Human Carcinoma Cells Express Receptors for Distinct Domains of Thrombospondin

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ABSTRACT

Thrombospondin (TSP), an adhesive glycoprotein, is incorporated into the extracellular matrix, mediates cell attachment and spreading, chemotaxis, haptotaxis, and may participate in the directed movement of cells in metastasis. Evidence from several model systems suggests that these functions may be mediated by different domains within the TSP molecule. Radioligand binding assays on 11B squamous carcinoma cells with 125I-TSP demonstrated the presence of 1.2 × 10^6 sites/cell with an apparent K_d of 74 nM. Binding studies using TSP fragments demonstrated that both the NH_2 terminal heparin-binding domain (HBD) and the COOH terminal fragment with a molecular weight of 140,000 (140K) retained the ability to bind 11B cells in a time-dependent, dose-dependent, saturable, and specific manner. The HBD bound to 11B cells with an apparent K_d of 1.2 μM at 1.4 × 10^5 sites/cell. Binding of intact TSP to 11B cells demonstrated half-maximal binding at 20 nM and a B_max of 9.1 × 10^5 sites/cell. The binding of 140K also showed a high degree of positive cooperativity with a Hill slope of +3.5, suggesting that binding one 140K molecule to cells leads to increased binding of additional 140K molecules. In addition, the HBD and 140K showed no cross-competition in binding assays. Therefore, it appears likely that these distinct TSP domains bind to separate sites on the cell surface. Neither vitronectin or the peptide RGDS were able to inhibit the binding of TSP or 140K to 11B cells. Based on these data, there appears to be more than one distinct receptor on 11B cells for TSP: one receptor class which mediates the binding of the HBD and a second receptor class which mediates the binding of the M, 140,000 fragment.

INTRODUCTION

TSP is an extracellular matrix glycoprotein of M, 450,000 first described as a major component of platelet α granules. It is synthesized and secreted by several different cell types including endothelial cells, fibroblasts, macrophages, smooth muscle cells, and epithelial cells. TSP has been implicated in the control of smooth muscle cell growth, and in the metastatic potential of squamous carcinoma cells. TSP has also been implicated in the attachment and spreading of melanoma cells. TSP also promotes melanoma cell chemotaxis and haptotaxis. Furthermore, a unique spatial and temporal distribution of TSP has been observed in the developing mouse embryo suggesting an important role for TSP in morphogenesis.

TSP is a trimeric molecule whose identical subunits are held together by intramolecular disulfide bonds. Extensive analysis of the TSP molecule has delineated a distinctive domain structure: the NH_2 terminus contains an HBD and the COOH terminus (140K) contains a region responsible for the binding of TSP to platelets, a conformationally sensitive Ca^{2+}-binding domain, and a portion that mediates the binding of TSP to fibronectin, fibrinogen, collagen V, laminin, and plasminogen (see Refs. 4 and 11 for review).

Several other extracellular matrix proteins, such as fibronectin and laminin, are composed of distinct structural domains. In many systems these structural domains appear to differentially promote cell adhesion, neurite outgrowth, chemotaxis, or haptotaxis. Similarly, the domains of TSP appear to mediate distinct cellular processes. The HBD of TSP has been shown to possess lectin-like activity, mediate melanoma cell chemotaxis, modulate the growth inhibitory response of smooth muscle cells to heparin, and may be important in TSP internalization and degradation by cells (6, 9, 16–19). The COOH-terminal domain has been implicated in the binding of TSP to platelets, the adhesion and spreading of carcinoma cells and keratinocytes to coated plastic, and melanoma cell attachment and haptotaxis (7–9, 20, 21).

Data from several investigators suggest that the HBD mediates the binding of TSP to cells through a cell surface-associated heparan sulfate proteoglycan and/or sulfated glycolipid, a binding site that may be distinct from that which recognizes the M, 140,000 COOH-terminal fragment of TSP. Several other cell surface molecules have also been proposed as receptors for TSP, including an RGD-dependent vitronectin receptor analogue on endothelial cells and platelet glycoprotein IV. The most compelling evidence for two different receptors for TSP comes from studies of melanoma cells. The M, 140,000 fragment supports attachment of G361 melanoma cells but not spreading. In contrast, the HBD appears to mediate G361 cell spreading but does not support attachment. The HBD also promotes melanoma cell chemotaxis, whereas the M, 140,000 fragment promotes haptotaxis. These differential effects of TSP domains on cell-substrate interactions and cell motility suggest that the binding of TSP to the cell surface is likely to be multifaceted and domain dependent.

