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Thrombospondins as key regulators of synaptogenesis in the central nervous system

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Abstract

Thrombospondins (TSPs) are a family of large, oligomeric multidomain glycoproteins that participate in a variety of biological functions as part of the extracellular matrix (ECM). Through their associations with a number of binding partners, TSPs mediate complex cell-cell and cell-matrix interactions in such diverse processes as angiogenesis, inflammation, osteogenesis, cell proliferation, and apoptosis. It was recently shown in the developing central nervous system (CNS) that TSPs promote the formation of new synapses, which are the unique cell-cell adhesions between neurons in the brain. This increase in synaptogenesis is mediated by the interaction between astrocyte-secreted TSPs and their neuronal receptor, calcium channel subunit $\alpha 2\delta$ -1. The cellular and molecular mechanisms that underlie induction of synaptogenesis via this interaction are yet to be fully elucidated. This review will focus on what is known about TSP and synapse formation during development, possible roles for TSP following brain injury, and what the previously established actions of TSP in other biological tissues may tell us about the mechanisms underlying TSP's functions in CNS synaptogenesis.

Keywords

synaptogenesis; astrocytes; neurons; injury-dependent synaptic plasticity

1. Introduction

Thrombospondins (TSPs) are secreted multidomain glycoproteins found throughout the body of vertebrates and lower metazoa (Adams, 2001; Bentley and Adams, 2010; Mosher and Adams, 2012). The TSP family consists of 2 subgroups organized by oligomerization state and domain structure: subgroups A and B. Subgroup A includes the trimeric TSP-1 and TSP-2, while the pentameric TSP-3, TSP-4 and TSP-5 comprise subgroup B (Lawler, 2002). Unlike other extracellular matrix (ECM) proteins such as collagen and laminin that play structural roles in the ECM, TSPs are primarily involved in regulating cell-cell and cell-matrix interactions (Bornstein, 2000). To do so TSPs act through a number of extracellular matrix proteins and cell surface receptors (Table 1) and control cytoskeletal dynamics, cell migration and cell attachment.

TSPs start to exert their effects as early as embryogenesis and are critical for the development of many organs in the body including the bones, muscles, heart and the brain (O'Shea et al., 1990a; Tucker et al., 1995). TSPs 1–4 have all been found in the brain (Iruela-Arispe et al., 1993; Lawler et al., 1993; O'Shea et al., 1990b). During early postnatal development they are primarily expressed by astrocytes, the predominant non-neuronal cell type in the CNS (Cahoy et al., 2008; Eroglu, 2009). Several studies in the last decade have

highlighted astrocytes and the TSPs that they secrete as major controllers of formation of neuronal synaptic connections in the developing nervous system (Christopherson et al., 2005; Hughes et al., 2010; Ullian et al., 2001).

Astrocytes are the most abundant cell type in the brain. They were originally viewed as merely the “glue” that filled in the space between neurons (Volterra and Meldolesi, 2005), but recent studies have shown them to be far more active participants in the development, maintenance and plasticity of the CNS than was previously thought. In fact, it has even been suggested that it may be astrocytic complexity which underlies the vast functional competency of the human brain (Oberheim et al., 2006). Astrocytes are often found in close apposition to the pre- and postsynaptic machinery of neurons at the excitatory (glutamatergic) connections, an arrangement that has come to be known as the “tripartite synapse” (Araque et al., 1999). Through this close contact with neurons, astrocytes can modulate the efficacy of synapses through release and uptake of neuroactive substances (Eroglu et al., 2008). As a complex process-bearing cell, a single astrocyte may contact and potentially coordinate the activity of up to 100,000 synapses at once (Bushong et al., 2003;2004).

Besides their structural and functional associations at excitatory synapses in the adult brain, astrocytes also play important roles in the regulation of synapse formation and elimination in the developing CNS (Christopherson et al., 2005; Ullian et al., 2001). Excitatory synaptogenesis in the mammalian CNS occurs primarily after birth. In rodents this synaptogenic period is during the second and third postnatal weeks. A host of neuronal cell-surface molecules and secreted signals contribute to synaptic organization and maturation (Kennedy and Ehlers, 2006; Ziv and Garner, 2004) but the cellular and molecular interactions that initiate this synaptogenic period are largely unknown. This period of synaptic development also closely correlates with the proliferation and differentiation of astrocytes (Ullian et al., 2004; Ullian et al., 2001). To determine the role of developing astrocytes in synapse development, Barres and colleagues used a purified retinal ganglion cell (RGC) culture system. When these retinal neurons were cultured in the complete absence of astrocytes (Meyer-Franke et al., 1995) they formed very few synapses and had low synaptic activity (Pfrieger and Barres, 1997). Conversely when the RGCs were cultured with astrocyte feeder layers or culture media that were conditioned by astrocytes, they had 3–7 fold higher number of synapses and over 10 fold more synaptic activity. These results showed that synaptogenesis is not only controlled by intrinsic mechanisms of neurons but are stimulated by astrocyte-secreted prosynaptogenic signals. Further investigation identified one of these signals as none other than thrombospondin-1 (Christopherson et al., 2005), the ECM molecule whose synthesis and secretion by astrocytes had been discovered nearly two decades earlier (Asch et al., 1986).

2. Thrombospondins promote excitatory synapse formation in the CNS

Using the purified RGC culture system described above, Christopherson and colleagues (2005) found that pure TSP-1 and TSP-2 mimicked the ability of astrocyte conditioned media (ACM) to increase the number of excitatory (glutamatergic) synapses formed by RGCs in culture. Furthermore, immunodepletion of TSP-2 from the ACM prevented astrocyte-induced synaptogenesis. These results showed that TSP-1 and TSP-2 are necessary and sufficient signals coming from astrocytes that stimulate excitatory synaptogenesis between RGCs. Electron microscopy (EM) was used to show that the TSP-1-induced synapses are ultrastructurally normal, indicating that TSP-1 is able to trigger the formation and proper alignment of pre- and postsynaptic specializations. However, whole-cell recordings revealed that though the synapses were presynaptically active with cycling synaptic vesicles, they were postsynaptically silent owing to a lack of functional 2-amino-3-

(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptors (Christopherson et al., 2005). These results hinted at the presence of additional signals in the ACM that can regulate different aspects of synaptogenesis (Ullian et al., 2004). Increased excitatory synaptogenesis in response to TSP-1 treatment was also seen in astrocyte-depleted hippocampal neuronal cultures (Hughes et al., 2010). However treatment of classical astrocyte-containing hippocampal cultures with TSP-1 did not lead to an increase in final synaptic density between hippocampal neurons, even though an increased rate of synaptogenesis during the early stages of synapse formation was observed (Xu et al., 2010). This difference is most likely due to the high levels of TSP-1 and TSP-2 that are secreted by astrocytes in the hippocampal culture (Xu et al., 2010), minimizing the impact of additional TSP-1 treatment. Secreted proteins from astrocytes were also shown to be involved in formation of inhibitory synapses that use gamma amino butyric acid (GABA) as the neurotransmitter (Elmariah et al., 2005a; 2005b), but TSP-1 was not crucial for this effect (Hughes et al., 2010).

Investigation of synaptic development in vivo in TSP-1 and TSP-2 double null mice confirmed the in vitro findings showing a role for TSPs in excitatory synaptogenesis, because TSP1/2-null mice had 30% fewer excitatory synapses in the cortex compared to their wild type littermates (Christopherson et al., 2005).

