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

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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

colorectal 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. Three-micrometer-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 K_S20.8, 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 adenocarcinoma 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.

Table 1
Distribution of Antibody Reactivities in Bronchioloalveolar Adenocarcinomas*

	0%	<5%	5%-25%	26%-50%	51%-75%	>75%
Mucinous bronchioloalveolar adenocarcinoma (n = 14)						
TTF	11 (79)	1 (7)	2 (14)	0 (0)	0 (0)	0 (0)
Cytokeratin 20	2 (14)	1 (7)	0 (0)	1 (7)	7 (50)	3 (21)
Cytokeratin 7	0 (0)	0 (0)	0 (0)	0 (0)	2 (14)	12 (86)
Villin	13 (93)	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)
Nonmucinous bronchioloalveolar adenocarcinoma (n = 26)						
TTF	2 (8)	0 (0)	0 (0)	1 (4)	1 (4)	22 (85)
Cytokeratin 20	25 (96)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)
Cytokeratin 7	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	25 (96)
Villin	26 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Colon mucinous adenocarcinoma (n = 11)						
TTF	11 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cytokeratin 20	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	10 (91)
Cytokeratin 7	10 (91)	0 (0)	0 (0)	1 (9)	0 (0)	0 (0)
Villin	0 (0)	0 (0)	0 (0)	1 (9)	3 (27)	7 (64)

TTF, thyroid transcription factor.
* Data are given as number (percentage).

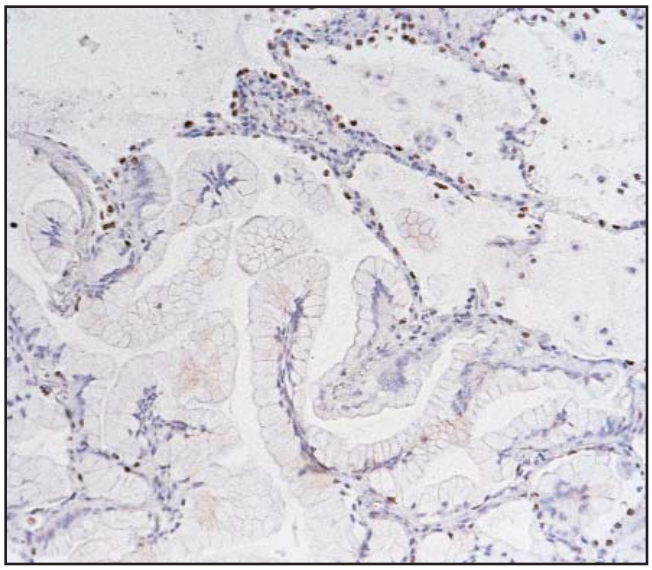

Image 1 Mucinous bronchioloalveolar adenocarcinoma. No reactivity (thyroid transcription factor, hematoxylin counterstain, ×12).

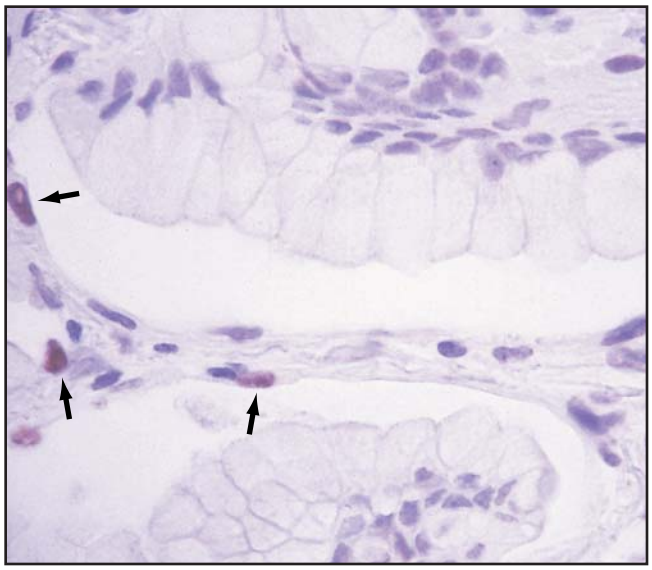

Image 2 Mucinous bronchioloalveolar adenocarcinoma. Higher magnification of Image 1. The mucinous neoplastic cells are nonreactive. Residual type II pneumocytes are reactive (arrows) (thyroid transcription factor, hematoxylin counterstain, ×64).

