

## COMPARISON OF THYROID TRANSCRIPTION FACTOR-1 EXPRESSION IN GASTRIC ADENOCARCINOMA AND NON-COHESIVE TYPE GASTRIC CARCINOMA: IS THERE ANY DIFFERENCE?

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### ABSTRACT

**Introduction:** Metastasis of an unknown primary site in liver, lung, lymph nodes and soft tissue is not rare, and in these patients, immunohistochemical findings may have an effect on the therapeutic implications and prognosis. Thyroid transcription factor-1 (TTF-1) is a transcription factor which plays an important role in the normal physiology of the thyroid and lungs. TTF-1 is highly specific and sensitive in the diagnosis of certain types of primary lung and thyroid carcinomas and immunohistochemical TTF-1 positivity may be used as a marker of malignancies metastasized from lung and thyroid. Although they are used extensively in daily pathological practice, detailed study of this antibody in some of neoplasms that may show difficulties in differential diagnosis is lacking. In this study, we aimed to determine the usefulness of TTF-1 positivity in the differential diagnosis of gastric carcinomas from lung malignancies.

**Materials and methods:** We examined TTF-1 expression in primary adenocarcinoma and non-cohesive carcinoma of the stomach. Surgical resection materials from 132 patients with gastric carcinoma were evaluated. The diagnosis of primary site was identified for all cases by clinical, radiological and/or histological correlation and the possibility of metastatic carcinoma was excluded. Immunohistochemical analysis was performed on the tissue-microarray block sections.

**Results.** Among 123 gastric carcinomas, 4 (3.25%) cases showed strong nuclear positivity with TTF-1. None of the 9 poorly cohesive gastric carcinomas stained for TTF-1.

**Conclusions:** In summary, our results show that TTF-1 positivity may be observed especially in gastric adenocarcinoma.

**Key words:** TTF-1, SPT24 clone, tissue microarrays, gastric carcinomas, immunohistochemistry.

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### Introduction

Metastasis in lymph node, soft tissue or organs such as liver or lung of unknown primary site showing similar carcinomas originating from primary organs are not uncommon and additional immunohistochemical analysis of this malignancy can provide very important basic information for the therapeutic management of the patient<sup>(1-5)</sup>. The identification of the primary focus of origin of

metastatic tumors mostly has therapeutic consequences. Primary lung and metastatic tumors may share similar cytopathological features leading to difficulties in differential diagnosis especially in routine cytology practice<sup>(5,6)</sup>.

Thyroid transcription factor-1 (TTF-1) is a kind of nuclear protein, and is one of the members of NKx2 family of homeodomain transcription factors. It was first identified interacting with the rat thyroglobulin gene as a DNA-binding activity

located in the thyroid. TTF-1 is a polypeptide that is encoded by a single gene locus consisting of 371 amino acids<sup>(1)</sup>. Firstly, the presence of TTF-1 was shown in the follicular epithelial cells of the thyroid, later it was found in the lung and other sites, including the parafollicular cells of the thyroid, the pituitary gland, parathyroid, Clara cells and specific areas of cerebrum<sup>(1-3)</sup>.

TTF1 plays a role in regulating genes in the thyroid, lung and brain. Thyroglobulin, thyrotropin receptor and thyroperoxidase are basic molecular targets of TTF-1 in the thyroid gland. It activates transcription of the genes that encode these proteins. TTF-1 promotes the transcription of the surfactant proteins A, B, C, D and the Clara cell secretory protein in the lung<sup>(3)</sup>. In cerebrum, the molecular targets of TTF-1 are not clear<sup>(1)</sup>. Positivity has been reported in ciliated metaplasia in the stomach and in non-ciliated cells in atrophic gastritis<sup>(3)</sup>. This positivity has been attributed to gastric bronco-pulmonary trans-determination.

Metastasis of unknown primary site in lymph nodes of soft tissue is not uncommon and immunohistochemical results of this metastatic focus can provide important information for the type of therapeutic management of the patient. The identification of the origin of metastatic tumor mostly has great effect on the prognostic value and therapeutic consequences. Positive TTF-1 staining in gastric adenocarcinoma is a rare entity (4-7). Primary adenocarcinomas of the gastrointestinal tract showing a diffuse TTF-1 positivity are extremely uncommon and TTF-1 positivity in the biopsy specimen could lead to misleading diagnosis of a metastatic lung tumor. Only a few case reports and a very low ratio of positivity were reported in series based on tissue-microarray paraffin blocks<sup>(4-7)</sup>. We decided to evaluate TTF-1 staining in gastric adenocarcinomas to estimate the reliability of the association of between TTF-1, lung and thyroid tumors.

