

CASE REPORT

Aberrant TTF-1 expression in papillary high-grade urothelial neoplasm: case report and literature review

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Abstract

We herein report the case of a 48-year-old man who developed synchronous advanced tumors in the lung and the bladder. The most striking feature of our case is that the otherwise typical bladder urothelial carcinoma showed focal areas (comprising less than 5% of the tumor mass) of nuclear positivity for TTF-1 (thyroid transcription factor-1). The different pattern of cytokeratin expression led us to consider them two independent primary tumors. Several recent reports have indicated that the type of clone used can influence the results of TTF-1 staining and can explain positivity in extrapulmonary and extrathyroid tumors.

Keywords: thyroid transcription factor-1, bladder carcinoma, lung adenocarcinoma.

□ Introduction

Thyroid transcription factor-1 (TTF-1) is a 38-kd homeodomain containing transcription factor expressed in the thyroid, in the lung (type II pneumocytes and Clara cells) and in some brain areas [1]. TTF-1 expression has been shown in pulmonary small cell carcinoma and adenocarcinoma, but not in squamous cell or neuroendocrine tumors [2] and it has been proposed as a useful marker to delineate the origin of metastatic adenocarcinomas [3]. Expression of TTF-1 has been described in small cell carcinomas of some locations, including the bladder [4–6], but has never been shown in Merkel cell carcinoma; another small cell tumor affecting the skin and that renders diagnostic difficulties with metastatic lesions from internal organs to the skin [7]. To our knowledge, this report is the first to describe the expression of TTF-1 in an urothelial cell carcinoma of classical type.

□ Patient, Methods and Results

We herein report the case of a 48-year-old man who consulted on asymptomatic hematuria. A urinary cytology disclosed an urothelial carcinoma with large atypical cells both isolated and in small papillary clusters (Figure 1).

During the preoperative assessment prior to the transurethral resection (TUR), the plain thoracic X-ray disclosed a suspicious nodule in the upper lobe of the left lung, which was later confirmed with a CT-scan (Figure 2).

Bronchoscopy confirmed the existence of a mass occluding the left upper lobe bronchus and the biopsy showed a large cell tumor suggestive of adenocarcinoma (Figure 3A) with the characteristic immunohisto-

chemical profile showing intense nuclear positivity in 80% of the tumor cells for TTF-1 (Figure 4A) and intense cytoplasmic cytokeratin 7 positivity in almost 100% of the cell.

Cytokeratin 20 was negative (Figure 5A). The bronchoscopy sample was small, but the tumor seemed well differentiated, despite high cytological grade. We found neither necrosis nor vascular invasion. The mitotic count in the samples was low (1–2/10 HPF).

During the assessment, the patient developed a pericardial effusion and the cytology of the pericardial fluid revealed involvement by adenocarcinoma. With a diagnosis of stage IV, lung carcinoma the patient was not considered amenable to surgery and was initially a candidate to palliative chemotherapy. However, before initiation of therapy, the urologists performed a TUR of the bladder mass to determine its nature.

The histological analysis of the samples revealed a papillary and solid classical high-grade urothelial cell carcinoma infiltrating the bladder smooth muscle layer (pT2 tumor) (Figure 3B). We found no vascular or lymphatic invasion and the mitotic count was 6/10 HPF.

Due to the history of the patient, we performed an immunohistochemical analysis of the bladder lesion and found intense positivity for both cytokeratin 7 and 20 in 50 and 75% of the tumor cells, respectively (Figure 5B), characteristic of bladder carcinoma.

To our surprise, we also found a focal, albeit intense nuclear reaction with TTF-1 in 5% of the tumor cells (Figure 3B).

As this positivity was less extensive than in the lung tumor and the cytokeratin profile was also different, tumors were considered separate lesions.

The patient was started on 285 mg Carboplatin and

4350 mg Gemcitabine, with a quite acceptable response and good tolerance.

The lung disease relapsed 5 months and the bladder tumor 12 months after diagnosis.

The bladder disease was excised again with a TUR and he was started on second line chemotherapy with

taxanes and today he is alive with lung disease 18 months after initial diagnosis.

For the morphological study, we used the classic histology technique of paraffin enclosure. Next, the prepared materials were stained with Hematoxylin–Eosin.

