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Immunohistochemical Study of Basement Membrane Antigens in Bronchioloalveolar Carcinoma

W. F. GRIGIONI, G. BIAGINI, S. GARBISA, A. D'ERRICO, M. MILANI, M. MASTRORILLI, V. VASI, V. VILLANACCI, G. GOZZETTI, and A. M. MANCINI

From the Departments of Pathology, Ultrastructural Pathology, and Surgery, the Institute of Histology, University of Bologna, Bologna, Italy, and the Institute of Histology, University of Padova, Padova, Italy

Bronchioloalveolar carcinoma (BAC), not yet completely defined as a biologic entity, has recently been classified into two different types. Immunohistochemical investigations, aimed at characterizing basement membrane (BM) behavior in the two types of BAC, revealed different distribution patterns. The first (Type I BAC) showed a linear staining for laminin and Type IV collagen similar to normal lung. Fibronectin was widely present in the septal interstitium and patchily distributed along the BM. The second (Type II BAC) showed a variable reaction for Type IV collagen and fibronectin, whereas laminin was absent or appeared as short, interrupted tracts around the epithelial neoplastic population, similar to conventional adenocarcinoma of the lung. These results suggest that only Type I BAC shows structural characteristics different from those of conventional adenocarcinoma of the lung. (Am J Pathol 1987, 128:217–224)

BRONCHIOLOALVEOLAR carcinoma (BAC) is a morphologic and clinical dilemma. BAC is usually considered a variant of peripheral lung adenocarcinoma (PLA), with a more diffuse and evident alveolar organization, and light- and electron- microscopic studies of BAC show many similarities with PLA, suggesting a common histogenesis.[1–4] The pleomorphic clinical aspects and the lack of any well-defined relationship between histologic features make it hard to consider BAC a defined biologic entity, easily distinguishable from conventional lung adenocarcinoma.[2,5]

Recently, histologic, clinical, and prognostic findings have suggested that BAC may be further subclassified into two main categories showing different biologic features,[6] although transitional aspects are also possible. These variable characteristics may account for the discordant opinions on BAC histogenesis and classification reported in the literature.[7–14]

If BACs are defined as all lung tumors characterized by an alveolar organization and a linear arrangement of neoplastic cells along a fibrovascular septum serving as a support,[15] two histotypes can be identified: 1) one, generally with a multiple nodular arrangement, mainly composed of mucinous cells on a preexistent septal alveolar stromal scaffold; 2) a second type organized as a single nodule, generally with nonmucinous neoplastic cells covering a thick fibrovascular septum where numerous lymphoid cells may be present.

In addition to histologic features, these two pathologic entities can also be differentiated on the basis of their tendency, particularly of the first type, to disseminate aerogenously within the lung, mimicking the clinical features of pneumonia, with important prognostic implications.[16–20]

These different morphologic features could express distinct biologic characteristics. As a rule, however, the two biologic entities are not easily separable. BACs with intermediate clinical and pathological patterns are also detectable and often difficult to distinguish from conventional lung adenocarcinoma.

It has been established that the invasive properties of cancer cells are linked to the morphofunctional relationship existing between carcinoma cells and the extracellular matrix microenvironment.[21,22] These interactions might influence tumoral cell behavior

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Address reprint requests to Prof. Walter Franco Grigioni, Instituto di Istologia ed Anatomia Patologica, Policlinico S. Orsola, Via Massarenti 9, 40138 Bologna, Italy.

mediating stroma–epithelium attachment and could also modulate morphogenesis and the organization of neoplastic tissues.[23–25]

Thus, immunohistochemical investigations, aimed at evaluating qualitative and quantitative characteristics of the extracellular matrix in BAC, could supply useful information on the clinical and biologic behavior of BAC and pinpoint objective characteristics distinguishing this tumor from conventional peripheral lung adenocarcinoma.

Materials and Methods

Among all cases of peripheral lung adenocarcinoma in the files of the Institute of Pathology of Bologna University, 12 cases of BAC and 4 cases of conventional (well to poorly differentiated) lung adenocarcinoma were selected. We accepted as BAC those tumors that, in all blocks examined, showed a neoplastic growth pattern characterized by an alveolar organization, with tumoral cells lying on fibroalveolar septa and histologically suggesting a growth of tumor cells on preexisting septa.

