Review Article

The role of thrombospondin-4 in cardiovascular diseases

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Abstract: Thrombospondin-4 is an extracellular matrix protein encoded by the human Thbs4 gene. TSP-4 plays a significant role in tissue remodeling, tissue proliferation, cell migration, angiogenesis, and synaptogenesis, and so forth. TSP-4 has several different functions that act differently in different tissues because TSP-4 has multiple different functional domains and can participate in different signaling pathways. Moreover, TSP-4 has unique features not shared by other TSP family members. Also, many previous studies have found that TSP-4 also plays an important role in the pathogenesis of many cardiovascular diseases, such as myocardial infarction, heart failure, hypertension, atherosclerosis, coronary artery disease and peripheral arterial disease. As a biomarker, TSP-4 can help the diagnosis and differential diagnosis of diseases and can also be used to assess disease progression and prognosis. Targeted activation or targeted inhibition of TSP-4 can also be used for the treatment of disease. Here, we review previous research papers on TSP-4, summarizing the structure, function, signaling pathways of TSP-4 and its roles in disease, especially its role in cardiovascular disease and its potential therapeutic value.

Keywords: TSP-4, cardiovascular disease, function, clinical value

Introduction

Thrombospondin-4 (TSP-4) is a protein encoded by the Thbs4 gene in humans and is a member of the TSP family. The TSPs are a family of five multidomain, calcium-binding matricellular glycoproteins that mediate cell-to-cell and cell-to-matrix interactions and are involved in tissue remodeling, which is associated with embryonic development [1-3], wound healing [3-6], synaptogenesis [1, 3, 7], and neoplasia [3, 8]. TSPs can be divided into two groups, subgroup A comprising TSP-1 and TSP-2 and subgroup B comprising TSP-3, TSP-4, and TSP-5. Compared with other family members, TSP-4 has some functions that are significantly different, even having functional antagonism with other family members. For example, TSP-4 can promote angiogenesis [3, 9-11], while TSP-1 and TSP-1 have potent anti-angiogenic effects [12, 13]. TSP-4 plays a role in the functions of basement membranes in various tissues. In adults, TSP-4 is detected in hearts [14-17], skeletal muscles [18, 19], and articular chondrocytes [20] and accumulates at the neuromuscular junction [1, 18]. Existing research indicates that TSP-4 is a critical regulator of tissue growth and remodeling.

Cardiac remodeling is a clinical manifestation of changes in related genomic expression that result in changes in molecular, cellular and interstitial changes and heart size, and is influenced by factors such as cardiac load or injury, hemodynamic load, and neurohormone activation [15, 21, 22]. Cardiac remodeling is generally accepted as a determinant of the clinical course of heart failure (HF) [21]. Previous research has shown that TSP-4 plays a very important role in myocardial remodeling and the cardiovascular system, it is expressed by endothelial cells and smooth muscle cells of large blood vessels, and it is expressed abundantly in capillaries [17, 23]. In addition, when there are cardiovascular diseases such as myocardial infarction (MI), HF, hypertension, arteriosclerosis, coronary artery disease (CAD) and peripheral arterial disease (PAD), they are often accompanied by changes in TSP-4 expression.
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In this review, we sum up the common role of TSP-4, especially regarding cardiovascular disease.

Structure

In the TSP family, TSP-1 and TSP-2 of subgroup A are homotrimers, while TSP-3, TSP-4 and TSP-5 of subgroup B are homopentamers [24]. In electron microscopy, TSP-4 is seen as a large central particle and is composed of five subunits which are attached by globular domains close to the N-terminal [24, 25]. The subunits of TSP-4 are similar in appearance to the TSP-1 subunits, but there is an obvious difference in that the TSP-4 subunits are smaller [26, 27].

TSPs have a complex multidomain structure, including the C-terminal domain, type III (Ca\(^{2+}\) binding) repeats, type II (EGF-like) repeats, the oligomerization domain, and the N-terminal domain (Figure 1A). Compared with TSP-1 and TSP-2, TSP-4 lacks type I (CD36 binding MMP inhibition) repeats and Von Willebrand factor type C domain and has an additional type II repeat [24, 28-30]. The signature domain of a TSP is its C-terminal domain, type III repeats and type II repeats, which are highly conserved among all TSPs [24, 28, 29, 31]. Calcium-binding is associated with type III repeat regions and contributes to changes in major conformations and functions of TSP-4 [24, 29]. N-terminal is less conservative and regulates the structure and stability of the coiled-coil region and binds heparin, but it is absent in TSP-5 [32]. TSP-4 can be enriched by heparin affinity chromatography [33]. Moreover, TSPs are connected via disulfide bridges into homotrimers, homopentamers, and heterooligomers [34] (Figure 1B).