In agreement with a role in the initiation of excitatory synaptogenesis in vivo, the expression of TSP-1 and TSP-2 by astrocytes peaks at the start of the synaptogenic period in the mouse brain (postnatal days 5–10), but is decreased in the adult brain (Christopherson et al., 2005), and is restricted to areas of ongoing neurogenesis (Hoffman et al., 1994). TSP-4, which was first identified in *Xenopus* embryos (Lawler et al., 1993), is also expressed in endothelial cells and smooth muscle cells of the brain vasculature (Stenina et al., 2003), in mammalian astrocytes in the mature CNS (Cahoy et al., 2008), and has been associated with axonal outgrowth and adhesion of retinal ganglion cells (Dunkle et al., 2007). Additionally, it is known to be present at the neuromuscular junction (Arber and Caroni, 1995), suggesting a possible role for TSP-4 in synapse formation in the peripheral nervous system.

All 5 of the known TSP family members induce synaptogenesis in cultured RGCs (Eroglu et al., 2009), and their synaptogenic activity was mapped to the epidermal growth factor (EGF)-like repeats of the TSP molecule. These EGF-like domains were known to bind to the Von Willebrand Factor A (VWF-A)-like domain of certain integrins (Pluskota et al., 2005), so Eroglu and colleagues decided to probe other cell-surface proteins that contained the VWF-A domain to look for potential TSP binding partners. Their search led them to discover the novel interaction between TSPs (TSP-1, TSP-2 and TSP-4) and the voltage-gated calcium channel (VGCC) subunit, $\alpha 2\delta$ -1 (Eroglu et al., 2009). Excitatory neurons including RGCs express high levels of $\alpha 2\delta$ -1, which is localized to synapses. Overexpression of $\alpha 2\delta$ -1 both in vitro and in vivo led to increases in synaptogenesis, while $\alpha 2\delta$ -1 knockdown resulted in loss of TSP-1-induced synapse formation in vitro. Closer inspection of the TSP-1- $\alpha 2\delta$ -1 association revealed that the extracellular component of $\alpha 2\delta$ -1 was responsible for its synaptogenic effects, and that its synaptogenic properties did not involve global changes in calcium channel expression levels or function despite the known roles of $\alpha 2\delta$ -1 in calcium channel function and trafficking (Eroglu et al., 2009).

$\alpha 2\delta$ -1 is the high affinity receptor for two commonly prescribed anti-epileptic, anti-neuropathic pain medications, gabapentin (Neurontin™) and pregabalin (Lyrica™) (Gee et al., 1996). Gabapentin and pregabalin were initially designed as hydrophobic gamma amino butyric acid (GABA) analogs to be used as anti-convulsants that would easily cross the blood brain barrier. However, further studies have shown that even though they possess anti-convulsant properties, they do not bind to canonical GABA receptors or transporters. A

study using a knock-in mouse that expresses a mutant $\alpha 2\delta$ -1 which cannot bind gabapentin or pregabalin has shown that $\alpha 2\delta$ -1 is the in vivo target for these drugs, and that these drugs mediate their therapeutic action through binding to $\alpha 2\delta$ -1 (Field et al., 2006). The cellular mechanisms underlying the mode of action of these drugs remain a mystery, because these drugs do not significantly affect calcium channel kinetics or function. Eroglu and colleagues showed that gabapentin blocks TSP- $\alpha 2\delta$ -1 binding and astrocyte/TSP-induced synapse formation in vitro (Eroglu et al., 2009). The drug similarly blocked synapse formation in vivo. In this experiment, neonatal mice were injected with either gabapentin or saline for the first postnatal week, which coincides with the initiation of synapse formation and the peak of TSP1/2 expression in the brain. There was a significant decrease in the density of excitatory synapses in the cerebral cortex of the gabapentin-injected mice in comparison to saline-injected control mice. These results showing that gabapentin can block new synapse formation in vivo and in vitro indicated that gabapentin's ability to block TSP/ $\alpha 2\delta$ -1 interaction and TSP-induced synapse formation might underlie its therapeutic function.

2.1 Potential mechanisms of thrombospondin function at the synapse

It has been proposed that the binding between the EGF-like domains of TSP and the VWF-A-like domain of $\alpha 2\delta$ -1 may act as an "on switch" for a synaptogenic signaling pathway. This may be achieved by the activation of a "synaptogenic signaling complex", which includes $\alpha 2\delta$ -1, upon binding of TSP. This complex may then nucleate a synaptic adhesion by the recruitment of cell adhesion and scaffolding proteins to the potential synaptic sites (Bolton and Eroglu, 2009). The binding of TSP to $\alpha 2\delta$ -1 may trigger a structural rearrangement in the latter, as it is known that VWF-A domains can switch a protein's structure from an inactive to an active state upon ligand binding (Whittaker and Hynes, 2002). The activated TSP/ $\alpha 2\delta$ -1 complex can then induce an intracellular signaling cascade that ultimately leads to the formation of a synapse (Fig. 1). Because gabapentin can inhibit the TSP- $\alpha 2\delta$ -1 interaction and thus inhibit TSP-induced synapse formation, it is possible that this drug arrests $\alpha 2\delta$ -1 in the inactive conformation (Fig. 1), thereby preventing formation of the complex. The inhibitory neurotransmitter GABA was also shown to inhibit TSP-2-induced synapse formation at high concentrations, raising the possibilities that GABA is the physiological ligand for $\alpha 2\delta$ -1 and that inhibitory neurotransmission in the brain may control the extent of excitatory connections (Eroglu et al., 2009). The nature of TSP- $\alpha 2\delta$ -1 downstream signaling and the constituents of the proposed synaptogenic signaling machinery are currently unknown. TSP-1 was recently found to bind to neuroligins (Xu et al., 2010), a family of postsynaptic adhesion molecules that are essential for CNS synapse function (Craig and Kang, 2007), thus it is possible that TSPs, $\alpha 2\delta$ -1 and neuroligins are components of the proposed synaptogenic signaling complex.

TSPs are known to control the actin cytoskeleton. Rearrangement of the actin cytoskeleton plays a pivotal role in synapse formation and remodeling as it is particularly linked to morphogenesis of the postsynaptic specializations of excitatory connections, dendritic spines (Svitkina et al., 2010). Spine number and morphology are controlled by signaling pathways initiated by regulatory Rho family GTPases, which are able to "switch on" signal transduction pathways when in a GTP-bound (i.e. active) state. Once triggered by GTPase-activating proteins (GAPs) or guanine exchange factors (GEFs), active GTPases including Rac1 and RhoA go on to induce downstream effectors including various actin polymerizing/depolymerizing factors (Hall, 1998). This pathway ultimately triggers reorganization of the actin cytoskeleton in dendrites, contributing to the initial contact and stabilization of synapses (Tashiro and Yuste, 2004). Previous studies in fibroblasts and myoblasts have shown that TSP-1 induces the formation of F-actin microspikes containing the actin-bundling protein, fascin (Adams, 1995; Adams and Schwartz, 2000). TSP-1's ability to alter cytoskeletal organization is mediated in part by the intracellular protein muskellin (Adams et

al., 1998) which acts as a trafficking protein to help move cargo along the actin cytoskeleton (Heisler et al., 2011).

The TSP- α 28-1 interaction is crucial for TSP's synaptogenic function; however, other previously described TSP receptors and signaling pathways are also present in neural tissues (Table 1) (Venstrom and Reichardt, 1993) and they may have additional functions at the synapse. For example, TSP-1 stimulates focal adhesion disassembly via its N-terminus binding to calreticulin [CRT]/low density lipoprotein-receptor related protein-1 [LRP-1], leading to inactivation of the small GTPase RhoA (Murphy-Ullrich, 2001; Orr et al., 2004; Sweetwyne and Murphy-Ullrich, 2012). Since RhoA signaling has been linked to synaptic plasticity (Murakoshi et al., 2011), CRT/LRP-1 signaling may thus provide a potential avenue for TSP-1 to regulate synaptic function.

