Differential Expression of Cytokeratins 7 and 20 and Thyroid Transcription Factor-1 in Bronchioloalveolar Carcinoma

An Immunohistochemical Study in Fine-Needle Aspiration Biopsy Specimens

Aylin Simsir, MD, Xiao-Jun Wei, MD, Herman Yee, MD, PhD, Andre Moreira, MD, PhD, and Joan Cangiarella, MD

Key Words: Bronchioloalveolar carcinoma; Immunohistochemistry; Cytokeratins; Thyroid transcription factor; Fine-needle aspiration

DOI: 10.1309/CM20WA20RW3T600L

Abstract

We studied the staining patterns of bronchioloalveolar carcinoma (BAC) with antibodies to cytokeratin (CK) 7, CK20, and thyroid transcription factor-1 (TTF-1) to determine the diagnostic usefulness of this panel in differentiating BAC from metastatic adenocarcinoma in material obtained by fine-needle aspiration biopsy (FNAB) of the lung.

We identified 16 cases of BAC. Of these, 6 were mucinous, 4 were nonmucinous, and 6 were mixed with focal mucinous differentiation. Immunohistochemical analysis with antibodies to CK7, CK20, and TTF-1 was performed on cell-block sections.

Of the 6 mucinous BACs, 4 (67%) were CK7+, CK20+, and TTF-1–. All 4 nonmucinous BACs were CK7+ and CK20–, and 2 (50%) were TTF-1+. All 6 mixed BACs were diffusely positive for CK7 and focally positive for CK20; 5 (83%) were TTF-1+.

Nonmucinous BACs display CK7, CK20, and TTF-1 immunoreactivity similar to conventional pulmonary adenocarcinoma. Mucinous and mixed BACs have an immunohistochemical phenotype that is different from that of conventional pulmonary adenocarcinoma. Knowledge of these staining patterns is crucial for distinguishing mucinous and mixed BACs from metastatic adenocarcinoma involving the lungs.

Differentiating primary pulmonary adenocarcinoma from adenocarcinoma metastatic to the lung is a common diagnostic problem, specifically when there are multiple lung nodules or if the patient has a history of extrapulmonary adenocarcinoma. In addition, a lung nodule might be the only obvious site of metastatic disease at initial examination. The distinction of primary from metastatic adenocarcinoma is especially challenging in bronchioloalveolar adenocarcinoma (BAC), which might manifest as multiple nodules indistinguishable radiographically from metastases. The mucinous subtype of BAC, in particular, histologically mimics mucinous adenocarcinomas originating from other sites such as the gastrointestinal tract. Conversely, colorectal adenocarcinoma might involve the lungs in a lepidic pattern, simulating BAC. Immunohistochemical analysis is a useful adjunct for ascertaining the site of origin in such cases.

Commonly used immunohistochemical markers for discriminating primary pulmonary adenocarcinoma from metastatic adenocarcinoma are cytokeratin (CK) 7, CK20, and thyroid transcription factor-1 (TTF-1). The majority of conventional pulmonary adenocarcinomas are positive for antibodies against TTF-1 and CK7 and negative for CK20. Despite numerous studies evaluating the pattern of immunostaining with CK7, CK20, and TTF-1 in adenocarcinomas originating from a wide variety of organ systems, only a few studies correlated the specific histologic type of pulmonary adenocarcinomas with the immunostaining profile. In fact, in many studies, BACs constituted not more than a handful of cases and usually were grouped with conventional pulmonary adenocarcinomas. Several investigators have shown that mucinous and nonmucinous BACs have different immunohistochemical staining patterns.
patterns. The majority of mucinous BACs are TTF-1−, and many display CK20 immunoreactivity, whereas nonmucinous BACs are similar to conventional pulmonary adenocarcinomas in their staining pattern. These findings have important clinical management implications in correctly identifying the site of origin of adenocarcinoma involving the lungs.

Radiologically guided fine-needle aspiration biopsy (FNAB) commonly is used as a diagnostic technique for the initial evaluation of pulmonary nodules. The distinction between primary adenocarcinoma of the lung and metastasis from another site is a frequently encountered diagnostic problem in the cytopathology laboratory. A review of the cytology literature revealed no reports describing the usefulness of CK7, CK20, and TTF-1 as a panel in the diagnosis of BAC by FNAB. We studied the staining patterns of antibodies to CK7, CK20, and TTF-1 on cell-block material from aspiration biopsies of histologically confirmed cases of BAC to confirm the results previously reported by others in surgical pathology and to document the usefulness of this panel to aid in distinguishing BAC from metastatic adenocarcinoma on often small amounts of material obtained by FNAB.

