# Pathophysiological roles of thrombospondin-4 in disease development

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**Abstract**

Thrombospondin-4 (TSP-4) belongs to the extracellular matrix glycoprotein family of thrombospondins (TSPs). The multidomain, pentameric structure of TSP-4 allows its interactions with numerous extracellular matrix components, proteins and signaling molecules that enable its modulation to various physiological and pathological processes. Characterization of TSP-4 expression under development and pathogenesis of disorders has yielded important insights into mechanisms underlying the unique role of TSP-4 in mediating various processes including cell-cell, cell-extracellular matrix interactions, cell migration, proliferation, tissue remodeling, angiogenesis, and synaptogenesis. Maladaptation of these processes in response to pathological insults and stress can accelerate the development of disorders including skeletal dysplasia, osteoporosis, degenerative joint disease, cardiovascular diseases, tumor progression/metastasis and neurological disorders. Overall, the diverse functions of TSP-4 suggest that it may be a potential marker or therapeutic target for prognosis, diagnosis, and treatment of various pathological conditions upon further investigations. This review article highlights recent findings on the role of TSP-4 in both physiological and pathological conditions with a focus on what sets it apart from other TSPs.

Keywords: Thrombospondin-4, calcium-binding, extracellular matrix glycoprotein, angiogenesis, inflammation, synaptogenesis, cardiovascular diseases, cancer growth, metastasis, nociception.

## 1. Introduction

Thrombospondins (TSPs) are a group of multimeric, extracellular matrix (ECM) glycoproteins that play an important role in regulating cell-cell and cell-matrix interactions [1-4]. Vertebrate TSPs comprise an evolutionarily conserved family of multidomain calcium-binding proteins that consists of five members assembled either as trimers (subgroup A, including TSP-1 and TSP-2) or pentamers (subgroup B, including TSP-3, TSP-4, and TSP-5, which is also known as cartilage oligomeric matrix protein [COMP]). Each TSP contains amino- and carboxy-terminal domains flanking different structural modules (for an extensive review of the domain architectures and structure of TSPs, please see [5, 6]. Briefly, the amino-terminal halves of TSPs vary in domain composition. But the carboxy-terminal regions are highly conserved that comprise of three (TSP-1 and TSP-2) to four (TSP-3, TSP-4 and TSP-5/COMP) type 2 epidermal growth factor-like (EGF-like) domains, thirteen calcium-binding type 3 repeats, and a carboxy-terminal globular domain that is homologous structurally to the L-type lectin (also known as the L-type lectin-like domain). This structural arrangement of the carboxy-terminal regions is characteristic for TSPs, and therefore collectively referred to as the signature domain [7, 8]. Upon calcium binding to the signature domain, TSP proteins undergo a conformational change [9-11] that exposes binding sites for other proteins and ECM molecules, enabling TSPs to interact with a variety of cell surface receptors, ECM proteins, and other signaling molecules [6, 8, 12-14]. This complex domain organization allows TSPs to play important and distinct roles in mediating diverse biological and pathological processes [14-18]. However, specific TSP family members also have unique activities based on their specific spatiotemporal distribution that underlies their roles in various processes, including the induction of

cell spreading and organization of actin-based protrusions, promoting cell migration, disassembly of focal adhesions, and modulation of cell proliferation and apoptosis [1].

## 2. TSP-4

Discovery of the *thbs4* gene can be traced back to its identification in the genome of the African clawed frog (Xenopus laevis) by Lawler et al. [19]. In 1995, human TSP-4 was first characterized as a homopentameric protein composed of five 140 kDa polypeptides [10]. Subsequently, expression of TSP-4 has been identified in various tissues/organs, including blood vessel wall [20], retina [21, 22], skin [23], umbilical tissue [24], liver [25], myocardium [26-29], neuromuscular junction [22], tendon and muscle [22, 30, 31], bone [32], dorsal root ganglia [33] and neural tissues, including astrocytes, in a mature brain and spinal cord [22, 34-37]. It is well known that TSP-4 is a multifunctional protein that plays a crucial role in angiogenesis, wound healing, bone formation, cardiovascular function, immune system modulation, cancer progression, neuroplasticity, neuroinflammation and nociception.

Figure 1 illustrates a schematic model depicting the domain organization of TSP-4. Unlike the subgroup A members, TSP-4 does not possess the unique features of the N-terminal domain found in TSP-1 and TSP-2. The N-terminal domain of TSP-4, like other subgroup B members, is connected to a coiled-coil region that contains the oligomerization site, in which five subunits are disulfide-linked to form a pentamer. Four Type 2 EGF-like domains are downstream of the coiled-coil region followed by a sequence of Type 3 calcium-binding repeats referring as the "calcium-binding wire module" [5, 6]. While calcium is crucial in maintaining the tertiary

structures of all TSPs, including TSP-4 [38, 39], significantly different calcium binding activities to the signature domains of TSP-4 contribute to the unique fine structure and inter-modular interactions of TSP-4 that are different from other TSPs [38, 40]. This suggests that TSP-4, while sharing some similar functions with other TSPs, may possess unique functionalities in mediating pathophysiological processes. For example, it has been reported that a single nucleotide polymorphism (SNP) in human *thbs4* gene, where proline is found at position 387 instead of alanine in more predominant forms, is associated with increased risk of premature myocardial infarction in patients [41, 42] probably due to increased calcium binding affinity at the third EGF-like repeat [39]. In contrast, SNP in human *thbs1* gene, which decreases calcium binding affinity of TSP-1 [39], is not associated with altered risk of premature myocardium infraction [43], but SNP in human *thbs2* gene has been found to associate with reduced risk of premature myocardial infraction in patients [43]. In addition to differential calcium binding, some other unique functions of TSP-4 could be potentially explained by its differential spatiotemporal distribution, binding to specific receptors, activation of different intracellular signaling pathways, and distinct interactions with other ECM components [16, 32, 44-47]. For example, TSP-4 exhibits a proangiogenic property that fundamentally differs from the antiangiogenic properties of subgroup A TSPs. This proangiogenic effect has been shown to be associated with the activation of the transforming growth factor-beta1 (TGF-$\beta$1) pathway [48, 49]. In addition, even though both subgroup B TSP-4 and TSP-5 proteins are induced in knee articular cartilage with the progression of osteoarthritis in patients, TSP-4 induction is more dramatic, and distributed in different articular cartilage zones at different stages of disease progression compared with that of TSP-5 [44]. Considering the diverse role of TSP-4 and its

implications in angiogenesis, cardiovascular disease, inflammation, synaptogenesis and nociception, this review highlights recent discoveries about the role of TSP-4 in relevant physiological and pathological activities. Hopefully, these novel discoveries can lead to new research directions to further characterizing TSP-4 as a potential target for development of better therapeutical interventions for these disorders, as well as a biomarker for diagnosis, prognosis and drug efficacy predictions.

## 3. Pathophysiological roles of TSP-4

While TSP-4 is highly expressed in early development stages, the expression level of TSP-4 at adulthood is relatively low under physiological conditions [16, 35-37, 44]. However, its expression level is highly upregulated in response to pathological insults and stress that can contribute to repair and regeneration processes, but unfortunately can also lead to the development of various abnormal conditions, including cardiovascular and neurological disorders [16, 23, 26, 32, 33, 35-37, 44, 50, 51]. This suggests that expression of TSP-4 is plastic and plays an important role in repair, regeneration, adaptation and unfortunately maladaptation processes that may underlie the development of various disorders (Figure. 2). In this review, we have summarized recent discoveries about the contributing role and underlying mechanisms of TSP-4 plasticity in mediating pathological processes, as well as its involvement in the development of various disease states. Our focus has been on highlighting the distinct effects of TSP-4 that differentiate it from other TSP molecules.

