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Expression of thrombospondin 1 (TSP 1) in patients with uterine smooth muscle tumors: An immunohistochemical study

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Abstract

Objective. Angiogenesis is an essential component for tumor development regulated by both proangiogenic and antiangiogenic factors. Thrombospondin 1 (TSP 1) suppresses angiogenesis by inhibiting endothelial cell proliferation and inducing endothelial cell apoptosis. The aim of this study was to compare the expression of TSP 1 in cases with leiomyoma, uterine smooth muscle tumor of uncertain malignant potential (STUMP) and leiomyosarcoma (LMS). Furthermore, we evaluated the prognostic relevance of TSP 1 in uterine LMS.

Methods. TSP 1 expression was investigated by immunohistochemistry from paraffin-embedded tissue in 26 patients with leiomyoma, in 24 patients with STUMP and in 21 patients with LMS. Standard immunohistochemical techniques were used to study the expression of TSP 1 in 5-μm-thick tumor sections. TSP 1 expression was correlated with survival using the Kaplan–Meier method and log-rank test for univariate analysis.

Results. TSP 1 was expressed in 77% of leiomyomas, in 13% of STUMP and in 24% of LMS. A statistically significant difference regarding the frequency of TSP 1 expression was observed between leiomyoma and LMS ($P < 0.05$) as well as between leiomyoma and STUMP ($P < 0.05$), but not between LMS and STUMP ($P > 0.05$). Furthermore, a statistically significant correlation between vascular space involvement and TSP 1 expression was observed in patients with uterine LMS, with patients without vascular space involvement having more frequently TSP 1 positive tumors ($P = 0.04$). No statistically significant correlation between TSP 1 and clinical stage, age and recurrence disease could be detected ($P > 0.05$).

Conclusions. We found that TSP 1 was more frequently expressed in leiomyoma compared to STUMP and LMS. Additionally, the statistically significant negative correlation between vascular space involvement and TSP 1 expression in patients with uterine LMS shows that TSP 1 might work as a predictive factor in patients with LMS. Further clinical studies are necessary to prove our results and to clarify the role of TSP 1 in uterine smooth muscle tumors.

Keywords: Thrombospondin 1 expression; Uterine leiomyosarcoma; Uterine smooth muscle tumor of uncertain malignant potential; Leiomyoma; Clinicopathologic parameters

Introduction

Angiogenesis, the growth of new capillaries from preexisting blood vessels, is essential for cancers to grow beyond minimal size [1]. There is convincing evidence that therapeutic inhibition of angiogenesis leads to inhibition of tumor growth and metastatic spread in several mouse tumor models [2,3]. In contrast to the large number of reports on tumor angiogenesis factors, much less is known about the expression and the biologic role of endogenous inhibitors of angiogenesis during carcinogenesis, tumor growth and tumor metastasis. Several naturally occurring inhibitors of tumor angiogenesis have been identified like thrombospondin 1 (TSP 1), angiostatin, endostatin and thrombospondin 2 (TSP 2) [2,4–6]. TSP 1 is a

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member of a family of matricellular proteins that are encoded by separate genes [7] and plays an important role in a variety of biologic processes, including cell–cell and cell–matrix interactions. An elevated secretion of thrombospondins is often observed in tumors and is sometimes considered as a predictive factor.

Several reports demonstrated that TSP 1 expression is inversely correlated with malignant progression in human lung, breast and bladder carcinoma cell lines [8,9]. However, the role of thrombospondin 1 in patients with uterine smooth muscle tumors is unclear.

Uterine leiomyomas are the most common benign smooth muscle tumors in women of reproductive age and occur in nearly 40% of women older than 35 years [10,11]. Smooth muscle tumors of uncertain malignant potential (STUMP) are characterized by a lower mitotic count and/or less nuclear atypia than uterine leiomyosarcomas [10,12]. Previous studies reported a favorable outcome in patients with STUMP [10,12]. Uterine leiomyosarcomas are rare tumors, accounting for only about 1.3% of all uterine malignancies, and they usually exhibit an extremely malignant clinical course. The risk of local recurrence and metastasis is high with reported 5-year survival rates ranging between 12 and 25% [10].

The aim of the present study was to compare the immunohistochemical profile of thrombospondin 1 in patients with leiomyomas, STUMP and LMS. Furthermore, we evaluated the correlation between TSP 1 expression and different clinicopathologic parameters in patients with uterine leiomyosarcomas.