According to Manning's criteria,[6] 3 cases were classified as Type I BAC and 9 as Type II BAC. Any tumors representing the transitional form between the two were excluded.

After lobectomy and pneumonectomy, 10–20 samples from neoplastic and adjacent normal lung were collected and fixed in 10% buffered formalin. All nearby lymph nodes were drawn and their location plotted on a map.

After routine staining, conventional adenocarcinomas and Type I and Type II BACs were also studied by immunohistochemical techniques.

Immunohistochemistry

The immunohistochemical method employed was the avidin–biotin peroxidase complex (ABC)[26,27] using antibodies against Type IV collagen, laminin, and fibronectin, kindly supplied by Dr. L. Liotta, of the National Institutes of Health, Bethesda, Maryland.

Sections were deparaffinized in xylene and rehydrated in alcohol, washed in distilled water, and rinsed in Tris-buffered saline (TBS) for 5 minutes. All sections were treated with 0.4% Pepsin in HCl 0.1 N for 2 hours at 37 C to unmask the extracellular antigenic sites,[28] and with a 0.3% solution of 30% H_2O_2 in TBS for half an hour to inhibit endogenous peroxidase activity, then washed in TBS for 20 minutes.

Sections were then incubated with goat serum (diluted 1:60) for 30 minutes and subsequently with primary antibodies—anti-Type IV collagen (1:500), anti-laminin (1:100), and anti-fibronectin (1:60)—overnight. After 20 minute's washing in TBS the sections were incubated with biotinylated antirabbit immunoglobulins for 30 minutes, washed in TBS for 20 minutes, reincubated by the ABC method for an hour in the dark, and again washed in TBS for 20 minutes. The developing agent used was 0.05%, 3.3′-diaminobenzidine-tetrahydrochloride (DAB-Sigma) in TBS, pH 7.4, with 0.01% H_2O_2 30%. Sections were subsequently counterstained in Mayer's hematoxylin and mounted in Permount.

Results

In our series, 3 cases of lung adenocarcinoma were classified as Type I and 9 as Type II BAC according to Manning's criteria.

