MINIREVIEW

The Role of Thrombospondin-1 in Tumor Progression

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The role of thrombospondin-1 (TSP-1) in tumor progression is both complex and controversial. It is clear from the literature that the function of TSP-1 in malignancy depends on the presence of other factors and the level of TSP-1 expression in the tumor tissue. High levels of TSP-1 secreted by tumors, which were engineered to overexpress TSP-1, inhibit tumor growth, while anti-sense inhibition of TSP-1 production in certain tumore also inhibits growth. Clearly, the presence of other factors in these experimental systems must be important. The role of TSP-1 in angiogenesis also depends on the levels of TSP-1, the presence and level of angiogenic stimulators such as basic fibroblast growth factor (bFGF), and the localization of TSP-1 in the tissue. Matrix-bound TSP-1 promotes capillary tube formation in the rat aorta model of angiogenesis, while TSP-1 inhibits bFGF- induced angiogenesis in the rat cornea model. The inhibitory effect also depends on the proteolytic state of TSP-1 since the amino terminus promotes angiogenesis in the cornea model, while the remaining140-kDa fragment inhibits bFGF-induced angiogenesis. Both the stimulatory and inhibitory effects of TSP-1 are likely due to upregulation of matrix-degrading enzymes and their inhibitors. These enzymes are critical for maintaining optimal matrix turnover during angiogenesis. These varied TSP-1-dependent mechanisms offer new targets for the development of anti-angiogenic therapeutics for the treatment of a variety of cancers, as well as other pathologies involving inappropriate angiogenesis such as diabetic retinopathy. [Exp Biol Med Vol. 226(8):726–733, 2001]

Key words: thrombospondin-1; adhesion; invasion; angiogenesis

Thrombospondin-1 (TSP-1) is a 450-kDa trimeric glycoprotein first described in 1971. The molecule is stored in high concentrations in the α granules of platelets, constitutes 25% of platelet-secreted protein (1), and participates in platelet aggregation and clot formation (2). Although a variety of other cells have been found to produce TSP-1, most of it is not free in circulation, but is strongly bound to the extracellular matrix (ECM). Numerous biological activities have been ascribed to TSP-1, including effects on cell adhesion, migration, proliferation, and angiogenesis. However, the precise biological role of TSP-1 has yet to be fully determined. In this updated review (3), we will briefly describe the functional roles of TSP-1 in tumor progression and we will propose a possible mechanism of action.

Expression and Localization of TSP-1

Numerous cell types have been shown to produce TSP-1 and incorporate it into the ECM. The expression of TSP-1 is higher in proliferating cells than in quiescent cells, and is regulated during cellular differentiation. By using immunostaining techniques, investigators found that TSP-1 can be synthesized and secreted by many cultured human tumor cell lines, including squamous carcinoma, melanoma, glioma, osteosarcoma, and breast adenocarcinoma (4–6). However, these transformed cells may not synthesize as much TSP-1 as normal cells, like normal fibroblasts (7). In a microarray analysis of breast cancer, it was found that metastatic cells express 3-fold less TSP-1 than normal cells (8). Thus, expression of TSP-1 may have different regulatory mechanisms in normal and transformed cell lines.

TSP-1 was significantly associated with bladder and gallbladder cancer recurrence and overall survival. Patients with low TSP-1 expression exhibited increased recurrence rates and decreased overall survival (9). In another study, 74.5% (29/39) of the T2 and T3 (TMN classification) gall-

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bladder cancers were TSP-1 positive, while none (0/14) of the T1 cancers showed TSP-1 expression ($I < 0.001$). Lymph node metastasis and venous involvement were frequently found in the TSP-1-positive cases (90.0% and 87.1%, respectively) of gallbladder adenocarcinoma ($I < 0.001$) (10). These findings suggested that TSP-1 plays an important and complex role in cancer cell growth and metastasis.

Varani *et al.* (11) found that the metastatic 11B squamous cell carcinoma cell line, which synthesized and secreted the highest level of TSP-1, formed the most undifferentiated tumors in athymic mice. Castle *et al.* (12) determined that when the same malignant cell line was transfected with a TSP-1 cDNA antisense expression vector to reduce TSP-1 synthesis, it appeared phenotypically more normal, decreased its rate of growth *in vitro*, and formed either no tumor or slow growing, highly differentiated tumors in athymic mice.

