Inhibition of Infiltration and Angiogenesis by Thrombospondin-1 in Papillary Thyroid Carcinoma

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ABSTRACT

Purpose: Angiogenesis is essential for tumor growth and is controlled by the balance between angiogenic and antiangiogenic factors. We studied the expression of angiogenic factors and antiangiogenic factors in papillary thyroid carcinoma.

Experimental Design: We investigated immunohistochemically the expression patterns and levels of angiogenic factor and its receptor, thrombospondin-1 (TSP-1) and CD36, and four angiogenic factors, vascular endothelial growth factor (VEGF), VEGF-C, angiopoietin-2 (Ang-2), and Tie-2, in the primary tumors of 75 papillary thyroid carcinoma patients. We also examined the microvessel count (MVC), using CD31 staining.

Results: VEGF expression strongly correlated with other angiogenic factors. The cytoplasm of cancer cells stained positive for all factors. Tie-2 and TSP-1 receptor also appeared in endothelia of microvessels. TSP-1 inversely correlated with the degree of invasion of the primary tumor to other adjacent organs and with MVC. A higher MVC correlated with poorer survival. To clarify the balance between angiogenic and antiangiogenic factors in the same tumor, we calculated the ratio of each angiogenic factor against TSP-1 as the antiangiogenic factor. The ratios VEGF/TSP-1, VEGF-C/TSP-1, and Ang-2/TSP-1 significantly correlated with a higher MVC. Furthermore, the ratios VEGF/TSP-1 and Ang-2/TSP-1 significantly correlated with the degree of infiltration.

Conclusions: To the best of our knowledge, this is the first report demonstrating that the balance between angiogenic and antiangiogenic factors correlates with distinct invasion to other organs and neovascularization of papillary thyroid carcinoma.

INTRODUCTION

Angiogenesis is essential for tumor growth and progression (1). Among many angiogenic factors, VEGF3 is considered the most important (2). Ang-2 is an angiogenic factor that binds to the Tie-2 receptor with an antagonistic effect for binding of Ang-1 and Tie-2 (3). Recently, Ang-2 has been reported to play a key role in initiating neovascularization before activation of VEGF (4, 5). Holash et al. (5) have reported that induced Ang-2 destabilized endothelium, smooth muscle, and the extracellular matrix and that VEGF led these unstable vessels to angiogenesis.

Antiangiogenic factors have also been identified, including TSP-1 (6), TSP-2 (7), angiotatin (8), endostatin (9), and vasostatin (10). TSP-1 is a high molecular weight protein and is recognized as a mediator of the components of the extracellular matrix (11). TSP-1 binds to some receptors, such as D51/CD61 (αβ integrin; Ref. 12). The Val-Thr-Cys-Gly sequence binds to CD36, so-called platelet GPIIb (13), and the COOH-terminal domain binds to a $M_\text{r}$ 80,000/105,000 receptor (14) to mediate each cell. TSP-1 is an antiangiogenic factor (15), but its biological role is still controversial. The antiangiogenic effects of TSP-1 have been reported in bladder cancer (16), squamous cell carcinoma (17, 18), breast cancer (19), and gastrointestinal tumors (19). However, TSP-1 has also been reported to be an angiogenic factor in cancers of the colon and rectum (20), pancreas (21), and breast (22) and a lymphangiogenic factor in gallbladder cancer (23). In addition, TSP-1 has been demonstrated to be an inhibitor of adhesion activity in breast cancer cells and a promoter of infiltration of cancer cells (24). Other studies have demonstrated that TSP-1 is an antitumor factor in melanoma (25) and small cell lung carcinoma (26), independent of angiogenesis. Thus, TSP-1 has been demonstrated to play diverse roles in each organ. CD36 and CD51 have also been investigated in some carcinoma tissues. CD36 has been demonstrated on breast cancer cells (22, 27, 28), cutaneous squamous cells (17), and colorectal cancer cells (29). Some authors observed no expression of CD36 on thyroid (30) or colon (31) cancer cells, but expression of CD51 has been demonstrated on breast (27) and thyroid (30) cancer cells. The patterns of expression vary in each organ, and the relationship between clinical data and the expression of CD36 was further investigated. We therefore investigated the effect of TSP-1 on cells through CD36 or CD51 and studied the expression levels of CD36.

In this study, we examined immunohistochemically the
expression patterns and levels of TSP-1 and CD36 in addition to four angiogenic factors, VEGF, VEGF-C, Ang-2, and Tie-2, in the primary tumors of papillary thyroid carcinoma patients. To clarify the roles of these factors in papillary thyroid carcinoma, we studied the correlation among the angiogenesis-related factors and the relationship between their expression and clinical data.

