Original Article

Simultaneous expression of TTF1 and GATA3 in a lung biopsy sample: confusion in diagnostic pathology

Lianhua Zhao1*, Chengyi Mao1*, He Xiao2, Ping Fu1, Hualiang Xiao1, Chuan Chen2, Ge Wang2

1Department of Pathology, 2Cancer Center, Daping Hospital, Army Medical University, No. 10 Changjiang Zhilu, Yuzhong District, Chongqing 400042, China. *Equal contributors.

Received June 11, 2019; Accepted July 26, 2019; Epub September 1, 2019; Published September 15, 2019

Abstract: Background: In daily work, pathologists often use TTF1 and GATA3 in the differential diagnosis of primary lung adenocarcinoma (TTF1+ GATA3-) and metastatic bladder cancer (or breast cancer) (TTF1- GATA3+). However, we encountered a small lung biopsy sample of TTF1+ GATA3+ (clinically suggesting both lung and bladder occupancy), and the dyeing results caused us great confusion; thus, we intended to determine the expressions of TTF1 and GATA3 in lung and bladder cancer by expanding the sample. Methods: The study included a complete case report and the tissue microarrays including pulmonary squamous cell carcinomas (n = 55), lung adenocarcinomas (n = 47), high-grade (n = 68) and low-grade (n = 43) urothelial carcinomas of the bladder. TTF1 and GATA3 immunohistochemical staining were performed on the tissue microarrays, and the relevant literature was retrieved. Results: Our staining results on tissue microarrays showed that TTF1 was expressed in pulmonary adenocarcinomas (44/47, 93.6%), squamous cell carcinomas (1/55, 1.8%), low-grade (1/43, 2.3%) and high-grade (2/68, 2.9%) urothelial carcinomas; GATA3 was only expressed in urothelial carcinomas of the bladder (high-grade: 48/68, 70.6%; low-grade: 42/43, 97.7%). Our literature search results showed that TTF1 could be expressed in a very small number of bladder urothelial carcinomas, and GATA3 could be expressed in a few primary lung squamous cell carcinomas and a very small number of primary lung adenocarcinomas. Conclusions: TTF1 and GATA3 are good markers in the differential diagnosis of primary non-small cell lung cancer (GATA3-) and metastatic urothelial carcinoma of the bladder (GATA3+). However, pathologists should pay attention to a few special cases: lung cancer may express GATA3, and urothelial carcinoma may express TTF1. In these cases, some additional immunohistochemical markers, such as napsin A and URO III, should be added to assist the diagnosis.

Keywords: TTF1, GATA3, tissue microarray, immunohistochemistry

Introduction

The identification of primary or metastatic lung cancer is often very difficult for pathologists, and immunohistochemical staining of some tissue-specific markers is often needed [1].

Thyroid transcription factor 1 (TTF1) is a tissue-specific transcription factor that plays an important role in the development of the thyroid, lung and diencephalon [2, 3]. In daily work, pathologists often use it to determine the origin (thyroid, lung, or diencephalon) of tumors [2, 3] and to differentiate lung adenocarcinoma (AC) from squamous cell carcinoma (SCC) [4].

GATA binding protein 3 (GATA3) is also a tissue-specific transcription factor widely used in the auxiliary pathological diagnosis of the primary site of metastatic tumors, especially metastatic breast cancer and bladder urothelial carcinoma (UC) [5].

Recently, our pathology department received a percutaneous lung biopsy sample, and meanwhile, an imaging examination suggested that the bladder wall was thickened. It is difficult to differentiate primary non-small cell lung carcinoma (NSCLC) from metastatic UC by hematoxylin and eosin staining (H&E) alone. Therefore, we performed immunohistochemical staining panels including TTF1 and GATA3. However, the staining results showed that both TTF1 and GATA3 were diffuse and strongly positive in the tumor cells.

The staining pattern of the tumor cells expressing both TTF1 and GATA3 aroused our great
interest. We had three questions about this case. (1) Is the primary site of the cancer the lung or bladder? (2) If it is primary lung cancer, can GATA3 be expressed? (3) If this case is metastatic bladder cancer, can TTF1 be expressed? To answer these questions, we designed this experiment and reviewed the relevant literature.

