Abstract. Thrombospondin (TSP)-1 and TSP-2 are matricellular proteins in the extracellular matrix (ECM), which serve a significant role in the pathological processes of various cardiovascular diseases (CVDs). The multiple effects of TSP-1 and TSP-2 are due to their ability to interact with various ligands, such as structural components of the ECM, cytokines, cellular receptors, growth factors, proteases and other stromal cell proteins. TSP-1 and TSP-2 regulate the structure and activity of the aforementioned ligands by interacting directly or indirectly with them, thereby regulating the activity of different types of cells in response to environmental stimuli. The pathological processes of numerous CVDs are associated with the degradation and remodeling of ECM components, and with cell migration, dysfunction and apoptosis, which may be regulated by TSP-1 and TSP-2 through different mechanisms. Therefore, investigating the role of TSP-1 and TSP-2 in different CVDs and the potential signaling pathways they are associated with may provide a new perspective on potential therapies for the treatment of CVDs. In the present review, the current understanding of the roles TSP-1 and TSP-2 serve in various CVDs were summarized. In addition, the interacting ligands and the potential pathways associated with these thrombospondins in CVDs are also discussed.

Contents
1. Introduction
2. The structure of TSP-1 and TSP-2
3. TSP-1 and TSP-2 in CVDs
4. Signal pathways associated with TSP-1 and TSP-2 in CVDs
5. Conclusions

1. Introduction
The extracellular matrix (ECM) serves a significant role in modulating tissue genesis and remodeling not only by connecting cells and providing support for them, but also by regulating connections between the cells, and between the cell and the matrix, inducing cell adhesion, motility and differentiation. In the cardiovascular system, the ECM participates in maintaining the structural continuity of the heart and vessels, providing physical support for cell adhesion, controlling cell growth and death, and regulating diastolic stiffness, as well as tissue repair or remodeling to the cardiovascular damage (1). A number of these functions are performed by a group of non-structural ECM proteins called matricellular proteins, which includes thrombospondins (TSPs), tenascins, peristin, osteopontin, CCN proteins and osteonectin (2).

As a family of matricellular proteins, TSPs may be secreted by various types of cells. A total of 5 members of the TSP family (TSP1-5) have been identified so far, and are divided into two subgroups, subgroup A and subgroup B, according to their structural differences. Subgroup A contains TSP-1 and TSP-2, which are trimeric and similar in structure, and subgroup B consists of TSP-3, TSP-4 and TSP-5, which are pentameric and smaller compared with those in subgroup A (3). TSP-1 and TSP-2 are the most studied thrombospondins. In the present review, the structure and the role of TSP-1 and TSP-2 in cardiovascular diseases (CVDs; Table I), and the potential pathways associated with these TSPs will be discussed.

2. The structure of TSP-1 and TSP-2
TSP-1 have a complex multidomain structure (Fig. 1), which can interact with various ligands, including ECM structural components, matricellular proteins, growth factors, receptors, proteases and cytokines (4). There is a total of 3 identical chains of TSP-1 or TSP-2 that may form a trimer with a disulfide-bond, which may be critical to some of their
functions (5). The monomer of TSP-1 and TSP-2 consists of an N-terminal domain, an interchain disulfide knot, a homologous procollagen region, 3 type III repeats, 3 type I repeats, 3 type II repeats of TSP-2 shares the same structure with TSP-1, however TSP-1 and TSP-2 in CVDs

3. TSP-1 and TSP-2 in CVDs

Due to their multidomain structure and the ability to interact with multiple ligands, TSP-1 and TSP-2 are active in various types of physiological and pathological processes. At present, there have been multiple studies concerning the role of TSP-1 and TSP-2 in various CVDs (Table I and Fig. 2), suggesting that they may become potential therapeutic targets. **Cardiac hypertrophy and heart failure.** Cardiac hypertrophy is primarily induced by chronic pressure overload, such as essential hypertension. Pathological features of cardiac hypertrophy include increased growth of the cardiomyocytes, proliferation of the cardiac fibroblasts and increased ECM deposition. There have been some reports on the role of TSP-1 and TSP-2 in cardiac hypertrophy. Compared with wild type mice, TSP-1-deficient mice exhibited enhanced early hypertrophy and late dilation when exposed to pressure overload (60). Despite this, TSP-1 (-/-) mice exhibited increased myocardial MMP-3 and -9 activation following pressure overload (60). In obese diabetic DB/DB mice, myocardial TSP-1 levels are significantly upregulated in the perivascular and interstitial space. In comparison with normal DB/DB mice, **DB/db TSP-1 (-/-) mice exhibited an enhanced LV dilation,**
**Table I. Role of TSP-1 and TSP-2 in various CVDs.**

