CASE SERIES

Thyroid transcription factor-1 expression in invasive and non-invasive urothelial carcinomas

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Abstract

Background: Thyroid transcription factor-1 (TTF-1) has been considered a sensitive marker for thyroid and lung tumors. Recent data have shown that a wide range of neoplasms may express TTF-1.

Case series: We performed an immunohistochemical study in a case series of 42 urothelial carcinomas (UCs) on tissue microarrays sections, in order to investigate how often UCs express the TTF-1 protein and the diagnostic utility of this marker. In addition, we sought to determine by immunohistochemistry if there is an association between TTF-1 expression and the expression of specific basal-like or luminal markers. Five out of the 42 cases (11.9 %) were positive for TTF-1. Three positive tumors concerned non-invasive papillary UCs. There was no association between TTF-1 expression and tumor grade (χ^2, p =0.419), stage (χ^2, p =0.550) or cytokeratin 5/6 (χ^2, p =0.330), cytokeratin 20 (χ^2, p =0.995) and estrogen receptors expression (χ^2, p =0.268).

Conclusions: UCs may show TTF-1 expression and pathologists should be aware of this phenomenon in order to avoid misdiagnosis, notably in metastatic disease. HIPPOKRATIA 2017, 21(3): 154-157.

Keywords: Thyroid transcription factor-1, urothelial neoplasm, immunohistochemistry, metastatic lung tumor, estrogen receptors, cytokeratin 5/6, cytokeratin 20

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Introduction

Thyroid transcription factor-1 (TTF-1) is a 38 kDa DNA-binding protein encoding expressed primarily in follicular cells and C-cells of the thyroid gland, Clara cells and pneumocytes type II of the lung and in some brain areas[1,2]. In the lung, TTF-1 operates as a promoter factor for the transcription of Clara cell secretory protein and surfactant proteins A, B, and C. In the thyroid gland, it acts as an activator of thyroglobulin, thyroperoxidase, and thyrotropin receptor genes[1,2].

TTF-1 expression in immunohistochemical studies is widely used as a marker for lung and thyroid gland tumors[1,3-5]. This is particularly important in the differential diagnosis between primary and secondary (metastatic) lung tumors since the lung is one of the most common sites of metastasis. Although TTF-1 has been considered a sensitive marker for lung tumors, especially for adenocarcinomas and small cell carcinomas, recent data supports that TTF-1 expression can be observed in a wide range of neoplasms[1]. In the literature, there is little evidence of its expression in urothelial carcinomas (UCs)[4,6].

Recently, we have encountered a case of a metastatic UC positive for TTF-1. A 64-year-old man with a previously diagnosed UC of the bladder presented with widespread lung and bone metastases. Palliative treatment included surgical removal of a thoracic wall metastasis and the histologic examination confirmed the presence of a high-grade carcinoma. The clinicopathological correlation was suggestive of metastatic urothelial carcinoma, although the observed TTF-1 immunoreactivity. This case prompted us to further investigate the frequency of TTF-1 positivity in randomly selected primary UC cases, and the possible correlation between this expression and several clinicopathological parameters or immunohistochemical expression of basal and luminal markers.

Case series

Formalin-fixed paraffin-embedded tissue samples

from 40 patients with primary urothelial carcinomas of the bladder, the ureter, and the renal pelvis were randomly selected. Tissue microarrays (TMA) blocks were constructed with a manual arrayer (Model I, Beecher Instruments, Sun Prairie, WI, USA). Each tumor was represented by three tissue cores, 1.5 mm in diameter. One case of an infiltrating urothelial carcinoma, high grade of the bladder has been tested twice, on primary tumor and on a recurrence that presented one year later. Two more cases of urothelial carcinomas were also included in the study and were tested in conventional slides. The first concerned a case of a non-invasive papillary urothelial carcinoma, low grade of the renal pelvis, and the second was the above mentioned metastatic to lung, urothelial carcinoma. Table 1 summarizes the histologic diagnosis, the grade and the location of the tumors included in the study. TMA also contained cores from placenta, tonsil, breast, renal, and colon tissue, as negative control markers, whereas cores from normal thyroid tissue were included to serve as positive control. These tissues helped for orientation during microscopy as well.

