The Value of Thyroid Transcription Factor-1 in Cytologic Preparations as a Marker for Metastatic Adenocarcinoma of Lung Origin

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Abstract

In tissue sections, thyroid transcription factor-1 (TTF-1) is a sensitive marker for adenocarcinomas of lung and thyroid origin. This immunohistochemical study evaluates the effectiveness of TTF-1 as a marker for pulmonary adenocarcinomas in paraffin sections of cell block preparations derived from effusion and fine-needle aspiration specimens. We evaluated 122 cell blocks including 8 primary and 39 metastatic pulmonary adenocarcinomas, 11 pulmonary neoplasms of other types, 50 specimens with nonpulmonary metastatic tumors, and 14 mesotheliomas. TTF-1 was reactive in 42 (89%) of 47 pulmonary adenocarcinomas. Only 1 of 4 pulmonary small cell/neuroendocrine tumors was TTF-1 positive, while 1 of 7 squamous cell carcinomas was weakly reactive. Of 50 metastatic tumors of nonpulmonary origin, focal weak reactivity was noted only for 1 metastatic ovarian carcinoma. All mesotheliomas were nonreactive. In cytologic preparations, TTF-1 is a highly selective marker for pulmonary adenocarcinoma and also can have a role in the distinction between pulmonary adenocarcinoma and mesothelioma.

Thyroid transcription factor-1 (TTF-1) is a nuclear protein that is selectively expressed in epithelial cells of thyroid and lung origin and in adenocarcinomas derived from these cells. In tissue biopsies and cytologic preparations, TTF-1 has been shown to be a sensitive marker for tumors of pulmonary origin. More recently, TTF-1 has been used to discriminate between pulmonary adenocarcinoma and malignant mesothelioma in pleural biopsy specimens. This study is the largest to date that evaluates the effectiveness of TTF-1 to discern the site of origin for neoplastic cells in cytologic specimens derived from fine-needle aspiration (FNA) or effusion fluids and also in the distinction between pulmonary adenocarcinoma and malignant mesothelioma in these preparations.

Materials and Methods

A total of 122 cell blocks containing various malignant tumors were identified in the files of the Cytology Division of Brigham & Women’s Hospital, Boston, MA. Cell blocks were prepared from the rinse fluids of FNA biopsies of primary lung carcinoma (14 cases), as well as from pleural, peritoneal, or pericardial fluids containing tumors of various types (total 108 cases). Primary lung carcinomas included 8 adenocarcinomas, 5 squamous cell carcinomas, and 1 neuroendocrine carcinoma. All fluids (from effusions) were diagnosed as “positive for metastatic carcinoma” (93 cases), mesothelioma (14 cases), or metastatic melanoma (1 case). Biopsy-documented primary sites of metastatic tumors included lung (44 cases [adenocarcinoma, 39; neuroendocrine carcinoma, 1; small cell carcinoma, 2; and squamous...
cell carcinoma, breast, gastrointestinal tract, pancreas, endometrium, ovary, prostate, skin, melanoma, and mesothelioma. The body cavity fluid and FNA specimens were processed into cell blocks using thromboplastin and plasma. The cell blocks were fixed in 10% neutral buffered formalin and embedded in paraffin.

Immunoperoxidase studies for TTF-1 were performed manually on 5-µm paraffin sections of the cell block following heat-induced epitope retrieval. Preliminary studies (on 54 cases) demonstrated that antigen retrieval using a 0.001-mol/L concentration of EDTA, pH 8.0, provided superior results compared with a 0.01-mol/L concentration of citrate buffer, pH 6.0. Deparaffinized sections were placed in a container of preheated 0.001-mol/L EDTA solution at pH 8.0, heated for 50 minutes in a steamer (model HS80; Black & Decker, Shelton, CT), then cooled for 20 minutes, washed under running water, placed in distilled water, and then treated with methanolic peroxide (5 parts methanol to 1 part 3% hydrogen peroxide) for 20 minutes. Slides were washed under running water, placed in distilled water and then in a 0.05-mol/L concentration of tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.6. Slides were incubated for 1 hour with monoclonal antibody specific for TTF-1 (clone 8G7G3/1, DAKO, Carpinteria, CA) at a dilution of 1:200, then washed and incubated with horseradish peroxidase–labeled polymer conjugated to goat antimouse immunoglobulin antibodies (Envision+ detection system, K4007, DAKO). Antibody localization was effected using a peroxidase reaction with DAB+ (3,3′-diaminobenzidine tetrahydrochloride) as the chromogen (Envision+ detection system). Staining intensity was enhanced with DAB enhancer (Zymed Laboratories, San Francisco, CA). Slides were counterstained with methyl green solution, dehydrated, and coverslipped. Only nuclear staining was regarded as a positive result. Intensity of staining was graded on a 0 to 3+ scale. A negative control slide, substituting Tris buffer for the monoclonal antibody, was evaluated for all immunoreactive cases. A positive control slide of TTF-1–positive lung adenocarcinoma was included in each run.