In contrast, others found the opposite result. Weinstat-Saslow *et al.* (13) observed that transfection of human MDA-MB-435 breast carcinoma cell line with TSP-1 cDNA to induce overexpression of TSP-1 reduced their capacity to grow and metastasize in athymic mice. Streit *et al.* (14) found similar results in two human cutaneous tumor cell lines. Stable overexpression of TSP-1 resulted in a significant inhibition of tumor growth by 50% to 75% after 4 weeks as compared with control tumors in nude mice. Also, extensive areas of necrosis were detected in TSP-1 overexpressing tumors. *In vitro*, the same cells showed no differences in anchorage-dependent and -independent cell growth. These studies suggest that TSP-1, by an indirect mechanism, activates expression of tumor genes that control invasion and growth. Moreover, another group showed that transfection with TSP-1 induced skin carcinoma suppression without affecting cell proliferation *in vitro* or *in vivo*. In this case, TSP-1 was postulated to act as a matrix barrier at the tumor/stroma border, which, probably by halting tumor vascularization, prevents tumor cell invasion (15).

Functional Roles of TSP-1 in Tumor Progression

Adhesion. The laboratory of George Tuszynski first showed that substratum-bound TSP-1 promoted adhesion of platelets and a variety of cells, regardless of species (16). After that, many studies have demonstrated that TSP-1 mediates cellular adhesion of numerous cell types including endothelial cells, fibroblasts, smooth muscle cells (SMC), keratinocytes, neurons, and several transformed cell lines such as melanoma, squamous carcinoma, and fibrosarcomas. Cell adhesion to ECM is crucial to several steps in tumor progression and metastasis.

TSP-1 has an Arg-Gly-Asp (RGD) integrin recognition sequence in the seventh type III repeat. The RGD peptide can inhibit adhesion to TSP-1 on cells expressing the integrin $\alpha_v\beta_3$. TSP-1 also interacts with other integrins including $\alpha_3\beta_1$ on neurons (17), $\alpha_4\beta_1$, and $\alpha_5\beta_1$ on activated T lymphocytes (18), and $\alpha_{II}\beta_{B3}$ and $\alpha_2\beta_1$ on activated platelets (19).

The $\alpha_3\beta_1$ integrin, with some cooperation of sulfated glyco-conjugates and $\alpha_4\beta_1$ integrin, mediates adhesion of MDA-MB-435 and MDA-MB-231 breast carcinoma cells to TSP-1. This β_1 integrin is maintained in an inactive or partially active state in these cell lines, but can be activated by exogenous stimuli including serum, insulin, IGF1, and ligation of CD98. It was found that $\alpha_3\beta_1$ is the dominant integrin for mediating adhesive activity of breast carcinoma cells to TSP-1 (20). For small cell lung carcinoma (SCLC) cell lines, it was found that $\alpha_3\beta_1$ integrin and sulfated glycolipids cooperate to mediate adhesion of SCLC cells to TSP-1 (21).

CD47 or IAP (integrin-associated protein) also binds to the adhesive motif Val-Val-Met (VVM). TSP-1 contains two VVM sequences (22). Overexpression in breast carcinoma cells of a carboxy-terminal deletion mutant of TSP-1 lacking the second VVM motif reversed the suppressive effect observed using native TSP-1, suggesting that the VVM motifs play a role in the increased tumorigenesis of MDA435 breast carcinoma cells (13).

CD36 was the first nonintegrin receptor for TSP-1 to be described. The binding sites that may mediate TSP-1 and CD36 interaction have been identified on both proteins. It is the CSVTCG domains within the type I repeats that bind CD36 (23). However, the binding constant is in the macromolar range indicating low affinity. CD36 is expressed in some breast carcinomas (24). Several tumor cells show dependence on this protein in binding to TSP-1 (25). Lysosomal integral membrane protein II (LIMPII) was identified as a member of the CD36 gene family. A recent study found that CD36 and LIMPII used the same conserved TSP-1-binding site and that LIMPII might function as an adhesion receptor for TSP-1 (26). Another study showed that CD36 cDNA transfection of Bowes melanoma cells did not increase cell attachment to type I repeats. It was suggested that CD36/TSP-1 interaction does not occur upon cell-matrix adhesion and haptotaxis (cell migration towards a substratum-bound ligand) (27).

