Correlation of serum thyroglobulin and anti-thyroglobulin antibody levels with pulmonary metastasis and bone metastasis in patients with thyroid cancer

Jun-Jun Tian1,2,*, Ran Tao1,2, Yin-Feng Shen1,2, Shao-You Xia3, Chen Li3

1. Department of General Surgery, Hubei Provincial Hospital of TCM, Wuhan 430061, China
2. Department of General Surgery, Hubei Academy of Traditional Chinese Medicine, Wuhan 430074, China
3. Department of General Surgery, Chinese PLA General Hospital, Beijing 100853, China

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ABSTRACT

Objective: To study the correlation of serum thyroglobulin (TG) and anti-thyroglobulin antibody (TGAb) levels with pulmonary metastasis and bone metastasis in patients with thyroid cancer. Methods: Patients with thyroid cancer who underwent surgical resection in our hospital were selected as the research subjects, and the patients without distant metastasis, with pulmonary metastasis and with bone metastasis were screened and enrolled in non-metastasis group, pulmonary metastasis group and bone metastasis group respectively. Serum was collected to determine the levels of TG and TGAb, and metastatic lesion tissue was collected to determine the expression proliferation-related molecules Bcl-2, Skp-2, caspase-3 and p27 as well as invasion molecules S100A4, MMP2, MMP13, SATB1 and Vimentin. Results: The positive rates of serum TGAb and TG of pulmonary metastasis group and bone metastasis group were significantly higher than those of non-metastasis group; S100A4, MMP2, MMP13, SATB1, Vimentin, Bcl-2 and Skp-2 mRNA contents in metastatic lesions of patients with positive serum TG and TGAb were significantly higher than those of patients with negative serum TG and TGAb while caspase-3 and p27 mRNA contents were significantly lower than those of patients with negative serum TG and TGAb. Conclusion: The increase of serum TG and TGAb contents is associated with the pulmonary metastasis and bone metastasis after thyroid carcinoma surgery, and has promoting effect on the proliferation and invasion of cancer cells in metastatic lesions.

1. Introduction

Thyroid cancer is the malignant head and neck tumor with the highest incidence in China, and the thyroid cancer detection rate is also rising with the development of ultrasound technology and fine needle aspiration biopsy technique in recent years. Papillary carcinomas and follicular carcinoma are the most common pathologic types of thyroid cancer and have high 5-year survival rate after surgery. In spite of this, some patients with thyroid cancer may have distant metastasis after surgery, and the bone metastasis and pulmonary metastasis are the most common[1,2]. At present, clinical indexes are still short for the evaluation of the risk of distant metastasis of thyroid cancer, and the diagnosis of distant metastasis mainly relies on imageological examination and pathological biopsy[3]. Thyroglobulin (TG) is the specific marker of thyroid tissue, the synthesis of TG and TGAb significantly reduce after surgical removal of the thyroid tissue, and the residual or distant metastasis of thyroid cancer can cause TG and TGAb synthesis maintained at a higher level[4,5]. In order to define the predictive value of serum TG and TGAb levels for postoperative distant metastasis of thyroid carcinoma, the correlation of serum thyroglobulin (TG) and anti-thyroglobulin antibody (TGAb) levels with pulmonary metastasis and bone metastasis in patients with thyroid cancer were analyzed in the following study.
2. Subjects and methods

2.1 Research subjects

Patients with thyroid cancer who underwent surgical resection in our hospital between February 2000 and February 2012 were selected as the research subjects, and all the patients received preoperative detection of serum thyroglobulin (TG) and anti-thyroglobulin antibody (TGAb) levels, received subtotal thyroidectomy and were diagnosed with thyroid cancer by postoperative pathology. Patients were followed up for 3 years after operation, and 108 patients without distant metastasis, 34 patients with pulmonary metastasis and 27 patients with bone metastasis were screened and enrolled in non-metastasis group, pulmonary metastasis group and bone metastasis group respectively. Non-metastasis group included 62 male cases and 46 female cases that were 40-57 years old; pulmonary metastasis group included 20 male cases and 14 female cases that were 42-54 years old; bone metastasis group included 15 male cases and 12 female cases that were 39-56 years old. There was no significant difference in the general data among the three groups.

2.2 Serum TG and TGAb level evaluation

5 mL peripheral blood sample was collected from three groups of patients after operation and centrifuged to get serum specimens, radioimmunoprecipitation kits were used to determine serum levels of TG and TGAb, and the following standard was referred to judge the contents of TG and TGAb: TG > 10 μg/L was judged as positive, and TGAb > 40 U/mL was judged as negative.

