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Thrombospondins and synaptogenesis★

Bin Wang, Weitao Guo, Yun Huang

Department of Orthopedics, Affiliated Hospital of Guangdong Medical College, Zhanjiang 524001, Guangdong Province, China

Abstract

Here, we review research on the mechanisms underlying the ability of thrombospondin to promote synaptogenesis and examine its role in central nervous system diseases and drug actions. Thrombospondin secreted by glial cells plays a critical role in synaptogenesis and maintains synapse stability. Thrombospondin regulates synaptogenesis through receptor α2δ-1 and neuroligin 1, and promotes the proliferation and differentiation of neural progenitor cells. It also participates in synaptic remodeling following injury and in the action of some nervous system drugs.

Key Words

thrombospondin; synapse; synaptogenesis; glial cell; α2δ-1; extracellular matrix; neural progenitor cells; Notch; opioid; regeneration; neural regeneration

Research Highlights

(1) This review summarizes the effects and underlying mechanisms of action of thrombospondin in synaptogenesis.
(2) Thrombospondin regulates synaptogenesis through receptor α2δ-1 and neuroligin 1, and promotes the proliferation and differentiation of neural progenitor cells.
(3) Thrombospondin also participates in synaptic remodeling following injury and in the action of some nervous system drugs.

Abbreviations

TSP, thrombospondin; NPCs, neural progenitor cells; CNS, central nervous system; RGCs, retinal ganglion cells; EGF, epidermal growth factor

Bin Wang★, Studying for master's degree, Attending physician, Department of Orthopedics, Affiliated Hospital of Guangdong Medical College, Zhanjiang 524001, Guangdong Province, China

Corresponding author: Weitao Guo, M.D., Chief physician, Department of Orthopedics, Affiliated Hospital of Guangdong Medical College, Zhanjiang 524001, Guangdong Province, China
guoweitao2000@sina.com

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INTRODUCTION

The occurrence and progression of nervous system injury and related diseases is a process involving changes in synapse number, structure and function. Therefore, the effective regulation of synapse formation and function is important for the treatment of nervous system diseases. Synaptic formation and remodeling involve multiple factors. Recent evidence indicates that astrocyte-secreted thrombospondin (TSP) can regulate synaptogenesis through receptor α2δ-1 and neuroligin 1, and promote the proliferation and differentiation of neural progenitor cells (NPCs). It also plays a role in synaptic remodeling post-injury, and modulates the action of some nervous system drugs[1-5]. Understanding the mechanisms of action of TSP on synaptogenesis should provide insight into the regulation of synaptic plasticity, and help in the development of treatments for nervous system disease and injury. However, it remains poorly understood how TSP modulates synaptogenesis. In the present study, we provide a review of the literature and summarize the effects and mechanisms of action of TSP on synaptogenesis. This should provide a reference for researchers examining the role of TSP in different types of synaptogenesis, including the downstream mechanisms that mediate these effects, as well as the *in vivo* regulation

and clinical application of this crucial protein.

ROLE OF TSP IN SYNAPTOGENESIS

The synapse is the key structure for signal communication between neurons. Large numbers of synapses relay electrical signals that mediate complex thoughts and actions, and synaptic morphological changes underlie learning and memory. Numerous ligands and cell surface molecules are involved in synapse formation and maturation[6]. However, the molecular mechanisms that regulate synaptogenesis remain poorly understood. Current studies have demonstrated that glial cells are critical for synapse formation and function[7-12]. Christopherson *et al* [13] reported that TSP secreted from astrocytes is a key regulator of synaptogenesis.

TSPs are large oligomeric extracellular matrix proteins. They bind a variety of membrane receptors, extracellular matrix components and cytokines to mediate interactions between cells and between cells and matrix[14-17]. TSPs play roles in cell attachment and migration, and can alter cell morphology, and inhibit angiopoiesis and tumor growth[18-20]. Astrocyte-secreted TSP distributes in synapses of the central nervous system (CNS) and the neuromuscular junction[21]. As extracellular matrix proteins, TSPs can induce cell adherence and alter signaling, thereby exhibiting the potential to induce synaptogenesis.

The TSP family consists of thrombospondins 1–5 (TSP3 and TSP5 are mainly expressed in bone and muscle, and have little association with synaptogenesis). TSP1 and TSP2, trimeric proteins, have similar structures and functional domains; TSP4, a pentameric protein, has a structure that differs from TSP1/TSP2 (Figure 1).

