Mucinous and Nonmucinous Bronchioloalveolar Adenocarcinomas Have Distinct Staining Patterns With Thyroid Transcription Factor and Cytokeratin 20 Antibodies

Neal S. Goldstein, MD, and Maria Thomas, MD

Key Words: Lung; Bronchioloalveolar; Adenocarcinoma; TTF; Cytokeratin 20; Cytokeratin 7; Mucinous villin

Abstract

We studied 14 mucinous and 26 nonmucinous bronchioloalveolar adenocarcinomas (BACs) with thyroid transcription factor (TTF), cytokeratin (CK) 7, CK20, and villin to characterize their staining patterns with these antibodies and identify staining differences between the neoplasms. We also stained 11 mucinous colon adenocarcinomas with the same antibodies to compare their reaction patterns with mucinous BACs. All pulmonary neoplasms were confirmed pulmonary primary BACs. Three (21%) of 14 mucinous neoplasms had weak TTF reactivity in fewer than 25% of neoplastic cell nuclei, and the other 11 (79%) were nonreactive. In contrast, 24 (92%) of 26 nonmucinous BACs were strongly TTF reactive. Eleven mucinous BACs (79%) had CK20 reactivity in more than 25% of neoplastic cells, whereas only 1 nonmucinous BAC (4%) had reactivity in fewer than 50% of the cells. One mucinous BAC (7%) had villin reactivity in approximately 10% of the neoplastic cells. All mucinous colon adenocarcinomas were diffusely reactive with CK20 and villin. Mucinous and nonmucinous BACs have disparate staining patterns with TTF and CK20. Mucinous BACs are usually TTF nonreactive and CK20 reactive, but nonreactive with villin, which distinguishes them from mucinous colon adenocarcinomas.

Thyroid transcription factor (TTF) is a 38-kd nuclear regulatory protein that binds to promoter regions and activates DNA transcription primarily in the cells of the thyroid, lung, and brain.1 In the lung, TTF activates the surfactant proteins A, B, and C and Clara cell secretory protein genes.2-5 It is expressed in type II pneumocytes and Clara cell of the lung of adults.6,7 Pulmonary and thyroid carcinoma cells also can express TTF.8-12 Among pulmonary non–small cell carcinomas, adenocarcinomas have the highest prevalence of TTF expression.8-11,13-19 Adenocarcinomas from sites other than the lung and thyroid are TTF nonreactive, making it a useful antibody for separating metastatic nonpulmonary adenocarcinoma from primary lung adenocarcinoma.11,13

The lung is a common site of metastatic mucinous adenocarcinomas, especially from neoplasms that originate in the gastrointestinal tract.20-26 Morphologically, metastatic mucinous adenocarcinomas can line alveolar walls in a “lepidic” pattern simulating primary mucinous bronchioloalveolar adenocarcinoma.24 Colorectal adenocarcinoma is the most common neoplasm that can mimic primary mucinous bronchioloalveolar adenocarcinoma.20-26 TTF would seem to be a useful antibody to positively identify mucinous bronchioloalveolar adenocarcinomas; however, we have encountered several TTF-nonreactive mucinous bronchioloalveolar adenocarcinomas in routine surgical pathology. Whether mucinous bronchioloalveolar adenocarcinomas have a different pattern of TTF reactivity than nonmucinous bronchioloalveolar adenocarcinomas has not been studied extensively. We found one study in which this was commented on as part of a large investigation of staining differences of several antibodies in the major types of pulmonary carcinoma.11

Cytokeratin 7 and 20 antibodies frequently are used to distinguish between primary pulmonary and metastatic
coloectral adenocarcinomas. Lung adenocarcinomas are considered cytokeratin 7 reactive and cytokeratin 20 nonreactive, whereas colorectal adenocarcinomas are cytokeratin 7 nonreactive and cytokeratin 20 reactive.\(^{27-30}\) It has been our anecdotal experience that mucinous bronchioloalveolar adenocarcinomas can have alternative patterns of cytokeratin 7 and 20 expression. Potential differences in cytokeratin 7 and 20 expression among bronchioloalveolar adenocarcinomas of different cell types also have not been studied extensively. The authors of one study found disparate patterns of reactivity between 5 mucinous and 5 nonmucinous bronchioloalveolar adenocarcinomas.\(^{31}\)

We examined the distribution of TTF, cytokeratin 7, and cytokeratin 20 staining in mucinous and nonmucinous bronchioloalveolar adenocarcinomas to characterize their patterns of reactivity and to confirm or refute the disparities reported by other authors. We also compared the immunoreactivity of mucinous bronchioloalveolar adenocarcinomas with primary mucinous colon adenocarcinomas to identify differences that would assist in their distinction.

