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Circulating Thrombospondin-2 and FGF-2 in Patients with Advanced Non-small Cell Lung Cancer: Correlation with Survival

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Abstract

Thrombospondin-2 (TSP-2) is an endogenous negative regulator of vascularization in human cancer. TSP-2 regulates angiogenesis through binding and sequestration of the proangiogenic fibroblast growth factor-2 (FGF-2). However, it is unclear whether TSP-2 and FGF-2 are related to prognosis in non-small cell lung cancer (NSCLC). To study this issue, we measured serum (Elisa) levels of TSP-2 and FGF-2 in 40 NSCLC patients (before chemotherapy) and 22 healthy subjects. Both TSP-2 and FGF-2 concentrations were elevated in the NSCLC group compared with control (TSP-2: 26.72 ± 8.00 *vs*. 18.64 ± 5.50 ng/ml, $p = 0.002$; FGF-2: 11.90 ± 5.80 *vs*. 7.26 ± 3.90 pg/ml, $p = 0.01$). Receiver-operating characteristic (ROC) curves were applied to find the cut-off serum levels of TSP-2 and FGF-2 (NSCLC *vs*. healthy: TSP-2 = 15.09 ng/ml, FGF-2 = 2.23 pg/ml). Patients before treatment with the TSP-2 level <24.15 ng/ml had a median survival of 23.7 months, but those with TSP-2 > 24.15 ng/ml had only 9 months' median survival ($p = 0.007$). Patients with FGF-2 level >11.21 pg/ml had significantly shorter survival than patients with FGF-2 < 11.21 pg/ml (7.5 months *vs*. 16 months, $p = 0.034$). We conclude that NSCLC patients have higher serum concentrations of TSP-2 and FGF-2 than healthy people. High levels of TSP-2 and FGF-2 may predict worse survival.

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Keywords
Fibroblast growth factor-2 • Lung cancer • Survival • Thrombospondin-2

1 Introduction

Thrombospondins are a group of extracellular glycoproteins that are increasingly being implicated in mechanisms relevant to cancerogenesis (Lawler and Lawler 2012). There are many reports of thrombospondin-1 (TSP-1), an inhibitor of angiogenesis. However, functions and properties of TSP-2 are not well understood. TSP-2 is also known to be an endogenous negative regulator of angiogenesis. The mechanisms by which TSP-2 inhibits angiogenesis can be broadly characterized as direct effects on vascular endothelial cells and indirect effects on the various growth factors, cytokines, and proteases that regulate angiogenesis (Zhang and Lawler 2007). They are also involved in the pathological tissue remodeling that is associated with atherosclerosis (Zhang and Lawler 2007). Recently, Golledge et al. (2013) reported the relation between serum TSP-2 and cardiovascular mortality in older men screened for abdominal aortic aneurysm. Other authors revealed that cancerous, but not stromal, TSP-2 contributes prognosis in pulmonary adenocarcinoma (Chijiwa et al. 2009). However, it is unclear whether serum levels of TSP-2 are related to prognosis in non-small cell lung cancer (NSCLC). Thrombospondins are promising sources of therapeutic agents to treat angiogenesis-driven diseases, including cancer. Colombo et al. (2010) reported that the fibroblast growth factor-2 (FGF-2) binding sequence of thrombospondins might serve as a template for the development of inhibitors of angiogenesis. According to several reports, FGF-2 is an attractive target for new antiangiogenic therapies (Beenken and Mohammadi 2009). As with some other angiogenesis pathways, FGF-2 pathway has been shown to be activated in lung cancer (Behrens et al. 2008). The precise role of this protein in pathogenesis of NSCLC is still unknown. Behrens et al. (2008) reported the overexpression of FGF-2 in NSCLC patients. There is no clear data on the serum concentrations of FGF-2 in NSCLC and their relationship with survival of patients.

2 Methods

The study was performed in conformity with the Declaration of Helsinki for Human Experimentation and the protocol was approved by an institutional ethics board. Written informed consent was obtained from all participants.

Serum samples obtained from 40 patients before treatment of NSCLC were analyzed (squamous cell carcinoma 21, others 19; mean age 63 ± 3 years; F/M-3/37). Serum samples were also obtained from 22 healthy volunteers (mean age 60 ± 4; F/M-2/20). Venous blood samples were drawn into tubes and centrifuged at 3,000× *g* for 10 min. After centrifugation, the serum samples were stored at −80 °C until use. The NSCLC group consisted of 16 patients at clinical Stage IIIB and 24 patients at stage IV of disease. For all patients, the diagnosis of NSCLC was confirmed by the histological examination of biopsy and cytological specimens taken during bronchoscopic examination (Pentax FB 18 V; Pentax Corporation, Tokyo, Japan).

