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The Role of TTF-1 in Differentiating Primary and Metastatic Lung Adenocarcinomas

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Thyroid transcription factor-1 (TTF-1) is a sensitive marker for pulmonary and thyroid adenocarcinomas. The aim of this work was to determine its usefulness in distinction between primary and metastatic lung adenocarcinomas. We have examined the expression of TTF-1 in 100 solitary pulmonary nodules. They included 50 stage I peripheral primary bronchial adenocarcinomas (30 men, 20 women, mean age: 60 years) and 50 metastatic pulmonary adenocarcinomas (21 men, 29 women, mean age: 57 years) of different origins, such as breast (13), colon (13), rectum (13), kidney (7), stomach (2), and thyroid gland (2). TTF-1 immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues. In primary bronchial adenocarcinomas we found immunopositivity in 46/50 cases, among them 30 cases showed strong nuclear immunostaining. In four primary adenocarcinoma cases the observed immunopositivity was localized to the cytoplasm. Out of the metastatic adenocarcinomas all but the 2 thyroid cancers were negative. Both thyroid tumors showed strong immunopositivity. Our results confirm that TTF-1 immunohistochemistry is a very sensitive and highly specific method in the differential diagnosis of primary and metastatic lung adenocarcinomas and should be used in the everyday clinical practice. (Pathology Oncology Research Vol 10, No 2, 85–88)

Keywords: TTF-1, immunohistochemistry, lung adenocarcinoma, metastasis

Introduction

Lung cancer is the leading cause of cancer death worldwide. Adenocarcinoma accounts for about 30% of lung cancers and shows a steadily increasing incidence, especially among women. Bronchial adenocarcinoma presenting as solitary pulmonary nodule often results in differential diagnostic problems with pulmonary metastasis from extrapulmonary adenocarcinomas such as of breast, gastric, colon or renal origin.

TTF-1 or thyroid transcription factor-1 is a tissue-specific homeodomain-containing transcription factor that plays an important role in the early differentiation and morphogenesis of the developing lung and thyroid gland. For example, in TTF-1$^{-/-}$ knockout mouse tracheoesophageal fistula and severe pulmonary hypoplasia develop. It regulates also the expression of surfactant apoproteins (A, B, C) and Clara cell antigen. During lung development, TTF-1 expression in terminal and respiratory cells is consistent from fetal through adult stages. In adults it is almost exclusively expressed in thyroid and pulmonary epithelial cells.

The expression of TTF-1 has also been found in malignant tumors highly selectively in lung and thyroid cancers. In lung cancer high frequency of TTF-1 expression has been observed in small cell carcinomas (85–90%) and in adenocarcinoma (75–80%), whereas squamous cell cancers and large cell carcinomas showed no expression, or at very low frequency.

The distinction between primary and metastatic lung adenocarcinoma could be difficult by routine histology. As primary bronchial adenocarcinomas often develop in the periphery of the lung, similarly to solitary pulmonary nodules caused by metastatic lung tumors, the difference in TTF-1 expression might be of clinical importance in distinguishing their primary or metastatic origin.

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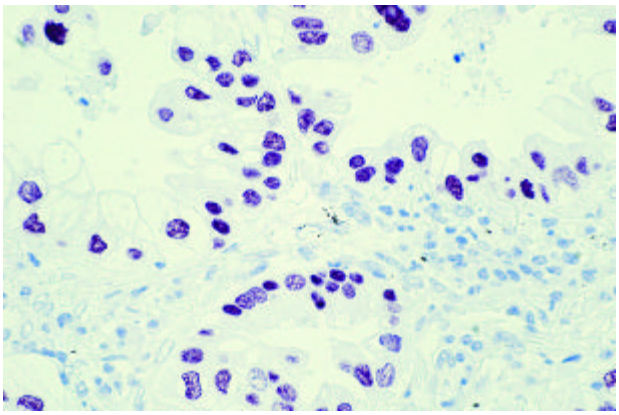

Figure 1. TTF-1 positive lung adenocarcinoma (x 500)

