Expression of Thyroid Transcription Factor-1, Cytokeratin 7, and Cytokeratin 20 in Bronchioloalveolar Carcinomas: an Immunohistochemical Evaluation of 67 Cases

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Distinguishing primary pulmonary adenocarcinoma from metastatic adenocarcinoma involving the lung is a common challenging task. The distinction between mucinous bronchioloalveolar carcinoma (BAC) and metastatic mucinous carcinoma of other sites, in particular, is difficult by routine histology. Immunohistochemical expression of thyroid transcription factor-1 (TTF-1), as well as cytokeratin 7 (CK 7) and cytokeratin 20 (CK 20), has proven diagnostic utility in discerning primary from metastatic neoplasms in the lung. Rigorous studies assessing the expression of these markers in BACs, particularly in regard to nonmucinous and mucinous subtypes, have not been performed. In this study, we evaluated the immunohistochemical expression of TTF-1, CK 7, and CK 20 in 67 BACs (48 nonmucinous, 12 mucinous, and 7 of mixed histology). Overall, 42 (63%) of the 67 BACs were positive for TTF-1. When stratified according to subtype, all 12 mucinous BACs were observed to be TTF-1 negative. This trend toward absence of TTF-1 expression in mucinous areas was also maintained among tumors with mixed histology. Sixty-three (94%) of 67 BACs were CK 7 positive, with no differences in expression observed upon subtype stratification. Three cases were noted to be positive for CK 20; all exhibited mucinous morphology. These results indicate that in contrast to the immunophenotypic profile exhibited by most pulmonary neoplasms, mucinous BACs are TTF-1 negative and may express CK 20. This suggests that in the context of differentiating mucinous BACs from extrapulmonary mucinous tumors metastatic to the lung, evaluation of TTF-1 and CK 20 expression may have limited diagnostic utility.

KEY WORDS: Bronchioloalveolar carcinoma, Cytokeratin 7, Cytokeratin 20, Thyroid transcription factor-1.

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Thyroid transcription factor-1 (TTF-1) is a tissue-specific transcription factor that is expressed by epithelial cells in the thyroid and lung (1, 2). Previous immunohistochemical studies using antibodies against TTF-1 have demonstrated immunoreactivity in a variety of tumors of pulmonary origin (3–13). Immunohistochemical positivity of bronchioloalveolar carcinomas (BACs) for TTF-1 has been well established, with such tumors addressed in most studies primarily as a subset of the general category of pulmonary adenocarcinomas (3, 6, 7, 9, 10). Expression of TTF-1 in the context of the mucinous and nonmucinous subtypes of BACs, however, has not been well described, though it has been suggested that mucinous pulmonary adenocarcinomas, including those of bronchioloalveolar type, tend to be TTF-1 negative (10).

Immunohistochemical expression of cytokeratins in pulmonary adenocarcinomas, particularly cytokeratin 7 (CK 7) and cytokeratin 20 (CK 20), has also been well studied, with the majority of such tumors exhibiting a characteristic CK 7–positive, CK 20–negative immunophenotype (14–21). This differential expression of CK 7 and CK 20 has been useful in the context of distinguishing primary adenocarcinomas of the lung from metastatic adenocarcinomas arising in other sites (16–19, 21). However, similar to the situation with studies involving TTF-1, few reports of CK 7 and CK 20 expression in pulmonary adenocarcinomas have addressed BACs specifically.

In the present study, we evaluated a large series of BACs to elucidate immunohistochemically the frequency of TTF-1, CK 7, and CK 20 expression,

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particularly in regard to the mucinous and nonmucinous subtypes of these tumors.