### 3.1. Angiogenesis

Angiogenesis plays a crucial role in physiological process such as growth, development, wound healing, and the formation of granulation tissue by increasing the supply of oxygen-rich blood to tissues and organs. The balance between proangiogenic and antiangiogenic activities is crucial for maintaining proper vasculature and homeostasis. However, dysregulation of these processes can lead to pathological angiogenesis that provides nutrients and oxygen to promote abnormal conditions such as cardiac hypertrophy, tumor growth and metastasis. TSP-4 has been shown to promote angiogenesis in various tissues and organs, including the heart, skeletal muscle, tendon and eye [15, 16, 30, 52], mainly through stimulating the formation of new blood vessels and promoting the proangiogenic function of endothelial cells [16, 48]. In addition, TSP-4 upregulation has been reported in several types of cancers, including breast cancer [53, 54], prostate cancer [55], gastric cancer [56-58], hepatocellular carcinoma [59, 60] and bladder cancer [61] that can enhance angiogenesis and promote cell migration and invasion activities.

The mechanism underlying the effects of TSP-4 in angiogenesis differ from that of well-studied subgroup A TSPs. The antiangiogenic effects of TSP-1 and TSP-2 are mediated through interactions of their respective type 1 domains with transmembrane glycoproteins CD36 and CD47. For TSP-1, this interaction results in suppression of a nitric oxide/c-GMP-dependent signaling pathway [62-65] or inhibition of gelatinolytic activities and the vascular endothelial growth factor (VEGF) pathway [66]. On the other hand, TSP-2 inhibits gelatinolytic activities and modulates directly or indirectly the Notch signaling pathway [65, 67-70]. In contrast, TSP-4 does not harbor the type 1 domains like the group A TSPs [5], but instead induces angiogenesis through a TGF-β1-dependent pathway [20, 48, 49].

Findings from studies using thbs4-deficient mice have confirmed that TSP-4 plays a role in promoting angiogenesis and skin wound healing by enhancing endothelial cell adhesion, migration, and proliferation [48]. The contrasting functions of TSP-4 compared to that of other TSP family members in the vascular wall support that TSP-4 proteins play distinct roles in angiogenesis and vascular biology.

Data from mechanistic studies indicate that TSP-4 mediates TGF-β1 induced angiogenesis in an unique manner that differs from other TSP family members. Specifically, TGF-β1 only induces TSP-4, but not TSP-1 nor TSP-3, expression in microvascular endothelial cells. TGF-β1 induced TSP-4 expression is stimulus-specific, only after stimulation with TGF-β1, but not fibroblast growth factor (bFGF). In addition, TSP-4 induction by TGF-β1 stimulation is cell-type specific, only occurs in microvascular endothelial cells, but not in macrovascular arterial endothelial cells nor in vascular smooth muscle cells. Thus, activation of this specific pathway is pathological stage-dependent, only occurring at the stage when the inducer (TGF-β1 in this case) is available and angiogenesis is needed, such as tissue remolding or tumor growth and metastasis. Interestingly, activation of this pathway is not involved in proliferation of endothelial cells as expected previously, supporting that a divergent pathway different from the specific effect of TSP-4 on angiogenesis mediates the cell proliferation effects of TGF-β1 [49].

Activation of this pathway, specifically TGF-β1 induction of TSP-4, is mediated through intracellular signaling molecules SMAD3 and P38 mitogen-activated protein kinase. Importantly, increased TSP-4 in turn provides positive feedback to the activation of SMAD3 and P38 to sustain the microenvironment of pathological angiogenesis [49, 71]. Inhibiting SMAD3 activities with inhibitors or knocking down SMAD3 with specific shRNA or siRNA results in a reduced level of TSP-4 inducted by

TGF-β1 stimulation [49]. Interestingly, even though SMAD3 is a known transcription factor involved in angiogenesis [72], TGF-β1 induced TSP-4 upregulation in microvascular endothelial cells is post-transcriptional and independent of protein synthesis. Thus, the detailed mechanism of this pathway in angiogenesis remains elusive and warrants further investigation [49].

### 3.2. Cardiovascular disorders

The ECM is critically involved in the development and progression of vascular disorders, ranging from angiogenesis to atherosclerosis, a condition in which fibrofatty lesions form in the artery wall, leading to reduced blood flow. These pathological conditions can increase the risk of myocardial hypertrophy, infarctions and stroke, as well as disabling peripheral artery diseases [73, 74]. Increasing body of evidence points to a contributory role of the *thbs4* gene in ECM remodeling within the vascular wall that plays a significant role in the initiation and progress of chronic myocadiac disorders including cardiac hypertrophy and diabetic complications. Rysa et al. [27] have analyzed time-dependent global changes in gene expression with DNA microarrays in spontaneously hypertensive rats and revealed that *thbs4* expression is increased with other genes encoding ECM proteins almost exclusively at the ending stage of diastolic heart failure developing from hypertrophic hearts. This finding supports that TSP-4 may play a critical role in mediating the remodeling process of a failing heart. A subsequent study investigating the contributory role of cardiac TSP-4 expression in heart failure has revealed that the expression of TSP-4 mRNA and protein increases rapidly in response to pressure load *in vivo*, occurring even before the onset of left ventricular hypertrophy and fibrosis [26]. In addition, findings from this study have suggested that TSP-4 might be an endothelial-specific

marker of pressure overload as its expression is limited exclusively to endothelial cells in the myocardium of hypertrophied hearts [26]. Interestingly, acute pressure overload to the heart induces *thbs4* gene upregulation in the left ventricle of young and old normotensive Wistar-Kyoto rats as well as spontaneously hypertensive rats with the induction being more dramatic in young normotensive and spontaneously hypertensive rats [26]. This indicates that the response of TSP-4 upregulation to pressure load is age-dependent and more plastic in young hearts. The contributory effects of TSP-4 to myocardium hypertrophy and heart failure are also confirmed by findings from a recent investigation [75] that TSP-4 expression is highly induced in human hypertrophic hearts as well as different cell populations of mouse hypertrophic hearts derived from Angiotensin II-induced stress. Together, these findings validate previous discoveries from DNA-microarray analyses that *thbs4* gene expression is dramatically elevated in human myocardium of ischemic and failing hearts [28, 29]. It is believed that increased TSP-4 plays a role in fibrosis resulting from chronic maladaptive response to cardiac stress.

In addition, while there is a high level of sexual dimorphism of gene expression in different cell populations, fibroblast-*thbs4* exhibits the greatest level of sexual dimorphism among the genes investigated [75]. These findings support that increased *thbs4* gene expression may play an important role in pathological remodeling of a hypertrophic heart in a sex- and cell-type specific manner. Among limited clinical data about TSP-4 expression in disease states, findings from a recent study [76] have demonstrated that plasma level of TSP-4 from patients with peripheral arterial diseases caused by atherosclerosis correlates positively with the severity of the disease, with the highest difference between patients with or without concomitant diabetes. Accordingly, the authors have suggested that plasma level

of TSP-4 can be used as a novel biomarker for atherosclerosis severity, especially among patients with diabetes.

### 3.3. Inflammation

To investigate the role of TSP-4 in inflammation, Rahman et al. [77] have examined the effects of TSP-4 on macrophage pro-inflammatory phenotype differentiation and apoptosis. Intraperitoneal injection of inflammatory agent lipopolysaccharide (LPS) into mice has led to peritonitis, a model of acute inflammation, that correlates with increased expression of TSP-4 in macrophages. Increased TSP-4 expression has also been observed in cultured macrophages treated with pro-inflammatory cytokines. The upregulation of TSP-4 promotes macrophage differentiation into pro-inflammatory phenotype, as supported by increased production of pro-inflammatory macrophage markers CD38 and Nos2 and a concurrent decrease in the expression of tissue-repair macrophage markers Egr2 and Arg1 [77]. It is worth noting that this contradicts the effects of LPS-induced TSP-1 increase, which has been linked to an elevation of Arg1 expression that promotes polarization of macrophages into an anti-inflammatory phenotype [78]. Increased TSP-4 expression also facilitates macrophage accumulation at inflammation sites, and promotes macrophage apoptosis, which can release pro-inflammatory factors at inflammatory sites to sustain a pro-inflammatory environment. Abolished TSP-4 expression in macrophages from *thbs4*-deficient mice leads to reduced expression of pro-inflammatory macrophage markers. Together, these findings support that TSP-4 plays a unique role in inflammation by promoting pro-inflammatory phenotype differentiation and apoptosis of macrophages at injury sites [77].