Materials and Methods

We searched the computerized records of the database of the New York University Hospitals (NYU Medical Center and Bellevue Hospital, New York, NY) for histologically confirmed cases of BAC in which a preoperative FNAB was performed. Clinical information was obtained from treating physicians to ensure that all cases included were primary lung tumors. All H&E-stained slides from resection specimens were reviewed to confirm the diagnosis of BAC according to the 1999 World Health Organization criteria. The World Health Organization defines bronchioloalveolar adenocarcinoma as a distinct type of well-differentiated pulmonary adenocarcinoma with a pure lepidic growth pattern and no evidence of stromal, vascular, or pleural invasion. BACs are further divided into mucinous, nonmucinous, and mixed (indeterminate) cell types. Common to all histologic subtypes is the proliferation of the neoplastic cells along the alveolar septa with preservation of the integrity of the terminal airway architecture.

For all patients, computed axial tomography–guided FNAB was performed by a radiologist using 22-gauge needles. The material obtained was expressed onto glass slides, smeared, air dried, and stained with modified Romanowsky stain (Newcomer Supply, Middleton, WI) or fixed in 95% alcohol and stained with the Papanicolaou stain (Surgipath Medical Industries, Richmond, IL). Additional material was placed into Cytorich Red (Tripath Care Technologies, TriPath Imaging, Burlington, NC) for cell-block processing and paraffin embedding. All cell blocks were stained with H&E. Immunohistochemical staining was performed using the avidin-biotin immunoperoxidase technique with monoclonal antibodies to CK7 (lyophilized, Ventana Medical Systems, Tucson, AZ), CK20 (dilution 1:50; DAKO, Carpinteria, CA), and TTF-1 (dilution 1:40; Neomarkers, Fremont, CA).

Nuclear staining was assessed for TTF-1 and cytoplasmic staining for CK7 and CK20. Immunoreactivity was evaluated semiquantitatively based on the intensity and estimated percentage of tumor cells that were stained. Intensity was quantified as follows: 1+, weak staining (detection required high magnification); 2+, moderate staining (detected readily at medium magnification); 3+, strong staining (detected readily at low magnification). The percentage of cells stained was qualified as follows: 0%, negative (absolute lack of staining); 1% to 25%, focal; 26% to 50%, intermediate; and greater than 50%, diffuse.

Histologically the cases were classified into pure mucinous (6 cases [38%]), pure nonmucinous (4 cases [25%]), and mixed (containing morphologic features of both; 6 cases [38%]) subtypes. The mucinous subtype contained tall, bland, columnar cells with basally oriented nuclei and abundant cytoplasmic mucin. The nonmucinous subtype showed uniform cuboidal or low columnar cells with eosinophilic or finely vacuolated cytoplasm lining intact alveolar septa. The mixed subtype contained both nonmucinous and mucinous epithelial cells. Cell blocks of aspiration biopsy specimens were classified similarly into 3 categories. Any cytology case that did not have adequate cellularity on the cell block sections to accurately represent the 3 subtypes of BAC in accordance with the histologic material was excluded from the study.

Results

We identified 16 cases. The patients ranged in age from 50 to 93 years, and 7 were men and 9 were women. Radiographically, 13 patients (81%) had a single pulmonary mass ranging from 1 to 5 cm, and 3 patients (19%) had multiple unilateral nodules ranging from 0.6 to 5.8 cm.

Overall, positivity (2+ to 3+) was noted for CK7 in 16 cases (100%), for CK20 in 11 cases (69%), and for TTF-1 in 8 cases (50%). The results of immunohistochemical staining according to histologic subtype are summarized in Table II.

Mucinous Subtype

Samples showing the mucinous subtype are illustrated in Image II. All cases showed diffuse 3+ cytoplasmic positivity for CK7. Diffuse 3+ cytoplasmic positivity for CK20...
was seen in 5 (83%) of 6 cases. TTF-1 was negative in 5 (83%) of 6 cases. One case with positive TTF-1 reactivity (>50% of cells) showed an intensity of 2+. In summary, 4 (67%) of 6 cases were CK7+, CK20+, and TTF-1–.

Nonmucinous Subtype

The nonmucinous subtype is illustrated in Image 2I. Diffuse cytoplasmic positivity (3+) for CK7 was noted in all 4 cases and positivity for TTF-1 (>50% of cells, 2+ to 3+) in 2 (50%). CK20 staining was negative in all cases.