**Materials and Methods**

Fourteen lobectomy or pneumonectomy specimens resected during January 1, 1990, through July 1, 2000, for mucinous bronchioloalveolar adenocarcinoma, in which the lung was firmly established as the primary origin of the neoplasm, were found in the files of the anatomic pathology department of William Beaumont Hospital, Royal Oak, MI. Twenty-six nonmucinous bronchioloalveolar adenocarcinoma lobectomy resection specimens accessioned during the same period as the mucinous bronchioloalveolar adenocarcinomas were selected randomly from a large pool of patients in which the lung was firmly established as the primary site of the neoplasm. Only bronchioloalveolar adenocarcinomas that had pure lepidic growth patterns, without central fibrosis, were used in the study.

The included cases were bona fide pulmonary bronchioloalveolar adenocarcinomas. Computer searches were performed by the William Beaumont Hospital Tumor Registry within its cancer database and its linked regional and national databases to establish that none of the patients had colonic, appendiceal, ovarian, breast, or pancreaticobiliary adenocarcinoma before or after the pulmonary resection. All patients had abdominal and pelvic computed tomography scans that showed no other carcinomas. Nine patients underwent complete colonoscopy in which no primary colonic adenocarcinomas were found. All 21 women in the study had normal-appearing appendices and ovaries on the computed tomography scans. All 5 women with mucinous and 12 of 16 women with nonmucinous bronchioloalveolar adenocarcinomas had a mammogram with a benign result obtained within 1 year of pulmonary resection.

The 14 mucinous bronchioloalveolar adenocarcinomas were composed of bland, tall, columnar cells with basally located nuclei and abundant apical mucinous cytoplasm. Five had a minor component of goblet mucin cells admixed with the columnar mucin cells.

The 26 nonmucinous bronchioloalveolar adenocarcinomas had slightly thickened alveolar septa from edema, inflammation, and minimal fibrosis. None were of the sclerosing type; all were devoid of a central fibrous region that contained small malignant tubules or acini. The neoplastic cells were of low cytologic grade and were a mixture of small cuboidal cells, columnar cells that had minimal eosinophilic cytoplasm, and columnar hobnail and peg cells.

Eleven primary colon mucinous adenocarcinomas were selected randomly from the files for the same period. All tumors had adenoma in the adjacent mucosa. Mucinous adenocarcinoma constituted more than 75% of the invasive component in all tumors.

**Immunohistochemical Staining**

One tumor block was selected from each case. Threemicrometer-thick consecutive sections were cut and placed onto charged slides. Sections were deparaffinized using sequential immersions into 2 xylene baths, 3 baths of decreasing alcohol concentrations, and 2 water baths, followed by a 1-minute wash in water. Slides were immersed in EDTA buffer (pH 7.0) and put into a commercial vegetable steamer at 95°C for 30 minutes. The slides were allowed to cool on the counter, remaining immersed in the heated EDTA buffer–filled containers for 5 minutes, followed by a 2-minute rinse with water while remaining in the containers. The slides were transferred into tris(hydroxymethyl)aminomethane–filled containers (pH 7.0) and allowed to undergo an additional 10 minutes of cooling on the countertop. They then were transferred to a commercial immunohistochemical autostainer (DAKO, Carpinteria, CA) and were washed with buffer, followed by a hydrogen peroxide incubation. The latter was rinsed off, and the primary antibody was applied. The primary antibody was incubated over the sections for 20 minutes at room temperature. After the primary antibody was washed off, the components of the Envision-plus detection system (DAKO) were applied, including an antimouse polymer, 2 distilled-water washes, and a final diaminobenzidine incubation for 4 minutes. Sections were counterstained with hematoxylin and coverslipped. A positive control slide containing known cytokeratin-reactive tissues was stained with each batch of simultaneously stained slides.