Materials and methods

50 primary peripheral bronchial adenocarcinomas of pathologic stage I, including 5 bronchiolo-alveolr carcinomas (30 men, 20 women, mean age: 60 years), and 50 metastatic lung adenocarcinomas (21 men, 29 women, mean age: 57 years) were studied. All tumors were surgical resection specimens, and were formalin-fixed and paraffin-embedded tissues. The primary lung adenocarcinomas were classified histologically according to the World Health Organization's criteria. Patients with mixed tumors, poorly differentiated or anaplastic tumors as well as those with more than one known primary tumor were excluded from the study. In the group of metastatic adenocarcinomas the primary sites were as follows: breast (13), colon (13), rectum (13), kidney (7), stomach (2), and thyroid gland (2).

4 µm tissue sections were deparaffinized in xylene and rehydrated. Immunohistochemical examination was performed using standard avidin-biotin-peroxidase complex method. Endogenous peroxidase was inhibited in 1% H_2O_2-methanol for 30 minutes at 37°C, and antigen retrieval was achieved by using 1% Vector (H3300) antigen retrieval solution and microwave heating (800 W for 20 minutes). The sections were incubated for 1 hour at room temperature with anti-TTF-1 monoclonal antibody (DAKO M3575) diluted 1:50. The antigen-antibody complexes were visualized using Streptavidin-HRP detection system (DAKO LSAB2, K0675) with either 3-amino-9-aethylcarbazole (AEC, HK 129-5K Biogenex) or 3,3'–diaminobenzidine tetrahydrochloride (DAB) as chromogen. All sections were then counterstained with Mayer's hemalaun, dehydrated, and coversliped. Type II pneumocytes served as positive internal controls and stained appropriately. Tumors were graded as negative (5%), weak positive (5–30%), and strong positive (30%), based on the percentage of positively stained tumor cells.

Results

In normal lung tissue intense nuclear staining was observed in type II pneumocytes as well as in Clara cells. In primary bronchial adenocarcinomas we found immunopositivity in 46/50 cases, among them 30 cases showed strong nuclear immunostaining (*Figure 1*). In four primary adenocarcinoma cases – among them two were bronchiolo-alveolar carcinomas – the observed immunopositivity was localized to the cytoplasm (*Table 1*).

In the group of metastatic adenocarcinomas all tumors except for carcinomas of thyroid gland origin were negative for TTF-1 (*Table 2*). In both thyroid cancer tissues an intense nuclear immunostaining was observed diffusely.

Discussion

TTF-1 is a tissue-specific transcription factor expressed in normal lung cells of terminal respiratory unit, such as type I and type II pneumocytes and small-sized bronchioles.[14,16] It has also been found in benign lung tumors, e.g. in sclerosing hemangioma and alveolar adenoma; both are suggested to derive from type II pneumocytes. In malignant lung tumors the level of TTF-1 expression varies extensively. It is controversial in pulmonary carcinoids, as in one study the absence of TTF-1 expression led to the hypothesis that these tumors could derive from a different stem cell line than small cell lung cancers,[23] in contrast with another observation, when immunopositivity was found in 69% of bronchial carcinoids with very high specificity.[4] Yatabe speculated that high percentage of TTF-1 expression in

Table 1. **TTF-1 expression in primary lung adenocarcinomas (n = 50)**

	TTF-1			
	Cases studied	–	+	++
Lung adenocarcinoma	50	4	16 (4*)	30

* cytoplasmic staining

Table 2. **TTF-1 expression in metastatic adenocarcinomas (n=50)**

	TTF-1			
	Cases studied	–	+	++
Breast	13	13	–	–
Colon	13	13	–	–
Rectum	13	13	–	–
Kidney	7	7	–	–
Stomach	2	2	–	–
Thyroid gland	2	–	–	2

