

# Thyroid transcription factor-1 expression in ovarian epithelial neoplasms

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**Thyroid transcription factor-1 (TTF-1) protein expression is widely used in the diagnosis of lung and thyroid carcinomas. Although there have been reports of TTF-1 immunoreactivity in tumors other than those originating in the lung or the thyroid, the expression of this marker has been studied in only a limited number of ovarian neoplasms. Our study examines the incidence of TTF-1 expression in a variety of ovarian epithelial neoplasms. Tissue microarrays of 138 ovarian serous carcinomas, 65 endometrioid adenocarcinomas, 35 mucinous adenocarcinomas, 30 mucinous neoplasms of low malignant potential, and 10 clear cell carcinomas were stained with anti-TTF1-antibody. In addition, whole tissue sections of 19 serous carcinomas, 5 endometrioid adenocarcinomas, 7 mucinous adenocarcinomas, and 3 clear cell carcinomas were stained. In the tissue microarrays, TTF-1 nuclear expression was demonstrated in 2 of 65 (3%) of the endometrioid adenocarcinomas; no nuclear immunoreactivity was identified in the remaining ovarian neoplasms. In the whole tissue sections, TTF-1 nuclear staining was present in 7 of 19 (37%) serous carcinomas, 1 of 5 (20%) endometrioid adenocarcinomas, and 1 of 3 (33%) clear cell carcinomas. In most of the positive cases, staining was focal, but in one endometrioid adenocarcinoma in the tissue microarray and in one serous and one clear cell carcinoma in the whole tissue sections, there was diffuse positivity. Overall, there was nuclear staining in 0.7% of tumors in the tissue microarray and 26% in the whole tissue sections. Although TTF-1 nuclear expression is generally considered to be a relatively specific marker for lung and thyroid neoplasms, the occasional immunoreactivity of ovarian carcinomas should be considered in the evaluation of neoplasms of unknown primary origin. It should also be taken into consideration when evaluating adenocarcinomas involving the lung in patients with a history of a gynecologic malignancy.**

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Thyroid transcription factor-1 (TTF-1) is a 38-kDa homeodomain-containing DNA-binding protein belonging to the *Nkx2* gene family. It was initially identified in follicular cells of the thyroid and found to regulate genes responsible for the production of thyroglobulin, thyroperoxidase, and thyrotropin receptor. Subsequently, TTF-1 was identified in respiratory epithelial cells, involving the regulation of surfactant and Clara cell secretory protein gene expression, as well as in areas of the developing brain.<sup>1,2</sup>

Immunoreactivity for TTF-1 has been found to be useful in the diagnosis of various lung and thyroid neoplasms and is widely used in surgical pathology.<sup>1,3,4</sup> Several studies have demonstrated the utility of TTF-1 immunoreactivity for determining if an adenocarcinoma of unknown primary is of pulmonary origin,<sup>3</sup> distinguishing pulmonary adenocarcinoma from mesothelioma<sup>5</sup> and distinguishing between pulmonary small cell carcinoma and Merkel cell carcinoma,<sup>6,7</sup> and in identifying brain metastases of pulmonary origin.<sup>8,9</sup>

While TTF-1 is a diagnostically useful marker, it is not 100% specific for lung and thyroid neoplasms. Expression of TTF-1 has also been reported in tumors other than those originating in the lung or thyroid; however, this marker has only been studied in a few gynecologic neoplasms.<sup>10–13</sup> We have previously reported TTF-1 immunoreactivity in 4% (1/28) of endocervical adenocarcinomas, 16% (6/32) of endometrial endometrioid adenocarcinomas, and

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23% (3/13) of endometrial serous carcinomas.<sup>14</sup> In this study, we evaluate the expression of TTF-1 in a variety of primary ovarian epithelial neoplasms.

## Materials and methods

Four pre-constructed tissue microarray paraffin blocks (0.6 mm per core) of 165 ovarian serous carcinomas (150 cases with 1 core each, 15 cases with 2 cores each), 74 endometrioid adenocarcinomas (2 cores each), 46 mucinous adenocarcinomas (2 cores each), 31 mucinous neoplasms of low malignant potential (2 cores each), and 15 clear cell carcinomas (2 cores each) were selected from the archival research material of the Department of Pathology at The University of Texas MD Anderson Cancer Center. Fifty-three cases were eliminated from the study due to insufficient material remaining in the blocks for evaluation, resulting in 138 ovarian serous carcinomas, 65 endometrioid adenocarcinomas, 35 mucinous adenocarcinomas, 30 mucinous neoplasms of low malignant potential, and 10 clear cell carcinomas for final evaluation. Whole tissue sections of 34 consecutive ovarian cancers from the archives of the Department of Pathology, Royal Group of Hospitals Trust, Belfast were also stained with TTF-1. These comprised 19 serous carcinomas, 5 endometrioid adenocarcinomas, 7 mucinous adenocarcinomas, and 3 clear cell carcinomas (whole tissue sections were different cases than the specimens included in the tissue microarray).

