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Adhesion of osteosarcoma cells to the 70-kDa core region of thrombospondin-1 is mediated by the α4β1 integrin

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Abstract

Thrombospondin-1 (TSP-1) is an extracellular glycoprotein that is involved in a variety of physiological processes such as tumor cell adhesion, invasion, and metastasis. It has been hypothesized that TSP-1 provides an adhesive matrix for osteosarcoma cells. Here we present data showing that TSP-1 can promote cell substrate adhesion to U2OS and SAOS cells through the α4β1 integrin. The dose-dependent adhesion to TSP-1 was inhibited by anti-integrin antibodies directed against the α4 or β1 subunit, but not by control antibodies against other integrins. To localize the potential α4β1-binding site within the TSP-1 molecule, the protein was subjected to limited proteolysis with chymotrypsin in the absence of calcium. The stable 70-kDa core fragment produced under these conditions promoted α4β1-dependent osteosarcoma cell adhesion in a manner similar to that of the intact protein. Moreover adhesion experiments with neutralizing antibodies revealed that the adhesion was totally dependent on the α4β1 interaction. Further blocking experiments with potential inhibitory peptides revealed that the α4β1-mediated adhesion was not influenced by peptides containing the RGD sequence. Attachment to the 70-kDa fragment was strongly inhibited by the CS-1 peptide, which represents the most active recognition domain for α4β1 integrin in fibronectin. The present data provide evidence that TSP-1 contains an α4β1 integrin-binding site within the 70-kDa core region.

Keywords: Thrombospondin-1; Osteosarcoma cells; α4β1 Integrin; Cell adhesion

Thrombospondin-1 (TSP-1) is the first member identified of a family of five related matrix glycoproteins. This 450-kDa glycoprotein was initially described as a major component of platelets [1]. Later on TSP-1 has been found in many normal and adult tissues, but it was also detected in body fluid, as an extracellular component of injured and remodelling tissue or detected in tumor, in situ. In vitro, a variety of cell types are able to synthesize TSP-1 including a number of different tumor cells [2].

TSP-1 has been implicated in several biological processes including involvement in platelet aggregation, regulation of cell proliferation, cell adhesion, tumor growth, and metastasis and angiogenesis [3]. The multifunctional effects of TSP-1 are probably the consequence of its modular structure. Each molecule consists of three identical subunits, where N- and C-terminal globular domains are flanking different structural domains. Common to all TSP proteins are epidermal growth factor-like type 2 repeats, and calcium-binding type 3 repeats. In addition TSP1 and TSP 2 contain a procollagen-like domain and properdin-like type 1 repeats [4]. This modular composition enables TSP-1 to bind to growth factors and enzymes and to interact with cells through different cell-binding sites. Different binding sites within the TSP-1 molecule can be mediated by a number of cell surface receptors, such as heparan sulfate and sulfatides, integrins, integrin associated protein IAP, CD 36, and the low density lipoprotein receptor-related protein [5–10]. Accumulating data indicate that each cell type has a unique adhesive phenotype and uses a different combination of TSP-1 receptors.

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The expression of VLA-4 (α4β1) integrin in several osteosarcoma cell lines [11] prompted us to investigate if this integrin participates in TSP-1-mediated cell adhesion. Only few investigations on TSP-mediated cell adhesion of osteosarcoma cell lines have been described, but with conflicting results. An antiadhesive function of TSP-1 was reported for osteosarcoma cell line MG-63, whereas in other investigations TSP-1 supported the adhesion of this cell line [12–14]. But in all cases the involvement of α4β1 integrin was never evaluated.

The α4β1 integrin is expressed mainly on leukocytes but also found in smooth muscle cells and tumor cells [15]. The two most effective ligands for VLA4-integrin are the adhesion receptor VCAM-1 and the extracellular matrix protein fibronectin. It appears that α4β1 plays a critical role during hematopoesis, in the control of inflammatory responses and in the process of tumor cell invasiveness [16]. Evidence for the role of α4β1 integrin in sarcoma but not in carcinoma extravasation was reported recently [17]. Based on the known effects of TSP-1 on tumor cell growth and metastasis formation we were interested to evaluate if this integrin mediates osteosarcoma cell interactions with TSP-1.

