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RESEARCH

Expression of Thyroid Transcription Factor-1 in Malignant Pleural Effusions

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Abstract Separating adenocarcinoma of the lung from non-pulmonary adenocarcinoma or malignant mesothelioma is difficult, especially in cytology specimens. Consequently, it is important to identify markers that may facilitate this distinction. Thyroid transcription factor-1 (TTF-1) is a homeodomain containing transcription factor expressed selectively in the thyroid, lung, and diencephalon. TTF-1 is also expressed in adenocarcinomas of the lung and is widely used as a pulmonary adenocarcinoma marker in surgical specimens. However, the utility of TTF-1 has rarely been investigated in cytology. In this study, we evaluated the expression of TTF-1 in malignant pleural effusions. The primary tumors included 26 pulmonary adenocarcinomas, 26 non-pulmonary adenocarcinomas (13 breast, 5 ovarian, 2 gastric, 2 prostatic, 1 esophageal, 1 colonic, 1 pancreatic and 1 renal) and 4 malignant mesotheliomas. Immunocytochemistry was performed on sections of cell blocks, using a mouse monoclonal TTF-1 antibody (clone 8G7G3/1) and a biotin-streptavidin detection system. Nuclear immunoreactivity for TTF-1 was detected in 19 pulmonary adenocarcinomas. All non-pulmonary adenocarcinomas and malignant mesotheliomas were negative. These data indicate that TTF-1 maintains its sensitivity (73%) and specificity (100%) in cell block preparations and is useful in separating adenocarcinoma of the lung from non-pulmonary adenocarcinoma and malignant mesothelioma in cytology specimens.

Keywords TTF-1 · Cytology · Pleural effusions · Lung · Adenocarcinoma · Malignant mesothelioma

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Introduction

Malignant pleural effusions are relatively common complications of both intra- and extrathoracic malignancies. The most common primary sites and/or tumor types for both men and women are, in descending order of frequency, lung, lymphoma/leukemia, gastrointestinal tract, genitourinary tract, melanoma and malignant mesothelioma [1]. The type of lung cancer most frequently found in pleural effusions is adenocarcinoma [1]. Unfortunately, adenocarcinoma of the lung can rarely be distinguished from non-pulmonary adenocarcinoma or malignant mesothelioma on cytological grounds alone. The patient's clinical history or the radiographic findings may sometimes be helpful. However, some patients have a history of multiple primary tumors and a malignant pleural effusion can also be the first sign of malignancy [2]. In addition, adenocarcinoma of the lung may invade the pleura and mimic malignant mesothelioma clinically [3]. For these reasons, it is important to identify markers that can aid in separating adenocarcinoma of the lung from non-pulmonary adenocarcinoma and malignant mesothelioma in cytology specimens.

Thyroid transcription factor-1 (TTF-1), also known as Nkx2.1, T/EBP (thyroid-specific-enhancer-binding protein) or TITF1, is a homeodomain containing transcription factor expressed selectively in the thyroid, lung, and diencephalon

[4]. In the lung, TTF-1 has been localized to alveolar Type II cells, cells of the bronchioloalveolar portals and non-ciliated bronchiolar epithelial (Clara) cells [5], where it is responsible for transcriptional activation of surfactant proteins A, B, and C, and Clara cell secretory protein [6, 7].

Neoplasms of thyroid [8, 9] and lung origin often retain expression of TTF-1. Among lung tumors, immunoreactive TTF-1 has been detected in alveolar adenoma [10], sclerosing hemangioma [11], adenocarcinoma [12, 13], carcinoid tumor [14], large cell neuroendocrine carcinoma [14], and small cell carcinoma [12, 14, 15]. Expression of TTF-1 in adenocarcinoma of the lung is of particular interest. Several studies have shown that TTF-1 can help separate adenocarcinoma of the lung from non-pulmonary adenocarcinoma or malignant mesothelioma in surgical specimens [13, 16–18]. However, the usefulness of TTF-1 has rarely been evaluated in cytology specimens [19–21].