Four micrometer thick sections were cut from the microarray and whole tissue section paraffin blocks and submitted for immunoperoxidase studies. The staining of the microarray sections was performed in a DAKO Auto Stainer (Carpinteria, CA, USA) using EnVision polymer (DAKO) and anti-TTF-1 antibody (1:25 dilution, 8G7G3/1, DAKO). To enhance immunostaining, a heat-induced epitope retrieval procedure was performed using a Black and Decker vegetable steamer (Shelton, CT, USA). The antigen antibody immunoreaction was visualized using diaminobenzidine (DAB) as a chromogen. The slides were counterstained with Mayer's hematoxylin.

In the other laboratory, four micrometer thick sections were cut from the paraffin wax-embedded blocks, floated onto sialinized slides, and dried

overnight at 37°C, before loading onto Vision Biosystem (Newcastle-upon-Tyne, England) Bond Max automated immunostainer. Heat-induced epitope retrieval was performed using retrieval ER 2 solution (Vision Biosystem) for 20 min. Immunolocalization using TTF-1 antibody (SPT 24 clone) (Novocastra, Newcastle-upon-Tyne, England) at a dilution of 1 in 200 was performed. The detection method used was a polymer detection system. DAB was used as the chromogen and sections were counterstained using hematoxylin (Vision Biosystem).

The anti-TTF-1-stained sections were reviewed, and the presence or absence of nuclear and cytoplasmic TTF-1 immunoreactivity was recorded. Results were recorded as negative, focally positive (<50% cells positive), or diffusely positive (50% or more cells positive).

## Results

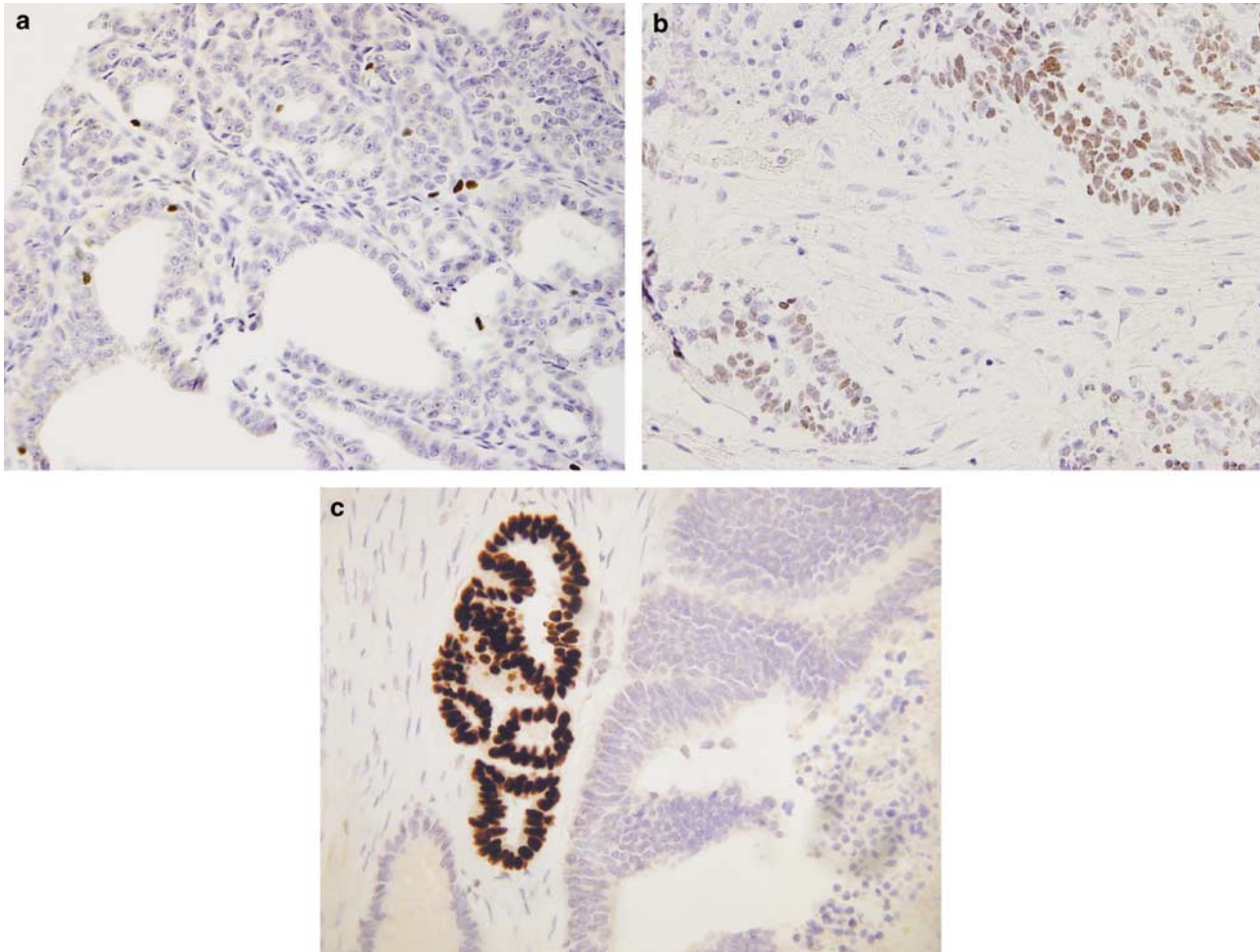
In the tissue microarrays, TTF-1 nuclear expression was seen in 2 of 65 (3%, one of two cores in each case) of the ovarian endometrioid adenocarcinomas (Table 1) (Figure 1). One case was focally positive (scattered positive nuclei) and one diffusely positive (approximately 60% of nuclei staining); none of the remaining ovarian neoplasms demonstrated any nuclear immunoreactivity for TTF-1. Cytoplasmic staining was noted in 1 of 65 (1.5%) of the ovarian endometrioid adenocarcinomas, 1 of 138 (0.7%) of the ovarian serous carcinomas, 2 of 35 (6%) of the ovarian mucinous adenocarcinomas, 1 of 10 (10%) of the ovarian clear cell carcinomas, and none of the ovarian mucinous neoplasms of low malignant potential.

