocyte differentiation (Table 1).

Occasionally in the solid area, the cells in cases #1 and #4 showed residual dense bodies containing degenerating lamellar bodies (Fig. 3B, inset). In the remaining 5 cases, the neoplastic cells in both the alveolar and solid components showed Clara cell granules. These membrane-bound granules were round, electron dense bodies with a mean diameter of 500 nm (range, 400-700 nm). In both of the cases with type II pneumocyte differentiation and Clara cell features, the frequency of these structures was generally diminished in solid zones compared to the alveolar component. In addition, the neoplastic cells contained short microvilli.

Discussion

The term bronchioloalveolar carcinoma (BAC) was first adopted by Liebow [10] to describe a specific subset of lung tumors with a linear spread of malignant cells growing along the pre-existing pulmonary parenchymal structures. The definition of BAC has been recently narrowed to a lepidic growth of malignant cells along the alveolar septa without stromal, vascular, or pleural invasion [9].

Our cases of BAC showed a predominant bronchioloalveolar, alveolar, growth pattern, but in addition, there were areas of stromal invasion characterized by solid nests and glands of malignant cells in a disrupted and fibrotic stroma. According to the WHO classification of lung tumors, solely based on light microscopic examination, such tumors should not be classified as BAC, but should be listed as "adenocarcinoma mixed bronchioloalveolar, solid and acinar," implying a BAC with bronchogenic differentiation or so-called dedifferentiation [9]. By adhering to this recently introduced classification, one might anticipate a

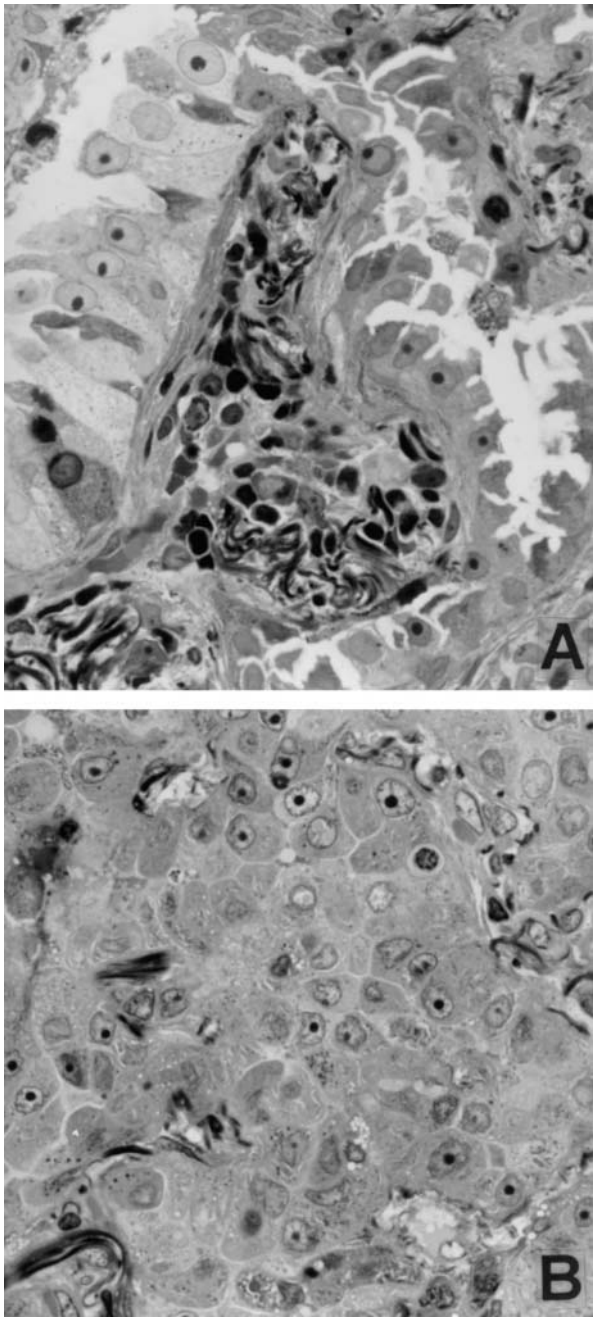


Fig. 1. Photomicrograph of plastic sections (1 μm), stained with toluidine blue from a bronchioloalveolar carcinoma showing well-differentiated "alveolar" growth pattern (panel A, x 540) and a second area with undifferentiated "solid" growth pattern (panel B, x 540).