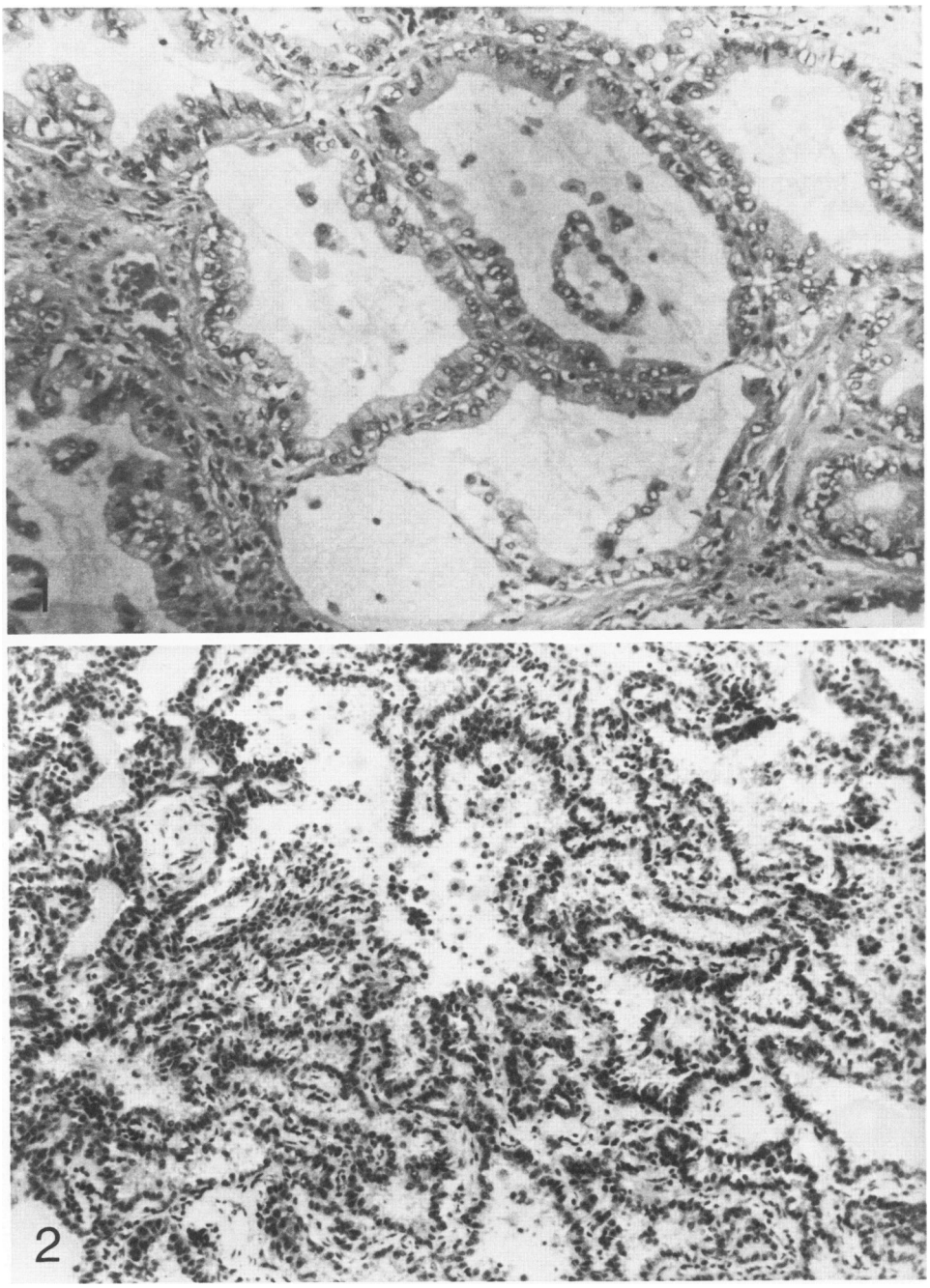
In the Type I BACs, commonly in the form of multiple nodules, the neoplastic elements were composed of muciparous, columnar cells lying on a septal–alveolar matrix and diffusing contiguously onto the adjacent bronchioli and alveolar ductules (Figure 1).

The Type II BACs, organized as a single nodule, ranging from 0.5 to several centimeters in diameter, showed a pleomorphic cytologic pattern: cuboidal or polyhedral cells, with large, polymorphous nuclei, and covered alveolar cavities delimited by thick stromal septa with some papillary projections (Figure 2). These septa, with many lymphoid and fibroblastic cells, seemed to fade gradually into the alveolar septa of the surrounding lung parenchyma.

In 2 cases, which also had scarring areas, BAC features were no longer detectable, and the carcinomatous cells were organized in glandular structures as in typical lung adenocarcinoma.

In the cases classified as conventional adenocarcinoma, the neoplastic tissue was organized in acinar and tubular structures, with cuboid–columnar epithelial cells, sometimes with muciparous features surrounded by a desmoplastic reaction. In all three groups of carcinomas, the immunohistochemical reaction for laminin, Type IV collagen, and fibronectin displayed different distribution patterns. Type I BAC showed a positive linear staining with anti-laminin and anti-Type IV collagen antibodies around neoplastic cells, forming a BM-like structure. These features resembled those observed in adjacent histologically normal lung (Figures 3–5).

Fibronectin was widely distributed in the interstitium, in association with collagen fibers, and fibronectin positivity with a patchy distribution was also seen at the BM level. Type II BAC and conventional adenocarcinoma showed a glycoprotein staining reaction that consistently differed from that of Type I



Figures 1 and 2—Bronchioloalveolar carcinoma. In Type I (**Figure 1**) (×75) histologic aspects suggest a linear arrangement of neoplastic cells along fibrovascular septa of the lung. In Type II (**Figure 2**) tumor cells cover thick fibrovascular septum (×50). (H&E)

BAC. Laminin was present as irregular tracts around the epithelial neoplastic population in all cases (Figure 6) but one. The antibodies against Type IV collagen and fibronectin revealed a variable staining from linear to spotty (Figures 7 and 8).In the stroma surrounding neoplastic cells, fibronectin was clearly evident.

