

Diversity of Function Is Inherent in Matricellular Proteins: An Appraisal of Thrombospondin 1

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OUR current appreciation of the involvement of thrombospondin 1 (TSP1)¹ in diverse biological processes (Fig. 1; 1, 3, 25) extends far beyond the role initially attributed to the protein in platelet aggregation and coagulation. This diversity of function has led to considerable confusion in the literature and skepticism among scientists that a single protein can subserve such protean, sometimes conflicting, functions. In this Commentary I will attempt to show that the ability of matricellular proteins, as defined below, to interact with a wide range of both matrix proteins and cell surface receptors can explain the complex biological functions of TSP1 and resolve many of the controversies regarding its mode of action. A conclusion of this analysis is that, even if allowances are made for some errors in experimentation and interpretation, the majority of the reported functions of TSP1, divergent as they appear to be, are likely to be correct.

The term "matricellular" is used in this analysis to refer to a group of modular, extracellular proteins whose functions are achieved by binding to matrix proteins as well as to cell surface receptors, or to other molecules such as cytokines and proteases that interact, in turn, with the cell surface. In addition to TSP1, this group is likely to include members of the tenascin protein family, SPARC/osteonectin and its relatives, and osteopontin. Although matricellular proteins can be associated with structural elements such as collagen fibrils or basement membranes, it is presumed that they do not contribute to the structural integrity of these elements. An association could, nevertheless, serve to sequester matricellular proteins, and provide a source of the proteins for subsequent recruitment to the cell surface. It should be noted that the distinction between structural matrix and matricellular proteins is not complete, since proteins such as fibronectin and laminin, which do serve as integral components of structural elements, also have adhesive functions and play biological roles that partially overlap those of matricellular proteins. Furthermore, matricellular proteins may participate in the formation of structural complexes under some circum-

stances, for example, the postulated role of osteopontin in the mineralization of bone matrix.

The Putative, Diverse Functions of TSP1 Cannot Be Explained by the Existence of Related TSPs, or Different Forms of TSP1

As information regarding TSP1 has accumulated, the large and bewildering number of important biological processes in which the protein has been implicated has become apparent (Fig. 1). Moreover, in some cases, contradictory effects have been ascribed to TSP1. Thus, experimental evidence indicates that TSP1 can be both adhesive and antiadhesive, can foster and retard metastatic spread, can stimulate and inhibit angiogenesis, and can increase and reduce proteolytic activity and fibrinolysis. Could all of these experimental results be valid, or might some effects be attributed to different TSPs or to different isoforms of TSP1? The TSP family currently consists of five members, TSP1-4 and TSP5/COMP (cartilage oligomeric matrix protein). Homotrimeric TSP1 and TSP2 are structurally similar but differ from pentameric TSP3, 4 and 5 (1-3). TSP1 and 2 are expressed widely in tissues of the developing and adult mouse at both the mRNA and protein levels (6, 14, 15), whereas the distribution of TSP3-5 mRNAs is thought to be more limited. This difference in tissue distribution, together with structural differences, indicates that the two

MOLECULAR FUNCTION	CELLULAR FUNCTION	BIOLOGICAL PROCESS
		EMBRYONIC DEVELOPMENT
		TISSUE DIFFERENTIATION
BINDING TO CELL-SURFACE RECEPTORS	CELL PROLIFERATION	NEURITE OUTGROWTH/ AXONAL GUIDANCE/ NERVE REGENERATION
BINDING OF CYTOKINES/ PROTEASES	ADHESION/CELL SHAPE	TUMOR GROWTH AND METASTASIS
EXTRACELLULAR PROTEIN INTERACTIONS	CHEMOTAXIS/ HAPTOTAXIS/ MIGRATION	ANGIOGENESIS
	PHAGOCYTOSIS	RESPONSE TO INJURY
		INFLAMMATION
		COAGULATION/ FIBRINOLYSIS

Figure 1. The diverse roles of TSP1. Biological processes in which TSP1 is implicated are listed in the right panel, cellular functions deduced from experiments in vitro are in the center panel, and molecular interactions determined with the purified protein are on the left.

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1. *Abbreviation used in this paper:* TSP1, thrombospondin 1.

subgroups subserve rather different biological functions.