For a subset of lung adenocarcinoma cases in which small numbers of immunoreactive cells were noted and/or staining intensity was not maximal, additional studies were performed using an alternative detection system that provided enhanced staining. Following antigen retrieval and methanolic-peroxide treatment, the slides were sequentially incubated with TTF-1 monoclonal antibody (1:3,000 dilution for 30 minutes), rabbit antimouse immunoglobulin antibody (1:150 dilution, 15 minutes; DAKO), and horseradish peroxidase–labeled polymer conjugated to goat antirabbit immunoglobulin antibodies (30 minutes; Envision+). Antibody localization was effected as previously described.

### Results

Immunoreactivity for TTF-1 was determined for neoplastic cells of various types in a total of 122 cases, which included cell blocks of FNA rinses of primary pulmonary neoplasms (14 cases), with the remainder (108 cases) representing cell blocks of pleural, peritoneal, or pericardial fluids. Results of these studies are summarized in **Table 1**. All cases interpreted as positive exhibited nuclear reactivity. Studies were readily interpretable since little to no background staining of histiocytes, mesothelial cells, or proteinaceous material was apparent. All control slides substituting Tris buffer for the primary antibody were negative.

All FNA samples (8/8) of primary lung adenocarcinomas were reactive for TTF-1, while similar preparations containing squamous cell carcinoma (5 cases) or neuroendocrine carcinoma (1 case) of lung were negative. Most body fluids containing metastatic pulmonary adenocarcinoma (34/39 cases [87%]) were reactive for TTF-1. The remaining 5 cases exhibited only cytoplasmic staining and were regarded as negative. In 1 case, both nuclear and cytoplasmic staining was noted. Overall results for primary and metastatic pulmonary adenocarcinomas demonstrated TTF-1 reactivity in 42 (89%) of 47 cases. Staining intensity was strong in most primary (2+ or 3+, 4 cases each) or metastatic pulmonary adenocarcinomas (1+, 1 case; 2+, 16 cases; 3+, 17 cases) **Table 2**. Generally, most tumor cells were reactive, although the...
**Image 1** Cell block sections of cases of lung adenocarcinoma (A, H&E, ×600) and mesothelioma (B, H&E, ×400). C, Low-power view of a case scored as 3+ to demonstrate the ease with which the slides can be interpreted given the low background (×200). Scoring system for thyroid transcription factor-1 staining (D, 1+; E, 2+; F, 3+; ×600, methyl green counterstain).
percentage of reactive cells varied. One of 7 cell blocks containing primary (5 cases) or metastatic (2 cases) squamous cell carcinoma of lung revealed weak staining (1+) for TTF-1 in 1 of the metastases. One of 3 fluids with metastatic small cell/neuroendocrine carcinoma of lung was strongly reactive (3+). All 14 mesotheliomas evaluated were TTF-1 negative.

For preparations containing metastatic adenocarcinoma from other sites (49 cases), weak reactivity (1+) of a small number of tumor cells was observed for only 1 specimen of ovarian adenocarcinoma. All cases of metastatic breast carcinoma were negative for TTF-1. One metastatic gastric tumor revealed cytoplasmic staining and was regarded as negative. In cases with metastatic carcinoma of breast, primary tumors were of the ductal type in 7 cases and lobular type in 1 case. Slides of the primary tumors were not available for review for the remaining 8 cases. A cell block containing metastatic melanoma was negative for TTF-1.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Staining Intensity for Thyroid Transcription Factor-1 in Cases of Primary and Metastatic Adenocarcinoma of Lung</th>
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<tr>
<td>Staining Intensity</td>
<td>Tumor Site</td>
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<td>Primary</td>
<td>8</td>
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<td>Metastatic</td>
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* Cytoplasmic staining only; interpreted as negative.

In 24 cases containing pulmonary adenocarcinoma in which staining intensity was not maximal and/or only small numbers of tumor cells were reactive, studies were repeated using an alternative 3-step detection system that provided enhanced staining. For 14 (88%) of 16 cases with 2+ staining, increased staining intensity was achieved by this method. Another 8 cases previously scored as 3+ did not show any darker staining. However, the majority of cases, even those with 3+ reactivity by the Envision+ system (mouse) revealed reactivity of more neoplastic cells. The level of nonspecific staining also increased using the enhanced detection system producing a blush of color over the blood, serum, mesothelial cells, and histiocytes. This was seen to some extent in 12 (50%) of 24 cases tested, including the 8 cases in which staining was initially strong. In addition, the enhanced method introduced additional cytoplasmic staining as part of this background in 2 cases. Since the final interpretation was not changed, and extremely clean preparations were obtained using the 2-step procedure (primary antibody followed by horseradish peroxidase–labeled polymer conjugated to goat antimouse immunoglobulin antibodies), the latter was regarded as the method of choice.