A novel receptor recognizing the CSVTCG in TSP-1 was identified in a lung carcinoma cell line. In 1993, our laboratory isolated a protein from lung carcinoma, specific for the CSVTCG residues in the type I repeats of TSP-1 by CSVTCG-Sepharose chromatography (28). It was cell surface-exposed on lung carcinoma cells. Anti-binding protein IgG and anti-CSVTCG peptide IgG inhibited lung carcinoma cell spreading and adhesion on TSP-1, but not on fibronectin and laminin. Anti-binding protein IgG inhibited human breast cancer progression in athymic mice. Immunohistochemical localization of this protein showed selective expression on invasive cancer cells and capillary endothelial cells (29). Arnoletti *et al.* (30) suggested that a reverse correlation existed between the degree of receptor staining and the overall survival of patients with squamous carcinoma of the head and neck.

TSP-1 also binds to heparin, sulfatide, and heparin sulfate proteoglycans (HSPGs) through an amino-terminal heparin-binding domain, through secondary heparin binding sites in the type I repeats, and possibly through other undefined sites (31). In addition, TSP-1 has been shown to bind polyhistidines with high affinity interaction (K_d = 5 nM), which is dependent on the number of consecutive histidines (32).

Motility and Invasion. TSP-1 has well-characterized effects on cell motility. TSP-1 can be both chemotactic and haptotactic to tumor cell motility. TSP-1-mediated chemotaxis was found to be partially dependent on a pertussis toxin- (PT) sensitive G-bind protein in A2058 melanoma cells. Procollagen domain and the CD47-binding domain (carboxyl terminal) are sensitive to PT. But responses to the type I and type III domains are not sensitive to PT. Thus, the residual chemotaxis to TSP-1 in the presence of PT might be mediated by the activities of the type I and III repeats (33).

Other evidence suggests that IAP (integrin-associated protein, CD47) stimulates $\alpha_2\beta_1$ integrin-mediated SMC migration via G-binding protein-mediated inhibition of extracellular signal-related kinase (ERK) activity and suppression of cyclic AMP levels. These two signaling pathways could modulate integrin and downstream components of cell motility (34). These authors also confirmed that peptide KRFYVVMWKK in the CD47-binding domain plays an important role in cell motility.

Pericellular proteolysis is crucial in tumor cell invasion. The plasminogen/plasmin system is one of the main protease systems involved in cancer progression. TSP-1 can bind to several proteases, including urokinase plasminogen activator (uPA), thrombin, plasmin, cathepsin G, and neutrophil elastase. Our laboratory showed that the adhesion and invasion of carcinoma cells in fibrin gels are potentiated by TSP-1 through a mechanism involving the plasminogen activator system and that this activity is due to the TSP-1-mediated upregulation of both uPA and plasminogen activator inhibitor-1 (PAI-1), a major inhibitor of cell-associated plasminogen activators. Also, the activation of TGFβ1 might be involved in the TSP-1-induced tumor cell invasion, since an antibody to TGFβ1 blocked TSP-1-dependent tumor cell invasion and uPA/PAI-1 production (35) (36).

The protein uPAR, a member of a family of cysteine-rich cell surface proteins, lacks a transmembrane domain. Experimental data have demonstrated that uPAR resulted in prevention of metastasis, tumor growth, and angiogenesis (37). Our laboratory showed that uPAR expression was upregulated more than 2-fold by either TSP-1 or $TGF\beta_1$. Breast tumor cell invasion was upregulated 7- to 8-fold by either TSP-1 or $TGF\beta_1$. Antibodies against uPA or uPAR neutralized the TSP-1 and $TGF\beta_1$-promoted breast tumor cell invasion (38). We also saw similar results with pancreatic tumor cells (39).

To determine the effect of tumor cell-produced TSP-1 in the regulation of the plasminogen/plasmin system and tumor cell invasion, TSP-1-transfected MDA-MB-435 breast cancer cells that overexpress TSP-1 ware studied. Tumor cell TSP-1 induced a 2- to 7-fold increase in uPAR and cell-associated uPA expression and a 50% to 65% increase in cell-associated uPA and plasmin activities. Also, tumor cell TSP-1 promoted cell invasion and decreased cell adhesion through upregulation of uPAR-controlled uPA and plasmin activities (40). Therefore, TSP-1 may play an important role in tumor cell adhesion and invasion by regulating the plasminogen/plasmin system.

