Cancerous, but not stromal, thrombospondin-2 contributes prognosis in pulmonary adenocarcinoma

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Received March 3, 2009; Accepted April 20, 2009

DOI: 10.3892/or_00000435

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Key words: thrombospondin-2, pulmonary adenocarcinoma, prognosis

Abstract. Thrombospondin (TSP)-2 is known to be an endogenous negative regulator of vascularization in human cancer. However, it is unclear whether TSP-2 expression is related to neovascularization and prognosis in non-small cell lung cancer. In this study, we quantitatively examined the expression of TSP-2 mRNA by real-time reverse transcription-polymerase chain reaction (RT-PCR) in 102 pulmonary adenocarcinomas. All 102 carcinoma specimens expressed TSP-2 mRNA. The expression of TSP-2 mRNA in carcinoma was significantly higher than normal lung tissues (p<0.0001, Kruskal-Wallis test). Sizes of tumors were significantly correlated with TSP-2 gene expression (p=0.0179, Kruskal-Wallis test). The TSP-2 expression levels of the stage II/III pulmonary carcinomas were significantly increased as compared to those of stage I (p=0.0136, Kruskal-Wallis test). Thirty-five patients with high TSP-2 mRNA expression showed poor prognosis in survival (p=0.0139, log-rank test). We examined TSP-2 protein localizations in the pulmonary adenocarcinoma overexpressing TSP-2 mRNA. The TSP-2 localizations were categorized in two patterns: cancerous TSP-2 expression pattern (TSP-2 expression in the cancerous cells) and non-cancerous TSP-2 expression pattern (TSP-2 expression in the stromal lymphoid cells). Pulmonary adenocarcinoma patients with cancerous TSP-2 expression pattern showed good prognosis (p=0.0220; Fisher's probability exact test). Non-cancerous TSP-2 expressions may reflect secondary reactions in the cancerous stroma. The stromal TSP-2 expression is not enough to suppress growth of pulmonary adenocarcinoma, while the cancerous TSP-2 expression directly inhibits growth of the carcinoma.

Introduction

Lung cancer is one of the most common malignant diseases in the world, and its prognosis is generally poor. Among several histological types of lung carcinoma, pulmonary adenocarcinoma makes up approximately half of the non-small cell lung cancer (NSCLC) and shows a variety of histopathological features. Surgical resection is currently the only method of possibly curing lung cancer; however, more than 50% of such patients who undergo a complete resection have a recurrence. Development of cancer growth and metastasis involves numerous biological steps, including angiogenesis in both the primary and the metastatic sites. A tumor with a rich vascularization is likely to grow more rapidly and have a high risk of metastasis.

TSP is a family of glycoproteins with at least five subtypes encoded by independent genes, in which TSP-1 and TSP-2 contain three properdin-like type-1 repeats, unlike other TSPs (1-6). TSP-2 is a 420-kDa homotrimeric extracellular matrix protein. TSP-2 has recently attracted attention as an endogenous negative regulator of angiogenesis in tumorigenesis (7,8). Since TSP-2 shows poor sequence homology to TSP-1 in the procollagen region but good matches in the type-1 repeats region, it has been suggested that the antiangiogenic activity of TSP-2 maps to the type-1 repeats (9). However, further functions and properties of TSP-2 are not well understood (7,10), while many studies have revealed those of TSP-1 (11-13). Human TSP-2 mRNA is expressed at high levels in aortic, cardiac, muscle, fetal, endocrine, immune and nervous tissues (10).

Some studies have reported that TSP expressions are related to tumor neovascularization and prognosis in various
neoplasms. We previously reported that TSP-2 gene expression detected by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) is significantly correlated with decreased vascularity in various tumor tissues including NSCLC (14-16). We have also reported good prognosis in patients with pulmonary adenocarcinoma expressing TSP-2 mRNA (14). On the other hand, Fontanini et al reported no statistical difference between TSP-2 mRNA expression and microvessel density in NSCLC by RT-PCR (17). Thus, it is still confusing whether TSP expression is related to neo-vasculization and prognosis in NSCLC.