In the current study, we analyzed the expression of TTF-1 in malignant pleural effusions, using immunocytochemical methods. Our data provide further evidence that TTF-1 is a useful aid in distinguishing adenocarcinoma of the lung from non-pulmonary adenocarcinoma and malignant mesothelioma in cytology specimens.

Materials and Methods

Materials

The study was approved by the Institutional Review Board of the University of South Florida, Tampa, Florida. Three consecutive years of cytopathology files were searched for cases of malignant pleural effusions at the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida. A total of 56 cases (52 cases of metastatic adenocarcinoma and 4 cases of malignant mesothelioma) with known primary sites and available cell blocks were selected for the study. Twenty-one patients were male (38%) and 35 patients were female (62%). The primary sites for metastatic adenocarcinomas were confirmed by chart review and are listed in Table 1. For male patients, the primary sites included the lung (58%), gastrointestinal tract (26%), and genitourinary tract (16%). For female patients, the primary sites included the lung (46%), breast (39%), and ovary (15%).

Cell blocks were prepared by the plasma/thrombin technique [22]. Briefly, pleural fluid specimens were centrifuged at 2000 rpm for 5 min. The supernatant was decanted and equal drops of plasma and thrombin were added to the sediment. The clot was placed onto a small piece of tissue paper, which was folded and placed in a cassette. The specimen was then fixed in 10% phosphate-buffered formalin and was embedded in paraffin.

Table 1 Primary sites for metastatic adenocarcinomas in malignant pleural effusions

Primary site	Number of cases
Lung	26 (50%)
Breast	13 (25%)
Ovary	5 (9%)
Stomach	2 (4%)
Prostate	2 (4%)
Esophagus	1 (2%)
Colon	1 (2%)
Pancreas	1 (2%)
Kidney	1 (2%)
Total	52 (100%)

Immunocytochemistry

The mouse monoclonal anti-TTF-1 antibody (clone 8G7G3/1) was purchased from Dako Corporation (Carpinteria, CA). For immunohistochemistry, 4-μm sections were cut from each paraffin block. Deparaffinized sections were pretreated with a microwave antigen retrieval system (Biogenex, San Ramon, CA) according to the manufacturer's protocol. After pretreatment, sections were incubated with a 1:500 dilution of the TTF-1 antibody. Antigen-antibody complexes were then visualized by a biotin-streptavidin detection system (Vectastain, Burlingame, CA) with 3,3′-diaminobenzidene as the chromogen. Sections of a pulmonary adenocarcinoma known to be positive for TTF-1 were used as a positive control. Negative controls were prepared by substituting the TTF-1 antibody with nonspecific mouse immunoglobulin G.

Nuclear immunoreactivity was assessed by two pathologists (AK, ALBG) independently with joint review and resolution of discrepancies. Staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) and the percentage of immunoreactive neoplastic cells was scored as 0 (0%), 1 (1% to 9%), 2 (10% to 49%), and 3 (50% to 100%). The sum of the intensity and percentage scores was used as the final staining score for each tumor (0 to 6). For statistical analysis, tumors having a final staining score of ≥3 were considered to be positive.

Statistical Analysis

Sensitivity and specificity were calculated using standard statistical methods [23]

Results

Nuclear immunoreactivity for TTF-1 was detected in 19 (73%) of the 26 metastatic pulmonary adenocarcinomas (Fig. 1). All 19 tumors with nuclear immunoreactivity had a final staining score of ≥3 and were considered positive