poor prognosis despite aggressive multimodality therapy, rather than the favorable prognosis that is generally associated with BAC [4]. Our results illustrate that malignant cells retain their ultrastructural BAC phenotype after stromal invasion. All of our cases are recent cases and none of them is qualified for a 5-yr evaluation, but the expected prognosis, purely after surgery, is excellent.

The definition of BAC as a strictly noninvasive carcinoma and its separation from mixed adenocarcinoma have prompted some authors to propose an intermediate category of BAC [1,11]. Castro et al [11] classified lung adenocarcinomas based on the percentage of the bronchioloalveolar component. They designated tumors as BAC, mixed adenocarcinoma, or solid/acinar adenocarcinoma if the tumors showed >75%, 50-75%, and <50% bronchioloalveolar pattern, respectively. Their results showed that despite stromal invasion, BAC and mixed adenocarcinoma had significantly better 5-yr survival than the solid/acinar adenocarcinoma. They suggested that an adenocarcinoma be designated as BAC when the tumor has more than 50% bronchioloalveolar growth pattern [11].

Similarly, Koga et al [1] classified their cases of lung adenocarcinomas based on the WHO classification with the interjection of an intermediate category of BAC with invasive growth when the

Fig. 2 (on page 228). Electron photomicrograph from the bronchioloalveolar carcinoma with "alveolar" pattern (panel A; x 6,600) and "solid" area (panel B; x 11,000) of the same tumor. In both areas, "alveolar" and "solid," the neoplastic cells contain Clara cell granules (arrow). The surface of the cells is lined with short microvilli. Nuclei are identified as "N," collagenous matrix as "C."
Fig. 3 (on page 229). Electron photomicrograph from the bronchiolo-alveolar carcinoma with "alveolar" region (panel A; x 7,000) and a "solid" growth area (panel B; x 13,5000) of the same tumor. The neoplastic cells in both panels (A and B) show lamellaeform inclusions (arrows), a characteristic feature of type II pneumocyte differentiation. The numbers of these inclusions appear to be decreased in the "solid" zone of growth. Nuclei are identified as "N." The inset (panel 3B) shows several lysosomes in the cytoplasm of a neoplastic cell in the "solid" area. The image is consistent with gradual autophagic degradation of lamellar bodies into dense residual body lysosomes (inset; x 38,000).

Fig. 2 (legend on page 227).