Based on these results, TTF-1 represents a highly selective marker for adenocarcinoma of lung origin and is a helpful diagnostic discriminant in the distinction between pulmonary adenocarcinoma and mesothelioma. Other primary neoplasms of lung such as small cell carcinoma also may be positive for TTF-1, although squamous cell carcinomas are typically nonreactive.
Discussion

TTF-1 is a tissue-specific transcription factor expressed in normal thyroid and lung, which has been shown to be commonly expressed in carcinomas of thyroid and lung origin. Bejarano et al., using a polyclonal antibody to TTF-1, reported expression in 35 (76%) of 46 tissue sections of lung adenocarcinomas, but in none of 48 nonpulmonary adenocarcinomas. In addition, Harlamert et al. showed TTF-1 to be useful for discriminating lung from breast adenocarcinoma on cytologic specimens prepared as cell blocks. In the latter study, lung adenocarcinomas showed nuclear reactivity for TTF-1 in 16 (76%) of 21 cases, and none of the breast carcinomas were reactive. We studied cytologic materials from a larger number of cases and included metastases from a broader range of primary sites. The sensitivity and specificity of TTF-1 staining for identifying malignant cells and identifying lung as the site of origin are 89% and 98%, respectively. The efficiency of our staining method is excellent, given the variability in tumor cell preservation in the cytologic preparations. Although the alternative 3-step method generally revealed reactivity of a greater number of cells and increased staining intensity, background staining was noted for some cases, and overall interpretation was not altered. Therefore, for routine studies, we have chosen to perform the 2-step technique using the mouse Envision+ detection system, which yields very clean preparations. One economic advantage of the 3-step method, however, is that the primary antibody can be used at a much higher dilution.

Several authors have identified TTF-1 reactivity in the majority of pulmonary and nonpulmonary small cell and large cell neuroendocrine carcinomas. In our limited sample of 5 small cell or neuroendocrine carcinomas metastatic to body fluids, only 1 case was reactive. This finding may reflect our limited sample or may be due to cellular degeneration, given the high rate of cell turnover and degree of background necrosis in these tumors.

TTF-1 staining in primary squamous cell carcinoma has been reported to be variable, ranging from 0% to 38%., Similarly in our cases of squamous cell carcinomas metastatic to body fluids, we could demonstrate TTF-1 staining in only a minority of cases (1/7 [14%]).

The distinction between malignant epithelioid mesothelioma and adenocarcinomas, particularly of lung origin, in body cavity fluids requires a panel of immunomarkers that typically includes antibodies reactive for adenocarcinoma or mesothelioma. Because TTF-1 is not expressed in normal mesothelium or in mesotheliomas, it has been used to distinguish epithelioid type mesotheliomas from lung carcinomas in pleural biopsies. Khoor et al. found reactivity in 158 (76.0%) of 208 adenocarcinomas and none of 95 malignant mesotheliomas using a monoclonal TTF-1 antibody. Di Loreto et al. reported reactivity in 19 (58%) of 33 lung adenocarcinomas, but in none of 24 mesothelioma tissue biopsies. Ordonez used a monoclonal antibody to show TTF-1 expression in 30 (75%) of 40 lung adenocarcinomas and none of 72 cases of mesothelioma in tissue biopsy specimens. Our studies demonstrate that TTF-1 represents an effective marker in this distinction as applied to cytologic (cell block) material. The sensitivity

Image A: A subset of lung adenocarcinoma cases that showed only small numbers of immunoreactive cells or weak staining intensity using the 2-step Envision+ method (DAKO, Carpinteria, CA) (A) were also evaluated using an alternative 3-step detection system (B) that provided enhanced reactivity but frequently yielded background staining (x400, methyl green counterstain).
and specificity of TTF-1 staining for detection of lung adenocarcinoma (distinguished from mesothelioma) are 89% and 100%, respectively.

Conclusions

TTF-1 is a sensitive and very highly selective marker of adenocarcinoma of pulmonary origin in cytologic preparations and also is effective in the distinction between malignant mesothelioma and pulmonary adenocarcinoma in pleural fluids. The methods described herein provide a reliable means of using this marker successfully as a diagnostic discriminant for routine evaluation of malignant cells in cell block preparations.

References

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