Proliferation. Recent work (41, 42) has identified several peptide sequences within the type I domain of TSP-1 molecule that inhibit aortic endothelial cell proliferation. Hugo *et al.* (43) have shown that these TSP-1 peptides are potent inhibitors of mesangial and glomerular endothelial cell proliferation *in vitro* and *in vivo*. *In vitro*, the TSP-1 influence on the proliferative activity was measured by [^{3}H] thymidine incorporation into the DNA. In the *in vivo* studies, experimental mesangial proliferative glomerulonephritis (anti-Thy model) was induced in Wistar rats. TSP-1 peptides were injected and renal biopsies were taken on Days 2 and 5. Double immunostaining using an antibody to proliferating cell nuclear antigen (PCNA), a marker of DNA synthesis, and an antibody to RECA-1 that is specific for endothelial cells showed significant decrease in proliferation from Day 2.

In another study by Iruela-Arispe *et al.* (44), it was shown that the proliferation of quiescent chicken endothelial cells was inhibited by TSP-1. The cells were stimulated to proliferate by addition of fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) and in the presence of different concentrations of TSP-1. During the treatment, cells were pulsed with [^{3}H] thymidine. Trichloroacetic acid precipitable counts of [^{3}H] thymidine were measured. To confirm that TSP-1 specifically mediated the suppression of endothelial cell proliferation, anti-TSP-1 antibodies were used. The antibodies neutralized the anti-proliferative effect of TSP-1.

Moreover, Yamashita *et al.* (45) have used carcinoma cell lines to evaluate the TSP-1 affect on proliferation rate. KIM-1 (human hepatocellular carcinoma) and CW-2 (human colon carcinoma) were used. Addition of exogenous purified TSP-1 to culture medium of CW-2 cells stimulated proliferation at 10 or 20 μg/ml dosage. In contrast, TSP-1 had no effect on KIM-1 cell proliferation. The prediction was that TSP-1 was already at saturated activity concentration in the KIM-1 cells because KIM-1 is a TSP-producing cell line and CW-2 is not. Therefore, depending on which cell line is studied, TSP-1 can stimulate proliferation.

Vascularity. In a report by Pazouki *et al.* (46), the biphasic effect of TSP-1 in the regulation of angiogenesis was studied. The density of the microvasculature in tissue sections was used as the index of the angiogenesis process. These authors assessed vascularity by four different methods, following immunostaining of histological sections with

antibodies to von Willebrand Factor (vWF). These methods were: average microvascular density (a-MVD), highest microvascular density (h-MVD), microvascular volume (MVV), and image analysis of stained area. a-MVD and h-MVD were assessed by counting the vessels that were contained within a grid covering an area of 0.476 mm^2 and the counts were expressed as number of vessels per square millimeter. To measure h-MVD, the tumor sections were scanned for the area of highest vascular density and from the number of vessels in five fields, the highest was taken. MVV was measured by point counting using an eyepiece graticule, which contained 100 points. Fifteen random fields (1500 points) were counted across each section. The results suggested that TSP-1 could have both stimulatory and inhibitory effects on angiogenesis, possibly dependent on the presence of other factors.

In another work by Kawahara *et al.* (47), the microvessel count was also used to determine tumor angiogenesis in cholangiocarcinoma (CCC), which is relatively hypovascular and hepatocellular carcinoma (HCC), which is often highly vascular. The T/N ratio, mRNA levels of TSP-1 and VEGF in tumor (T) versus the surrounding noncancerous tissue (N), was identified. High T/N ratio of TSP-1 together with low T/N ratio of VEGF mRNA levels was associated with poor vascularity in CCC. In contrast, both low and high vascularity HCC showed similar T/N ratios of TSP-1 mRNA levels, which did not correlate with hypervascularity in HCC. Light microscopy was used to count the average number of microvessels in five random areas, each field having an area of 1.035 mm^2. These findings suggest that no major conclusions can be drawn by counting microvessels around tumor sections. Schor *et al.* (48) have assessed the vascularity value as an index of angiogenesis. Their findings suggested that statistically significant differences in vascularity values are most likely to arise from failure to optimize the staining protocol, such as the pretreatment of the sections and the endothelial markers used. Further studies with *in vivo* angiogenesis models are required to better understand the phenomenon.