Abnormal TSP-4 upregulation also plays a critical role in mediating cancer inflammation and growth. TSP-4 expression is increased in breast cancer tissue compared with that in adjacent noncancerous tissue of patients. Deletion of *thbs4* from mouse breast cancer xenografts leads to reduced inflammation in breast cancer as evidenced by diminished expression of macrophage maker CD68 and proinflammatory markers CD38 and Ccl2. Together, these data support that TSP-4 expression is induced mainly in macrophages in cancer microenvironment that plays a critical role in promoting macrophage infiltration and inflammation. Apparently, TSP-4 mediates proinflammatory effects of TGF-β1 in cancer microenvironment by promoting macrophage infiltration, cancer inflammation and growth as supported by findings that *thbs4* knockout in mice leads to reduced TGF-β1-induced macrophage infiltration in breast cancer xenografts [79].

TSP-4 is also a mediator of hyperglycemia-induced inflammation and cancer growth through a distinct pathway different from that activated by TGF-β1 [79]. TSP-4 expression is induced in both cancerous and noncancerous tissues of diabetic patients with higher level of induction in cancerous tissues. In addition, TSP-4 expression is highly increased in breast cancer xenografts from both type-1 and type-2 diabetic mouse models [79]. Interestingly, hyperglycemia-induced TSP-4 regulates expression of macrophage markers and pro-inflammatory cytokines differently compared with that of TGF-β1-induced TSP-4. In addition, the increased TSP-4 expression is mainly co-localized with tumor-associated macrophage markers with only a small portion co-localized with endothelial cell markers [79]. This observation stands in contrast to the upregulation of TSP-1 induced by glucose, which is mainly observed in endothelial cells [80, 81]. These findings indicate that elevated levels of TSP-4 triggered by hyperglycemia play a role in mediating cancer

inflammation and growth through a distinct pathway that is independent of the effects of TGF-β1 and other TSPs.

Recent findings from mechanistic investigations shed some new light on intracellular signaling pathways linked to the effects of TSP-4 on cancer inflammation and progression. Chou et al. [61] have reported that TSP-4 can promote the migration and invasion of cancer cells by enhancing expression of matrix metalloproteinase 2 through activation of the AKT signaling pathway in bladder cancer progression and metastasis. Activation of the AKT signaling pathway is also reported as a result of TSP-4 interactions with the transmembrane receptor integrin α2 in gallbladder cancer cells that induce phosphorylation of heat shock factor 1 and TGF-β1 upregulation, which in turn provides a positive feedback signaling cascade to increase TSP-4 expression to maintain malignant progression of cancer cells [82].

Together, findings from these studies support that TSP-4 is a critical mediator for activation of different signaling pathways related to inflammation and cancer progression in a cell-type and disease-state specific manner. Since inflammatory processes are closely associated with the etiology of numerous cancers and neurological disorders [83-85], the involvement of TSP-4 in these processes underscores its unique role in pathogenesis of these inflammation triggered disorders, including cancer growth/metastasis, neuroinflammatory and degenerative diseases.

### 3.4. Immune system

TSP-4 is also implicated in modulation of the immune system. In a study conducted by Ponda and Breslow [86] using rodents and cell cultures, it has been

discovered that TSP-4 interacts with a peptide derived from domain 5 of high-molecular-weight kininogen. This interaction facilitates chemotaxis by accelerating the acute migration of immune cells from local inflammation sites to lymphocytes. Neutralizing antibodies against TSP-4 can effectively inhibit this activity. Thus, TSP-4 establishes a connection between inflammation and adaptive immunity, highlighting its role in immune response regulation. This result further supports the notion that interfering with interactions between TSP-4 and domain 5 of high-molecular-weight kininogen may serve as an site-specific target for chemotaxis modulation.

High throughput microarray analysis comparing gene expression profiles between bone marrow lesions collected from total knee replacement of advanced osteoarthritis (OA) patients and normal bone of non-OA control patients has revealed that *thbs4* is one of the most highly up-regulated genes in advanced OA patients. However, it is not clear if this increase underlines the immune response to inflammation, or pre-synaptic hypersensitivity and pain state development (see section 3.6) associated with advanced OA [87]. This change at the DNA level is confirmed at the protein level. Ruthard et al. [88] have reported that TSP-4 proteins are increased in serum of most OA patients as supported by findings that IgG autoantibodies against TSP-4 (and other ECM proteins) are found in these serum samples, but not so in serum samples from healthy donors. This implies that TSP-4 is likely involved in the pathogenesis of OA in human patients. The authors suggest that detecting TSP-4 autoantibodies in serum samples may serve as a biomarker for OA diagnosis. In another clinical study, serum proteomic analysis reveals that TSP-4 is significantly increased in serum samples collected from patients with active systemic lupus erythematosus (SLE), a complex systemic autoimmune disorder,

compared to that from patients in remission and healthy control volunteers [89]. Although the exact pathological role of TSP-4 in SLE development remains elusive, there is a notable positive correlation between serum TSP-4 levels and the disease stage activity of SLE. Therefore, the authors propose that serum TSP-4 level can be used to monitor the disease stage activity of SLE.

### 3.5. Bone formation and regeneration

The physiological process of bone remodeling is dynamic and well-coordinated that regulates and maintains a balance between bone resorption and formation [90, 91]. Adult skeleton maintains its functional integrity and strength through a continuous process of repair called the bone-remodeling cycle [92]. While osteoclasts, osteoblasts, and osteocytes are well-known as essential bone cells involved in remodeling, recent findings suggest that the ECM also plays a significant role in bone remodeling, as well as in skeletal abnormalities and tumor bone metastasis [93]. The ECM in bone regulates critical processes such as cell adhesion, differentiation, proliferation, growth factor responsiveness that ultimately influence the functional properties of bone maturation and regeneration [94, 95].

Although TSP-4 is relatively less characterized compared to other members of the TSP family in the skeletal system, some studies have revealed the unique versatility of TSP-4 in interacting with various ECM components, modulating ECM-cell interactions and osteocyte differentiation in bone remodeling [16, 96, 97]. Findings from a recent study have revealed that the localization and expression patterns of TSP-4 differ from that of TSP-5 within the cartilage tissue throughout the developmental stages of endochondral bone formation and during fracture healing process [32]. TSP-4 is exclusively expressed in the hypertrophic chondrocyte zone

of transient cartilage during endochondral ossification, whereas TSP-5 is observed throughout all zones of transient cartilage. During fracture healing, TSP-4, but not TSP-5, is sparsely detected in the periosteum. Therefore, the differential spatiotemporal distribution of TSP-4 in cartilage tissue during bone formation and remolding support a specific role of TSP-4 in these processes [32].

Using a proteomic approach, Sanchez and colleagues [98] have compared the secretome of osteoblasts obtained from sclerotic and non-sclerotic regions of subchondral bone of osteoarthritis patients. They have found that sclerotic osteoblasts secrete less TSP-4 protein than non-sclerotic osteoblasts. This implies that the role of TSP-4 in remodeling and maintenance of the bone matrix may be compromised in the pathological process of sclerosis in subchondral bone of osteoarthritis patients [98]. In contrast, findings from a recent clinical study have revealed that TSP-4 expression is increased in articular cartilage in the knee of osteoarthritis patients that correlates with the severity of osteoarthritis [99]. With its potential proangiogenic properties and its ability to interact with various ECM proteins, TSP-4 has emerged as an important target for future investigations regarding the mechanisms of its functional role in bone remodeling under these pathophysiological conditions. This in turn can lead to development of target specific interventions or biomarkers for disorders involving TSP-4 dysregulations in skeletal abnormalities.

### 3.6. Synaptogenesis

TSP-4 has also been implicated in the regulation of synaptic function in the central nervous system [35, 100-103]. While its role in regulating synapse formation and stabilization under normal physiological conditions is not yet well

defined, a recent study [104] has revealed that TSP-4 expression is induced after astrocyte-neuron interactions in vitro following the activation of astrocytic sphingosine-1-phosphate receptor 1 that promotes astrocyte morphological complexity and morphogenesis. The authors suggest that this neuronal contact induced TSP-4 expression is likely involved in neural circuit assembly and synaptogenesis.