| CVD type               | TSP-1 Effect                                                                                     | (Refs.) | TSP-2 Effect                                                                                     | (Refs.) |
|------------------------|-------------------------------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------|---------|
| MI                     | TSP-1 polymorphism associated with MI                                                            | (56-58) | TSP-2 polymorphism promotes MI                                                                  | (54)    |
|                        | TSP-1 expression increases in patients with MI                                                   | (47)    | Hypoxia induces TSP-2 expression in cardiomyocyte progenitor cells                               | (52)    |
|                        | TSP-1 protects the myocardium from fibrotic remodeling in MI                                     | (50)    | -                                                                                               | -       |
|                        | TSP-1 decreases following PCI associated with adverse cardiac events                              | (48)    | -                                                                                               | -       |
|                        | Ischemia/reperfusion accelerate the induction of TSP-1 in rat MI model                           | (49)    | -                                                                                               | -       |
| Cardiac hypertrophy    | TSP-1 protects pressure-overloaded myocardium                                                     | (60)    | TSP-2 absence leads to age-associated dilated cardiomyopathy                                    | (62)    |
|                        | TSP-1 overexpression in the diabetic heart inhibits chamber dilation                              | (61)    | TSP-2 absence enhances cardiomyocyte damage and matrix disruption in cardiomyopathy             | (63)    |
|                        |                                                                                                 | -       | TSP-2 prevents cardiac injury and dysfunction in viral myocarditis                               | (64)    |
| Heart failure          | TSP-1 expression decreases in failing hearts                                                      | (66,67) | Increased TSP-2 related to CHF-associated mortality and all-cause mortality among patients with CAD with CHF | (71)    |
|                        | miRNA-18 and miRNA-19 modulate TSP-1 expression in age-associated HF                              | (69)    | High serum TSP-2 levels correlate with poor prognosis in patients with HF                         | (72,73) |
|                        | Oral anticoagulation therapy causes the decrease of TSP-1 in patients with HF                    | (70)    | -                                                                                               | -       |
| Valvular disease       | Not available                                                                                    |         | TSP-2 increases in human fibrosclerotic and stenotic aortic valves                               | (75)    |
| Cerebral and carotid artery disorder | Fluavastatin inhibits intimal hyperplasia after carotid artery ligation in WT but not Thbs1-null mice | (77)    | TSP-2 small interfering RNA inhibits vascular response to the injury in rat carotid balloon angioplasty model | (82)    |
|                        | TSP-1 expression increases in platelets from patients with CAD                                   | (76)    | TSP-2 deficiency leads to an impaired recovery following a stroke                                | (78,83) |
|                        | TSP-1 increases following stroke, and TSP-1 deficiency leads to an impaired recovery following stroke | (78,83) | TSP-2 increases in ischemic brain and may lead to spontaneous angiogenesis                         | (79)    |
|                        | TSP-1 is highly expressed in the ischemic brain                                                  | (79)    | Altered TSP-2 expression following spontaneous intracerebral hemorrhage is associated with angiogenesis | (81)    |
|                        | Altered TSP-1 expression following spontaneous intracerebral hemorrhage is related to angiogenesis | (81)    | -                                                                                               | -       |
| Atherosclerosis        | TSP-1 increases in VSMC of human atherosclerotic lesions                                           | (89)    | TSP-2 was absent from the endothelium inside the atheromatous plaque                              | (97)    |
|                        | TSP-1 increases in large arteries of diabetic animals and decreases in microvascular ECs         | (89,93,94) | -                                                                                               | -       |
|                        | Proatherogenic flow initiates EC apoptosis and arterial stiffening via TSP-1                     | (40,84) | -                                                                                               | -       |
| CVD type      | TSP-1                                                                 | (Refs.) | TSP-2                                                                 | (Refs.) |
|--------------|------------------------------------------------------------------------|---------|------------------------------------------------------------------------|---------|
| Angiogenesis | TSP-1 inhibits tumor angiogenesis                                       | (22,100, 101) | TSP-2 inhibits the proliferation of microvascular ECs                     | (110, 111) |
|              | TSP-1 overexpression in diabetes leads to an impaired angiogenesis      | (102)   | TSP-2 deficiency promotes angiogenesis                                   | (112, 113) |
|              | TSP-1 may inhibit angiogenic responses in the ischemic retina           | (103)   | TSP-2 limits angiogenesis by decreasing gelatinolytic activity *in situ* | (114)   |
|              | TSP-1 deficiency contributes to enhanced neovascularization in the eye  | (104-108) | TSP-2 overexpression result in an inhibition of vascularization in rheumatoid arthritis | (115)   |
|              | TSP-1 downregulation in EC enhances angiogenesis                       | (98,99) | TSP-2 expression in the wounds of aged mice impairs the rate of wound healing | (116)   |
| Arterial restenosis | TSP-1 expression by VSMCs is an early response to injury             | (118)   | Increased vessel density in TSP2-/- mice but not in TSP1-/- animals      | (117)   |
|              | TSP-1 is not a major component of the ECM in human restenotic tissues  | (121)   | TSP-2 silencing of aortic SMCs improved cell attachment but did not affect cell migration or proliferation | (122)   |
|              | TSP-1 and β1 integrin interaction is related to platelet-stimulated SMC proliferation | (120)   | -                                                                      | -       |
|              | TSP-1 may reverse the inward remodeling of resistance arteries from hypertension rat | (119)   | -                                                                      | -       |
| Other CVDs   | TSP-1 promotes pulmonary hypertension associated with hypoxia          | (123)   | TSP-2 deficient mice exhibit a bleeding diathesis despite normal blood coagulation | (127)   |
|              | TSP-1 deficiency accelerates aortic aneurysm progression               | (124)   | TSP-2 deficient mice exhibit an altered foreign body reaction characterized by increased vascularity | (128)   |
which was associated with mild non-progressive systolic dysfunction, and TSP-1 could incorporate into the matrix and inhibit leptin-induced MMP-2 activation (61). These previous studies suggest that TSP-1 is upregulated in the diabetic heart and prevents chamber dilation by exerting matrix-preserving actions on the cardiac fibroblasts.