To address this question we carried out cell adhesion experiments with human osteosarcoma cell lines U2OS and SAOS. The results obtained demonstrate that TSP-1 and its 70-kDa-core region promotes cell adhesion of both osteosarcoma cell lines in a dose-dependent manner. The high level of cell adhesion to the 70-kDa fragment suggested an important binding site within this part of the molecule. Using anti-integrin antibodies and inhibitory peptides, we could show that the α4β1 integrin is involved in the adhesion of osteosarcoma cell lines on TSP-1 and the essential recognition site for this integrin is located within the 70-kDa region.

Materials and methods

Reagents and antibodies. Heparin and 3-(4,5-dimethylthiazol-2-yl-dipenyltetrazolimbromide) (MTT) were obtained from Sigma (Munich, Germany). Culture medium, trypsin/EDTA, penicillin, and streptomycin were supplied by Gibco (Grand Island, NY). Synthetic peptides GRGDSP and its control peptide GRGESP were from Bachem (Bubendorf, Switzerland), the fibronectin CS-1 fragment (EILDVPST) and its control peptide (EILEVPST) from Peninsula Labs (Belmont, USA). The following monoclonal antibodies (mAbs; all of mouse origin) were used: anti-α2 mAb P1E6 (Dako, Carpinteria, USA), anti-αvβ3 mAb 609 (DPC Biermann, Bad Nauheim, Germany), anti-α4 mAb HP2.1 (Immunotech, Marseille, France), anti-α4 mAb 44H6 (Dianova, Hamburg, Germany), anti-α4 mAb Lia1/2 (Beckman, Krefeld, Germany), anti-α5 mAb SAM1, anti-αv mAb AMF7, anti-β1 mAb K20, anti-β3 mAb SZ21, anti-CD36 mAb FA6.152, and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG were from Bibby-Dunn (Asbach, Germany).

Cell culture. U2OS and SAOS cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained in medium RPMI-1640 containing 10% fetal calf serum (FCS) (Gibco, Grand Island, NY), 2 mM L-glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B at 37 °C in a humidified 5% CO_2 incubator. Cells were subcultured in 25 cm^2 tissue culture flasks (Falcon Plastics, Oxnard, CA) using 0.05% trypsin/0.02% EDTA. Human monocytes were kindly supplied by Dr. T. Engel (Institute of Clinical Chemistry).

Thrombospondin and isolation of the 70-kDa fragment. TSP-1 was isolated from human platelets according to previously published procedures using chromatography on heparin-Sepharose (Pharmacia, Freiburg, Germany) and gel filtration chromatography on Bio-Gel A 0.5 (Bio-Rad, Munich, Germany) [18]. To prepare the trimeric 70-kDa fragment, purified TSP-1 was treated with chymotrypsin at an enzyme/substrate ratio of 100:1 in the presence of 5 mM EDTA for 30–60 min at room temperature. Under these conditions two stable fragments are produced, the monomeric N-terminal 25-kDa heparin-binding domain and the trimeric 70-kDa fragment [19]. The digest was applied to a QFF-Sepharose column, where the 70-kDa fragment was bound, whereas the 25-kDa fragment was found in the flowthrough fraction. The 70-kDa fragment was eluted from the column with Tris buffered saline containing 300 mM NaCl. The 25-kDa heparin-binding domain was applied to a heparin-Sepharose column and eluted thereof with 0.55 M NaCl. The purity of TSP-1 and its fragments was confirmed by SDS polyacrylamide gel electrophoresis.