Importantly, Gan and Sudhof [105] have reported that TSP-4 is one of the critical circulating factors that promote synaptogenesis and synaptic connectivity during brain development in juveniles. In this study, blood from young mice was introduced into aged mice that resulted in enhancement of brain synaptic connectivity. Remarkably, this increase in connectivity correlated with a reversal of age-related cognitive impairments. *In vitro* studies confirmed that blood from young, but not old, mice could increase synapse numbers and synaptic connectivity in neurons derived from human embryonic stem cells. These findings support that TSP-4 plays a critical role in brain function development, and reduced TSP-4 expression is highly likely contributing to reduced synapse formation and transmission in an aging brain that may contribute to age-related cognitive impairments [105].

Synaptopathological roles of TSP-4 have also been implicated by findings from a large number of studies revealing that expression of TSP-4, but not other TSPs, is induced after mechanical injuries to the nervous system that plays a critical role in promoting aberrant excitatory synaptogenesis [22, 33, 37, 51, 106]. Increased excitatory synapses can cause hyperexcitability of neuronal circuitries that underscores the pathology of some neurological disorders, including epilepsy [35, 107], substance abuse [36] and neuropathic pain [51, 100, 103, 108-110]. This

is confirmed by findings that deletion of the *thbs4* gene or treatments with TSP-4 antisense oligodeoxynucleotides as well as neutralizing antibodies result in blockade of nerve injury induced excitatory synaptogenesis and pain states in a spinal nerve ligation injury model [37, 111]. Findings from mechanistic studies have indicated that TSP-4 interacts with its neuronal receptor, the voltage-gated calcium channel alpha-2-delta-1 ($Ca_v\alpha_2\delta_1$) subunit protein, to promote abnormal excitatory synapse formation/maintenance in the brain [35] and spinal cord [110, 111]. Increased $Ca_v\alpha_2\delta_1$ expression results in elevated excitatory synapses and neuron hyperexcitability in the central nervous system [35, 112] that is associated with epilepsy- and pain-like disorders [107, 112]. This $Ca_v\alpha_2\delta_1$ induced hyperexcitability is diminished in *thbs4* knockout mice [111]. Conversely, deletion of the $Ca_v\alpha_2\delta_1$ gene from neurons in mice leads to blockade of TSP-4 induced excitatory synaptogenesis [103, 111, 113] and pain states [111].

In order to explore the $Ca_v\alpha_2\delta_1$ binding domain of TSP-4, five recombinant TSP-4 truncation constructs were created: the N-terminal domain deletion construct encoding the EGF-like domains, type-3 calcium-binding domain, and the C-terminal domain; the EGF-like domain construct encoding EGF-like repeats (EGF-LIKE); the C-terminal domain construct encoding the type-3 calcium-binding domain and the C-terminal domain; the N-terminal domain alone construct; and the N-terminal domain plus the coiled-coil domain construct [110]. Findings from binding studies have revealed that only TSP-4 recombinant proteins containing the EGF-like or coiled-coil domains exhibit similar binging affinity as the wild-type TSP-4 to $Ca_v\alpha_2\delta_1$ proteins, suggesting that these domains contain major $Ca_v\alpha_2\delta_1$ binding sites. However, only recombinant proteins containing EGF-LIKE can cause significant increases in excitatory synapse numbers in cocultures of DRG and spinal cord neurons, as well

as behavioral hypersensitivity in naïve rats that mimics neuropathic pain states induced by peripheral nerve injuries. Blocking interactions between TSP-4 EGF-LIKE and $Ca_v\alpha_2\delta_1$ can reverse aberrant excitatory synaptogenesis and behavioral hypersensitivity induced by TSP-4 recombinant proteins containing EGF-LIKE [110]. These findings are consistent with earlier findings in the brain [35] and confirm that the EGF-like domain of TSP-4 is the molecular determinant in mediating excitatory synaptogenesis and behavioral hypersensitivity through its interaction with $Ca_v\alpha_2\delta_1$.

The mechanism underlying TSP-4 induced synaptogenesis remains elusive. TSP-4 has been shown to interact directly or indirectly with several proteins involved in synapse formation and plasticity, including Neurexin, Neuroligin, the small Rho GTPase, Ras-related C3 botulinum toxin substrate 1 (Rac1), and the N-Methyl-D-Aspartate (NMDA) receptor. It is noteworthy that all members of the TSP family are capable of inducing synaptogenesis [35]. However, TSP-4 may exhibit greater efficacy than other TSPs in promoting neurite outgrowth [22]. TSP binding to $Ca_v\alpha_2\delta_1$ is believed to be an initiating step of a series of synaptogenic cascade events that contribute to spinogenesis and excitatory synapse formation [113]. For example, TSP-4 and $Ca_v\alpha_2\delta_1$ interaction can activate a down-stream intracellular signaling pathway through activation of Rac1 [113]. In addition, TSPs have been shown to interact with synaptogenic Neuroligin 1 proteins [114], which are critical in recruitment and retention of NMDA receptors at post-synaptic terminals of excitatory synapses through interactions between its specific extracellular domain and the NMDA receptor GluN1 subunit [115]. Furthermore, $Ca_v\alpha_2\delta_1$ family members have been found to bind directly to Neurexin, the prominent trans-synaptic adhesion partners of Neuroligins [116]. Chen et al. [117] have reported that the C-terminal domain of $Ca_v\alpha_2\delta_1$ can interact with and form a heteromeric complex with

NMDA receptors that promotes synaptic targeting of NMDA receptors. Taken together, these findings support that the interaction between TSP-4 and $Ca_v\alpha_2\delta_1$ is crucial in triggering a complex network of protein interactions that ultimately promote abnormal synapse formation and spinogenesis, which is critical in mediating central sensitization associated with behaviorally hypersensitive disorders such as epilepsy and pain.

### 3.7. Ion channels

Even though $Ca_v\alpha_2\delta_1$ is a structure subunit of high-voltage gated calcium channels (HVGCC) [118], synaptogenesis induced by TSP-4 and $Ca_v\alpha_2\delta_1$ interactions seems not likely related to modulation of HVGCC since TSP-4 induced synaptogenesis is not blocked by specific antagonists to different HVGCC [35, 119]. However, TSP-4 induced synaptogenesis is sensitive to blockade of T-type low-voltage gated calcium channel (LVGCC) functions by specific antagonists [103]. The mechanism involving T-type LVGCC in TSP-4 induced synaptogenesis is not yet understood. A recent study has revealed that inhibiting T-type LVGCC in mice can attenuate trigeminal nerve injury-induced orofacial neuropathic pain states [120]. Whether this effect is mediated through blocking TSP-4 induced synapse formation and stabilization remains to be determined.

In addition, findings from several studies have reported that TSP-4 plays a role in modulating ion channel functions. For example, Imoto et al. [121] have revealed that TSP-4 significantly inhibits L-type HVGCC and voltage-dependent $K^+$ channel (Kv) currents. The latter can lead to prolongation of action potential duration in isolated ventricular myocytes from rats [121]. Since TSP-4 binds to extracellular adhesion molecule integrins [122], which are known to interact with Kv to

determine the gating property of Kv channels [123], the authors have hypothesized that TSP-4 may inhibit the activity of Kv channels by altering their binding property to integrins. Reduced Kv activity is also linked to injury-induced peripheral nerve hyperexcitability that can lead to pain states [124]. Electrophysiologic studies in dorsal root ganglionic neurons have revealed that TSP-4 inhibits N- and L-type HVGCC currents, but increases T-type LVGCC currents, most likely through interactions with $Ca_v\alpha_2\delta_1$ [125]. These changes may play a role in modulating sensory neuron hypersensitivity after peripheral nerve injury.