**MATERIALS AND METHODS**

The subjects of this study were 25 recurrent papillary thyroid carcinoma patients with distant metastases and 50 non-recurrent patients who were treated in our hospital. As disease-free patients, we selected at random two nonrecurrent patients for each recurrent patient matched according to age, sex, the TNM classification of the postoperative diagnosis, and the surgical method used. All patients were euthyroid before primary operations. All patients had received thyroxin to suppress serum thyroid-stimulating hormone during follow-up after the initial surgical treatment. The dose of thyroxin necessary to maintain the serum thyroid-stimulating hormone level under the lower limit for each patient was determined by measuring the level every 6 months during follow-up. Distant recurrence of disease was identified by echography, chest X-rays, computed tomography, and/or thallium or radioiodine scintigraphy during follow-up.

For this study, we used embedded paraffin sections of the primary tumor from each patient, which were resected at each initial surgical procedure. Informed consent was obtained from all enrolled patients. We determined the clinical stage and TNM classification of the primary tumor according to the International Union Against Cancer classification (32).

**Immunohistochemical Procedures.** The paraffin-embedded sections were cut into sections 4 µm thick and fixed on aminopropyltriethoxysilane-coated glass slides (Matsunami, Osaka, Japan). After deparaffinization by standard methods, normal rabbit serum was applied, and the sections were incubated for 10 min at room temperature to block nonspecific binding of the antibody. The slides were then incubated for 2 h at room temperature with each of the following primary antibodies: VEGF antibody, a mouse monoclonal IgG2a antibody (sc-7269; Santa Cruz Biotechnology, Santa Cruz, CA), at a concentration of 10 µg/ml; VEGF-C antibody, a mouse polyclonal antibody (sc-9047; Santa Cruz Biotechnology), at a concentration of 20 µg/ml; Ang-2 antibody, a goat polyclonal antibody (sc-7016; Santa Cruz Biotechnology), at a concentration of 10 µg/ml; and Tie-2 antibody, a rabbit polyclonal antibody (sc-324; Santa Cruz Biotechnology), at a concentration of 4 µg/ml. CD31 antibody (Clone JC/70A, mouse monoclonal antibody; Neo Markers, Fremont, CA) was used to determine the intratumoral MVC. TSP-1 monoclonal antibody (MAB054; Chemicon International, Inc., CA) was used at 2 µg/ml, and CD36 monoclonal antibody (MAB1204; Chemicon, Temecula, CA) was used at a 200-fold dilution. After incubation, the slides were washed in PBS for 15 min, and each secondary antibody (mouse, rabbit, and goat) was applied, using the LSAB kit (DAKO Corp., Carpinteria, CA) according to the manufacturer’s protocol. After washing, the color was developed with 5-bromo-4-chloro-3-indoxyl phosphate and nitroblue tetrazolium chloride (DAKO). The slides were then washed in distilled water for 5 min and mounted.

We used normal mouse serum instead of primary antibody in control studies, which were performed for all slides (data not shown).

**Analysis of Immunohistochemical Detection.** Immunostaining was evaluated by two repeated stainings of the same specimen. We blinded ourselves to the characteristics of the patients, the extent of the tumor, and prognostic scores. Cells in each tissue section were evaluated as positive when they showed a distinctly specific stain when compared with the cells of negative control sections. Two investigators then independently decided the level of intensity of positive cells of each slide. Only in the case of discordance between the investigators did they discuss the results and together decide the intensity. For these immunohistochemical analyses, except for that of CD31, we evaluated both the intensity and distribution of stained cells in each section. To evaluate the intensity of staining, we divided the stained cells in the largest population of the section into four categories based on degree of staining (from 0 to 3). To evaluate the distribution of stained cells, the sections were divided into four categories based on percentage of stained cells: 0 (no stained cells present), 1 (<30% of the entire population of cells stained), 2 (<60% of the population stained), and 3 (≥60% of the population stained). Multiplication of both scores produced a final score ranging from 0 to 9; the results are presented as the mean ± SD.

The MVC was determined in CD31-positive vessels. Initially, the areas with the highest vascularity in a section were detected by scanning the tumor at a low power (×40), and identified areas considered as hot spots of microvascularization in the section were observed at a high power (×400). Individual microvessels were counted in three views. The mean MVC obtained from the three views was used for analysis.