TSP-2 is also closely associated with cardiac hypertrophy. Data suggests that older TSP-2 (-/-) mice are associated with an enhanced dilated cardiomyopathy characteristic as impaired systolic function as well as increased cardiac dilatation and myocardial fibrosis, indicating that TSP-2 deficiency leads to an age-associated dilated cardiomyopathy (62). Compared to wild-type mice, TSP-2-knockout mice display increased mortality accompanied by decreasing cardiac function, increased cardiomyocyte apoptosis and ECM damage in a doxorubicin-induced cardiomyopathy mouse model (63). The absence of TSP-2 also results in decreased systolic function and enhanced cardiac dilatation in human Coxsackie virus B3 (CVB3)-induced myocarditis (64). Previous data also identified that TSP-2 expression is activated uniquely in hypertrophic hearts that may develop heart failure, which may be an early-stage molecular program of heart failure (65).

Abnormal myocardium remodeling leads to myocardial overload. If not treated promptly, long-term myocardial overload may progress into heart failure. From the perspective of pathology, heart failure is associated with abnormal inflammation, coagulation activation and endothelial dysfunction. TSP-1 and TSP-2 also participate in some of these changes. Previous studies have revealed that TSP-1 expression is decreased in failing hearts, which may be associated with ventricular dilatation (66,67). Treatment of cardiomyocytes with a TSP1-derived peptide that activates cd47 leads to increased cardiomyocyte hypertrophy in a cA2+ and calmodulin protein kinase II dependent manner, indicating that TSP-1 may contribute to LV hypertrophy and heart failure (68). Using aged mouse models with failure-resistant and failure-prone characteristics, a previous study identified that micro(mi)RNA-18 and miRNA-19 may modulate TSP-1 expression and cardiac ECM protein levels in age-associated heart failure; therefore, decreased miRNA-18/19 and increased TSP-1 levels may contribute to the identification of failure-prone hearts (69). TSP-1 levels in patients with heart failure may also be decreased due to oral anticoagulation therapy, which is used to prevent thromboembolic events (70).

Elevated TSP-2 is primarily associated with poor prognosis in patients with heart failure. Among patients with coronary heart disease with symptomatic congestive heart failure (CHF), circulating TSP-2 is increased, which is associated with increased 3-year CHF-associated death, all-cause mortality and recurrent hospitalization risk (71). In patients with preserved ejection fraction heart failure, high serum levels of TSP-2 are associated with poor prognosis (72,73). TSP-2 overexpression in wild-type mouse hearts led to decreased cardiac inflammation and improved cardiac function after CVB3 infection, suggesting that TSP-2 may mitigate against cardiac injury, inflammation, and dysfunction during acute viral myocarditis (64).

Table I. continued.

| CVD type | TSP-1 | TSP-2 |
|---------|-------|-------|
| Hypertrophic heart failure | Increases systolic function | Elevates in acute Kawasaki disease |
| Acute myocardial infarction | Regulates migration and adhesion of mononuclear cells | Elevates in acute Kawasaki disease |
| Acute aortic dissection | Increases in plasma of patients with acute aortic dissection | Elevates in plasma of patients with acute aortic dissection |

Valvular disease. Calcific aortic valve disease (CAVD) is a progressive disorder manifesting as sclerotic stiffening and
valvular thickening, eventually leading to aortic stenosis. The pathological process of CAVD is accompanied by inflammatory cell infiltration, lipid accumulation, fibrosis, ECM disorder, angiogenesis and nodular calcification (74). In fibrotic and stenotic aortic valves, the mRNA levels of TSP-2 are increased 4.9-fold (P=0.037) and 4.8-fold (P=0.001), respectively (75). TSP-1 can also be detected in the fibrotic and stenotic valves, but the expression of TSP-1 is not significantly different, indicating that CAVD was associated with TSP-2 upregulation in aortic cusps (75). However, evidence suggesting an association between TSP-1 and valvular diseases is limited, and the specific role of TSP-2 in the pathological process of valvular disease requires further study.

Cerebral and carotid artery disorder. Cerebral and carotid artery disease are important subgroups of peripheral vascular diseases, which have high mortality rates worldwide. TSP-1 and TSP-2 may also serve a role in cerebral and carotid artery disease. In symptomatic patients with carotid artery diseases, TSP-1 expression on the surface of circulating platelets is significantly increased (76). Compared with wild-type mice, TSP-1 (-/-) mice exhibit a decreased response to fluvastatin in inhibiting intimal hyperplasia following carotid artery ligation, indicating that TSP-1 upregulation in aortic cusps (75). However, evidence suggesting an association between TSP-1 and valvular diseases is limited, and the specific role of TSP-2 in the pathological process of valvular disease requires further study.