## 4. Conclusion

In conclusion, TSP-4 is an ECM glycoprotein that plays a critical role in mediating various physiological processes including cell-cell, and cell-matrix protein communication/adhesion, cell migration, proliferation, angiogenesis, tissue remodeling, and synaptogenesis. Maladaptive TSP-4 expression in response to various pathological stimuli and stress, such as inflammation, metabolic disorders, myocardium overload, injury to the central and peripheral nervous systems, neurodegenerative diseases and cancerous microenvironment, can lead to pathological processes including inflammation, fibrosis, aberrant angiogenesis, myocardium hypertrophy, and excitatory synapse formation. This in turn can accelerate the development of various disorders including degenerative joint disease, tumor progression/metastasis, cardiovascular diseases, inflammation, epilepsy and neuropathic pain (Figure 2). Findings summarized here provide new insights into the unique pathophysiological roles of TSP-4 and associated underlying mechanisms in mediating these pathological disorders, which in turn can provide helpful guidelines for future research directions that can eventually lead to

development of specific interventions and biomarkers for diagnosis and prognosis of these disorders.

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## References


[1] Bornstein, P., *Thrombospondins as matricellular modulators of cell function.* J Clin Invest, 2001. **107**(8): p. 929-34.

[2] Stenina, O.I., E.J. Topol, and E.F. Plow, *Thrombospondins, their polymorphisms, and cardiovascular disease.* Arterioscler Thromb Vasc Biol, 2007. **27**(9): p. 1886-94.

[3] Kazerounian, S., K.O. Yee, and J. Lawler, *Thrombospondins in cancer.* Cell Mol Life Sci, 2008. **65**(5): p. 700-12.

[4] Maemets-Allas, K., et al., *Stimulation with THBS4 activates pathways that regulate proliferation, migration and inflammation in primary human keratinocytes.* Biochem Biophys Res Commun, 2023. **642**: p. 97-106.

[5] Adams, J.C. and J. Lawler, *The thrombospondins.* Cold Spring Harb Perspect Biol, 2011. **3**(10): p. a009712.

[6] Carminati, L., et al., *Controlled extracellular proteolysis of thrombospondins.* Matrix Biol, 2023. **119**: p. 82-100.

[7] Adams, J. and J. Lawler, *Extracellular matrix: the thrombospondin family.* Curr Biol, 1993. **3**(3): p. 188-90.

[8] Kvansakul, M., J.C. Adams, and E. Hohenester, *Structure of a thrombospondin C-terminal fragment reveals a novel calcium core in the type 3 repeats.* EMBO J, 2004. **23**(6): p. 1223-33.

[9] Chen, H., et al., *Cartilage oligomeric matrix protein is a calcium-binding protein, and a mutation in its type 3 repeats causes conformational changes.* J Biol Chem, 2000. **275**(34): p. 26538-44.

[10] Lawler, J., et al., *Characterization of human thrombospondin-4.* J Biol Chem, 1995. **270**(6): p. 2809-14.

[11] Qabar, A., et al., *Thrombospondin 3 is a pentameric molecule held together by interchain disulfide linkage involving two cysteine residues.* J Biol Chem, 1995. **270**(21): p. 12725-9.

[12] Hogg, P.J., D.A. Owensby, and C.N. Chesterman, *Thrombospondin 1 is a tight-binding competitive inhibitor of neutrophil cathepsin G. Determination of the kinetic mechanism of inhibition and localization of cathepsin G binding to the thrombospondin 1 type 3 repeats.* J Biol Chem, 1993. **268**(29): p. 21811-8.

[13] Hogg, P.J., et al., *Thrombospondin is a tight-binding competitive inhibitor of neutrophil elastase.* J Biol Chem, 1993. **268**(10): p. 7139-46.

[14] Murphy-Ullrich, J.E., *Thrombospondin 1 and Its Diverse Roles as a Regulator of Extracellular Matrix in Fibrotic Disease.* J Histochem Cytochem, 2019. **67**(9): p. 683-699.

[15] Frolova, E.G., et al., *Thrombospondin-4 regulates fibrosis and remodeling of the myocardium in response to pressure overload.* FASEB J, 2012. **26**(6): p. 2363-73.

[16] Stenina-Adognravi, O. and E.F. Plow, *Thrombospondin-4 in tissue remodeling.* Matrix Biol, 2019. **75-76**: p. 300-313.

[17] Murphy-Ullrich, J.E., *Thrombospondin-1 Signaling Through the Calreticulin/LDL Receptor Related Protein 1 Axis: Functions and Possible Roles in Glaucoma.* Front Cell Dev Biol, 2022. **10**: p. 898772.

[18] Stenina-Adognravi, O., S. Muppala, and J. Gajeton, *Thrombospondins and remodeling of the tumor microenvironment.* Vessel Plus, 2018. **2**.

[19] Lawler, J., et al., *Identification and characterization of thrombospondin-4, a new member of the thrombospondin gene family.* J Cell Biol, 1993. **120**(4): p. 1059-67.

[20] Stenina, O.I., et al., *Thrombospondin-4 and its variants: expression and differential effects on endothelial cells.* Circulation, 2003. **108**(12): p. 1514-9.

[21] Dunkle, E.T., F. Zaucke, and D.O. Clegg, *Thrombospondin-4 and matrix three-dimensionality in axon outgrowth and adhesion in the developing retina.* Exp Eye Res, 2007. **84**(4): p. 707-17.

[22] Arber, S. and P. Caroni, *Thrombospondin-4, an extracellular matrix protein expressed in the developing and adult nervous system promotes neurite outgrowth.* J Cell Biol, 1995. **131**(4): p. 1083-94.

[23] Klaas, M., et al., *Thrombospondin-4 Is a Soluble Dermal Inflammatory Signal That Selectively Promotes Fibroblast Migration and Keratinocyte Proliferation for Skin Regeneration and Wound Healing.* Front Cell Dev Biol, 2021. **9**: p. 745637.

[24] Koh, S., et al., *Human Umbilical Tissue-Derived Cells Promote Synapse Formation and Neurite Outgrowth via Thrombospondin Family Proteins.* J Neurosci, 2015. **35**(47): p. 15649-65.

[25] Klaas, M., et al., *The alterations in the extracellular matrix composition guide the repair of damaged liver tissue.* Sci Rep, 2016. **6**: p. 27398.

[26] Mustonen, E., et al., *Thrombospondin-4 expression is rapidly upregulated by cardiac overload.* Biochem Biophys Res Commun, 2008. **373**(2): p. 186-91.

[27] Rysa, J., et al., *Distinct upregulation of extracellular matrix genes in transition from hypertrophy to hypertensive heart failure.* Hypertension, 2005. **45**(5): p. 927-33.

[28] Tan, F.L., et al., *The gene expression fingerprint of human heart failure.* Proc Natl Acad Sci U S A, 2002. **99**(17): p. 11387-92.

[29] Gabrielsen, A., et al., *Gene expression signals involved in ischemic injury, extracellular matrix composition and fibrosis defined by global mRNA profiling of the human left ventricular myocardium.* J Mol Cell Cardiol, 2007. **42**(4): p. 870-83.

[30] Frolova, E.G., et al., *Control of organization and function of muscle and tendon by thrombospondin-4.* Matrix Biol, 2014. **37**: p. 35-48.

[31] Hauser, N., et al., *Tendon extracellular matrix contains pentameric thrombospondin-4 (TSP-4).* FEBS Lett, 1995. **368**(2): p. 307-10.

[32] Andres Sastre, E., et al., *Spatiotemporal distribution of thrombospondin-4 and -5 in cartilage during endochondral bone formation and repair.* Bone, 2021. **150**: p. 115999.

[33] Valder, C.R., et al., *Coupling gene chip analyses and rat genetic variances in identifying potential target genes that may contribute to neuropathic allodynia development.* J Neurochem, 2003. **87**(3): p. 560-73.

[34] Eroglu, C., *The role of astrocyte-secreted matricellular proteins in central nervous system development and function.* J Cell Commun Signal, 2009. **3**(3-4): p. 167-76.

[35] Eroglu, C., et al., *Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis.* Cell, 2009. **139**(2): p. 380-92.