Discussion

BAC is a heterogeneous entity, from a histogenetic, clinical, and prognostic point of view. Some authors have suggested that BAC is a variant of typical lung adenocarcinoma, from which it differs only in its architectural organization. The wide range of variants present in this neoplasm suggests that a heterogeneous group of tumors is arbitrarily assembled under a single nosologic entity.

By criteria suggested by the World Health Organization (WHO), only tumors originating from the alveoli or respiratory bronchioli and spreading contiguously to other alveoli, via preexisting septal alveolar primers, can be classified as BAC.[15] These character-

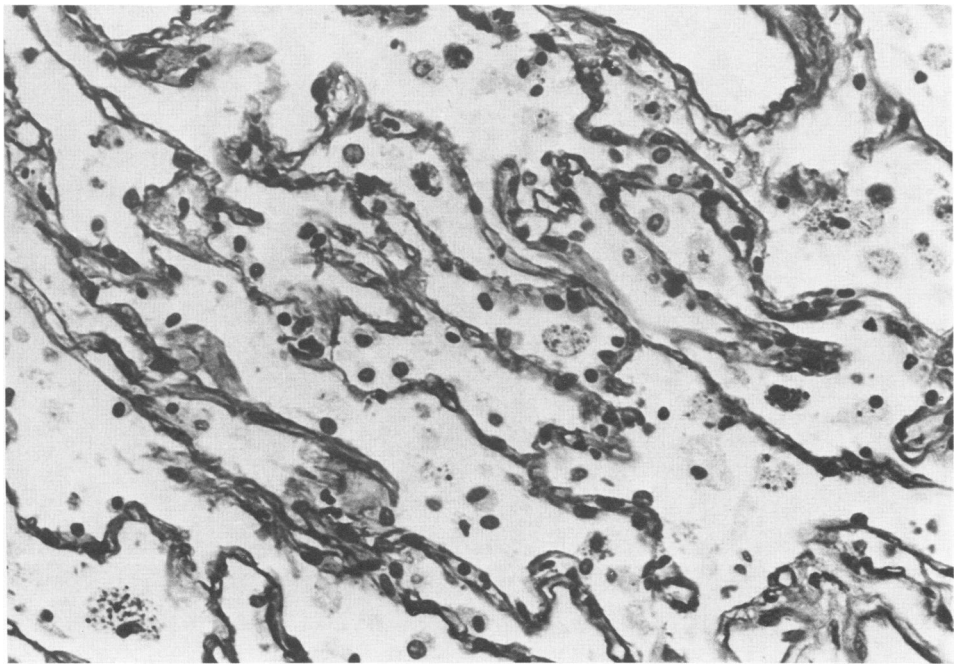


Figure 3—Normal lung. A continuous positivity is present along the alveolar septa and around blood vessles with anti-Type IV collagen antibody. (×50)