Cell adhesion assay. Ninety-six-well polysorb immunoplates (Nunc, Wiesbaden, Germany) were coated with TSP-1 diluted in Tris–buffered saline (TBS; 20 mM Tris, pH 7.4, 150 mM NaCl) in the presence of 2 mM calcium at 4 °C overnight. Plates were blocked with 2% bovine serum albumin (BSA) in TBS for 1 h at 37 °C. After a washing step with 0.1% BSA in TBS the adhesion assays were performed. Sarcoma cells were harvested from just confluent cultures, using 5 mM EDTA in PBS, washed twice in serum-free medium and resuspended to a density of 3.5×10^5 cells/ml in RPMI containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+}. Aliquots of the cell suspension (2×10^4 cells) were added to each well in a final volume of 50 μl and cells were allowed to attach to the wells for up to 30 min at 37 °C. After this attachment period and a washing step to eliminate unattached cells, attached cells were incubated with 100 μl RPMI medium containing MTT (50 μg/ml) for additional 4 h at 37 °C [20]. The dye containing medium was aspirated and 100 μl of 50% *N*,*N*-dimethylformamide in 10% SDS was added to each well to solubilize the bound formazan. Plates were read at 550 nm (test wavelength) and 630 (reference wavelength) in a Dynatech MR 7000 microplate reader (Denkendorf, Germany). In some experiments adherent cells were detached with trypsin and cells counted in a Coulter ZM counter (Coulter Electronics, Krefeld, Germany). To determine which integrins are responsible for osteosarcoma cell adhesion, blocking experiments were performed with anti-integrin monoclonal antibodies or with synthetic peptides. In this case cells were preincubated for 30 min with the appropriate monoclonal antibody or with the potential inhibitory peptides before the adhesion assay was performed.

Flow cytometry analysis. Sarcoma cells were isolated with 5 mM EDTA in PBS and washed twice in cold washing buffer PBS/3% FCS/2% NaN_3, pH 7. Cells (5×10^5 in 20 μl) were mixed with 20 μl of saturating concentrations of mAbs and incubated on ice for 40 min. After two washing steps to remove unbound mAbs, 20 μl of a 1:40 dilution of FITC labelled goat anti-mouse IgG was added to the resuspended cells and incubated in the dark for another 30 min on ice. The stained cells were washed twice, resuspended in washing buffer containing propidium iodide (50 μg/ml) for exclusion of cell debris and analysed by a fluorescence-activated cell sorter (FACScan) using Lysis-II software (Becton Dickinson, Heidelberg, Germany). For each sample 1×10^4 cells were collected at a flow rate of 1×10^3 cells/s. Cells were gated using dot blots with the forward light scatter and the F1-3 characteristics. Determination was performed in duplicate of three experiments. Data are expressed in fluorescence intensity (MFI) units [21] as calculated by the formula: MFI units (mean fluorescence channel × % positive cells of experimental sample) – (mean fluorescence channel × % positive cells of control sample).

Results and discussion

Cell adhesion plays a fundamental role in tumor progression and metastasis and is mediated by a variety of distinct receptor/ligand interactions. TSP-1 is counted to a group of extracellular matrix proteins, like SPARC and tenascin, described to influence cell function by modulating cell–matrix interaction such as cell adhesion [3]. In some cases TSP-1 seems to have a clear adhesive in other cases anti-adhesive properties. This diversity may be explained partially by the ability of TSP-1 to bind different cell surface receptors. There are accumulating data indicating that each cell type is using a special combination of cell-surface receptors. Some of those TSP-1/receptor interactions are well characterized, but nearly nothing is known about the TSP-1/α4β1 interactions.

The α4β1 integrin mediates osteosarcoma cell adhesion to TSP-1

Two osteosarcoma cell lines, SAOS and U2OS, were used to investigate the adhesive properties of TSP-1. Both cell lines were attached to TSP-1-coated microwells. The adhesion process was concentration-dependent and saturable (Fig. 1). Maximum adhesion was observed at a TSP-1 concentration of 1.2 μg/cm^2 culture dish area which is identical to a coating concentration of 5 μg/ml later on used. During the normal adhesion assay conditions the osteosarcoma cells remained rounded, whereas after a longer incubation time, 1–2 h, spreading was observed (unpublished observation). Attachment was specific for TSP-1 as coating with BSA as a control substrate did not promote a significant attachment (less then 10%) and none of the cells did spread. In addition attachment was prevented when TSP-1 was treated with 5 mM EDTA prior to coating or when adhesion was performed in the absence of divalent cations. Therefore adhesion experiments were always performed with RPMI medium containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+}.