[36] Walker, C.D., W.C. Risher, and M.L. Risher, *Regulation of Synaptic Development by Astrocyte Signaling Factors and Their Emerging Roles in Substance Abuse.* Cells, 2020. **9**(2).

[37] Kim, D.S., et al., *Thrombospondin-4 contributes to spinal sensitization and neuropathic pain states.* J Neurosci, 2012. **32**(26): p. 8977-87.

[38] Misenheimer, T.M. and D.F. Mosher, *Biophysical characterization of the signature domains of thrombospondin-4 and thrombospondin-2.* J Biol Chem, 2005. **280**(50): p. 41229-35.

[39] Stenina, O.I., et al., *Polymorphisms A387P in thrombospondin-4 and N700S in thrombospondin-1 perturb calcium binding sites.* FASEB J, 2005. **19**(13): p. 1893-5.

[40] Carlson, C.B., et al., *Structure of the calcium-rich signature domain of human thrombospondin-2.* Nat Struct Mol Biol, 2005. **12**(10): p. 910-4.

[41] Cui, J., et al., *Gender dependent association of thrombospondin-4 A387P polymorphism with myocardial infarction.* Arterioscler Thromb Vasc Biol, 2004. **24**(11): p. e183-4.

[42] Topol, E.J., et al., *Single nucleotide polymorphisms in multiple novel thrombospondin genes may be associated with familial premature myocardial infarction.* Circulation, 2001. **104**(22): p. 2641-4.

[43] Boekholdt, S.M., et al., *Thrombospondin-2 polymorphism is associated with a reduced risk of premature myocardial infarction.* Arterioscler Thromb Vasc Biol, 2002. **22**(12): p. e24-7.

[44] Maly, K., et al., *COMP and TSP-4: Functional Roles in Articular Cartilage and Relevance in Osteoarthritis.* Int J Mol Sci, 2021. **22**(5).

[45] Gebauer, J.M., et al., *COMP and TSP-4 interact specifically with the novel GXKGHR motif only found in fibrillar collagens.* Sci Rep, 2018. **8**(1): p. 17187.

[46] Forbes, T., A.G. Pauza, and J.C. Adams, *In the balance: how do thrombospondins contribute to the cellular pathophysiology of cardiovascular disease?* Am J Physiol Cell Physiol, 2021. **321**(5): p. C826-C845.

[47] Narouz-Ott, L., et al., *Thrombospondin-4 binds specifically to both collagenous and non-collagenous extracellular matrix proteins via its C-terminal domains.* J Biol Chem, 2000. **275**(47): p. 37110-7.

[48] Muppala, S., et al., *Proangiogenic Properties of Thrombospondin-4.* Arterioscler Thromb Vasc Biol, 2015. **35**(9): p. 1975-86.

[49] Muppala, S., et al., *Thrombospondin-4 mediates TGF-beta-induced angiogenesis.* Oncogene, 2017. **36**(36): p. 5189-5198.

[50] Pan, B., et al., *Painful nerve injury upregulates thrombospondin-4 expression in dorsal root ganglia.* J Neurosci Res, 2015. **93**(3): p. 443-53.

[51] Crosby, N.D., et al., *Thrombospondin-4 and excitatory synaptogenesis promote spinal sensitization after painful mechanical joint injury.* Exp Neurol, 2015. **264**: p. 111-20.

[52] Ge, H., et al., *MicroRNA 148a-3p promotes Thrombospondin-4 expression and enhances angiogenesis during tendinopathy development by inhibiting Kruppel-like factor 6.* Biochem Biophys Res Commun, 2018. **502**(2): p. 276-282.

[53] McCart Reed, A.E., et al., *Thrombospondin-4 expression is activated during the stromal response to invasive breast cancer.* Virchows Arch, 2013. **463**(4): p. 535-45.

[54] Curtis, C., et al., *The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups.* Nature, 2012. **486**(7403): p. 346-52.

[55] Liu, J., et al., *Reciprocal regulation of long noncoding RNAs THBS4-003 and THBS4 control migration and invasion in prostate cancer cell lines.* Mol Med Rep, 2016. **14**(2): p. 1451-8.

[56] Kuroda, K., et al., *The clinicopathological significance of Thrombospondin-4 expression in the tumor microenvironment of gastric cancer.* PLoS One, 2019. **14**(11): p. e0224727.

[57] Chen, X., et al., *THBS4 predicts poor outcomes and promotes proliferation and metastasis in gastric cancer.* J Physiol Biochem, 2019. **75**(1): p. 117-123.

[58] Cho, J.Y., et al., *Gene expression signature-based prognostic risk score in gastric cancer.* Clin Cancer Res, 2011. **17**(7): p. 1850-7.

[59] Su, F., et al., *Over-expression of Thrombospondin 4 correlates with loss of miR-142 and contributes to migration and vascular invasion of advanced hepatocellular carcinoma.* Oncotarget, 2017. **8**(14): p. 23277-23288.

[60] Wu, H., et al., *Thrombospondin-4 expression as a prognostic marker in hepatocellular carcinoma.* Gene, 2019. **696**: p. 219-224.

[61] Chou, K.Y., et al., *Thrombospondin-4 promotes bladder cancer cell migration and invasion via MMP2 production.* J Cell Mol Med, 2021. **25**(13): p. 6046-55.

[62] Dawson, D.W., et al., *CD36 mediates the In vitro inhibitory effects of thrombospondin-1 on endothelial cells.* J Cell Biol, 1997. **138**(3): p. 707-17.

[63] Isenberg, J.S., et al., *CD47 is necessary for inhibition of nitric oxide-stimulated vascular cell responses by thrombospondin-1.* J Biol Chem, 2006. **281**(36): p. 26069-80.

[64] Isenberg, J.S., W.A. Frazier, and D.D. Roberts, *Thrombospondin-1: a physiological regulator of nitric oxide signaling.* Cell Mol Life Sci, 2008. **65**(5): p. 728-42.

[65] Zhang, K., et al., *Role of thrombospondin-1 and thrombospondin-2 in cardiovascular diseases (Review).* Int J Mol Med, 2020. **45**(5): p. 1275-1293.

[66] Rodriguez-Manzaneque, J.C., et al., *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**(22): p. 12485-90.

[67] Krady, M.M., et al., *Thrombospondin-2 modulates extracellular matrix remodeling during physiological angiogenesis.* Am J Pathol, 2008. **173**(3): p. 879-91.

[68] Pan, W., et al., *TSP2 acts as a suppresser of cell invasion, migration and angiogenesis in medulloblastoma by inhibiting the Notch signaling pathway.* Brain Res, 2019. **1718**: p. 223-230.

[69] Wang, M.M., *Notch signaling and Notch signaling modifiers.* Int J Biochem Cell Biol, 2011. **43**(11): p. 1550-62.

[70] Meng, H., et al., *Thrombospondin 2 potentiates notch3/jagged1 signaling.* J Biol Chem, 2009. **284**(12): p. 7866-74.

[71] Qian, W., et al., *Thrombospondin-4 critically controls transforming growth factor beta1 induced hypertrophic scar formation.* J Cell Physiol, 2018. **234**(1): p. 731-739.

[72] Arderiu, G., et al., *PAR2-SMAD3 in microvascular endothelial cells is indispensable for vascular stability via tissue factor signaling.* J Mol Cell Biol, 2016. **8**(3): p. 255-70.

[73] Arroyo, A.G. and M.L. Iruela-Arispe, *Extracellular matrix, inflammation, and the angiogenic response.* Cardiovasc Res, 2010. **86**(2): p. 226-35.

[74] Libby, P., et al., *Atherosclerosis.* Nat Rev Dis Primers, 2019. **5**(1): p. 56.

[75] McLellan, M.A., et al., *High-Resolution Transcriptomic Profiling of the Heart During Chronic Stress Reveals Cellular Drivers of Cardiac Fibrosis and Hypertrophy.* Circulation, 2020. **142**(15): p. 1448-1463.