small cell lung cancer (SCLC) might be an atavistic feature – dated from TTF-1 function during lung development – as it is expressed only in neuroendocrine tumors of pulmonary origin and not in extrapulmonary ones.[22] Contrarily, Ordonez has observed TTF-1 positivity also in extrapulmonary small cell carcinoma, such as of GI, urinary bladder and uterine cervix origin.[18] TTF-1 has been highly selectively found in lung and thyroid cancers, however, medullary thyroid cancers known to possess neuroendocrin differentiation was found TTF-1 negative.[23] Sturm reported 100% specificity of TTF-1 for large cell neuroendocrine carcinoma.[20] When combined with p63 immunohistochemistry TTF-1 was found to be helpful in the differential diagnosis of SCLC (TTF-1^+/$p63^-$) and undifferentiated squamous cell carcinoma (TTF-1^-/$p63^+$).[21]

Distinguishing primary adenocarcinoma (ADC) from metastatic ADC in the lung is often a challenging task, especially when the tumor appears as solitary pulmonary nodule. In primary lung ADC most authors describe TTF-1 immunopositivity higher than 75%, however, within this group important differences could be observed.[11] Goldstein has studied 40 bronchiolo-alveolar carcinomas and observed frequent (92%) and strong TTF-1 expression in nonmucinous tumors, whereas it was weak, and was found only in 21% of the mucinous counterpart.[8,13] TTF-1 immunohistochemistry was also found to be very specific in distinguishing primary signet-ring cell carcinoma of the lung and of extrapulmonary origin, such as breast, stomach, and colon.[5,15] In primary and metastatic lung ADC the combination of TTF-1 with antibody against surfactant apoprotein A did not increase the sensitivity of the investigations over the use of TTF-1 alone.[23] In lung ADC the TTF-1 expression was observed not to be affected by the differentiation state. In the study of Yatabe et al TTF-1 positivity was more often found in females, non-smokers, and related significantly with negative p53 staining, less frequent Rb loss, and preserved p27 expression.[22] These findings led to the hypothesis that molecular pathogenesis of TTF-1 positive ADC differs from TTF-1 negative ADC. TTF-1 staining was found to be very reliable in distinguishing brain metastasis from pulmonary or extrapulmonary site especially when dealing with adenocarcinomas and large-cell carcinomas.[19]

TTF-1 expression was observed as a highly sensitive and specific immunomarker for distinguishing pulmonary and extrapulmonary adenocarcinomas also in malignant pleural effusion fluid.[2,9,12,17] TTF-1 immunopositivity is characteristic for lung adenocarcinomas and thyroid cancers, but as the latter is rarely metastasize to the serosal surfaces, therefore TTF1 is an important marker for malignant pleural fluid of pulmonary origin. Besides, in case of TTF-1 positivity – in addition to the clinical picture – the use of the highly sensitive and specific thyroglobulin immunostaining could be helpful in discriminating thyroid carcinomas from primary lung cancer.

In many investigations the specificity of TTF-1 for pulmonary ADC versus mesothelioma was found to be 100%.[1]

Recently, strong expression of TTF-1 was found to predict better survival in non-small cell lung cancer, moreover, it was observed together with decreased VEGF-C, neuroendocrine gene, plasminogen activator urokinase receptor and cathepsin L.[6,10]

In our study four out of fifty primary lung adenocarcinoma showed immunopositivity that was localized to the cytoplasm. The significance of this observation is still questionable. Fujita investigated six human lung adenocarcinoma cell lines, and out of those six five showed cytoplasmic staining.[7] Bejarano analyzed the diagnostic value of this pattern of immunoreactivity. He found that from 361 tumors of 29 different organ sites 6,3 % presented with cytoplasmic staining. It was also observed in non-neoplastic liver tissue, therefore he concluded that cytoplasmic TTF-1 staining is a nonspecific finding and should be disregarded for diagnostic purposes.[3]

In the present work we have studied the expression of TTF-1 in 50 primary and 50 metastatic lung adenocarcinomas. We found that TTF-1 expression is very valuable in distinguishing primary lung adenocarcinomas from those arising in different organs. Our results confirm that TTF-1 immunohistochemistry is a very sensitive and highly specific method and should be used in the everyday clinical practice.

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