## Materials and methods

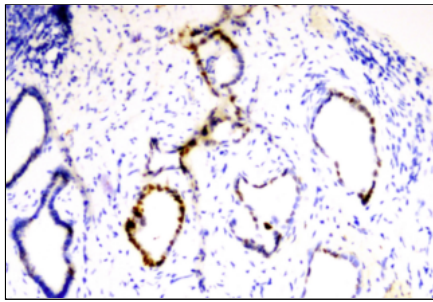
132 cases with gastric adenocarcinoma were evaluated retrospectively in our study. After examining the tumor contained in haematoxylin-eosin stained slides, appropriate areas were selected, taken from donor paraffin block and embedded in new multiple tissue-microarray paraffin block. Sections of 4- $\mu$ m-thickness were taken from the paraffin blocks and placed on positively charged poly-L-lysine coated slides. Immunohistochemical

study was performed on the multiple tissue-microarray (4\*6 arrays) paraffin blocks. Antigen retrieval was performed using the citrate buffer solution (pH 6.0) following the manufacturer protocol. All immunohistochemical staining process, including the deparaffinization of the slides, was performed on Benchmark XT® (Ventana Medical Syst., USA) automated stainer using the iView DAB detection kit (Ventana, AZ) with the TTF-1 antibody (Novocastra Laboratories Ltd., UK, monoclonal antibody clone SPT24 dilution 1:25).

The results of TTF-1 immunohistochemical staining were based only on the nuclear type positivity of tumor cells. Only 2 of 132 gastric carcinoma cases were diagnosed on endoscopic biopsies and 130 were total or subtotal gastrectomy materials. Mayers' haematoxylin was used as a counterstain after immunohistochemical staining protocol. Lung adenocarcinoma and normal thyroid follicular tissues were used as external control tissue on every section and a negative control solution that did not contain primary antibody was used as negative control in every session. Immunohistochemical stains were scored using a two-tiered scoring system: positive or negative. Positive results were based on moderate and strong nuclear positivity and gastric carcinomas with more than 1% positive cells being considered positive for TTF-1 antibody. GraphPad Prism Software (GraphPad Software, Inc. CA, USA) was used for statistical analysis and p values were calculated using Fisher's exact test.

## Results

123 of 132 (93%) tumors were classical adenocarcinoma with various differentiations and only in 9 patients were histological types of tumors with poorly cohesive type carcinoma (including mixed adenocarcinoma with >50% diffuse / signet ring cell features) identified. There were 4 tumors of 132 that were stained positively with TTF-1 staining (Figure 1) and overall positivity for TTF-1 was 3,03% (Table 1). Focal, mild nuclear staining of less than 1% of the nuclei were seen in two cases, but both of these cases were accepted as negative. All of the four TTF-1 positive cases had classical adenocarcinoma and the ratio of TTF-1 positivity in all classical adenocarcinoma of the stomach was 3.25% (Figure 2). There were no statistical significant differences relating to age, sex and TNM stage in TTF-1 positive and negative cases.

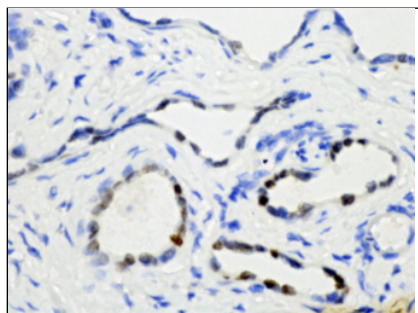


**Figure 1:** Positive glands (center and right side) with nuclear stained with TTF-1 and negative glands nuclei (left side) were seen (x100 / TTF-1 antibody, immunohistochemistry).

*TTF-1: thyroid transcription factor-1*

Tumor type	TTF-1 (+)	TTF-1 (-)	Total	%
Adenocarcinoma	4	119	123	3.02
Poorly-Cohesive carcinoma	0	9	9	0
Total patients	4	128	132	

**Table 1:** The results of nuclear TTF1 immunohistochemical staining in all cases with various types of gastric carcinoma.



**Figure 2:** In high magnification, positive glands stained nuclear were seen and note negative stromal nuclei around the glands. (x400 / TTF-1 antibody, immunohistochemistry).