Also relevant to TSP-1's signaling capabilities are its synaptogenic EGF-like repeats, which were discovered to induce EGFR transactivation in epithelial cells (Liu et al., 2009). Specifically, activation of EGFR/ErbB2 by TSP-1 resulted in alterations in endothelial barrier function, with disruption of cell-cell junctions (Garg et al., 2011). EGFRs are also known to bind to and influence the actin cytoskeleton (Rijken et al., 1991) and play a role in neurite outgrowth (Goldshmit et al., 2004). Recent studies have also linked TSP-2 to the Notch signaling pathway, which is known to play a major role in development and homeostasis of multiple organ systems including the CNS (Meng et al., 2009; 2010).

The interactions between TSP and these various signaling molecules surely lie at the crux of CNS development and synaptogenesis. There is also a distinct possibility that these pathways are critical for another type of synaptic organization, which occurs following injury to the CNS.

3. Thrombospondin and the matricellular protein response to CNS injury

Reorganization of synaptic networks is a hallmark of many neurological disorders including but not limited to traumatic brain injury (TBI), stroke, epilepsy, and Alzheimer's disease (AD) (Cavazos and Cross, 2006; Hu et al., 2004; Perez-Cruz et al., 2011; Rossini and Dal Forno, 2004). For example in stroke, ischemic insult results in widespread dendritic beading and spine loss that is transient when residual blood flow is available, but this damage can become permanent when energy reserves are severely compromised (Risher et al., 2010). Loss of spines, which are normally present postsynaptically at the majority of synapses in the brain (Harris and Kater, 1994), may result in alteration of function (Zhang and Murphy, 2007) or even cortical remapping (Sigler et al., 2009). Promotion of new synaptogenesis is an attractive target for stroke therapy (Chen et al., 2002; Nudo, 2003), as restoration of synaptic circuits has been shown to improve functional recovery after stroke (Adkins et al., 2008; Jones et al., 2009).

3.1 The role of thrombospondin in recovery from stroke and other brain injuries

Considering their critical roles in developmental synaptogenesis, it is perhaps not surprising to find that many matricellular proteins have been shown to be upregulated after CNS injury. Increased TSP-1 originating from brain macrophages was detected after cortical lesioning, with a reported stimulatory effect on neurite outgrowth (Chamak et al., 1994). Spinal cord injury results in a dramatic increase in TSP-1 expression (Benton et al., 2008), while TSP-1 and TSP-2 expression levels are significantly increased following stroke (Lin et al., 2003; Zhou et al., 2010), where their upregulation is primarily driven by purinergic signaling in astrocytes (Tran and Neary, 2006). Liauw and colleagues (2008) further investigated the role of TSP in stroke recovery. Occlusion of the distal middle cerebral artery and common carotid artery confirmed an increase in TSP-1 and 2 mRNA and protein levels that

colocalized primarily to astrocytes. Focal ischemia in both wild type and TSP-1/2 double null (KO) mice revealed significant deficits in synaptic recovery and axonal sprouting in the TSP KO mice. These morphological deficits correlated to decreased functional recovery as assessed by the tongue protrusion test of motor function despite similar infarct size and blood vessel density between KO and wild type (Liauw et al., 2008), suggesting that TSP-1/2 deficiency did not significantly impact angiogenesis. Though these findings suggest that TSP is beneficial in terms of synapse recovery, it has been reported that inhibition of nitric oxide signaling by TSP-1 limits tissue reperfusion following stroke (Isenberg et al., 2009) and that blocking TSP-1 signaling may actually improve stroke outcome (Maxhimer et al., 2009). Further studies are needed to determine if the synaptogenic potential of TSP outweighs its negative regulatory effects in the ischemic brain.

TSP's ability to alter synaptic remodeling after cortical injury is similarly crucial for developmental plasticity. Eroglu and colleagues (2009) used a "barrel cortex plasticity" assay to investigate the role of TSP in injury-dependent plasticity in the developing CNS. Mouse whiskers project to the brain as a topographically ordered "somatotopic" map (Erzurumlu et al., 2006), which is organized into "barrels" in the primary somatosensory cortex. Lesion of the whiskers results in structural changes in the associated barrel and its neighbours, thereby providing a direct measure of injury-dependent cortical plasticity. The investigators administered gabapentin after whisker removal to preclude the effects of TSP-induced synaptogenesis. This resulted in an atypical barrel cortex plasticity following whisker lesioning compared to saline-injected controls (Eroglu et al., 2009). This result directly implicated TSP-1/2 to be involved in cortical synaptic remodeling and plasticity after peripheral injury. Aberrant cortical remodeling also occurred following whisker lesioning in TSP-1/2 KO mice, further confirming that TSP-induced synapse formation is required for proper barrel rewiring following injury.

The actions of TSP during cortical injury may also involve the regulation of matrix metalloproteinases (MMPs). As their name implies, MMPs are capable of cleaving components of the ECM, resulting in ECM remodeling and changes in cell behavior (Ethell and Ethell, 2007). In stroke, MMPs are responsible for breaking down the basal lamina of cerebral blood vessels, resulting in disruption of the blood brain barrier (BBB) and edema formation (Montaner et al., 2001). TSP-1 induces upregulation of MMP-9 in endothelial cells (Qian et al., 1997), and MMP-9 has in turn been shown to stimulate the release of vascular endothelial growth factor (VEGF) from the ECM, triggering the so-called "angiogenic switch" (Bergers et al., 2000). Alternatively, TSP-1 can inhibit this process via suppression of MMP-9 activation, resulting in decreased VEGF secretion which contributes to TSP's role as an inhibitor of angiogenesis (Rodriguez-Manzanique et al., 2001). TSP-1/2 regulate MMP-2 clearance via binding to LRP1 (Emonard et al., 2004; Yang et al., 2001), while TSP-1 also regulates the expression of tissue inhibitors of metalloproteinases (TIMPs), indicating that TSP determines the precise balance of MMPs and thus influences the organization of the ECM (John et al., 2009). TSP-2 has a similar association with MMP-2, granting the TSP family tight control over the expression of these versatile proteases which can remodel the ECM according to biological needs (Agah et al., 2002; Krady et al., 2008). In the heart, MMPs mediated by TSP are crucial for myocardial remodeling after injury (Schellings et al., 2009). In the brain, MMPs have been implicated in the establishment of synaptic networks (Ethell and Ethell, 2007), as deficits in MMP-9 manifest in impairments in long-term potentiation (LTP) and behavior (Nagy et al., 2006). Thus, the timing of MMP expression regulated by TSP is critical to different biological processes, as increased induction of MMPs may be important for development while a decrease in MMP expression may represent a mechanism for TSP to reduce brain injury.