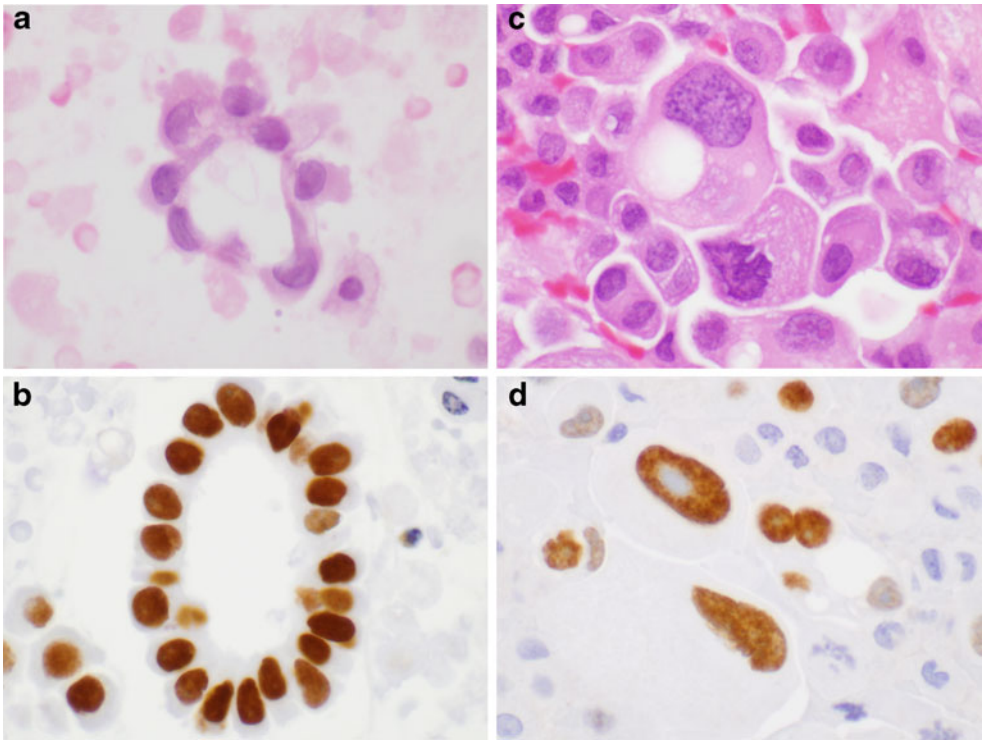

Fig. 1 TTF-1 expression in adenocarcinomas of lung origin in pleural effusions (cell block preparations). Low-grade pulmonary adenocarcinoma with hematoxylin and eosin stain (**a**) and nuclear immunoreactivity for TTF-1 (**b**). High-grade pulmonary adenocarcinoma with hematoxylin and eosin stain (**c**) and nuclear immunoreactivity for TTF-1 (**d**). Original magnification x600 (**a-d**)

(Table 2). Non-pulmonary adenocarcinomas and malignant mesotheliomas were uniformly devoid of any staining. The sensitivity of TTF-1 for adenocarcinoma of the lung was 73% and the specificity of TTF-1 for adenocarcinoma of the lung versus non-pulmonary adenocarcinoma and malignant mesothelioma were 100%.

Discussion

We analyzed the expression of TTF-1 in malignant pleural effusions caused by pulmonary and non-pulmonary adenocarcinomas and malignant mesotheliomas. Adenocarcinomas are known to be the largest group of malignant pleural effusions. In 1985, Johnston published a review of 584 consecutive malignant pleural effusions; adenocarcinomas comprised 47.4% of the cases [1]. In Johnston's study, the most frequent primary organ site among males was the lung, followed by the gastrointestinal and genitourinary tracts. Among female patients, the order of frequency was breast, female genital tract (usually ovary), lung, and gastrointestinal tract. In our study, the lung was the single most common primary site among not only men, but also women. The reason for this minor discrepancy is not entirely clear. It may be the consequence of local practice variations such as differences in populations served by the two medical centers. Alternatively, it may reflect recent increases in lung cancer among women [24].

