MINIREVIEW

Expression of Thrombospondin-1 in Cancer: A Role in Tumor Progression (44008)

XIAOHUA QIAN‡ AND GEORGE P. TUSZYNSKI*,†,‡,[1]

Departments of Surgery, Medicine,† and Pathology,‡ Medical College of Pennsylvania and Hahnemann University, Philadelphia, Pennsylvania 19129*

Abstract. **Thrombospondin-1 (TSP-1), a trimeric high molecular weight glycoprotein, is one of the major secreted proteins of human platelets and an extracellular matrix component of a variety of cells including vascular endothelial cells and tumor cells. TSP-1 has been shown to be highly expressed in human malignant tissues and present in higher than normal levels in the plasma of cancer patients. TSP-1 has also been shown to promote hematogenous tumor spread, tumor cell adhesion and invasion, and angiogenesis. Overall these studies provide compelling evidence for the conclusion that TSP-1 plays an important role in tumor progression.**

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Tumor progression is a complex, multistep process involving tumor cell invasion, growth, angiogenesis, and hematogenous spread. These steps are mediated in part by tumor cell–extracellular matrix (ECM) interactions. In this review, we briefly describe the experimental evidence suggesting a role for thrombospondin-1 (TSP-1), a multifunctional platelet and extracellular matrix protein, in tumor progression. Our focus will primarily be on the work and interpretation of our laboratory which has provided evidence in support of the hypothesis that TSP-1 promotes tumor progression.

TSP-1

Thrombospondin (TSP), now referred to as TSP-1 because of the discovery of related genes (see below), was first identified in 1971 by Baenziger *et al.* as a high–molecular weight glycoprotein released from thrombin-stimulated platelets (1). Subsequent characterization of this protein by Lawler and others revealed that TSP-1 is composed of three identical disulfide-linked chains each consisting of 1152 amino acids (MW 145,000) (2, 3). Further analysis of the structure of TSP-1 molecule obtained by cloning of TSP-1 cDNA, isolated from human endothelial cells (3) and fibroblasts (4), revealed that each polypeptide chain is composed primarily of domains consisting of repeating homologous amino acid sequences. These domains are (i) NH_2-terminal globular domain; (ii) a procollagen homology domain; (iii) the type I or properdin repeat domain, consisting of three repeating sequences homologous to sequences found in properdin; (iv) the type II repeat domain, consisting of three repeating sequences homologous to those in epidermal growth factor; (v) the type III repeat domain, consisting of seven repeating Ca^{2+}-binding sequences, homologous to Ca^{2+}-binding sequences found in calmodulin, and (vi) a COOH-terminal globular domain (3, 5). These distinct domains interact with different cell surface receptors as well as other macromolecules such as collagens, and mediate a variety of cellular processes in-

[1] To whom requests for reprints should be addressed at Department of Surgery, Medicine and Pathology, Medical College of Pennsylvania and Hahnemann University, 2900 Queen Lane, Philadelphia, PA 19129.

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cluding cell attachment, migration, proliferation, and differentiation (5). These domains, together with their proposed functions and putative cellular receptors, are summarized in the schematic diagram shown in Figure 1.

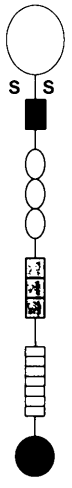
TSP-1 Is an Adhesive Protein

Among the diverse cellular activities mediated by TSP-1, cellular adhesion has been described most extensively and is still considered one of the primary physiological functions of TSP-1. In 1987, our laboratory first showed that substratum-bound TSP-1 promoted adhesion of platelets and a variety of cells regardless of species (6). Subsequently, many studies from different laboratories have demonstrated that TSP-1 mediates cellular adhesion of numerous cell types including endothelial cells, fibroblasts, smooth muscle cells, keratinocytes, neurons, and several transformed cell lines such as melanoma, squamous carcinoma, and fibrosarcomas (7–9). These studies have subsequently led to the identification of cell adhesive motifs in the TSP-1 molecule and the cellular receptors for TSP-1. Currently, four motifs in TSP-1 have been recognized as adhesive sites (Figure 1): (i) the N-terminal heparin binding domain interacts with cell surface heparan sulfate proteoglycans, and mediates the aggregation of platelets (10) and adhesion of chinese hamster ovary cells (11); (ii) the CSVTCG (Cys-Ser-Val-Thr-Cys-Gly) sequences within the type I repeats bind CD36 (12) and/or the CSVTCG receptor, recently identified and isolated from tumor cell membranes (13). This domain promotes the adhesion of a variety of cells including endothelial cells and tumor cells (14). In addition, the type I repeat domain has recently been shown to participate in the activation of transforming growth factor–β1 (TGFβ1) *in vitro* (15), and neurite outgrowth which is mediated by the interaction with integrin $\alpha_3\beta_1$ (16); (iii) the RGD (Arg-Gly-Asp) sequence in the last of the type III repeats mediates adhesion by interaction with $\alpha_v\beta_3$ integrin receptors (also referred to as the vitronectin receptor, or CD 51) (17); and (iv) the RFYVVMWK and IRVVM sequences in the C-terminal domain promote adhesion most likely by binding to a putative 52-kDa receptor (18).