In this study, we examined in detail and quantitatively the gene expression of TSP-2 by real-time PCR in 102 cases of pulmonary adenocarcinoma obtained by complete surgical resection. We discuss herein the relationship between cancerous or non-cancerous expressions of TSP-2 and clinicopathological features in pulmonary adenocarcinoma.

Materials and methods

Patients. One hundred and two pulmonary adenocarcinoma specimens were obtained from surgical specimens with the patients' informed consent from October 1985 to November 1995. Tissues were immediately frozen and stored at -80°C until analysis. Surgical specimens were also processed for routine histopathological analysis. The pathological features of the samples were classified according to the WHO histological criteria (18).

The patient consisted of 56 men and 46 women with a mean age of 62.9±9.52 years. Tumor status was T1 in 46 patients, T2 in 44, T3 in 8 and T4 in 4. Fifty-seven patients had no lymph node metastasis (N0), whereas 43 patients had lymph node metastasis (N1 in 14, N2 in 28 and N3 in 1) and 2 patients had unknown N status (Nx).hide the rest of the text...
not significantly correlated with distant metastasis found after surgery (p=0.0918, Table I). The high TSP-2 pulmonary adenocarcinoma patients (35 patients, TSP-2 levels: higher than mean level) significantly showed poorer prognosis than the low TSP-2 patients (p=0.0139, log-rank test, Fig. 1).

Correlations between prognosis and TSP-2 localization in pulmonary adenocarcinoma. We immunohistochemically

<table>
<thead>
<tr>
<th>Type (n)</th>
<th>TSP-2 expression</th>
</tr>
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<tr>
<td>Pulmonary adenocarcinoma (102)</td>
<td>1.976±2.267a</td>
</tr>
<tr>
<td>Lung tissue (20)</td>
<td>0.539±0.712a</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;60 (37)</td>
<td>1.518±1.395</td>
</tr>
<tr>
<td>≥60 (65)</td>
<td>2.237±2.613</td>
</tr>
<tr>
<td>Gender</td>
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</tr>
<tr>
<td>Male (56)</td>
<td>1.974±2.387</td>
</tr>
<tr>
<td>Female (46)</td>
<td>1.979±2.138</td>
</tr>
<tr>
<td>T status</td>
<td></td>
</tr>
<tr>
<td>T1+T2 (90)</td>
<td>1.778±1.888b</td>
</tr>
<tr>
<td>T3+T4 (12)</td>
<td>3.464±3.951b</td>
</tr>
<tr>
<td>N status</td>
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<tr>
<td>N0 (57)</td>
<td>1.797±2.377</td>
</tr>
<tr>
<td>N1+N2+N3 (43)</td>
<td>2.248±2.160</td>
</tr>
<tr>
<td>Stage</td>
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</tr>
<tr>
<td>I (55)</td>
<td>1.512±1.535c</td>
</tr>
<tr>
<td>II+III (47)</td>
<td>2.520±2.821c</td>
</tr>
<tr>
<td>Distant metastasis found after surgery</td>
<td></td>
</tr>
<tr>
<td>Yes (40)</td>
<td>2.596±3.127</td>
</tr>
<tr>
<td>No (62)</td>
<td>1.577±1.358</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test; ‘p<0.0001; ‘p=0.0179; ‘p=0.0136.

Figure 1. Overall survival of 102 completely resected patients with pulmonary adenocarcinoma, stratified by TSP-2 mRNA expression.

Figure 2. Immunohistochemical expression of TSP-2. Cases with high TSP-2 expression level and good prognosis (alive >2 years) showed strong cancerous expression [(A) x25] but not in lymphocytes [(B) x138]. Cases with high TSP-2 expression level but poor prognosis (died within 2 years) showed low expression in tumor cells but high levels in lymphocytes [(C) x138].

confirmed the localization of TSP-2 protein in pulmonary adenocarcinoma. TSP-2 protein localizations were categorized as two patterns: cancerous TSP-2 expression pattern (TSP-2 expression in the cancerous cells, Fig. 2A and B) and non-
cancerous (stromal) TSP-2 expression pattern (TSP-2 expression in the lymphoid cells, Fig. 2C). The patients alive more than 2 years (14 patients) predominantly showed cancerous TSP-2 pattern (p=0.0322, Fisher’s probability exact test, Table II), while the patients who died within 2 years (8 patients) predominantly showed the stromal TSP-2 pattern (p=0.0220).