Atherosclerosis. Atherosclerosis is characterized by thickening, hardening and decreased elasticity of the arterial wall. Lipid levels, endothelial cell injury, inflammation and the migration of vascular smooth muscle cells (VSMC) are considered as several fundamental pathological processes of atherosclerosis. Previous evidence suggested that TSP-1 can interact with some of the aforementioned factors and further regulate the pathological process of atherosclerosis through various mechanisms, while the association between TSP-2 and atherosclerosis requires further investigation. Following partial carotid ligation, disturbed blood flow induced arterial stiffening through collagen deposition. Compared with wild type carotid arteries, TSP-1 knockout animals have significantly decreased arterial stiffening, indicating that disturbed
flow may promote arterial stiffening through TSP-1 (84). Conversely, proatherogenic flow conditions may induce endothelial apoptosis via TSP-1 (40). The absence of TSP-1 accelerates the maturation of the atherosclerotic plaque in apolipoprotein E (ApoE−/−) mice, indicating that TSP-1 may function as an inhibitor of atherosclerosis (85,86). TSP-1 may also interact with lipoproteins. In hypercholesterolemic atherosclerotic rabbits, the overexpressed TSP-1 secreted by injured arteries may bind to very-low-density lipoprotein (VLDL), which may promote its incorporation into nascent atherosclerotic plaques, simultaneously delivering VLDL cholesterol into the lesions (87,88). These results indicate that TSP-1 may serve different roles in different pathological stages of atherosclerosis. Therefore, it is necessary to further investigate the specific role of TSP-1 in atherosclerosis.

An important pathological process of atherosclerosis is the migration of media smooth muscle cells (SMCs) into the intima and hyperplasia. The expression of TSP-1 has been demonstrated to increase in VSMC in human atherosclerotic lesions (89), which may contribute to inflammation and atherogenesis. Hypoxia induces the migration of the coronary artery SMCs, which is elicited by TSP-1 (90,91). An additional study identified that TSP modulates SMCs migration, which may accelerate atherosclerotic lesion development during vascular injury or inflammation (92). TSP-1 may also modulate the interaction between diabetes and atherosclerosis. Evidence reveals that TSP-1 expression is increased in large arteries of diabetic animals however, the protein levels of TSP-1 in microvascular endothelial cells are decreased when exposed to high glucose levels (89,93,94). In a hyperglycemic ApoE−/− mouse model, lack of TSP-1 prevented atherogenic lesion formation (95). The expression of TSP-1 is increased in hypoxic pulmonary hypertension rats, which may contribute to the pathogenesis of hypoxic pulmonary vascular remodeling (96).

Compared with TSP-1, there is limited research on the association between TSP-2 and atherosclerosis. In atherosclerotic specimens, TSP-2 mRNA was absent from intraplaque microvessels and endothelial cells lining the atheromatous plaque (97). Therefore, the specific mechanism of TSP-2 in
the pathological process of atherosclerosis requires further investigation.

Angiogenesis. Angiogenesis is a fundamental physiological process associated with tissue repair following injury, which also promotes tumor progression. This process is tightly modulated by various growth factors and the interaction between cells and the ECM. TSP-1 and TSP-2 have been revealed to regulate angiogenesis by interacting with specific growth factors, cells and ECM. Previous evidence indicates that downregulation of endothelial cell TSP-1 causes an enhancement of in vitro angiogenesis (98). In vitro and in vivo models indicated that factor XIII, a clotting factor, may also promote angiogenesis by downregulating TSP-1 and stimulate endothelial cell proliferation and migration (99). In TSP-1-deficient animals, tumor burden and vasculature increase markedly, and TSP-1 overexpression resulted in decreased tumor diameter and fewer tumor capillaries, indicating that TSP-1 may inhibit tumor angiogenesis (22,100). The inhibitory effect of TSP-1 on tumors may be accomplished via cross-talk with endothelial cells (101). The overexpression of TSP1-CD47 signaling in diabetes is associated with endothelial cell dysfunction, which leads to impaired angiogenesis (102). In the ischemic retina, glia-derived TSP-1 may inhibit angiogenic responses (103), and deficiency of TSP-1 contributes to enhanced neovascularization in the eye (104-108).

Similar to TSP-1, TSP-2 can also inhibit angiogenesis and tumor growth, even with greater potency compared with that of TSP-1 (109). In vitro experiments indicated that TSP-2 inhibits proliferation of microvascular endothelial cells (110,111), and the absence of TSP-2 is associated with enhanced angiogenesis, partly due to the altered endothelial cell and ECM interactions (112,113). Decreasing gelatinolytic activity in situ leads to TSP-2-limited angiogenesis (114). In rheumatoid arthritis, TSP-2 overexpression also inhibits vascularization (115).

In older mice, the delay of TSP-2 and MMP2 expression in wounds may promote the impaired rate of wound healing (116). TSP-2 gene knockout mice exhibited increased blood vessel density, but no such alteration was observed in TSP-1-deficient animals (117). This evidence indicates the role of TSP-2 in anti-angiogenesis.