**Materials and methods**

**A clinical case**

A 54-year-old male patient was admitted to our hospital with pain in his left shoulder and waist for more than 1 month and lower abdominal pain for more than half a month. The magnetic resonance imaging (MRI) examination of his neck and lumbar vertebrae at the local hospital showed abnormal signal shadows of the T3, L1-5, and S2 vertebrae, so multiple metastatic tumors were considered. Positron emission tomography/computed tomography (PET/CT) was performed in our hospital. The results showed the following: (1) the left posterior wall of the bladder was thickened, and the left ureter was dilated with hydronephrosis, so it was suggested that further cystoscopy should be performed; (2) there were multiple space-occupying lesions in both lungs, with the largest one located in the posterior basal segment of the lower left lobe (the maximum diameter was approximately 1.9 cm); (3) multiple hepatic metastases, especially in the upper segment of the left lateral lobe, with a maximum diameter of 1.3 cm; (4) multiple lymph node metastasis occurred in the left neck, retroperitoneum and left iliac perivessel; (5) multiple bone metastases were found in both the trunk and upper limbs. Subsequently, a percutaneous biopsy of the left lower lung was performed to confirm the diagnosis.

A histopathological examination showed that the tumor cells formed papillary structures, with the fibrovascular axis lined by multilayered tumor cells, accompanied by polarity disorder (Figure 1A). The tumor cells were round or short columnar with clear boundaries, the cytoplasm was eosinophilic or transparent, the nuclei were round or oval with different sizes, the chromatin was delicate, small nucleoli could be seen, and a few mitotic figures (approximately 4/10 HPF) could be seen. According to the clinical data (PET/CT suggested space-occupying lesions of the bladder and lung) and HE morphology, we could not determine the original location of the tumor, so the immunohistochemical markers, including TTF1 and GATA3, were measured in the biopsy specimen. The results showed that both TTF1 and GATA3 were strongly expressed in the tumor cells.
TTF1 and GATA3 in urothelial and lung carcinomas

In total, approximately 40% of the tumor cells were TTF1-positive (Figure 1B), and up to 80% of the tumor cells expressed GATA3 (Figure 1C). The cytokeratins 34βE12 (Figure 1D), 5/6 (Figure 1E), 7, 17, and 19 (Figure 1F) were positive, and cytokeratins 14 and 20 were negative. Napsin-A and Uroplakin III were negative. The proliferative index (Ki67) was approximately 60%.

The above immunohistochemical results brought us great confusion in judging the primary location of the tumor, so we searched the relevant literature. Only a small number of documents were retrieved, and the results showed that: GATA3 could be expressed in a few NSCLC cases and TTF1 could also be expressed in bladder UC; therefore, it was still not possible to make a definite diagnosis.

We decided to further explore the expression of GATA3 in NSCLC and TTF1 in bladder UC in our own series of cases.

The patient was informed about the study, and he agreed to include the case report in the study.

Clinical samples

The study was performed on tissue microarrays composed of formalin-fixed paraffin-embedded tissue from 102 cases of NSCLC and 111 cases of bladder UC, obtained from surgical resection in our hospital from 2011 to 2017. All cases were re-diagnosed by two highly experienced pathologists using the criteria of the WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart (Fourth edition) and WHO Classification of Tumours of the Urinary System and Male Genital Organs (Fourth edition). In short, invasive adenocarcinoma of the lung (Note: In this article, it is abbreviated as AC) is a malignant epithelial tumor with glandular differentiation, mucin production or pneumocyte marker expression (currently, the most commonly used are TTF1 and napsin-A). Squamous cell carcinoma (SCC) is a malignant epithelial tumor that either shows keratinization and/or intercellular bridges, or is morphologically undifferentiated non-small cell carcinoma that expresses immunohistochemical markers of squamous cell differentiation (i.e. p40, P63, CK5, CK5/6, of which P40 has higher specificity than others). Infiltrating urothelial carcinoma (Note: In this article, it is abbreviated as UC) is characterized by a propensity for divergent differentiation. Defining histological criterion is invasion beyond the basement membrane. The grading of the cases is based on cytological disorder, which is defined as abnormalities in nuclear size, shape, and nuclear chromatin at low and medium magnification. Mitoses may or may not be present. In high-grade UC, cellular disorder, nuclear size variation, and irregular and pleomorphic nuclei are readily apparent at low to intermediate magnification. Irregular prominent nucleoli and numerous mitoses, including irregular forms, are typical.

The Clinical Ethics Committee of Daping Hospital, Army Medical University approved this study.

Immunohistochemical staining

Unstained slides (4 μm thick) were prepared from prepared tissue microarrays. Immunohistochemical labeling with monoclonal mouse antibodies to TTF1 (Maxim, Fuzhou, China; clone 8G7G3/1; prediluted) and GATA3 (Maxim, Fuzhou, China; clone L50-823; prediluted) were performed using the conventional method, as described previously. For each immunostaining run, positive and negative controls were included. Specifically, the positive controls were the following: lung AC for TTF1 and UC for GATA3. The negative controls consisted of incubation with a secondary antibody only.