Angiogenesis. Angiogenesis, the process by which new blood vessels are formed from pre-existing vessels, is an important event in physiologic or pathologic conditions. Endothelial cells and pericytes, which form capillaries, carry all genetic information to form the whole capillary network. Angiogenic and anti-angiogenic molecules released by accessory cells control neovascularization, the migration and proliferation of endothelial cells, and their morphogenetic differentiation in capillaries and the remodeling of ECM. In physiologic conditions, these steps are highly regulated. In contrast, in many diseases, including cancer, the formation of capillary network is due to an imbalance of positive versus negative angiogenic factors produced by tumor and host infiltrating cells. These angiogenic modulators include VEGF, basic FGF, transforming growth factor α (TGF-α), TGF-β, TSP-1, angiostatin, and endostatin, among others.

Rapid progress in the area of angiogenesis research is identifying more and more angiogenic molecules. Despite this progress, a major problem remains the difficulty of finding suitable methods for characterizing the angiogenic response. At first, the different *in vitro* assays give cell line-specific results that do not represent the *in vivo* reality (49). There are also many *in vivo* tests that have been developed, like the mouse corneal angiogenesis assay, the chick chorio-allantoic membrane assay, and matrigel plug assay. These have their own limitations in regards to the difficulty of quantitating the response and distinguishing it from the nonspecific host response (50).

The role of TSP-1 in angiogenesis is controversial. For example, some consider TSP-1 a stimulator of tumor progression and angiogenesis (51, 52). Qian *et al.* (52) showed that TSP-1 modulates proteolytic enzyme levels in bovine aortic endothelial cells (BAE) and that these enzymes are responsible for the proteolytic degradation of the ECM. The proteolytic degradation was accomplished in part by the activity of matrix metalloproteinases (MMPs). Destruction and compositional alterations of the ECM are key processes in angiogenesis. The two well-known angiogenesis assays that were used were the endothelial cell tube formation in collagen gel and the modified Boyden chamber invasion assay. Endothelial cells, when cultured between two layers of collagen gel, are induced to form tubular networks that approximate the endothelial cell morphogenic differentiation occurring during angiogenesis. TSP-1 incorporated in the collagen gels had a biphasic effect, showing potentiation at concentrations <10 μg/ml and inhibition at concentrations of 15 μg/ml or higher. It was also shown that TSP-1 increased the production of MMP-9 and had no effect on MMP-1 and -3 levels (52). In Figure 1, these results are schematically represented.

The effect of TSP-1 on BAE cell invasion was tested using the modified Boyden chamber assay in the presence or absence of antibody to TSP-1. The chambers have poly-

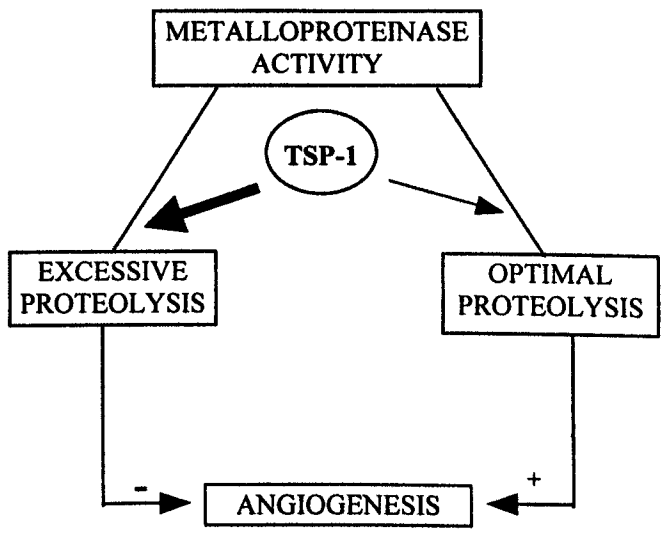

Figure 1. The proposed role of TSP-1 in modulating proteolysis during angiogenesis.

carbonate filters, 8-μm pore size, and are coated with 100 μg of type I collagen. The lower compartment of the chamber had 600 μl of serum-free medium containing 0.1% bovine serum albumin, and the upper compartment had 50,000 cells suspended in 400 μl of the same medium. Varying concentrations of TSP-1 were placed in the lower compartment and the neutralizing antibodies were placed in the upper compartment. Invasive cells adhering to the undersurface of the filter were fixed, stained, and counted. TSP-1 promoted cell invasion by a mechanism involving MMP-9 activity of endothelial cells, as the addition of anti-MMP-9 antibody inhibited the TSP-1 induced invasion by about 80% (52). These results indicate that endothelial cell motility is mediated by TSP-1 upregulated MMP-9 activity.