TSP may also be involved in the inflammatory response to CNS injury. Inflammation is a hallmark of nearly all neurological disorders, highlighted by the induction of microglia, monocytes, cytokines and other mediators of cell injury (Wee Yong, 2010). In the immune system, TSP-1 modulates the adhesion and aggregation of monocytes/macrophages via interactions with CD36 and CD47 (Yamauchi et al., 2002b). Accordingly, decreased inflammation and increased white matter sparing following spinal cord injury were seen when the TSP-1 receptor CD47 was knocked out, though curiously this protection did not extend to TSP-1 knockout mice (Myers et al., 2011). In addition, TSP-1 enhances the release of the inflammatory cytokines IL-6 and IL-10 via CD36 (Yamauchi et al., 2002a). There is growing evidence for cytokines playing key roles in synaptic plasticity and neurogenesis (Bilbo and Schwarz, 2009; McAfoose and Baune, 2009), such as IL-6's importance to the modulation of LTP, a form of synaptic plasticity associated with learning and memory (Balschun et al., 2004). However, TSP-1 can also suppress IL-10 release by interacting with latent transforming growth factor beta-1 (TGF- β 1) (Yamauchi et al., 2002a). Indeed, TSP-1 is known to activate latent TGF- β 1 (Schultz-Cherry et al., 1994; Schultz-Cherry and Murphy-Ullrich, 1993). Given the upregulation of TGF- β 1 after cortical injury (Makwana et al., 2007), it is a strong possibility that the interaction between TSP and TGF- β 1 may play important roles in the injured brain. Moreover, TGF- β 1 is also linked to multiple aspects of synapse formation and plasticity particularly in the peripheral nervous system (PNS) (Banyai et al., 2010; Feng and Ko, 2008; Packard et al., 2003). TGF- β 1 released by Schwann cells (the major glial cell type in the PNS) promotes synapse formation at the neuromuscular junction (Feng and Ko, 2008) potentially through binding to the synaptogenic proteoglycan agrin.

3.2 Altered thrombospondin expression in neurological disease

Abnormalities in the expression of TSP and other matricellular proteins may underlie cortical dysfunction in neurological disorders that are characterized by aberrant synaptic networks. For example, altered spine morphology and reduced synaptic density in Down's syndrome (DS), the most common genetic form of mental retardation, have recently been linked to decreased TSP expression in DS astrocytes (Garcia et al., 2010).

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in which massive synapse loss occurs in the early clinical stage (Masliah et al., 1991; Sze et al., 1997). This synaptic loss shows high correlation with the cognitive decline presented by AD patients (Terry et al., 1991). TSP-1 expression is markedly decreased in vulnerable neuronal populations in the brains of AD patients (Buee et al., 1992), making any synaptogenically-driven repair mechanism unlikely to succeed. Synaptic disorganization is also a hallmark of epilepsy, in which aberrant synaptogenesis following seizures may actually contribute to hyperexcitability and thus worsen epileptic discharges (Cavazos and Cross, 2006). Seizure activity induces widespread alterations in the localization and synthesis of ECM molecules in a so-called 'matrix response' driven primarily by astrocytes (Hoffman et al., 1998). The finding that the anti-convulsant drug gabapentin inhibits TSP-induced synaptogenesis (Eroglu et al., 2009) may have direct implications for the drug's mechanism of action, suggesting that the prevention of aberrant synaptic remodeling can be therapeutic. Future research will be necessary to determine if alterations in the expression of TSPs and their known binding partners may be beneficial in the treatment of brain injury and neurological disease.

4. Conclusions

TSPs are a family of matricellular proteins with functions in numerous organ systems (Murphy-Ullrich and Iozzo, 2012). Recent findings demonstrated a critical role for TSPs 1, 2 and 4 in synaptogenesis. Expressed primarily by astrocytes in the CNS, TSPs 1 and 2

promote CNS synaptogenesis during development and participate in synaptic repair after brain injury. Perhaps owing to these synaptogenic properties, elevated TSP expression in specific brain regions of human versus nonhuman primates has been suggested to underlie the distinct cognitive abilities of humans (Caceres et al., 2007). The list of known binding partners and functions of TSP is long and rapidly growing. Better characterization of TSP and its downstream signaling pathways should facilitate our understanding of the complex process of synapse formation.

Abbreviations

ACM	astrocyte conditioned media
AD	Alzheimer's disease
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
CNS	central nervous system
CRT	calreticulin
DS	Down's syndrome
ECM	extracellular matrix
EGF	epidermal growth factor
EM	electron microscopy
GABA	gamma amino butyric acid
GEF	guanine exchange factor
GAP	GTPase-activating protein
KO	knockout
LRP-1	low density lipoprotein-receptor related protein-1
LTP	long-term potentiation
MMP	matrix metalloproteinase
NMDA	<i>N</i> -Methyl-D-aspartate
P38MAPK	p38 mitogen-activated protein kinase
PNS	Peripheral Nervous System
RGC	retinal ganglion cell
TBI	traumatic brain injury
TGF-β1	transforming growth factor beta-1
TIMP	tissue inhibitor of metalloproteinase
TSP	thrombospondin
VEGF	vascular endothelial growth factor
VGCC	voltage-gated calcium channel
VWF-A	Von Willebrand Factor A

References

- Ables JL, Breunig JJ, Eisch AJ, Rakic P. Not(ch) just development: Notch signalling in the adult brain. *Nat. Rev. Neurosci.* 2011; 12:269–283. [PubMed: 21505516]

- Abumrad NA, Ajmal M, Pothakos K, Robinson JK. CD36 expression and brain function: does CD36 deficiency impact learning ability? *Prostaglandins Other Lipid Mediat.* 2005; 77:77–83. [PubMed: 16099393]
- Adams JC. Formation of stable microspikes containing actin and the 55 kDa actin bundling protein, fascin, is a consequence of cell adhesion to thrombospondin-1: implications for the anti-adhesive activities of thrombospondin-1. *J. Cell Sci.* 1995; 108(Pt 5):1977–1990. [PubMed: 7657718]
- Adams JC. Thrombospondins: multifunctional regulators of cell interactions. *Annu. Rev. Cell Dev. Biol.* 2001; 17:25–51. [PubMed: 11687483]
- Adams JC, Schwartz MA. Stimulation of fascin spikes by thrombospondin-1 is mediated by the GTPases Rac and Cdc42. *J. Cell. Biol.* 2000; 150:807–822. [PubMed: 10953005]
- Adams JC, Seed B, Lawler J. Musklin, a novel intracellular mediator of cell adhesive and cytoskeletal responses to thrombospondin-1. *EMBO J.* 1998; 17:4964–4974. [PubMed: 9724633]
- Adkins DL, Hsu JE, Jones TA. Motor cortical stimulation promotes synaptic plasticity and behavioral improvements following sensorimotor cortex lesions. *Exp. Neurol.* 2008; 212:14–28. [PubMed: 18448100]
- Agah A, Kyriakides TR, Lawler J, Bornstein P. The lack of thrombospondin-1 (TSP1) dictates the course of wound healing in double-TSP1/TSP2-null mice. *Am. J. Pathol.* 2002; 161:831–839. [PubMed: 12213711]
- Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 1999; 22:208–215. [PubMed: 10322493]
- Arber S, Caroni P. Thrombospondin-4, an extracellular matrix protein expressed in the developing and adult nervous system promotes neurite outgrowth. *J. Cell. Biol.* 1995; 131:1083–1094. [PubMed: 7490284]
- Asch AS, Leung LL, Shapiro J, Nachman RL. Human brain glial cells synthesize thrombospondin. *Proc. Natl. Acad. Sci. U. S. A.* 1986; 83:2904–2908. [PubMed: 2939460]
- Asch AS, Silbiger S, Heimer E, Nachman RL. Thrombospondin sequence motif (CSVTCG) is responsible for CD36 binding. *Biochem. Biophys. Res. Commun.* 1992; 182:1208–1217. [PubMed: 1371676]
- Balschun D, Wetzel W, Del Rey A, Pitossi F, Schneider H, Zuschratter W, Besedovsky HO. Interleukin-6: a cytokine to forget. *FASEB J.* 2004; 18:1788–1790. [PubMed: 15345694]
- Banyai L, Sonderegger P, Patthy L. Agrin binds BMP2, BMP4 and TGFβ1. *PLoS One.* 2010; 5:e10758. [PubMed: 20505824]
- Bentley AA, Adams JC. The evolution of thrombospondins and their ligand-binding activities. *Mol. Biol. Evol.* 2010; 27:2187–2197. [PubMed: 20427418]
- Benton RL, Maddie MA, Worth CA, Mahoney ET, Hagg T, Whittemore SR. Transcriptomic screening of microvascular endothelial cells implicates novel molecular regulators of vascular dysfunction after spinal cord injury. *J. Cereb. Blood Flow Metab.* 2008; 28:1771–1785. [PubMed: 18612314]
- Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat. Cell Biol.* 2000; 2:737–744. [PubMed: 11025665]
- Beumer K, Matthies HJ, Bradshaw A, Broadie K. Integrins regulate DLG/FAS2 via a CaM kinase II-dependent pathway to mediate synapse elaboration and stabilization during postembryonic development. *Development.* 2002; 129:3381–3391. [PubMed: 12091308]
- Bilbo SD, Schwarz JM. Early-life programming of later-life brain and behavior: a critical role for the immune system. *Front Behav Neurosci.* 2009; 3:14. [PubMed: 19738918]
- Blake SM, Strasser V, Andrade N, Duit S, Hofbauer R, Schneider WJ, Nimpf J. Thrombospondin-1 binds to ApoER2 and VLDL receptor and functions in postnatal neuronal migration. *EMBO J.* 2008; 27:3069–3080. [PubMed: 18946489]
- Bolton MM, Eroglu C. Look who is weaving the neural web: glial control of synapse formation. *Curr. Opin. Neurobiol.* 2009; 19:491–497. [PubMed: 19879129]
- Bornstein P. Matricellular proteins: an overview. *Matrix Biol.* 2000; 19:555–556. [PubMed: 11102745]