[76] Zierfuss, B., et al., *Thrombospondin-4 increases with the severity of peripheral arterial disease and is associated with diabetes.* Heart Vessels, 2020. **35**(1): p. 52-58.

[77] Rahman, M.T., et al., *Effects of thrombospondin-4 on pro-inflammatory phenotype differentiation and apoptosis in macrophages.* Cell Death Dis, 2020. **11**(1): p. 53.

[78] Chen, X., et al., *Dental Follicle Stem Cells Ameliorate Lipopolysaccharide-Induced Inflammation by Secreting TGF-beta3 and TSP-1 to Elicit Macrophage M2 Polarization.* Cell Physiol Biochem, 2018. **51**(5): p. 2290-2308.

[79] Muppala, S., et al., *Thrombospondin-4 mediates hyperglycemia- and TGF-beta-induced inflammation in breast cancer.* Int J Cancer, 2021. **148**(8): p. 2010-2022.

[80] Dabir, P., et al., *Aryl hydrocarbon receptor is activated by glucose and regulates the thrombospondin-1 gene promoter in endothelial cells.* Circ Res, 2008. **102**(12): p. 1558-65.

[81] Gutierrez, L.S. and J. Gutierrez, *Thrombospondin 1 in Metabolic Diseases.* Front Endocrinol (Lausanne), 2021. **12**: p. 638536.

[82] Shi, Y., et al., *Thrombospondin 4/integrin alpha2/HSF1 axis promotes proliferation and cancer stem-like traits of gallbladder cancer by enhancing reciprocal crosstalk between cancer-associated fibroblasts and tumor cells.* J Exp Clin Cancer Res, 2021. **40**(1): p. 14.

[83] Stephenson, J., et al., *Inflammation in CNS neurodegenerative diseases.* Immunology, 2018. **154**(2): p. 204-219.

[84] Kwon, H.S. and S.H. Koh, *Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes.* Transl Neurodegener, 2020. **9**(1): p. 42.

[85] Fuster-Matanzo, A., et al., *Role of neuroinflammation in adult neurogenesis and Alzheimer disease: therapeutic approaches.* Mediators Inflamm, 2013. **2013**: p. 260925.

[86] Ponda, M.P. and J.L. Breslow, *Serum stimulation of CCR7 chemotaxis due to coagulation factor XIIa-dependent production of high-molecular-weight kininogen domain 5.* Proc Natl Acad Sci U S A, 2016. **113**(45): p. E7059-E7068.

[87] Kuttapitiya, A., et al., *Microarray analysis of bone marrow lesions in osteoarthritis demonstrates upregulation of genes implicated in osteochondral turnover, neurogenesis and inflammation.* Ann Rheum Dis, 2017. **76**(10): p. 1764-1773.

[88] Ruthard, J., et al., *Identification of antibodies against extracellular matrix proteins in human osteoarthritis.* Biochem Biophys Res Commun, 2018. **503**(3): p. 1273-1277.

[89] Zhong, L., et al., *Serum proteomics study reveals candidate biomarkers for systemic lupus erythematosus.* Int J Clin Exp Pathol, 2017. **10**(10): p. 10681-10694.

[90] Siddiqui, J.A. and N.C. Partridge, *Physiological Bone Remodeling: Systemic Regulation and Growth Factor Involvement.* Physiology (Bethesda), 2016. **31**(3): p. 233-45.

[91] Bolamperti, S., I. Villa, and A. Rubinacci, *Bone remodeling: an operational process ensuring survival and bone mechanical competence.* Bone Res, 2022. **10**(1): p. 48.

[92] Raggatt, L.J. and N.C. Partridge, *Cellular and molecular mechanisms of bone remodeling.* J Biol Chem, 2010. **285**(33): p. 25103-8.

[93] Alcorta-Sevillano, N., et al., *Deciphering the Relevance of Bone ECM Signaling.* Cells, 2020. **9**(12).

[94] Kjaer, M., *Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading.* Physiol Rev, 2004. **84**(2): p. 649-98.

[95] Lin, X., et al., *The Bone Extracellular Matrix in Bone Formation and Regeneration.* Front Pharmacol, 2020. **11**: p. 757.

[96] Adams, J.C. and F.M. Watt, *Regulation of development and differentiation by the extracellular matrix.* Development, 1993. **117**(4): p. 1183-98.

[97] Tucker, R.P., J.C. Adams, and J. Lawler, *Thrombospondin-4 is expressed by early osteogenic tissues in the chick embryo.* Dev Dyn, 1995. **203**(4): p. 477-90.

[98] Sanchez, C., et al., *Comparison of secretome from osteoblasts derived from sclerotic versus non-sclerotic subchondral bone in OA: A pilot study.* PLoS One, 2018. **13**(3): p. e0194591.

[99] Maly, K., et al., *The Expression of Thrombospondin-4 Correlates with Disease Severity in Osteoarthritic Knee Cartilage.* Int J Mol Sci, 2019. **20**(2).

[100] Crosby, N.D. and B.A. Winkelstein, *Spinal Astrocytic Thrombospondin-4 Induced by Excitatory Neuronal Signaling Mediates Pain After Facet Capsule Injury.* Ann Biomed Eng, 2016. **44**(11): p. 3215-3224.

[101] Christopherson, K.S., et al., *Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis.* Cell, 2005. **120**(3): p. 421-33.

[102] Gonzalez-Calvo, I., et al., *Synapse Formation and Function Across Species: Ancient Roles for CCP, CUB, and TSP-1 Structural Domains.* Front Neurosci, 2022. **16**: p. 866444.

[103] Yu, Y.P., et al., *Gabapentin prevents synaptogenesis between sensory and spinal cord neurons induced by thrombospondin-4 acting on pre-synaptic Cav alpha2 delta1 subunits and involving T-type Ca(2+) channels.* Br J Pharmacol, 2018. **175**(12): p. 2348-2361.

[104] Singh, S.K., T. Kordula, and S. Spiegel, *Neuronal contact upregulates astrocytic sphingosine-1-phosphate receptor 1 to coordinate astrocyte-neuron cross communication.* Glia, 2022. **70**(4): p. 712-727.

[105] Gan, K.J. and T.C. Sudhof, *Specific factors in blood from young but not old mice directly promote synapse formation and NMDA-receptor recruitment.* Proc Natl Acad Sci U S A, 2019. **116**(25): p. 12524-12533.

[106] Andersson, D., et al., *Plasticity response in the contralesional hemisphere after subtle neurotrauma: gene expression profiling after partial deafferentation of the hippocampus.* PLoS One, 2013. **8**(7): p. e70699.

[107] Faria, L.C., et al., *Epileptiform activity and behavioral arrests in mice overexpressing the calcium channel subunit alpha2delta-1.* Neurobiol Dis, 2017. **102**: p. 70-80.

[108] Li, K.W., et al., *Trigeminal nerve injury-induced thrombospondin-4 up-regulation contributes to orofacial neuropathic pain states in a rat model.* European Journal of Pain, 2014. **18**(4): p. 489-495.

[109] Park, J., et al., *Synaptic ultrastructure changes in trigeminocervical complex posttrigeminal nerve injury.* J Comp Neurol, 2016. **524**(2): p. 309-22.

[110] Park, J.F., et al., *The EGF-LIKE domain of thrombospondin-4 is a key determinant in the development of pain states due to increased excitatory synaptogenesis.* J Biol Chem, 2018. **293**(42): p. 16453-16463.

[111] Park, J., et al., *Central Mechanisms Mediating Thrombospondin-4-induced Pain States.* J Biol Chem, 2016. **291**(25): p. 13335-48.

[112] Li, C.Y., et al., *Calcium channel alpha2delta1 subunit mediates spinal hyperexcitability in pain modulation.* Pain, 2006. **125**(1-2): p. 20-34.

[113] Risher, W.C., et al., *Thrombospondin receptor alpha2delta-1 promotes synaptogenesis and spinogenesis via postsynaptic Rac1.* J Cell Biol, 2018. **217**(10): p. 3747-3765.

[114] Xu, J., N. Xiao, and J. Xia, *Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1.* Nat Neurosci, 2010. **13**(1): p. 22-4.