Dawson *et al.* (53) went a step further and modified the TSP-1 type I repeat domain by substituting three L-amino acids by their D-enantiomers. The substitution of any of the 3 L-amino acids conferred on the peptide a potent anti-angiogenic activity. The ability of the peptides to block endothelial cell migration was used to measure the *in vitro* anti-angiogenic potency of the peptides. *In vivo,* a corneal neovascularization assay was performed on rats and mice. A Hydron-Sucralfate pellet is implanted into surgically created pocket in the avascular cornea, approximately 1.5 mm from the surrounding vascular limbus of rats and 0.3 to 0.5 mm from the vascular limbus of mice. The Hydron pellets have either bFGF alone as a control, or bFGF with different concentrations of the tested peptides. Vessel ingrowth was measured by slit lamp microscopy. Both the rat and mice experiments showed remarkable inhibition of bFGF-induced neovascularization.

Taraboletti and colleagues (54) reported that the amino terminus and whole molecule stimulated angiogenesis in the rabbit cornea model, while the 140-kDa fragment missing the amino terminus had no activity (54). However, both the whole molecule and the 140-kDa fragment inhibited bFGF-induced angiogenesis. The authors interpret their results as indicating that the angiogenic potential of TSP-1 depends on its state of proteolytic fragmentation and the capacity of these fragments to induce metalloproteinase enzyme activity in endothelial cells by means of specific receptors. These results are in agreement with our published work (55) showing that TSP-1 can induce MMP-9 in endothelial cells and that this activity can regulate the angiogenic potential of TSP-1.

In another more recent work by Taraboletti *et al.* (56), the 25-kDa heparin-binding fragment of TSP-1 is more efficient than the whole molecule to induce angiogenesis. The fragments were tested in the rabbit cornea assay where inflammatory reactions and growing of capillaries can be easily monitored. TSP-1 alone showed a specific proangiogenic response that was abrogated by an antibody to TSP-1. In the presence of 200 ng of FGF-2, the 25-kDa fragment elicited a strong angiogenic response, while the 140-kDa carboxy-terminal fragment of TSP-1 completely blocked the neovascular response induced by FGF-2. This dual role of TSP-1 might rely on the association of TSP-1 with other molecules that can mask distinct TSP-1 domains.

The corneal neovascularization assay was recently performed in CD36-null and p53-null mice (57). CD36 has been proposed as the transmembrane receptor of TSP-1. TSP-1 was found not to block corneal neovascularization induced by bFGF in mice null for CD36. These mice were not genetically insensitive to inhibitors of angiogenesis, as the function of the inhibitor angiostatin was not affected by the lack of CD36. TSP-1 inhibitory effect was p53 independent, as TSP-1 could inhibit neovascularization in the corneas of p53-null mice. These data suggested that CD36 was essential for the anti-angiogenic activity of TSP-1 *in vivo.*

The anti-angiogenic activity of TSP-1 and its domains was also evaluated in the chorioallantoic membrane (CAM) angiogenesis assay (44). This method is based on the vertical growth of new capillary vessels into a collagen gel pellet placed on the CAM. The collagen gel was supplemented with an angiogenic factor such as FGF-2 or VEGF in the presence or absence of test peptides. The extent of the angiogenic response was measured by use of fluorescein isothiocyanate-Dextran injected into the circulation of the CAM. The CAM assay results showed that only peptides derived from the second and third type I repeats of TSP-1 were angioinhibitory.

Activation of TGF-β1 by TSP-1

TGF-β1 is a 25-kDa dimeric protein that functions as a potent growth differentiation regulatory cytokine involved in diverse tissue remodeling processes such as wound healing, tissue morphogenesis, angiogenesis, and tumor development. TGF-β1 is produced by virtually all cell types in a latent form and it requires activation in order to interact with its specific receptors, the transmembrane serine/threonine kinases and to elicit a biological response (58). Although TGF-β1 has a strong angiogenic effect *in vivo*, its *in vitro* effects on the angiogenic properties of endothelial cells are complex depending on the concentration of TGF-β1, matrix composition, and the presence of other cytokines (59, 60). For example Iruela-Arispe and Sage (61) showed that TGF-β1 inhibited the proliferation of endothelial cells in subconfluent monolayers, but it promoted the growth of cells that formed cords. Madri and coworkers (62) proposed a possible mechanism for these differential effects of TGF-β1 by showing that the profile of TGF-β1 receptors expressed by endothelial cells determines the response of endothelial cells to TGF-β1.