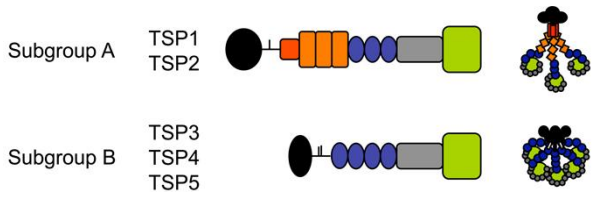

Figure 1 Domain structure of thrombospondins (TSPs)[1].

N-terminal domain (black), procollagen (red) and properdin-like repeats (orange), epidermal growth factor-like repeats (blue), calcium binding repeats (grey) and C-terminal L-lectin like globular domain (green).

These family members have different coding genes. Although TSPs are expressed in the brain, their effects on central nerve cells remain unknown[22]. Christopherson *et al* [13] found that astrocyte-secreted TSP1 and TSP2 can significantly increase the quantity of synapses in retinal ganglion cells (RGCs) cultured *in vitro* (Figure 2). In addition, the number of excitatory synapses is significantly reduced in TSP1/TSP2 knockout mice. Similar effects of TSP1 were found on hippocampal neurons cultured *in vitro*[3]. In addition, TSP1 can promote neuronal migration[23] and axonal overgrowth[24].

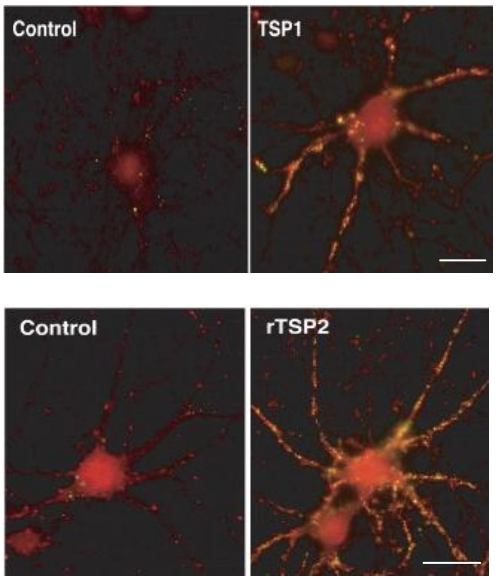

Figure 2 Thrombospondin (TSP) promotes synaptogenesis in retinal ganglial cells (RGCs; immunofluorescence, scale bar: 30 μm)[13].

Red: synaptotagmin; green: postsynaptic density protein 95.

The quantity of synapses generated in RGCs cultured in the presence of TSP1 (TSP1 group) was significantly greater than in RGCs cultured in the absence of astrocytes (control group).

The quantity of synapses generated in RGCs cultured in the presence of recombinant TSP2 (rTSP2 group) was significantly greater than in RGCs cultured in the absence of astrocytes (control group).

In contrast to other TSPs, TSP4 is only expressed by mature astrocytes (postnatal 17 days)[18]. TSP4 represents the mature TSP isoform in the CNS. It is essential for regulating synaptic formation and plasticity. Interestingly, TSP4 is also found in the neuromuscular junction, indicating a possible role in neuromuscular junction structure[22]. A comparison of TSP gene expression among primates showed that TSP2 and TSP4 levels are relatively higher in humans[25]. It is likely that high TSP expression may promote synaptogenesis in the human brain, resulting in higher cognition in humans.

CHARACTERISTICS OF TSP IN SYNAPTOGENESIS

TSP can promote presynaptic and postsynaptic remode-

ling, but TSP expression is significantly reduced in the CNS of adult rodents, and correspondingly, synapse formation and plasticity is significantly limited[26]. This indicates that there is a specific time window in which TSP promotes synaptogenesis in the CNS, *i.e*. TSP1 and TSP2 are highly expressed during development, but downregulated after maturation[13, 27-28]. This suggests that TSP1 and TSP2 regulate synaptogenesis in a narrow developmental window, and that they are not essential for maintaining synapses in the mature organism. However, high TSP expression is found in the mature newt, goldfish and other organisms with strong neural regeneration capacity[29].

While TSP1 and TSP2-induced synapses have normal ultrastructure and presynaptic membrane function, the postsynaptic membranes lack a-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid receptors, which are silenced[13, 27]. This silencing is common in early development of excitatory synapses in the CNS[30]. Christopherson *et al* [13] cultured RGCs with astrocytes or astrocyte conditioned medium and found a-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid receptor labeling in the postsynaptic membrane. The authors hypothesized that astrocytes produce a signaling molecule that can promote the formation of functional synapses.