Although the extent to which the functions of TSP1 and TSP2 are related or overlapping is uncertain, a distinction between the two proteins is suggested by their different tissue distribution (14, 15), by the observation that TSP1, but not TSP2, is transiently induced during osteogenic differentiation of MC3T3-E1 cells (28), and by the finding that the promoter sequences of the mouse TSP1 and TSP2 genes are different (2). The latter finding is compatible with the demonstration that the TSP1 gene is highly responsive to growth factors, whereas the TSP2 gene is not (2). A direct demonstration of a functional difference between TSP1 and TSP2 was recently reported by Pellerin et al. (22), who found that TSP2 inhibited the spreading of bovine adrenocortical cells, whereas TSP1 did not. Southern blot analysis and in situ chromosomal localization studies are consistent with a single genetic locus for TSP1 in mice and humans. Nevertheless, isoforms could result from alternate splicing, as has been documented for fibronectin, tenascin, and many other extracellular proteins. Typically, Northern blot analyses reveal only a single 6-kb band for TSP1, but alternate splice forms might be generated in a cell- or tissue-specific manner and thus might be difficult to detect. To date, only one report of unpublished data supports the existence of a low molecular weight form of TSP1, which could be the result of alternate splicing (10). Therefore, the process, if it exists, is probably not common. A second candidate for an alternate splice form of TSP1, the 140-kD fragment of hamster TSP1 that has been shown to function as an inhibitor of angiogenesis (11), is more likely to result from limited proteolysis of the intact protein.

Heterogeneity in TSP1 could also be introduced by the formation of heterotrimers between TSP1 and TSP2. A recent study presented evidence for heterotrimeric TSP1/TSP2 molecules, based on their isolation by type-specific antibodies (21). The validity of these results depends critically on the ability to exclude association of TSP1 and TSP2 homotrimeric molecules prior to immunoprecipitation. Since association of TSPs may be highly dependent

on the presence of other proteins, ionic strength, and other factors, suitable control experiments could be difficult to design. The experiments of O'Rourke et al. (21) will therefore require confirmation. Some indication that heterotrimeric TSP1/TSP2 molecules, if present, might not be common is provided by the findings that mRNAs for TSP1 and TSP2 are separated spatially and temporally during mouse development (14), and that immunofluorescence studies show distinctly different patterns for TSP1 and TSP2 in cultures of differentiating embryonal carcinoma cells (16). Thus, although some early studies of the immunolocalization of TSP1, which were performed before it was known that related TSPs existed, might have confused the different TSPs, it seems unlikely that any of the properties of TSP1 listed in Fig. 1 can be ascribed exclusively to another TSP family member, or to a different isoform of TSP1. It remains a possibility that some contradictory effects of TSP1 can be explained by experimental variables. For example, the activity of soluble TSP1 could differ from that of TSP1 bound to a substratum.

The Participation of TSP1 in Diverse and Complex Biological Processes Can Be Explained by an Extensive Repertoire of Molecular and Cellular Interactions

If it is the case that the complex functions of TSP1 cannot be attributed to different family members or isoforms, how can they be achieved? TSP1 binds and activates latent TGF β 1, and serves as a competitive inhibitor of several serine proteases (Fig. 2; 12, 27). Since the conformation of the Ca²⁺-binding repeats in TSP1, and thus the affinity for binding to integrins and proteases, can be influenced by Ca²⁺ concentration (12, 29), the ionic milieu of the cell could also regulate TSP1 function. The interaction of TSP1 with proteins that form structural elements (collagens, proteoglycans, fibronectin, laminins) might serve to present TSP1 to the cell surface, and might also modulate the interaction of those proteins with their own receptors. The effects of these interactions are likely to influence cell proliferation, adhesion, and migration in different

