Chapter 13
Thrombospondins: Endogenous Inhibitors of Angiogenesis

Paul Bornstein

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Abstract: The thrombospondin (TSP) gene family consists of five members, two of which, TSP1 and TSP2, have been shown to play important roles in the regulation of angiogenesis. While TSP1 and TSP2 are secreted into the extracellular environment, they do not play structural roles but rather function to regulate cellular behavior by interaction with numerous cell-surface receptors, proteases, cytokines, and other bioactive proteins. As a consequence, these TSPs are termed matricellular proteins. TSP1, but not TSP2, is capable of activating latent TGFβ, and can thereby stimulate the production of extracellular matrix. Matricellular TSPs inhibit angiogenesis both by causing apoptosis of endothelial cells (EC) and by inhibiting their proliferation. However, under some circumstances TSP1 has also been shown to be proangiogenic. TSPs interact with matrix metalloproteinases (MMPs) 2 and 9 and function as clearance factors by directing these MMPs to the scavenger receptor, LRP1, and thence to lysosomal degradation. As a consequence, TSPs function to regulate cell adhesion. TSP1 also inhibits nitric oxide-stimulation of EC proliferation by interaction with the CD47/integrin-associated protein receptor. Finally, by virtue of their ability to inhibit angiogenesis, TSPs have the potential to inhibit tumor growth and metastases, a property that may have clinical applications.

Introduction

Thrombospondins (TSPs) comprise a small family of extracellular proteins that are present in all vertebrates and in some invertebrates [1,2]. The first member of the family, TSP1, was shown to be released from thrombin-treated platelets and was initially termed thrombin-sensitive protein. It was subsequently renamed thrombospondin to indicate that its release from the α granules of platelets in response to thrombin treatment did not necessarily require proteolysis of the protein. Vertebrates express five paralogous genes whose translation products are assembled as homotrimers of 145kDa chains (TSP1 and TSP2) or homopentamers of ~110kDa chains (TSPs 3–5; Fig. 13.1). TSP5 was first identified in cartilage and is still referred to as cartilage oligomeric matrix protein (COMP) in many publications. There is some evidence that TSP4 and TSP5 exist as both homo- and heteropentamers [3], but these findings require confirmation.

The structures of the five thrombospondin monomers are shown schematically in Fig. 13.1. Trimeric TSPs are composed of a globular, heparin-binding NH2-terminal (N-terminal) domain followed by a procollagen homology domain, three type I thrombospondin or properdin repeats, three type II or EGF-like repeats, seven type III or Ca2+-binding repeats and a COOH-terminal (C-terminal) globular domain. The three monomers are linked by interchain disulfide bonds that are placed between the N-terminal domain and the type I repeats. Pentameric TSPs lack the procollagen homology domain and type I repeats and contain four instead of three type II repeats (Fig. 13.1). The numbers of amino acids in the pentameric N-terminal domains also differ substantially among the three proteins, whereas TSP1 and TSP2 have N-terminal domains that are very similar in size.

The macromolecular structure of TSP1 has been studied by rotary-shadowing electron microscopy and by X-ray crystallography. Rotary shadowing reveals a bola-like structure in which a large globule, representing the three N-terminal domains, is connected by strands to each of the three individual C-terminal globules [4]. This structure is supported by the crystal structures of the three type I repeats of TSP1 [5] and of a fragment consisting of the three type II and type III repeats and the C-terminal globule of TSP2 [6]. Several reviews provide additional information regarding the structures of the thrombospondins [7–9].
Thrombospondins as Matricellular Proteins

Thrombospondins are frequently referred to as ‘extracellular matrix’ proteins. While this is valid in the sense that these proteins are secreted and function in the extracellular environment in close association with macromolecular structural elements such as collagen fibrils, there is no good evidence that thrombospondins actually serve in a structural capacity. TSP5 represents a possible exception in that although the phenotype of the TSP5 knockout mouse is grossly normal [10], mutations in the THBS5 gene are responsible for the dwarfing syndrome, pseudoachondroplasia and multiple epiphyseal dysplasia [11]. Instead, TSP1 and TSP2 function as extracellular modulators of cellular function. These properties are achieved by the ability of these proteins to interact with a wide variety of cell-surface signaling receptors, as well as with growth factors, cytokines, and other bioactive molecules such as matrix metalloproteinases (MMPs).