The requirement of divalent cations argues for the involvement of integrins in the adhesion process [22]. To determine which integrin receptor at the cell surface is involved, fluorescence-activated cell sorting was performed. As shown in Fig. 2 very high α4 and 1 expression was observed for both cell lines. All U2OS and SAOS cells showed β1 immunoreactivity and all U2OS and nearly all SAOS cells (98%) were α4 positive. Although 76% of SAOS and 96% of U2OS were α5 positive this subunit was expressed moderately. Weak mean fluorescence intensity of αv and β3 was detected in both cell lines, although 55% of U2OS and 73% of SAOS were positive for αv and 41 % for SAOS for β3. In U2OS cells the β3 subunit was hardly expressed (only 18% expressed). Very weak α2 expression was recorded in SAOS cells and no expression was found in U2OS cells. In addition we also examined the osteosarcoma cells for the expression of the 88-kDa transmembrane protein CD36, originally found on platelets and monocytes, that has been reported to function as an adhesive receptor for collagen, TSP-1, and malaria-infected erythrocytes [23]. In both osteosarcoma cell lines only a low expression of CD36 expression was detected.

In order to identify the involvement of integrins or of the non-integrin receptor CD36 in the adhesion process,

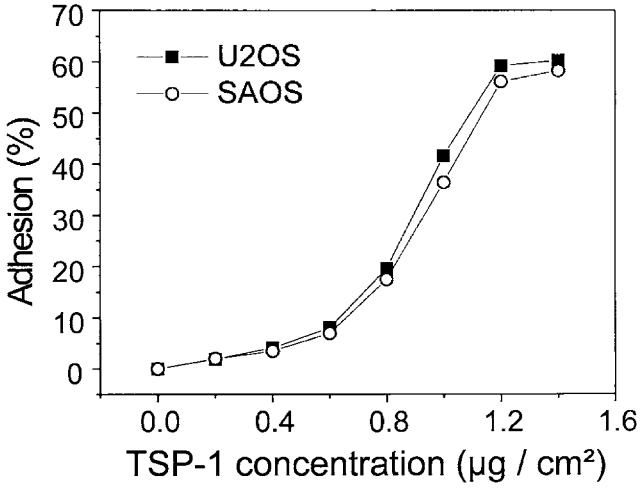

Fig. 1. Concentration-dependent adhesion of osteosarcoma cell lines U2OS and SAOS to TSP-1. Wells were coated with increasing concentration of TSP-1 overnight at 4 °C. After 30 min at 37 °C attached cells were quantitated by counting cells attached with a Coulter counter. Values are expressed as percentage of cell adhesion and each point denotes the mean of quadruplicate determinations of a representative experiment.

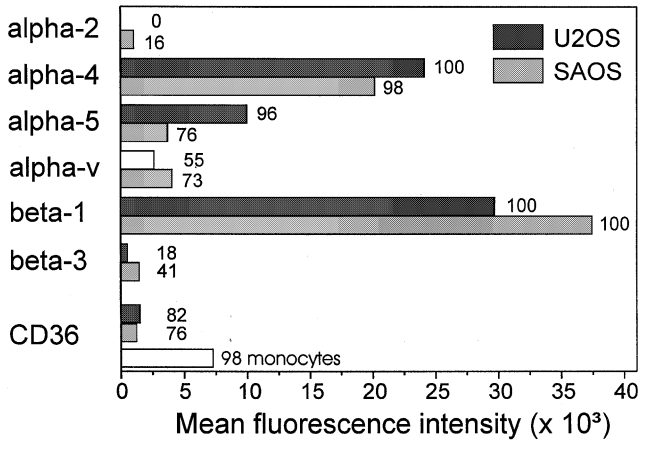

Fig. 2. Integrin expression by U2OS and SAOS osteosarcoma cells. Cells were incubated with anti-integrin monoclonal antibodies directed to different subunits. The cells were washed and incubated with FITC-conjugated goat anti-mouse IgG, washed again, and analysed by using fluorocytometry. The expression of CD36 by monocytes is included as control. Mean fluorescence intensity units are indicated by bars and mean percentage of positive cells is shown by figures.