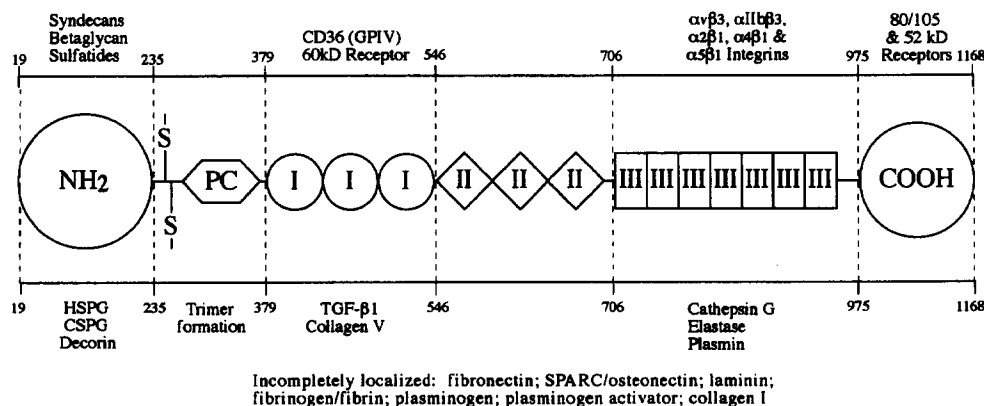


Figure 2. Location of ligand-binding sites for cell surface receptors (*top*) and protein-protein interactions (*bottom*) within the domain structure of TSP1. The structural plan of TSP1 consists of an NH₂-terminal domain, a type I procollagen homology region (PC), three type I (TSP or properdin) repeats, three type II (EGF-like) repeats, seven type III (Ca²⁺-binding) repeats, and a COOH-terminal domain. In several instances (52- and 60-kD receptors, TGF- β 1, pro-

teases), specific peptide sequences within these domains have been implicated in these interactions. The last type III repeat contains an RGD sequence but the assignment of all integrin-binding sites to the type III repeats has not been established. A single chain in the homotrimeric protein is shown. The numbers indicate amino acids, with 1 representing the initiating methionine in the signal peptide. HSPG and CSPG: heparan and chondroitin sulfate proteoglycans, respectively.

ways that are dependent on cellular and extracellular matrix context.

TSP1 also binds to a disconcertingly large number of cell surface molecules that include proteoglycans, integrins and non-integrin receptors (Fig. 2). The signaling pathways that are affected, and the intracellular metabolic consequences of such interactions, are essentially unknown. It is likely that different cells in different locations will express a distinct repertoire of receptors. Furthermore, it seems possible that some biological effects require the engagement of more than one receptor by a single TSP1 molecule. Thus, the presence at the cell surface of a molecule that binds to only a subset of these receptors could modulate the functional consequences of TSP1 action. Viewed in this light, the participation of TSP1 in the diverse biological processes listed in Fig. 1, and its conflicting effects, are not surprising and are a direct consequence of the multiple interactions summarized in Fig. 2.

What Role Does TSP1 Play in Cell Proliferation?

The gene that encodes TSP1 is rapidly induced by PDGF, serum, and other growth factors (17); nevertheless, a role for a matricellular protein in the regulation of cell growth seems surprising. There is little doubt that TSP1, either alone or in concert with PDGF or EGF, can stimulate proliferation of smooth muscle cells, mesangial cells, and fibroblasts (3, 18, 20). This effect could result from the binding and presentation of growth factors to their receptors, but such interactions remain to be demonstrated. In contrast, TSP1 either has no direct effect or inhibits endothelial cell growth (20, 30, 32). Since TSP1 binds and activates latent TGF β 1, cell-specific effects might reflect a differential response to this multifunctional growth factor. Equally relevant are recent findings which show that, in mesangial cells, TSP1 stimulates the secretion of PDGF and EGF (18). The existence of such a positive feedback loop could explain the interdependence of cellular responses such as proliferation, chemotaxis, and migration on TSP1 and growth factors. The lack of response of most endothelial cells to PDGF could also account for the failure of TSP1 to stimulate endothelial cell growth. Finally, the differential effects of modification of protease activity, and the display of different subsets of receptors, could contribute to cell-specific effects of TSP1.

What Role Does TSP1 Play in the Neoplastic Process and in Angiogenesis?

Neoplasia, comprising neoplastic conversion, malignant progression, tumor growth, and metastasis, is a highly complex process that is species, cell, and tissue dependent. Given its mode of action at the cell surface, it seems unlikely that TSP1 plays a direct role in the initial steps in this process. It is also unnecessary to postulate, a priori, that effects of TSP1 will be found to be concordant in the two latter steps, i.e., tumor growth and metastasis. The case for a positive role for TSP1 in metastatic potential can be made as follows. TSP1 serves as an adhesion factor for many neoplastic cells and stimulates chemotaxis and haptotaxis. In the presence of TSP1, microemboli consisting of tumor cells and platelets are more likely to attach to endothelial cells and, aided by the ability of TSP1 to foster