Mixed Subtype

Samples showing the mixed subtype are illustrated in Image 3I. All 6 cases showed diffuse cytoplasmic positivity (3+) for CK7. CK20 positivity was noted in all cases (2+); however, the number of cells stained ranged from 10% to 90%. TTF-1 positivity was noted in 5 cases (83%), with positivity detected mainly in the nonmucinous component (>50% of cells, 1+ to 2+).

Discussion

BACs commonly are multifocal and, thus, radiologically they mimic metastatic neoplasms. Mucinous BACs in particular tend to spread aerogenously, forming satellite pulmonary nodules; thus, metastatic adenocarcinoma frequently is the initial clinical diagnosis.1,2,21 Distinction of mucinous BACs from metastatic adenocarcinoma on morphologic grounds, both cytologically and histologically, can be difficult owing to overlapping cytologic features with metastatic mucinous adenocarcinomas of gastrointestinal, pancreatic, and ovarian origins. Similarly, metastatic mucinous adenocarcinomas, especially from a gastrointestinal primary site, can line alveolar walls in a lepidic pattern and histologically simulate primary mucinous BACs.1,2 Colorectal adenocarcinoma is notorious in this respect. Thus, immunohistochemical analysis is a useful adjunct in the differential diagnosis.

As the use of radiologically guided FNAB has gained popularity for the initial evaluation of pulmonary masses, distinguishing primary pulmonary adenocarcinomas from metastases has become a routine task of the cytopathology laboratory. A panel composed of CK7, CK20, and TTF-1 immunostains has been shown to be beneficial in distinguishing primary pulmonary adenocarcinomas from metastatic adenocarcinomas on histologic material.3,8,11-13,20,22 The vast majority of conventional pulmonary adenocarcinomas are reported to be TTF-1+ and CK7+ and CK20–. The number of BACs in these studies is few, with even fewer cases when BACs are subtyped into mucinous and nonmucinous categories. Only a few investigators specifically targeted BACs with respect to CK7, CK20, and TTF-1 immunoreactivity patterns.16-19 To the best of our knowledge, these results have not been replicated in material obtained by FNAB.

TTF-1 is a 38-kd, tissue-specific nuclear protein that activates DNA transcription in the embryonal and mature thyroid and lung epithelial cells.23-25 In the adult lungs, TTF-1 is expressed by type II pneumocytes and Clara cells.24 Numerous studies have shown TTF-1 positivity in primary pulmonary small and non–small cell carcinomas.3,4,11,12,14,15,21,23-25 The frequency of immunoreactivity varies according to the histologic type of the tumor. Among non–small cell carcinomas, adenocarcinomas have the highest rate of TTF-1 positivity, with close to 80% immunoreactivity.4,10-12,15,25 In general, TTF-1 immunoreactivity ranges from 25% to 100% in BACs.9,14,10,16,17,25 This wide range reflects the shortcomings of some of these studies in which the number of BACs was low, and correlation with the specific histologic subtype of BAC was not done. When BACs are divided into mucinous, nonmucinous, and mixed subtypes, the differences are striking.

In the present study, 2 (50%) of 4 nonmucinous BACs were positive for TTF-1, whereas 5 (83%) of 6 mucinous BACs were negative. In the mixed BAC category, 5 (83%) of 6 cases were positive for TTF-1, with positivity confined mostly to the nonmucinous component. TTF-1 positivity in nonmucinous BACs in the present series was lower than the previously reported range of 75% to 92%.16,17 The low rate of TTF-1 positivity in mucinous adenocarcinomas, on the other hand, is in agreement with findings reported in the literature. We identified 3 studies in which mucinous BACs were reported separately; TTF-1 positivity in these series ranged from 0% to 21%.10,16,17 Lau et al16 reported TTF-1 positivity in 86% of mixed BACs and commented that in such tumors, the trend toward TTF-1 negativity was maintained in the mucinous component. This observation is similar to our findings. The preferential expression of TTF-1

---

**Table 1**

<table>
<thead>
<tr>
<th>Cytokeratin 7</th>
<th>Cytokeratin 20</th>
<th>TTF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucinous (n = 6)</td>
<td>6 (100); 3+</td>
<td>5 (83); 3+</td>
</tr>
<tr>
<td>Nonmucinous (n = 4)</td>
<td>4 (100); 3+</td>
<td>0 (0); 0</td>
</tr>
<tr>
<td>Mixed (n = 6)</td>
<td>6 (100); 3+</td>
<td>6 (100); 3+</td>
</tr>
</tbody>
</table>

TTF-1, thyroid transcription factor-1; 2+, moderate staining (detected readily at medium magnification); 3+, strong staining (detected readily at low magnification). Data are given as number (percentage) positive; staining intensity.