Immunohistochemistry for TTF-1 (clone SPT24 Novocastra, Newcastle Upon Tyne, UK), estrogen receptors (ER)(clone 6 F11, Leica Biosystems, Newcastle Upon Tyne, UK), cytokeratin (CK) 20 (clone Ks 20.8, DakoCytomation, Glostrup, Denmark) and CK5/6 (clone D5/16B4, DakoCytomation, Glostrup, Denmark) antigens was performed on four-micrometer unstained sections from TMAs and conventional slides. All the immunostains were performed on 4 micron thick sections, using the Bond Max and Bond III autostainers (Leica Microsystems, Wetzlar, Germany). Specifically, for TTF-1 four-micrometer deparaffinized and rehydrated in xylene and alcohol sections from TMAs and conventional slides were used. Heat-induced epitope retrieval (HIER) was performed using ethylenediaminetetraacetic acid (EDTA) buffer at pH 9.0 for 20 minutes. Endogenous peroxidases were inactivated by treatment with "Peroxidase block" (BOND Polymer Refine Detection Kit; Bond™, Newcastle, Upon Tyne, UK) for ten minutes. Immunostaining was performed at Leica BOND-MAX autostainer using the monoclonal antibody to TTF-1, clone SPT24 (dilution 1:80, Novocastra™, Newcastle, Upon Tyne, UK) with an incubation time of 20 minutes. Incubation in "Post Primary Block" and in "NovoLink Polymer" (secondary antibody [serum] for the primary antibody) was followed with an incubation time of 30 minutes for each step (BOND Polymer Refine Detection Kit). DAB chromogen substrates were applied for eight minutes (BOND Polymer Refine Detection Kit). Mayer's Hematoxylin was used as a counter-stain.

The immunohistochemical stains for TTF-1, CK5, CK20 were scored as positive if the neoplastic cells were immunoreacted in a percentage of >5 % of the cells, regardless of the intensity of positivity. TTF-1 expression in the vast majority of the neoplastic cells (≥80 % of the cells), as it is usually observed in lung adenocarcinoma, was characterized as high. ER positivity evaluated according to the established criteria[7].

Statistical analysis was performed using the IBM SPSS Statistics for Windows (IBM SPSS, IBM Corp., Armonk, NY, USA) version 20.0 and p values were calculated using chi-squared test. Statistical significance was set at $p < 0.05$.

Out of 42 cases of urothelial carcinomas studied, five were positive for TTF-1 (11.9 %) (Table 1). Those five cases concerned two cases of non-invasive papillary urothelial carcinomas, low grade (Figure 1A, B), one case of non-invasive papillary urothelial carcinoma, high grade (Figure 1C), a case of infiltrating urothelial carcinoma, high grade and the metastatic one. Both the specimens from the primary tumor and the recurrence of the infiltrating, high grade, urothelial carcinoma (Figure 1D, E) expressed TTF-1. Of note, the metastatic urothelial carcinoma showed a high TTF-1 expression (positive cells >80 %) (Figure 1F), as usually is observed in lung or thyroid carcinomas. It is mentioned that heterogeneity in the expression between the three cores from the same tumor was observed. Two cases showed very weak immunoreactivity in a few scattered cells (positive cells <5 %) and they were consequently considered negative. There was no statistical association between TTF-1 expression and patients' gender, tumor size (χ^2, $p = 0.679$), grade (χ^2, $p = 0.419$), infiltrating (χ^2, $p = 0.845$), multifocal status (χ^2, $p = 0.774$), and stage (χ^2, $p = 0.550$). CK5/6, CK20, and ER were evaluated in cases with adequate material and were found positive in a percentage of 50 % (14/29), 41.9 % (13/31), and 14.3 % (5/34), respectively. No association was found between TTF-1 and CK5/6 (χ^2, $p = 0.330$), CK20 (χ^2, $p = 0.995$) or ER expression (χ^2, $p = 0.268$). However, it is emphasized that these results may be biased due to the small number of cases included in the

Table 1: Pathological characteristics of the tumors included in the case series and thyroid transcription factor-1 (TTF-1) expression.

