Multi-author Review
Thrombospondins: from structure to therapeutics

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Thrombospondins: from structure to therapeutics

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Introduction (Part of a Multi-author Review)

Following its discovery in 1971 as a major protein released by activated platelets, thrombospondin-1 (TSP1) spent several decades in search of a physiological function. Early studies indicated that it modulates platelet aggregation, but when TSP1 null mice were first described in 1998, no defect in platelet function was apparent [1]. Furthermore, the null mice appeared relatively normal, with minor defects in skeletal development, fertility, and lung homeostasis. Nevertheless, TSP1 is expressed in various tissues during embryonic development with a complex choreography, and most cells in culture express and secrete TSP1 in response to specific growth factors. In healthy adults, TSP1 is expressed at low levels in most tissues apart from megakaryocytes and platelets, but rodent and human pathology studies revealed intriguing changes in TSP1 expression in a number of disease states. In general, increased TSP1 expression is associated with tissue injury or remodeling.

Functional studies revealed that TSP1 can positively or negatively regulate adhesion, motility, proliferation, and survival of various cell types. Furthermore, TSP1 binds avidly to several growth factors and proteases and mediates activation of latent transforming growth factor-β. TSP1 also interacts with a bewildering number of cell surface receptors and other extracellular matrix proteins, some of which were shown to mediate specific cellular responses to TSP1. Thus, many cells both produce TSP1 and respond to it. The high degree of sequence conservation for TSP1 from fish to birds to humans argued that some important function maintained this protein throughout evolution of vertebrates; but what drives that evolutionary pressure?

Genome sequencing studies further revealed that TSP1 belongs to a small family of five secreted proteins. As discussed in the review by Carlson et al. TSP2 bears the most similarity to TSP1, both of which are trimeric glycoproteins. The other three thrombospondins belong to a second subclass of pentameric proteins. All thrombospondins share a highly conserved C-terminal signature domain. These domains of TSP1 and TSP2 have been crystallized, and Carlson et al. review the structural features of this domain that have been identified and the functional implications of this structural data to other members of the TSP family. Structures for recombinant N-terminal domain of TSP1 and its central type 1 repeats have also been solved, and Carlson et al. present a new model for the intact protein that incorporates these data and previous low-resolution structural and conformational studies of TSP1. This model may help us to understand how the reported sequence polymorphisms and point mutations in thrombospondins cause disease.

Although TSP5 (also known as cartilage oligomeric matrix protein or COMP) was the last member to join this family, it was the first to have a clearly defined role in human disease. Mutations in COMP are an autosomal dominant cause of dwarfism. A variety of structural mutations in COMP have been found in patients with two types of skeletal dysplasias, pseudoachondroplasia and multiple epiphyseal dysplasia. Posey et al. discuss the distribution of these mutations in COMP and several new *in vitro* and murine models that are beginning to provide insights into how mutations in COMP lead to defective growth of long bones.

Beginning with three independent descriptions in 1990 of inhibitory activities of TSP1 for endothelial cells [2–4], TSP1 became widely known as an angiogenesis inhibitor. TSP1 potently inhibits endothelial cell growth and motility stimulated by specific growth factors and under certain conditions induces apoptosis of these cells. The anti-angiogenic activities of TSP1 were initially mapped to its type 1 repeats and are mediated in part via interaction of this domain of TSP1 with the scavenger receptor and fatty acid transporter CD36. TSP2 also contains type 1 repeats and shares a similar anti-angiogenic activity. *In vivo* studies demonstrated the ability of TSP1 to inhibit developmental angiogenesis, such as in the chick chorioallantoic membrane, and pathological angiogenesis of tumors [5, 6]. The latter observation led to clinical investigations of TSP1 expression in various cancers, which showed consistent loss of TSP1 expression during malignant progression of certain cancers. This loss was linked to alterations in specific oncogenes and tumor suppressor genes such as p53, Myc, and Ras that control TSP1 expression [7–9]. Recent work reviewed by Kazerounian et al. shed additional light on the role of TSP1 in the pathogenesis of cancer and some interesting therapeutic strategies using TSP1-based agents to treat existing tumors. A drug developed by Abbott Laboratories that was based on an anti-angiogenic peptide from the type 1 repeats of TSP1 is now in phase II clinical trials [10]. In addition to presenting an overview of the role of TSP1 and other thrombospondins in cancer, Kazerounian et al. describe how recombinant type 1 repeats of TSP1 can be used to regulate tumor growth and angiogenesis.