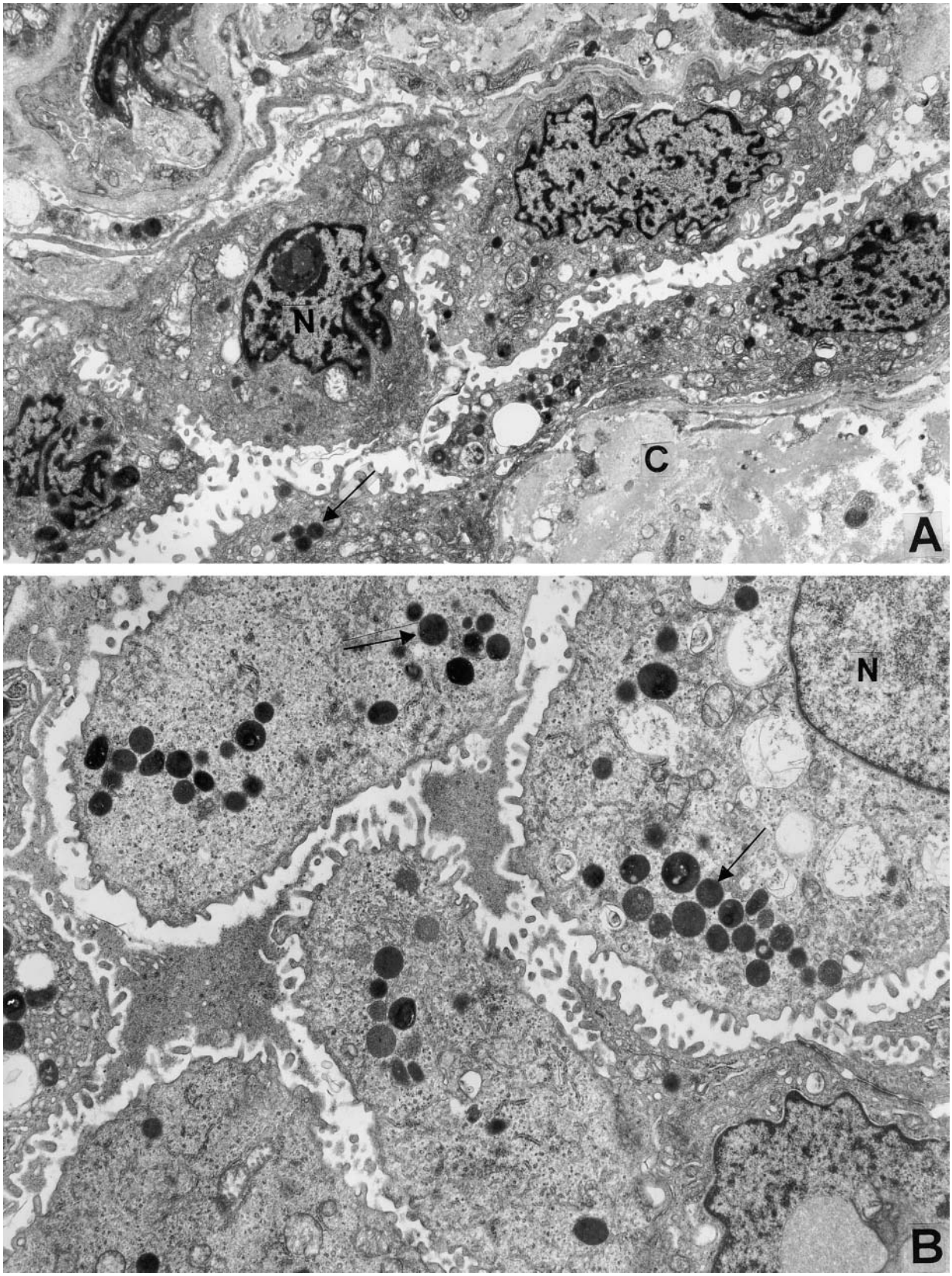
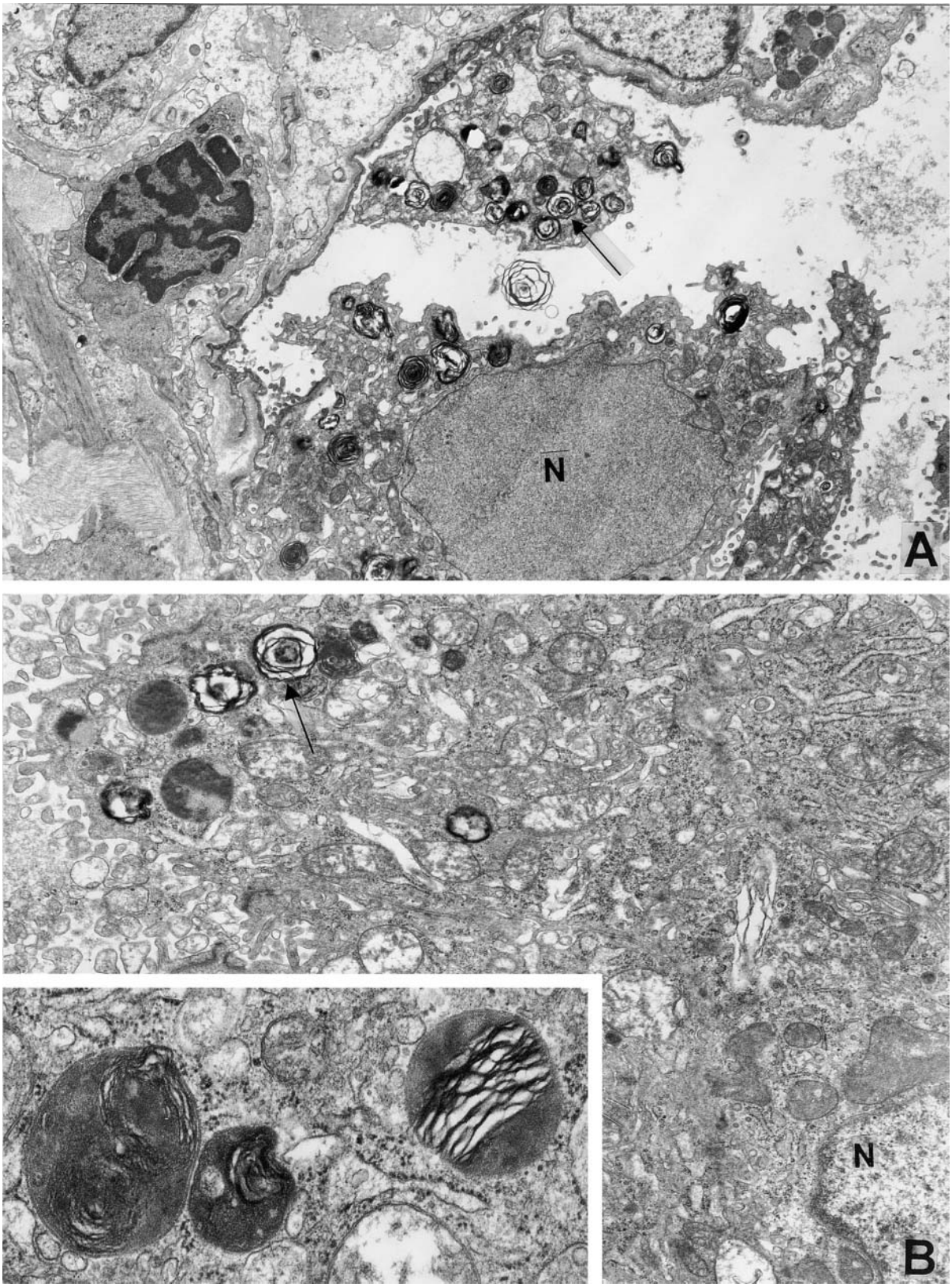