Arterial restenosis. Restenosis of the arteries following cardiovascular surgery, such as PCI, is a major problem, which leads to a poor prognosis. The pathological process of arterial restenosis is similar to atherosclerosis to a certain extent, including endothelial injury, migration and proliferating of VSMCs into the intima. Similar to atherosclerosis, the precise role of TSP-1 in the pathological process of arterial restenosis is difficult to define. In the balloon catheter injury rat model, TSP was markedly increased in the thickening arterial wall, and the TSP antigen in thickening arterial wall is primary secreted by VSMCs (118). In rat resistance arteries, TSP-1 was able to reverse the pathological inward remodeling caused by spontaneous hypertension, indicating that TSP-1 may act as an inhibitor of arterial restenosis (119). A previous study identified that the interaction of TSP-1 and β1 integrin is associated with platelet-stimulated SMC proliferation (120). However, there is also evidence revealing that TSP-1 is not a major component of ECM in human restenotic tissues, even in the presence of hypercellularity or ongoing cellular proliferation (121).

In human aortic SMCs, TSP-2 silencing caused by siRNA improves cell attachment but does not affect cell proliferation and migration, suggesting that TSP-2 also participates in the pathological process of arterial restenosis (122), which represents a novel hypothesis.

Other CVDs. In addition to the aforementioned major CVDs, TSP-1 and TSP-2 also serve important roles in a number of other CVDs. Evidence indicated that TSP-1 may contribute to the pathogenesis of pulmonary hypertension associated with hypoxia (123). TSP-1 deficiency contributes markedly to maladaptive remodeling of the ECM, causing an acceleration of aortic aneurysm progression (124). During the abdominal aortic aneurysm development, TSP-1 regulates the adhesion and migration of mononuclear cells and promotes vascular inflammation (125). During autologous proangiogenic cell therapy, TSP-1-derived peptide RFYYVWMK may interact with priming CD34+ cells and enhance the vascular engraftment (126). TSP-2 (+) mice exhibit a blebbing diathesis even if they have normal blood coagulation and no thrombocytopenia (127), and an altered foreign body reaction characterized by an enhanced vascularity (128,129). The plasma TSP-2 level is elevated in acute Kawasaki disease, which may be a novel predictor for intravenous immunoglobulin resistance (130). In a TSP-2-knockout mouse model, significantly increased endothelial cell density and reduced fibrosis were observed in the peri-graft region during the cardiac cell transplantation (131). These studies suggest that TSP-1 and TSP-2 also function in other CVDs, such as pulmonary hypertension, aortic aneurysm progression and acute Kawasaki disease.

4. Signal pathways associated with TSP-1 and TSP-2 in CVDs

Due to their multidomain structure, TSP-1 and TSP-2 can specifically bind to numerous types of different ligands. Therefore, they are involved in various signal pathways regulating cellular activities and ECM components in CVDs (Tables II and III). A comprehensive description of these pathways may facilitate the understanding of the role TSP-1 and TSP-2 serve in the pathological processes of multiple CVDs at the molecular level, which may provide certain potential therapeutic strategies.

ECM-receptor interaction. Interactions between various cells and the ECM cause direct or indirect modulation of numerous cellular activities, such as proliferation, adhesion, migration, differentiation and apoptosis, which contributes markedly to numerous CVDs. TSP-1 binds to HSPG with high affinity, which promotes human melanoma cell migration (132). At the sites of inflammation, TSP1 binding to tumor-specific glycoprotein 6 may regulate hyaluronan metabolism, indicating a critical role of TSP-1 in mediating cellular interactions with hyaluronan (9). During the vascular smooth muscle inflammatory response, TSP-1 and TSP-2 may bind to versican and negatively modulate the ECM component (11). In certain circumstances, TSP-1 can bind to LIMPII and promote cell adhesion (27). Previous data indicated that TSP-1 may bind
Table II. Signal pathways associated with TSP-1 in CVDs.