A consequence of these properties is that the functions of TSP1 and TSP2 are highly complex and context-dependent, that is they are subject to differences that reflect the tissue and cellular environments in which these proteins are expressed. As a general rule, these proteins are not prevalent in normal adult animals, but their genes are induced during development and growth, and in response to injury. These characteristics have led to the application of the term ‘matricellular proteins’ to TSP1 and TSP2, as well as to SPARC, osteopontin, and to some members of the tenascin and CCN (Cyr, Connective tissue growth factor, Nov) families, which are unrelated structurally but function similarly to thrombospondins [12, 13]. However, it should be noted that while matricellular proteins do not appear to play structural roles, structural proteins such as the collagens and fibronectin are clearly capable of engaging signaling receptors, and can thereby affect cell function.

Since relatively little is known concerning the functions of the pentameric thrombospondins, and particularly in regard to their role in angiogenesis if any, this chapter will focus on TSP1 and TSP2.

The Functions of Thrombospondins in Wound Healing

As matricellular proteins, thrombospondins are involved in a wide range of functions, including a role in synapse formation in the central nervous system [14, 15], but their participation in wound healing, and in the organization of the extracellular matrix (ECM), are of particular relevance to their functions as modulators of angiogenesis. Studies of wound healing in mice have presented investigators with an apparent paradox. Although the intrinsic functional properties of TSP1 and TSP2 proteins are quite similar, the phenotypes of mice that lack functional TSP1 or TSP2 genes (knockout mice) are very different [16, 17]. This paradox can be resolved by the realization that the promoter sequences upstream from the start of transcription in the two genes differ considerably. As a result, the spatial patterns of synthesis of the two encoded proteins in different cells and tissues, and the temporal program of synthesis during development, growth, and in response to injury, are also quite different. This dichotomy can best be illustrated by a study of excisional skin wound healing in TSP1-null, TSP2-null, and TSP1/TSP2 double-null mice. Mice that lack a functional TSP2 gene close skin wounds more rapidly and with less scarring. Histological examination of the wound bed as a function of time revealed prolonged vascularity and an abnormal organization of collagen fibers in its granulation tissue [18]. These findings reflect the known properties of TSP2, as indicated by the phenotype of TSP2 knockout mice [17]. By contrast, wound healing was delayed in TSP1-null mice and was accompanied by a reduction in blood vessels and inflammatory cells, relative to that in TSP2-null mice. [19].

Surprisingly, the healing response in TSP1/TSP2 double-null mice resembled that in TSP1-null animals, despite the fact that double-null mice also lack TSP2 [19]. These findings can be explained by the fact that TSP1 is normally released from platelets and secreted by inflammatory cells early in the wound healing process, and is strongly chemotactic for neutrophils and monocytes [20, 21] that are needed for the normal progression of the healing process. Thus, the presence or absence of TSP1 dictates the course of wound healing. A similar conclusion was reached by the use of antisense TSP1 oligonucleotides in wildtype (WT) mice [22]. The importance of a normal spatial and temporal expression of TSP1 is further demonstrated by the finding that over-expression of TSP1

Fig. 13.1. A schematic representation of the structures of the individual chains of the trimeric thrombospondins 1 and 2, and the pentameric thrombospondins 3–5. In the trimeric proteins, the NH2-terminal heparin-binding domain is followed by an interchain disulfide knot linking all three chains, a procollagen domain (PC) that is homologous to sequences in the N-propeptides of types I-III procollagens, three type I repeats, also known as properdin or TSR domains, three type II or EGF-like repeats, seven type III or calcium-binding repeats, and a globular COOH-terminal domain. The pentameric thrombospondins differ from the trimeric proteins in that the sizes of the pentameric N-terminal domains vary considerably among the three proteins. These proteins also lack type I repeats, and contain four type three repeats. (Reproduced with permission from Bornstein, P. ‘Matricellular Proteins’ in G.J. Laurent and S.D. Shapiro, Eds, Encyclopedia of Respiratory Medicine, London, UK. Elsevier. 2006, pp 175–183)