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The thrombospondins

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Abstract

Thrombospondin-1 (TSP-1) was studied in the 1980s as a major component of platelet α -granules released upon platelet activation and also as a cell adhesion molecule. In 1993, we published a short review that discussed the exciting identification by molecular cloning of four additional vertebrate gene products related to TSP-1 [Current Biology 3 (1993) 188]. We put forward a structurally based classification for the newly identified proteins and discussed the functional and evolutionary implications of the new gene family. Since that time, the depth and breadth of knowledge on vertebrate TSPs and their functions in cells and tissues in health and disease has expanded into important new areas. Of particular interest is the new knowledge on the complex, domain and cell-type specific effects of TSPs on cell-signaling and cell-adhesion behaviour, the roles of TSP-1 and TSP-2 as anti-angiogenic agents, the roles of TSP-1 and TSP-2 in wound-healing, and associations of point mutations and polymorphisms in TSP-1, TSP-4 and TSP-5/COMP with human genetic diseases. The TSP family also now includes invertebrate members. In this article, we give the 2004 view on TSPs and our perspectives on the significant challenges that remain. Other articles in this issue discuss the functions of vertebrate TSPs in depth.

Keywords

Thrombospondin-1; Extracellular glycoproteins; TSR

1. Introduction

Thrombospondins (TSPs) are multidomain, calcium-binding extracellular glycoproteins of animals. Most attention has focused on establishing their properties and functions in vertebrates, where TSPs were first identified. The prototypic member of the family is thrombospondin-1 (TSP-1), which has functions in platelet aggregation, inflammatory response and the regulation of angiogenesis during wound repair and tumor growth. Many of these properties are shared by TSP-2, which also has complex and interesting roles in the assembly of connective tissue extracellular matrix (ECM) (reviewed in Lawler, 2000; Bornstein, Armstrong, Hankenson, Kyriakides, & Yang, 2000). The other TSP that has been studied in depth, TSP-5/COMP (cartilage oligomeric matrix protein), is principally expressed in cartilage and certain other connective tissues and has roles in chondrocyte attachment, differentiation and cartilage ECM assembly (reviewed in Unger & Hecht, 2001). Little is known about the biology of TSP-3 and TSP-4. The analysis of gene knockout mice has established that TSP-1, TSP-2 and TSP-5/COMP each have distinct and non-redundant roles

in specific tissues (Kyriakides, Zhu, Smith, Bain, & Yang, 1998; Lawler, Sunday, Thibert, Duquette, & George, 1998; Svensson et al., 2002). These distinct roles are due to differences in the transcriptional regulation of each gene and also differences in the functional capabilities of individual family members. For example, the phenotypic abnormalities in the lungs of TSP-1-null mice appear to result from defective activation of transforming growth factor- β , an activity that is unique to TSP-1 (Schultz-Cherry et al., 1995; Crawford et al., 1998). However, there also appear to be fundamental commonalities in the cell and tissue mechanisms that underlie TSP functions: all the vertebrate TSPs that have been analysed experimentally support cell attachment in a calcium-dependent manner and bind to other ECM glycoproteins and proteoglycans. Depending on the cell-type and the TSP family member, additional activities include induction of cytoskeletal organisation and cell migration, modulation of cell proliferation and also indirect effects on cell function through regulation of extracellular proteases and activation or inhibition of cytokines and growth factors (reviewed in Adams, 2001) (Fig. 1).