Function

As an intercellular protein, most of the functions of TSP-4 play a role in the intercellular substance. TSP-4 regulates the composition of the extracellular matrix (ECM) in its predominantly enriched sites, including muscles and tendons. TSP-4 may mediate the binding of the collagen fibril assembly to the cell surface [41]. Moreover, TSP-4 deficiency leads to changes in the composition and physiological function of these tissues [42] and plays a critical role in cardiovascular disease (Figure 2).

Previous studies have revealed that TSP-4 also plays an indispensable role in the migration, adhesion, remodeling, regeneration and inflammation, proliferation, and promotion of nervous system development [1, 4, 5, 43].

Remodeling and regeneration

The TSP family underlies tissue remodeling and regeneration, whose secretion is often induced by stress [44]. As a member of the TSP family, the earliest and most reported function of TSP-4 is tissue remodeling and regeneration.

The upregulation of TSP-4 expression often occurs during tissue damage, regeneration, and remodeling [45, 46]. TSP-4 can alter the composition of the matrix proteins and, as such, TSP-4 is involved in the remodeling process [47, 48]. In cardiac cell stroma, TSP-4 binds and regulates structural ECM proteins during tissue damage [49], a process that promotes adaptive ECM remodeling. Moreover, cardiomyocytes regulate cardiac fibrosis by transcriptionally controlling TSP-4, which in turn regulates the activation of cardiac fibroblasts [50]. Also, TSP-4 can stimulate collagen gene expression and myofibroblast transformation.
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Through the Toll-like receptor four signaling pathway, thereby promoting skin fibrosis [51]. TSP plays a role in limb regeneration in vivo [52]. Moreover, the overexpression of TSP-4 activates non-hepatocytes around the portal area of the damaged liver to promote liver regeneration [53].

Proliferation

As with tissue remodeling and tissue regeneration, proliferation is one of the main functions of TSP-4, especially in the cardiovascular system. The expression of TSP-4 by vascular cells regulates the proliferation of endothelial cells and smooth muscle cells, thereby affecting the vessel wall [23]. Recombinant TSP-4 binds to integrin α₂ and gabapentin receptor α₂δ₁ to accelerate the proliferation of vascular smooth muscle cells [54] and microvascular endothelial cells [9], and the proliferation of endothelial cells isolated from TSP-4 knockout (KO) mice is significantly slowed down [10]. The SNP of TSP-4 significantly alters its ability to support endothelial cell proliferation [55]. Moreover, the C-terminal peptide of TSP-4 (C21) can stimulate erythroid cell proliferation [56]. C21 stimulates thymidine erythrocytes and promotes the cell culture of cord blood erythrocytes and bovine skin fibroblasts [57].

Migration and adhesion

Many studies have shown that TSP-4 is involved in cell adhesion and migration [58-60]. TSP-4 is essential for cell adhesion and cell migration in neurons and vascular smooth muscle cells [61]. TSP-4 regulates adhesion molecules and axonal growth promoting molecules in ECM [2]. Previous studies have revealed that fusion proteins containing C21 support myoblast adhesion [43]. It was found that TSP-4 regulates the adhesion of macrophages to vascular smooth muscle cells [62] and the migration of vascular smooth muscle cells [54] by directly interacting with macrophages or by binding to integrin α₂ and gabapentin receptor α₂δ₁ [9]. TSP-4 with an SNP mutation also has a significant change in its capacity to support endothelial cell adhesion [55]. It has also been reported that the migration and invasion of prostate cancer cells are inhibited by silencing IncRNA TSP-4 [63].

Angiogenesis

Angiogenesis is an important process for growth and development as well as wound healing and granulation tissue formation. At the same time, it is also a basic step in the transition of a tumor from a benign state to a malignant state.

TSP-4 affects a variety of endothelial cell-associated responses, including the transformati

Figure 2. The role of thrombospondin-4 (TSP-4) in cardiovascular diseases. The green arrow defines the promoting effect, the red arrow defines the suppression effect, and the black straight line defines the unknown.
Inflammation

Inflammation is a complex biological response that protects the body from harmful stimuli. TSP-4 is important for the regulation of vascular inflammation [66].