- Buee L, Hof PR, Roberts DD, Delacourte A, Morrison JH, Fillit HM. Immunohistochemical identification of thrombospondin in normal human brain and in Alzheimer's disease. *Am. J. Pathol.* 1992; 141:783–788. [PubMed: 1415477]
- Bushong EA, Martone ME, Ellisman MH. Examination of the relationship between astrocyte morphology and laminar boundaries in the molecular layer of adult dentate gyrus. *J. Comp. Neurol.* 2003; 462:241–251. [PubMed: 12794746]
- Bushong EA, Martone ME, Ellisman MH. Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *Int. J. Dev. Neurosci.* 2004; 22:73–86. [PubMed: 15036382]
- Caceres M, Suwyn C, Maddox M, Thomas JW, Preuss TM. Increased cortical expression of two synaptogenic thrombospondins in human brain evolution. *Cereb. Cortex.* 2007; 17:2312–2321. [PubMed: 17182969]
- Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ, Barres BA. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* 2008; 28:264–278. [PubMed: 18171944]
- Cavazos JE, Cross DJ. The role of synaptic reorganization in mesial temporal lobe epilepsy. *Epilepsy Behav.* 2006; 8:483–493. [PubMed: 16500154]
- Chamak B, Morandi V, Mallat M. Brain macrophages stimulate neurite growth and regeneration by secreting thrombospondin. *J. Neurosci. Res.* 1994; 38:221–233. [PubMed: 8078107]
- Chen R, Cohen LG, Hallett M. Nervous system reorganization following injury. *Neuroscience.* 2002; 111:761–773. [PubMed: 12031403]
- Christopherson KS, Ullian EM, Stokes CC, Mullen CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell.* 2005; 120:421–433. [PubMed: 15707899]
- Cole GJ, Loewy A, Glaser L. Neuronal cell-cell adhesion depends on interactions of N-CAM with heparin-like molecules. *Nature.* 1986; 320:445–447. [PubMed: 3083264]
- Craig AM, Kang Y. Neurexin-neuroligin signaling in synapse development. *Curr. Opin. Neurobiol.* 2007; 17:43–52. [PubMed: 17275284]
- Dunkle ET, Zaucke F, Clegg DO. Thrombospondin-4 and matrix three-dimensionality in axon outgrowth and adhesion in the developing retina. *Exp. Eye Res.* 2007; 84:707–717. [PubMed: 17320079]
- Elmariash SB, Hughes EG, Oh EJ, Balice-Gordon RJ. Neurotrophin signaling among neurons and glia during formation of tripartite synapses. *Neuron Glia Biol.* 2005a; 1:1–11. [PubMed: 16528406]
- Elmariash SB, Oh EJ, Hughes EG, Balice-Gordon RJ. Astrocytes regulate inhibitory synapse formation via Trk-mediated modulation of postsynaptic GABAA receptors. *J. Neurosci.* 2005b; 25:3638–3650. [PubMed: 15814795]
- Emonard H, Bellon G, Troeberg L, Berton A, Robinet A, Henriot P, Marbaix E, Kirkegaard K, Patthy L, Eeckhout Y, Nagase H, Hornebeck W, Courttoy PJ. Low density lipoprotein receptor-related protein mediates endocytic clearance of pro-MMP-2.TIMP-2 complex through a thrombospondin-independent mechanism. *J. Biol. Chem.* 2004; 279:54944–54951. [PubMed: 15489233]
- Eroglu C. The role of astrocyte-secreted extracellular proteins in central nervous system development and function. *J. Cell Commun. Signal.* 2009; 3:167–176. [PubMed: 19904629]
- Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell.* 2009; 139:380–392. [PubMed: 19818485]
- Eroglu, C.; Barres, BA.; Stevens, B. Glia as active participants in the development and function of synapses. In: Hell, JW.; Ehlers, MD., editors. *Structural and functional organization of the synapse*. 1st ed.. New York: Springer; 2008. p. 683–714.
- Erzurumlu RS, Chen ZF, Jacquin MF. Molecular determinants of the face map development in the trigeminal brainstem. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 2006; 288:121–134. [PubMed: 16432893]