2. Structure

The known TSPs fall into two subgroups, termed A and B, according to their oligomerisation status and molecular architecture (Adams & Lawler, 1993). Of the vertebrate TSPs, TSP-1 and TSP-2 form homotrimers and TSP-3, TSP-4 and TSP-5/COMP form homopentamers (Fig. 2) (Adams, 2001). *Drosophila* TSP is pentameric and two TSPs, one each of the subgroup A and B forms, have been identified in *Ciona intestinalis* (Adams et al., 2003). Each TSP subunit contains multiple domains and a coiled-coil oligomerisation region. The hallmark of all TSPs is the presence in the carboxy-terminal half of each polypeptide of a variable number of EGF-like domains that are contiguous with seven so-called TSP type 3 repeats and a C-terminal region of globular character (Fig. 2). This cassette of domains, around 650 amino acids long, is the most highly-conserved region of each subunit and forms an effective search tool for identification of novel TSPs in diverse organisms. The amino-terminal regions are more varied, in sequence and in structure, between the individual TSPs (e.g., TSP-1 and TSP-2 are 25% identical in the amino-terminal domains and also each contain a von Willebrand factor type C domain; TSP-5/COMP lacks a distinct amino-terminal domain). However, a common feature of four of the vertebrate TSPs and both the *Ciona* TSPs is an amino-terminal domain predicted to have a fold similar to the laminin G domain (Beckmann, Hanke, Bork, & Reich, 1998). Interestingly, the insect TSPs have a distinctive amino-terminal domain in terms of sequence (Fig. 2), yet this also functions as a heparin-binding domain (Adams et al., 2003). A distinctive aspect of the TSP subgroup A structure is the presence of so-called thrombospondin type 1 domains, also known as properdin domains. These domains have a novel antiparallel three-stranded structure and link TSP-1, TSP-2 and *Ciona* TSP-A to an extensive domain superfamily of extracellular proteins, termed the thrombospondin repeat (TSR) superfamily. Many members of this family have been identified through genome sequencing projects; those that have been studied experimentally have been found to function in cell interactions, neuronal guidance and extracellular proteolysis (Adams & Tucker, 2000; Tan et al., 2002 and see articles by Tucker and Apte in this issue). In TSP-1 and TSP-2, the type 1 domains have specific functions in signaling inhibition of angiogenesis by binding to CD36 and also support attachment of multiple cell types (see article by Lawler and Detmar in this issue).

A unique feature of pentameric TSPs is a central hydrophobic channel, 7.3 nm long and 0.2–0.6 nm in diameter, that is formed by the assembly of the coiled-coil oligomerisation domains into an α -helical bundle. This structure has similarities to ion channel models and has been shown to bind vitamin D and all-*trans* retinoic acid within the channel (Guo, Bozic, Malashkevich, Kammerer, & Schutless, 1998). It has been proposed that an additional function of subgroup B TSPs could be to store hydrophobic signaling molecules and facilitate their

delivery at cell-surfaces. Considering that lipophilic compounds such as ecdysone have major roles in insect developmental transitions, this might also be a function of the insect TSPs.

3. Phylogeny of thrombospondins

The animal kingdom is traditionally classified according to the presence or absence of a body cavity (coelom) and by processes of early embryonic development that lead to the formation of the mouth of the adult organism (protostome versus deuterostome). In common with laminins and collagens, thrombospondins are present in both protostome and deuterostome animals. The known protostomal representatives include single TSPs encoded in the genomes of the insects *Drosophila melanogaster* and *Anopheles gambiae* and a partial sequence from cDNA in the silkworm *Bombyx mori*. *Drosophila* TSP has been experimentally demonstrated to assemble as a pentamer (Adams et al., 2003). It is expected that TSP(s) could be present in other bilaterian protostomes or simpler animals. However, the *Caenorhabditis elegans* genome does not encode a TSP. ESTs obtained from the cnidarian *Hydra vulgaris* have been designated as TSP-like sequences (GenBank CF777333, CD285621), however, because these are TSR sequences, this assignment should be viewed with caution until longer *Hydra* cDNA sequences that demonstrably encode TSR in combination with EGF domains, TSP type 3 repeats and a carboxy-terminal domain have been assembled. With regard to the appearance of TSPs during the evolution of bilaterians, it will be of future interest to establish whether TSPs are present in other protostome phyla (e.g., annelids, molluscs).