The prevalence of TTF reactivity in pulmonary adenocarcinomas is approximately 70% with a reported range of 52% to 78%.[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.[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.[18] Extensive TTF

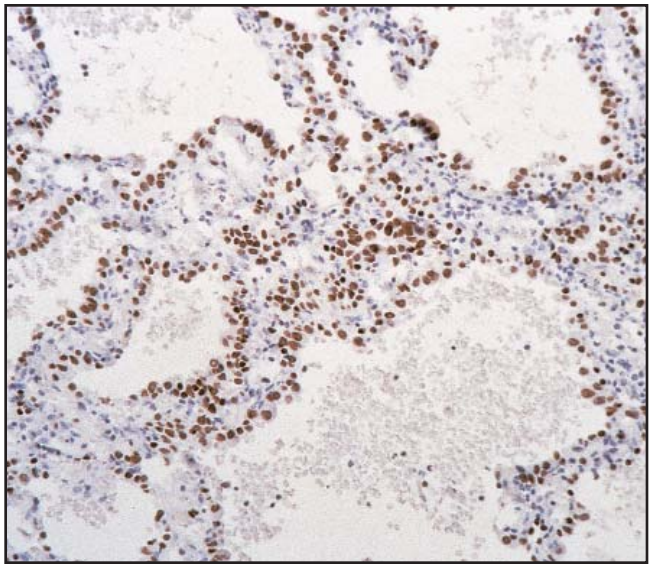

Image 3 Nonmucinous bronchioloalveolar adenocarcinoma. Diffuse, strong reactivity (thyroid transcription factor, hematoxylin counterstain, ×12).

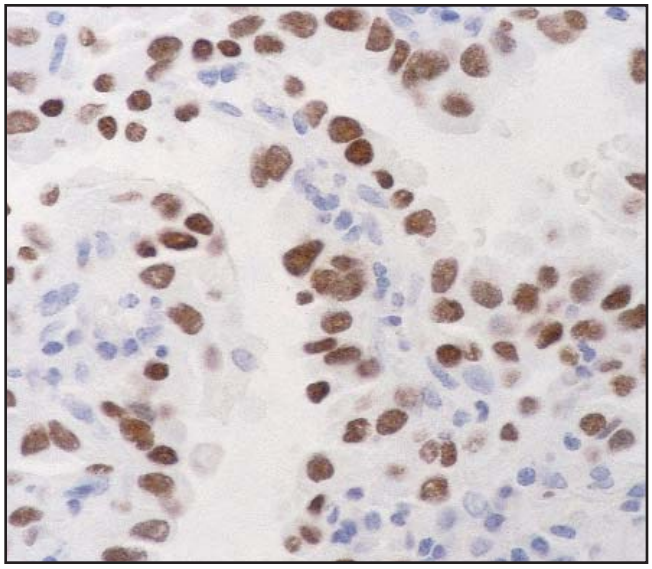

Image 4 Nonmucinous bronchioloalveolar adenocarcinoma. Higher magnification of Image 3. Almost every cell is strongly reactive (thyroid transcription factor, hematoxylin counterstain, ×64).

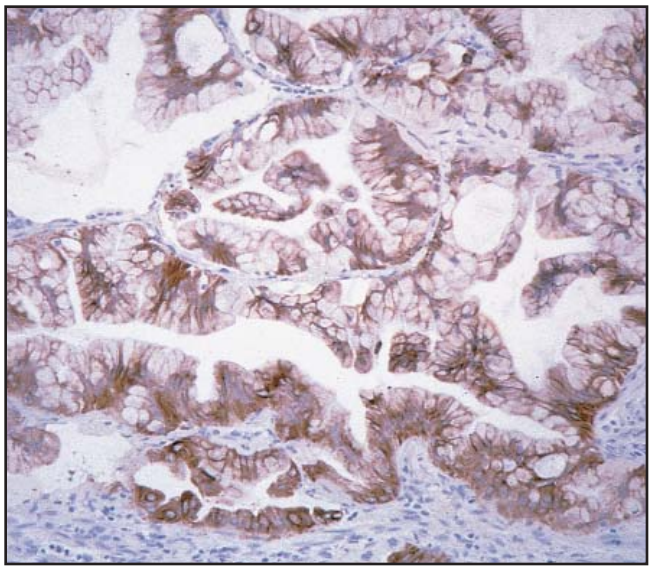

Image 5 Mucinous bronchioloalveolar adenocarcinoma. Extensive reactivity of the neoplastic cells (cytokeratin 20, hematoxylin counterstain, ×12).