In the whole tissue sections, 7 of 19 (37%) serous carcinomas exhibited nuclear TTF-1 positivity (Table 2) (Figure 2). In one case, there was diffuse immunoreactivity with close to 100% of nuclei staining. In the other six cases, there was focal staining of scattered nuclei. There was focal nuclear staining of one endometrioid adenocarcinoma (scattered positive nuclei) and diffuse nuclear positivity of one clear cell carcinoma (close to 100% of nuclei staining) (Figure 3). None of the mucinous carcinomas were positive. There was cytoplasmic staining of one serous carcinoma in the whole tissue sections.

**Table 1** TTF-1 expression of ovarian neoplasms in tissue microarrays

Ovarian neoplasms	Serous carcinoma (%)	Endometrioid AdenoCa (%)	Mucinous AdenoCa (%)	Mucinous neoplasm of LMP (%)	Clear cell carcinoma (%)
TTF-1 positive (nuclear)	0 (0)	2 (3)	0 (0)	0 (0)	0 (0)
TTF-1 Cytopl staining	1 (0.7)	1 (1.5)	2 (6)	0 (0)	1 (10)
Total cases	138	65	35	30	10

AdenoCa, adenocarcinoma; Cytopl, cytoplasmic; LMP, low malignant potential; TTF-1, thyroid transcription factor-1.



**Figure 1** Ovarian endometrioid adenocarcinomas with focal (a) and diffuse (b) nuclear expression of TTF-1 in tissue microarray; focal nuclear staining in whole section (c).

**Table 2** TTF-1 expression of ovarian neoplasms in whole tissue sections

Ovarian neoplasms	Serous carcinoma (%)	Endometrioid AdenoCa (%)	Mucinous AdenoCa (%)	Clear cell carcinoma (%)
TTF-1 positive (nuclear)	7 (37)	1 (20)	0 (0)	1 (33)
TTF-1 Cytopl staining	1 (5)	0 (0)	0 (0)	0 (0)
Total cases	19	5	7	3

AdenoCa, adenocarcinoma; Cytopl, cytoplasmic; TTF-1, thyroid transcription factor-1.