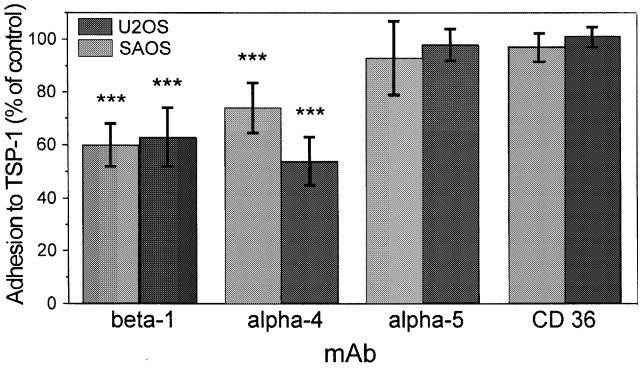

Fig. 3. Integrin α4β1 involved in U2OS and SAOS cell adhesion to TSP-1. Cells were preincubated with 40 μg/ml antibody for 15 min at 37 °C prior the cells were added to wells coated with TSP-1 (5 μg/ml). In control experiments no antibody was present. Data are expressed as percentage of control adhesion. Error bars indicate SD; significance to control was assayed using Student's t test: $^{***}p \leqslant 0.005$.

cell adhesion experiments were performed in the presence of neutralizing mAbs. The results demonstrate a most prominent inhibition with mAb anti-integrin β1 (about 40%) and anti-integrin α4 (up to 50%) but no or less than 10% inhibition in the presence of mAb anti-integrin α5 (Fig. 3). The result strongly supports that α4β1 integrin participates in the adhesion process to TSP-1. In addition no influence was observed with mAbs anti-integrin αvβ3 (LM609) and mAbs anti-integrin α2 (data not shown). Moreover adhesion experiments with the blocking anti-CD 36 monoclonal antibody FA6-152 was without effect in the adhesion experiments (Fig. 3). Adhesion assays in the presence of higher concentrations of anti-α4 mAb HP2.1 did not further increase the inhibitory influence, suggesting that other molecules may be involved in the adhesion process of TSP-1 with osteosarcoma cell lines, but which has not been investigated further on.

The 70-kDa chymotryptic fragment of TSP-1 promotes α4β1-dependent cell adhesion

To localize the potential α4β1-binding domain within the TSP-1 molecule we included in our adhesion experiment a 70-kDa chymotryptic fragment of TSP-1 as adhesive substrate. Chymotryptic digestion of TSP-1 in the presence of EDTA produces two stable fragments, the monomeric N-terminal 25-kDa heparin-binding domain and the trimeric 70-kDa central core region, which contains the procollagen-like domain as well as type 1 and type 2 repeats [19]. But this fragment does not contain the RGDA sequence at the C-terminus of the type 3 calcium-binding repeats and the cell-binding domain found in the C-terminal globular region. The rationale for use of the 70-kDa core region of TSP-1 was based on previous studies by Yabkovitz et al. [24], which described α4β1 and α5β1 as novel TSP-1 receptors on activated T-cells. Blocking of CD4+ T cell adhesion to intact TSP-1 was partly obtained with anti-TSP mAb A4.1. The binding epitope for this antibody lies within the amino terminal central stalk like region [19]. Therefore the 70-kDa fragment of TSP-1 was tested for activity and was found to promote dose-dependent adhesion of both osteosarcoma cell lines, reaching about 78% of the value obtained with the native protein. Comparable to native TSP-1 the maximum adhesion was obtained at a concentration of 5–10 μg/ml (Fig. 4A). Adhesion of both cell lines to the 70-kDa fragment was entirely dependent on the presence of the α4β1 integrin. Evidence could be obtained in cell adhesion experiments with integrin blocking antibodies. Adhesion of both cell lines to the 70-kDa fragment was inhibited over 88% by the addition of either anti-α4 (HP2/2); 44 H6) or anti-β1 (Lia1/2) mAbs (Fig. 4B). As shown for both cell lines their dose-dependent susceptibility to inhibition by anti-β1 mAb Lia1/2 was nearly identical, whereas there was some differences in the inhibitory influence of anti-α4 mAb HP2/1 observed in both cell lines. Half maximal inhibition of adhesion could be achieved at 5 μg/ml with the mAb against the β subunit, whereas 0.8 μg/ml of anti-α4 mAb HP2/2 was necessary for U2OS cells to obtain the same inhibition and 2 μg/ml for SAOS cells (Fig. 4C). Complete inhibition was archived with 3 μg/ml of HP 2/1 and about 10 μg/ml of Lia 1/2. The adhesion process between these osteosarcoma cell lines and the 70-kDa TSP-1 fragment is clearly shown to be entirely α4β1-mediated.

