Original Article

Mining expression and prognosis of TACSTD2 and SPP1 in thyroid carcinoma by using Oncomine and Kaplan-Meier plotter databases

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Abstract: Objective: To further investigate TACSTD2 and SPP1 gene mRNA expression in thyroid carcinoma and their association with patients’ prognosis and clinical pathological characteristics by data mining of the Oncomine and Kaplan-Meier plotter databases. Methods: TACSTD2 and SPP1 mRNA expression data from thyroid carcinoma were retrieved from the Oncomine database and further analyzed by comparing tumor tissue with corresponding normal tissue. The prognostic significance of TACSTD2 and SPP1 mRNA in thyroid carcinoma were evaluated by the online survival analysis tool “Kaplan-Meier Plotter” (KM plotter) database. Fifty-one patients with thyroid carcinoma who received surgery were retrospectively analyzed. The TACSTD2 and SPP1 protein expression in cancer tissue and normal thyroid tissue were examined by immunohistochemical assay. The correlation between TACSTD2, SPP1 protein expression and patients’ clinicopathological characteristics were also evaluated. Results: Four analyses compared the TACSTD2 gene expression between thyroid cancer and normal tissue were included in the present work. The pooled results showed that TACSTD2 mRNA expression levels in thyroid carcinoma tissue was significantly higher than that of normal thyroid tissue (p=0.003). For SPP1, the combined analysis from 8 analyses demonstrated that SPP1 mRNA level in thyroid carcinoma was up regulated compared to normal tissue with statistical significance (p<0.05). A significant difference of overall survival (OS) between TACSTD2 high and low expression groups was found in thyroid carcinoma patients (HR=0.17, 95% CI: 0.04-0.74, p=0.0078). However, for the SPP1 gene the difference was not statistically different between high and low mRNA expression groups (HR=0.062, 95% CI: 0.23-1.71, p=0.35). TACSTD2 interacted with TGFBI, MAP3K11, RAB13, MMP2, CHD1, KRT19, KRTAP5-2, ZNF92, C1QBP, and CYP8B1, which are related to cell proliferation and cellular protein metabolic pathways. The protein coded by SPP1 interacted with FGF2, RUNX2, IGF1, INS, VEGFA, IL6, MMP13, PTH, SP7, and BMP2, which are related to epithelial cell to mesenchymal cell transition (EMT), cell proliferation and negative regulation of apoptotic processes. Immunohistochemical analysis showed that TACSTD2 positive expression was correlated with the tumor capsular invasion (p<0.05) and lymph node metastasis (p<0.05). SPP1 positive expression was correlated with lymph node metastasis (p<0.05). Conclusion: TACSTD2 and SPP1 mRNA expression were up-regulated in cancer tissue compared to normal thyroid tissue in thyroid carcinoma. High level TACSTD2 mRNA was associated with poor prognosis.

Keywords: Data mining, TACSTD2, SPP1, prognosis, thyroid carcinoma

Introduction

Thyroid cancer is one of the most commonly diagnosed head and neck malignant tumors [1]. Although the incidence of thyroid cancer accounts for only 5% of thyroid nodules and 1% of total solid malignant tumors, it is the most common cancer in the endocrine system [2]. At present, the molecular mechanism of thyroid cancer is not fully understood. Most studies believe that it is related to the genetic background and acquired environmental factors of the patients [3]. The genetic background includes family history of malignant tumors, genetic polymorphism of some metabolic enzymes [4-6]. The acquired factors are mainly related to radiation [7], abnormal iodine uptake and other factors [8, 9]. Early thyroid cancer patients can be treated by surgery, and the prognosis is good. The 5-year survival rate after operation can reach more than 90%, but the prognosis of patients with advanced disease is poor. Th-
Therefore, it is particularly important to explore the prognostic factors of thyroid cancer.

Transmembrane glycoprotein TACSTD2, also known as Trop-2, is an important signal transduction molecule regulating the growth of cancer cells [10]. Recent studies have shown that TACSTD2 is overexpressed in breast cancer, non-small cell lung cancer, head and neck squamous cell carcinoma and other malignant solid tumors [11, 12]. It plays an important role in the proliferation and invasion of tumor cells, and is associated with metastasis and poor prognosis of tumors. The SPP1 gene located on chromosome 4q13, is a secretory phosphorylated glycoprotein with various biological activities. It can promote cell chemotaxis, adhesion, migration, proliferation and induce angiogenesis, promote the degradation of the extracellular matrix, mediate the escape of cancer cells, inhibit apoptosis of cancer cells, stimulate signal transduction pathways, and it is closely related to the migration and metastasis of cancer cells [13, 14].