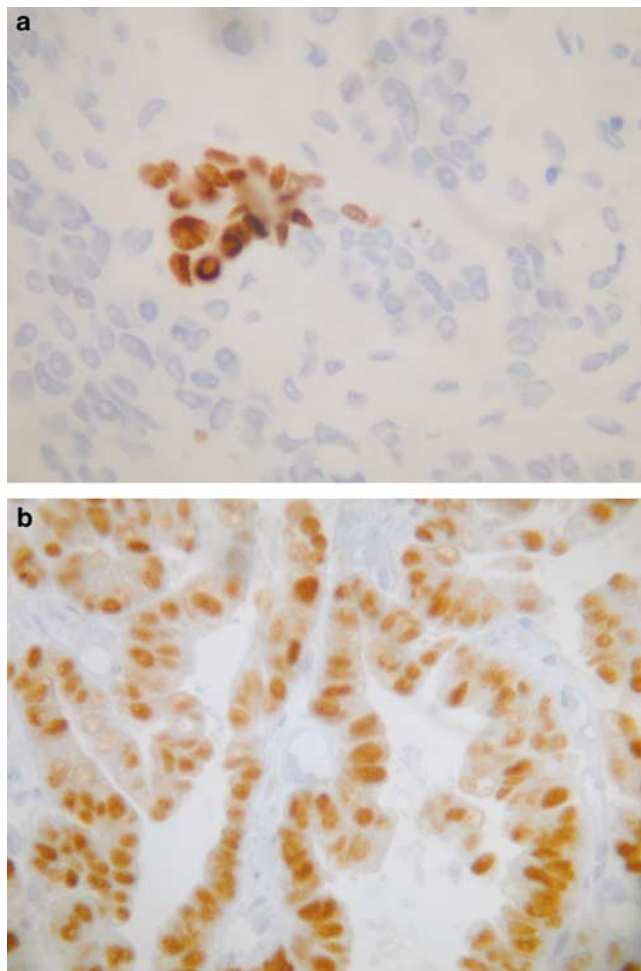
Overall, 0.7% of tumors in the tissue microarrays and 26% in the whole tissue sections exhibited some degree of nuclear TTF-1 positivity.

## Discussion

Immunohistochemistry can be an important adjunctive component in the evaluation of a neoplasm, whether primary or metastatic. TTF-1 has been found to be a sensitive and relatively specific marker for thyroid and pulmonary neoplasms. In the thyroid, tumors of follicular derivation including

follicular adenomas, follicular carcinomas, papillary carcinomas, medullary carcinomas, and some anaplastic carcinomas are immunoreactive.<sup>1</sup> In the lung, TTF-1 expression is common in primary adenocarcinomas<sup>1,3,4</sup> as well as in neuroendocrine tumors, including small cell carcinoma, carcinoid tumor, atypical carcinoid tumor, and large cell neuroendocrine carcinoma.<sup>15-18</sup>

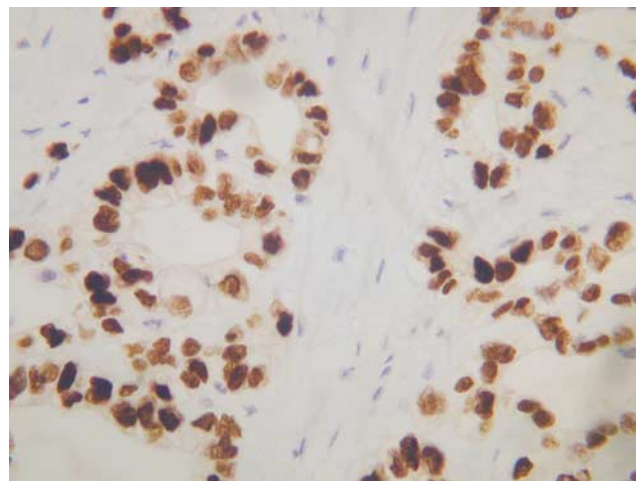
However, in spite of the relatively high specificity of TTF-1 for thyroid and pulmonary neoplasms, immunoreactivity has also been reported in tumors other than those originating in these organs. TTF-1 expression is not uncommon in extrapulmonary