We undertook a series of binding experiments to examine in greater detail the binding of TSP and TSP fragments to 11B squamous carcinoma cells. Both the HBD and the COOH-terminus 140K retained the ability to bind to 11B cells in a time-dependent, dose-dependent, saturable, and specific manner indicating the presence of specific receptors on the cell surface. The HBD bound to 11B cells with approximately the same number of sites/cell as intact TSP but with a lower affinity. The 140K demonstrated strong positive cooperativity in binding suggesting that the binding of this domain to cell surface receptors proceeds via a complex and interactive mechanism. Additionally, cross-competition experiments demonstrated that the HBD and 140K appeared to bind to independent sites on the cell surface. Therefore, the interaction of TSP with the carcinoma cell surface can occur through either of two molecular domains, each of which may, in turn, differentially affect the mechanism of TSP action.
MATERIALS AND METHODS

Reagents and Materials. Heparin (from porcine intestinal mucosa, grade I-A) BSA (98–99%, essentially fatty acid- and globulin-free), hirudin, chondroitin sulfate, desmertan sulfate, and aprotinin were purchased from Sigma Chemical Co. (St. Louis, MO). Heparin-Sepharose was purchased from Pharmacia (Uppsala, Sweden). Na\textsuperscript{25}I (carrier free) was purchased from ICN Biomedicals (Irvine, CA). Vitronectin was purified according to the procedure of Yatohgo et al. (28) and its homogeneity verified by SDS-PAGE and immunoblotting with an antivitronectin antibody (Chemicon, El Segundo, CA). Thrombin (5002 units/ml) was a generous gift of Dr. John Fenton (New York State Department of Health, Albany, NY).

Purification of TSP and Fragments. TSP was isolated from thrombin-stimulated platelets essentially as described previously (29). The recombinant HBD was purified from Escherichia coli containing plasmid HBD5 utilizing ion exchange and heparin-Sepharose affinity chromatography as described before (30). The 140K was generated from intact TSP by proteolytic digestion with thrombin. Thrombin (16 units/mg TSP) was added to TSP in 20 mM 4-morpholinepropanesulfonic acid-0.15 M NaCl-1 mm Ca\textsuperscript{2+}-20% sucrose, pH 7.6. The reaction mixture was incubated at 37°C for 8 h before being stopped with 50 units hirudin and 10 μg/ml aprotinin. Heparin-Sepharose affinity chromatography was used to separate the 140K from intact TSP and the heparin-binding domain since the latter two components bind tightly to heparin-Sepharose, whereas 140K did not. Purified proteins were concentrated further, when necessary, by Centricon 10 or 30 ultrafiltration (Amicon, Danvers, MA). TSP, the rHBD, and the 140K were purified to ≥95% homogeneity as judged by SDS-PAGE analysis.

Iodination of TSP and TSP Fragments. Iodination of TSP was carried out by the iodogen method according to that described by Fraker and Speck (31) to a specific activity of ~3 x 10\textsuperscript{6} cpm/μg. Previously, 125I-TSP labeled in this manner has been shown to retain biological activity in cell adhesion assays (32). Iodination of the 140K and the rHBD was performed using Bolton-Hunter reagent (New England Nuclear, Boston, MA) to a specific activity between 0.5 and 2.5 x 10\textsuperscript{6} cpm/μg. The 125I-rHBD was further purified on a heparin-Sepharose column to ensure that this reagent retained heparin-binding activity. A Sephadex G-25 column (Sigma) was used to separate iodinated proteins from free iodine. SDS-PAGE followed by autoradiographic analysis of iodinated reagents verified that no degradation had occurred during iodination. Additionally, ≥95% of incorporated counts were precipitable with trichloroacetic acid.

Cell Culture and Harvesting. UM-SCC-11B squamous carcinoma cells were grown in monolayer culture in Eagle’s MEM containing 10% calf bovine serum, MEM nonessential amino acids, 100 units/ml penicillin, and 100 μg/ml streptomycin (32). Cells were harvested at 80% confluency by incubating plates with 10 ml cold phosphate-buffered saline containing 1 mM EDTA, pH 7.4, for 10 min. Cells were pipeted off the plates, pooled, and centrifuged at 1000 rpm for 6 min at 4°C. The cells were washed three times in cold phosphate-buffered saline, pH 7.4, before being resuspended in blocking buffer for binding assays (see below).