A large-scale analysis of single-nucleotide polymorphisms associated with familial premature coronary artery disease published in 2001 has refocused interest on the role of thrombospondins in vascular biology [11]. Remarkably, polymorphisms in TSP1, TSP2, and TSP4 all showed significant correlations with premature myocardial infarction in this cohort. The polymorphisms in TSP1 and TSP4 result in single amino acid substitutions that alter conformational and functional properties of the respective proteins and presumably confer an increased proatherogenic or prothrombotic activity. The exact mechanism for this altered activity and the molecular and cellular targets of TSP1 and TSP4 in the vasculature that lead to this pathogenesis remain to be defined.

TSP1 null mice have recently provided a number of important insights into the role of this protein in vascular physiology and pathology. Although the TSP1 null mice fare well in the protected environment of a vivarium, subjecting these mice to specific stresses reveals several clear vascular phenotypes. Returning to its role in platelets, Bonnefoy et al. discuss recent evidence that TSP1 controls the function of von Willebrand factor by regulating its multimer size. TSP1 protects von Willebrand factor from degradation by the plasma protease ADAMTS-13. In TSP1 null mice, this manifests as defective thrombus adherence, and a normal phenotype could be restored by providing exogenous TSP1 or neutralizing ADAMTS-13.

Isenberg et al. recently identified a second pathway through which TSP1 modulates platelet function. TSP1 binding to its cell receptors CD47 or CD36 elicits signals that potently antagonize physiological responses to nitric oxide (NO) in vascular cells. NO plays an important role in limiting the aggregation and adhesion of platelets [12]. This activity is mediated by the NO-activated soluble guanylate cyclase in platelets, and the resulting elevation in cytoplasmic cGMP activates cGMP-dependent protein kinase to control platelet adhesion and aggregation via several targets, including VASP and Rap1. TSP1 binding to CD47 simultaneously inhibits soluble guanylate cyclase and cGMP-dependent protein kinase activities. TSP1 binding to CD36 further inhibits this pathway by controlling activation of the nitric oxide synthase isoform NOS3. Thus, TSP1 controls multiple steps in the NO/cGMP signaling cascade.

Although TSP1 null platelets exhibit normal aggregation under standard conditions, a clear defect is revealed when exogenous NO is provided. In the presence of physiological NO levels, TSP1 null platelets require much higher levels of thrombin to induce aggregation. Thus, TSP1 released from platelets plays an important role in hemostasis to support platelet aggregation by overcoming the physiological antithrombotic activity of NO.

In addition to platelets, NO is a major regulator of vascular smooth muscle physiology [12]. NO produced by endothelial cells induces relaxation of vascular smooth muscle to increase blood flow. Under ischemic conditions, reduction of nitrite further increases NO-mediated relaxation to restore blood flow [13]. TSP1 null mice show a roughly twofold greater increase in skeletal muscle blood oxygen levels in response to NO. Following ischemic injury, TSP1 and CD47 null mice show enhanced tissue survival and acute maintenance of tissue perfusion. Limiting bleeding through increasing vasoconstriction and enhancing platelet function in hemostasis could provide the evolutionary pressure to maintain TSP1 in vertebrates. However, in association with modern medical procedures and diseases of aging, where tissue TSP1 levels are elevated, these activities of TSP1 may have become a liability.

Knowing that TSP1/CD47 signaling limits survival of ischemia also provides an opportunity to develop new

therapies. Isenberg et al. review several approaches to overcome the deleterious effects of this pathway on surgical ischemia, skin grafting, and vascular insufficiencies associated with age and diet.

Transgenic mice lacking specific thrombospondins, over-expressing thrombospondins in specific tissues, or expressing mutated COMP have begun to reveal some secrets of this interesting gene family. One key insight has been recognizing that these mice must be subjected to specific physiological stresses to reveal the functions of each thrombospondin. In addition to the studies reviewed here, readers are referred to a number of additional published disease models that have utilized these mice but could not be addressed in this multi-author review or cited here due to space limitations. This is an exciting time for thrombospondin research, and additional therapeutics may emerge from ongoing studies that will benefit diseases ranging from cancer to cardiovascular disease to aging. I want to thank all of the authors for contributing their time and insights in preparing these reviews.

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