**Statistical Analysis.** For statistical analysis, the F test and Spearman’s rank correlation test were used, and P < 0.05 was considered significant.

**RESULTS**

The characteristics of the patients enrolled in this study are shown in Table 1. The median age and primary tumor size were 57 years and 3.0 cm, respectively. Fifty-one of the patients were women (68%), and 24 were men (32%). There were 24 patients with pEX2 (infiltrating to adjacent organs except muscles), and three with poorly differentiated carcinoma of the primary tumor. Among the patients with recurrent tumors, metastatic sites appeared in the lung (53.8%), bone (30.8%), pleura (7.7%), liver (3.8%), and brain (3.8%). The disease-free and overall survival of recurrent patients were 24 and 86 months, respectively. The overall survival of the entire patient population was 94 months. There was no significant difference in duration of follow-up between the recurrent group and nonrecurrent group. Among all of the patients, there were 47 well patients, 15 surviving patients with disease, and 10 patients who died of the disease.

**Immunohistochemical Study.** Positive staining for VEGF, VEGF-C, Ang-2, and Tie-2 in papillary carcinoma was found in the cytoplasm of the cancer cells. However, Ang-2 and Tie-2 also appeared in the endothelia of microvessels. In 75
patients, the percentages of cells positive for VEGF, VEGF-C, Ang-2, and Tie-2 were 96, 88, 98.7, and 100%, respectively. The immunohistochemical scores for VEGF, VEGF-C, Ang-2, and Tie-2 were 5.31/1006, 2.3, 5.08/1006, 2.9, 5.64/1006, 2.4, and 6.4/1006, 2.1, respectively. The mean MVC for all of the investigated tumors was 16.75/1006, 2.9. Fig. 1 shows examples of immunohistochemical staining for Ang-2, Tie-2, and CD31. Strong positive staining for TSP-1 and CD36 was also seen in the cytoplasm of the cancer cells. TSP-1 and CD36 also appeared in the endothelia of microvessels. The percentage of positive cells was 100% for both TSP-1 and CD36. The immunohistochemical scores for TSP-1 and CD36 in all patients were 6.41/1006, 1.8, and 5.89/1006, 2.0, respectively. Fig. 2 shows examples of staining for TSP-1 and CD36. Fig. 3 shows examples of staining for CD31, TSP-1, CD36, and Tie-2 in the endothelia of adjacent tissues.

Correlation among the Expression Levels of Angiogenesis-related Factors. VEGF expression showed a significant correlation with all other factors except TSP-1 (Table 2 and Fig. 4). VEGF-C expression showed a significant correlation with Ang-2 and Tie-2. Ang-2 expression was significantly correlated with Tie-2. The expression of TSP-1 correlated with that of CD36 and inversely with MVC. Fig. 4 shows examples of the correlations between VEGF and VEGF-C and between TSP-1 and MVC.

Relationship between Clinical Data and the Expression of Angiogenesis-related Factors. Table 3 shows the relationship between clinical data and each angiogenesis-related factor. In the presence of lymph node involvement, there was a significant correlation with the expression of VEGF-C. The degree of

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**Table 1** Characteristics of the enrolled patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>57 (11–74)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>57/50</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>3.0 (0.8–13)</td>
</tr>
<tr>
<td>No. with poor differentiation of primary tumor</td>
<td>3</td>
</tr>
<tr>
<td>Metastatic sites (n)</td>
<td>1</td>
</tr>
<tr>
<td>Outcome</td>
<td>Alive and well: 47</td>
</tr>
<tr>
<td>Overall survival (months)</td>
<td>Alive with disease: 15</td>
</tr>
<tr>
<td>No. with poor differentiation of primary tumor</td>
<td>3</td>
</tr>
<tr>
<td>Disease-free survival (months)</td>
<td>Alive with disease: 15</td>
</tr>
<tr>
<td>metastasis (n)</td>
<td>Alive with disease: 15</td>
</tr>
<tr>
<td>Disease-free survival (months)</td>
<td>Alive with disease: 15</td>
</tr>
</tbody>
</table>

*P* values: NS, not significant.

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EX of the primary tumor inversely correlated with the expression of TSP-1. The overall survival correlated with stronger expression of CD36 and with a lower MVC score. Other clinical factors, such as age, gender, and tumor size, did not correlate with the expression of the angiogenesis-related factors investigated. In addition, no relationship was found between the expression levels of any of the angiogenic factors and recurrence.