The existence of these various adhesive domains and their putative receptors suggests that TSP-1–mediated cell-substratum adhesion is diverse and cell type dependent. For example, bovine aortic endothelial cells have been shown to attach poorly to TSP-1, with little spreading and focal contact formation (19), while primary cultures of human umbilical vein endothelial cells (HUVEC) attach well to TSP-1 (8). In addition, Adams recently reported that H9c2 myoblasts, which were adherent and spreading on a TSP-1 substrate, did not assemble focal adhesion contacts, forming instead microspike structures as evidenced by the different organization of cytoskeletal proteins in the adhesion contacts (20). These studies also suggest that TSP-1 may support cell adhesion which facilitates cell movement through the ECM, rather than stably anchoring the cell to the matrix.

TSP-1 Gene Family

It is now known that TSP-1, originally characterized from platelet-released proteins, is only one mem-



Domains	Receptors or Binding Proteins	Functions	References
N-terminal	Heparan sulfate proteoglycans	Platelet aggregation, adhesion endocytic degradation	10, 11, 42, 43
Procollagen homology	Matrix, plasma proteins	Trimer assembly	3, 5
3 Type I repeats (properdin) CSVTCG	CD36 CSVTCG-receptor Integrin $\alpha_3\beta_1$	Adhesion chemotaxis & haptotaxis TGF-β_1 activation Neurite outgrowth	12, 13, 14, 15, 17
3 Type II repeats (EGF-like)	?	?	3, 5
7 Type III repeats RGD	Integrin $\alpha_v\beta_3$,	Ca^{++} binding adhesion	17
C-terminal	52 kDa protein 105/80 kDa protein	Adhesion Chemotaxis & haptotaxis	18, 52

Figure 1. The domain structure of TSP-1 and its putative receptors and cellular functions. TSP-1 is a disulfide-bonded homotrimer, the domain structure of one isochain is shown schematically on the left of the table. The putative cellular receptors and functions of TSP-1 corresponding to each domain are listed.

ber of a family of structurally related proteins encoded by different genes, which include at least four new members designated TSP-2, TSP-3, TSP-4, and TSP-5/COMP (cartilage oligomeric matrix protein) (21, 22). TSP-1 and TSP-2 have similar domain structures but differ in the regulation of their gene expression. The TSP-1 gene is an early response gene, the transcription of which can be rapidly induced by serum and growth factors such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and TGFβ1 (23–25), while the TSP-2 gene is not rapidly upregulated by serum (25). TSP-3, TSP-4, and TSP-5/COMP genes encode for smaller proteins which lack the procollagen homology region and type I repeats (21). In addition, they have been shown to form pentamers (26–28), whereas TSP-1 and TSP-2 are homotrimers. In contrast to TSP-1 and TSP-2, which are expressed in a wide variety of tissues (29–31), TSP-3, TSP-4, and TSP-5/COMP have more tissue-specific distributions: TSP-3 is highly expressed in lung and skin (32); TSP-4 in cardiac and skeletal muscle (33); and TSP-5 in cartilage and tendon (28, 34). Recently, a corticotropin-induced secreted protein (CISP) was purified from bovine adrenocortical cells and shown to be related to TSP-2 (35). Since the physiological functions of these new members of the TSP gene family have yet to be determined, this review briefly summarizes the current state of our knowledge on the expression, localization, and functions of TSP-1 in cancer.