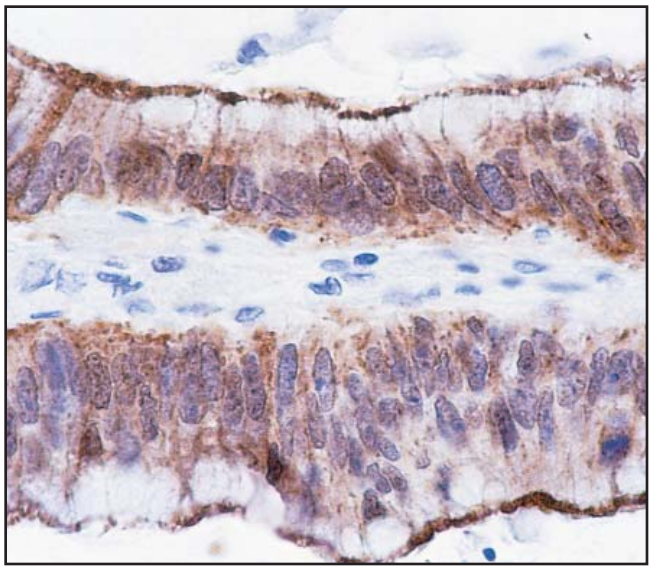

Image 6 Mucinous colon adenocarcinoma. There is diffuse basilar cytoplasmic and brush border reactivity (villin, hematoxylin counterstain, ×64).

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]

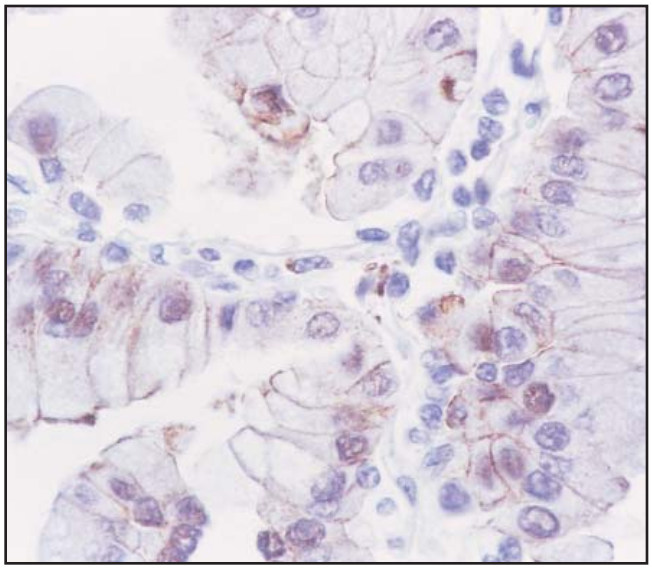

Image 7 Mucinous bronchioloalveolar adenocarcinoma. Focal reactivity. Staining is predominantly membranous and focally cytoplasmic (villin, hematoxylin counterstain, ×58).

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 misconclusion 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.

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References

1. Guazzi S, Price M, De Felice M, et al. Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. *EMBO J*. 1990;9:3631-3639.
2. Zhang L, Whitsett JA, Stripp BR. Regulation of Clara cell secretory protein gene transcription by thyroid transcription factor-1. *Biochim Biophys Acta*. 1997;1350:359-367.
3. Kelly SE, Bachurski CJ, Burhans MS, et al. Transcription of the lung-specific surfactant protein C gene is mediated by thyroid transcription factor 1. *J Biol Chem*. 1996;271:6881-6888.
4. Bruno MD, Bohinski RJ, Huelsman KM, et al. Lung cell–specific expression of the murine surfactant protein A (SP-A) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. *J Biol Chem*. 1995; 270:6531-6536.
5. Bohinski RJ, Di Lauro R, Whitsett JA. The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. *Mol Cell Biol*. 1994;14:5671-5681.
6. Lazzaro D, Price M, De Felice M, et al. The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. *Development*. 1991;113:1093-1104.
7. Stahlman MT, Gray ME, Whitsett JA. Expression of thyroid transcription factor-1 (TTF-1) in fetal and neonatal human lung. *J Histochem Cytochem*. 1996;44:673-678.
8. Bejarano PA, Baughman RP, Biddinger PW, et al. Surfactant proteins and thyroid transcription factor-1 in pulmonary and breast carcinomas. *Mod Pathol*. 1996;9:445-452.
9. Fabbro D, Di Loreto C, Stamerra O, et al. *TTF*-1 gene expression in human lung tumours. *Eur J Cancer*. 1996; 32A:512-517.
10. Holzinger A, Dingle S, Bejarano PA, et al. Monoclonal antibody to thyroid transcription factor-1: production, characterization, and usefulness in tumor diagnosis. *Hybridoma*. 1996;15:49-53.
11. Kaufmann 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.
12. Ordonez NG. Thyroid transcription factor-1 is a marker of lung and thyroid carcinomas. *Adv Anat Pathol*. 2000;7:123-127.
13. Ordonez NG. Value of thyroid transcription factor-1, E-cadherin, BG8, WT1, and CD44S immunostaining in distinguishing epithelial pleural mesothelioma from pulmonary and nonpulmonary adenocarcinoma. *Am J Surg Pathol*. 2000;24:598-606.
14. Khoor A, Whitsett JA, Stahlman MT, et al. Utility of surfactant protein B precursor and thyroid transcription factor 1 in differentiating adenocarcinoma of the lung from malignant mesothelioma. *Hum Pathol*. 1999;30:695-700.
15. Puglisi F, Barbone F, Damante G, et al. Prognostic value of thyroid transcription factor-1 in primary, resected, non–small cell lung carcinoma. *Mod Pathol*. 1999;12:318-324.
16. Bohinski RJ, Bejarano PA, Balko G, et al. Determination of lung as the primary site of cerebral metastatic adenocarcinomas using monoclonal antibody to thyroid transcription factor-1. *J Neurooncol*. 1998;40:227-231.
17. Harlamert HA, Mira J, Bejarano PA, et al. Thyroid transcription factor-1 and cytokeratins 7 and 20 in pulmonary and breast carcinoma. *Acta Cytol*. 1998;42:1382-1388.
18. Di Loreto C, Puglisi F, Di Lauro V, et al. TTF-1 protein expression in pleural malignant mesotheliomas and adenocarcinomas of the lung. *Cancer Lett*. 1998;124:73-78.
19. Di Loreto C, Di Lauro V, Puglisi F, et al. Immunocytochemical expression of tissue specific transcription factor-1 in lung carcinoma. *J Clin Pathol*. 1997;50:30-32.
20. Rosenblatt MB, Lisa JR, Collier F. Primary and metastatic bronciolo-alveolar carcinoma. *Dis Chest*. 1967;52:147-152.
21. Rosenblatt MB, Lisa JR, Collier F. Criteria for the histologic diagnosis of bronchogenic carcinoma. *Dis Chest*. 1967;51:587-595.
22. Graeme-Cook F, Mark EJ. Pulmonary mucinous cystic tumors of borderline malignancy. *Hum Pathol*. 1991;22:185-190.
23. Whitsett JA, Glasser SW. Regulation of surfactant protein gene transcription. *Biochim Biophys Acta*. 1998;1408:303-311.