The expression of TSP-4 is significantly upregulated in inflammation [45]. Thbs4 encodes a glycoprotein involved in the inflammatory response [67]. TSP-4 is involved in the inflammatory response to neuropathic pain [68]. Cells with the TSP-4 A387P mutation release excess H$_2$O$_2$ and interleukin-8 [69]. Moreover, TSP-4 inhibition significantly reduces the number of macrophages in the lesion, inhibits endothelial cell activation, and reduces other markers of vascular wall inflammation [62].

Stabilization

TSP-4 has also been found to be stabilizing in muscle cells. TSP-4 has a protective effect on the heart and skeletal muscle. TSP-4 activates the adaptive endoplasmic reticulum (ER) stress response, induces ER expansion, and enhances muscle membrane stability [70]. In myofibers, TSP-4 selectively enhances partial vesicle trafficking to increase integrin and dystroglycan attachment complexes [31] to stabilize muscle fiber membranes [71]. Moreover, TSP-4 has been shown to maintain the stability of cartilage ECM by responding to substrate stiffness and mechanical changes in tendons and muscle tissue [72].

Adipogenesis

Adipogenesis is the process of cell differentiation by which pre-adipocytes become adipocytes. TSP-4 is a putative exercise-induced and obesity-associated myokine in mice [73]. The upregulation of TSP-4 in human pre-adipocytes is compared to mature adipocytes [74]. Therefore, TSP-4 may be involved in early adipogenesis events. Moreover, TSP-4 mediates the interaction between muscle fibers, fat cells, and ECM, so it promotes the spread of fat cells in muscle tissue [19].

Nervous system

The increased expression of TSPs during human brain evolution leads to changes in synaptic organization and plasticity, and contributes to human unique cognitive abilities and our special susceptibility to neurodegenerative diseases [7, 75].

TSP-4 accumulates in neuromuscular junctions and specific synaptic-rich structures in adults [1]. TSP-4 binds to the specific cell integrin receptors of ECM and activates intracellular signal transduction pathways leading to synaptogenesis [76]. In addition to synaptogenesis, TSP-4 increases dendritic dendrites and synaptic transmission to enhance synaptic connections [77], and TSP-4 is related to synaptic rearrangement and plasticity [78]. TSP-4 secreted by astrocytes promotes neuronal migration into the olfactory bulb [79]. When the TSP-4 of NG$_2$ cells is overexpressed, it can promote the spontaneous neuronal differentiation of NG$_2$ cells [80].

After a cortical injury, TSP-4 activates the subependymal ventricular zone (SVZ) notch to activate downstream signals, thereby promoting glial cell production [81]. The increased expression of TSP-4 in the spinal cord after spinal cord injury [82] and small joint injury [83] induces excitatory synapse development and promotes the transduction of articular stress in joints [84] and may lead to excessive spinal excitability in the development and maintenance of persistent neuropathic pain. Infraorbital nerve injury leads to the upregulation of TSP-4 in the trigeminal spinal complex, resulting in orofacial neuropathic pain [85]. After peripheral nerve injury, the expression of TSP-4 is increased in sensory neurons [86], and the appearance of hypersensitivity is promoted by the $\alpha_2\delta_1$ calcium subunit [87].

Signal pathway

ER stress response

The ER stress response is the most widely studied and reported TSP-4 signaling pathway. TSPs play a prominent role in the process of tissue remodeling of the disease, enhancing the ER function and ER protection by regulating the mechanism of action by activating transcription factor 6α (Atf6α) [88]. TSP-4 mediates pathological protection by up-regulating the Atf6α pathway, which is at least partially effective [49]. The transport of TSP-4 through the secretory pathway can increase cardiac and muscle fiber membrane stability [70]. The type III repeat and C-terminal domains of the wild-type
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and calcium-binding mutant TSP-4 interact with Atf6α and then induce adaptive ER stress, which can cause intracellular vesicle expansion [44, 89].

**TGF-β**

In cardiomyocytes, the study revealed that levels of TSP-4 increased after stimulation with the TGF-β ligand [6]. One study outlined a new pathway by which TGF-β regulates angiogenesis. TSP-4 levels are up-regulated by SMAD3 from the TGF-β signal in microvascular endothelial cells [10].

**PI3K-Akt**

Thbs4 is one of the leading genes that can enrich the PI3K-Akt pathway, and TSP-4 is also a product of activated fibroblasts, while TSP-4 can stimulate fibroblasts via the PI3K-Akt pathway in turn [51]. In the progression of gastric cancer, TSP-4 is down-regulated by fibroblast growth factor receptor two via the PI3K-Akt pathway [90].