To clarify the balance of expression of angiogenic and antiangiogenic factors in the same tumor, we calculated the ratio of each angiogenic factor versus TSP-1 as the antiangiogenic factor. Table 4 shows the relationship between each ratio and the clinical data. The increased ratio of VEGF to TSP-1 significantly correlated with a higher degree of EX and a higher MVC. The increased ratio of VEGF-C to TSP-1 correlated with a higher MVC. The increased ratio of Ang-2 to TSP-1 correlated with a higher EX and a higher MVC.

**DISCUSSION**

Angiogenesis, which is essential for tumor growth and progression (1), does not involve a single pathway, but is a complex of many factors and signal transduction systems. Within this angiogenesis complex, the balance between angiogenic and antiangiogenic factors is important (33). In the present study, we investigated the expression and distribution of TSP and CD36 in papillary thyroid carcinomas to detect the expression of two antiangiogenic factors and four angiogenic factors: VEGF, VEGF-C, Tie-2, and Ang-2. All of these factors appeared in the cytoplasm of the tumor, demonstrating production by thyroid cancer cells. It is generally recognized that cancer cells produce VEGF and VEGF-C (34, 35). Ang-2 is expressed in the cytoplasm of cancer cells in hepatocellular carcinoma (36), Kaposi’s sarcoma (37), and gastric carcinoma (38). Its expression is localized in the endothelial cells of glioblastoma (39), astrocytoma (40), and non-small cell lung cancer (41), but not in the cancer cells. Tie-2 has been reported in the cytoplasm of cancer cells in glioma (5) and Kaposi’s sarcoma (37), but its expression is localized in intratumoral endothelial cells in gastric cancer (38), glioblastoma (39), and breast cancer (42). In our study, Tie-2 was present in the cytoplasm of both cancer and endothelial cells. Some investigators have reported TSP-1 expression in the cytoplasm of thyroidhyperplasia (43) and breast (27, 28), colorectal (44), and prostate cancer (45) cells. However, there have also been many reports of TSP-1 expression only in cells adjacent to the stromal tissue of the tumor and/or in the intratumoral stromal tissue in bladder (16), mouth (18), pancreas (21), and thyroid cancer (30). CD36 has been identified in the cytoplasm of cancer cells of cutaneous squamous cell carcinoma (17), breast cancer (27, 28), and colon cancer (29), and also in the endothelial cells of colon cancer (29, 31). Therefore, the reported expression sites of these angiogenesis-related factors vary. In our study, CD36 appeared in the cytoplasm of both cancer cells and endothelial cells.

Regarding the relationship among the factors studied, VEGF was closely related to the other angiogenic factors. Among the antiangiogenic factors, two factors were closely related to each other. MVC showed a significant positive correlation with the expression of VEGF and an inverse correlation with TSP-1. These results indicate that VEGF is a major angiogenic factor.
genic factor (2) and that TSP-1 should be recognized as an antiangiogenic factor (15).

In the present study, there was no correlation between the clinical data, such as age, gender, and tumor size, and the expression levels of angiogenesis-related factors. In papillary thyroid carcinoma, these factors have been considered independent prognostic factors (46), but they were not related to the expression levels of angiogenic factors. The presence of node involvement was closely related to higher expression of VEGF-C. In a recent study, it was clearly demonstrated that VEGF-C promoted lymph node metastasis and distant metastasis (35). TSP-1 has been reported as an antiangiogenic factor (16–19, 31). In thyroid cancer, Bunone et al. (47) reported that a decrease in TSP-1 enabled hematogenous spread. In our findings, TSP-1 showed a significant inhibitory effect on the MVC of tumors.

Some studies have shown that TSP-1 suppresses tumor growth through antiangiogenic effects in cutaneous squamous cell carcinoma (17), breast carcinoma (48), thyroid cancer (49), and melanoma (50). In other studies, TSP-1 has been demonstrated as an antitumor factor in melanoma (25) and small cell lung carcinoma (26), independent of angiogenesis. In other studies of the relationship between tumor invasion and TSP-1, however, TSP-1 has been reported to promote invasion through up-regulation of the plasminogen/plasmin system (24, 28, 50).

In our study, higher expression of TSP-1 inversely correlated with EX. Whether this inverse result is derived from a property of each organ should be investigated. As for overall survival, a higher MVC significantly correlated with poorer survival, as in our and other previous studies (51, 52). In other studies, the expression of TSP-1 correlated with a good prognosis (18, 44), but there was no relationship in the results of the present study similar to the relationship reported by Gasparini et al. (53).