2.3 Metastatic lesion tissue collection and molecule detection

After imageological examination confirmed pulmonary metastasis or bone metastasis, biopsy was conducted to confirm the nature of metastases, meantime metastatic lesion tissue was collected to extract RNA for fluorescence quantitative PCR amplification, the amplified genes included the Bcl-2, Skp-2, caspase-3, p27, S100A4, MMP2, MMP13, SATB1 and Vimentin, and the amplification curve was referred to calculate S100A4, MMP2, MMP13, SATB1 and Vimentin mRNA levels.

2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis was by t test, count data analysis was by chi-square test and \( P < 0.05 \) meant statistical significance in differences.

3. Results

3.1 Serum TGAb and TG levels

The number of cases with positive serum TGAb and TG of non-metastasis group were 17 cases (15.74%) and 22 cases (20.37%) respectively, the number of cases with positive serum TGAb and TG of pulmonary metastasis group were 19 cases (55.88%) and 22 cases (64.71%) respectively, and the number of cases with positive serum TGAb and TG of bone metastasis group were 16 cases (59.26%) and 19 cases (70.37%) respectively. After chi-square test, the positive rates of serum TGAb and TG of pulmonary metastasis group and bone metastasis group were significantly higher than those of non-metastasis group.

3.2 Invasion molecule expression in metastatic lesions of patients with different serum TGAb and TG levels

Analysis of S100A4, MMP2 and MMP13 as well as SATB1 and Vimentin expression in metastatic lesions of patients with different serum TGAb and TG levels was shown in Table 1: S100A4, MMP2, MMP13, SATB1 and Vimentin mRNA contents in metastatic lesions.

<table>
<thead>
<tr>
<th>TGAb level</th>
<th>n</th>
<th>S100A4 pathway</th>
<th>MMP2 pathway</th>
<th>MMP13 pathway</th>
<th>SATB1 pathway</th>
<th>Vimentin pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>35</td>
<td>2.35±0.31</td>
<td>1.97±0.25</td>
<td>2.16±0.23</td>
<td>2.64±0.30</td>
<td>1.78±0.16</td>
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<tr>
<td>Negative</td>
<td>26</td>
<td>1.06±0.17</td>
<td>0.98±0.11</td>
<td>0.94±0.12</td>
<td>1.04±0.14</td>
<td>1.02±0.13</td>
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<td>T</td>
<td>13.582</td>
<td>&lt;0.05</td>
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<table>
<thead>
<tr>
<th>TG level</th>
<th>n</th>
<th>S100A4 pathway</th>
<th>MMP2 pathway</th>
<th>MMP13 pathway</th>
<th>SATB1 pathway</th>
<th>Vimentin pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>41</td>
<td>2.39±0.34</td>
<td>2.05±0.24</td>
<td>1.92±0.20</td>
<td>2.79±0.35</td>
<td>1.89±0.17</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>1.03±0.16</td>
<td>0.93±0.11</td>
<td>1.06±0.14</td>
<td>0.99±0.11</td>
<td>0.97±0.13</td>
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<td>T</td>
<td>14.128</td>
<td>&lt;0.05</td>
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of patients with positive serum TGAb were significantly higher than those of patients with negative serum TGAb; analysis of S100A4, MMP2 and MMP13 as well as SATB1 and Vimentin expression in metastatic lesions of patients with different serum TG levels was shown in Table 2: S100A4, MMP2, MMP13, SATB1 and Vimentin mRNA contents in metastatic lesions of patients with positive serum TG were significantly higher than those of patients with negative serum TG. Differences in S100A4, MMP2, MMP13, SATB1 and Vimentin mRNA contents in metastatic lesions were statistically significant between patients with different serum TGAb and TG levels ($P<0.05$).

### 3.3 Proliferation molecule expression in metastatic lesions of patients with different serum TGAb and TG levels

Analysis of Bcl-2, Skp-2, caspase-3 and p27 expression in metastatic lesions of patients with different serum TGAb levels was shown in Table 3: Bcl-2 and Skp-2 mRNA contents in metastatic lesions of patients with positive serum TGAb were significantly higher than those of patients with negative serum TGAb while caspase-3 and p27 mRNA contents were significantly lower than those of patients with negative serum TGAb; analysis of Bcl-2, Skp-2, caspase-3 and p27 expression in metastatic lesions of patients with different serum TG levels was shown in Table 4: Bcl-2 and Skp-2 mRNA contents in metastatic lesions of patients with positive serum TG were significantly higher than those of patients with negative serum TG while caspase-3 and p27 mRNA contents were significantly lower than those of patients with negative serum TG. Differences in Bcl-2, Skp-2, caspase-3 and p27 mRNA contents in metastatic lesions were statistically significant between patients with different serum TGAb and TG levels ($P<0.05$).

