Usefulness of Cdx2 in Separating Mucinous Bronchioloalveolar Adenocarcinoma of the Lung From Metastatic Mucinous Colorectal Adenocarcinoma

Reda S. Saad, MD, PhD,* Patrick Cho, MD, Jan F. Silverman, MD, and Yulin Liu, MD, PhD

Key Words: Cdx2; Thyroid transcription factor-1; TTF-1; Mucinous bronchioloalveolar carcinoma; Colorectal carcinoma; Lung cancer

Abstract

We studied the diagnostic value of Cdx2 to distinguish mucinous bronchioloalveolar carcinoma from mucinous colorectal adenocarcinoma metastatic to the lung. We retrieved 92 via the hospital computer system, including 30 mucinous bronchioloalveolar carcinomas, 32 nonmucinous bronchioloalveolar carcinomas, and 30 mucinous colorectal adenocarcinomas metastatic to the lung. All cases were confirmed by clinical history and surgical resection with occasional immunohistochemical studies. Cases were stained with antibodies against Cdx2, thyroid transcription factor-1 (TTF-1), cytokeratin (CK) 7, and CK20. Bronchioloalveolar carcinoma, mucinous type, showed positive staining for Cdx2, TTF-1, CK7, and CK20 in 0 (0%), 5 (17%), 30 (100%), and 18 (60%) of 30 cases, respectively; nonmucinous tumors were positive in 0 (0%), 30 (94%), 32 (100%), and 0 (0%) of 32 cases, respectively. For colorectal adenocarcinoma, the positive staining for Cdx2, TTF-1, CK7, and CK20 was 29 (97%), 0 (0%), 7 (23%), and 29 (97%) of 30 cases, respectively. Our results demonstrated Cdx2 as a sensitive and specific marker for differentiating metastatic colorectal adenocarcinoma from mucinous bronchioloalveolar adenocarcinoma.

Because the lung is a common site for primary and metastatic adenocarcinomas, defining the origin of a carcinoma in a patient with a pulmonary nodule occasionally can be a diagnostic dilemma. The differentiation between primary and metastatic pulmonary adenocarcinoma is crucial for therapeutic and prognostic purposes. Metastatic lesions can be treated by wedge resection in contrast with lobectomy, which is recommended for primary tumors. In addition, lung metastases from colorectal carcinomas can be resected with improved survival or even cure. Identifying the characteristic histologic features of colorectal adenocarcinoma, such as dirty necrosis and elongated and pencil-like nuclei, might be difficult in small biopsy specimens; these features also can be seen in pulmonary adenocarcinoma. In addition, bronchioloalveolar carcinoma can manifest as multiple lung nodules similar to cancer metastatic to the lung, while metastatic colorectal adenocarcinoma can line the alveolar walls in a lepidic pattern mimicking primary mucinous bronchioloalveolar carcinoma.

Immunohistochemical markers serve an important role in the differential diagnosis between primary and metastatic lung lesions. Among the markers used to identify primary lung tumors are thyroid transcription factor-1 (TTF-1), cytokeratin (CK) 7, and CK20. Several reports have shown that the predominant CK profile of lung adenocarcinoma (CK7+/CK20−) is distinctly different from that of colonic adenocarcinoma (CK7−/CK20+). However, both colorectal adenocarcinoma and mucinous bronchioloalveolar carcinoma are reported to be CK7+/CK20+ and TTF-1−. Because of these immunophenotypic similarities, the differentiation between mucinous bronchioloalveolar carcinoma and metastatic mucinous colorectal carcinoma can be a challenge.
Cdx2, a member of Cdx homeobox genes, is a nuclear transcription factor that has an essential role as a regulatory protein for proliferation and differentiation of intestinal epithelial cells in fetal and adult tissues.16 Cdx2 is expressed specifically in colonic and small intestinal mucosa and has been implicated in disorders involving abnormal intestinal differentiation and neoplasia.17,18 The use of Cdx2 as an immunohistochemical marker has been described recently in studies of human gastric and colonic cancer.19-21

In the present study, we evaluated the immunohistochemical expression of Cdx2 in a series of mucinous bronchioloalveolar carcinomas and metastatic mucinous colorectal adenocarcinomas to determine whether Cdx2 expression is a reliable marker to identify the colorectal origin of a lung metastasis. We also examined the distribution of TTF-1, CK7, and CK20 staining in mucinous and nonmucinous bronchioloalveolar carcinomas to study their patterns of reactivity.