In the deuterostomes, TSPs have principally been studied in vertebrates, a subgroup of the chordate phylum. Five TSPs are encoded in the human genome and four to five TSPs have been identified in other vertebrates, including mouse, chicken, *Xenopus lae-vis* and *Danio rerio*. Other chordate subphyla include the cephalochordates and urochordates. The recently completed genome sequence of the urochordate *C. intestinalis* allowed a clear view of the status of TSPs in an organism widely regarded as representing the simplest modern form of the chordate genome and body plan (Satoh, Satou, Davidson, & Levine, 2003). Interestingly, although *Ciona* is considered to have diverged from the ancestral chordate before the whole genome duplications that took place during the evolution of modern vertebrates (Dehal et al., 2002; Panopoulou et al., 2003), not one but two distinct TSP-encoding sequences were identified within the genome and are supported by EST data. One of these corresponds to a subgroup B TSP. The other has the major domains equivalent to TSP-1 and TSP-2 and oligomerises through an unusual oligomerisation domain (Adams et al., 2003; Haase, Monk, & Adams, in preparation). Thus, the subgroup A form of TSP, associated in vertebrates with functions in platelet aggregation and wound healing, arose before the evolution of the blood clotting or fibrinolysis cascades (Jiang & Doolittle, 2003). It will be interesting to trace the evolution of oligomerisation within the family more extensively, by establishing whether single or multiple TSP genes are present in the other deuterostome phyla, the echinoderms and hemichordates.

4. Expression, synthesis and degradation

The expression of TSPs has mainly been studied in vertebrates. TSPs are expressed during development, during which each TSP has a specific spatiotemporal pattern of expression. Most adult tissues co-express one or more TSPs and major alterations occur during pathophysiological conditions. For example, TSP-2 production is upregulated in fibroblasts during granulation of skin wounds (Kyriakides, Tam, & Bornstein, 1999). TSP-1 and TSP-2 are highly expressed by stromal fibroblasts and endothelial cells within tumors (Brown et al., 1999; Hawighorst et al., 2001). TSP-4 transcripts are upregulated in the skeletal muscle of Duchenne muscular dystrophy patients (Chen, Zhao, Borup, & Hoffman, 2000). TSP-5/COMP and COMP fragments in serum and synovial fluid have been correlated with osteoarthritis,

joint injury and cartilage degradation (Neidhart et al., 1997; Clark et al., 1999). In *Drosophila* embryogenesis, D-TSP is transcribed during germband extension in a segmental pattern corresponding to developing apodeme cells and a subset of myoblasts and is also present in the wing and leg imaginal discs (Adams et al., 2003).

Protein synthesis and degradation have been mainly studied with regard to TSP-1 and TSP-2. TSP-1 is synthesised by many cell types in culture, because of the presence of a serum-response element in the promoter. Synthesis is highest in subconfluent and proliferative cells. TSP-1 mRNA expression is increased in response to growth factors PDGF, bFGF and TGF β 1, heat shock and hypoxia, and is down-regulated in response to IL-1 β and TNF α (reviewed in Adams, Tucker, & Lawler, 1995). Interestingly, in view of the role of TSP-1 as an inhibitor of angiogenesis, TSP-1 transcription is down-regulated by a number of dominant transforming oncogenes including c-fos, c-jun, v-src and Ras (Mettouchi et al., 1994; Watnick, Cheng, Rangarajan, Ince, & Weinberg, 2003). The complex relationship between expression of TSP-1, TSP-2 and tumor angiogenesis is discussed in detail in the article by Lawler and Detmar.

TSP-1 can be degraded either extracellularly or intracellularly. In the vascular system of healthy individuals, the plasma concentration of TSP is very low (around 180 ng/ml for TSP-1) but can be dramatically and locally increased when TSP-1 is released from the α -granules of activated platelets. This TSP-1 contributes to platelet aggregation by binding to platelet surfaces and by its incorporation into the fibrin clot. Subsequently, this TSP is a substrate for cleavage by thrombin and Factor XIIIa during fibrinolysis and clot resolution. TSP-1 is also a substrate for the cathepsins and elastases released by leucocytes and neutrophils during inflammatory response, but there is little information on the fragments produced in vivo or their biological activities (Adams, Tucker, & Lawler, 1995; Hogg, 1994; Crawford et al., 1998). Presumably, other TSPs are also susceptible to these proteases. COMP/TSP-5 is a substrate for matrix metalloproteinases 19 and 20 and an ADAMTS (Dickinson et al., 2003; Stracke et al., 2000) and the binding of TSP-2 to MMP-2 regulates and localises enzyme activity (Yang, Strickland, & Bornstein, 2001).