Cell-binding Assays. Binding assays were performed in 1.5-ml microfuge tubes in a final volume of 0.2 ml. Both microfuge tubes and cells were incubated in blocking buffer (MEM containing 1% BSA) for 45 min to block nonspecific binding. Cells (2 x 10\textsuperscript{5}/sample) and binding buffer (MEM containing 1% BSA) with or without 100-fold excess (w/w) cold competitor were then preincubated for 30 min at 4°C. The peptides GRGDS (Calbiochem, La Jolla, CA) and RGES (Peninsula Labs, Belmont, CA) were used for cold competition at a final concentration of 1 mM. When heparin was used for competition, the 125I-ligand was preincubated with 100-fold excess (w/w) heparin for 30 min at 4°C. The binding assay was initiated by adding the 125I-ligand to each sample and incubating the tubes for 45 min at 4°C with gentle mixing. At the end of the assay period, bound counts were separated from unbound counts by layering each sample over a 20% sucrose cushion in binding buffer and centrifugation at 10,000 x g for 2.5 min. Cell pellets were frozen in an ethanol-dry ice bath, excised, and counted on an LKB 1261 Multigamma gamma counter (LKB, Bromma, Sweden).

RESULTS

The interaction of TSP with 11B squamous carcinoma cells was examined in radioligand binding assays. These experiments were performed on 11B cells in suspension, in contrast to an earlier report (32) in which binding was conducted on monolayer cultures, in order to ensure that TSP bound to cell surface-associated molecules and not extracellular matrix components laid down by cells. Initial studies revealed that cells harvested with trypsin, as compared to cells harvested with EDTA, exhibited an 80% decrease in TSP binding. TSP binding after trypsinization did not increase even after allowing cells to recover for 2 h at 37°C (data not shown). Consequently, all binding studies were performed with EDTA-harvested cells.

Specific binding of intact TSP to 11B cells is shown in Fig. 1. Specific binding of TSP to cells increases as the concentration of input TSP increases in the concentration range of 2–350 nM TSP. Binding data were fit to either a one-site or two-site model using the GraphPad computer program and goodness-of-fit was assessed. The data points best fit a two-site model of binding in which the second binding site represented a large component

![Graph](https://via.placeholder.com/150)
to examine the relationship between the structural domains of TSP and their interaction with the cell surface since functional data indicated that different TSP domains mediated unique functional responses. Time course binding studies were carried out with the rHBD and 140K to examine the kinetics of binding to 11B cells. Previous work has shown that the rHBD of TSP was identical to the native HBD in both heparin and cell binding assays (30). The rHBD demonstrated saturable binding to cells within 10 min (Fig. 2A). Similarly, 140K demonstrated saturable binding within 20 min (Fig. 2B). Thus, both fragments exhibited binding kinetics consistent with a time dependence relevant to physiological processes.

Specific binding of the rHBD to 11B cells was dose dependent and saturable (Fig. 3). Specificity of binding was demonstrated by competition with 100-fold excess, unlabeled rHBD. Binding could also be competed for by the addition of 100-fold excess unlabeled TSP or heparin (Table 1). Specific HBD binding increased in a dose-dependent manner with increasing ligand in the concentration range of 40 nM–2 μM. Nonlinear regression analysis of specific binding using the GraphPad computer program demonstrated that the data best fit a one-site model of binding (r² 0.99) with a Kₐ of 1.2 μM and 1.4 × 10⁶ sites/cell.