The stage of disease was determined according to the TNM system (Goldstraw et al. 2007). To establish the disease stage, the following investigations were applied in each patient: physical examination, X-ray and CT of the chest, and ultrasonography of the abdomen. All patients received four cycles of chemotherapy (21-day cycle; cisplatin at a dose of 30 mg/m^2 on Days 1, 2, and 3 and gemcitabine at a dose of 1,000 mg/m^2 on Days 1 and 8 of the cycle). The response to therapy was evaluated by diagnostic imaging techniques, including X-ray and CT of the chest, and then the overall response to therapy was analyzed according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse et al. 2000).

Serum samples were assayed for the levels of TSP-2 and FGF-2 by a quantitative sandwich enzyme immunoassay technique (Quantikine HS, R&D System, Minneapolis, MN) according

to the manufacturer's instructions. The minimum detectable levels of TSP-2 and FGF-2 were 0.025 ng/ml and 0.22 pg/ml, respectively. All specimens were assayed in duplicates.

The Shapiro-Wilk test was used for data distribution analysis. All parametrical data was calculated by *t*-test. We used the Mann-Whitney U and Wilcoxon tests for the features inconsistent with the normal data distribution. The Spearman rank test was used to calculate correlations between the parameters. Receiver-operating characteristics (ROC) curves were constructed to find the cut-off levels of TSP-2 and FGF-2. Overall survival was calculated using the Kaplan-Meier Method. The significance of the difference in survival rates was determined by the log-rank test. A value of $p < 0.05$ was considered to indicate statistical significance. Statistica 10.0 software (StatSoft Inc., Tulsa, USA) was used for all analyses.

3 Results

There were no significant differences in age or gender between the patients and healthy subjects. The mean serum TSP-2 and FGF-2 levels were higher in NSCLC group than in healthy people (TSP-2: 26.72 ± 8.00 *vs.* 18.64 ± 5.50 ng/ml, $p = 0.002$; FGF-2: 11.90 ± 5.80 *vs.* 7.26 ± 3.90 pg/ml, $p = 0.01$) (Fig. 1a, b). There were no correlations between the concentrations of TSP-2 and FGF-2.

We constructed ROC curves of serum TSP-2 and FGF-2 to determine the cut-off values (Fig. 2). Specificity and sensitivity of serum TSP-2 in NSCLC patients relative to healthy people were 67 and 96 %, respectively, at a cut-off value of 15.01 ng/ml. Specificity and sensitivity of serum FGF-2 in NSCLC group relative to healthy subjects were 86 and 95 %, respectively, at a cut-off value of 2.24 pg/ml. The area under the curve for serum TSP-2 and FGF-2 were 0.81 and 0.66, respectively.

There were no correlation between the levels of TSP-2 or FGF-2 and the stage of tumor (TSP-2 IIIB *vs.* TSP-2 IV: 25.51 ± 8.10 *vs.* 27.81 ± 8.20 ng/ml, respectively, $p = 0.32$; FGF-2 IIIB *vs.* FGF-2 IV: 9.20 ± 6.00 *vs.* 13.71 ± 9.30 pg/ml, respectively, $p = 0.067$).

We did not find a significant relationship between pretreatment concentrations of TSP-2 or FGF-2, and the effect of chemotherapy. After treatment we found a partial response (PR) in 18 (45 %), stabilisation (SD) in 10 (25 %) and progressive disease (PD) in 12 (30 %) patients (TSP-2: PR *vs.* SD *vs.* PD – 25.10 ± 8.50 *vs.* 25.24 ± 10.40 *vs.* 29.49 ± 6.60 ng/ml, respectively, $p = 0.23$; FGF-2: PR *vs.* SD *vs.* PD – 12.19 ± 11.00 *vs.* 12.76 ± 6.90 *vs.* 10.04 ± 7.20 pg/ml, respectively, $p = 0.13$).

