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Role of Thrombospondin 1 in Liver Diseases

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Abstract

Thrombospondin 1 (TSP1) is a matricellular glycoprotein and can be secreted by many cell types. Through binding to extracellular proteins and/or cell surface receptors, TSP1 modulates a variety of cellular functions. Since its discovery in 1971, TSP1 has been found to play important roles in multiple biological processes including angiogenesis, apoptosis, latent TGF- β activation and immune regulation. TSP1 also involves in regulating many organs' functions. However, the role of TSP1 in liver diseases has not been extensively addressed. In this review, we summarize the findings about the possible role that TSP1 plays in chronic liver diseases focusing on non-alcoholic fatty liver diseases, liver fibrosis and hepatocellular carcinoma.

Keywords

TSP1; TGF- β ; angiogenesis; NAFLD; liver fibrosis; liver cancer

Introduction

Thrombospondins are a family of secreted multifunctional proteins consisting of thrombospondin 1–5. Thrombospondin 1 (TSP1) was the first member of the family and discovered in 1971. TSP1 is a homotrimeric glycoprotein of 450 kDa with multiple functional domains (1–5). The structure of TSP1 is composed of three polypeptide chains, which are connected by disulfide bonds (6). This structure is comprised of different regions that are beneficial to the overall function of this extracellular protein (Figure 1). The amino-terminal domain (NTD) of TSP1 plays a role in heparin binding that regulates both removal and uptake of TSP1 (7). It also regulates cell adhesion and chemotaxis. The procollagen region (PC) is involved in angiogenesis suppression (2). There are then three repeats (Type I, Type II, and type III). These repeats play a role in cellular adhesion, migration, growth, and angiogenesis (2, 6). The final region of TSP1 is the carboxy-terminal domain. This domain

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest

plays a role in cell adhesion, migration, platelet accumulation, and regulating nitric oxide signaling (2, 6, 8).

In many cell types, TSP1 is secreted to extracellular space, where it can bind to many receptors (e.g. integrins, CD36 and CD47) and elicits cellular functions. CD36 is one of TSP1's receptors. It exists on many cell types such as endothelial cells, vascular smooth muscle cells, adipocytes, macrophages, tubular cells, and podocytes (9–11). CD36 interacts with the type 1 repeats of TSP1 to regulate cellular functions in many ways. It activates the p38 MAPK pathway and induces endothelial cell apoptosis, which negatively regulates angiogenesis (12–14). TSP1 and CD36 interaction also activates p38MAPK pathway in kidney podocytes and involves in podocyte apoptosis (11). On macrophages, TSP1 binds to CD36 and leads to macrophage activation (10). In platelets, TSP1 activates CD36-dependent pathways including JNK and p38MAPK and regulates platelet functions (15). In addition to CD36, CD47 is another receptor for TSP1. CD47 expresses on many cell types including endothelial cells, smooth muscle cells, epithelial cells, neutrophils, erythrocyte, or chondrocyte (16, 17). CD47 activation by the C-terminal domain of TSP1, suppresses intracellular nitric oxide and downstream cyclic GMP (cGMP) signaling (18, 19), which are dependent on eNOS activation. In addition, effects of CD47 activation contribute to elevated reactive oxidative species (ROS) in a number of tissues including kidneys (20). Through these interactions, CD47 regulates inflammation, cell adhesion, and survival in response to various stressors (16, 17, 21).

TSP1 has been shown to play a role in many diseases. For example, our studies and others have shown that TSP1 is a major regulator for latent TGF- β activation and contributes to the development of diabetic nephropathy (22–25). We found that high glucose treatment (mimic diabetes condition) stimulates TSP1 expression in kidney mesangial cells through decreased cGMP/cGMP-dependent protein kinase pathway-mediated transcriptional suppression and increased expression of transcription factor-Upstream Stimulatory Factor 2 (USF2) (24, 26). Increased TSP1 can then activate latent TGF- β . This activation is through binding of the KRFLK sequence in the type 1 repeats domain of TSP1 to the LSKL sequence of latency associated peptide from the latent TGF- β (27). The activated TGF- β further stimulates mesangial cell to produce excessive amount of extracellular matrix protein in the kidney such as collagen IV, leading to kidney fibrosis. In addition, TSP1 plays a role in organ ischemia reperfusion injury (19, 28, 29). Recent studies demonstrate that TSP1 inhibits renal tubular epithelial cell recovery after ischemia reperfusion injury through inhibition of proliferation/self-renewal in a CD47 dependent manner (30). TSP1 also plays a role in cancer metastasis (31) and inflammation (10, 32, 33) in a CD36-dependent pathway. Taken together, the role of this extracellular protein in disease is widespread; it can have much clinical significance in hepatic function as well. Therefore, in this review, we focus on TSP1 and its role in chronic liver diseases.