Table 2 Thyroid transcription factor-1 (TTF-1) immunoreactivity in pulmonary and non-pulmonary adenocarcinoma and malignant mesothelioma

Tumor type	TTF-1 positive
Pulmonary adenocarcinoma (n=26)	19 (73%)
Non-pulmonary adenocarcinoma (n=26)	0
Malignant mesothelioma (n=4)	0

TTF-1 is a tissue-specific transcription factor required for branching morphogenesis and epithelial cell differentiation during lung development [6, 7]. In the mature lung, TTF-1 is expressed in alveolar Type II cells, cells of the bronchioloalveolar portals and Clara cells [5]. In these cell types, expression of TTF-1 parallels that of surfactant proteins A, B, and C, and Clara cell secretory protein, which are among its transcriptional targets [25–27]. TTF-1 is also expressed in the majority of adenocarcinomas of the lung with the expression linked to more favorable prognosis in some but not all studies [28–30]. Recent reports have suggested that amplification and resultant overexpression of the TTF-1 gene contribute to increased proliferation and survival of lung cancer cells [31, 32]. Therefore, TTF-1 is now considered as a lung cancer-specific oncogene [32].

The utility of TTF-1 as a pulmonary adenocarcinoma marker is well established in surgical pathology [13, 16–18]. On the other hand, the current report is one of the few studies that have evaluated the role of TTF-1 in the field of cytology [19–21]. In the various surgical pathology studies,

TTF-1 immunoreactivity has been detected in 58%–76% of pulmonary adenocarcinomas [13, 16, 33–35]. TTF-1 expression has been observed in adenocarcinomas with acinar, papillary, bronchioloalveolar, and solid growth patterns [13]. Results of a recent study have indicated that papillary adenocarcinoma shows the strongest correlation with TTF-1 expression [36]. Conversely, mucinous bronchioloalveolar carcinoma shows low expression of TTF-1 [37]. In previous cytology studies, immunoreactive TTF-1 has been detected in 79–89% of pulmonary adenocarcinomas [19–21]. These reports do not offer any explanation for the higher expression of TTF-1 in cytology versus surgical samples. In the current study, 73% of pulmonary adenocarcinomas expressed immunoreactive TTF-1. Our results seem to be more in line with results of the surgical pathology studies and are more likely to be reproducible in routine cytology practice.

In the context of separating adenocarcinomas of the lung from non-pulmonary adenocarcinomas and malignant mesotheliomas in surgical specimens, TTF-1 has demonstrated outstanding specificity for lung primaries. With the exception of carcinomas of thyroid origin, TTF-1 immunoreactivity has rarely been detected in non-pulmonary adenocarcinomas [16, 38, 39]. Bejarano et al. have observed focal TTF-1 staining in 1 of 66 gastric and 1 of 8 endometrial adenocarcinomas [16]. More recently, TTF-1 immunoreactivity has been detected in occasional ovarian carcinomas [38, 39]. All malignant mesotheliomas studied thus far have been negative for TTF-1 [13, 33, 35]. As far as publications in the field of cytology are concerned, Hecht et al. have noted a single case of metastatic ovarian carcinoma with focal weak TTF-1 immunoreactivity in 50 metastatic carcinomas of non-pulmonary origin [20]. No TTF-1 staining has been observed in non-pulmonary adenocarcinomas or malignant mesotheliomas by Afify et al. and Ng et al. [19, 21]. Likewise, no TTF-1 immunoreactivity was detected in non-pulmonary adenocarcinomas or malignant mesotheliomas in our study. These findings suggest that TTF-1 is highly specific for adenocarcinomas of the lung not only in surgical specimens, but also in cytology preparations.

In summary, we analyzed the expression of TTF-1 in malignant pleural effusions. Immunoreactivity was detected in 73% of pulmonary adenocarcinomas, whereas non-pulmonary adenocarcinomas and malignant mesotheliomas were uniformly negative. These data validate the use of TTF-1 as a pulmonary adenocarcinoma marker in cell block preparations.

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