However, the correlation between both TACSTD2 and SPP1 expression and thyroid carcinoma in patient prognosis is not completely clear. In our present work, we evaluated the correlation between TACSTD2, SPP1 expression and thyroid carcinoma patient prognosis by deep data mining of the Oncomine and Kaplan-Meier plotter databases.

Material and methods

Study design

We first analyzed the expression of TACSTD2 and SPP1 mRNA in human normal thyroid tissue in BioGPS (http://biogps.org/goto=welcome) database, and then analyzed the differential expression of TACSTD2 and SPP1 mRNA between cancer tissue and normal tissue of thyroid carcinoma patients in Oncomine (https://www.oncomine.org/) database of oncogene expression chip. The expression of TACSTD2 and SPP1 was divided into two groups, and the prognosis of patients with TACSTD2 and SPP1 high and low expression was evaluated through the Kaplan-Meier Plotter (http://kmplot.com/analysis/) database. The protein interaction database STRING (https://string-db.org/cgi/input.pl) was used to predict the protein-protein interaction network for TACSTD2 and SPP1. The correlation between TACSTD2 and SPP1 mRNA expression and clinical pathological characteristics of the patients were evaluated by immunohistochemical assay.

Data extraction from oncomine database and analysis

Oncomine database (https://www.oncomine.org/) is a gene web-based database and integrated data mining platform, in which data can be screened and mined according to need. From the Oncomine database, we retrieved TACSTD2 and SPP1 related data sets with the following restrictions: (1) tumor type: thyroid carcinoma; (2) tissue comparison: normal thyroid tissue vs thyroid carcinoma tissue; (3) data type: mRNA; (4) significance: P<0.0001; (5) Gene fold change: >2; (6) gene sequencing: the first 10%.

Survival analysis by Kaplan-Meier plotter

The prognosis (overall survival, OS) of TACSTD2 and SPP1 mRNA expression of thyroid carcinoma patients was analyzed by Kaplan-Meier plotter (www.kmplot.com). This database provides the online survival analysis of lung cancer, ovarian cancer, gastric cancer and breast cancer. In order to evaluate prognostic significant of TACSTD2 and SPP1 expression in thyroid cancer patients’ survival, we divided the patient data into two cohorts according to the median TACSTD2 and SPP1 mRNA expression (high versus low expression). The overall survival of the thyroid carcinoma patients were compared between TACSTD2 and SPP1 high and low expression groups.

Immunohistochemical assay

Fifty one cases of thyroid carcinoma who received surgery were retrospectively analyzed. The TACSTD2 and SPP1 protein expression in cancer tissue and normal thyroid tissue were examined by immunohistochemical assay. Paraffin sections were dewaxed and hydrated. Antigen was repaired after soaking and rinsing in distilled water. Sections were rinsed in PBS and dripped with non-immune serum. After incubation at room temperature for 10 minutes, the antigen was washed off. Antibody incubation was done at room temperature for
Figure 1. TACSTD2 and SPP1 mRNA relative expression in thyroid tissue from human beings (A: TACSTD2 mRNA relative expression level was 5.20; B: SPP1 mRNA relative expression level was 8.10).
60 minutes. The negative control was replaced by PBS. After PBS was washed, biotin-labeled antibodies were dripped on and incubated at room temperature for 10 minutes. PBS was rinsed and dripped with Streptomyces antibiot ic-peroxidase solution, incubated at room temperature for 30 minutes. After PBS wash, DAB color developer was applied, and sealed with neutral gum.

**Statistical analysis**

Data was analyzed by Stata 11.0 software. The measurement data was expressed by $X \pm s$ and compared by student-t test. The counted data were expressed by rate and compared by $\chi^2$-test. A proportional hazard ratio was used for survival analysis through a log-rank test and survival curve. P<0.05 was considered statistically different.

**Results**

**TACSTD2 and SPP1 mRNA relative expression**

TACSTD2 and SPP1 mRNA relative expression level of normal thyroid tissue from human beings were screened from the BioGPS database (http://biogps.org/#goto=welcome). The relative TACSTD2 and SPP1 mRNA expression levels were 5.20 and 8.10 from normal thyroid tissue in human beings, Figure 1.