Expression of TSP1 in liver

One major source of TSP1 in mammalian is platelet, which can rapidly release TSP-1 from their α -granules after activation. TSP-1 can also be produced by many types of cells including hepatocytes, stellate cells, megakaryocytes, vascular smooth muscle cells,

fibroblast, endothelial cells, epithelial cells, and keratinocytes (7, 18, 34–38). In the liver, during murine embryogenesis, megakaryocytes are the sources of TSP1 transcripts in this organ detected as early as day 12. High steady-state levels of mRNA of TSP1 were observed on days 11–13, with a significant decrease thereafter as shown by using RNase protection assays (39). In adult liver, TSP-1 is expressed at very low or nearly undetectable levels under physiological conditions (40). However, TSP1 can be markedly upregulated under pathophysiological conditions in the liver. For example, hepatocytes and endothelial cells from normal liver tissue showed minimal staining for TSP1; while in liver tissue from the congenital hepatic fibrosis patients, hepatocytes displayed stronger staining for TSP-1 than in normal tissue (41). Congenital hepatic fibrosis-derived stellate cells also secrete more TSP1 (41). In an *in vitro* model of non-alcoholic fatty liver disease, free fatty acid treated hepatocytes had increased intracellular lipid accumulation, associated with increased pro-inflammatory cytokines and TSP1 expression (42). In partial hepatectomy mouse model, an immediate and transient TSP1 expression was induced, majorly from endothelial cells and activated hepatic stellate cells (43). TSP1 expression was also increased in stellate cells isolated from alcohol-treated rats or in liver from carbon tetrachloride (CCl₄) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet induced fibrosis mouse models (44). Importantly, in liver samples from human patients with alcohol cirrhosis, non-alcoholic steatohepatitis related cirrhosis or other fibrosis, TSP1 levels were upregulated (44). Taken together, these data indicate that under different pathologic conditions, TSP1 is upregulated in different liver cell types to a varied extent. These data also provide strong evidence that TSP1 may play an important role in the development of chronic liver diseases.

Role of TSP1 in non-alcoholic fatty liver disease

With the epidemic burden of obesity and metabolic diseases, non-alcoholic fatty liver disease (NAFLD) is becoming a most common hepatic disease worldwide (45). NAFLD is defined histologically as lipid accumulation in no more than 5% of hepatocytes with no evidence of excess alcohol intake, hepatitis B or C infection, autoimmune hepatitis, iron overload, drugs or toxins intake. NAFLD is a progressive disease ranging from steatosis to steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC). The role of TSP1 in NAFLD is largely unknown.

TSP1 is involved in tissue injury and inflammatory diseases such as in rheumatoid arthritis and inflammatory joint disease (46–53). Recently, TSP1 has been identified as an adipokine that is up-regulated in visceral adipose tissue (AT) from obese human subjects and correlated with AT inflammation and insulin resistance (54). By using whole body TSP1 deficient mice, studies from our laboratory revealed a novel role for TSP1 in stimulating macrophage recruitment and activation in AT that contributes to inflammation and insulin resistance resulting from high fat diet-induced obesity (DIO) (33), which was supported by two latter reports (55, 56). Moreover, we found that TSP1 deficiency also protected mice from obesity-induced kidney inflammation and damage in DIO model (57). Importantly, these protective effects of TSP1 deficiency were observed even though TSP1 deficient mice exhibited similar levels of obesity as wild type controls (33). Moreover, in our unpublished results, we found that TSP1 deficiency reduced lipid accumulation as well as TNF-alpha production in liver from high fat diet fed mice, suggesting that TSP1 stimulates liver steatosis and

steatohepatitis and plays a role in NAFLD (Figure 2). This hypothesis is supported by a study showing that in an *in vitro* NAFLD model, free fatty acid treatment induced TSP1 expression in hepatocytes, which was associated with fat accumulation and inflammatory and fibrogenic responses in these cells (42). In addition, aerobic swimming training prevented high-fat diet induced NAFLD in mouse model through regulation of lipid metabolism in liver such as down-regulation of TSP1's receptor-CD36 expression in liver (58). However, the detailed cellular and molecular mechanisms of the involvement of TSP1 in NAFLD are not clear and need further extensive studies.

Role of TSP1 in liver fibrosis

Liver fibrosis is a dynamic wound-healing response of the liver to many causes of chronic injuries including viral infection, alcohol or non-alcoholic fatty liver diseases. It is a complex event involving inflammatory damage, extracellular matrix deposition, hepatocyte death, the progressive fibrosis and remodeling (59–61). Classically, the activation and proliferation of hepatic stellate cells (HSCs), which represents the major extracellular matrix (ECM)-producing cells, plays a key role in liver fibrosis. In addition to HSC, other cell types in the liver such as immune cells, sinusoidal endothelial cells, and hepatocytes also participate in this process by responding to several key cytokines (62). These cytokines include TGF- β , PDGF, vascular endothelial growth factor A (VEGFA), connective tissue growth factor, and nerve growth factor et al. (42). Among these cytokines, the pro-fibrotic effect of TGF- β has been very well studied. TGF- β induces hepatic stellate cell activation to become myofibroblast phenotype to produce more collagen and leads to liver fibrosis (63).