MATERIALS AND METHODS

Sixty-seven cases of BAC were retrieved from the archives of the Department of Anatomic Pathology at Cedars-Sinai Medical Center, Los Angeles, California. All cases were surgically resected (wedge biopsy, lobectomy, or pneumonectomy) between January 1990 and February 2001. Medical records were reviewed to verify that the cases were of primary lung origin and did not represent metastatic disease from primary tumors at other sites. All hematoxylin and eosin–stained slides containing tumor (range, 2–6; mean, 4.1) for each case were reviewed and the diagnoses of BAC according to World Health Organization criteria (22) confirmed. In accordance with the WHO definition of BAC (22), all tumors exhibited a purely lepidic growth pattern of cells along intact alveolar septa, with no evidence of stromal, vascular, or pleural invasion. The 67 cases were classified into nonmucinous (n = 48) and mucinous (n = 12) subtypes, as well as those that were of mixed histology, displaying morphologic features of both (n = 7). The nonmucinous BACs (Fig. 1A) were characterized by a proliferation of uniform cuboidal, low columnar, or peg-shaped cells lining intact, slightly thickened alveolar walls, whereas the mucinous BACs (Fig. 2A) were composed of cytologically bland, tall columnar cells with apical cytoplasmic mucin and basally oriented nuclei. The BACs of mixed histology (Fig. 3A) exhibited areas with both nonmucinous and mucinous epithelial cells, as described above. A few of these cases displayed focal areas of interstitial expansion with a mild lymphocytic infiltrate and a minimal amount of fibrosis (Fig. 3A). The underlying alveolar architecture was otherwise intact, and the tumors lacked features suggestive of invasion, such as cytologic atypia or an acinar, tubular, or solid growth pattern. As there was no overt evidence of stromal invasion and the cases otherwise conformed to the morphologic definition of BAC, we elected to include these cases in the study.

Immunoperoxidase studies were performed on 4-μm, formalin-fixed, paraffin-embedded tissue sections that were routinely deparaffinized in xylene and rehydrated in graded ethanols. Sections used for TTF-1 analysis were pretreated with Nuclear Decloaker (Biocare Medical, Walnut Creek, CA) at pH 9.5 and then underwent 4 minutes of pressure cooker treatment. Sections used for CK 7 and CK 20 were pretreated with citrate buffer (pH 6.0) and water bath immersion at 99° C (20 min in; 20 min out). Primary antibody source and dilutions were as follows: TTF-1 (Biocare Medical; Clone 8G78G3/1; 1:100 dilution); CK 7 (DAKO; Clone OV-TL 12/30; 1:30 dilution); CK 20 (DAKO; Clone Ks20.8; 1:300 dilution). Sections were subjected to incubation with primary antibodies in a