Materials and methods

Tissue collection

This retrospective study included 71 cases of uterine smooth muscle tumors treated between 1990 and 2000 at the Department of Gynecology and Obstetrics of the University Hospital Vienna. Due to the rarity of LMS and STUMP, case selection was not consecutive. Pathologic diagnosis of the tumors was performed using criteria in literature [10,13]. Microscopic characteristics such as mitotic activity, coagulative tumor cell necrosis and nuclear atypia were analyzed.

According to this definition, of the 71 cases of smooth muscle tumors of the uterus, 26 were diagnosed as leiomyomas, 24 as smooth muscle tumors of uncertain malignant potential (STUMP) and 21 as leiomyosarcomas.

All patients with leiomyosarcomas were staged, retrospectively, according to a modified staging system of the International Federation of Gynecology and Obstetrics (FIGO) for endometrial cancer.

Serial sections were prepared for hematoxylin and eosin staining and immunohistochemistry. An experienced pathologist reviewed all slides. Clinical information, including follow-up data, was obtained from the database of the department of Gynecology and Obstetrics. The institutional board approved this retrospective data analysis.

Immunohistochemistry

Immunohistochemical analysis for TSP 1 was performed on formalin-fixed and paraffin-embedded sections. Immunohistochemical staining was performed by the biotin–streptavidin–peroxidase method. A mouse monoclonal antibody against activated TSP 1 (Ab4, Labvision, Newmarket, United Kingdom, dilution 1:50) was used as the primary antibody.

In brief, tissue sections were dewaxed, deparaffinized in xylene, rehydrated through a series of graded alcohols and washed in water. Antigen retrieval was undertaken by immersing the slides for 10 min in a 300 mL solution of 0.1% trypsin and 0.1% calcium chloride. The slides were rinsed in running water, bathed in TBS buffer for 5 min and covered with 0.03% hydrogen peroxide for a further 5 min to reduce nonspecific staining. The slides were then incubated with a 1:50 dilution of a mouse monoclonal primary antibody for TSP 1 (Ab4, Labvision, Newmarket, United Kingdom) at room temperature in a humid chamber for 30 min. After further washing in buffer for 5 min, the secondary-peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulins was applied for 30 min. The sections were washed in buffer and TSP 1 antigen visualized using 3,3-diaminobezidinetetrahydrochloride chromogen and hematoxylin counterstain. Slides were dehydrated through graded alcohols and coverslips applied.

Positive controls were sections known to express the investigated antibodies. Negative controls were obtained by omitting the primary antibodies.

Interpretation of slides

The immunohistochemical interpretation of all slides was performed by an experienced pathologist. TSP 1 staining intensity was recorded as weak, moderate and strong based on the extracellular staining in intratumoral or immediate peritumoral areas. The interpretation of immunohistochemical staining was expressed as follows: intensity was described as 0 (no staining) to 3 (strong staining) and the percentage of staining was recorded as 0 (no staining), 1+ (positive staining in ≤30% of cells), 2+ (positive staining in 31–50% of the cells) and 3+ (positive staining in >51% of the cells).

Statistical analysis

Chi-square tests were used to compare the frequency distributions of TSP 1 expression between the analyzed groups (leiomyoma, STUMP and LMS) and to compare the frequency distributions of binary outcome variables between TSP 1 positive and TSP 1 negative LMS. The end point of overall survival was used for analysis. Survival probabilities were calculated by the product limit method of Kaplan and Meier [14]. Univariate analysis was performed using the log-rank test. P values of less than 0.05 were considered statistically significant. The SPSS system (SPSS Inc., Chicago, IL, USA) was used for the calculations.

Results

Expression of TSP 1 protein in leiomyoma, STUMP and LMS

The frequency of thrombospondin 1 in leiomyoma, STUMP and LMS is shown in Table 1. TSP 1 was expressed in 20/26 (77%) leiomyomas, in 3/24 (13%) STUMP and in 5/21 (24%) LMS. A statistically significant difference regarding the frequency of TSP 1 expression was observed between leiomyoma and LMS ($P = 0.001$) as well as between leiomyoma

Table 1
Frequency and staining intensity of TSP 1 protein in uterine smooth muscle tumors (LMS, STUMP, leiomyoma)

	LMS $n = 21$	STUMP $n = 24$	Leiomyoma $n = 26$
TSP 1			
Positive	5	3	20
Negative	16	21	6
Staining intensity for TSP 1			
− (0% staining)	16	21	6
+ (staining in ≤30%)	5	3	12
++ (staining in 31–50%)	0	0	8
+++ (staining in >51%)	0	0	0

The staining intensity was evaluated to the percentage of stained cells: − negative (no staining), + weak positive (positive staining in ≤30% of tumor cells), ++ moderate positive ((positive staining in 31–50% of the cells) and +++ strong positive (positive staining in >51% of the tumor cells).