MECHANISM OF TSP REGULATION OF SYNAPTOGENESIS

After removing astrocytes from the culture system, RGC synapse number was significantly reduced, possibly because TSPs not only promote synaptogenesis, but also maintain synaptic stability[13]. In addition, the presynaptic membrane protein synaptotagmin and the postsynaptic membrane protein postsynaptic density protein 95, as well as synapse-associated protein 102 and Homer protein, were not influenced by TSPs, indicating that TSPs do not regulate the synthesis or degradation of these proteins, but instead function by regulating their transport or cellular localization. Therefore, we assume that TSPs bind synaptic regions to induce synaptogenesis by affecting the localization of cell surface receptors. Many known TSP receptors aggregate at the synapse, including integrin-related protein CD47, various types of integrins and low-density lipoprotein receptor-related protein 1. TSP may activate downstream presynaptic and/or postsynaptic signaling cascades to induce synaptogenesis by binding one or several receptors.

Eroglu *et al* [1] reported that no known TSP receptor could induce synaptogenesis. They found that TSP binds von Willebrand factor repeat sequences, which are present in α2. Therefore, they assumed that α2δ may be the TSP receptor. α2δ-1 overexpression in nerve cells of transgenic mice increased the number of excitatory synapses, and blocking the receptor inhibited synaptic plasticity in the sensory cortex of neonatal mice in response to sensory nerve deprivation by whisker trimming[1]. In addition, gabapentin, an antagonist of α2δ-1, can greatly reduce synaptogenesis in the brain of wild-type mice[1]. All the above findings demonstrate the importance of the TSP-α2δ-1 interaction in synaptogenesis. Further study showed that epidermal growth factor (EGF)-like region of TSP can directly bind α2δ-1 to stimulate synaptogenesis[1], demonstrating that α2δ-1 is the neuronal TSP receptor. α2δ-1 knockout using siRNA showed that α2δ-1 is essential for TSP-induced synaptogenesis[1]. α2δ-4 gene disruption in mice results in the lack of ribbon synapses in photoreceptor cells[31]. α2δ-3 mutation produces a deficiency in synaptic transmission and presynaptic membrane structure in *Drosophila* neuromuscular junction[32-33]. These results indicate that α2δ family members are involved in synaptogenesis.

Most neuronal synapses have the α2δ TSP receptor, but this receptor is independent of the activity of voltage-gated calcium channels during synaptogenesis[1]. Then, how does α2δ participate in the regulation of synaptogenesis? Kurshan *et al* [33] proposed that α2δ may play a role through cytoskeletal rearrangements. However, the mechanism by which the α2δ ligand participates in synaptogenesis remains unclear. With a short intracellular region, δ is not essential for synaptogenesis, so there may be another transmembrane protein which interacts with α2δ and relays signals to cells. However, it is unknown if this proposed interaction mediates the effects observed in previous studies[1, 33] (Figure 3).

Xu *et al* [27] cultured hippocampal neurons, and found that TSP1 exhibited effects similar to neurexin in inducing synaptogenesis. Neurexin-induced synapses also lack the AMPA receptor, and the binding intensity with which TSP1 binds neuroligin1 is similar to that for TSP1 binding to the low density lipoprotein receptor[27]. This may imply that they share a common mechanism. In hippocampal neurons cultured with the neuroligin1 extracellular domain and in hippocampal neurons subjected to RNAi-mediated neuroligin1 knockout, the ability of TSP1 to promote synaptogenesis is inhibited[35-36], indicating that neuroligin1 mediates the effects of TSP1 on synaptogenesis. TSP is a polymeric protein; thus, its function in inducing synaptogenesis may involve neuroligin1 aggregation. However, further investigation is required to determine whether neuroligin1 also binds neurexins when binding TSP to induce presynaptic membrane differentiation.

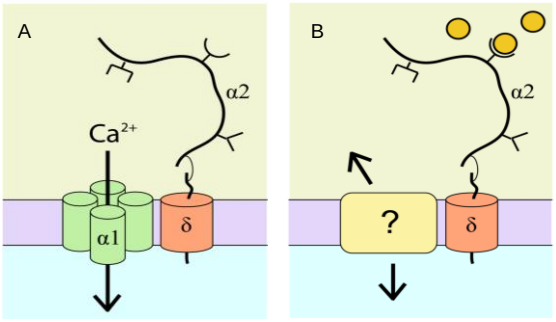

Figure 3 α2δ regulates synaptogenesis through a novel pathway[34].