The two pathologists independently interpreted the results under a microscope. The intensity (1+ to 3+) and percentage of tumor nuclear staining were used as the evaluation results. Positivity was defined as a staining intensity of at least 1+ in at least 5% of tumor cells.

Statistical analysis

The chi-squared test \( (\chi^2) \) for related proportions was used to assess the differences between the two methods in the same group. Fisher's exact test was used to assess the differences in categorical outcomes between the independent groups. All analyses were performed using
SPSS software (version 18.0; SPSS, IL, USA). A $P$-value < 0.05 was considered statistically significant.

**Results**

The study included tissue microarrays from 102 cases of NSCLC (SCC: 55 and AC: 47) and 111 cases of bladder UC (high-grade UC: 68 and low-grade UC: 43) (Figure 2A).

The results of the immunohistochemical staining of TTF1 showed that the total expression rate was 44.1% in 102 cases of NSCLC, 93.6% (44/47) in AC and 1.8% (1/55) in SCC, and the difference of the positive rate was statistically significant ($\chi^2 = 86.626$, $P < 0.001$). The average percentage of TTF1 positive was $81.11 \pm 4.31\%$.

The total expression rate of TTF1 in bladder cancer was 2.70% (3/111), of which the low-grade UC was 2.3% (1/43) and the high-grade UC was 2.9% (2/68) (Fisher’s exact test probability, $P = 1.00$). The percentages of the positive cells in the 3 cases were 20% (high-grade), 30% (low-grade) (Figure 2B) and 70% (high-grade), respectively.

The total expression rate of GATA3 in bladder cancer was 81.1% (90/111), of which the low-grade UC was 97.7% (42/43) (Figure 2C) and the high-grade UC was 70.6% (48/68). The difference in the positive rate was statistically significant ($\chi^2 = 12.599$, $P < 0.001$). The average percentage of GATA3 positive was $66.72 \pm 2.50\%$, of which the low-grade UC was $77.38 \pm 2.34\%$ and the high-grade was $57.40 \pm 3.70\%$.

GATA3 was not expressed in the tissue microarrays of the 102 cases of NSCLC.

**Discussion**

The lungs are one of the most common target organs for malignant tumor metastasis, and the probability of malignant tumors metastasizing to the lung is approximately 25% [6]. Therefore, in the daily work of pathologists, any lung biopsy samples need to exclude the possibility of metastatic tumors, but this process is usually difficult and often requires immunohistochemistry [1, 4].

TTF1 is a member of the homologous transcription factor Nkx2 family [2]. TTF1 expression is of high sensitivity for thyroid and lung tumors [2], especially lung AC, with a reported positive rate ranging from approximately 81.3% up to 93% [7-9] (Table 1). In our experience, approximately 93.6% of lung ACs are TTF1-positive. However, the reported positive rate of lung SCCs ranges from 0% to 8% [7-9] (Table 1). In our experiment, only one out of 55 cases (1.8%) of SCCs was TTF1 positive. We speculate that the difference of TTF1 expression in lung SCCs is related to the inconsistent interpretation criteria. Currently, the pathologist’s diagnostic criterion is that, under the appropriate clinical background, tumors that are focally positive for TTF1 should be diagnosed as lung AC, not SCC, but the SCC marker P40 requires more than 50% of tumor cells, and local positivity for P40 cannot diagnose SCC [4]. According to this principle, previously diagnosed TTF1-positive SCC should now be modified to AC. In other words, when the immunohistochemical method is needed to determine whether it is lung SCC or...
TTF1 and GATA3 in urothelial and lung carcinomas