In addition further cell adhesion experiments with putative blocking reagents, heparin, and the soluble synthetic peptide GRGDSP, and with the neutralizing mAb anti-CD36 were performed to find out if other potential cell-binding sites within the native molecule or the central core fragment posses any influence on cell adhesion. Attachment of cells was insensitive to heparin and could not be prevented by the RGD antagonist and its negative control peptide (Fig. 5A). The CSVTCG sequence located in the type I repeats of TSP-1 has been suggested to be the primary CD36 binding site [25]. Neutralizing mAb anti-CD36 did not affect attachment of osteosarcoma cells to the 70-kDa fragment (Fig. 5A) and confirm the results obtained with the intact TSP-1 molecule that this non-integrin receptor is not involved in the adhesion process.

The integrin α4β1 interacts on the surface of endothelial cells with its counterreceptor, the vascular cell adhesion molecule 1 (VCAM-1) [26]. A second ligand is the extracellular matrix glycoprotein fibronectin [15,16]. Within fibronectin α4β1 recognizes several sequences in the central part of the molecule. The most active adhesive sequence within the III CS domain of fibronectin is circumscribed by the CS-1 peptide and contains the tripeptide Leu–Asp–Val (LDV) as minimal active site [27]. To assess a possible relationship between the

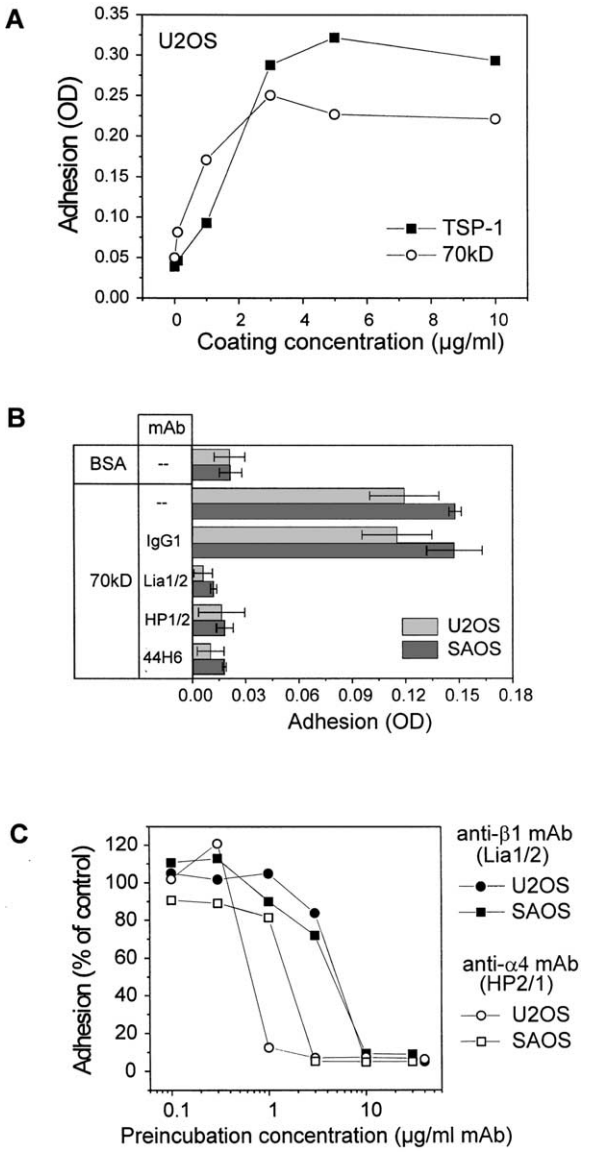

Fig. 4. The 70-kDa fragment of TSP-1 promotes the α4β1-dependent cell adhesion of osteosarcoma cells. (A) Comparison of cell adhesion of U2OS cells to intact TSP-1 and the purified 70-kDa fragment. Wells were coated with increasing concentrations of intact TSP-1 or the 70-kDa TSP-1 fragment and cell adhesion was performed in the presence of RPMI medium containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+}. Values are shown as mean absorbance at 550 nm. (B) Adhesion experiments with blocking antibodies indicate that the 70-kDa chymotryptic fragment of TSP-1 