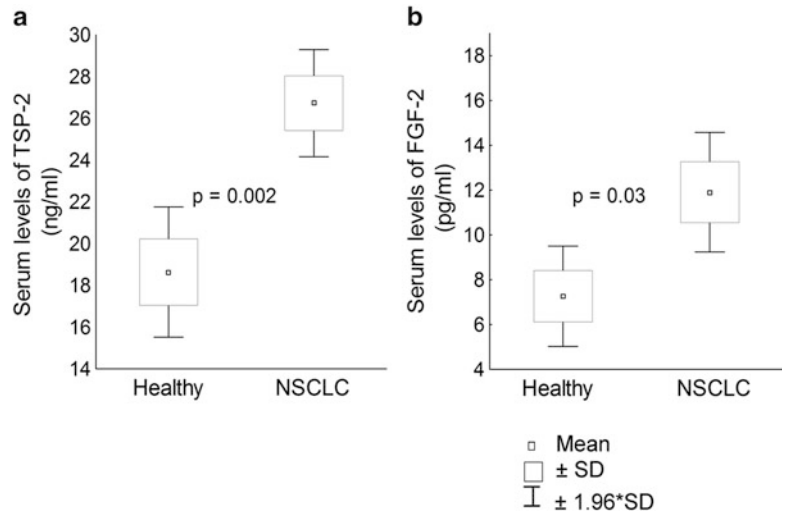

Fig. 1 **Serum concentrations of thrombospondin-2 (TSP-2) (a) and of fibroblast growth factor-2 (FGF-2) (b)** in non-small cell lung cancer (NSCLC) patients and healthy control subjects

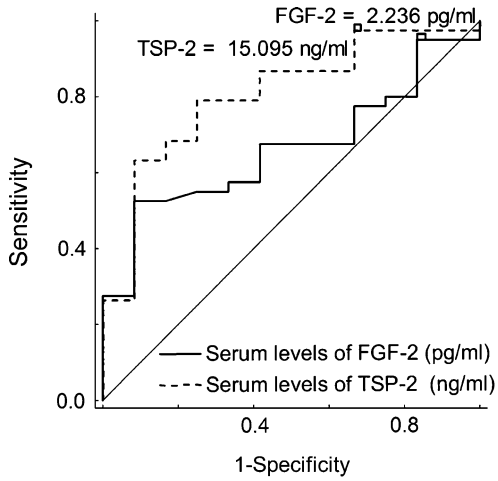

Fig. 2 Receiver operating characteristic (ROC) curves for serum thrombospondin-2 (TSP-2) and fibroblast growth factor-2 (FGF-2) in differentiating non-small cell lung cancer (NSCLC) patients from healthy people

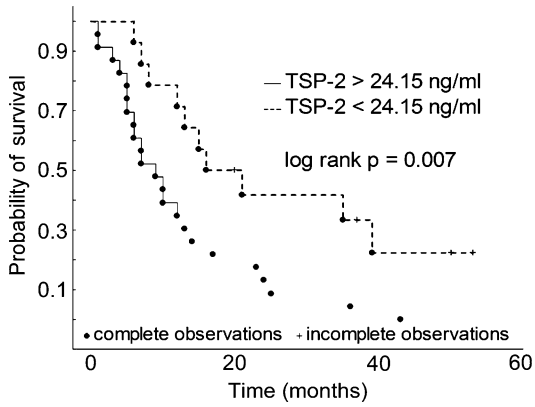

Fig. 3 Kaplan-Meier survival curve. The *solid line* represents survival of patients with thrombospondin-2 (TSP2) >24.15 ng/ml and the *broken line* of those with TSP2 <24.15 ng/ml. Patients with the TSP2 level >24.15 ng/ml had significantly shorter survival

The mean overall survival of all patients was 15.7 ± 13.0 months. The patients with elevated serum TSP-2 levels (TSP2 level >24.15 ng/ml) had a significantly shorter overall survival than those with lower serum TSP-2 levels (TSP2 level <24.15 ng/ml: 9.0 months *vs*. 23.7 months) (Fig. 3). The patients with FGF-2 levels >11.21 pg/ml had a significantly shorter survival than patients with FGF-2 <11.21 pg/ml (7.5 months *vs*. 16.0 months, respectively) (Fig. 4).