TSP-1 and TSP-2 are also degraded intracellularly through endocytosis and lysosomal proteolysis. The balance between ECM retention of TSP or uptake and degradation by cells depends on the array of TSP receptors expressed by particular cell types. Uptake is mediated by binding of the laminin-G-like TSP amino-terminal domain to heparan sulphate proteoglycans and the low-density lipoprotein receptor-related protein, in a complex with calreticulin (Godyna, Liao, Popa, Stefansson, & Argraves, 1995; Orr et al., 2003 and see the article by Murphy-Ullrich et al. in this issue). It remains to be tested whether this mode of regulation applies to other TSPs that have the laminin-G-like amino-terminal domain, or whether insect TSPs, with the distinct amino-terminal domain, have a longer retention time in ECM.

5. Possible medical applications

The clinical significance of TSPs is now a substantial and important area of current research pertaining to both human genetic and acquired diseases. It has been appreciated since the mid-1990s that coding-region mutations (point mutations and small deletions) of TSP-5/COMP are causal in pseudoachondroplastic dysplasia (PSACH, OMIM 117170), an autosomal dominant bone dysplasia caused by premature arrest of bone growth and laxity of joints. These mutations, which cluster within the type 3 repeats and carboxy-terminus, lead to a deficiency of TSP-5 in cartilage ECM due to decreased calcium-binding and an aberrant conformation that impairs the progression of this TSP and other cartilage ECM components through the chondrocyte secretory pathway. The disease, its molecular basis and current views on therapeutic possibilities are discussed in the article by Hecht.

More recently, single-nucleotide polymorphisms (SNPs) that affect the coding regions of TSP-1 and TSP-4 have emerged as novel risk factors correlated with familial premature myocardial infarction (Topol et al., 2001). Interestingly, a SNP that alters the 3'-untranslated region of TSP-2 is correlated with a protective effect (Topol et al., 2001; Boekholdt et al., 2002). These important findings provide a new focus for research into the roles of TSPs in the cardiovascular system and are discussed in the article by Plow and co-authors.

A third focus of interest for innovative clinical practice is the development of reagents based on the TSR of TSP-1 and TSP-2 as inhibitors of tumor angiogenesis. Several active peptide motifs have been identified in the TSR that bind CD36 and possibly glycosaminoglycans to signal endothelial cell apoptosis (Lawler, 2000). The article by Lawler and Detmar discusses the latest developments in this area. Also of interest are the emerging roles of TSP-1 and TSP-2 in wound-healing, which include the regulation of TGF- β activity by a peptide motif RFK that is present in the type 1 repeats of TSP-1 but not TSP-2 (Schultz-Cherry et al., 1995). The significance of modulatory adhesion molecules in wound-healing and the potential for new approaches to accelerate wound-repair or minimise scarring and foreign body reaction are discussed in the articles of Schwarzbauer and Bornstein.

6. Future prospects

Our knowledge and understanding of the functions and mechanisms of action of TSPs has increased immensely over the last 10 years. Yet, open questions remain and new frontiers offer new challenges for the future. The roles of TSPs in regulating cell behaviour and phenotype have been of constant interest for a number of years and there remains much to be learnt concerning the mechanisms by which TSPs regulate cell-signaling processes, and the interplay between extracellular deposition and cell-mediated uptake of TSPs. Mechanisms for cross-talk between TSP, other ECM components acting through integrins, and growth-factor signaling pathways also remain to be elucidated. For example, inhibition of angiogenesis may involve crosstalk between signals initiated by TSP-1 and TSP-2 and the vascular endothelial growth factor pathway. To date, the bulk of research activity has focused on TSP-1 and TSP-2 (Fig. 1). We anticipate that greater attention to the properties of pentameric TSPs will lead to an understanding of the full spectrum of functions of vertebrate TSPs.