| Domains          | Interacting molecules | Associated signal pathway | Effect                                                                 | (Refs.) | Inhibitors (Refs.) |
|------------------|-----------------------|---------------------------|----------------------------------------------------------------------|---------|-------------------|
| N-terminal       | Heparan sulfate       | Phagosome                 | Endocytosis of TSP-1 by the vascular endothelial cells               | (8)     | Heparinase III (8) |
|                  | HSPG                  | Phagosome                 | Mediates binding and degradation of TSP-1 in ECs                     | (159)   | Heparin (159)     |
|                  | Sulfatides            | ECM-receptor interaction  | Promotes cell adhesion                                              | (132)   | Heparin and dextran sulfates (129) |
|                  | TSG-6                 | ECM-receptor interaction  | Mediates cellular interactions with hyaluronan                      | (9)     | Heparin (9)       |
|                  | LRP                   | Phagosome                 | Internalization and degradation of TSP-1                            | (160,161)|                  |
|                  |                       | ECM-receptor interaction  | Participate in cell signaling with cell surface calreticulin        | (10,13,14)|                  |
|                  | Versican              | ECM-receptor interaction  | Inhibits VSMC inflammatory response                                  | (11)    | Heparin (11)      |
|                  | Integrin α3β1         | ECM-receptor interaction  | Inhibits angiogenesis, Mediates cell motility, Stimulates cell adhesion and spreading | (17,15,16) |                  |
|                  | Integrin α4β1         | ECM-receptor interaction  | Mediates adhesion of T cells                                         | (134)   |                  |
|                  | Integrin α6β1         | ECM-receptor interaction  | Mediates adhesion of microvascular endothelial to immobilized TSP-1 | (19)    |                  |
|                  | Calreticulin          | Focal adhesion            | Induces focal adhesion disassembly and cell migration                | (10,13,14)|                  |
|                  | PDGF                  | PI3K-AKT pathway          | Mediates VSMC proliferation and migration                            | (138)   | Protein disulphide isomerase and heparin (138) |
| Type I repeats   | MMP2                  | ECM homeostasis           | Inhibits MMP2 activity and regulate collagen homeostasis            | (20)    |                  |
| domain           | MMP9                  | ECM homeostasis           | Regulates collagen homeostasis                                       | (20)    |                  |
|                  |                       | PI3K-AKT pathway          | Modulates EC invasion and morphogenesis                              | (139)   |                  |
|                  | CD36                  | ECM-receptor interaction  | Increases EC apoptosis and anti-angiogenic activity                  | (15,25) |                  |
|                  |                       | PI3K-AKT pathway          | Promotes cell adhesion of monocytes/macrophages                      | (147)   |                  |
|                  |                       | Phagosome                 | Inhibits the NO signal transduction                                  | (143)   |                  |
|                  | LIMPII                | ECM-receptor interaction  | Promotes cell adhesion in some circumstances                        | (27)    | LIMPII antibody (27) |
|                  | β1 integrin           | ECM-receptor interaction  | Promotes adhesion of cells that express β1 integrin                 | (28,135)| Alpha-subunit antagonists (28) |
|                  | Latent TGF-β          | TGF-β pathway             | Stimulates endothelial cell tubulogenesis                            | (153)   | Tsp-2 (45)        |
|                  |                       |                           | Recruits inflammatory cells, stimulate angiogenesis, and deposit new matrix | (45,154,155)|                  |
|                  |                       |                           | Increases myofibroblast differentiation                              | (156)   |                  |
| Domains | Interacting molecules | Associated signal pathway | Effect | (Refs.) | Inhibitors | (Refs.) |
|---------|----------------------|---------------------------|--------|---------|-----------|---------|
| CD148   | PI3K-AKT pathway     | Negative regulation of growth factor signals, suppressing cell proliferation and transformation | (33,34) | -       | -         | -       |
| Type II repeats domain | EGFR     | PI3K-AKT pathway | Increases cell migration | (140) | -       | -         |
| β1 integrin | ECM-receptor interaction | Promotes adhesion of the cells that express β1 integrin | (28,135) | B1 blocking antibody, disintegrins | (28) |
| Type III repeats domain | Integrin αIIβ3 | ECM-receptor interaction | Promotes TSP-1 binding with platelet | (136) | -       | -         |
| Integrin αvβ3 | PI3K-AKT pathway | Trigger caspase-independent cell death | (142) | -       | -         | -       |
| Integrin αvβ3 | ECM-receptor interaction | Promote TSP-1 binding with the platelets | (136) | -       | -         | -       |
| FGF2 | PI3K-AKT pathway | Inhibits apoptosis | (43) | Calcium and heparin | (6,36) |
| Calcium | Calcium pathway | Promotes cell spreading and cell adhesion to immobilized TSP-1 | (18,42) | Heparin | (42) |
| C-terminal domain | CD47 | ECM-receptor interaction | Inhibit cell adhesion of monocytes/macrophages | (147) | -       | -         |
| C-terminal domain | CD47 | ECM-receptor interaction | Promote platelet adhesion on inflamed vascular endothelium | (144) | -       | -         |
| Collagen I | ECM homeostasis | PI3K-AKT pathway | Inhibit cGMP synthesis and NO signaling | (143,145) | -       | -         |
| Unknown | DBP | DBP-C5a pathway | Promote pulmonary arterial vasculopathy | (152) | -       | -         |
| Unknown | ADAMTS1 | ADAMTS1-TSP1 pathway | Prevents vascular diseases | (38) | -       | -         |
| Unknown | ADAMTS1 | ADAMTS1-TSP1 pathway | Maintains the balance between the vasodilation and the vasoconstriction | (163) | -       | -         |
to calreticulin (CRT) on the cell surface and induce focal adhesion disassembly, as well as cell migration through the association of CRT with lipoprotein LRP (10,13,133).

Integrins are a family of glycosylated, heterodimeric transmembrane receptors that consist of α and β subunits, which provide a physical link between the ECM and the cytoskeleton. Previous studies identified that TSP-1 and TSP-2 may also interact with various types of integrins. Binding of integrin α3β1 to TSP-1 mediates efficient migration of ECs, indicating that the binding of TSP-1 and integrin α3β1 stimulates cell adhesion and migration (15,16). Despite this, integrin α3β1 binding to TSP-1 can also mediate cell motility and inhibit angiogenesis (15,17). Studies have demonstrated that αβ1 integrin mediates CD47-stimulated sickle red blood cells adhesion to immobilized TSP-1 and modulate T cell behavior (18,134). In addition, the N-terminal domain of TSP-1 is also a ligand for αβ1 integrin, which modulates the adhesion of human microvascular endothelial to immobilized TSP-1 and TSP-2 (19). TSP-1 and TSP-2 may also interact with β1 integrin, contributing to the adhesion of cells that express β1 integrin (28,135). The type III repeats of TSP-1 and TSP-2 may interact with integrin αIIβ3 and αvβ3, promoting their binding with platelets (136). A previous study has demonstrated that TSP-2 may also contribute to anti-angiogenesis in diabetes myocardium (137). These results indicate the important role of the interaction between integrins and TSPs in the ECM-receptor interaction.