The balance between angiogenic and antiangiogenic factors has been recognized as important in this angiogenesis complex (33). The concept has been described by Prehn (54), and it has been shown that fibrosarcomas counterbalance their own secretion of TSP-1 by overproducing angiogenic factors (25). Recently, Filleur et al. (33) demonstrated that tumor resistance to the antiangiogenic effect of TSP-1 develops as a result of in vivo outgrowth of tumor cell variants that secrete increased amounts of angiogenic factors (VEGF), which counterbalance the inhibitory effect of TSP-1 on neovascularization. Morelli et al. (19) reported that the expression of VEGF was elevated in approximately half of the sera from gastric and breast cancer patients and that these sera stimulated endothelial cell growth, whereas sera that inhibited endothelial growth contained high levels of TSP. On the basis of these findings, we studied the effect of the balance between angiogenic and antiangiogenic factors on clinical factors. Two clinical factors, EX and MVC, were affected by the balance between angiogenic and antiangiogenic factors. Two clinical factors, EX and MVC, were affected by the balance between angiogenic and antiangiogenic factors. Two clinical factors, EX and MVC, were affected by the balance between angiogenic and antiangiogenic factors. Two clinical factors, EX and MVC, were affected by the balance between angiogenic and antiangiogenic factors.

To the best of our knowledge, this is the first report

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**Table 2** Correlation among the expression levels of the factors

<table>
<thead>
<tr>
<th>Analyzed by Spearman’s rank correlation coefficient.</th>
<th>VEGF-C</th>
<th>Ang-2</th>
<th>Tie-2</th>
<th>TSP-1</th>
<th>CD36</th>
<th>MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VEGF</strong></td>
<td>P &lt; 0.0001</td>
<td>P = 0.0004</td>
<td>P = 0.0026</td>
<td>NS</td>
<td>P = 0.0247</td>
<td>P = 0.0236</td>
</tr>
<tr>
<td>r = 0.603</td>
<td></td>
<td></td>
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<tr>
<td><strong>VEGF-C</strong></td>
<td>P &lt; 0.0001</td>
<td>P = 0.0074</td>
<td>P = 0.0001</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>r = 0.572</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Ang-2</strong></td>
<td>P = 0.004</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r = 0.572</td>
<td></td>
<td></td>
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<tr>
<td><strong>Tie-2</strong></td>
<td>NS</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r = 0.335</td>
<td></td>
<td></td>
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<tr>
<td><strong>TSP-1</strong></td>
<td>P = 0.026</td>
<td>P = 0.044</td>
<td></td>
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<tr>
<td>r = 0.258</td>
<td></td>
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<tr>
<td><strong>CD36</strong></td>
<td>NS</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
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</tbody>
</table>

*NS, not significant.*
Table 3 Relationship between clinical data and the expression level of each factor
Analyzed by Spearman’s rank correlation coefficient or by F test. Other clinical factors, such as age, gender, primary tumor size, and presence of recurrence, were not significantly correlated with any factors.

<table>
<thead>
<tr>
<th></th>
<th>VEGF-A</th>
<th>VEGF-C</th>
<th>Ang-2</th>
<th>Tie-2</th>
<th>TSP-1</th>
<th>CD36</th>
<th>MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node involvement&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>P = 0.0191</td>
<td>r = 0.273</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EX</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.0095</td>
<td>r = −0.302</td>
<td>NS</td>
</tr>
<tr>
<td>Overall survival</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.0473</td>
<td>P = 0.0279</td>
<td>r = 0.231</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lymph node involvement, presence of metastatic lymph nodes among dissected lymph nodes at initial operation.
<sup>b</sup> NS, not significant.

Table 4 Relationship between the ratio of each factor and the clinical data
Analyzed by Spearman’s rank correlation coefficient or by F test. Other clinical factors, such as age, gender, primary tumor size, presence of lymph node involvement, presence of recurrence, and overall survival, were not significantly correlated with each ratio.

<table>
<thead>
<tr>
<th>VEGF/TSP</th>
<th>VEGF-C/TSP</th>
<th>Ang-2/TSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>EX</td>
<td>P = 0.0056</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MVC</td>
<td>P = 0.0097</td>
<td>r = 0.318</td>
</tr>
</tbody>
</table>

<sup>a</sup> NS, not significant.

showing that the balance between angiogenic and antiangiogenic factors is closely related to distinct invasion to other adjacent organs and neovascularization of the primary tumor.

REFERENCES


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