(A) α2δ combines with α1 to regulate the properties of voltage-gated calcium channels. α2δ consists of two domains, the extracellular α2 domain and the transmembrane domain δ, which are linked by disulfide bonds.

(B) A possible model for α2δ in regulating synaptogenesis: thrombospondins (extracellular circles) bind to the von Willebrand factor interaction motif of α2 (open half circle), causing a conformational change in α2δ. "?" indicates unclear mechanism.

Astrocytes can induce NPCs to differentiate into neurons by secreting soluble molecules[37-38]. Lu *et al* [3] found that TSP1 deficiency greatly affects the self-renewal capacity of NPCs *in vitro* and *in vitro*. As a soluble factor secreted by astrocytes, TSP1 induces NPCs to differentiate into neurons and inhibits their differentiation into oligodendrocytes. TSP1 may influence cell fate through interactions with membrane proteins. Integrins can regulate NPC proliferation through Galectin-1/β1[39], while CD47 promotes nerve development through Cdc42 and Rac[40]. In addition, the Notch1 signaling pathway modulates the proliferation and differentiation of NPCs[41-42]. TSP1 binds Notch1 by complexing with Jagged1[43] and/or Contactin[44] to inhibit NPC differentiation into oligodendrocytes, thereby promoting their differentiation into nerve cells. TSP1 may directly bind EGF-like repeats and/or indirectly regulate nerve maturation through ApoER2 and the low density lipoprotein receptor. Collectively, these observations indicate that TSP1 promotes NPC proliferation and their differentiation into neurons, thereby increasing neuronal number, which benefits synaptogenesis. In addition, TSP can mediate de-adhesion between the cell and matrix in certain states[45], which may promote synaptogenesis. In summary, TSP promotes synaptogenesis by interacting with growth factors, extracellular matrix, adhesion molecules and receptors.

TSP IN CENTRAL NERVOUS SYSTEM DISEASES

Synaptogenesis defects can result in various nervous system diseases and motor dysfunction. Astrocyte-secreted TSPs are important for nerve cell development, migration and synaptogenesis *in vivo* and *in vitro*. Are TSPs also involved in the pathogenesis of epilepsy, stroke or trauma-induced neurological dysfunction? After nervous system injury, TSP expression increases in response to purine signals and mechanical irritation[46]. For example, TSP1 and TSP2 levels are elevated after stroke[47], and α2δ-1 and TSP4 gene expression is upregulated after spinal cord injury[48-49]. Furthermore, increased α2δ-1 expression enhances excitatory synaptic transmission and elevates the neuropathic pain threshold[50-51]. Reduced TSP1 levels are associated with neuropathological manifestations in Down's syndrome patients[52]. These findings demonstrate that TSP1 is associated with the occurrence and progression of nervous system diseases. Liauw *et al* [4] studied the role of TSP1/2 in synaptogenesis and synaptic functioning following stroke. They established a model of focal cerebral ischemia in 8 to 12-week-old wild-type and TSP1/2 knockout mice by unilateral occlusion of the middle cerebral and common carotid arteries. TSP1 and TSP2 mRNA and protein expression increased in wild-type mice, mainly in glial fibrillary acidic protein-positive astrocytes. In addition, a comparison of angiopoiesis, synaptic density, axon sprouting, infarction size and functional recovery showed that TSP1/2 knockout mice had impaired recovery compared with wild-type mice. Another study reported that TSP upregulation following stroke contributes to angiopoiesis after ischemia[47]. However, there was no difference in infarction size or blood vessel density between wild-type and TSP1/2-knockout mice, indicating that angiopoiesis is not influenced by TSPs, although synaptic density and axon sprouting are significantly reduced in TSP1/2-knockout mice[4]. In adult human brain, TSP1 and TSP2 levels are very low, but reactive astrocytes and microglia express these proteins[47, 53]. Scars mainly comprised glial cells form in lesions after brain tissue injury, increasing TSP1 and TSP2 levels, which may induce epilepsy.