Like TSP-1, TGF-β1 is found in the platelet α granules and it can be released upon platelet degranulation (63). The observation that TSP-1 and TGF-β1 colocalized in the ECM of osteoblasts suggests the possible interaction between these two molecules (64). Indeed, a number of similarities can be found between the biological effects of TGF-β1 and those of TSP-1: Inhibition of endothelial cell proliferation *in vitro* (65) (66), stimulation of angiogenesis *in vivo* through recruitment of inflammatory cells (67), promoting effects

on capillary tube formation and stabilization (68), upregulation of uPA and PAI-1 in cancer cells (35, 36) and upregulation of PAI-1 in endothelial cells (66), and stimulation of fibroblast and SMC proliferation (69, 70). These findings raise the possibility that TSP-1 and TGF-β1 may mutually interact in the cellular events that contribute to the angiogenic response. Consistent with this concept is the recent work of Crawford *et al.* (71) who showed that TSP-1 is responsible for a significant proportion of the activation of TGF-β1 *in vivo*. However, another group found that TSP-1, either alone or in the presence of cultured SMC (a cell type known to activate latent TGF-β1 *in vitro* and *in vivo*) is unable to activate latent TGF-β1. The conclusion was that any TSP-1-mediated activation of TGF-β1 must depend on additional factor(s) (72).

Postulated Mechanism of TSP-1 Mediated Angiogenesis

We have formulated the following hypothesis for the mechanism of TSP-1 mediated endothelial cell invasion and angiogenesis. The salient features of this hypothesis are presented schematically in Figure 2. TSP-1 and latent TGF-β1 are synthesized by growing endothelial cells or are available from activated platelets and stromal cells. Latent TGF-β1 is either directly activated by TSP-1 or by additional factors on the cell surface. Active TGF-β1 either signals the cell directly or in association with a TSP-1 receptor to upregulate the expression of a group of genes important for matrix turnover such as MMP-9, its inhibitor, uPA/ PAI-1, TSP-1, and collagen. These series of reactions can be amplified by an autocrine feedback mechanism involving TSP-1 and TGF-β1 synthesis since in other systems, TGF-β1 upregulates its own synthesis as well as that of TSP-1 (73).

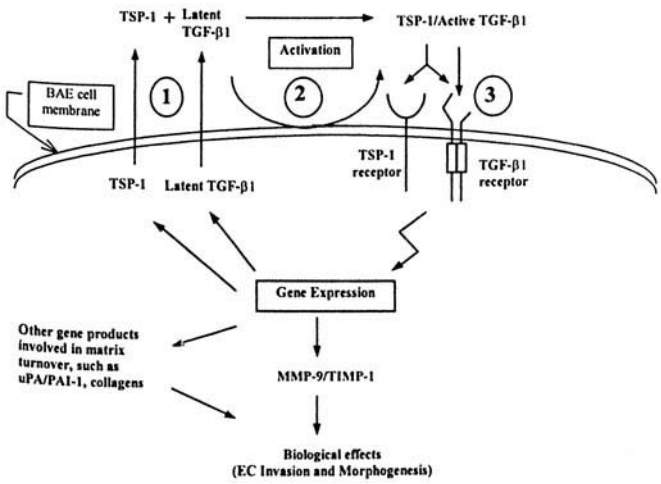

Figure 2. Mechanism for role of TSP-1 in angiogenesis and cancer invasion. TSP-1 is synthesized by growing tumor and endothelial cells or is available from activated platelets and stromal cells. TSP-1 upregulates metalloproteinase activity of endothelial and tumor cells by a mechanism mediated by TGF-β1. TGF-β1 signals the cell directly or in association with a TSP-1 receptor to upregulate the expression of a group of genes important for matrix turnover such as MMP-9 and its inhibitor, TIMP-1. The extent of TSP-1 upregulated metalloproteinase activity, which is dependent on the level of TSP-1 (see Fig. 1), determines whether TSP-1 will promote or inhibit tumor invasion and angiogenesis.

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