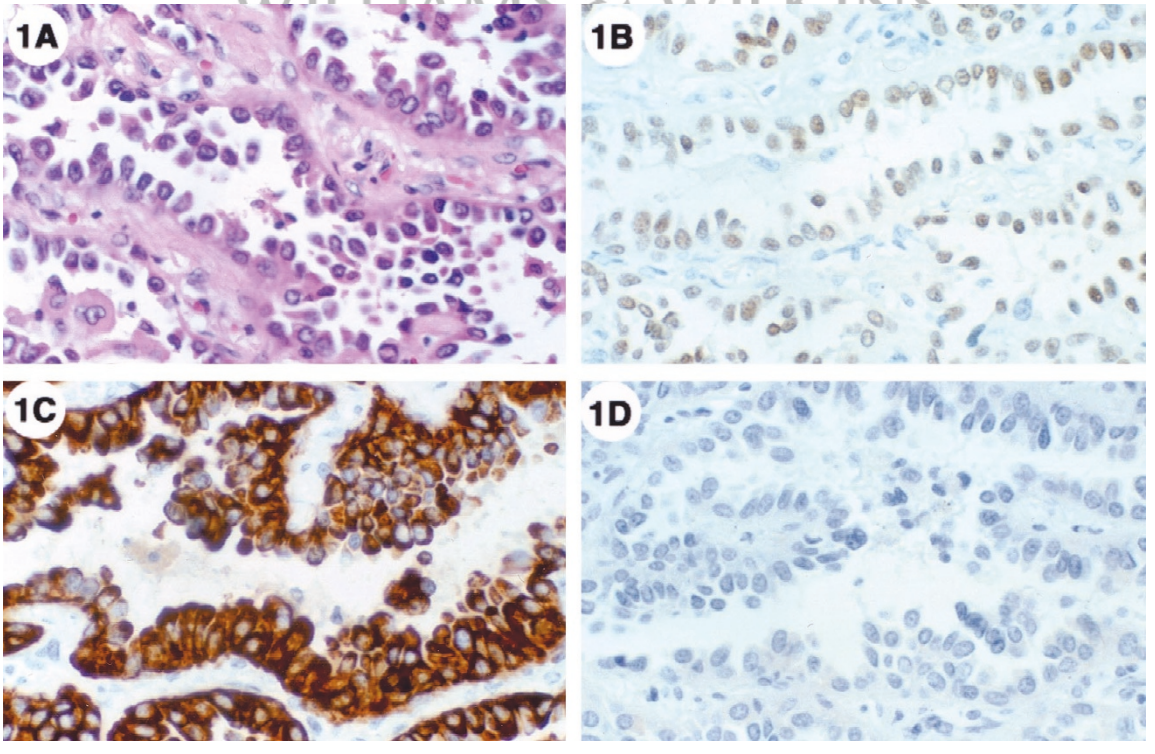

FIGURE 1. Nonmucinous bronchioloalveolar carcinoma (original magnifications, 400×). **A,** hematoxylin and eosin staining. **B,** nuclear TTF-1 immunoreactivity. **C,** positive CK 7 expression in tumor cells. **D,** lack of CK 20 staining.

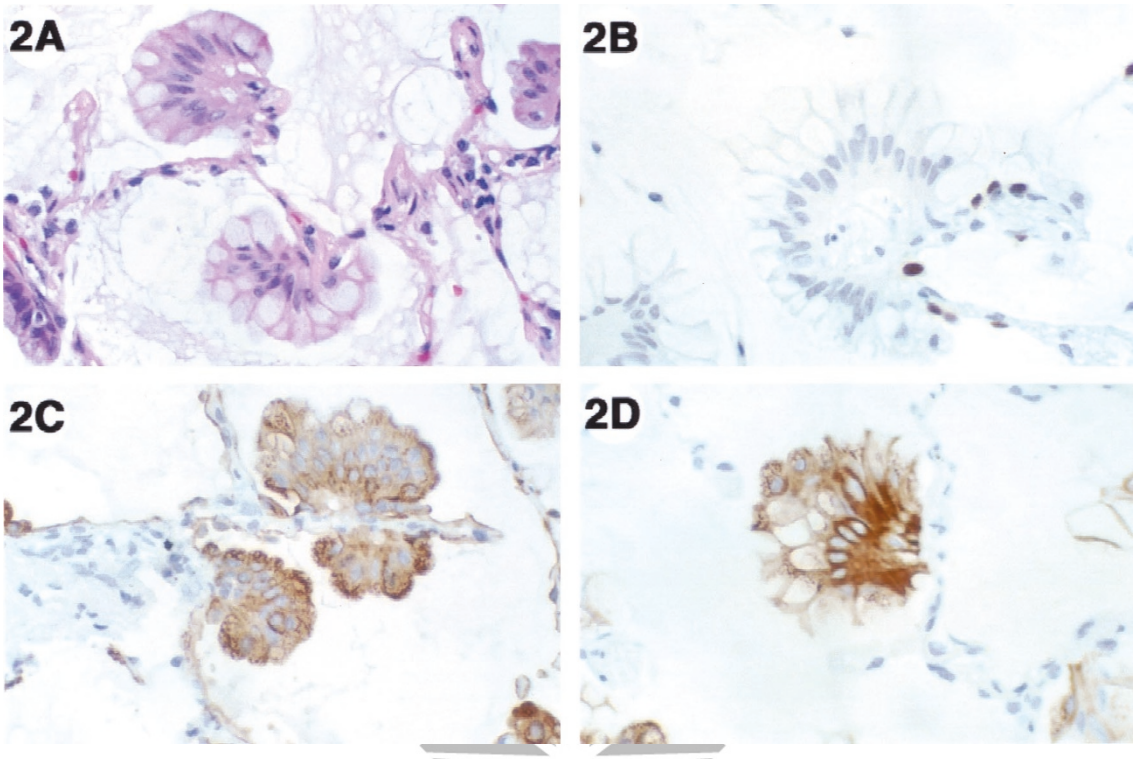

FIGURE 2. Mucinous bronchioloalveolar carcinoma (original magnifications, 400×). **A,** hematoxylin and eosin staining. **B,** absence of TTF-1 immunoreactivity. Note nuclear TTF-1 expression in non-neoplastic alveolar epithelial cells. Strong immunoreactivity for CK 7 (**C**) and CK 20 (**D**).