Materials and Methods

Case Selection

The institutional review board of Allegheny General Hospital, Pittsburgh, PA, approved the study. We included 62 unselected cases of primary bronchioloalveolar adenocarcinoma and 30 cases of mucinous colorectal carcinoma metastatic to the lung from patients who underwent surgical resection during a 5-year period at Allegheny General Hospital (January 1996 to December 2000). Histologic diagnoses of the lung carcinomas were determined according to World Health Organization criteria22 that defined bronchioloalveolar carcinoma as adenocarcinoma with a pure bronchioloalveolar growth pattern and no evidence of vascular, pleural, or stromal invasion. Cases of bronchioloalveolar carcinoma included 32 of the nonmucinous subtype and 30 of the mucinous subtype.

Tissue samples from the specimens were fixed in 10% buffered formalin, processed, and stained with H&E. All cases were reviewed to confirm the diagnosis. One paraffin block with the maximum amount of tumor and proper fixation was chosen from each case for immunohistochemical studies.

Immunohistochemical Analysis

We stained 4-µm-thick sections from the paraffin-embedded tissue blocks for Cdx2, TTF-1, CK7, and CK20. Table I gives the characteristics, pretreatment, dilutions, incubation periods, localization, and sources of the antibodies. The tissue sections were mounted on coated slides and dried 1 hour at 60°C. The sections were deparaffinized in xylene and rehydrated in a descending series of ethanol solutions.

After preincubation with 1% hydrogen peroxide for 10 minutes to block the endogenous peroxidase activity, the antibodies were incubated at appropriate dilutions for periods and temperatures as indicated in Table 1. An automated stainer (Ventana Immunostainer, Ventana Medical Systems, Tucson, AZ) and a basic diaminobenzidine immunohistochemical detection system (Ventana Medical Systems) were used for the rest of the procedure. The sections were counterstained with Mayer hematoxylin, dehydrated, cleared in xylene, and mounted. Benign bronchioloalveolar cells served as an internal positive control sample for TTF-1 in the specimens. Sections of benign colonic mucosa served as a positive control sample for Cdx2. For negative control samples, the primary antibody was omitted for each run.

Microscopic Evaluation

Three observers (R.S.S., P.C., and Y.L.) blindly assessed the immunostaining without knowledge of the previous clinical, radiologic, or histopathologic diagnoses. For TTF-1 and Cdx2, only nuclear staining was considered positive. Intensity was graded on a 3-tiered scale (1+, negative to weak; 2+, moderate; and 3+, strong). The percentage of staining was recorded as follows: 0% to 10%, 11% to 25%, 26% to 50%, 51% to 75%, and more than 75%. Cases that stained 0% to 10% or showed diffuse 1+ staining were considered negative and others as positive. After the cases were evaluated, the diagnoses were revealed and staining patterns of the various tumors compared. Interobserver variability was resolved by consensus of the slide in question under a multiheaded microscope.

Table I

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Pretreatment</th>
<th>Dilution</th>
<th>Incubation Time (min)*</th>
<th>Localization</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdx2</td>
<td>Cdx-88</td>
<td>Microwave†</td>
<td>1:200</td>
<td>60</td>
<td>Nuclei</td>
<td>BioGenex, San Ramon, CA</td>
</tr>
<tr>
<td>TTF-1</td>
<td>8G7G3/1</td>
<td>Microwave†</td>
<td>1:150</td>
<td>60</td>
<td>Nuclei</td>
<td>DakoCytomation, Carpinteria, CA</td>
</tr>
<tr>
<td>CK7</td>
<td>OV-TL 12/30</td>
<td>Protease 1</td>
<td>1:200</td>
<td>32</td>
<td>Cytoplasm</td>
<td>DakoCytomation</td>
</tr>
<tr>
<td>CK20</td>
<td>KS20.8</td>
<td>Protease 1</td>
<td>1:50</td>
<td>32</td>
<td>Cytoplasm</td>
<td>DakoCytomation</td>
</tr>
</tbody>
</table>

CK, cytokeratin; TTF-1, thyroid transcription factor-1.
† All at room temperature.
* In citrate buffer.
Statistical Analysis

Statistical analysis was performed to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the investigated markers for colorectal carcinoma and lung tumors. For Cdx2, false-positive cases included metastatic adenocarcinomas other than colorectal carcinomas expressing Cdx2, and false-negative cases included colorectal carcinomas that did not express Cdx2. For TTF-1, false-positive cases included metastatic adenocarcinomas other than lung tumors expressing TTF-1, and false-negative cases included lung adenocarcinomas that did not express TTF-1.