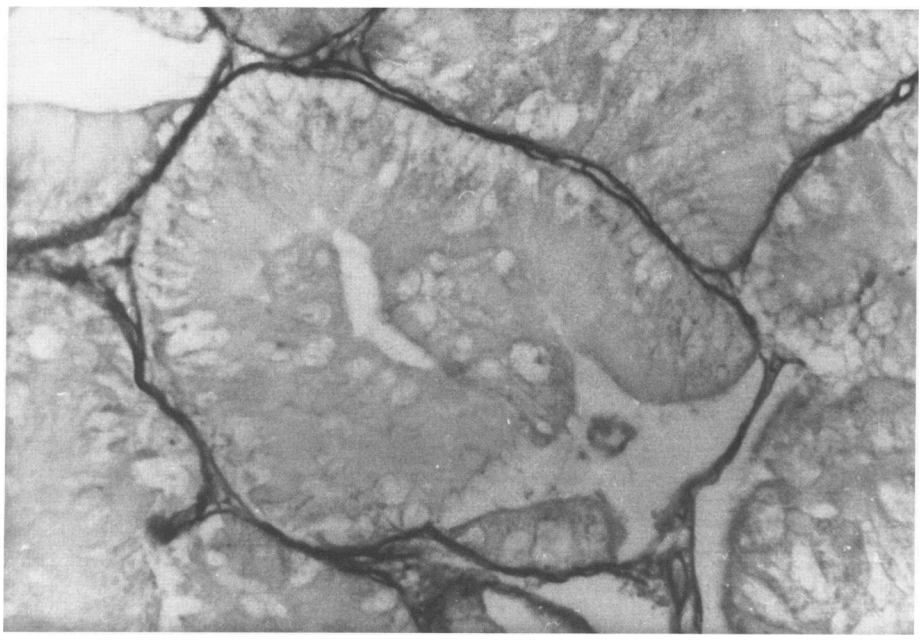


Figure 4—Type I BAC. Staining for laminin: a linear, continuous positivity is detectable around neoplastic cells. (×75)

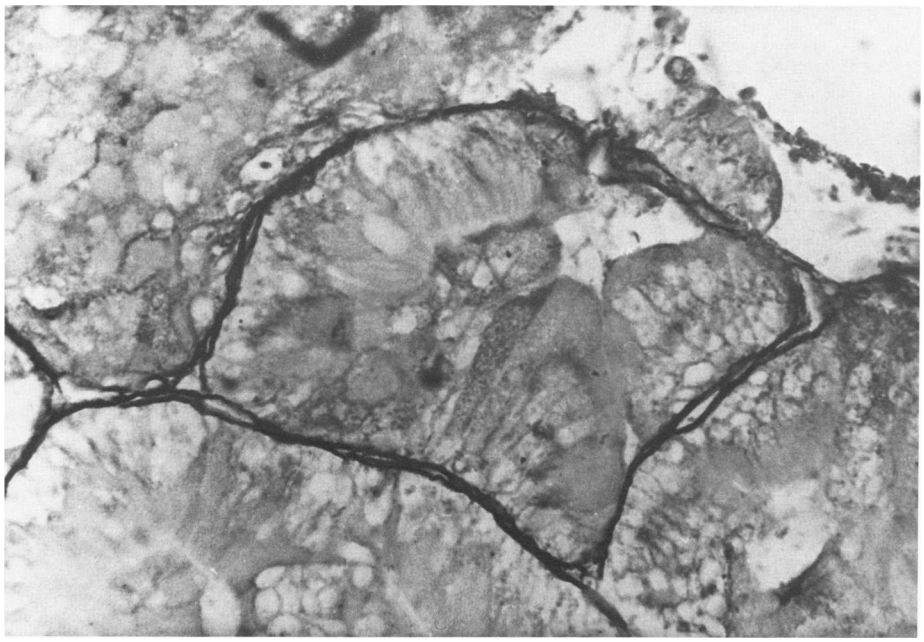


Figure 5—Type I BAC. Linear staining underlying the neoplastic epithelium, obtained with anti-Type IV collagen antibody. (×75)

istics are not always easy to assess objectively in routine surgical pathology where lobectomies or pneumonectomies are frequently performed in advanced stages of the neoplasia.

Furthermore, peripheral lung adenocarcinoma with morphologic features supporting a growth on fibrovascular septa may vary in histologic features and prognosis.[2,16]

Recently, some authors[6] have suggested that BAC may be further subclassified into two main categories showing different clinicopathologic aspects, although transitional aspects are also possible.

Our investigation also supports the view that peripheral lung adenocarcinoma, with histologic architecture suggestive of BAC, can easily be subclassified into two main types.

Furthermore, conventional lung adenocarcinoma may likewise display some histologic features of BAC. Immunohistochemical analyses on extracellular matrix glycoproteins reveal clear qualitative and quantitative differences with regard to the presence of BM antigens (Type IV collagen, laminin, and fibronectin) in the different groups.

Type I BAC shows a positive staining for Type IV collagen, laminin, and fibronectin with a distribution similar to that of the normal lung, suggesting that in all the fields examined neoplastic elements are arranged so as to cover preexisting alveolar septa.

On the other hand, marked differences from the normal lung are detected in Type II BAC. In this neoplasm laminin is often absent or present in an irregular, spotted fashion, whereas Type IV collagen shows a variable positivity. In Type II BAC fibronectin is increased, parallel to the increase in septal stroma.

These structural extracellular matrix modifications seem to indicate that in Type II BAC the stroma is synthesized *de novo* by carcinomatous cells, with many similarities to the conventional adenocarcinoma.