**PI3K-AKT pathway.** The PI3K-Akt pathway can be activated by various cellular stimuli, such as growth factors, and regulates numerous fundamental cellular functions, such as cell proliferation, migration and apoptosis. These cellular activities are critical for the pathological process of CVDs. TSP-1 and TSP-2 also participate in a number of these activities through the PI3K-Akt pathway. The N-terminal domain of TSP-1 can interact with platelet-derived growth factor, leading to modulation of VSMC proliferation and migration (138). In addition to the ability to degrade collagen, studies suggest that MMP-9 may also release vascular endothelial growth factor to participate in modulating the invasion and the morphogenesis of endothelial cells, which can also be modulated by TSP-1 (139). The type II repeats of TSP-1 interacts with EGFR and increases cell migration (140). The type III repeat domain of TSP-1 and TSP-2 may interact with integrin αIIβ3 and αvβ3, promoting SMC migration (141). Binding of TSP-1 and TSP-2 to FGF2 inhibits apoptosis (43) and triggers caspase-independent cell death (142).

CD36 is a multi-ligand receptor that participates in various pathological processes of CVDs, such as the formation of atherosclerosis. CD47 is a glycoprotein on numerous types of cell surfaces, which serves an important regulatory role in immune response and inflammation. The binding of TSP-1 and CD36 inhibits angiogenesis through promoting endothelial cell apoptosis and inhibiting nitric oxide (NO) signal transduction (15,25,143). The C-terminal domain of TSP-1 and TSP-2 can interact with CD47, which may promote cell migration and adhesion (18,42,144), inhibit cyclic guanosine monophosphate synthesis, nitric oxide (NO) signaling (143,145) and cell cycle progression in ECs (146). Previous data revealed that the binding of TSP-1 and CD47 may also inhibit angiogenesis, blood
| Domains             | Interacting molecules | Associated signal pathways | Effect                                      | (Refs.) | Inhibitors          | (Refs.) |
|---------------------|-----------------------|----------------------------|---------------------------------------------|---------|---------------------|---------|
| N-terminal domain   | LRP                   | Phagosome                  | Internalization and degradation of TSP-2    | (160,161) | Heparin             | (157)  |
|                     |                       | Calreticulin Focal adhesion| Participates in cell signaling with cell surface calreticulin | (10,13,14) |                      |         |
|                     | Integrin α4β1         | ECM-receptor interaction   | Mediates adhesion of T cells                | (134)   |                     |         |
|                     | Integrin α6β1         | ECM-receptor interaction   | Mediates adhesion of microvascular endothelium to immobilized TSP-2 | (19)    |                     |         |
|                     | Versican              | ECM-receptor interaction   | Inhibits VSMC inflammatory response         | (11)    | Heparin             | (11)   |
|                     |                       |                            | (significantly weaker compared with TSP-1)  |         |                     |         |
| Type I repeats      | MMP2                  | ECM homeostasis            | Inhibition of MMP2 activity                 | (20)    |                     |         |
|                     | MMP9                  | ECM homeostasis            | Mediation of collagen fibrillogenesis       | (113)   |                     |         |
|                     | CD36                  | ECM homeostasis            | Regulate collagen homeostasis               | (20)    |                     |         |
|                     | β1 integrin           | ECM-receptor interaction   | Promotes adhesion of the cells that express β1 integrin | (28)    | β1 blocking antibody, disintegrins | (28)   |
| Type II repeats     | EGFR                  | PI3K-AKT pathway           | Increases cell migration                    | (140)   |                     |         |
| Type III repeats    | Calcium               | Calcium pathway            | Inhibits vascular diseases                  | (38)    |                     |         |
| C-terminal domain   | Integrin αIIβ3        | ECM-receptor interaction   | Promotes TSP-1 binding with platelets       | (136)   |                     |         |
|                     | Integrin αvβ3         | ECM-receptor interaction   | Promotes TSP-1 binding with platelets       | (136)   |                     |         |
|                     | FGF2                  | PI3K-AKT pathway           | Antiangiogenic activity                     | (43)    | Calcium and heparin | (36)   |
|                     | Calcium               | Calcium pathway            | Inhibits vascular diseases                  | (38)    |                     |         |
|                     | CD47                  | ECM-receptor interaction   | Promotes the cell adhesion to immobilized TSP-2 | (18)    |                     |         |
|                     | Unknown               | PI3K-AKT pathway           | Promotes a pro-angiogenic phenotype via the regulation of the oxidative stress | (165)   |                     |         |
flow, and adhesion of monocytes and macrophages (147-150), which may promote foam cell formation (151), pulmonary arterial vasculopathy (152) and LV heart failure (68). Through these mechanisms, TSP-1 and TSP-2 serve a significant role in numerous CVDs.