Table 1. TTF1 expression reported in tumors of the lung and bladder

<table>
<thead>
<tr>
<th>Clone</th>
<th>Lung SCC</th>
<th>Lung AC</th>
<th>Primary UC</th>
<th>Metastatic UC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>8G7G3/1</td>
<td>1/28 (3.6)</td>
<td>60/71 (84.5)</td>
<td>-</td>
<td>-</td>
<td>GT G, 2015 [9]</td>
</tr>
<tr>
<td>8G7G3/1</td>
<td>3/49 (6.1)</td>
<td>161/198 (81.3)</td>
<td>-</td>
<td>-</td>
<td>R N, 2015 [11]</td>
</tr>
<tr>
<td>8G7G3/1</td>
<td>0/201 (0)</td>
<td>380/429 (89)</td>
<td>-</td>
<td>-</td>
<td>H V, 2018 [10]</td>
</tr>
<tr>
<td>8G7G3/1</td>
<td>-</td>
<td>-</td>
<td>0/11 (0)</td>
<td>O K, 2000 [15]</td>
<td></td>
</tr>
<tr>
<td>8G7G3/1</td>
<td>-</td>
<td>-</td>
<td>5/98 (5.1)</td>
<td>Matoso A, 2010 [12]</td>
<td></td>
</tr>
<tr>
<td>8G7G3/1</td>
<td>-</td>
<td>-</td>
<td>1/30 (3.3)</td>
<td>MJ F-A, 2011 [13]</td>
<td></td>
</tr>
<tr>
<td>SPT24</td>
<td>12/201 (6%)</td>
<td>397/429 (93)</td>
<td>-</td>
<td>-</td>
<td>H V, 2018 [10]</td>
</tr>
<tr>
<td>SPT24</td>
<td>-</td>
<td>-</td>
<td>5/42 (11.9)</td>
<td>S, 2017 [14]</td>
<td></td>
</tr>
<tr>
<td>SPT24</td>
<td>-</td>
<td>-</td>
<td>5/98 (5.1)</td>
<td>Matoso A, 2010 [12]</td>
<td></td>
</tr>
<tr>
<td>SP141</td>
<td>16/197 (8%)</td>
<td>388/419 (93)</td>
<td>-</td>
<td>-</td>
<td>H V, 2018 [10]</td>
</tr>
</tbody>
</table>

SCC squamous cell carcinoma, AC adenocarcinoma, UC urothelial carcinoma.

Table 2. GATA3 expression reported in tumors of the lung and bladder

<table>
<thead>
<tr>
<th>Clone</th>
<th>Lung SCC</th>
<th>Lung AC</th>
<th>Primary UC</th>
<th>Metastatic UC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>L50-B23</td>
<td>9/74 (12)</td>
<td>6/71 (8)</td>
<td>49/54 (90.7)</td>
<td>-</td>
<td>M M, 2014 [18]</td>
</tr>
<tr>
<td>L50-B23</td>
<td>40/202 (20)</td>
<td>9/431 (2)</td>
<td>-</td>
<td>8/8 (100)</td>
<td>H V, 2018 [10]</td>
</tr>
<tr>
<td>D13C9</td>
<td>-</td>
<td>49/95 (51.6)</td>
<td>-</td>
<td>-</td>
<td>T H, 2017 [19]</td>
</tr>
<tr>
<td>sc-268</td>
<td>0/49</td>
<td>0/61</td>
<td>62/72 (86)</td>
<td>-</td>
<td>H L, 2012 [5]</td>
</tr>
</tbody>
</table>

SCC squamous cell carcinoma, AC adenocarcinoma, UC urothelial carcinoma.

AC, as long as TTF1 is positive (whether localized or diffuse), it should be diagnosed as lung AC.

TTF1 is also widely used for the differential diagnosis of primary and secondary pulmonary carcinomas, because it is specifically expressed in primary pulmonary ACs but almost never in colorectal cancer, renal cell carcinomas, and so on [2]. Bladder cancer can occasionally metastasize to the lungs.

Our results showed that 3 of the 111 cases of UC expressed TTF1 (2.70%) (antibody clone 8G7G3/1) including 1 case of low-grade UC with 30% positive cells, and 2 cases of high-grade UC, with 20% and 70% positive cells, respectively. Because our 111 cases did not include lung metastatic UC, we searched the literature for information about TTF1 expression in bladder cancer, including both primary and metastatic cases, and five articles were retrieved (Table 1). Matoso et al. [10] found that 5.1% (5/98) primary bladder UC were TTF1-positive (antibody clone 8G7G3/1 and SPT 24): 3/98 showed diffuse strong positive staining and 2/98 focal positive staining.

Fernández-Aceñero et al. [11] found 1 case out of 30 (3.3%) primary UC cases focally expressing TTF1 (antibody clone 8G7G3/1). Sotiriou et al. [12] reported 4 primary UCs (2 for low-grade and 2 for high-grade) that were weakly to moderately focally immunoreactivity for TTF1, as well as 1 metastatic UC with dense and diffuse immunoreactivity for TTF1, from 42 cases (antibody clone SPT 24). Kaufmann et al. [13] reported that TTF1 (antibody clone 8G7G3/1) was not expressed in 11 cases of metastatic UC; however, TTF1 (antibody clone SPT24) was expressed in 1 of 8 cases of metastatic UC reported by Halla Vidarsdottir [14] (the case was also positive for CK7, CK20, p40, p63, and GATA3).