Only Type I BAC seems to grow on fibrovascular septa in which the distribution of extracellular matrix glycoproteins is identical to that of the normal lung. So, in line with WHO criteria, only this type of tumor should be considered a true bronchioloalveolar carcinoma.

However, these aspects are not conclusive evidence that in this tumor the scaffold is "preexisting" and not synthesized *de novo.*

As a general rule, the extracellular matrix, particularly the BM, is synthesized by epithelial and endothelial cells in association with microenvironmental stimuli. Also, neoplastic cells of epithelial origin can produce BM, depending only on their degree of cellular differentiation and not on the staging of the neoplasia. Invasive and metastatic carcinomas can, in fact, produce a BM qualitatively and quantitatively identical to the BM of the normal epithelium.[29,30] In view of this, well-differentiated cells of the peripheral lung adenocarcinoma may synthesize BM in a way similar to that of the normal lung, making it impossible to establish whether lung septal stroma existed

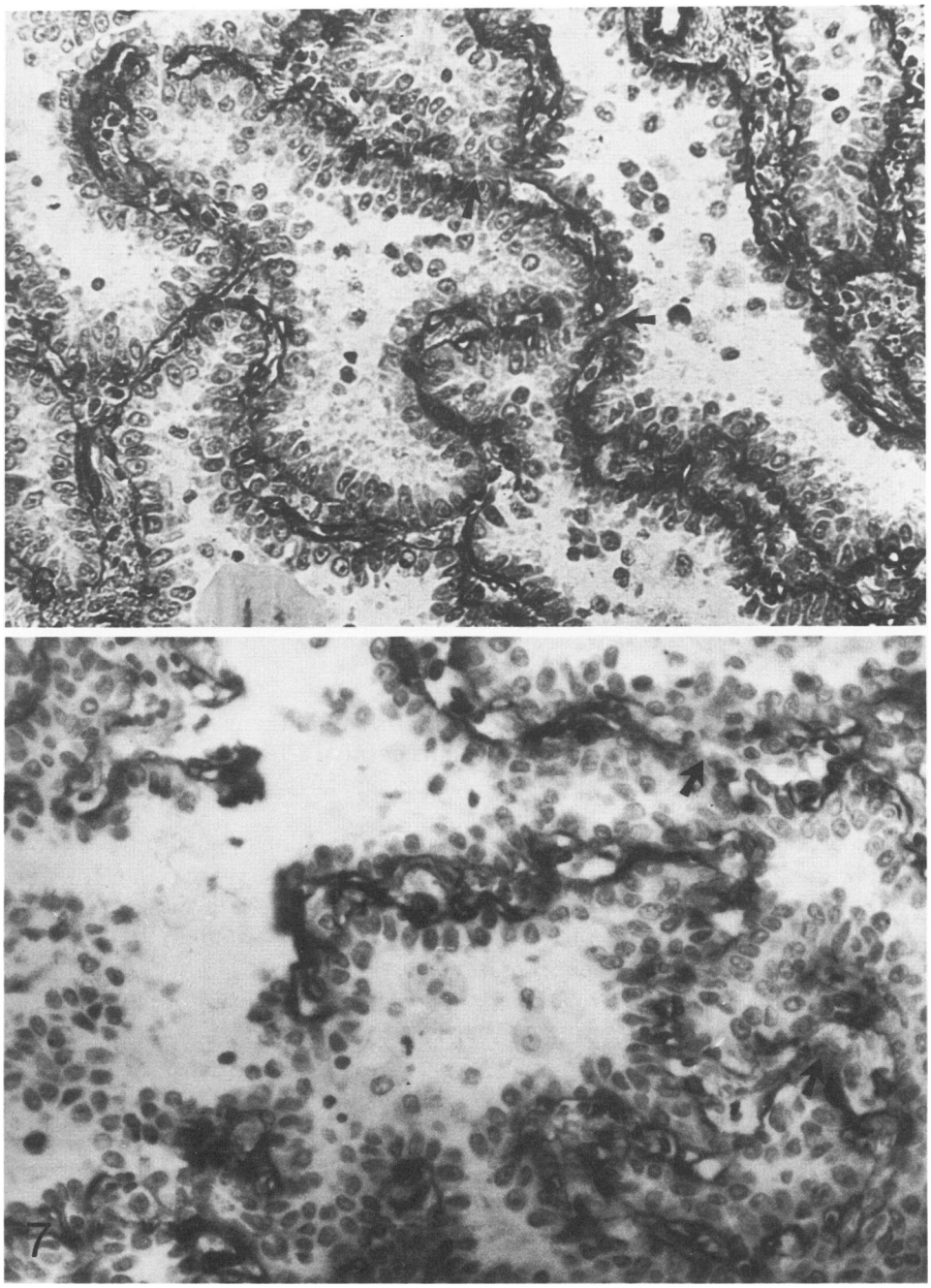


Figure 6 and 7—Type II BAC. Irregular positivity (*arrows*) around carcinomatous cells for both laminin (**Figure 6**) and Type IV collagen (**Figure 7**). (×50)