Fig. 3 (legend on page 227).



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tumor had more than 50% BA pattern. Their results suggest that BAC-invasive type should be classified independently from the mixed subtype in the WHO criteria, since the former has a better prognosis [1].

Ultrastructurally, non-mucinous BACs show either lamellar bodies or Clara cell granules indicative of type II pneumocyte or Clara cell differentiation, respectively [5-8]. Kitamura et al [8] illustrated that the cells comprising the postulated precursor lesion of BAC (ie, atypical adenomatous hyperplasia), predominantly contain lamellar bodies, whereas the cells comprising "overt" BAC contain both lamellar bodies and Clara cell granules. They attributed this phenotypic change to Clara cell metaplasia [8].

In two of our cases of BAC, the malignant cells revealed lamellar bodies in the alveolar and solid components (Fig. 3). Occasionally, the lamellar bodies in the solid part were in the process of degradation in lysosomal residual bodies (Fig. 3B, inset). We believe that these modified lamellar bodies point to degradative transformation by an intracellular autophagic system, rather than to Clara cell metaplasia. In the other 5 cases of BAC, the neoplastic cells revealed Clara cell granules in both the alveolar and solid parts (Fig. 2).

In summary, we have observed that the malignant cells in the poorly-differentiated, solid component of our cases, although not readily recognizable as BAC by light microscopy, show ultrastructural features of the well-differentiated, non-invasive, alveolar component. Accepting the dictum, "lung carcinomas are classified according to the best-differentiated component and graded by the most poorly differentiated component," as proposed by the WHO [12], we feel that our cases are BAC, despite poorly-differentiated solid growth with invasive features. Due to the serious therapeutic implications (eg, withholding chemotherapy, in case of BAC) or implementing chemotherapy in case of non-BAC, we urge pathologists to perform electron microscopy when there is difficulty in the classification of pulmonary adenocarcinomas.

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