- Ethell IM, Ethell DW. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. *J. Neurosci. Res.* 2007; 85:2813–2823. [PubMed: 17387691]
- Faber-Elman A, Lavie V, Schwartz I, Shaltiel S, Schwartz M. Vitronectin overrides a negative effect of TNF-alpha on astrocyte migration. *FASEB J.* 1995; 9:1605–1613. [PubMed: 8529840]
- Feng Z, Ko CP. Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-beta1. *J. Neurosci.* 2008; 28:9599–9609. [PubMed: 18815246]
- Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, Bramwell S, Corradini L, England S, Winks J, Kinloch RA, Hendrich J, Dolphin AC, Webb T, Williams D. Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:17537–17542. [PubMed: 17088553]
- Gao AG, Lindberg FP, Finn MB, Blystone SD, Brown EJ, Frazier WA. Integrin-associated protein is a receptor for the C-terminal domain of thrombospondin. *J. Biol. Chem.* 1996; 271:21–24. [PubMed: 8550562]
- Garcia O, Torres M, Helguera P, Coskun P, Busciglio J. A role for thrombospondin-1 deficits in astrocyte-mediated spine and synaptic pathology in Down's syndrome. *PLoS One.* 2010; 5:e14200. [PubMed: 21152035]
- Garg P, Yang S, Liu A, Pallero MA, Buchsbaum DJ, Mosher DF, Murphy-Ullrich JE, Goldblum SE. Thrombospondin-1 opens the paracellular pathway in pulmonary microvascular endothelia through EGFR/ErbB2 activation. *American journal of physiology. Lung cellular and molecular physiology.* 2011; 301:L79–L90. [PubMed: 21531776]
- Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J. Biol. Chem.* 1996; 271:5768–5776. [PubMed: 8621444]
- Goicoechea S, Orr AW, Pallero MA, Eggleton P, Murphy-Ullrich JE. Thrombospondin mediates focal adhesion disassembly through interactions with cell surface calreticulin. *J. Biol. Chem.* 2000; 275:36358–36368. [PubMed: 10964924]
- Goldshmit Y, Walters CE, Scott HJ, Greenhalgh CJ, Turnley AM. SOCS2 induces neurite outgrowth by regulation of epidermal growth factor receptor activation. *J. Biol. Chem.* 2004; 279:16349–16355. [PubMed: 14764607]
- Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell.* 2004; 119:1013–1026. [PubMed: 15620359]
- Guo N, Krutzsch HC, Inman JK, Roberts DD. Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. *Cancer Res.* 1997; 57:1735–1742. [PubMed: 9135017]
- Hall A. Rho GTPases and the actin cytoskeleton. *Science.* 1998; 279:509–514. [PubMed: 9438836]
- Harris KM, Kater SB. Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annu. Rev. Neurosci.* 1994; 17:341–371. [PubMed: 8210179]
- Heisler FF, Loebrich S, Pechmann Y, Maier N, Zivkovic AR, Tokito M, Hausrat TJ, Schweizer M, Bähring R, Holzbaur EL, Schmitz D, Kneussel M. Muskelein Regulates Actin Filament- and Microtubule-Based GABA(A) Receptor Transport in Neurons. *Neuron.* 2011; 70:66–81. [PubMed: 21482357]
- Herz J, Chen Y. Reelin, lipoprotein receptors and synaptic plasticity. *Nat. Rev. Neurosci.* 2006; 7:850–859. [PubMed: 17053810]
- Hoffman JR, Dixit VM, O'Shea KS. Expression of thrombospondin in the adult nervous system. *J. Comp. Neurol.* 1994; 340:126–139. [PubMed: 8176001]
- Hoffman KB, Pinkstaff JK, Gall CM, Lynch G. Seizure induced synthesis of fibronectin is rapid and age dependent: implications for long-term potentiation and sprouting. *Brain Res.* 1998; 812:209–215. [PubMed: 9813331]
- Hu B, Liu C, Bramlett H, Sick TJ, Alonso OF, Chen S, Dietrich WD. Changes in trkB-ERK1/2-CREB/Elk-1 pathways in hippocampal mossy fiber organization after traumatic brain injury. *J. Cereb. Blood Flow Metab.* 2004; 24:934–943. [PubMed: 15362724]

- Hughes EG, Elmariah SB, Balice-Gordon RJ. Astrocyte secreted proteins selectively increase hippocampal GABAergic axon length, branching, and synaptogenesis. *Mol. Cell. Neurosci.* 2010; 43:136–145. [PubMed: 19850128]
- Iruela-Arispe ML, Liska DJ, Sage EH, Bornstein P. Differential expression of thrombospondin 1, 2, and 3 during murine development. *Dev. Dyn.* 1993; 197:40–56. [PubMed: 8400410]
- Iruela-Arispe ML, Lombardo M, Kruttsch HC, Lawler J, Roberts DD. Inhibition of angiogenesis by thrombospondin-1 is mediated by 2 independent regions within the type 1 repeats. *Circulation.* 1999; 100:1423–1431. [PubMed: 10500044]
- Isenberg JS, Annis DS, Pendrak ML, Ptaszynska M, Frazier WA, Mosher DF, Roberts DD. Differential interactions of thrombospondin-1, -2, and -4 with CD47 and effects on cGMP signaling and ischemic injury responses. *J. Biol. Chem.* 2009; 284:1116–1125. [PubMed: 19004835]
- John AS, Hu X, Rothman VL, Tuszyński GP. Thrombospondin-1 (TSP-1) up-regulates tissue inhibitor of metalloproteinase-1 (TIMP-1) production in human tumor cells: exploring the functional significance in tumor cell invasion. *Exp. Mol. Pathol.* 2009; 87:184–188. [PubMed: 19747478]
- Jones TA, Allred RP, Adkins DL, Hsu JE, O'Bryant A, Maldonado MA. Remodeling the brain with behavioral experience after stroke. *Stroke.* 2009; 40:S136–S138. [PubMed: 19064784]
- Kanda S, Shono T, Tomasini-Johansson B, Klint P, Saito Y. Role of thrombospondin-1-derived peptide, 4N1K, in FGF-2-induced angiogenesis. *Exp. Cell Res.* 1999; 252:262–272. [PubMed: 10527617]
- Kennedy MJ, Ehlers MD. Organelles and trafficking machinery for postsynaptic plasticity. *Annu. Rev. Neurosci.* 2006; 29:325–362. [PubMed: 16776589]
- Kinoshita A, Shah T, Tangredi MM, Strickland DK, Hyman BT. The intracellular domain of the low density lipoprotein receptor-related protein modulates transactivation mediated by amyloid precursor protein and Fe65. *J. Biol. Chem.* 2003; 278:41182–41188. [PubMed: 12888553]
- Krady MM, Zeng J, Yu J, MacLauchlan S, Skokos EA, Tian W, Bornstein P, Sessa WC, Kyriakides TR. Thrombospondin-2 modulates extracellular matrix remodeling during physiological angiogenesis. *Am. J. Pathol.* 2008; 173:879–891. [PubMed: 18688033]
- Lawler J. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. *J. Cell. Mol. Med.* 2002; 6:1–12. [PubMed: 12003665]
- Lawler J, Duquette M, Whittaker CA, Adams JC, McHenry K, DeSimone DW. Identification and characterization of thrombospondin-4, a new member of the thrombospondin gene family. *J. Cell. Biol.* 1993; 120:1059–1067. [PubMed: 8432726]
- Lawler J, Hynes RO. An integrin receptor on normal and thrombasthenic platelets that binds thrombospondin. *Blood.* 1989; 74:2022–2027. [PubMed: 2478219]
- Lawler J, McHenry K, Duquette M, Derick L. Characterization of human thrombospondin-4. *J. Biol. Chem.* 1995; 270:2809–2814. [PubMed: 7852353]
- Liauw J, Hoang S, Choi M, Eroglu C, Sun GH, Percy M, Wildman-Tobriner B, Bliss T, Guzman RG, Barres BA, Steinberg GK. Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. *J. Cereb. Blood Flow Metab.* 2008; 28:1722–1732. [PubMed: 18594557]
- Lin TN, Kim GM, Chen JJ, Cheung WM, He YY, Hsu CY. Differential regulation of thrombospondin-1 and thrombospondin-2 after focal cerebral ischemia/reperfusion. *Stroke.* 2003; 34:177–186. [PubMed: 12511771]
- Liu A, Garg P, Yang S, Gong P, Pallero MA, Annis DS, Liu Y, Passaniti A, Mann D, Mosher DF, Murphy-Ullrich JE, Goldblum SE. Epidermal growth factor-like repeats of thrombospondins activate phospholipase Cgamma and increase epithelial cell migration through indirect epidermal growth factor receptor activation. *J. Biol. Chem.* 2009; 284:6389–6402. [PubMed: 19129184]
- Makwana M, Jones LL, Cuthill D, Heuer H, Bohatschek M, Hristova M, Friedrichsen S, Ormsby I, Bueringer D, Koppius A, Bauer K, Doetschman T, Raivich G. Endogenous transforming growth factor beta 1 suppresses inflammation and promotes survival in adult CNS. *J. Neurosci.* 2007; 27:11201–11213. [PubMed: 17942715]