conversion of plasminogen to plasmin, traverse capillary and lymphatic endothelia (31). To the extent that TSP1 can also stimulate proliferation of such tumor cells, growth of metastatic foci would be fostered. The demonstration of serum- and anchorage-independent growth in clones of NIH 3T3 cells expressing high levels of TSP1, and neutralization of the effect by monoclonal antibodies to TSP1 (4), and the reversal of the malignant phenotype of squamous carcinoma cells by antisense RNA-mediated reduction in TSP1 (5), favor such a model. On the other hand, a negative correlation between metastatic potential and expression of TSP1 was recently found in several tumor cell lines (34), and expression of a TSP1 cDNA in human breast carcinoma cells reduced tumor growth and metastases in nude mice (33). A resolution of these apparently conflicting conclusions can be found in differences in the nature and number of TSP1 receptors characteristic of the various tumor cell lines, in their ability to activate and respond to TGF β or proteases, and/or to initiate a positive regulatory loop in which endogenous growth factors are secreted in response to TSP1.

Regulation of neovascularization as a factor in control of tumor growth has received considerable attention (9). An inhibitory role for TSP1 was demonstrated in the angiogenic response to basic FGF and to conditioned medium from psoriatic keratinocytes, as determined by the rodent corneal assay (11, 19, 30), and in the formation of cords and tubes by endothelial cells in vitro (13). The finding that p53, a prominent tumor suppressor gene, positively regulates TSP1 expression (7) provides supportive evidence for such a role. In contrast, the addition of TSP1 to aortic explants, embedded in a collagen or fibrin gel, stimulated microvessel formation (20). This effect was attributed not to stimulation of endothelial proliferation but to growth of myofibroblasts, which presumably produce angiogenic factors. A major consequence of activation of TGF- β by TSP1 is stimulation of PAI-1 production by many cells (26). PAI-1 could affect at least two steps that are important in angiogenesis: disruption of basement membranes and cell-matrix interactions. Thus, regulation of tumor growth by TSP1 reflects the complex interplay of positive and negative influences of the protein on neoplastic cells and on associated stromal and vascular cells. These effects, in turn, can be attributed to the various interactions of TSP1 with cells in the neoplasm, and with stromal elements which could serve either to present TSP1 to cells or to sequester it from them (Fig. 2).

Perspectives

Given its multiple interactions, the pleotropic, sometimes conflicting, functions of TSP1 can be seen as an inherent consequence of its structure. As a matricellular protein, TSP1 functions as an integrator of the complex information imparted by extracellular protein motifs and cell surface receptors. The specificity of its function would seem to be dictated by the combinatorial arrangement of available cell-surface and extracellular ligands. In effect, the complexity of the pericellular environment provides an explanation for the diverse functions of TSP1.

If, indeed, TSP1 participates in the many complex functions described in Fig. 1, one would expect that an animal

lacking the protein would not be viable. Nevertheless, a preliminary report indicates that mice with a targeted disruption of the TSP1 gene display at most a very subtle phenotype (Polverini, P. J., L. A. DiPietro, V. M. Dixit, R. O. Hynes, and J. Lawler. 1995. Thrombospondin 1 knockout mice show delayed organization and prolonged neovascularization of skin wounds. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 9:272a.), and mice lacking tenascin C are apparently normal (24). How can one explain such a paradox? Even though some proteins may be produced in locations where they have no function because it is more economical for an organism to waste superfluous protein than it is to regulate expression tightly (8), I do not believe that this phenomenon provides the complete answer. Nor do I believe that many of the functions ascribed to TSP1 and tenascin C are based on artifacts of experimentation *in vitro*. It seems likely that one or more defects will be found in these "null" mice when they can be subjected to the correct critical tests. Furthermore, the sudden loss of TSP1 in a postnatal animal could have very different, and more serious, consequences. The functions described for TSP1 in this Commentary are almost all in the nature of regulatory or modulating effects. Compensatory adjustments for lack of TSP1 might therefore be made in an animal deprived of the protein on a genetic basis, a process that could be termed "genetic adaptation". A related phenomenon is the known ability of genetic modifiers, present in different murine genetic backgrounds, to affect the penetrance of mutant phenotypes differentially. If these considerations have validity, targeted disruption of other matricellular protein genes may also prove, on initial assessment, to have relatively innocuous consequences.

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