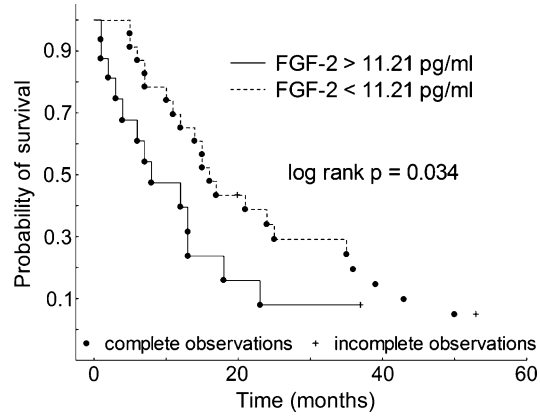

Fig. 4 Kaplan-Meier survival curve. The *solid line* represents survival of patients with fibroblast growth factor-2 (FGF-2) >11.21 pg/ml and the *broken line* of those patients with FGF-2 <11.21 pg/ml. Patients with FGF-2 level >11.21 pg/ml had significantly shorter survival

4 Discussion

TSP-2 has recently attracted attention as an endogenous negative regulator of angiogenesis in tumorigenesis (Lawler 2000). However, functions and properties of TSP-2 are not well understood. Fontanini et al. (1999) reported no statistical differences between TSP-2 mRNA expression and microvessel density in NSCLC by RT-PCR. The opposite results were presented by Tokunaga et al. (1999). They reported that TSP-2 gene expression is correlated with decreased vascularity in NSCLC. Moreover, Chijiwa et al. (2009) reported that patients with pulmonary adenocarcinoma and a pattern of cancerous TSP-2 expression have a good prognosis. On the other hand, pulmonary adenocarcinoma patients with non-cancerous TSP-2 expressions pattern showed poor prognosis (Chijiwa et al. 2009). In a study of Chijiwa et al. (2009), cases with high TSP-2 expression level and good prognosis showed its strong cancerous expression, but not in lymphocytes. On the contrary, cases with high TSP-2 expression, but poor prognosis, showed its low expression in tumor cells, but high levels in lymphocytes (Chijiwa et al. 2009). In the present study, we showed a higher serum level of TSP-2

in NSCLC patients compared with healthy subjects. Moreover, patients with higher levels of TSP-2 had shorter survival. A possible explanation for TSP-2 increased levels in NSCLC group is its releasing from blood cells, endothelial cells, or other cells involved in the pathological tissue remodeling of cancer stroma (Zhang and Lawler 2007). Our results are in accord with those of Chijiwa et al. (2009). They revealed that the stromal TSP-2 expression is not enough to suppress growth of NSCLC, while the cancerous TSP-2 expression directly inhibits growth of the tumor (Chijiwa et al. 2009).

TSP-2 acts at the interface between the cell surface and extracellular matrix to provide contextual cues that regulate matrix structure and cellular phenotype during tumor growth (Lawler and Detmar 2004). One of the main regulators of matrix metalloproteinases production and invasiveness in human cancer cell lines is FGF (Corn et al. 2013).

FGF-2 belongs to a family of ubiquitously expressed ligands that bind to the extracellular domain of FGFRs (FGF Receptors), initiating a signal transduction cascade that promotes cell proliferation, motility, and angiogenesis (Ribatti et al. 2007; Mohammadi et al. 2005). Elevated levels of FGF-2 have been detected in NSCLC cell lines, but the precise role of these molecules in the pathogenesis and progression of this tumor is still unknown (Kuhn et al. 2004).

The first study investigating serum levels of FGF-2 in NSCLC was reported by Brattström et al. (1998). They demonstrated elevated serum of FGF-2 in NSCLC patients (Brattström et al. 1998). We confirmed that findings because NSCLC patients had higher levels of FGF-2 in serum than healthy people. In the present study, patients with higher FGF-2 serum levels had shorter survival than those with lower concentration of FGF-2 (7.5 months *vs*. 16 months). These observations are different from the study of Brattström et al. (1998) who found that elevated FGF-2 at diagnosis was a significant favorable prognostic factor for survival. However, these results disagree with other studies. In a study by Ueno et al. (2001) in 60 NSCLC patients, serum FGF-2 levels did not differ between the clinical stages of NSCLC and showed no correlation with survival. Neither did we find any correlation between the levels of TSP-2, FGF-2 and the stage of tumor. However, our observations on survival are compatible with some other works (Brattström et al. 2002, 2004; Joensuu et al. 2002). In the present study the elevated FGF-2 levels correlated with poor survival in NSCLC.

In summary, data on the prognostic implications of TSP-2 and FGF-2 in serum of NSCLC patients are ambiguous. We believe that the determination of concentrations of TSP-2 and FGF-2 in serum may have a practical significance in predicting survival of patients with lung cancer. Further large scale studies are still needed to define the role of these markers in NSCLC.

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Conflicts of Interest The authors had no conflicts of interest to declare in relation to this article.

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