**Figure 2** Ovarian serous carcinoma with focal (a) and diffuse (b) nuclear expression of TTF-1 in whole sections.

small cell carcinomas and large cell neuroendocrine carcinomas of other primary sites, including the gastrointestinal tract, prostate, bladder, breast, and female genital tract.<sup>6,19–23</sup> TTF-1 positivity has also been reported in 2 of 27 ependymomas arising in the third ventricle,<sup>24</sup> 4 of 5 invasive ductal carcinomas of the breast,<sup>25</sup> 1 of 66 gastric cancers,<sup>12</sup> 4 of 41 colorectal adenocarcinomas,<sup>26</sup> and 4 of 7 colonic adenocarcinomas.<sup>27</sup> In the gynecologic tract, TTF-1 expression has been noted in 2 of 166 ovarian carcinomas (1 mixed serous and endometrioid adenocarcinoma and 1 serous carcinoma) in a tissue microarray study,<sup>10</sup> 1 of 16 ovarian cancers (cell block of cytology fluid),<sup>11</sup> 1 of 8 endometrial cancers,<sup>12</sup> and 1 of 14 primary peritoneal serous carcinomas.<sup>13</sup> In a previous study, we identified TTF-1 immunoreactivity in 1 of 28 (4%) endocervical adenocarcinomas, 6 of 32 (19%) endometrial endometrioid adenocarcinomas, and 3 of 13 (23%) endometrial serous carcinomas.<sup>14</sup>

In the current study, our aim was to study the expression of TTF-1 in a variety of ovarian epithelial neoplasms including endometrioid adenocarcinomas, serous carcinomas, mucinous adenocarcinomas, clear



**Figure 3** Ovarian clear cell carcinoma exhibiting diffuse nuclear TTF-1 immunoreactivity in whole section.

cell carcinomas, and mucinous neoplasms of low malignant potential. Similar to the previous studies of carcinomas of the gynecologic tract, we identified nuclear TTF-1 immunoreactivity in a significant percentage of tumors. Two endometrioid adenocarcinomas exhibited nuclear positivity in the tissue microarray, while other neoplasms were negative (overall percentage of positive cases 0.7%). Not surprisingly, there was a much higher rate of positivity in the whole tissue sections with 26% of the tumors exhibiting nuclear staining, including two cases with positivity close to 100% of the nuclei.

The differences in percentage of tumors staining between the two study groups can be attributed to a couple of factors. In the tissue microarray group, we examined one to two cores of tumor at 0.6 mm per core vs whole tissue sections in the other group. Therefore, the difference in volume of tumor examined between the two groups was substantial, and a sampling error may account for the variability in TTF-1 expression. Secondly, two different anti-TTF-1 antibody clones were used, the 8G7G3/1 clone (DAKO) in the microarray group and the SPT24 clone (Novocastra) in the whole tissue sections. Previous studies have shown that the SPT24 clone has a higher sensitivity for TTF-1 protein than the 8G7G3/1 clone,<sup>26,27</sup> which may have led to a higher percentage of cases staining for TTF-1 in the whole tissue sections.

Cytoplasmic expression of TTF-1 was identified in a small number of cases. Cytoplasmic TTF-1 immunoreactivity has previously been reported in 23 of 361 (6.3%) primary and metastatic tumors from multiple organ sites (lung, colon, breast, larynx, orbital bone),<sup>28</sup> and is considered nonspecific. However, others have suggested that cytoplasmic TTF-1 may be useful in the diagnosis of hepatocellular carcinoma.<sup>29,30</sup>

The expression of TTF-1 by ovarian carcinomas should be kept in mind while evaluating a patient

with a neoplasm of unknown primary site or a carcinoma involving the lung in a patient with a history of a gynecologic malignancy. Moreover, lung carcinomas may metastasize to the ovary and mimic an ovarian primary.<sup>31</sup> In such instances, other markers in addition to TTF-1 may be useful in establishing the origin of the tumor. Surfactant protein A has been negative in the ovarian carcinomas that have been studied, but it is positive in 40–73% of pulmonary primaries.<sup>32,33</sup> Nuclear WT-1 expression may be of utility in distinguishing serous carcinoma from carcinomas of the lung.<sup>34,35</sup> Estrogen and progesterone receptors are positive in many ovarian carcinomas, but should be used with caution as they also can be expressed by some pulmonary adenocarcinomas.<sup>36,37</sup>

In summary, although TTF-1 is considered a relatively sensitive and specific marker for lung and thyroid neoplasms, it can occasionally be expressed in tumors from other sites, including the ovary. Most of these neoplasms exhibit focal staining, but occasionally there is diffuse immunoreactivity. As with any other marker, TTF-1 should be interpreted in the context of the clinical setting, radiologic findings, morphologic features, and the results of other markers.

## Disclosure/conflict of interest

None.

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