DAKO Auto-Stainer, with antibody localization using a standardized two step method using the DAKO EnVision + System, horseradish peroxidase, and 3,3'-diaminobenzidine as a chromogen. Appropriate positive and negative tissue controls were used throughout.

Nuclear staining was assessed for TTF-1, whereas cytoplasmic staining was evaluated for CK 7 and CK 20. Immunoreactivity was evaluated according to the intensity of tumor cell staining (0–3+), as well as according to the percentage of tumor cells that were stained. A particular tumor was considered positive if >10% of the tumor cells reacted with any intensity.

RESULTS

The immunohistochemical results are summarized in Table 1. TTF-1 positivity was observed in 42 (63%) of 67 BACs. No TTF-1 immunostaining was detectable in 12 mucinous BACs (Fig. 2B), in contrast to the positive staining present in 36 (75%) of 48 BACs of nonmucinous subtype (Fig. 1B). Seven BACs displayed mixed features of both mucinous and nonmucinous subtypes. Five (71%) of the 7 cases showed TTF-1 expression limited to the nonmucinous component of the tumor (Fig. 3C), with absence of staining in the mucinous component (Fig. 3B). One case displayed TTF-1 immunoreac-

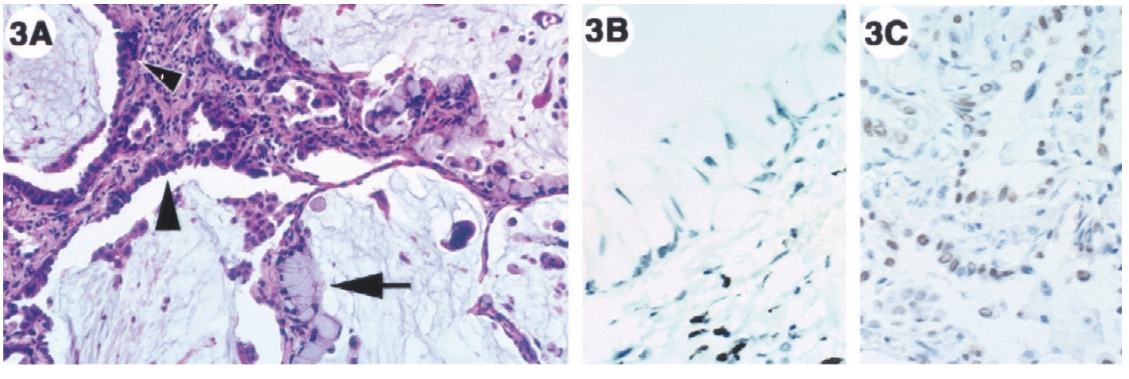

FIGURE 3. **A,** bronchioloalveolar carcinoma with mixed mucinous (***arrow***) and nonmucinous (***arrowheads***) morphology (original magnification, 200×). **B,** absence of TTF-1 expression in mucinous areas (original magnification, 400×). **C,** nuclear TTF-1 immunoreactivity in nonmucinous component of tumor (original magnification, 200×).

TABLE 1. Thyroid Transcription Factor-1, Cytokeratin 7, and Cytokeratin 20 Immunoreactivity in Bronchioloalveolar Carcinomas

Tumor Type	*N*	TTF-1, no. (%)	CK 7, no. (%)	CK 20, no. (%)
Nonmucinous	48	36 (75)	46 (96)	0 (0)
Mucinous	12	0 (0)	10 (83)	3 (25)
Mixed	7	6 (86)	7 (100)	0 (0)
Total BACs	67	42 (63)	63 (94)	3 (4)

tivity in both the mucinous and nonmucinous areas, whereas an additional case showed absence of a positive reaction in both.

CK 7 was consistently expressed by nearly all BACs (94%), irrespective of subtype (Figs. 1C, 2C). In contrast, BACs were typically negative for CK 20 (Fig. 1D). Only 3 (4%) of 67 BACs demonstrated CK 20 expression; all were of mucinous subtype (Fig. 2D). Of note, focal staining for CK 20 that did not meet our criteria for a positive reaction was observed in an additional three cases of mucinous BAC.

DISCUSSION

TTF-1 expression has been well demonstrated in a number of tumors of pulmonary origin, though the frequency of immunoreactivity tends to vary according to tumor histology (1, 3–5, 7, 13). In the present study, TTF-1 positivity was observed in 63% of BACs. Previous investigators have shown the frequency of TTF-1 staining in such tumors to range anywhere from 25–100%, though the number of cases in each particular series has been relatively small (3, 6, 7, 9, 10).