Primary antibodies used were TTF-1 (clone 8G7G3/1, 1:400 dilution; DAKO), cytokeratin 7 (clone OV-TL-12/30,
1:800 dilution; DAKO), cytokeratin 20 (clone Ks20.8, 1:500 dilution; DAKO), and villin (clone 1D2C3, 1:200 dilution; Immuno Tech, Marseille, France).

The percentage of reactive invasive adenocarcinoma cells was quantified as follows: 0%; less than 5%; 5% to 25%; 26% to 50%; 51% to 75%; or more than 75%. Intensity of stained cells was quantified into 1 of 3 categories: 1+, weak staining, required high magnification to detect; 2+, moderate staining, reactivity could be seen easily at medium magnification and, with careful, slow inspection, using dim light at low magnification; 3+, strong staining that was seen easily at low magnification.

Results

Three (21%) of 14 mucinous bronchioloalveolar adenocarcinomas had TTF reactivity of weak intensity in 25% or fewer neoplastic cell nuclei [Table 1]. Admixed benign type II pneumocytes at the periphery of the neoplasms had strong TTF expression in these 3 neoplasms. Eleven mucinous neoplasms (79%) were TTF nonreactive [Image 1 and Image 2]. In contrast, 24 (92%) of 26 nonmucinous bronchioloalveolar adenocarcinomas were strongly TTF reactive [Image 3 and Image 4]. Twenty-three nonmucinous neoplasms (88%) had TTF reactivity in more than 50% of the neoplastic cells. Two (8%) of 26 nonmucinous bronchioloalveolar adenocarcinomas were TTF nonreactive.

Eleven (79%) of the mucinous bronchioloalveolar adenocarcinomas had strong cytokeratin 20 reactivity in more than 25% of the neoplastic cells; 10 (71%) had reactivity in more than 50% of the neoplastic cells [Image 5]. Three mucinous neoplasms (21%) were cytokeratin 20 nonreactive or had reactivity in fewer than 5% of cells. In contrast, only 1 nonmucinous bronchioloalveolar adenocarcinoma (4%) had cytokeratin 20 reactivity that was limited to fewer than 50% of the neoplastic cells. The other 25 (96%) of nonmucinous neoplasms were cytokeratin 20 nonreactive. All 11 (100%) of the mucinous colon adenocarcinomas were extensively cytokeratin 20 reactive.

All of the mucinous and nonmucinous bronchioloalveolar adenocarcinomas had strong cytokeratin 7 reactivity in more than 50% of the neoplastic cells. In contrast, 10 (91%) of 11 mucinous colon adenocarcinomas were cytokeratin 7 nonreactive, and 1 neoplasm (9%) had reactivity in 26% to 50% of the cells.

All 11 colon mucinous adenocarcinomas were reactive with villin in a cytoplasmic and apical (brush border) pattern [Image 6]. Ten (91%) had reactivity in more than 50% of the neoplastic cells. Thirteen (93%) of the mucinous bronchioloalveolar adenocarcinomas were nonreactive with villin. One mucinous bronchioloalveolar adenocarcinoma (7%) had reactivity in 5% to 25% of the neoplastic cells in cytoplasmic and membrane patterns [Image 7]. No apical pattern of staining was seen. All nonmucinous bronchioloalveolar adenocarcinomas were villin nonreactive.

Discussion

Mucinous and nonmucinous bronchioloalveolar adenocarcinomas have a similar architectural growth pattern along unaltered or minimally thickened alveolar septa. Despite the growth similarity, the results of the present study suggest that the cells constituting mucinous and nonmucinous bronchioloalveolar adenocarcinomas have disparate patterns of TTF and cytokeratin 20 expression.

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<th>Table 1 Distribution of Antibody Reactivities in Bronchioloalveolar Adenocarcinomas*</th>
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<td>Nonmucinous bronchioloalveolar adenocarcinoma (n = 26)</td>
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TTF, thyroid transcription factor.
* Data are given as number (percentage).
The prevalence of TTF reactivity in pulmonary adenocarcinomas is approximately 70% with a reported range of 52% to 78%.\textsuperscript{11,13-15,17-19} Within the adenocarcinoma group, nonmucinous bronchioloalveolar adenocarcinomas have the highest prevalence of TTF reactivity. We found that 92% of 26 nonmucinous bronchioloalveolar adenocarcinomas were TTF positive, which is similar to the prevalences of 71% of 14 neoplasms, 83% of 6 neoplasms, and 86% of 29 neoplasms reported by other authors.\textsuperscript{11,14,18} TTF reactivity in nonmucinous neoplasms usually is extensive; 85% of the neoplasms had TTF reactivity in more than 75% of the cell nuclei in the present study, and another study reported that a mean of 70% (range, 30%-90%) of bronchioloalveolar adenocarcinoma cells were TTF reactive.\textsuperscript{18} Extensive TTF
reactivity in most nonmucinous bronchioloalveolar adenocarcinomas is not surprising because TTF normally is expressed in type II pneumocytes and Clara cell of the lung, and it only follows that neoplasms that are composed of cells with differentiation toward these two cell types also express TTF.6,7