- Maslah E, Terry RD, Alford M, DeTeresa R, Hansen LA. Cortical and subcortical patterns of synaptophysinlike immunoreactivity in Alzheimer's disease. *Am. J. Pathol.* 1991; 138:235–246. [PubMed: 1899001]
- Maxhimer JB, Shih HB, Isenberg JS, Miller TW, Roberts DD. Thrombospondin-1/CD47 blockade following ischemia-reperfusion injury is tissue protective. *Plast. Reconstr. Surg.* 2009; 124:1880–1889. [PubMed: 19952644]
- McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. *Neuroscience and biobehavioral reviews.* 2009; 33:355–366. [PubMed: 18996146]
- Meng H, Zhang X, Hankenson KD, Wang MM. Thrombospondin 2 potentiates notch3/jagged1 signaling. *J. Biol. Chem.* 2009; 284:7866–7874. [PubMed: 19147503]
- Meng H, Zhang X, Lee SJ, Strickland DK, Lawrence DA, Wang MM. Low density lipoprotein receptor-related protein-1 (LRP1) regulates thrombospondin-2 (TSP2) enhancement of Notch3 signaling. *J. Biol. Chem.* 2010; 285:23047–23055. [PubMed: 20472562]
- Meyer-Franke A, Kaplan MR, Pfrieger FW, Barres BA. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron.* 1995; 15:805–819. [PubMed: 7576630]
- Mikhailenko I, Kounnas MZ, Strickland DK. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor mediates the cellular internalization and degradation of thrombospondin. A process facilitated by cell-surface proteoglycans. *J. Biol. Chem.* 1995; 270:9543–9549. [PubMed: 7721883]
- Montaner J, Alvarez-Sabin J, Molina CA, Angles A, Abilleira S, Arenillas J, Monasterio J. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. *Stroke.* 2001; 32:2762–2767. [PubMed: 11739970]
- Mosher DF, Adams JC. Adhesion-modulating/matricellular ECM protein families: a structural, functional and evolutionary appraisal. *Matrix Biol.* 2012 In press, This Issue.
- Murakoshi H, Wang H, Yasuda R. Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature.* 2011; 472:100–104. [PubMed: 21423166]
- Murphy-Ullrich JE. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? *J. Clin. Invest.* 2001; 107:785–790. [PubMed: 11285293]
- Murphy-Ullrich JE, Iozzo RV. Thematic Mini-Review Series: Thrombospondins in physiology and disease, new tricks for an old dog. *Matrix Biol.* 2012 In press, This issue.
- Murphy-Ullrich JE, Schultz-Cherry S, Hook M. Transforming growth factor-beta complexes with thrombospondin. *Mol. Biol. Cell.* 1992; 3:181–188. [PubMed: 1550960]
- Myers SA, DeVries WH, Andres KR, Gruenthal MJ, Benton RL, Hoying JB, Hagg T, Whittemore SR. CD47 knockout mice exhibit improved recovery from spinal cord injury. *Neurobiol. Dis.* 2011; 42:21–34. [PubMed: 21168495]
- Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. *J. Neurosci.* 2006; 26:1923–1934. [PubMed: 16481424]
- Nudo RJ. Functional and structural plasticity in motor cortex: implications for stroke recovery. *Phys. Med. Rehabil. Clin. N. Am.* 2003; 14:S57–S76. [PubMed: 12625638]
- O'Shea KS, Liu LH, Kinnunen LH, Dixit VM. Role of the extracellular matrix protein thrombospondin in the early development of the mouse embryo. *J. Cell. Biol.* 1990a; 111:2713–2723. [PubMed: 2277082]
- O'Shea KS, Rheinheimer JS, Dixit VM. Deposition and role of thrombospondin in the histogenesis of the cerebellar cortex. *J. Cell. Biol.* 1990b; 110:1275–1283. [PubMed: 2182649]
- Oberheim NA, Wang X, Goldman S, Nedergaard M. Astrocytic complexity distinguishes the human brain. *Trends Neurosci.* 2006; 29:547–553. [PubMed: 16938356]
- Ohnishi H, Kaneko Y, Okazawa H, Miyashita M, Sato R, Hayashi A, Tada K, Nagata S, Takahashi M, Matozaki T. Differential localization of Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 and CD47 and its molecular mechanisms in cultured hippocampal neurons. *J. Neurosci.* 2005; 25:2702–2711. [PubMed: 15758180]

- Orr AW, Pallero MA, Xiong WC, Murphy-Ullrich JE. Thrombospondin induces RhoA inactivation through FAK-dependent signaling to stimulate focal adhesion disassembly. *J. Biol. Chem.* 2004; 279:48983–48992. [PubMed: 15371459]
- Packard M, Mathew D, Budnik V. Wnts and TGF beta in synaptogenesis: old friends signalling at new places. *Nat. Rev. Neurosci.* 2003; 4:113–120. [PubMed: 12563282]
- Perez-Cruz C, Nolte MW, van Gaalen MM, Rustay NR, Termont A, Tanghe A, Kirchhoff F, Ebert U. Reduced Spine Density in Specific Regions of CA1 Pyramidal Neurons in Two Transgenic Mouse Models of Alzheimer's Disease. *J. Neurosci.* 2011; 31:3926–3934. [PubMed: 21389247]
- Pfriegeer FW, Barres BA. Synaptic efficacy enhanced by glial cells in vitro. *Science.* 1997; 277:1684–1687. [PubMed: 9287225]
- Pluskota E, Stenina OI, Krukovets I, Szpak D, Topol EJ, Plow EF. Mechanism and effect of thrombospondin-4 polymorphisms on neutrophil function. *Blood.* 2005; 106:3970–3978. [PubMed: 16099885]
- Qian X, Wang TN, Rothman VL, Nicosia RF, Tuszynski GP. Thrombospondin-1 modulates angiogenesis in vitro by up-regulation of matrix metalloproteinase-9 in endothelial cells. *Exp. Cell Res.* 1997; 235:403–412. [PubMed: 9299165]
- Rijken PJ, Hage WJ, van Bergen en Henegouwen PM, Verkleij AJ, Boonstra J. Epidermal growth factor induces rapid reorganization of the actin microfilament system in human A431 cells. *J. Cell Sci.* 1991; 100(Pt 3):491–499. [PubMed: 1808202]
- Risher WC, Ard D, Yuan J, Kirov SA. Recurrent spontaneous spreading depolarizations facilitate acute dendritic injury in the ischemic penumbra. *J. Neurosci.* 2010; 30:9859–9868. [PubMed: 20660268]
- Rodriguez-Manzanique JC, Lane TF, Ortega MA, Hynes RO, Lawler J, Iruela-Arispe ML. Thrombospondin-1 suppresses spontaneous tumor growth and inhibits activation of matrix metalloproteinase-9 and mobilization of vascular endothelial growth factor. *Proc. Natl. Acad. Sci. U. S. A.* 2001; 98:12485–12490. [PubMed: 11606713]
- Rossini PM, Dal Forno G. Neuronal post-stroke plasticity in the adult. *Restor. Neurol. Neurosci.* 2004; 22:193–206. [PubMed: 15502265]
- Schellings MW, van Almen GC, Sage EH, Heymans S. Thrombospondins in the heart: potential functions in cardiac remodeling. *J. Cell Commun. Signal.* 2009; 3:201–213. [PubMed: 19798592]
- Schultz-Cherry S, Lawler J, Murphy-Ullrich JE. The type 1 repeats of thrombospondin 1 activate latent transforming growth factor-beta. *J. Biol. Chem.* 1994; 269:26783–26788. [PubMed: 7929414]
- Schultz-Cherry S, Murphy-Ullrich JE. Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. *J. Cell. Biol.* 1993; 122:923–932. [PubMed: 8349738]
- Sigler A, Mohajerani MH, Murphy TH. Imaging rapid redistribution of sensory-evoked depolarization through existing cortical pathways after targeted stroke in mice. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:11759–11764. [PubMed: 19571005]
- Stenina OI, Desai SY, Krukovets I, Kight K, Janigro D, Topol EJ, Plow EF. Thrombospondin-4 and its variants: expression and differential effects on endothelial cells. *Circulation.* 2003; 108:1514–1519. [PubMed: 12952849]
- Sun X, Mosher DF, Rapraeger A. Heparan sulfate-mediated binding of epithelial cell surface proteoglycan to thrombospondin. *J. Biol. Chem.* 1989; 264:2885–2889. [PubMed: 2521631]
- Svitkina T, Lin WH, Webb DJ, Yasuda R, Wayman GA, Van Aelst L, Soderling SH. Regulation of the postsynaptic cytoskeleton: roles in development, plasticity, and disorders. *J. Neurosci.* 2010; 30:14937–14942. [PubMed: 21068295]
- Sweetwyne MT, Murphy-Ullrich JE. Thrombospondin1 in tissue repair and fibrosis: TGF- β -dependent and independent mechanisms. *Matrix Biol.* 2012 In press, This issue.
- Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 1997; 56:933–944. [PubMed: 9258263]