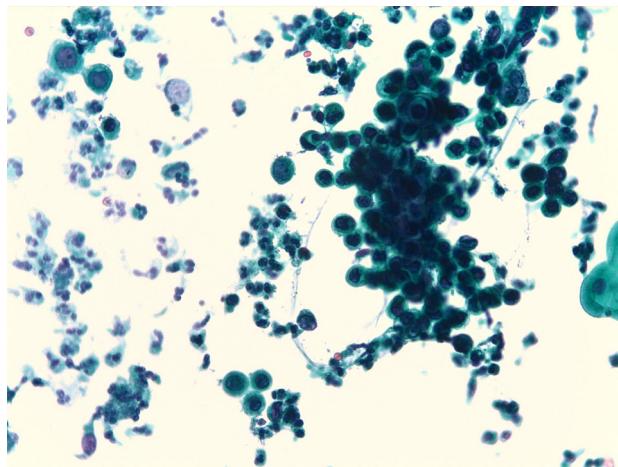


Figure 1 – Urinary cytology showing atypical urothelial cells with occasional papillary growths (Papanicolaou stain, $\times 200$).

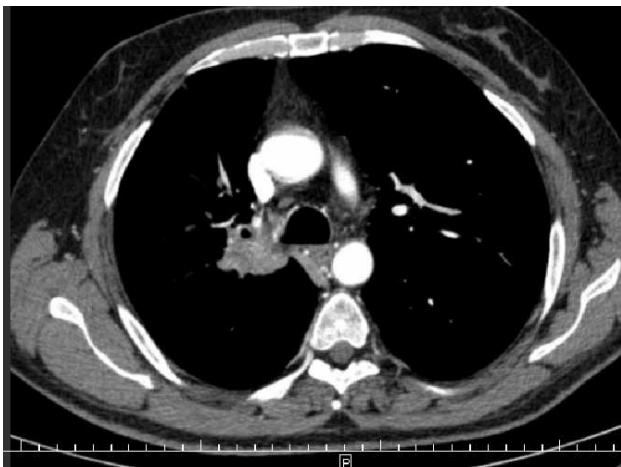
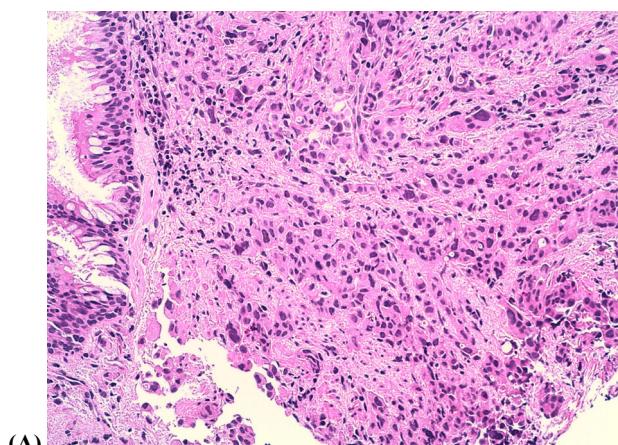
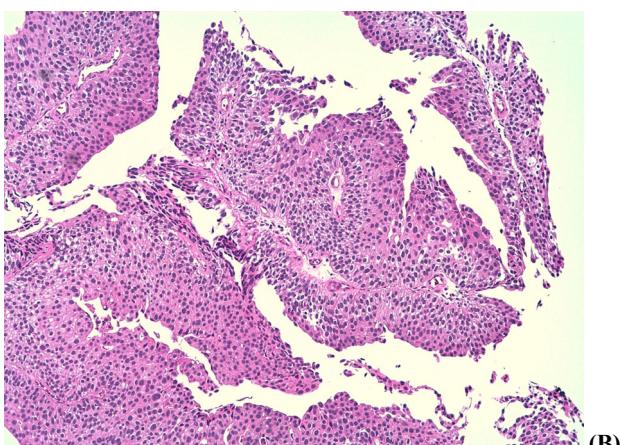


Figure 2 – The CT scan confirms the nodule in the left lung upper lobe.