**ERK-MAPK**

When TSP-4 is overexpressed, the ERK/MAPK signaling pathway is inhibited, which promotes the differentiation of polydendrocytes, while the level of neuronal markers in polydendrocytes increases significantly. This suggests that TSP-4 plays a role in the cell fate determination of polydendrocytes via the ERK-MAPK signaling pathway [80].

**Cardiovascular diseases**

TSPs and their SNPs play an important role in cardiovascular pathology [66]. Interestingly, the A387P SNP of TSP-4 promotes Ca\(^{2+}\) binding, but the N700S SNP of TSP-1 leads to decreased Ca\(^{2+}\) binding production [38]. Both TSP mutations, although different in mechanism, cause cardiovascular disease.

**Myocardial infarction**

Studies have shown that TSP-4 activates ER stress and selectively inhibits TGF-β in myocytes, which is a protective mode for cardiomyopathy and reduces early mortality after MI [6]. It was initially reported that elevated levels of TSP-4 mRNA in rats after MI [91] and the A387P variant of TSP-4 show a strong association of MI [92]. Later, some studies found that the A387P variant of TSP-4 is a determining factor in the development of MI at any age [36, 93]. There are also some research results that are different. A study shows that the TSP-4 A387P polymorphism is only associated with MI in females, especially female homozygous status [35], while another study has limited the homozygosity of the TSP-4 A387P variant to older women [39]. Moreover, the TSP-4 A387P SNP was also confirmed to be significantly associated with myocardial infarction in men [94]. For studies in specific countries, two studies suggest that the TSP-4 A387P polymorphism is a significant and independent risk factor for MI in Americans [95] and Egyptians [96]. Another study adds that existing evidence suggests that TSP-4 polymorphisms other than A387P in the study are not associated with MI [40]. There was an opposite finding that showed no significant association between the TSP-4 A387P polymorphism and MI in the population they studied [37].

**Ventricular hypertrophy and heart failure**

TSP-4 is associated with myocardial remodeling after hypertensive heart disease [97], which may lead to ventricular hypertrophy. Moreover, pathological ventricular hypertrophy often leads to ventricular dilatation, making the heart unable to effectively pump blood, leading to HF. Moreover, TSP-4 plays a vital role in regulating the remodeling of HF [22]. Animal experiments have revealed that Thbs4\(^{-/-}\) has significant deficiencies in adapting to chronic stress overload [98]. Also, the overexpression of TSP-4 was also found in rats with monocrotaline-induced pulmonary hypertension [99], end-stage dilated cardiomyopathy [14], and volume overload HF caused by aorta-tubular heart [100].

**Hypertension**

Long-term hypertension is one of the key risk factors for CAD, HF, and other cardiovascular diseases. In hypertension, TSP-4 regulates the progression of cardiac hypertrophy, affects aortic aneurysm formation, and alters endothelium-dependent resistance to arterial relaxation [17]. Moreover, TSP-4 was identified as having a critical role in causing cardiac hypertrophy and aortic dissection in Ang II-induced hypertension [48]. TSP-4 is significantly increased in the small mesenteric arteries of hypertension.
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Mice with specific destruction of TSP-4 in the heart significantly reduced the adaptation to pressure overload [49].

**Coronary artery disease**

CAD is caused by a decrease in myocardial blood flow due to various factors. Initially, a study found a significant association between CAD and the A387P SNP of TSP-4 in the US population [102]. Subsequently, more research confirmed this finding and showed that the A387P SNP of TSP-4 is associated with an increased risk of CAD in the US population [95, 103, 104]. In contrast, there was no significant relationship between the A387P mutations in TSP-4 and CAD in the target population in one study [37].

**Peripheral artery disease**

Atherosclerosis usually does not have any symptoms at first, but in severe cases, it can cause CAD, stroke, peripheral arterial disease, and kidney problems. TSP-4 is enriched in atherosclerotic lesions and areas prone to pathogenesis [105], and TSP-4 is also involved in the development of atherosclerosis [106]. As mentioned earlier, TSP-4 affects the recruitment of macrophages by affecting endothelial cells [62]. The A387P SNP of TSP-4 has a significant effect on the structure, and inhibits the adhesion and proliferation of endothelial cells [23]. The mutation is consistent with the phenotype that induces atherosclerosis and thrombosis [55], which may be responsible for atherosclerosis. Curcumin has anti-atherosclerotic effects, and one of its possible mechanisms is to prevent an oxidized low-density lipoprotein-induced decrease in TSP-4 expression [107]. Conversely, in one study, a decrease in TSP-4 levels was observed in atherosclerotic lesions [108].