[115] Budreck, E.C., et al., *Neuroligin-1 controls synaptic abundance of NMDA-type glutamate receptors through extracellular coupling.* Proc Natl Acad Sci U S A, 2013. **110**(2): p. 725-30.

[116] Tong, X.J., et al., *Retrograde Synaptic Inhibition Is Mediated by alpha-Neurexin Binding to the alpha2delta Subunits of N-Type Calcium Channels.* Neuron, 2017. **95**(2): p. 326-340 e5.

[117] Chen, J., et al., *The alpha2delta-1-NMDA Receptor Complex Is Critically Involved in Neuropathic Pain Development and Gabapentin Therapeutic Actions.* Cell Rep, 2018. **22**(9): p. 2307-2321.

[118] Dolphin, A.C., *The alpha2delta subunits of voltage-gated calcium channels.* Biochim Biophys Acta, 2013. **1828**(7): p. 1541-9.

[119] Yu, Y.P., et al., *Gabapentin prevents synaptogenesis between sensory and spinal cord neurons induced by thrombospondin-4 acting on pre-synaptic Ca(v) alpha(2) delta(1) subunits and involving T-type Ca(2+) channels.* Br J Pharmacol, 2018. **175**(12): p. 2348-2361.

[120] Montera, M., et al., *Trigeminal neuropathic pain is alleviated by inhibition of Ca(v)3.3 T-type calcium channels in mice.* Channels (Austin), 2021. **15**(1): p. 31-37.

[121] Imoto, K., et al., *Thrombospondin-4 induces prolongation of action potential duration in rat isolated ventricular myocytes.* J Vet Med Sci, 2020. **82**(6): p. 707-712.

[122] Pluskota, E., et al., *Mechanism and effect of thrombospondin-4 polymorphisms on neutrophil function.* Blood, 2005. **106**(12): p. 3970-8.

[123] Becchetti, A., G. Petroni, and A. Arcangeli, *Ion Channel Conformations Regulate Integrin-Dependent Signaling.* Trends Cell Biol, 2019. **29**(4): p. 298-307.

[124] Alles, S.R.A. and P.A. Smith, *Peripheral Voltage-Gated Cation Channels in Neuropathic Pain and Their Potential as Therapeutic Targets.* Front Pain Res (Lausanne), 2021. **2**: p. 750583.

[125] Pan, B., et al., *Thrombospondin-4 divergently regulates voltage-gated Ca2+ channel subtypes in sensory neurons after nerve injury.* Pain, 2016. **157**(9): p. 2068-80.

**Figure legends**

Figure 1. Domain structure of thrombospondin-4 (TSP-4). N-terminal domain of TSP-4 is followed by a coiled-coil region that contains the oligomerization site. In this site, five subunits become disulfide-linked. Four Type 2 EGF-like domains are downstream of the coiled-coil region followed by a sequence of Type 3 calcium-binding repeats.

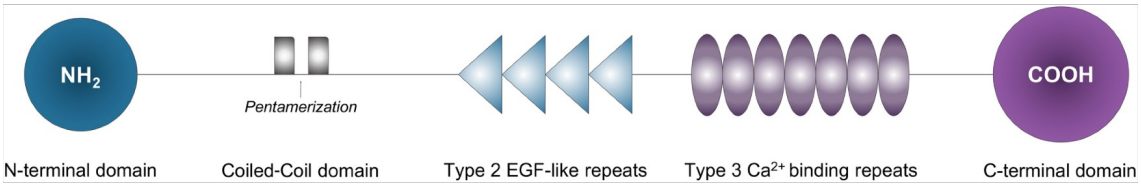
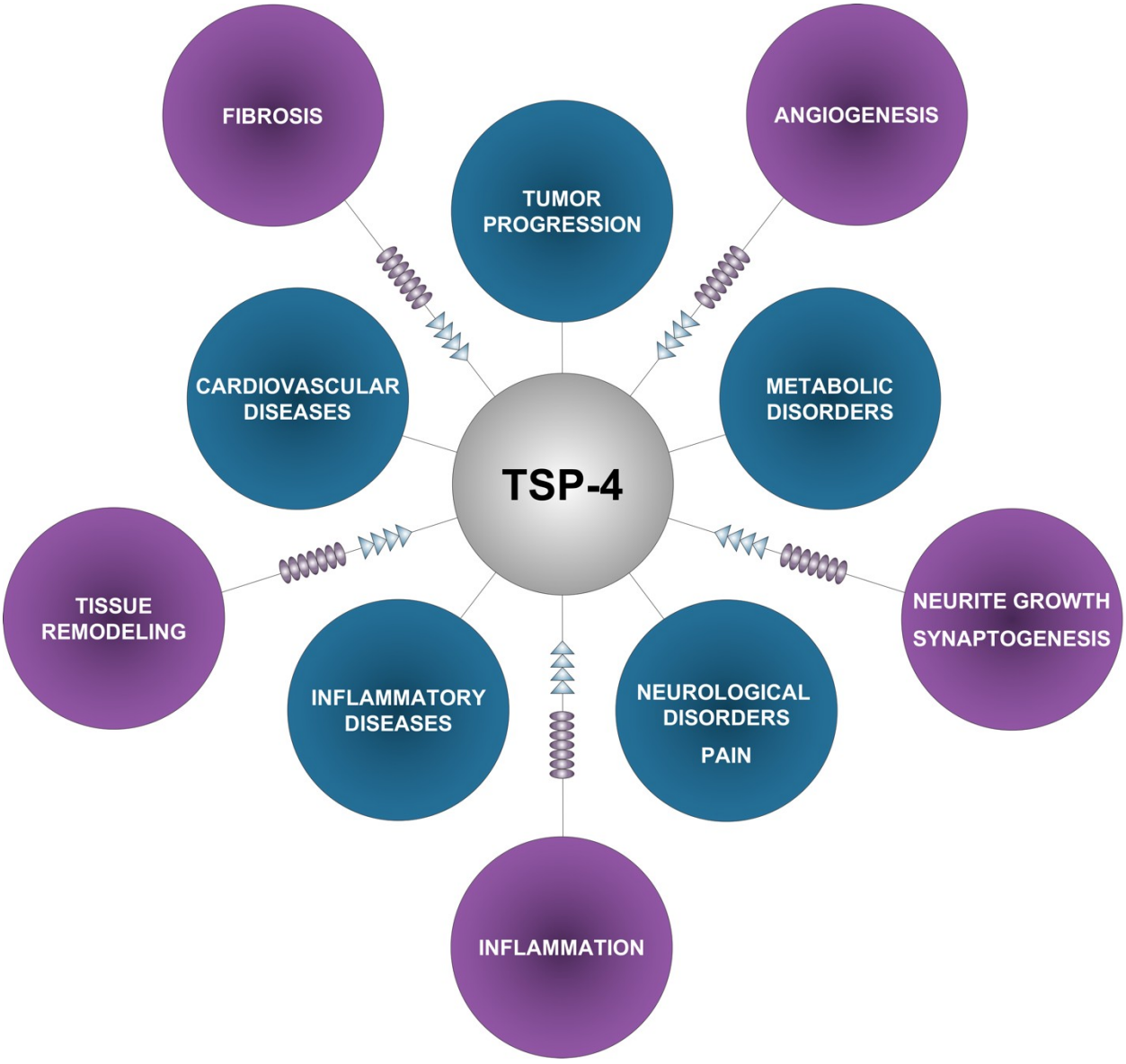



Figure 2. Thrombospondin-4 (TSP-4) has been implicated in various pathophysiological conditions. Purple circles - Some of the functional roles of TSP-4. Blue circles - Some of the pathological disorders mediated by TSP-4 dysregulation.


FIBROSIS
TUMOR PROGRESSION
ANGIOGENESIS
CARDIOVASCULAR DISEASES
TSP-4
METABOLIC DISORDERS
TISSUE REMODELING
NEURITE GROWTH
SYNAPTOGENESIS
INFLAMMATORY DISEASES
NEUROLOGICAL DISORDERS
PAIN
INFLAMMATION