BACs can be classified into mucinous and nonmucinous subtypes, also known as Type 1 and Type 2, respectively (23, 24). Type 1, or mucinous, BACs display goblet or mucin-producing cells, whereas Type 2, or nonmucinous, BACs show predominantly Clara cell or Type II pneumocyte differentiation. Tumors that exhibit both mucinous and nonmucinous histologic patterns have also been described (25, 26).

When classified into different subtypes, the current study demonstrated TTF-1 positivity in 75% of the nonmucinous BACs and complete absence of staining in all mucinous BACs examined. Interestingly, in those tumors that exhibited areas of both mucinous and nonmucinous histology, the trend toward nonexpression of TTF-1 in the mucinous component was maintained, with six of seven such tumors lacking TTF-1 immunoreactivity in mucinous areas. Kaufmann and Dietel (10) noted a similar absence of TTF-1 staining in their examination of mucinous pulmonary carcinomas, five of which were BACs.

Previous examination of CK 7 and CK 20 expression in pulmonary adenocarcinomas has demonstrated CK 7 positivity and CK 20 negativity in the vast majority of cases studied (14–21). In the present analysis, most BACs were observed to be CK 7 positive and CK 20 negative, a pattern of immunoreactivity similar to that defined for pulmonary adenocarcinomas in general. When evaluated by subtype, however, CK 20 immunoreactivity was noted in 25% of the mucinous BACs studied. In contrast, CK 20 expression was conspicuously absent in the nonmucinous BACs. This represents an interesting finding, as adenocarcinomas of the lung in general are regarded as tumors that are CK 20 negative (15–21). Although the possibility exists that these CK 20–positive cases represent metastatic mucinous tumors from extrapulmonary sites such as the gastrointestinal tract, thus explaining their CK 20 expression, our review of the clinical records supports classification of these cases as primary lung tumors.

Few reports have analyzed patterns of CK 7 and CK 20 immunoreactivity in the context of BACs. Tan *et al.* (19) observed a CK 7–positive, CK 20–negative immunophenotype in four of four BACs, two of which showed ultrastructural evidence of mucinous differentiation. Comparable results were reported by Ritter *et al.* (20), who noted a CK 7–positive, CK 20–negative staining pattern in five of five nonmucinous BACs. However, similar to the results of our study, CK 20 expression was observed in four of five mucinous BACs. Although the exact reasons for CK 20 immunoreactivity in pulmonary mucinous BACs are not clear, a similar phenomenon has been observed in ovarian neoplasms. Although most ovarian carcinomas exhibit a CK 7–positive, CK 20–negative immunophenotype, ovarian mucinous neoplasms in particular tend to express CK 20 (15, 17, 18, 21, 27, 28).

The histologic distinction between primary pulmonary adenocarcinomas and adenocarcinomas metastatic to the lung can be a difficult task, with significant clinical ramifications. In this regard, immunohistochemical evaluation of CK 7 and CK 20 expression patterns has been a useful method of accurately classifying these entities (16–19, 21). Similarly, the diagnostic utility of TTF-1 expression in tumors of pulmonary origin has been in distinguishing such tumors from metastatic tumors of similar histology (4, 10, 13). Metastatic mucin-producing carcinomas, particularly those of enteric, pancreatic, and ovarian origin, may closely simulate the morphology of mucinous BACs (24, 30–32). The consistent lack of TTF-1 immunopositivity, as well as the occasional identification of CK 20 in mucinous BACs, as demonstrated in this study, suggests that these markers would not be of value in the context of differentiating primary pulmonary from metastatic mucinous tumors.

In summary, immunohistochemical expression of TTF-1 was noted in the majority of a large series of BACs. As with other pulmonary adenocarcinomas, the BACs in this study also tended to exhibit a

CK 7–positive and CK 20–negative immunophenotype. However, BACs of mucinous morphology were notable for their conspicuous absence of TTF-1 immunoreactivity and occasional expression of CK 20. These findings imply that evaluation of TTF-1 and CK 20 expression has limited clinical utility in the context of differentiating primary pulmonary from metastatic mucinous neoplasms.[29]

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