TSP PARTICIPATES IN THE ACTION OF NERVOUS SYSTEM DRUGS

TSP is not only involved in the pathogenesis of nervous system diseases; it also holds promise for their treatment. Studies show that excitatory synapse formation underlies neuropathic pain and epileptic pathophysiology. Activated astrocytes play important roles in the treatment of epilepsy and spinal cord peripheral nerve injury- induced neuropathic pain[54]. Astrocyte-secreted TSPs may be involved in the repair of synapses after CNS injury.

The TSP receptor α2δ-1 is a high-affinity receptor for two anti-epileptic drugs, gabapentin and pregabalin[55]. α2δ-1

gene knockout mice cannot bind gabapentin or pregabalin, indicating the treatment effects of these drugs are mediated through α2δ-1[56]. Gabapentin and pregabalin do not influence single signal dynamics of calcium channels and exhibit only minimal effects on synaptic transmission[57]. Then, what are the mechanisms of action of these drugs? Eroglu *et al* [1] found that gabapentin binding to α2δ-1 restricts the conformation of the von willebrand factor-A domain in the receptor, thereby inhibiting the TSP-α2δ-1 interaction, and interfering with synaptogenesis. This may be the mechanism of action of gabapentin and pregabalin.

Opioids can modulate the functioning of spinal cord synapses and dendrites[58-59]. Ikeda *et al* [5] studied the influence of opioids on neurons and glial cells *in vivo* and *in vitro*, and found that morphine reduced TSP1 levels in astrocytes, and suppressed synaptogenesis and axonal growth. Extracellular signal-regulated kinase activation can increase TSP1 levels in astrocytes[5]. Therefore, morphine likely reduces TSP1 expression by inhibiting the phosphorylation and activation of extracellular signal-regulated kinase[60]. In addition, morphine may influence transforming growth factor levels *via* extracellular signal-regulated kinase, and transforming growth factor can induce TSP1 expression in various types of cells, including glial cells[61-62]. TSP1 has EGF-like repeats, which can directly or indirectly inhibit negative feedback of the EGF receptor through a matrix metalloproteinase-dependent mechanism[63], reducing TSP1 expression in astrocytes. The EGF receptor is an important receptor for morphine. Long-term morphine treatment can inhibit synaptogenesis and axonal overgrowth, indicating that opioids may interfere with the ability of TSP1 to promote synaptogenesis and stability.

TSPs are involved in the mechanism of action of nervous system drugs. This indicates that the mechanism of action of TSPs shares similarities with neuroregulatory mechanisms. They regulate the nervous system through a number of common pathways, possibly involving those affecting synaptic function. Studies of the underlying mechanisms may provide important insight into the functioning of neuroactive compounds.

DISCUSSION

Astrocyte-secreted TSPs can promote synaptogenesis *in vivo* and *in vitro*. An understanding of the underlying mechanisms may provide a basis for the modulation of synapse formation, structure and function, and advance the clinical treatment of nervous system diseases. There are some issues to be resolved. First, TSP1 and TSP2 levels change with time in the CNS; thus, it is critical to determine whether exogenous TSP1 or TSP2 can restore the synaptogenetic capacity of the mature nervous system or enhance synaptogenesis after nerve injury. In addition, methods for regulating TSP secretion by astrocytes are still lacking. Third, the signaling components downstream of TSPs, as well as related receptors and binding ligands, requires clarification. And it remains uncertain whether the effects of TSPs are only limited to glutamatergic synapses or can be extended to inhibitory GABAergic synapses. Moreover, further studies are needed to determine how different TSPs regulate synaptogenesis (the effects of TSP subtypes are different because of their mechanisms of action or distribution) in specific brain regions. The intracellular molecular signals and mechanisms by which TSP-α2δ-1 regulates CNS synaptogenesis require further investigation. α2δ-1 is highly expressed in skeletal muscle, myocardium and bone, in addition to the CNS[64], so it is also necessary to explore whether TSP-α2δ-1 participates in the functioning of those tissues. Finally, studies should focus on the effects of TSP deficiency and overexpression on nervous system function, as related to disease and treatment. Future studies should focus on the *in vivo* role of TSPs to confirm their role in synaptogenesis, complex behaviors and neural circuitry for the treatment of neurological disease and injury.

Astrocyte-secreted TSPs are key regulators of synaptogenesis, acting through α2δ-1 and neuroligin 1, and promote the proliferation and differentiation of neural progenitor cells. TSPs also participate in synaptic remodeling following injury and in the action of some nervous system drugs.

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Conflicts of interest: None declared.

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