One study found a significant increase in aneurysm formation rates in Thbs4−/− mice treated with ANG II, which was not observed in hypertensive WT mice or untreated Thbs4−/− mice [17]. It is speculated that TSP-4 has no obvious effect under physiological conditions, but it provides a major protective effect under pathological load conditions to prevent aortic aneurysms caused by hypertension [48]. Aortic aneurysms show significant differences in the etiology based on the location of the human abdominal aortic aneurysm, which exhibits a strong positive correlation with male gender [17, 65]. The protective effect of TSP-4 on aortic aneurysms needs to be further examined to determine the role of aneurysm location, age, gender and the effects of diabetes.

A recent study has found that TSP-4 levels are positively correlated with the severity of PAD and show an unknown correlation with diabetes [109], which may lead to arteriosclerosis. At present, related research on PAD other than atherosclerosis is still relatively lacking.

**Clinical value**

**Biomarker**

Different levels of TSP-4 expression in tissues can be used to distinguish between different cell sources. TSP-4 is one of the specific markers used to identify articular cartilage [20] and to distinguish between proper tendon progenitor cells and peritenon progenitors [110]. In mice, SVZ astrocytes express high levels of TSP-4 as opposed to cortical astrocytes expressing low levels of TSP-4 [81]. Furthermore, the differential regulation of TSP-4 has potential value as an in vitro biomarker for inducing signal transduction [111].

TSP-4 can be used for the identification and diagnosis of specific pathological conditions and diseases. TSP-4 is a known CAD marker [112] and a cardiac overload-specific endothelial-specific marker [15]. TSP-4 may be a new marker for atherosclerosis, especially in the subgroup of diabetic patients [109]. Another study found that TSP-4 expression was increased in osteoarthritis and correlated with the severity of osteoarthritis [46], suggesting that TSP-4 can be used as a biomarker for osteoarthritis. Currently, the detection of IgG isotype autoantibodies against TSP-4 can be used to support the diagnosis of osteoarthritis [113]. Also, TSP-4 was identified as a potential biomarker for locoism [114] and myopathy associated with S-adenosyl homocysteine hydrolase deficiency [115]. Similarly, TSP-4 and its degree of methylation can serve as important tumor markers.

**Assessment**

TSP-4-labeled innervated tendon, together with laminin labeled with the basement membrane, can assess the size and distribution of muscle...
fibers [18]. The level of TSP-4 expression can be used to assess the mean of the phenotypic state of the meniscus cells [116]. The overexpression and polymorphism of TSP-4 are associated with vascular invasion in advanced cancer and can be used to assess the risk and prognosis of hepatic cancer [11] and gastric cancer [117].

**Therapy**

In addition to its use in diagnostics, TSP-4 can also be used as a therapeutic target for some diseases.

As an ECM scaffold at the tendon junction, TSP-4 may be used for tendon strengthening and repair [118, 119]. By targeting KLF6 to promote endothelial cell angiogenesis, TSP-4 contributes to the treatment of tendon lesions [120]. Also, TSP-4 has been identified as a potential therapeutic target for muscular dystrophy [71]. TSP-4 plays an important role in the progression of hepatic cancer [121], and targeting TSP-4 is a promising therapeutic strategy for the treatment of advanced hepatic cancer [11]. In a rat model, TSP-4 in bone marrow stromal cells promotes endothelial cell proliferation and migration as well as tube formation, and post-stroke injection can promote the recovery of neural function [64].

Understanding the timing and role of TSP-4 overexpression in persistent neuropathic pain after an injury is critical for designing TSP-4-based therapies [83]. Controlling the interaction between TSP4-mediated intracellular calcium signaling in peripheral sensory neurons [122] and blocking the EGF-like domain of TSP4 and Caαδ [32] may be targeted for the development of analgesic drugs for neuropathic pain.

**Conclusion**

According to this review, it is easy to find that the research on TSP-4 is still insufficient compared to the well-studied TSP-1 and TSP-2, especially in terms of specific molecular mechanisms associated with ligands and signaling pathways. In the cardiovascular system, TSP-4 inhibits MI, HF, hypertension, and promotes PAD, while its A387P mutation increases the risk of MI, CAD, and PAD. Moreover, the impact of TSP-4 on key etiological mechanisms such as inflammation and vascular remodeling in major aneurysms requires further investigation. Through further research on TSP-4, TSP-4 may become an effective therapeutic target for the aforementioned cardiovascular diseases.

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**Disclosure of conflict of interest**

None.

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