In contrast, we found that mucinous bronchioloalveolar adenocarcinomas rarely expressed TTF. We found only one study in which the authors separately reported TTF staining results in mucinous bronchioloalveolar adenocarcinomas, and they also noted no reactivity in the 5 studied cases.11 The reason mucinous bronchioloalveolar adenocarcinoma cells are TTF nonreactive is unknown. Possibly it is a reflection of the function of TTF and the cells in which it is normally expressed. TTF binds and activates genes controlling surfactant proteins A, B, and C and Clara cell secretory protein.2-5

Cytokeratin 20 also had disparate expression in nonmucinous and mucinous bronchioloalveolar adenocarcinomas. Twenty-five (96%) of 26 nonmucinous neoplasms were cytokeratin 20 nonreactive, whereas 11 (79%) of 14 mucinous neoplasms were cytokeratin 20 positive. These results support the findings of the group of authors who first called attention to these differences.31 These authors found that all of 5 studied nonmucinous bronchioloalveolar adenocarcinomas were cytokeratin 20 nonreactive, whereas 4 (80%) of 5 mucinous neoplasms were cytokeratin 20 positive.

Villin is a tissue-specific, actin-binding protein that interacts with and stabilizes the microvillar actin core bundles of intestinal and renal brush borders.32 It is found in almost all colon adenocarcinomas and in a minority of pulmonary mucinous adenocarcinomas and bronchioloalveolar adenocarcinomas.33-37 Although a significant percentage of pulmonary adenocarcinomas are villin reactive, they have not been reported to have the diffuse, strong, apical (brush border) pattern of staining seen in colon adenocarcinomas.

The importance of these findings rests in the use of these antibodies for distinguishing between primary mucinous bronchioloalveolar adenocarcinomas and pulmonary metastases of mucinous adenocarcinoma, of which the colon is the most common primary site.20-26 Cytokeratin 20 and TTF do not seem to be useful for distinguishing between
these neoplasms. The cytokeratin 20–positive, TTF-negative pattern typically is considered supportive of colorectal adenocarcinoma, and, without due consideration, a mucinous bronchioloalveolar adenocarcinoma could be interpreted incorrectly as metastatic colorectal mucinous adenocarcinoma. The significance of this misconception is obvious if it is extended into the interpretation of lung biopsy specimens, in which a primary mucinous bronchioloalveolar adenocarcinoma could be interpreted incorrectly as metastatic colorectal adenocarcinoma and a patient is inappropriately denied surgery, or the incorrect chemotherapy is instituted. Cytokeratin 7, if extensively reactive, and villin, if it is nonreactive or reactive in a cytoplasmic pattern, are supportive of a primary mucinous bronchioloalveolar adenocarcinoma. Conversely, strong villin reactivity in a brush border pattern would be supportive of a metastatic colon mucinous adenocarcinoma.

Mucinous and nonmucinous bronchioloalveolar adenocarcinomas have disparate staining patterns with TTF and cytokeratin 20. Nonmucinous bronchioloalveolar adenocarcinomas are usually TTF reactive and cytokeratin 20 nonreactive. Conversely, mucinous bronchioloalveolar adenocarcinomas are usually TTF nonreactive and cytokeratin 20 reactive. Mucinous bronchioloalveolar adenocarcinomas are nonreactive with villin or have a cytoplasmic staining pattern, while mucinous colon adenocarcinomas have a strong brush border (apical) pattern of reactivity. These results should be kept in mind when pathologists consider whether a mucinous neoplasm is primary or metastatic.

From the Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak, MI.

Address reprint requests to Dr Goldstein: Dept of Anatomic Pathology, William Beaumont Hospital, 3601 W Thirteen Mile Rd, Royal Oak, MI 48073.

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