**TACSTD2 and SPP1 mRNA expression in multiple cancers**

Four hundred forty-nine and 431 studies were included in the Oncomine database from multiple types of cancers, comparing cancer versus normal tissue for TACSTD2 and SPP1 mRNA expression, respectively (Figure 2). Four analy-
Comparison of TACSTD2 Across 4 Analyses

<table>
<thead>
<tr>
<th>Median Rank</th>
<th>p-Value</th>
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</thead>
<tbody>
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<td>69.5</td>
<td>2.35E-6</td>
<td>TACSTD2</td>
</tr>
</tbody>
</table>

Legend
1. Tall Cell Variant Thyroid Gland Papillary Carcinoma vs. Normal
2. Thyroid Gland Papillary Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006
3. Thyroid Gland Papillary Carcinoma vs. Normal
   He Thyroid, Proc Natl Acad Sci U S A, 2005
4. Thyroid Gland Papillary Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006

Comparison of SPP1 Across 8 Analyses

<table>
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</table>

Legend
1. Follicular Variant Thyroid Gland Papillary Carcinoma vs. Normal
2. Tall Cell Variant Thyroid Gland Papillary Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006
3. Thyroid Gland Follicular Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006
4. Thyroid Gland Oncocytic Follicular Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006
5. Thyroid Gland Papillary Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006
6. Thyroid Gland Undifferentiated (Anaplastic) Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006
7. Thyroid Gland Papillary Carcinoma vs. Normal
   He Thyroid, Proc Natl Acad Sci U S A, 2005
8. Thyroid Gland Papillary Carcinoma vs. Normal
   Giordano Thyroid, Clin Cancer Res, 2006

Figure 3. TACSTD2 and SPP1 mRNA expression in thyroid carcinoma comparing cancer tissue to normal tissue.
TACSTD2 and SPP1 expression in thyroid carcinoma

The pooled results showed that TACSTD2 mRNA expression level in thyroid carcinoma tissue was significant higher than that of normal thyroid tissue (p<0.05), Figure 3. For SPP1, the combined analysis from 8 analysis (3 studies) [15-17] demonstrated that SPP1 mRNA level in thyroid carcinoma was up regulated compared to normal tissue with statistical difference (p<0.05), Figure 3.

**TACSTD2 and SPP1 mRNA expression and prognosis**

The prognostic significance of TACSTD2 and SPP1 mRNA expression and thyroid carcinoma patient prognosis were analyzed by Kaplan-Meier Plotter. A significant difference of overall survival (OS) between TACSTD2 high and low expression groups was found in thyroid carcinoma patients (HR=0.17, 95% CI: 0.04-0.74, p=0.0078). However, for the SPP1 gene the difference was not statistically different between high and low mRNA expression groups (HR=0.062, 95% CI: 0.23-1.71, p=0.35), Figure 4.

**TACSTD2 and SPP1 protein-protein interaction networks**

The possible protein-protein interaction of TACSTD2 and SPP1 were analyzed through the STRING database. The results showed that TACSTD2 interacted with TGFB1, MAP3K11, RAB13, MMP2, CHD1, KRT19, KRTAP5-2, ZNF92, C1QBP, and CYP8B1, etc. (Figure 5). Gene ontology (GO) analysis showed that most of the above proteins were related to cell proliferation and cellular protein metabolic processes. The protein coded by SPP1 interacted with FGF2, RUNX2, IGF1, INS, VEGFA, IL6, MMP13, PTH, SP7, BMP2 and etc. (Figure 6). Most of these proteins are related to the epithelial cell to mesenchymal cell transition (EMT), cell proliferation and negative regulation of apoptotic processes.

**Immunohistochemical analysis**

The positive expression rate of TACSTD2 in cancer tissue was significantly higher than that of normal thyroid tissue (62.7% vs 15.7%, p<0.05). For SPP1, the positive expression rate in thyroid cancer tissue and normal tissue were 68.6% and 23.5%, respectively, with significant statistical difference (p<0.05), Table 1.