Discussion

There is increasing evidence that TSP-2 plays a major role in the regulation of primary angiogenesis (5,6). TSP-2 is considered to promote apoptosis and inhibit proliferation of microvessel endothelial cells (21,22). In this study, we showed that gene expression levels of TSP-2 were obviously increased in the tumor. The TSP-2 expression levels were significantly correlated with sizes of tumor and pathological stages. Moreover, enhanced TSP-2 expression levels were correlated with worse prognosis in pulmonary adenocarcinoma. We previously reported that TSP-2 gene expression was significantly correlated with better prognosis in pulmonary adenocarcinoma (14). The results presented here appear to be contradictory to the previous study. In the previous study, gene expressions of TSP-2 were evaluated by conventional and non-quantitative RT-PCR methods. In this study, we used the quantitative real-time RT-PCR procedures. We also analyzed localization of TSP-2 in the tumor. We showed here that cancerous TSP-2 expression patterns were correlated with better prognosis in pulmonary adenocarcinoma, whereas stromal TSP-2 expression patterns were correlated with worse prognosis. The contradictory results obtained by studies with bulk materials did not reflect thees TSP-2 localizations. We were able to explain in detail the TSP-2 expression in prognosis of pulmonary adenocarcinoma in this study.

Streit et al reported that squamous cell carcinoma cell lines transfected with murine TSP-2 showed inhibited dermal microvascular endothelial cell migration in vitro and intra-dermal tumor growth in vivo (4). In our previous reports, colon cancer cell lines transfected with human TSP-2 showed decreased proliferation of microvascular endothelial cells in vitro and the down-regulated expression of matrix metalloproteinas (MMP)-2 and 9 mRNA (23,24). Pancreatic cancer cell lines transfected with TSP-2 showed fewer invasions in vitro through the down-regulation of MMP-9 and urokinase type plasminogen activator activities (25). In malignant melanoma xenografts, the overexpression of TSP-2 inhibited hematogenous metastasis in vivo (26). In these results, tumor cells secreting TSP-2 show fewer invasive properties and result in better prognosis because of the anti-angiogenic effect of TSP-2.

The stromal up-regulation of the endogenous angiogenesis inhibitor TSP-2 is supposed to play a protective role in multistep carcinogenesis as part of host anti-tumor defense mechanisms (27). Stromal TSP-2 expressed in the dermis has been shown to inhibit angiogenesis in response to a foreign body implant (28). The TSP-2 expression was highly up-regulated in the mesenchymal tumor stroma throughout the consecutive stages of skin tumorigenesis (27). TSP-2 deficiency dramatically enhanced the susceptibility to experimental skin carcinogenesis. TSP-2 deficiency was also associated with increased tumor angiogenesis and decreased tumor cell apoptosis. Vascularity in the cancer stoma is regulated by the balance between various angiogenic and angio-inhibitory factors. Therefore, the stromal TSP-2 overexpression means a reactive phenomenon due to a highly angiogenic tumor progression, which could lead to a worse prognosis.

It is difficult to distinguish whether TSP-2 is overexpressed in the tumor cells or reactively expressed in the mesenchymal tumor stroma by the quantitative real-time PCR with bulk materials. In this study, TSP-2 mRNA were detected in all the cases whereas in only half of the cases by conventional RT-PCR in our previous study (14). It may explain why the correlations between prognosis and the expressions of TSP-1 and -2 are still controversial (14,17,29,30). We plan to study how the cancerous TSP-2 overexpression effects the tumor invasion and angiogenesis by TSP-2 transfectants of pulmonary adenocarcinoma cell lines.

Acknowledgements

We are grateful to Johbu Itoh PhD and Mr. Yuichi Tada (Department of Pathology, Tokai University), and Toshiyuki Suganuma MD (Yokosuka General Hospital Uwamachi) for their technical assistance and helpful discussion.

References