1 For cytokeratin 20 staining, 10% to 90% of cells showed positive staining.

2 TTF-1 positivity was positive mainly in the nonmucinous component.
Image 11 Mucinous bronchioloalveolar carcinoma. A, Aspiration smear shows flat monolayered sheets of bland-appearing mucinous cells (modified Romanowsky stain, ×40). B-D, Cell-block preparation shows cytoplasmic positivity for cytokeratin (CK) 7 (B, ×40) and CK20 (C, ×40) and negativity for thyroid transcription factor-1 (D, ×40). E, Histologic sections show mucin-containing columnar cells lining the alveolar spaces (H&E, ×40).
Image 2i Nonmucinous bronchioloalveolar carcinoma. A, Aspiration smear shows monolayered sheets and clusters of uniform cells with oval nuclei and fine chromatin (modified Romanowsky stain, ×40). B-D, Cell-block preparation shows cytoplasmic positivity for cytokeratin (CK) 7 (B, ×40), negativity for CK20 (C, ×40), and nuclear positivity for thyroid transcription factor-1 (D, ×40). E, The characteristic lepidic growth pattern of columnar and cuboidal cells is noted on the histologic specimen (H&E, ×40).
Mixed bronchioloalveolar carcinoma (BAC).  

**A.** Aspiration smear shows clusters of cells with scant cytoplasm typical of BAC and focal cells with abundant mucinous cytoplasm (modified Romanowsky stain, ×40).  

**B-D.** Cell-block preparation shows positivity for cytokeratin (CK) 7 (B, ×40), focal positivity for CK20 in the mucinous component (C, ×40), and positivity for thyroid transcription factor-1 (D, ×40).  

**E.** The histologic specimen shows BAC with focal mucinous differentiation (H&E, ×40).
in nonmucinous BACs is in keeping with the normal localization of this protein in type II pneumocytes and Clara cells. Nonmucinous BACs originate from type II pneumocytes or Clara cells, whereas mucinous BACs most likely originate from the bronchiolar lining cells that have undergone mucinous (goblet cell) metaplasia.

In addition to TTF-1, mucinous BACs also have aberrant CK20 expression that differs from conventional pulmonary adenocarcinomas and nonmucinous BACs. We found that all mucinous BACs were CK7+, similar to conventional pulmonary adenocarcinomas; however, the majority (5/6 [83%]) also displayed CK20 reactivity. This is similar to results from other series that found a CK20+ phenotype in 25%, 79%, 80%, and 89% of mucinous BACs. CK7 reactivity in these series ranged from 80% to 100%. CK7 and CK20 expression in nonmucinous BACs closely mirrors that of conventional adenocarcinomas in that the majority are reactive for CK7 (range, 96%-100%) and nonreactive for CK20 (range, 71%-100%).

It is noteworthy that in addition to ours, only 1 other study evaluated mixed BACs as a separate category for CK7 and CK20 expression. All mixed cases showed a staining pattern similar to that of conventional pulmonary adenocarcinomas and nonmucinous BACs. All (100%) were CK7+ and CK20−. In contrast, in our experience, CK7 and CK20 were positive in all cases of mixed BACs, a pattern similar to that for mucinous BACs; however, the number of cells with immunoreactivity for CK20 was as low as 10% in some cases.

One shortcoming of the present study is the small number of cases included in each subcategory of BAC. Despite this limitation, our findings confirm the aberrant expression of CK20 and TTF-1 in mucinous BACs as reported by others.

BACs constitute a heterogeneous group in regard to their immunohistochemical profile. Nonmucinous BACs are similar to conventional pulmonary adenocarcinomas in CK7, CK20, and TTF-1 immunoreactivity. In contrast, most mucinous BACs are positive for CK7 and CK20 and negative for TTF-1. Mixed BACs display an overlapping pattern; they usually are CK7+, CK20+, and TTF-1+. This panel performs equally well on a small amount of material obtained by FNAB as a tissue biopsy or excision specimen. It is crucial to be familiar with the disparate expression of these antibodies in BACs when determining whether a mucinous adenocarcinoma in the lung is primary or secondary.

References

10. Kaufman O, Dietel M. Thyroid transcription factor-1 is the superior immunohistochemical marker for pulmonary adenocarcinomas and large cell carcinomas compared to surfactant proteins A and B. Histopathology. 2000;36:8-16.

From the Department of Pathology, New York University School of Medicine–Bellevue Hospital Center, New York, NY.

Address reprint requests to Dr Simsir: New York University Medical Center, 530 First Ave, Skirball-West Tower, Suite 10U, New York, NY 10016.