promotes the α4β1-dependent cell adhesion. Cells were preincubated with 40 μg/ml mouse IgG, anti-α4 mAb (HP2/1; 44H6), or anti-β1 mAb (Lia1/2) and cell adhesion performed with wells coated with the 70-kDa TSP-1 fragment (5 μg/ml). Control experiments were performed in the absence of any additive (−) or in the presence of mouse IgG. In addition, adherence to wells coated with bovine serum albumin (BSA) (20 mg/ml) are shown. Values represent the absorbance averaged from two experiments in triplicate wells and values are mean absorbance ± SD. (C) Dose-dependent inhibition of U2OS and SAOS cell adhesion to the 70-kDa TSP-1 fragment. Cell adhesion experiments were performed in the presence of increasing concentrations of anti-α4 mAb (HP2/1) or anti-β1 (Lia1/2) under the same experimental conditions as described in (B). Results are shown as percentage of attached cells relative to control (in the presence of mouse IgG).

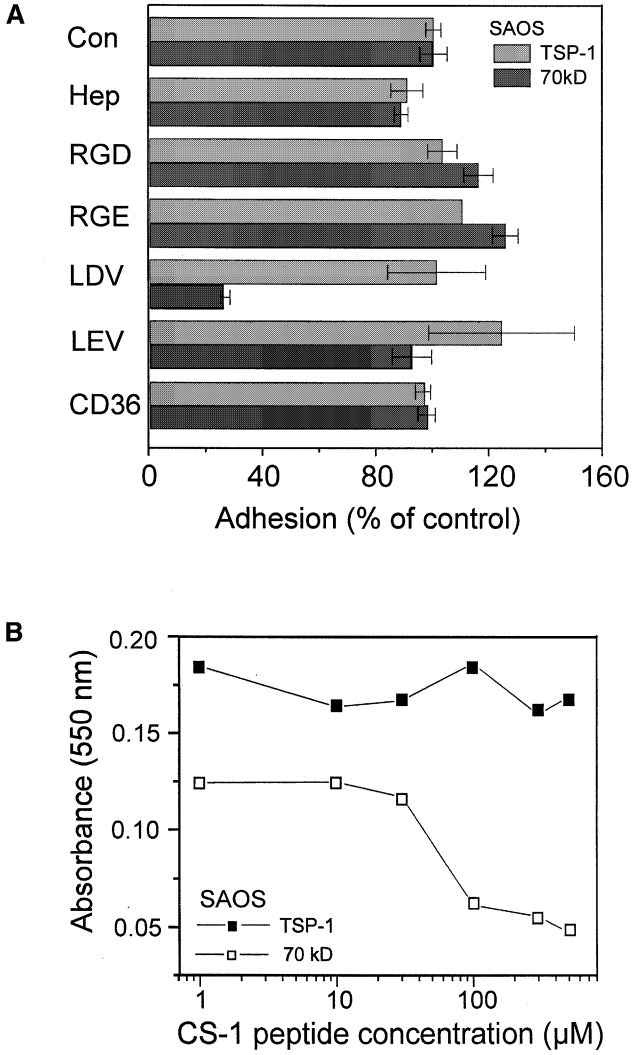

Fig. 5. Effect of CS-1 peptide on intact TSP-1 and 70-kDa fragment-mediated cell adhesion. (A) Cells were allowed to adhere on wells coated with 5 μg/ml of intact TSP-1 or the 70-kDa fragment and in addition to BSA (20 mg/ml)-coated wells. Prior to plating SAOS cells were incubated either with heparin (300 μg/ml), anti-CD36 mAb (FA6.152; 40 μg/ml), or with the following peptides (500 μM): RGD (GRGDSP) and its control RGE (GRGESP); CS-1 peptide LDV (EILDVSPT) and its control LEV (EILEVSPT). In the control no peptide was present. The amount of adherence on BSA-coated wells was subtracted from the obtained values and the results expressed as percentage of attached cells relative to control ± SD. (B) Dose-dependent inhibition of cell adhesion to the 70-kDa TSP-1 fragment in response the CS-1 peptide. Cell adhesion of SAOS cells can be inhibited with increasing concentrations of CS-1 peptide (EILDVSP) to wells coated with the 70-kDa TSP-1 fragment, but not to native TSP-1. Values are given as mean absorbance at 550 nm.