Production of TSP-1 by Normal and Tumor Cell Lines

TSP-1 was thought to be only a platelet protein until 1981, when McPherson *et al.* first demonstrated that bovine aortic endothelial cell synthesized and secreted TSP-1 in culture (36). Since then, numerous cell types, including those of both epithelial and mesenchymal origins, have been shown to produce TSP-1 and incorporate it into the ECM (37). In general, the expression of TSP-1 is higher in proliferating cells than in quiescent cells (38), and is regulated during cellular differentiation (39, 40). For example, Castle *et al.* demonstrated that synthesis of TSP-1 mRNA and protein was induced during retinoic acid–mediated differentiation of neuroblastoma cells (39). Secreted TSP-1, which is usually cell surface– and/or ECM–associated (41), is probably degraded by low-density lipoprotein receptor (LRP)–mediated endocytosis (42, 43).

Using immunolabeling techniques such as immunofluorescence, immunoprecipitation, and enzyme-linked immunosorbent assay (ELISA), many investigators have also shown that TSP-1 can be synthesized and secreted by many cultured human tumor cell lines, including those derived from squamous carcinoma, melanoma, glioma (44), osteosarcoma (45), and breast adenocarcinoma (46). However, these neoplastically transformed cells may not synthesize as much TSP-1 as normal cells. For example, Mosher reported that SV-40 transformed fibroblasts and fibrosarcoma cells secreted TSP-1 at rates of 8.2 $\mu g/10^6$ cells/24 hr and 4.9 $\mu g/10^6$ cells/24 hr, respectively, while normal fibroblasts secreted 32 $\mu g/10^6$ cells/24 hr, or four times as much as transformed fibroblasts (47). Similarly, Ray-Chaudhury *et al.* demonstrated that the tumorigenic endothelial cell lines transformed by polyoma T oncogenes produced little TSP-1 and did not respond to TGFβ1 regulation, while normal capillary and aortic endothelial cells constitutively synthesized significant amounts of TSP-1, which was upregulated by TGFβ1 (48). These studies indicate different regulatory mechanisms underlying the expression of TSP-1 in normal and transformed cell lines.

The biological function of tumor cell–produced TSP-1 has been shown to have either a promoting or inhibitory role in tumor progression. In a series of elegant experiments, Varani *et al.* demonstrated that the metastatic potential of a group of squamous carcinoma cell lines directly correlated with their capacity to synthesize TSP-1 (49). These authors 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. Moreover, Castle *et al.* confirmed these observations by showing 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 (50). By contrast, Wienstat-Saslow *et al.* observed that transfection of human MDA-MB-435 breast carcinoma cell line with TSP-1 cDNA to induce over expression of TSP-1 in these cells reduced their capacity to grow and metastasize in athymic mice (51). One possible explanation for these contradictory observations could be that they are due to differences in the capacity of the various tumor cell lines to bind TSP-1 rather than their capacity to synthesize TSP-1. It is well known that different cell lines express different TSP-1 receptors with varying affinities for TSP-1 and that the cellular functions of TSP-1 are mediated by these specific cell surface receptors and/or binding proteins (5, 9). For example in the squamous cell carcinoma study (49), metastatic cell line 11B was not only shown to produce the most TSP-1 but also to bind the highest amount of TSP-1 on its cell surface by means of a putative 80/105-kDa tumor cell receptor specific for the C-terminal domain of TSP-1 (52). However, it was not determined whether the TSP-1–transfected MDA-MB-435 breast carcinoma cells in the Wienstat-Saslow *et al.* study (51) were capable of binding the overexpressed TSP-1 or whether any of the overexpressed

TSP-1 functioned as a competitive inhibitor for endogenous TSP-1–mediated adhesive interactions operative during tumor progression.