24. Rosai J. Respiratory tract. In: Rosai J, ed. *Ackerman's Surgical Pathology*. St Louis, MO: Mosby; 1996:289-434.
25. Moran CA. Pulmonary system. In: Weidner N, ed. *The Difficult Diagnosis in Surgical Pathology*. Philadelphia, PA: Saunders; 1996:94-142.
26. Colby TV, Koss MN, Travis WD. Bronchioloalveolar carcinoma. In: Rosai J, Sobin LH, eds. *Tumors of the Lower Respiratory Tract*. Washington, DC: Armed Forces Institute of Pathology; 1995:203-234.
27. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol*. 2000;13:962-972.
28. Miettinen M. Keratin 20: immunohistochemical marker for gastrointestinal, urothelial, and Merkel cell carcinomas. *Mod Pathol*. 1995;8:384-388.
29. Loy TS, Calaluce RD. Utility of cytokeratin immunostaining in separating pulmonary adenocarcinomas from colonic adenocarcinomas. *Am J Clin Pathol*. 1994;102:764-767.
30. Wang NP, Zee S, Zarbo R, et al. Coordinate expression of cytokeratins 7 and 20 defines unique subsets of carcinomas. *Appl Immunohistochem*. 1995;3:99-107.
31. Ritter JH, Boucher LD, Wick MR. Peripheral pulmonary adenocarcinomas with bronchioloalveolar features: immunophenotypes correlate with histologic patterns. *Mod Pathol*. 1998;11:566-572.
32. Friederich E, Vancompernolle K, Louvard D, et al. Villin function in the organization of the actin cytoskeleton: correlation of in vivo effects to its biochemical activities in vitro. *J Biol Chem*. 1999;274:26751-26760.
33. Bacchi CE, Gown AM. Distribution and pattern of expression of villin, a gastrointestinal-associated cytoskeletal protein, in human carcinomas: a study employing paraffin-embedded tissue. *Lab Invest*. 1991;64:418-424.
34. Tan J, Sidhu G, Greco MA, et al. Villin, cytokeratin 7, and cytokeratin 20 expression in pulmonary adenocarcinoma with ultrastructural evidence of microvilli with rootlets. *Hum Pathol*. 1998;29:390-396.
35. Nambu Y, Iannettoni MD, Orringer MB, et al. Unique expression patterns and alterations in the intestinal protein villin in primary and metastatic pulmonary adenocarcinomas. *Mol Carcinog*. 1998;23:234-242.
36. Moll R, Robine S, Dudouet B, et al. Villin: a cytoskeletal protein and a differentiation marker expressed in some human adenocarcinomas. *Virchows Arch B Cell Pathol Incl Mol Pathol*. 1987;54:155-169.
37. Savera AT, Torres FX, Lindin MD, et al. Primary versus metastatic pulmonary adenocarcinoma: an immunohistochemical study using villin and cytokeratins 7 and 20. *Appl Immunohistochem*. 1996;4:86-94.