Binding of 140K to 11B cells was distinct from the binding observed with either intact TSP or the HBD. The dose responsiveness of specific 140K binding increased biphasically over the concentration range of 1–60 nM 140K (Fig. 4). Nonlinear regression analysis with the GraphPad program indicated that

![Graph](image_url)

**Fig. 2.** Time course of rHBD and 140K binding to 11B carcinoma cells. A, cells (1 × 10⁶) were incubated with ¹²⁵I-rHBD (600 nM) at 4°C with gentle mixing. At indicated time points, 100-μl aliquots were removed and bound cpm separated from unbound cpm as described in “Materials and Methods.” Each point represents an average value from 2 independent experiments and was assayed in duplicate. B, cells (1 × 10⁶) were incubated with ¹²⁵I-140K (5 nM) at 4°C with gentle mixing. At indicated time points, 100-μl aliquots were removed and bound cpm were separated from unbound cpm as described. Each point represents the mean from 2 experiments with all time points performed in duplicate.

of nonspecific binding at high ligand concentrations (r² 1.00). Comparing the fit of the data to a one-site versus a two-site model in this manner and performing an F test to determine goodness-of-fit, a P value of <0.05 was obtained suggesting that the two-site model was a better fit. Binding parameter for the high affinity site of Kₐ = 74 nM representing 1.2 × 10⁶ sites/cell were estimated by the computer using this best fit model for the interaction of intact TSP with 11B cells. When the fit of the binding data was compared for a one-site versus a two-site model, in which the second binding site was assumed to represent specific binding, it was not possible to statistically distinguish between these two models (P < 0.25). Therefore, it appears that the binding of TSP to cells is best described by a two-site model in which the second binding site is of very low affinity/high capacity. TSP self-association/polymerization at high ligand input values has previously been noted in solid phase binding assays (34) and could account for binding at this secondary site. Additionally, we have determined that TSP binding is divalent cation dependent with half-maximal binding observed at approximately 100 μM Ca²⁺/Mg²⁺.

Binding studies were initiated with TSP fragments in order

* R. Yabkowitz and V. M. Dixit, unpublished observations.

### Table 1 Inhibitors of binding of TSP fragments to 11B cells

<table>
<thead>
<tr>
<th>Treatment or competitor</th>
<th>¹²⁵I-rHBD</th>
<th>¹²⁵I-140K</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSP</td>
<td>100²</td>
<td>100</td>
</tr>
<tr>
<td>rHBD</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>140K</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>Heparin</td>
<td>109</td>
<td>0</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>0</td>
<td>ND²</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>1</td>
<td>ND</td>
</tr>
</tbody>
</table>

² Binding assays were performed as described in “Materials and Methods.” Radiolabeled ligand (from 1–800 nM) was incubated with 11B cells in the presence or absence of a 100-fold excess of the indicated competitor. All points were performed in duplicate.

² Inhibition specific binding (%). Values were calculated as follows:

\[
f_mol\ ligand\ bound\ in\ absence\ of\ competitor \times 100
\]

\[
\frac{100 + \left(\frac{fmol\ ligand\ bound\ in\ presence\ of\ competitor}{fmol\ bound\ in\ absence\ of\ competitor}\right)}{fmol\ bound\ in\ absence\ of\ competitor}
\]

where 100% inhibition is defined by ¹²⁵I-ligand binding in the presence of 100-fold excess unlabeled TSP.

² ND, not determined.

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* R. Yabkowitz and V. M. Dixit, unpublished observations.
Together with the large differential in the number of binding cells. These results revealed that binding of the HBD did not inhibit binding of the rHBD but not the 140K to cells binding of the 140K to cells. The differential ability of heparin sulfate proteoglycan as has been suggested by others (19, 22, binding site for the HBD of TSP may, therefore, be a heparan
sulfate, dermatan sulfate significantly inhibited specific rHBD binding to the cell surface. The computer estimated that half-maximal binding was achieved at 36 nM with a $B_{\text{max}}$ of $1.9 \times 10^5$ sites/cell. Attempts to fit the data to either a one-site or two-site model of binding resulted in nonconvergence thus confirming that the best fit of the data is to a cooperative binding model.

The binding sites for the HBD and 140K on the 11B cell surface might be contained within a single cell surface molecule or, alternatively, each domain might independently bind to distinct cell surface molecules. Cross-competition binding studies were conducted to determine whether the HBD and 140K influenced the binding of its counterpart to 11B carcinoma cells (Table 1). The binding of $^{125}$I-rHBD to 11B cells was not inhibited by 100-fold excess 140K. Likewise, 100-fold excess rHBD was unable to compete for the binding of $^{125}$I-140K to cells. These results revealed that binding of the HBD did not modulate the binding of the 140K to cells and vice versa. Together with the large differential in the number of binding sites/cell for each domain, these data indicated that the HBD and 140K bound to distinct molecules on the cell surface.