Localization of TSP-1 and Its Receptors in Human Tumor Tissues

Compared with the number of studies on TSP-1 synthesis by tumor cell lines *in vitro*, relatively few reports in the literature have described the expression and localization of TSP-1 in human malignant tissues. Breast tumors have been the most studied probably because Pratt and colleagues first showed that cytosolic homogenates of malignant tumors contained 10–150 times more TSP-1 than extracts from benign tumors (53). Wong *et al.* then immunolocalized TSP-1 in 48 malignant breast tumors as well as 30 normal and benign breast tissues (54). These authors found that 96% (46/48) of the malignant carcinomas showed strong TSP-1 staining in the desmoplastic stroma juxtaposed to tumor cells or at the basement membrane associated with the malignant ductal epithelium. By contrast, normal breast tissue and benign breast lesions showed no TSP-1 staining apart from some staining of the large distended cysts of fibrocystic disease and faint stromal staining in some of the fibroadenomas. Furthermore, these authors observed no major difference in the staining pattern for other adhesive matrix proteins such as laminin, fibronectin, and collagens type I, III, and IV between malignant and benign breast tissues, suggesting that only TSP-1 was differentially expressed in normal and tumor tissue. Clezardin and co-workers also observed strong immunostaining for TSP-1 in the desmoplastic stroma but not in the epithelial tumor cells of invasive ductal breast carcinomas, and the basement membrane surrounding ducts containing *in situ* carcinoma (55). Using *in situ* hybridization to localize the expression of TSP-1 mRNA, the authors concluded that the fibroblasts present in the desmoplastic stroma and the myoepithelial cells surrounding *in situ* carcinomas were the sources of the overexpressed TSP-1 in human neoplastic breast tissues. A recent study from our laboratory further confirmed the above patterns of expression of TSP-1 in human normal, benign, and malignant breast tissues (56). All these studies suggest that the increased expression of TSP-1 correlates with neoplastic transformation, at least in malignant breast tumors, and may contribute to and/or result from the persistent desmoplastic stromal reaction of some solid tumors. However, the high expression of TSP-1 in tissues is not exclusively found in malignant tumors. Transient high expression of TSP-1 has also been found in tissues during early wound healing (57) and embryonic development (58), suggesting a common dynamic role of TSP-1 in both normal and malignant tissues.

The immunohistochemical localization of TSP-1 receptors in breast and head and neck carcinoma has provided limited but more clear evidence suggesting the involvement of TSP-1 and its receptors in tumor progression. Clezardin *et al.* showed that CD51, the vitronectin receptor, is moderately expressed in invasive ductal carcinoma, while no immunoreactivity was observed for CD36 (55). By contrast, these authors found that CD36 becomes selectively expressed in normal lactating ducts. These authors also found that CD51 was expressed in most of the lobular carcinoma cells examined while CD36 only co-distributed in a small subpopulation of these tumors. We recently demonstrated that the CSVTCG-receptor for TSP-1 was highly expressed in malignant epithelial cells of all the invasive breast ductal carcinoma and skin squamous carcinoma investigated but absent in normal epithelial cells (56, 59). In addition to tumor cells, capillary endothelium was found focally immuno-positive for this receptor only in regions proximal to carcinoma. Moreover, using the computer-assisted image analysis to quantify the immunohistochemical staining of CSVTCG-receptor, Arnoletti *et al.* observed a reverse correlation between the degree of receptor staining and the overall survival of patients with squamous carcinoma of the head and neck (59). Taken together, these results suggest that increased expression of TSP-1 in tumor stroma may promote tumor cell attachment, migration, and tumor angiogenesis through its interaction with selectively expressed TSP-1 receptors on tumor cells and endothelial cells during the processes of tumor progression.

Functions of TSP-1 in Tumor Progression

Hematogenous Spread. The first direct evidence suggesting that TSP-1 may play a significant role in tumor metastasis came from an experimental metastatic study performed in our laboratory showing that TSP-1, injected iv together with mouse sarcoma cells, greatly potentiated lung colony formation (60). The mechanism of this TSP-1 potentiating effect on experimental tumor metastasis involved the adhesive activity of TSP-1 since in subsequent studies we were able to show that antibodies against the CSVTCG adhesive domain of TSP-1 blocked B16-F10 melanoma cell metastasis in the same murine model of experimental metastasis (14). Moreover, the presence of high blood levels of TSP-1 in patients with gastrointestinal, breast, lung and gynecological cancer is consistent with the view that TSP-1 may play a role in hematogenous cancer spread (61, 62). Further evidence for a role for TSP-1 in hematogenous cancer spread was obtained by Incardona *et al.* (63), who demonstrated that human breast adenocarcinoma MCF-7 cells, which do not synthesize much TSP-1 but possess a large number of cell surface receptors for TSP-1, utilized TSP-1 to attach to human endothelial cells and

form tumor cell aggregates. These results strongly suggest that tumor cell–associated TSP-1 may promote hematogenous spread of tumor cells by mediating tumor cell–endothelial cells and tumor cell–tumor cell interactions.