Results

Table 2 and Table 3 show the percentages of cases that stained for TTF-1, Cdx2, CK7, and CK20 in bronchioloalveolar and metastatic colorectal carcinomas.

Thyroid Transcription Factor-1

TTF-1 immunoreactivity demonstrated a fine granular and diffuse staining pattern confined to the nuclei. In nonmucinous bronchioloalveolar carcinoma, TTF-1 demonstrated strong (3+) and diffuse nuclear staining in more than 75% of the cells in 22 (69%) of 32 cases and in 51% to 75% of cells in 8 cases (25%) Image 1Al. In mucinous bronchioloalveolar carcinoma, TTF-1 was negative in the majority of cases (25/30 [83%]) Image 1Bl. TTF-1 was only positive in 5 cases (17%); 2 cases (7%) showed strong (3+) staining (51%-75% of cells); and 3 cases (10%) demonstrated moderate (2+) staining of 26% to 50% of cells. TTF-1 was negative in all 30 cases (100%) of metastatic colorectal adenocarcinomas. No cytoplasmic reactivity was identified in any of the cases. The sensitivity, specificity, PPV, and NPV of TTF-1 to differentiate nonmucinous bronchioloalveolar adenocarcinoma from metastatic colorectal adenocarcinoma were 94%, 100%, 100%, and 94%, respectively, with 97% accuracy, and for the mucinous subtype were 17%, 100%, 100%, and 55%, respectively, with 58% accuracy.

CK7 and CK20

The CK7+/CK20− immunophenotype was expressed in all 32 cases of nonmucinous bronchioloalveolar carcinoma (100%), in 12 (40%) of 30 cases of mucinous bronchioloalveolar carcinoma, and in only 1 (3%) of 30 cases of metastatic colorectal carcinoma. The CK7−/CK20+ immunophenotype was expressed by 22 (73%) of 30 metastatic colorectal carcinomas and was not observed in any of the primary bronchioloalveolar carcinomas. The CK7+/CK20+ immunophenotype was expressed in 18 (60%) of 30 mucinous bronchioloalveolar carcinomas

| Table 2 | Cases Staining With Various Percentages of Positive Cells in Primary Bronchioloalveolar Carcinoma and Metastatic Colorectal Adenocarcinomas* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| *Negative* | 11%-25% | 26%-50% | 51%-75% | 76%-100% | Total |
| Nonmucinous bronchioloalveolar carcinoma (n = 32) | | | | | |
| TTF-1 | 2 (6) | 0 (0) | 8 (25) | 22 (69) | 30 (94) |
| Cdx2 | 32 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Mucinous bronchioloalveolar carcinoma (n = 30) | | | | | |
| TTF-1 | 25 (83) | 0 (0) | 3 (10) | 2 (7) | 5 (17) |
| Cdx2 | 30 (100)‡ | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Metastatic colorectal carcinoma (n = 30) | | | | | |
| TTF-1 | 30 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Cdx2 | 1 (3) | 3 (10) | 5 (17) | 21 (70) | 29 (97) |

* TTF-1, thyroid transcription factor-1.
† Positive cases defined as 10% of neoplastic cells staining.
‡ Of 30 cases, 3 (10%) showed focal weak Cdx2 staining (≤10% of cells).

| Table 3 | Results for CK7 and CK20* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| CK7+/CK20− | CK7+/CK20+ | CK7−/CK20+ |
| Nonmucinous bronchioloalveolar carcinoma (n = 32) | 32 (100) | 0 (0) | 0 (0) |
| Mucinous bronchioloalveolar carcinoma (n = 30) | 12 (40) | 18 (60) | 0 (0) |
| Metastatic colorectal carcinoma (n = 30) | 1 (3) | 7 (23) | 22 (73) |

CK, cytokeratin; +, positive; −, negative.
* Data are given as number (percentage).
Image 2A and in 7 (23%) of 30 metastatic colorectal adenocarcinomas, which was not helpful in this differential diagnosis Image 3A. However, CK20 reactivity was diffuse in metastatic colorectal carcinoma and mainly focal in mucinous bronchioloalveolar carcinoma. None of the nonmucinous bronchioloalveolar carcinomas demonstrated the CK7+/CK20+ immunophenotype.