binding motif for α4β1 of TSP-1 or its 70-kDa fragment and the adhesive sequence of fibronectin, we performed adhesion assays in the presence of soluble CS-1 peptide. Whereas the active CS-1 peptide (EILDVPST) prevented attachment of U2OS cells to 70-kDa coated wells, the control peptide (EILEVPST) had no inhibitory activity (Fig. 5A). Using increasing doses a 75%

inhibition was obtained in the presence of 500 μM of the CS-1 peptide (Fig. 5B). In contrast to the 70-kDa fragment no marginal influence was observed in the adhesion process of osteosarcoma cells to the native TSP-1 molecule (Fig. 5A).

Taken together these results confirm and extend the initial results with anti-integrin mAbs of the specific role of α4β1 and demonstrate that indeed the recognition site of the α4β1 integrin is located within the 70-kDa central stalk-like region of the TSP-1 molecule.

Known binding sites for α4β1 integrin found in fibronectin and VCAM-1 include the binding sequences LDVP, IDSP, or EDV. In the 70-kDa fragment of TSP-1 none of these binding sites are present and yet an inhibitory effect of the CS-1 peptide was observed. There exists an LDV motif in the N-terminal heparin-binding domain (25-kDa fragment), but this globular domain is cleaved from the intact molecule during digestion with chymotrypsin and displayed no activity in adhesion experiments (unpublished observation). A similar inhibitory effect of the CS-1 peptide was recently reported for osteopontin another ligand for the α4β1 integrin [28]. The blocking CS-1 peptide specifically interfered with binding to α4β1 integrin although this adhesive protein does not contain binding sequences found in fibronectin and VCAM-1. Further analysis revealed that two novel binding sites not related to the binding motif found in the CS-1 peptide are responsible for the interaction with the α4β1 integrin [29]. These results demonstrate that the α4β1 integrin recognizes a wide variety of motifs and therefore shows a broader ligand specificity than most other members of the integrin family. Further work will be necessary to identify the recognition sequence for α4β1 integrin within the 70-kDa fragment of TSP-1.

The functional role of the TSP-1/α4β1 interaction remains to be determined. In a large number of investigations the contribution of α4β1 integrin in the trafficking process of leukocytes into tissue during the normal inflammatory response as well as in pathological situations such as during the development of atherosclerotic lesions has been described [16,30,31]. Depending on the stage of tumor progression the expression of α4β1 integrin may also influence the metastatic process of tumor cells. Induction of α4β1 integrin in primary tumors resulted in reduced metastasis formation whereas after entering blood circulation expression of α4β1 on tumor cells derived from melanoma, sarcoma, or lymphomas rather promoted metastasis formation [32, and references cited therein]). In most of these cases VCAM-1 or fibronectin has been reported to be the preferential ligand for α4β1. But the expression of TSP-1 in a variety of cells and tissue and the here-reported specific interaction with the α4β1 integrin argues for a participation of TSP-1 in these processes.

In conclusion, we found that osteosarcoma cells show a prominent expression of α4β1 integrin. Adhesion experiments demonstrated that this integrin is an adhesion receptor for TSP-1. Specific fragmentation of TSP-1 with chymotrypsin allowed us to locate the α4β1-binding site within a central 70-kDa core region of the molecule and inhibition experiments with blocking antibodies and with synthetic peptides suggest a specific binding recognition sequence for the α4β1 integrin. In addition to the identification of this binding motif the relevance of the TSP-1/α4β1 interaction during the process of tumor progression and metastasis is a further interesting question emerging from our results.

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