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Mini review

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 α2δ-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.

Contents

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-this issue). 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 (O'Shea et al., 1990b; Iruela-Arispe et al., 1993; Lawler et al., 1993). During early postnatal development they are primarily expressed by astrocytes, the

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, *N*-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.

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Table 1
Binding partners of TSP and their known functions in the CNS.

Receptor	TSP interaction site	CNS functions
α2δ-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)
Neuroligin (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)

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 (Ullian et al., 2001; Christopherson et al., 2005; Hughes et al., 2010).

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 (Ullian et al., 2001; Christopherson et al., 2005). 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 (Ziv and Garner, 2004; Kennedy and Ehlers, 2006) 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., 2001; Ullian et al., 2004). 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 pro-synaptogenic 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 et al. (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