**TACSTD2 and SPP1 expression and patient clinicopathological features**

TACSTD2 positive expression was correlated with tumor capsular invasion (p<0.05) and lymph node metastasis (p<0.05). SPP1 positive expression was correlated with lymph node metastasis (p<0.05). However, TACSTD2 and SPP1 expression was not correlated with age, gender, pathology type nor prognostic index (all p>0.05), Table 2.
Discussion

Thyroid cancer accounts for more than 90% of human endocrine gland malignant tumors [19]. The International Organization for Cancer estimates that in 2010, there were about 44,670 new cases of thyroid cancer worldwide and about 1,690 deaths from this disease. Globally, 122,800 new cases of differentiated thyroid cancer are diagnosed each year, the majority of which occur in young adults, with annual increase of about 4% [20].

TACSTD2, tumor associated calcium signal transducer 2, also known as Trop2 gene, is 1.8 Kb in length and encodes 323 amino acids [21]. TACSTD2 protein is a phosphorylated glycoprotein with a single transmembrane region on the cell surface. It contains an EGF-like repeat region, a thyroglobulin-like repeat region and a transmembrane region. There are serine and tyrosine phosphorylation sites and a PIP2 binding sequence at the carboxyl end. Recent studies have found that TACSTD2 is highly expressed in various epithelial cancers, but very low or not expressed in normal epithelial tissues [11, 12, 22]. Overexpression of TACSTD2 can promote the proliferation, and invasion of some cancer cells, including ovarian cancer. Conversely, silencing the TACSTD2 gene can inhibit the gr-
TACSTD2 and SPP1 expression in thyroid carcinoma

The median overall survival of thyroid carcinoma patients with elevated TACSTD2 levels were significantly lower than that of lower TACSTD2 level cases. This indicated that TACSTD2 mRNA level can potentially be a biomarker for poor prognosis of thyroid carcinoma patients.

The SPP1 encoding protein is a chemokine-like protein, rich in sialic acid and secreting non-collagen type. It is a phosphorylated acidic glycoprotein containing arginine, glycine, and aspartic acid (RGD) residues. SPP1 encoding protein is associated with the occurrence, development, metastasis and prognosis of various tumors [25, 26]. In our work, we found that SPP1 mRNA levels in thyroid carcinoma was up regulated compared to normal tissue. The median overall survival of thyroid carcinoma patients with elevated TACSTD2 levels were significant lower than that of lower TACSTD2 level cases. This indicated that TACSTD2 mRNA level can potentially be a biomarker for poor prognosis of thyroid carcinoma patients.

### Table 1. TACSTD2 and SPP1 protein expression in thyroid carcinoma (n, %)

<table>
<thead>
<tr>
<th></th>
<th>TACSTD2</th>
<th>SPP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer tissue</td>
<td>32 (62.7)</td>
<td>35 (68.6)</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>8 (15.7)</td>
<td>12 (23.5)</td>
</tr>
<tr>
<td>Chi-square</td>
<td>15.83</td>
<td>20.87</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
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</table>

Figure 6. Protein-protein interactions of SPP1.

The protein-protein interactions of SPP1 are shown in the network diagram.
TACSTD2 and SPP1 expression in thyroid carcinoma

Table 2. Correlation between TACSTD2, SPP1 protein expression and patient clinical pathological feature

<table>
<thead>
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<th>TACSTD2</th>
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<td>χ²</td>
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<td>Female</td>
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</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>12</td>
<td>1.15</td>
<td>0.28</td>
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<tr>
<td>Age (year)</td>
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<tr>
<td>&lt;45</td>
<td>30</td>
<td>17</td>
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<td>≥45</td>
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<td>15</td>
<td>4.19</td>
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<td>Papillary</td>
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<tr>
<td>Follicular</td>
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<td>11</td>
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<tr>
<td>Tumor capsular invasion</td>
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<tr>
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<td>Negative</td>
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<td>≥66</td>
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<td>Lymph node metastasis</td>
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<tr>
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<td>18</td>
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normal tissue with statistical differences. This indicated that SPP1 may play an important role in the development of thyroid carcinoma.

Conclusion

In our present work, we investigate TACSTD2 and SPP1 gene mRNA expression in thyroid carcinoma and their association with patient prognosis and clinical pathological outcome by data mining of the Oncomine and Kaplan-Meier plotter databases. According to the present evidence, TACSTD2 and SPP1 mRNA expression levels were up-regulated in cancer tissue compared to normal thyroid tissue in thyroid carcinoma. High level TACSTD2 mRNA was associated with poor prognosis.

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TACSTD2 and SPP1 expression in thyroid carcinoma