With the development of many gene knockout mice, the interactions between TSPs and other ECM components and the consequences for ECM structure can be analysed to new levels of precision. Such studies have uncovered insights into the roles of TSP-1 and TSP-2 in wound healing and foreign body reaction (Kyriakides, Leach, Hoffman, Ratner, & Bornstein, 1999). The knockout mice also provide an *in vivo* platform for evaluation of the physiological significance of the extensive data that is rapidly accumulating from cDNA microarray experiments. In many contexts, TSPs appear to have a regulatory function during tissue remodeling. Thus, we anticipate that up or down-regulation of TSPs in various experimental systems will be commonplace. The importance of these changes can be evaluated in the knockout mice. In the future, we would expect to see applications of all this fundamental information in the development of ECM materials for bioengineering and regenerative medicine.

The analysis of TSP function in invertebrates will bring a new perspective to our understanding of the biological roles of TSPs and may extend and deepen our understanding of core functions of TSPs mediated by the highly-conserved carboxy-terminal region. The link between TSP polymorphisms and human genetic predisposition to premature coronary artery disease is engendering a new appraisal of the roles of multiple TSPs in the cardiovascular system and also focuses attention on the functions of the carboxy-regions. Overall, we anticipate that future analysis of TSP function will be facilitated by detailed knowledge of the structure of these

proteins. Within the next few years, the structure of all of the domains of the TSPs will be determined by X-ray crystallography or NMR. These data will have important implications for understanding the functions of the wild-type proteins and the effects of various naturally occurring mutations and polymorphisms. This new knowledge will unlock the potential to translate fundamental research findings on TSPs into the clinical context.

Abbreviations

ECM	extracellular matrix
EST	expressed sequence tag
TSP	thrombospondin
TSR	thrombospondin type 1 repeat

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	TSP-1	TSP-2	TSP-3	TSP-4	TSP-5/COMP
Calcium-binding	+	+	+	+	+
Cell Attachment	+	+	+	+	+
Cell Spreading/	+	+	NT	+	-/+
Cytoskeletal organisation*					
Cell Motility*	+	+	NT	NT	NT
Cell Proliferation*	S/I	S/I	NT	NT	NT
Neurite outgrowth	+	NT	NT	+	NT
Apoptosis*	+	+	NT	NT	NT
Phagocytosis*	+	NT	NT	NT	NT
Binding other ECM components	+	+	NT	+	+
Connective ECM assembly	-	+	NT	NT	+
Binding/Regulation of Proteases*	+	+	NT	NT	+
<u>Activation of TGF-β</u>	+	-	NT	NT	NT
Platelet aggregation	+	+	NT	NT	NT
Cell-cell Interactions*	+	+	NT	+	NT
<u>Inhibition of Angiogenesis</u>	+	+	NT	NT	-

Fig. 1.

Functions of TSPs. Key: experimentally demonstrated function (+), or absence of activity (-).

For proliferation, S: stimulation, I: inhibition and depends on the cell-type. NT: not tested.

Underlined properties are known to be mediated by the type 1 repeats and are therefore unlikely to be shared in pentameric TSPs by the same mechanism. (*): indicates function is cell-type dependent, either because of cell-binding to different TSP domains or because of the interaction of different cell-type-specific receptors with the same TSP domain. Cell-cell interactions include binding of parasitised red blood cells to endothelium, binding of T cells to activated endothelium, macrophage interactions with apoptotic cells, and neurite outgrowth on appropriate cell layers.

Fig. 2.

Domain architecture of TSPs. The diagram shows the domain composition of the various forms of TSPs. Red box indicates the cassette of domains that is conserved in all TSPs. Major distinct domain functions are listed underneath subgroup A and suggest several possibilities for clinical translation (see text).