Location	**TTF1 positive tumors**				**Positive/ Total**
	Non-invasive papillary urothelial carcinoma		**Infiltrating urothelial carcinoma**		
	Low grade	High grade	Low grade	High grade	
Bladder	2/16	1/11	-	1/10	4/37
Ureter	0/1	-	0/1	-	0/2
Renal Pelvis	0/1	-	0/1	-	0/2
Metastatic bladder carcinoma	-	-	-	1/1	1/1
Positive/Total	2/18	1/11	0/2	2/11	5/42

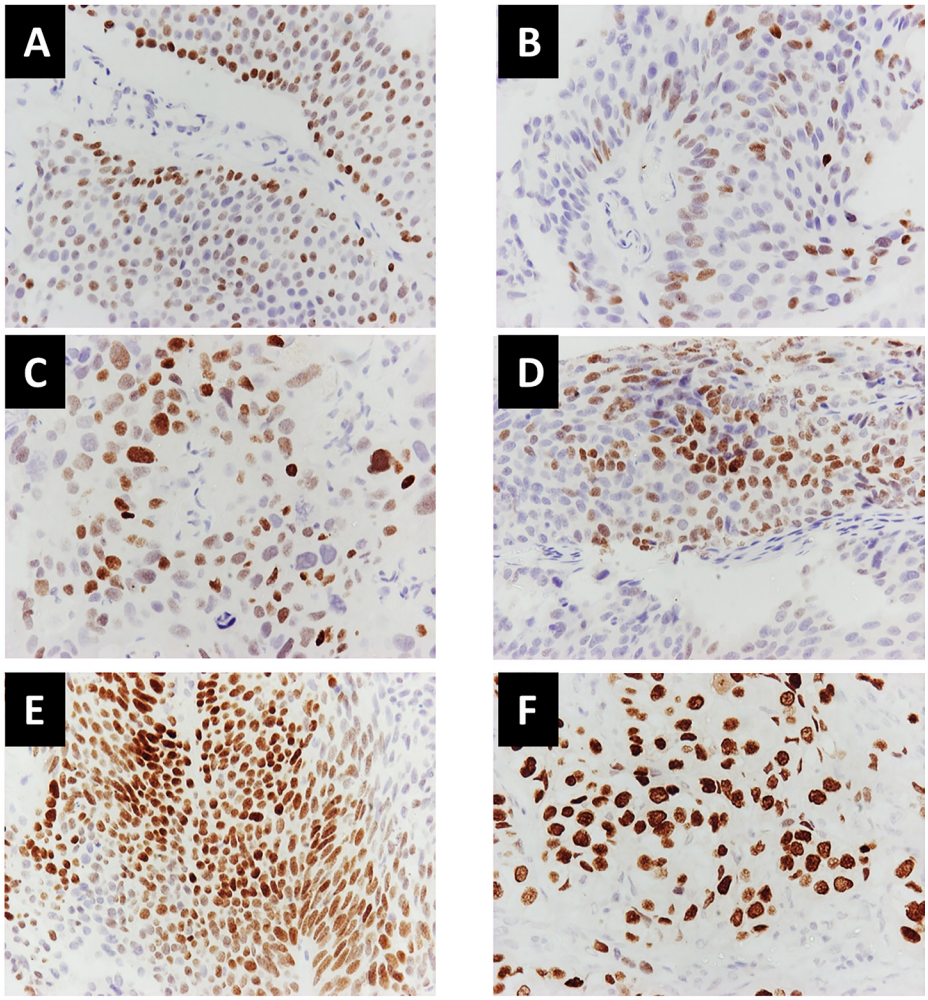

Figure 1: Thyroid transcription factor-1 (TTF-1) expression in urothelial carcinomas. Non-invasive papillary urothelial carcinomas, low grade (A, B) and high grade (C), with weak to moderate and focally dense immunoreactivity for TTF-1. Infiltrating urothelial carcinoma with weak to moderate and focally dense immunoreactivity for TTF-1 on the primary tumor (D) and on the recurrence (E). Metastatic urothelial carcinoma with dense and diffuse immunoreactivity for TTF-1 (F) (all specimens: Immunohistochemistry, x 400).

study. Only one TTF-1 positive tumor was CK5/6 positive. The majority of ER-negative UCs was TTF-1 negative as well. Of note, all ER-positive cases were of high grade.

Discussion

A considerable number of studies have shown that various types of tumors of other sites, besides lung and thyroid gland, also express TTF-1[4,8-13]. To the best of our knowledge, two case series in the English literature have reported in total six cases of infiltrating urothelial carcinomas of the bladder with TTF-1 expression[4,6] (Table 2). In the first study, the researchers compared the sensitivity in detecting pulmonary and extrapulmonary tumors of the two most commonly used clones, namely 8G7G3/1 and SPT24[4]. Both clones detected five positive tumors in a total number of 98 cases of infiltrating urothelial carcinomas of the bladder (5.1 %). In the second study, 30 cases of infiltrating urothelial carcinomas of the bladder were tested and only one of them expressed TTF-1 (3.3

Table 2: Studies in the literature that investigated thyroid transcription factor-1 (TTF-1) expression in urothelial carcinomas.

Authors (year)	Antibody clone	TTF-1 positivity (%)