TGF- β is secreted by many cell types as a latent form. It must be activated before it can elicit its cellular functions. There are many factors that can activate latent TGF- β (64). TSP1 is one of the endogenous activators for latent TGF- β activation. It induces latent TGF- β activation through its type 1 repeats domain (KRFK sequence) (23). Moreover, LSKL peptide has been identified to be a TSP1 antagonist to block TSP1-mediated TGF- β activation (27). This TSP1-mediated TGF- β activation plays an important role in tissue injury and fibrosis including diabetes-associated glomerulosclerosis, heart fibrogenic alteration, and liver fibrosis (25, 65–70). Increased TSP1 expression and TGF- β levels have been found in the livers from patients suffered from the congenital hepatic fibrosis (41). An *in vitro* study also showed that TSP1 activates latent TGF- β secreted by hepatic stellate cells (71). In addition, bile acid stimulated TSP1 expression in hepatocytes, leading to TGF- β activation, hepatic stellate cells activation and fibrogenesis (72). Hepatitis C virus (HCV) core protein induced intrahepatic TSP1 expression and TGF- β activation in HCV core transgenic mouse model *in vivo* as well as in the co-culture of hepatoma and stellate cells *in vitro* (73). Together, these studies from different disease models suggest that TSP1-mediated TGF- β activation contributes to liver fibrosis. This was further supported by a study showing that injection of the LSKL peptide to block TSP1-mediated TGF- β activation reduced dimethyl-nitrosamine induced liver damage and fibrosis (70), suggesting that blocking the TSP1-mediated TGF- β activation may serve as a novel therapeutic target for liver fibrosis (59).

In addition to its role in mediating TGF- β activation as above stated, TSP1 is also a known modulator of angiogenesis (74). Hepatic angiogenesis is closely related to fibrosis in both clinical and experimental conditions (75). The cross-talk among hepatic sinusoidal endothelial cells, stellate cells and hepatocytes plays an important role in angiogenesis process during liver regeneration and fibrosis development (76). Studies demonstrated that pathological angiogenesis and sinusoidal remodeling may act as the initiators of liver fibrosis (77, 78) and the angiogenesis pathway has been targeted for treatment of liver fibrosis (59). There is evidence showing that TSP1 correlated with liver angiogenesis in experimental liver fibrosis (79). TSP1 expression in liver was positively correlated with the severity of liver fibrosis as well as angiogenesis in rat cirrhosis model induced by diethyl nitrosamine (79), suggesting that TSP1 might contribute to liver fibrosis not only as an activator of TGF- β , but also as an modulator of angiogenesis (as illustrated in Figure 3). In addition, studies showed that TSP1 through interaction with its receptor CD47 induces defenestration of liver sinusoidal endothelial cells and contributing to liver fibrogenesis (80).

Role of TSP1 in liver cancer

There are very limited data on the role of TSP1 in hepatocellular carcinoma (HCC), the third leading cancer death worldwide (81). In human samples, increased TSP1 expression was found in HCC cells, in endothelial cells and in fibroblasts surrounding the tumor. TSP1 expression was also increased in stromal cells surrounding the tumor (82–84). Moreover, studies from Poon et al demonstrated that higher TSP1 levels were associated with the presence of venous invasion and advancing tumor staging, and were linked to poor survival in HCC patients(84). A positive correlation of TSP1 and VEGF levels in HCC was demonstrated in their study, suggesting that TSP1 may stimulate tumor invasion and progression through a proangiogenesis role in HCC, similarly as found in pancreatic cancer (85), colorectal cancer and cutaneous melanoma (86, 87). However, this finding is in contrast to the general concept of anit-angiogenesis role of TSP1 played in other types of cancer such as bladder cancer and lung carcinoma (88, 89). The inhibitory effect of TSP1 on angiogenesis has been demonstrated through either direct regulation of endothelial cell migration, proliferation and apoptosis or by antagonizing VEGF activity (review paper (13)).

In addition to TSP1's role in regulating angiogenesis in HCC, interestingly, a recent study by Lee et al demonstrated that blockade of a TSP1 signaling receptor-CD47 suppressed HCC growth *in vivo* through blocking the cathepsin-S/protease-activated receptor-2 signaling(90), suggesting the contribution of increased TSP1 signaling to HCC development and progression. However, there are some controversial findings existed. One study found no difference in TSP1 expression between normal and HCC liver samples (91); while another study found that TSP1 levels were greater in normal tissue over that of hepatocellular carcinoma (HCC) (92). In addition, other study demonstrated no positive correlation of TSP1 expression levels with tumor grades and angiogenesis in HCC patients (93). With these controversial findings, future studies are needed to solve these discrepancies. More studies are required to further understand the signaling pathways of TSP1 in HCC development and progression.

Conclusion

TSP1 is a multifunctional matricellular protein. It is a known anti-angiogenesis factor and also a key molecule to activate latent GF- β . Previous studies demonstrated that TSP1 is an important player in liver pathophysiology. Future studies are needed to further determine the cellular and molecular mechanisms by which TSP1 contributes to chronic liver diseases. This may lead to the identification of novel therapeutic targets to delay or stop the progression of chronic liver diseases.

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Figure 1.
TSP1 structure and related receptors/ligands

Figure 2.
Proposed role of TSP1 in obesity-associated non-alcoholic fatty liver disease

Figure 3.
Proposed role of TSP1 in liver fibrosis