- Tashiro A, Yuste R. Regulation of dendritic spine motility and stability by Rac1 and Rho kinase: evidence for two forms of spine motility. *Mol. Cell. Neurosci.* 2004; 26:429–440. [PubMed: 15234347]
- Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* 1991; 30:572–580. [PubMed: 1789684]
- Tran MD, Neary JT. Purinergic signaling induces thrombospondin-1 expression in astrocytes. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:9321–9326. [PubMed: 16754856]
- Tucker RP, Adams JC, Lawler J. Thrombospondin-4 is expressed by early osteogenic tissues in the chick embryo. *Dev. Dyn.* 1995; 203:477–490. [PubMed: 7496039]
- Ullian EM, Christopherson KS, Barres BA. Role for glia in synaptogenesis. *Glia.* 2004; 47:209–216. [PubMed: 15252809]
- Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. Control of synapse number by glia. *Science.* 2001; 291:657–661. [PubMed: 11158678]
- Venstrom KA, Reichardt LF. Extracellular matrix. 2: Role of extracellular matrix molecules and their receptors in the nervous system. *FASEB J.* 1993; 7:996–1003. [PubMed: 8370483]
- Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 2005; 6:626–640. [PubMed: 16025096]
- Wee Yong V. Inflammation in neurological disorders: a help or a hindrance? *Neuroscientist.* 2010; 16:408–420. [PubMed: 20817918]
- Whittaker CA, Hynes RO. Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. *Mol. Biol. Cell.* 2002; 13:3369–3387. [PubMed: 12388743]
- Xu J, Xiao N, Xia J. Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1. *Nat. Neurosci.* 2010; 13:22–24. [PubMed: 19915562]
- Yamauchi Y, Kuroki M, Imakiire T, Abe H, Uchida H, Beppu R, Yamashita Y, Shirakusa T. Thrombospondin-1 differentially regulates release of IL-6 and IL-10 by human monocytic cell line U937. *Biochem. Biophys. Res. Commun.* 2002a; 290:1551–1557. [PubMed: 11820799]
- Yamauchi Y, Kuroki M, Imakiire T, Uno K, Abe H, Beppu R, Yamashita Y, Shirakusa T. Opposite effects of thrombospondin-1 via CD36 and CD47 on homotypic aggregation of monocytic cells. *Matrix Biol.* 2002b; 21:441–448. [PubMed: 12225809]
- Yang Z, Strickland DK, Bornstein P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. *J. Biol. Chem.* 2001; 276:8403–8408. [PubMed: 11113133]
- Zhang S, Murphy TH. Imaging the impact of cortical microcirculation on synaptic structure and sensory-evoked hemodynamic responses in vivo. *PLoS Biol.* 2007; 5:e119. [PubMed: 17456007]
- Zhou HJ, Zhang HN, Tang T, Zhong JH, Qi Y, Luo JK, Lin Y, Yang QD, Li XQ. Alteration of thrombospondin-1 and -2 in rat brains following experimental intracerebral hemorrhage. Laboratory investigation. *J. Neurosurg.* 2010; 113:820–825. [PubMed: 20136391]
- Ziv NE, Garner CC. Cellular and molecular mechanisms of presynaptic assembly. *Nat. Rev. Neurosci.* 2004; 5:385–399. [PubMed: 15100721]

Figure 1. The model for a "synaptogenic signaling complex" activated by TSP that potentially regulates prosynaptogenic-signaling pathways

(A) The $\alpha 2$ portion of the neuronal calcium channel subunit $\alpha 2\delta - 1$ (blue) is entirely extracellular while the $\delta - 1$ (purple) portion contains a transmembrane domain. Astrocyte-secreted TSP binds to the VWF-A domain of $\alpha 2\delta - 1$ via its EGF-like repeats (red) to cause a conformational change in the molecule from closed to open. The activated $\alpha 2\delta - 1$ then recruits an as yet unidentified signaling partner(s) (binding region unknown) to form a "synaptogenic signaling complex" which exerts multiple effects known to influence synaptogenesis through interactions with a variety of signaling molecules. TSP is involved in modulating the reorganization of the ECM by interacting with TGF- $\beta 1$ as well as regulating the balance between MMPs and TIMPs. TSP influences the actin cytoskeleton via EGFRs. The synaptogenic complex may also induce clustering of various neurotransmitter receptors and scaffolding proteins via neuroligins, promoting adhesion between a presynaptic axon and postsynaptic dendrite. (B) Binding of either gabapentin or GABA keeps $\alpha 2\delta - 1$ in its inactive closed state. TSP cannot bind to $\alpha 2\delta - 1$ in this conformation, thereby preventing the downstream signaling pathways that promote synaptogenesis.

Table 1

Binding partners of TSP and their known functions in the CNS.

Receptor	TSP Interaction Site	CNS Functions
$\alpha 2\delta$ -1 (Eroglu et al., 2009)	Type 2 EGF-like repeats (Eroglu et al., 2009)	Synapse formation (Eroglu et al., 2009)
ApoER2 (Blake et al., 2008)	unknown	Reelin signaling; neuronal migration (Herz and Chen, 2006)
CD36 (Asch et al., 1992)	Type 1 repeats (Guo et al., 1997)	Microglial activation; brain lipid metabolism (Abumrad et al., 2005)
CD47/IAP (Gao et al., 1996)	C-terminal domain (Kanda et al., 1999)	Neurite development (Ohnishi et al., 2005)
Heparin (Lawler et al., 1995)	N-terminal domain (Lawler et al., 1995)	Cell-cell recognition and adhesion (Cole et al., 1986)
HSPG (Sun et al., 1989)	Type 1 repeats (Iruela-Arispe et al., 1999)	Cell adhesion; astrocyte migration (Faber-Elman et al., 1995)
Integrin (Lawler and Hynes, 1989)	N-terminal domain; Type 3 repeats (Bentley and Adams, 2010)	Neuronal migration; synapse architecture and function (Beumer et al., 2002)
Latent TGF- β (Murphy-Ullrich et al., 1992)	Type 1 repeats (Schultz-Cherry et al., 1994)	Activation of TGF- β ; Cytoskeletal stability; mobilization of synaptic machinery (Packard et al., 2003)
LRP1/CRT (Mikhailenko et al., 1995)	N-terminal domain (Goicoechea et al., 2000)	Endocytosis of MMPs (Emonard et al., 2004); Notch signaling (Kinoshita et al., 2003)
Neuroigin (Xu et al., 2010)	unknown	Synapse formation (Graf et al., 2004)
Notch (Meng et al., 2009)	unknown	Neural progenitor cell proliferation and differentiation; neuronal morphology (Ables et al., 2011)
VLDLR (Blake et al., 2008)	unknown	Reelin signaling; neuronal migration (Herz and Chen, 2006)