Cdx2 Expression

Cdx2 was expressed in 29 (97%) of 30 metastatic colorectal adenocarcinomas. In positive cases, the immunoreactivity was predominantly nuclear with occasional faint cytoplasmic reactivity (Image 3). The majority of the cases (21/30 [70%]) demonstrated strong (3+) and diffuse immunostaining in more than 75% of the cells (Image 2); 5 cases showed strong staining (3+) of 51% to 75% of the cells; and 3 cases showed moderate staining (2+) of 26% to 50% of the cells. No immunoreactivity was observed in any of the 62 bronchioloalveolar carcinomas of the lung. However, 3 cases of mucinous bronchioloalveolar carcinoma demonstrated weak (1+) and/or focal staining (<10%), considered as negative for the present study. The sensitivity, specificity, PPV, and NPV of Cdx2 to differentiate metastatic colorectal adenocarcinoma from

**Image 11** Bronchioloalveolar carcinoma. A, Nonmucinous subtype demonstrating strong thyroid transcription factor-1 (TTF-1) positivity (TTF-1, original magnification ×250). B, Mucinous subtype with negative staining. Note that benign pneumocytes served as a positive internal control (TTF-1, original magnification ×400).

**Image 21** Mucinous bronchioloalveolar carcinoma of the lung. A, Strong and diffuse cytokeratin 20 immunoreactivity (original magnification ×250). B, Negative staining for Cdx2 (original magnification ×400).
bronchioalveolar carcinomas were 97%, 100%, 100%, and 98%, respectively, with 99% accuracy.

**Discussion**

Primary lung carcinomas, particularly mucinous bronchioalveolar carcinomas, might be indistinguishable from metastatic colorectal carcinoma. Immunohistochemical analysis has been used as an ancillary tool to better define the origin of the lung cancer. The most widely used immunohistochemical markers used to confirm the origin of lung carcinoma are TTF-1 and the CK7-CK20 expression profile.

TTF-1, a member of NKx2 homeobox genes, is a transcription factor required for normal development of the thyroid gland and lung alveolar cells.\(^2^3\) TTF-1 is expressed in about 70% to 80% of pulmonary adenocarcinomas.\(^2^4^-^3^0\) Similar to previous reports,\(^1^5^,^2^9^-^3^0\) our study showed that nonmucinous bronchioalveolar carcinoma has the highest prevalence of TTF-1 reactivity, because 94% (30/32) demonstrated TTF-1 staining. TTF-1 reactivity in nonmucinous carcinoma is usually extensive. In the present study, TTF-1 stained more than 75% of the cell nuclei in 22 (69%) of 32 and more than 50% in 8 (25%) of 32 positive cases. The explanation of the intense TTF-1 staining in nonmucinous bronchioalveolar carcinoma is that these tumors arise from type II pneumocytes and Clara cells, which normally express TTF-1.\(^3^1^-^3^2\) In contrast, we found that mucinous bronchioalveolar carcinoma rarely expressed TTF-1 (5/30 [17%]). Other studies have reported that TTF-1 is of limited sensitivity in mucinous bronchioalveolar carcinoma, with expression in only 10% to 21% of cases,\(^1^3^-^1^5^,^3^0\) similar to our results.

Another frequently used immunohistochemical profile to confirm the origin of a metastatic adenocarcinoma is defining the CK7-CK20 phenotype, because lung adenocarcinomas are CK7+/CK20−, whereas colorectal adenocarcinomas usually are CK7−/CK20+. Although characteristic, this profile is not specific because tumors of other origins can share the CK7−/CK20+ phenotype.\(^4^,^3^2\) Furthermore, CK20 immunoreactivity frequently is restricted to subsets of tumor cells, and small biopsy specimens might show false-negative results. In the present study, 1 case of metastatic colorectal carcinoma demonstrated a CK7+/CK20− profile, leading to misclassification of the lesion as a primary lung adenocarcinoma. Mucinous bronchioloalveolar carcinoma and metastatic colorectal carcinoma can coexpress CK7 and CK20. A recent study showed that CK7 expression, in addition to CK20 expression, in colorectal carcinoma depends mainly on the site, with a high percentage (74%) in rectal adenocarcinoma.\(^1^2\) This pattern also can be identified in 20% of conventional lung adenocarcinomas.\(^3^3\) The present study showed that 23% of colorectal adenocarcinomas (7/30) and 60% of mucinous bronchioloalveolar carcinomas (18/30) showed a CK7+/CK20+ profile. This immunostaining pattern might be misleading because it is seen in tumors of different origins, such as pancreatic and bladder adenocarcinomas and cholangiocarcinomas.\(^3^4^-^3^5\)