*TTF-1: thyroid transcription factor-1*

**Discussion**

The most common sites of cancer metastasis are the liver and the lung. Because of this, TTF-1 is used as a reliable marker to distinguish between primary malignancy and metastatic malignancy of the lung, especially when the pathologist is dealing with an adenocarcinoma in light microscopical examination<sup>(5-7)</sup>. TTF-1 is also a very useful antibody in the differential diagnosis of the adenocarcinoma located in liver.

Nuclear positivity of TTF-1 antibody is consistent with metastatic adenocarcinoma originating possibly from the lung or thyroid.

Holzinger et al.<sup>(8)</sup> produced a TTF-1 monoclonal antibody clone named 8G7G3/1 and they reported positive nuclear staining of this antibody in pulmonary adenocarcinomas and small cell carcinomas of the lung, and negative results in breast and colon adenocarcinomas. Then, this antibody was considered a very specific marker for follicular malignant tumors of the thyroid and for small cell carcinoma and adenocarcinoma of lung in histopathological as well as cytological specimens and became very popular among pathologists as a useful marker of lung and thyroid malignancy<sup>(2,3,5-8)</sup>. Finally Alkushi et al.<sup>(9)</sup> reported a case showing nuclear TTF-1 antibody positivity in a papillary serous carcinoma located in the uterus in their study of genital adenocarcinomas.

In addition to specific lung and thyroid malignant tumor, the positivity of TTF-1 antibody has also been demonstrated in very different types of tumors such as ependymoma<sup>(11)</sup>, multiform glioblastoma<sup>(12)</sup>, endocervical adenocarcinoma, serous and endometrioid carcinoma of ovary, mucinous carcinoma of the ovary<sup>(13-15)</sup>, primary and metastatic colonic adenocarcinoma<sup>(16)</sup>, atrophic gastritis<sup>(4)</sup>, prostatic acinar adenocarcinoma<sup>(17)</sup>, and malignant melanoma of the skin<sup>(18)</sup>.

Primary adenocarcinomas of the gastrointestinal tract including colorectum and stomach showing a diffuse immunohistochemical positivity of TTF-1 antibody are quite common and this positivity of TTF-1 antibody in the biopsy specimen could lead to misleading diagnosis of a metastatic lung tumor in gastrointestinal system or organ. Pegolo et al.<sup>(6)</sup>, in a 62-year-old woman and in a 66-year-old man, reported clinical and pathological features of 2 confusing cases. The former patient had gastric adenocarcinoma with liver metastasis; the latter patient had adenocarcinoma in the gallbladder discovered incidentally during routine screening for colorectal cancer. Both of the neoplasms, gastric adenocarcinoma and colorectal adenocarcinoma, showed a diffuse TTF-1 positivity and both the patients had no evidence of lung carcinoma. Neither a gastric adenocarcinoma with diffuse TTF-1 expression nor a TTF-1-positive gallbladder adenocarcinoma had been described before this report<sup>(6)</sup>. Matoso et al.<sup>(7)</sup> found TTF-1 positivity in only one case (0.9%) of 110 gastric carcinomas.

They also found that TTF-1 positivity of the bladder carcinoma is not very rare (5.1%). Additionally, they found positivity rates of 1.2%, 1.8% and 2.5% in prostate carcinoma, salivary gland carcinoma and colon carcinoma respectively. As previously stated, TTF-1 positivity in gastric carcinomas was 3.06% in our cases and this ratio is higher than TTF-1 positivity in the manuscript published by Matoso.

## Conclusion

Positive staining with TTF-1 in gastric adenocarcinomas is very rare according to current published manuscripts. TTF-1 positivity was detected in approximately 3% (4 of 132 cases) of stomach carcinomas. Because 3% positivity is not negligible, TTF-1 positivity can be misleading when TTF-1 is positive in gastric endoscopic biopsy or gastric resection specimens. The pathologist should keep in mind the possibility of TTF-1 positivity that this nuclear staining may not have originated only from the lung. Similar to TTF-1 positivity seen in other organs or systems, gastric classical adenocarcinomas can also be stained positively with TTF-1 antibody. The possibility of the presence of TTF-1 positivity in stomach intestinal type adenocarcinoma in addition to lung and thyroid tumors should not be forgotten. Otherwise, there were no stained tumor nuclei in diffuse type of gastric carcinoma and larger series are needed to clarify the results related with staining difference in different types of carcinoma.

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