beforehand or, on the contrary, was synthesized *de novo*. Although this criterion proposed in the classification of the bronchioloalveolar carcinoma proves difficult to assess in routine surgical pathology, the different features of the extracellular matrix of peripheral lung adenocarcinoma could explain its different biologic behavior.

The BM represents a natural basal support for the epithelial cells. The cell support attachment is mediated by several macromolecules such as cell receptors, stromal glycoproteins (fibronectin, laminin), and serum spreading factors.[31]

These glycoproteins seem to interact with intracellular microfilaments, causing actin binding and stimulation of cell motility.[32]

Furthermore, specific peptide fragments of fibronectin and laminin can promote the adhesion and direct migration of epithelial cells.[33] Hence, different

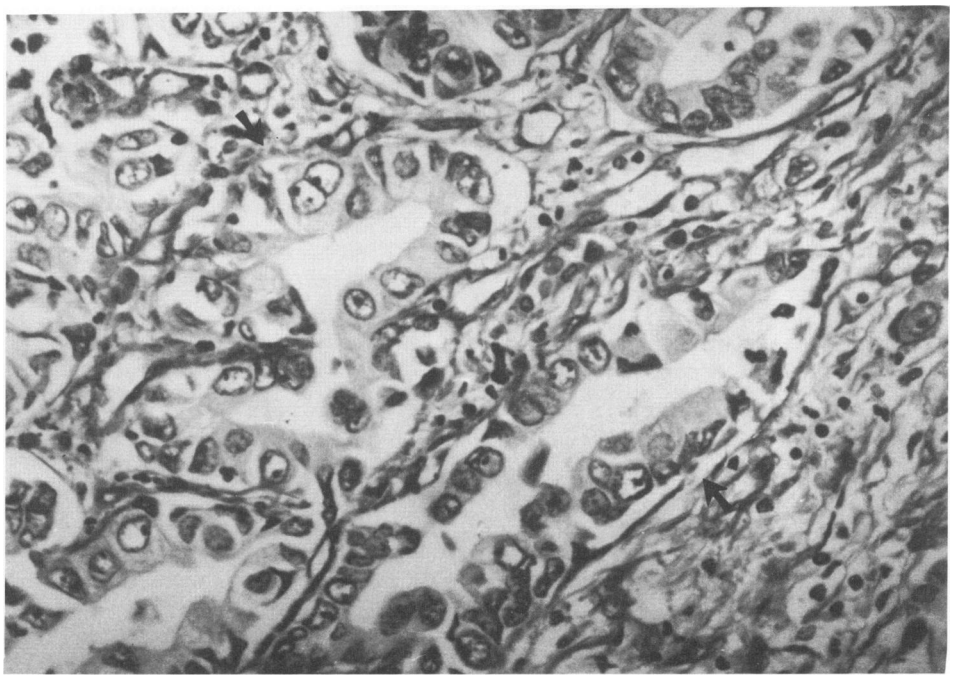


Figure 8—Conventional adenocarcinoma. An irregular positivity for Type IV collagen can be observed. (×75)

transmembrane arrangements between the intracellular microfilaments (actin, α-actin, vinculin) and the extracellular matrix might produce functional changes in cell adhesion and migration properties.[34]

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