To summarize the expression patterns for the 12 TTF1-positive UC (11 primary, 1 metastatic) cases reported above [10, 11, 13, 14]: 5/12 cases were of diffuse and strong positivity, the other 7 cases were focally immunoreactive. The reported expression rate ranged from 0 to 5.1% (antibody clone 8G7G3/1). The positive rate of TTF1 (antibody clone 8G7G3/1) in our cases was 2.70% (3/111), 1/111 was diffuse and strongly positive, and 2/111 were focal.
positive. However, Sotiriou et al. [12] reported that the positive rate of TTF1 (antibody clone SPT 24) was as high as 11.9% (5/42). We speculate that the clone number of the antibody leads to the difference in the results, and Halla Vidarsdottir et al. [8] confirmed this view: their results showed that the SPT24 clone was less specific but more sensitive for the detection of lung AC compared with the 8G7G3/1 clone. Therefore, when using TTF1 to distinguish primary lung AC from non pulmonary tumors, pathologists should pay attention to the antibody clone in use.

GATA3 is a sensitive and specific marker of breast and bladder cancer [5]. Pathologists routinely use it as part of screening panels for metastatic tumors of unknown origin, especially when suspecting metastatic breast or UC. Previous studies have reported that the sensitivity of GATA3 to UC is 80% or higher, regardless of primary or metastatic UC [5, 12-17] (Table 2). The staining result of our cases was similar to those reported above: the total expression rate of GATA3 in UC was 81.1% (90/111).

Research by Markku Miettinen and colleagues [16] showed that GATA3 was also a useful marker for renal and germ cell tumors, mesotheliomas, and paragangliomas. The results also showed that a few lung SCCs and ACs express GATA3 (antibody clone L50-823), with the expression rates of 12% (9/74) and 8% (6/71), respectively. Halla Vidarsdottir et al. [14] reported similar results: the expression rate of GATA3 (antibody clone L50-823) in lung SCC was 20% (40/202), and it was 2% (9/431) in lung ACs. Although Liu Haiyar et al. [5] (antibody clone sc-268) reported lung SCC (49 cases) and ACs (61 cases) that expressed GATA3, Alex Chang et al. [15] (antibody clone L50-823) reported that lung SCC (25 cases) did not express GATA3. None of our lung SCCs (47 cases) or ACs (55 cases) expressed GATA3 (antibody clone L50-823).

These results, including the above-mentioned and ours, were based on tissue microarrays. However, based on resected lung ACs from 95 cases, the results of Toshihiro Hashiguchi et al. [17] showed that 70 cases presented GATA3 (antibody clone D13C9) expression in at least one field, and 49 cases (51.6%) were classified as having a high GATA3 expression group based on the 6.8% threshold. Survival analysis showed that high GATA3 expression was associated with worse overall survival and disease-free survival compared with low GATA3 expression.

Therefore, the differential expression of GATA3 may be related to the antibody clone number, the selected sample (tissue microarray or surgical sample) and the cutoff value used by different authors. Pathologists should pay attention to the above aspects in their daily work.

In summary, most UC does not express TTF1, and the majority of NSCLC does not express GATA3, except for a few cases. In other words, very few primary and metastatic UCs express TTF1, and few primary lung ACs and SCCs also express GATA3. Therefore, in most cases, TTF1 and GATA3 are extremely useful markers that can be used to distinguish NSCLC from metastatic UC, but in rare cases, pathologists need to synthesize the results of clinical, imaging or other immunohistochemical markers to make final diagnoses (distinguish primary lung carcinoma from metastatic UC).

Based on the Report from the International Society of Urologic Pathology Consensus Conference, positivity for GATA3, CK20, and either high-molecular weight cytokeratin (HMWCK) or cytokeratin (CK) 5/6 is of value in proving urothelial differentiation in the appropriate morphologic and clinical context [18, 19]. Our case expressed GATA3, HMWCK and CK5/6 (suggesting urothelial differentiation), and combined with the morphological and clinical characteristics, we finally made the diagnosis of lung metastasis of UC. Unfortunately, the patient refused all treatment and died five months later.

Disclosure of conflict of interest
None.

Address correspondence to: Drs. Ge Wang and Chuan Chen, Cancer Center, Daping Hospital, Army Medical University, No. 10 Changjiang Zhilu, Yuzhong District, Chongqing 400042, China. Tel: +86-23-68757171; Fax: +86-23-68757161; E-mail: wangge70@hotmail.com (GW); sinkriver@126.com (CC)

References
TTF1 and GATA3 in urothelial and lung carcinomas