Additional binding experiments were carried out to further characterize the HBD binding site on 11B cells. Both TSP and heparin completely inhibited the specific binding of $^{125}$I-rHBD to cells (Table 1). In contrast, neither chondroitin sulfate or dermatan sulfate significantly inhibited specific rHBD binding to cells (0 and 1% inhibition, respectively). The 11B cell surface binding site for the HBD of TSP may, therefore, be a heparan sulfate proteoglycan as has been suggested by others (19, 22, 35). As would be expected, heparin did not compete for the binding of the 140K to cells. The differential ability of heparin to inhibit binding of the rHBD but not the 140K to cells provided further evidence that the cell surface binding sites for these domains are likely to be distinct molecules.

A summary of the binding parameters of 11B carcinoma cells for intact TSP, the HBD, and the 140K is presented in Table 2. TSP bound to 11B cells with an apparent $K_s$ of 74 nM, an intermediate value between the binding affinities observed for the HBD and 140K. It is probable that the binding of intact TSP to cells is modulated by complex ligand-receptor interactions that cannot be entirely explained by the interactions of the isolated individual TSP domains with cells alone. Although the number of binding sites/cell for intact TSP and the HBD was very similar, their apparent respective $K_s$ values differed by a factor of 20. This discrepancy suggested that conformational restraints on the HBD may be present in the intact TSP molecule, possibly as a result of the trimeric nature of TSP, which are lost after the HBD is isolated.

Although many receptors for extracellular matrix proteins have been shown to bind their respective ligands via an RGD-dependent mechanism (36-41), the role of the RGD sequence

Table 2 Summary of binding parameters for TSP or TSP fragments to 11B cells

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_s$ (nM)</th>
<th>No. of binding sites/cell x $10^3$</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSP</td>
<td>74 (±26)$^a$</td>
<td>12.0 (±4.5)</td>
<td>10</td>
</tr>
<tr>
<td>rHBD</td>
<td>1200 (±138)</td>
<td>14.0 (±0.8)</td>
<td>7</td>
</tr>
<tr>
<td>140K</td>
<td>36$^b$ (±5)</td>
<td>1.9 (±0.4)</td>
<td>13</td>
</tr>
</tbody>
</table>

$^a$ Numbers in parentheses, SE.

$^b$ $EC_50$ value, concentration of 140K that yields half-maximal binding.
in TSP remains ambiguous. Previous work demonstrated no effect of a synthetic RGD peptide on 11B squamous carcinoma cell attachment to TSP-coated plastic (21). To examine further the interaction between TSP binding to the cell surface and RGD peptides, binding experiments were conducted using 1 mM RGDS or RGES to compete for the binding of 125I-TSP to 11B cells. From the binding data (Fig. 5A), it was clear that neither RGDS nor the control peptide, RGES, was capable of competing for the binding of TSP to 11B cells. Vitronectin, which contains an RGDS sequence that has been demonstrated to be functionally relevant (39), also did not inhibit 125I-TSP binding to cells. Since the RGD sequence of TSP resides in the 140K, binding of 125I-140K to 11B cells was also examined in the presence of excess RGDS, RGES, or vitronectin (Fig. 5B). The peptides RGDS and RGES did not inhibit the binding of 140K to cells. Similarly, vitronectin was unable to compete for the binding of 140K to cells. These results suggested that the RGD sequence of TSP does not participate in the binding of TSP to the 11B cell surface and that the 11B cell 140K receptor is not the previously described vitronectin receptor (25).

DISCUSSION

Human 11B squamous carcinoma cells express at least two distinct binding sites for TSP. Although the domain structure of TSP has been well documented, the interaction of the TSP domains with the cell surface remains unclear. Here, we have shown that the NH2-terminus heparin-binding domain binds to 11B cells with an apparent Kd of 1.2 μM at 1.4 x 10⁶ sites/cell, whereas the COOH-terminus 140K domain binds to 11B cells with half-maximal binding reached at 36 nM and a Bmax of 1.9 x 10⁶ sites/cell. Therefore, the accumulating evidence that functionally differentiates between the HBD and 140K domain of TSP in distinct cellular processes is reiterated in the interaction of each domain with distinct cell surface receptors.