Previous studies have correlated the metastatic potential of tumor cells with their capacity to aggregate platelets (64). These platelet-tumor aggregates have been shown to form during the initial arrest of tumor cells in the capillary bed and play an important role in the mechanisms of hematogenous tumor implantation. The mechanism of this tumor-promoted platelet aggregation is varied since platelets can be aggregated by tumor-derived platelet agonists such as ADP and thrombin. Our laboratory was the first to show that exogenously added TSP-1 promoted thrombin- and ADP-stimulated platelet aggregation (65). Further support for a direct role for TSP-1 in platelet aggregation was obtained by Kehrel *et al.* (66), who showed that TSP-1 promoted collagen-induced platelet aggregation and demonstrated that a lack of functional TSP-1 was the cause of a hemorrhagic diathesis in a patient with defective collagen-induced platelet aggregation. Direct demonstration that TSP mediates tumor platelet aggregation was obtained by Katagiri *et al.* (67), who showed that TSP-1 promoted HMV-I human melanoma cell–induced platelet aggregation. Subsequently, Clezardin *et al.* (68) showed that osteosarcoma cell surface bound TSP-1 mediates tumor-platelet aggregation. These data directly support the hypothesis that both tumor- and platelet-associated TSP-1 mediate platelet-tumor cell interactions that occur during the initial phases of tumor cell metastasis culminating in the arrest of tumor cells in the capillary bed.

Growth. TSP-1 has been found highly expressed in the stroma of malignant breast tumor tissues as described above and other solid tumors which are characterized by a strong desmoplastic reaction such as found in pancreatic adenocarcinoma (our unpublished observations). These findings not only indicate that TSP-1 is produced by stromal cells, but also suggest that TSP-1 may contribute to the remarkable proliferation of interstitial connective tissue observed in these tumors. This hypothesis is supported by several studies *in vitro* showing that TSP-1 stimulates proliferation of fibroblasts (69, 70) and facilitates growth of smooth muscle cells in response to epidermal growth factor (EGF) (71). In fact, TSP-1 has also been shown to promote directly tumor cell growth. In a transfection study, Castle *et al.* demonstrated that overexpression of TSP-1 in NIH 3T3 cell lines resulted in serum and anchorage independent growth *in vitro,* suggesting a growth-supportive role of TSP-1 for transformed cells (72). More recently, Boukerche *et al.* also concluded that TSP can modulate tumor cell growth *in vivo* based on their observations that anti–TSP-1 antibodies inhibited the growth of human melanoma cells (M_3Da) in athymic mice (73). Therefore, TSP-1 may support tumor progression by stimulating both stromal cell proliferation and tumor cell growth.

Motility and Invasion. Several lines of evidence support the hypothesis that the stromal-rich TSP-1 in malignant tissue has a promoting effect on tumor cell motility and invasion. Using a Boyden chamber cell motility assay, Taraboletti *et al.* first showed that TSP-1 induced chemotaxis and haptotaxis of human melanoma cells (74). Similarly, Yabkowitz *et al.* observed TSP-1–mediated chemotaxis and haptotaxis in metastatic squamous carcinoma cells but not in nonmetastatic tumor cell lines (75). Recent results in our laboratory have shown that the adhesion and invasion of carcinoma cells in fibrin gels are potentiated by TSP-1 through a mechanism involving the plasminogen activator system (76). We have determined that this activity is due to the TSP-1–mediated upregulation of both urokinase type plasminogen activator (uPA) and plasminogen activator inhibitor–1 (PAI-1), a major inhibitor of cell-associated plasminogen activators (77, 78). We believe that, as proposed by others (79), upregulation of PAI-1 and uPA is critical for the efficiency of the invasive process, since it allows a controlled degradation of the extracellular matrix not only by tumor cells but also by endothelial cells. In addition, our studies have shown that TSP-1 induces tumor cell invasion through a mechanism involving the activation of endogenous TGFβ1, since tumor cell invasion and uPA/PAI-1 production can be blocked with an anti-TGFβ1 antibody (77, 78).