**TGF-β pathway.** A wide range of different cellular functions, such as cell proliferation, differentiation, migration and apoptosis, can also be modulated by TGF-β, a member of the transforming growth factor superfamily, which is a group of secreted cytokines. Studies also revealed that TSP-1 regulates the above cellular activities through the TGF-β pathway. Previous data suggested that the type I repeats of TSP-1 may bind and activate latent TGF-β. The activated TGF-β can further stimulate new matrix deposition and angiogenesis (45,153-155), promote inflammatory response via recruitment of inflammatory cells and increase myofibroblast differentiation (156) through the TGF-β pathway.

TSP-2 may also bind to latent TGF-β. However, TSP-2 cannot activate latent TGF-β. In addition, due to this reason, TSP-1 and TSP-2 can regulate the activity of TGF-β and modulate the downstream pathways by competitively binding to it (45).

**ECM homeostasis.** Numerous pathological processes of CVDs are accompanied by the destruction of ECM homeostasis. For example, excessive accumulation of type I and type III collagen is a significant feature of cardiac hypertrophy, which is due to the higher collagen synthesis capacity compared with the degradation ability. MMP2 and MMP9 serve crucial roles in maintaining ECM homeostasis. Evidence revealed that TSP-1 and TSP-2 may interact with MMP2 and MMP9, which can inhibit their activity and regulate collagen homeostasis (20,157,158).

In addition, there is also evidence revealing that collagens can interact with TSP-1 directly. The C-terminal domain of TSP-1 may bind to collagen I, contributing to fibroblast homeostasis (156). These results suggest that TSP-1 and TSP-2 contribute markedly to ECM homeostasis.

**Phagosome pathway.** Phagocytosis of TSP-1 serves a critical role in tissue remodeling and inflammation in CVDs, which is mediated by various ligands. In vascular endothelial cells, the heparan sulfate proteoglycans expressed on the cell surface are associated with the process of binding and endocytosis of TSP-1, which leads to its lysosomal degradation (8). Evidence revealed that the HSPG on the endothelial cells may mediate the binding and degradation of TSP-1 (159). Studies suggest that LRP may also function in mediating phagocytosis of TSP-1 in certain types of cells (160,161), indicating that LRP may serve a significant role in the catabolism of TSP-1 in vivo. The binding of TSP-1 and CD36 has been demonstrated to promote the internalization of oxidized LDL, fatty acids and phospholipids, leading to inhibition of atherosclerosis (162). However, little is known on the specific role of TSP-2 in the phagosome pathway, and requires further study.

**Calcium pathway.** Calcium is an indispensable ion involved in numerous physiological processes in the human body. It participates in maintaining the biopotentials on both sides of the cell membrane, maintaining normal muscle expansion
and relaxation, nerve conduction and vasoconstriction. TSP-1 and TSP-2 can bind to calcium and affect the function of modulating physiological activities. Using a simulated model, previous studies have identified that the change between fully calcium-loaded and calcium-depleted TSP1-Sig1 may modulate its interactions, which may become a novel therapeutic target (38,163). Binding of TSP-2 and FGF2 can be inhibited by calcium, indicating that calcium can affect cell function via intervening in interactions between other molecules (36).

Other pathways. In addition to the aforementioned pathways, TSP-1 and TSP-2 also interact with numerous other ligands. During the coagulation reaction, TSP-1 can interact with the vitamin D-binding protein, contributing to the chemotaxis of coagulation factor C5a (164). TSP-2 can interact with cytochrome p450 1B1, promoting angiogenesis through the regulation of oxidative stress (165). In addition, as an important gas signal in the cardiovascular system, NO can negatively regulate TSP-2 transcription and induce angiogenesis (166).

A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) is a type of metalloproteinase which has been demonstrated to be associated with numerous CVDs. Studies have identified that ADAMTS1 contributes to wound closure and inhibits the angiogenesis via interaction with TSP-1 and TSP-2 (167). Evidence has revealed that there is a close association between ADAMTS7 and CVDs. TSP-1 and TSP-2 interaction with ADAMTS7 promotes the pathological processes of atherosclerosis, coronary artery disease (168-172), aortic aneurysm (173) and vascular remodeling (174-176) through interacting with TSP-1 and TSP-2. Conversely, there is also evidence revealing that ADAMTS7 may inhibit LV reverse remodeling following MI (177-179), suggesting ADAMTS7 may be a critical regulator in CVDs.

5. Conclusions

The present review suggests that TSP-1 and TSP-2 serve significant roles in the pathological process of numerous CVDs, and their multi-domain structural features and ability to bind to different ligands may also provide novel targets for the treatment of different CVDs at the molecular level.

However, there are two limitations of the present study. Firstly, although both TSP-1 and TSP-2 have a similar multi-domain structure, both bind to different ligands and serve different roles. There is limited research into the specific role of TSP-2 in the pathogenesis of numerous CVDs, indicating that more research is required. Secondly, numerous novel ligands remain to be identified. Fortunately, with the development of new large-scale techniques, including array-based surface plasmon resonance, new-generation yeast two-hybrid and numerous novel computational methods, novel TSP-1 and TSP-2 ligands may be identified (4). Identification of these ligands may contribute to determination of the interaction networks of TSP-1 and TSP-2, which may provide an improved understanding of their role in CVDs.

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Authors’ contributions

KZ, ML and LY carried out literature search and acquisition of references. KZ, ZL and GF were involved in the conception and design of the manuscript. ZL performed manuscript review and gave final approval of the version to be published. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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