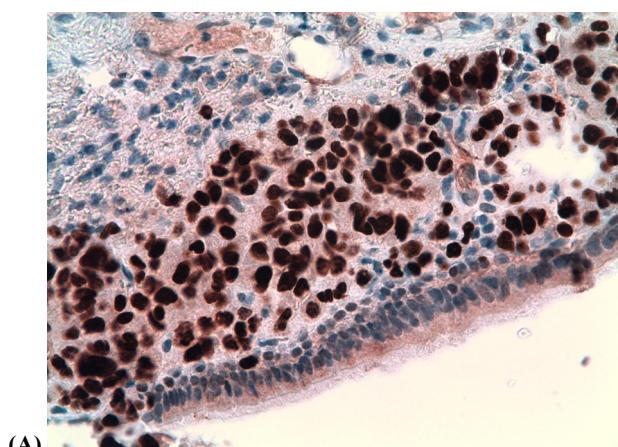


(A)

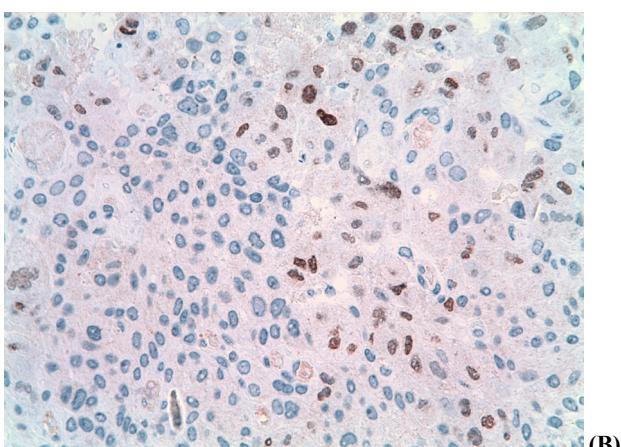


(B)

Figure 3 – (A) Medium power view of the lung biopsy, showing a diffuse growth of large cells with nuclear pleomorphism, showing some features suggestive of adenocarcinoma ($\times 200$); (B) Papillary urothelial cell carcinoma ($\times 100$). Hematoxylin–Eosin stain.



(A)



(B)

Figure 4 – (A) TTF-1 positivity in the lung tumor. Note positivity in the normal pseudostratified epithelium overlying the tumor; (B) Focal intense positivity for TTF-1 in the deep infiltrating areas of the bladder tumor. Immunohistochemical reaction for TTF-1, Dako Denmark, $\times 200$.

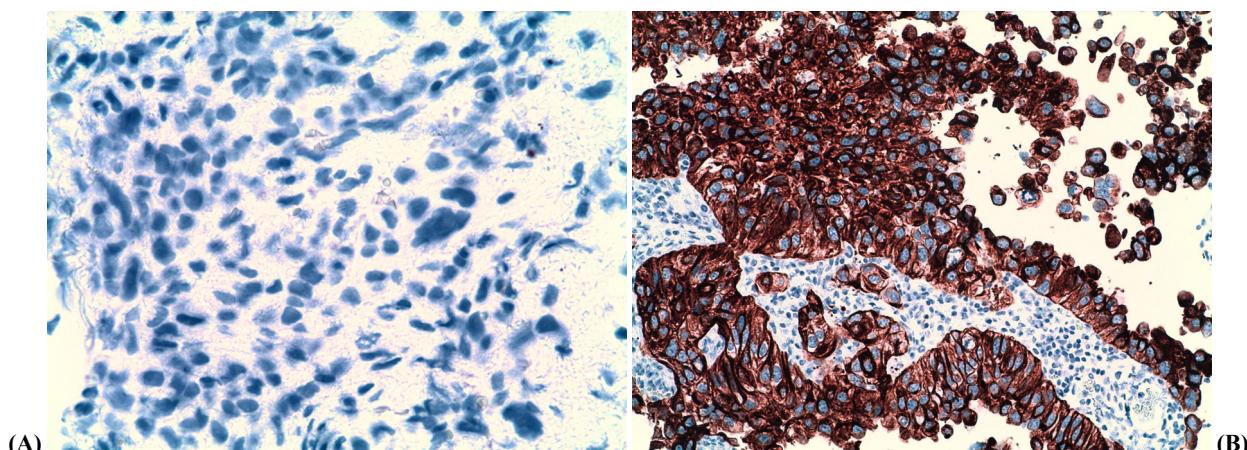


Figure 5 – (A) Negative cytokeratin 20 in the lung tumor; (B) Positive cytokeratin 20 in the bladder tumor. Immunohistochemical reaction for CK20, Dako Denmark, ×200.