Few studies have attempted to correlate CK20 expression with histologic subtypes of lung adenocarcinoma.\(^1^3^-^1^5^,^3^5\) Goldstein and Thomas\(^1^5\) and Shah et al\(^3^5\) found that 79% and 89%, respectively, of mucinous bronchioloalveolar carcinomas were CK20+. The present study demonstrated a smaller percentage (60% [18/30]) of mucinous bronchioloalveolar carcinomas that were CK20+. Lau et al\(^3^0\)
reported only 25% of mucinous bronchioloalveolar carcinomas to be CK20+. The reason for this discrepancy might be the threshold cutoff or using a different antigen retrieval technique (all the investigators used the same antibody).

Although mucinous and nonmucinous bronchioloalveolar carcinomas might have similar architectural growth patterns, the results of the present study confirmed that mucinous and nonmucinous bronchioloalveolar carcinomas have different staining patterns with TTF-1 and CK20, similar to the findings of previous studies. Nonmucinous bronchioloalveolar carcinomas usually are reactive for TTF-1 and nonreactive for CK20. Conversely, mucinous bronchioloalveolar carcinomas usually are TTF-1+ and CK20+, and this could lead to a misclassification of the carcinoma. These findings suggest that other immunohistochemical markers are needed to distinguish primary mucinous bronchioloalveolar carcinoma from pulmonary metastases of mucinous colorectal carcinoma.

Villin is an actin-binding protein that interacts with and stabilizes the microvillin actin core bundles of the intestinal and renal brush borders. Villin has been expressed in the majority of colon adenocarcinomas and in a minority of pulmonary mucinous adenocarcinomas and bronchioloalveolar adenocarcinomas. Strong villin reactivity in a brush border pattern is supportive of a metastatic colon mucinous adenocarcinoma, while no reactivity or cytoplasmic reactivity associated with CK7 positivity is supportive of a primary mucinous bronchioloalveolar carcinoma.

Cdx2 is a transcription factor that is expressed in normal and neoplastic intestinal epithelium. Similar to the findings in previous studies, in the present study, Cdx2 immunostained 29 (97%) of 30 cases of mucinous colorectal adenocarcinomas metastatic to the lung, and it was negative in all mucinous and nonmucinous types of bronchioloalveolar carcinomas. Therefore, Cdx2 is a useful immunohistochemical marker for the differential diagnosis of primary vs metastatic adenocarcinomas in the lung and in the workup of metastases from an unknown primary site. This study also showed that Cdx2 is helpful in the distinction between mucinous bronchioloalveolar carcinoma and mucinous colorectal carcinoma metastatic to the lung that can be difficult to differentiate based on histologic grounds. However, a few cases of colorectal carcinoma have been reported to be negative for Cdx2, and these tumors were poorly differentiated carcinomas.

Recent studies have reported Cdx2 positivity in gastric, hepatobiliary, pancreatic, and ovarian mucinous adenocarcinomas. The possibility of Cdx2 immunoreactivity in metastatic tumors deriving from the aforementioned primary sites should be kept in mind. However, in gastrointestinal adenocarcinomas other than colorectal, staining usually is positive in a low percentage (20%-30%) of cases and usually is focal and less intense than that seen in colorectal carcinoma. Also, a recent study found that a majority of pancreatic adenocarcinomas were Cdx2-. Therefore, strong and extensive nuclear Cdx2 immunostaining in the appropriate clinical context (male sex or female without an ovarian mass) favors colorectal adenocarcinomas.

Cdx2 identified almost all cases (29/30 [97%]) of colorectal carcinoma metastatic to the lung. Our study showed that in the context of the differential diagnosis of adenocarcinomas in the lung (especially the mucinous type), Cdx2 should be added to the existing panel of immunohistochemical markers to aid in the differential diagnosis of metastatic colorectal carcinoma vs primary mucinous bronchioloalveolar carcinoma.

References


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