Although the precise mechanisms of TSP-1–mediated tumor cell migration and invasion have yet to be elucidated, it is evident that TSP-1–mediated tumor cell attachment may also contribute to its modulation of cell migration. Wang *et al.* recently showed that TSP-1 promoted head and neck squamous carcinoma cell invasion *in vitro,* which was mediated by the CSVTCG receptor, one of the TSP-1 adhesion receptors expressed on tumor cells (80). Additional evidence has recently been obtained in our laboratory in support for the hypothesis that the invasiveness of a given tumor cell line may correlate with its capacity to interact with TSP-1. For instance, we investigated the TSP-1–mediated invasiveness of three human breast adenocarcinoma cell lines (MDA-MB-231, SKBR-3, MCF-7) and one benign mammary epithelial tumor cell line (MCF-10A) using a Boyden chamber invasion assay (81). We found that all the metastatic cell lines, which highly expressed the CSVTCG receptor for TSP-1, had the highest capacity to invade the collagen matrix in response to TSP-1, whereas the MCF-10A cell line which expressed undetectable CSVTCG-receptor by Northern and Western blot analysis, did

not invade in response to TSP-1. These data suggest a clear correlation between the expression of CSVTCG receptor for TSP-1 in breast tumor cells and the invasive capacity of the tumor cells and underscores the conclusion that the capacity of a tumor cell to interact with TSP-1 determines whether TSP-1 can modulate its metastatic potential.

Angiogenesis. It is generally accepted that angiogenesis, the development of new capillaries from preexisting blood vessels, is essential for the growth and progression of solid tumors (82). Angiogenesis is a complex process which is regulated by soluble angiogenic regulators such as bFGF, vascular endothelial growth factor (VEGF), and TGFβ1, as well as insoluble regulators such as extracellular matrix proteins. Available evidence indicates that extracellular matrix proteins are able to modulate the response of endothelial cells to soluble growth factors (83). As an extracellular matrix protein associated with the vascular basement membrane (84), TSP-1 has been shown to play a role in angiogenesis. TSP-1 has been proposed to be either an angiogenic inhibitor or promoter.

The conclusion that TSP-1 is an inhibitor of angiogenesis has been made by Bouck and co-workers on the basis of results obtained using the rat cornea assay of angiogenesis (85). In this assay TSP-1 (0.125 μg) was placed together with bFGF (0.05 μg) into a slow-release pellet and the pellet implanted into the avascular rat cornea. These authors found that TSP-1 inhibited the bFGF induced formation of microvessels. In contrast, BenEzra *et al.* recently reported that TSP-1 (2 μg/pellet) potentiated the angiogenic effect of both bFGF (0.5 μg/pellet) and lipopolysaccharide (LPS) in a rabbit cornea angiogenesis assay by 5-fold (86). One possible explanation for this discrepancy can be found in the amounts of TSP-1 and bFGF implanted in the pellets in these different studies. Similarly, there are conflicting observations regarding the effect of TSP-1 on endothelial cell migration, an important process during angiogenesis. Using a Boyden chamber cell migration assay, Taraboletti *et al.* (87) demonstrated that at concentrations of 5–50 μg/ml, TSP-1 alone, or together with bFGF, stimulated the motility of endothelial cells in a dose-dependent manner. In contrast, Tolsma *et al.* reported that at lower concentrations (around 2.5 μg/ml), TSP-1 inhibited the bFGF-induced endothelial cell migration *in vitro* (88). From these observations, it appears that the effect of TSP-1 on angiogenesis is concentration-dependent: at low concentrations, TSP-1 displays inhibitory effects on angiogenesis, whereas at high concentrations, TSP-1 stimulates angiogenesis. It is known that in circulation, the normal TSP-1 concentration is about 0.05–0.4 μg/ml in plasma (61, 89), whereas after the activation of the coagulation system, it increases to 10–30 μg/ml in serum (90, 91). It has been evident that under certain pathological conditions such as in the presence of malignant tumors, the plasma TSP-1 concentration increases significantly as well as the amount of TSP-1 expressed in tumor tissues (61, 62, 53). Therefore, it is likely that TSP-1 exerts its pro-angiogenic effect in the elevated concentration range usually seen in cancer patients or in other pathological conditions where high levels of TSP-1 are produced. However, the timing of the component events of angiogenesis in relation to the levels and localization of TSP-1 may be critical factors determining the activity of TSP-1 during the angiogenic process.