The method used in the immunohistochemical study was the automatized methodology marketed by Dako and performed in the Autostainer PlusLink. In this method, antigen retrieval is performed with a commercial Target Retrieval Solution (also by Dako). In short, the steps of the process are as follows: deparaffinising, alcohol rehydrating, endogenous peroxidase inhibition, incubation with peroxide in 3% distilled water, incubation with the primary antibody (the negative control), in the optimal solution, incubation with the secondary biotinylated species specific antibody (serum) for the primary antibody, incubation with the peroxidated Streptavidin, chromogen developing (DAB – 3,3'-diaminobenzidine) in the dark, counterstaining with Mayer's Hematoxylin, for 15–30 seconds, alcohol dehydrating, with increasing concentrations, xylol clarification, and finally mounting with Canada Balm. The result is visualizing the investigated antigens, with the EnVision technology, that determines a brown solution at their levels (negative nuclei are stained in light blue with Hematoxylin).

Markers used and their main characteristics are summarized in Table 1.

Table 1 – Summary of the features of the immunohistochemical reagents used

Name	Manufacturer	Clone	Species	Dilution
TTF-1		8G7G3/1		
CK7	Dako	OV-TL 12/30	Mouse	Prediluted
CK20		Ks20.8		

For each used antibody, we used both external positive control and external negative control, using the same work technique. We also followed, on diagnosis specimens, the presence of internal positive control. If this internal positive control is present, there is no need for an external positive control.

Discussion

The thyroid transcription factor-1 (TTF-1) is a 38-kd homeodomain containing transcription factor expressed in the thyroid, in the lung and in some brain areas [1]. TTF-1 is expressed in small cell carcinoma of the lung and lung adenocarcinomas, but not in squamous cell carcinoma or neuroendocrine tumors. TTF-1 has been

proposed as a useful immunohistochemical marker to determine the pulmonary or thyroid origin of tumors, mainly in metastatic lesions and small cell carcinomas. However, recent reports have rendered doubts over this diagnostic utility, for some series have shown a varying rate of reactivity mainly in extrapulmonary small cell carcinomas, ranging from 10% to 42% [4, 5]. TTF-1 remains useful for the differential diagnosis of mesothelioma and pulmonary adenocarcinoma and also to distinguish cutaneous metastasis from small cell carcinomas from Merkel cell carcinoma, for the latter are consistently negative with this marker [7].

We have found no previous references in MEDLINE to the expression of TTF-1 in urothelial carcinoma. The only related reference is a comment on a report by Jones TD *et al.* that reviewed 44 cases of small cell carcinoma of the bladder and described no positivity for TTF-1 in the classical urothelial cell carcinoma areas found adjacent to the foci of small cell carcinoma in some samples. Alijo Serrano F *et al.* found positivity in large and small cell neuroendocrine carcinomas of the bladder, but apparently not in classic urothelial cell areas [6]. In this sense, our report is unique for it is the first to describe TTF-1 positivity in the deep infiltrating areas of a papillary urothelial cell carcinoma. We feel this is fairly relevant for the daily practice for urothelial and lung lesions often coexist, for they are related to common risk factors (mainly smoking) [9]. Luckily, in this case the comparison of the extent and intensity of the reactivity is not comparable in both samples. When confronted to this case, we thought the positive areas might represent metastasis from a pulmonary primary tumor. It is well known that benign tumors can be the location of metastasis from other malignant tumors, a fact that has been described in leiomyomas and also in ovarian benign tumors [10]. However, a thorough review of the slides revealed that these cells were positive for both cytokeratin 20 and 7, a data consistent with an urothelial origin, while the pulmonary tumor was only positive with cytokeratin 7.

A recent report by Matoso A *et al.* [11] has shown a different pattern of TTF-1 expression according to the clone employed. In this report the authors compared the

expression of the clones SPT24 and 8G7G3/1 and found that the latter was more frequently expressed in extra-pulmonary lesions, including bladder carcinoma. As we have employed the 8G7G3/1, the relative lack of specificity of this clone could be a reason for the aberrant expression of TTF-1 in the urothelial cell carcinoma. Unluckily we do not have the SPT24 clone to investigate this possibility.

Conclusions

To report a case of urothelial cell carcinoma displaying a positive reaction with TTF-1 in a patient with a synchronous lung adenocarcinoma. This reaction can render diagnostic doubts about the possible metastatic nature of the lesion, mainly in small samples. A comparison of the reactivity extent and intensity and also the expression of other immunohistochemical markers can help diagnosis.

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