Matoso et al (2010)[4]	8G7G3/1 and SPT24	5/98 (5.1)*
Fernández-Aceñero et al (2011)[6]	8G7G3/1	1/30 (3.3)
Kaufmann et al (2000)[3]	8G7G3/1	0/11 (0)
Current study (2016)	SPT24	5/42 (11.9)

TTF-1: thyroid transcription factor-1, *: same results for both clones.

%), using 8G7G3/1 clone[6] (Table 2). A third study failed to find any TTF-1 positive urothelial carcinoma in a total number of 11 cases, using 8G7G3/1 clone[3] (Table 2). Previous studies have reported that the SPT24 clone is more sensitive compared to 8G7G3/1 clone in detecting both extrapulmonary and pulmonary tumors[8,10]. The only study which compared TTF-1 expression in urothelial carcinomas with both clones, failed to find any difference[4] and the authors argued that previously reported differences may be due to technical parameters[4].

CK5/6 is considered a protein of squamous and basal cell differentiation. In normal urothelium, CK5-expressing basal cells can differentiate into CK20-expressing superficial umbrella cells[14]. These markers along with estrogen receptors (ER) were included in previous studies that tried to distinguish basal-like and luminal bladder carcinoma subtypes[15]. In this study, immunohistochemistry was used to define surrogate molecular subtypes. The TTF-1 expression does not seem to be a characteristic of a particular subtype, emphasizing however that this result may be biased due to the limited number of TTF-1 positive cases.

In conclusion, TTF-1 should not be considered a specific marker for lung and/or thyroid tumors. Although immunostaining against TTF-1 is a useful tool for the distinction between primary and secondary (metastatic) lung tumors, the TTF-1 expression should be interpreted very cautiously, since other malignant tumors (e.g., UC) may express TTF-1 in their primary and metastatic sites. The frequency and the epidemiological, clinical and pathological characteristics of TTF-1 positive urothelial carcinomas need further investigation.

Conflict of interest

Authors declare no conflict of interest.

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