The hypothesis that TSP-1 is an angiogenic agonist is further supported by the observation that matrix-bound TSP-1 promotes microvessel formation in a rat aorta explant model (69). In this study, TSP-1 was

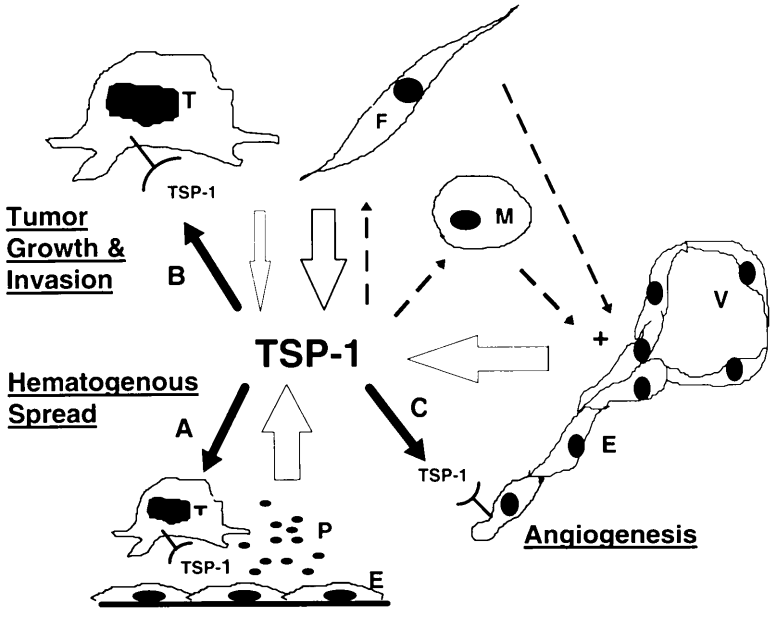


Figure 2. Schematic illustration of roles of TSP-1 in tumor progression. TSP-1, thrombospondin-1; T, tumor cells; F, fibroblasts; E, endothelial cells; M, macrophages/monocytes; P, platelets; V, microvessels; Y, TSP-1 receptors. ⇨: The size of the arrow denotes the relative abundance of TSP-1 produced by the different cell types, TSP-1 in tumor tissue and plasma is produced less likely by tumor cells, but more likely by tumor stromal fibroblasts, proliferating or activated endothelial cells, and activated platelets. →: The functions of TSP-1 in tumor progression are (A) mediation of tumor cell hematogenous spread; (B) supporting tumor growth and invasion; (C) promoting tumor angiogenesis. --▶: TSP-1 promotes angiogenesis by indirect mechanisms involving fibroblasts and inflammatory cells.

shown to stimulate nonendothelial cells, which in turn produce angiogenic factors, suggesting that TSP-1 could also function as a paracrine modulator in the process of angiogenesis. In addition, several observations from different laboratories also suggest that TSP-1 is a promoter of angiogenesis: (i) increased expression of TSP-1 is localized immunohistochemically in newly formed microvessels of healing wounds (57); (ii) activated macrophages, which stimulate angiogenesis *in vivo,* up-regulate production of TSP-1 6-fold compared with unstimulated macrophages, which are not angiogenic (92); (iii) proliferating endothelial cells produce more TSP-1 than quiescent endothelial cells (93); (iv) TSP-1 promotes and stabilizes endothelial cell tube network formation in an *in vitro* collagen gel angiogenesis assay (94); and (v) virus-transformed endothelial cells, which express little or no TSP-1 mRNA and protein, are incapable of forming endothelial cell tube networks *in vitro* and normal microvessels *in vivo,* but regain the capacity to form microvessels when transfected with TSP-1 cDNA (48, 95, 96). Taken together these observations strongly suggest that TSP-1, which is highly expressed in malignant tumor stroma, can effectively modulate tumor angiogenesis and subsequent tumor progression.

Summary

TSP-1 is a platelet and matrix protein, which is highly expressed in malignant tissues and presents in higher than normal levels in the plasma of cancer patients. The evidence thus far suggests that TSP-1 promotes the process of metastasis by the following mechanisms as summarized schematically in Figure 2: (A) TSP-1 mediates the hematogenous arrest of tumor cells by its direct adhesive activity on platelet-tumor emboli and the vessel wall, as well as its inhibitory effects on fibrinolysis; (B) TSP-1 supports tumor growth and tumor cell invasion by up-regulating the balanced matrix proteolysis system such as the uPA-PAI-1 system, in concert with modulating tumor cell-ECM interactions mediated by tumor cell–specific adhesion receptors; (C) TSP-1 promotes tumor angiogenesis by directly modulating endothelial cell migration and morphogenesis, and indirectly stimulating tumor stromal cells and attracting inflammatory cells. Further investigation is underway to reveal the molecular mechanisms of these TSP-1–mediated processes.

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