### 4. Discussion

The common pathologic types of thyroid cancer are papillary carcinoma and follicular carcinoma, and they are with better long-term prognosis and higher survival rate after surgical resection. In spite of this, there are still some patients with thyroid cancer who will develop distant metastasis in organs and tissues within five years after operation[6,7]. At present, there is still a shortage of effective indicators to predict the distant metastasis of thyroid cancer. Thyroglobulin (TG) is the main component of thyroid follicular cells and extracellular matrix, is strongly tissue-specific, and is not expressed in non-thyroid tissue[8]. The TG generation significantly decreases, and serum TG level significantly reduces after patients with thyroid cancer receive surgical resection; if the thyroid carcinoma tissue is not completely removed, there will be thyroid cancer stem cell residual in the remaining thyroid tissue, which can cause abnormal TG synthesis, and also stimulate the production of autoantibodies TGAb[9,10]. In order to define the value of serum TG and TGAb contents for postoperative distant metastasis of thyroid carcinoma, the relationship of distant metastasis of thyroid cancer with serum TG and TGAb contents were analyzed in the study, and the results showed that the positive rates of serum TGAb and TG of pulmonary metastasis group and bone metastasis group were significantly higher than those of non-metastasis group. This indicates that the continued positive expression of TG and TGAb in serum after thyroid cancer resection is closely related to the postoperative long-term distant metastasis of thyroid carcinoma.

Thyroid cancer cell metastasis to the distance depends on a variety of migration and invasion-related molecules, and the biological pathways mediated by S100A4 and SATB1 have been proven to be closely associated with thyroid cancer cell invasion. S100A4 is a member of the S100 protein family, which has the double helix structure of the EF, and can be targeted to regulate a variety of matrix metalloproteinases (MMPs) to promote the degradation of extracellular matrix and basement membrane. MMP9 and MMP13 are two MMPs controlled by the S100A4, and the high expression of the S100A4 in the thyroid cancer cells can increase the expression of MMP9 and MMP13 and promote cell invasion[11,12]. SATB1 is a transcription factor that contains two CUT sequences and can form similar PDZ domain, which regulates the growth and differentiation of cells; in thyroid cancer cells, PDZ can be targeted to adjust the expression of mesenchymal marker molecule Vimentin, promote the
cellular epithelial-mesenchymal transition, and make the cells obtain strong invasion ability[13,14]. In the study, analysis of the expression of above invasion molecules in thyroid carcinoma metastases proved that S100A4, MMP2, MMP13, SATB1 and Vimentin mRNA contents in metastatic lesions of patients with positive serum TG and TGAb were significantly higher than those of patients with negative serum TG and TGAb. This indicates that the elevated serum TG and TGAb levels in patients with thyroid cancer after operation are associated with enhancement of thyroid cancer invasion ability.

After distant metastasis of thyroid cancer, the cancer cells in metastatic lesions are with strong proliferation ability, and the constant cell proliferation can result in the constant development of metastatic lesions. The proliferation of cancer cells is regulated by a variety of anti-apoptotic and pro-apoptotic molecules, and the increased expression of anti-apoptotic molecules and the decreased expression of pro-apoptotic molecules can promote cell proliferation. The Bcl-2 is a very important molecule that regulates mitochondrial apoptosis, which can close the transition pore on the mitochondrial membrane and reduce the release of cytochrome C so as to inhibit the activation of apoptotic executive molecule caspase-3[15]; Skp2 is a necessary factor for cell cycle progression from G1 phase to S phase, which can antagonize the negative cell cycle-regulating effect mediated by tumor suppression gene p27, and then promote the cell cycle process and cell proliferation[16]. In the study, analysis of the expression of above proliferation-related molecules showed that Bcl-2 and Skp-2 mRNA contents in metastatic lesions of patients with positive serum TG and TGAb while caspase-3 and p27 mRNA contents were significantly lower than those of patients with negative serum TG and TGAb. This indicates that the elevated serum TG and TGAb levels in patients with thyroid cancer after operation are associated with the enhancement of cell proliferation capacity in thyroid cancer metastases.

To sum up, the elevated serum TG and TGAb levels are associated with postoperative pulmonary metastasis and bone metastasis of thyroid carcinoma, and have promoting effect on the cancer cell proliferation and invasion in the metastatic lesions.

References