The HBD has been implicated in the lectin-like activity of TSP, cell spreading, melanoma cell chemotaxis, and TSP degradation by endothelial cells (6, 9, 16–19). The HBD may also mediate the killing of 11B carcinoma cells by monocytes (42). Several lines of evidence have pointed to a heparan sulfate proteoglycan as a candidate for a TSP receptor (19, 22, 35). Our data also suggest that a heparan sulfate proteoglycan may serve as a receptor for the HBD. Binding of the HBD is inhibited by heparin but not by either chondroitin or dermatan sulfate. The binding parameters for the HBD (1.4 x 10⁶ sites/cell) suggest that the majority of TSP binding to cells is due to the interaction of the HBD with its receptor at the cell surface. Although intact TSP contains three HBDs (one at the NH2 terminus of each monomer), there is no evidence for binding cooperativity. The binding of one HBD does not appear to influence the binding of another HBD moiety.

The 140K of TSP has been shown to be involved in the initial attachment of 11B carcinoma cells to TSP, melanoma cell haptotaxis, and platelet binding (9, 20, 21). The attachment of 11B carcinoma cells to a substratum is promoted by the 140K as well as intact TSP (21). The concentration of TSP or 140K that promotes half-maximal attachment is approximately 11 nM, a ligand concentration consistent with the range of ligand concentrations used in these binding studies and similar to the 140K concentration at which half-maximal cell binding is observed, approximately 30 nM. Therefore, the dose range within which significant receptor occupancy occurs correlates well with the dose range for TSP-mediated 11B cell attachment. Binding of 140K to 11B cells was characterized by positive cooperativity (Hill coefficient of +3.5) reaching half-maximal binding at 36 nM 140K and representing a maximum of 2 x 10⁶ sites/cell. Changes in receptor conformation as a result of ligand binding at low 140K concentrations could account for this cooperative interaction. Recently, Frelinger et al. (43) demonstrated that ligand occupancy of platelet glycoprotein IIb-IIIa resulted in conformational changes leading to an increased number of binding sites for a monoclonal antibody recognizing this adhesion receptor. The complex nature of the interactions between TSP domains and their respective receptors on the 11B cell surface suggests further that intact TSP binding to the cell surface may involve several interconnected mechanisms. Further information concerning the structure and spatial organization of TSP receptors may clarify the pathways involved in TSP binding to cells.

Recently, some data have been presented that the TSP receptor on endothelial cells is similar to the previously described endothelial cell vitronectin receptor (25). In our experiments, there is no inhibition of TSP binding to 11B cells by a 100-fold excess of vitronectin. Neither does vitronectin inhibit the binding of the 140K of TSP (which contains the RGD sequence) to cells. These results are consistent with other data that demonstrated that neither vitronectin nor anti-vitronectin antibodies inhibited TSP binding to cells or cell attachment to TSP, respectively (8, 19). Further, preliminary results from our laboratory confirm the absence of the β3α6 vitronectin receptor from 11B carcinoma cells (data not shown). Additionally, the peptide RGDS, which inhibits the attachment of cells to vitronectin-coated substrates (44) and mediates vitronectin receptor binding in affinity chromatography purification (39), had no effect on the binding of TSP or 140K to 11B cells. The inability of RGD peptides to inhibit TSP-mediated cell attachment or TSP binding has been previously reported (8, 21, 27, 45). Based on these results, it appears unlikely that TSP binds to 11B cells via its RGD sequence or to a vitronectin receptor analogue on these cells.

Thrombospondin has been shown to modulate cellular growth and differentiation and has been linked to increased tumor cell metastasis (7). The ability of TSP to bind plasminogen and plasminogen activator (46) may play a direct role in cellular growth control or metastasis by increasing local concentrations of extracellular proteases. It is not known whether the effects of TSP on cellular processes are mediated solely through intact TSP, but accumulating evidence suggests that TSP fragments may play some role as well. The domain structure of TSP is well characterized and has led to numerous studies which have been able to functionally distinguish between the HBD and the COOH-terminus 140K domain. We have been able to further distinguish between these TSP domains with respect to their binding to squamous carcinoma cells. In alignment with functionally distinct TSP domains, there may also exist different intracellular pathways, i.e., for signal transduction, associated with each domain. The purification of TSP receptors will undoubtedly provide a first step in dissecting the molecular events which regulate the diverse effects of TSP on cells.

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CARCINOMA CELL THROMBOSPONDIN RECEPTORS


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