Role of the S100 protein family in liver disease (Review)

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Abstract. Liver disease is a significant health challenge worldwide and comprises liver fibrosis and cirrhosis, viral hepatitis, fatty liver, non-alcoholic fatty liver disease, alcoholic liver disease and hepatocellular carcinoma (HCC). Due to the lack of effective treatments, the prognosis of end-stage liver disease (including advanced liver cirrhosis and HCC) is often poor. S100 proteins are a type of Ca^{2+} binding protein, which are expressed in a cell-specific manner in vertebrates. These proteins are involved in numerous functions, such as serving as intracellular Ca^{2+} sensors, transduction of Ca^{2+} signals and regulation of extracellular factors that affect cellular activity by binding to a range of membrane receptors. Evidence has shown that S100 proteins serve key roles in the occurrence and development of liver disease and can be used as potential therapeutic targets or diagnosis markers. For example, certain studies have suggested that blocking S100 protein expression may be an innovative treatment strategy. The present review focuses on the functions of the S100 protein family in liver disease.

Contents

1. Introduction

As the largest gland in the human body, the liver of an adult weighs ~ 1.5 kg (1). The liver is involved in multiple physiological processes of the human body, including lipid (2), fatty acid and glucose metabolism (3), immune response (4), detoxification and secretion of growth factors (such as hepatocyte growth factor, TGF-β and -α and acidic fibroblast growth factor) (5) and cytokines (including IL-6 and -10) (6,7). Liver disease presents a notable burden worldwide, with 1.5 billion people worldwide suffering from chronic liver disease in 2017, most commonly associated with non-alcoholic fatty liver disease (NAFLD; 60%), hepatitis B (29%) and C virus (9%; HBV and HCV, respectively) and alcoholic liver disease (ALD; 2%) (8,9). Liver disease primarily comprises viral hepatitis, fatty liver, NAFLD, ALD, liver cirrhosis and hepatocellular carcinoma (HCC). Current data indicate that both acute and chronic liver disease are prevalent worldwide, resulting in significant morbidity and mortality (8). Approximately 2 million deaths occur worldwide each year (10). In 2017, there were 2.14 million liver-associated deaths, representing an 11.4% increase since 2012 (10). In China, liver disease affects ~300 million people (11). Over the past 20 years, only the United States and Japan, have made progress in decreasing the burden of viral hepatitis (12). Moreover, statistics from China revealed that viral hepatitis is largely under control, especially in urban areas, which is attributed to government efforts to contain HBV and HCV infection and expanded programs for systematic immunization (13). However, the improvement of living standards has led to an increased incidence of other types of liver disease, such as metabolic liver disease; the prevalence of liver disease, including NAFLD and ALD, is increasing year by year, which will lead to further end-stage liver disease, such as advanced liver cirrhosis and HCC (13). The prevalence of advanced ALD 2-5% in at-risk populations, with notable differences according to age, sex and drinking history (14). The global prevalence of NAFLD is ~25% (15). However, end-stage liver disease (such as advanced liver cirrhosis and HCC) cannot be cured using current treatments.

S100 proteins are a type of Ca^{2+} binding protein that are expressed in a cell-specific manner in vertebrates. It have been reported to participate in numerous different pathways, and serve a key role in multiple cellular processes, including proliferation, apoptosis, differentiation and inflammation (16). Previous studies have confirmed that S100 protein expression levels are altered in a variety of diseases, including central nervous system, cardiac and rheumatic system disease, as well as cancer (such as breast cancer and melanoma) (17-21). Moreover, S100 proteins can be detected in a variety of body fluids, including serum, sputum and urine, as well as

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in feces (22,23), which suggests that the S100 protein family may be a potential biomarker of certain diseases (such as lupus nephritis and rheumatoid arthritis) (17,21,24). The liver and S100 proteins are involved in numerous physiological processes, such as proliferation and inflammation (16,25,26). In addition, certain members of the S100 protein family (such as S100A4) serve key roles in the occurrence and development of liver disease (27,28). Previous studies have shown that S100 proteins are diagnostic and prognostic markers in HCC (29,30). To the best of our knowledge, however, the role of S100 proteins in liver disease has not been systematically reviewed. The present review summarizes the function of S100 proteins in the pathogenesis of liver disease and their potential as diagnostic and therapeutic targets.

2. S100 protein family

The Ca^{2+}-binding S100 protein was first discovered in the cow brain in 1965 (31). The S100 protein is a unique type of Ca^{2+}-binding protein, and the family of proteins is named for its solubility in 100% ammonium sulfate (31). S100 proteins are only expressed in vertebrates (32). At present, >20 human S100 proteins have been identified, most of them with low molecular mass (~10-14 kDa) (16), of which 19 members (S100 protein, group A) are located on the 1q21 chromosome (33). Certain S100 proteins are presented in Table I. Among other genes, S100A11P is located on chromosome 7q22-q3, S100B on chromosome 21q22, S100G on chromosome Xp22, S100P on chromosome 4p16 and S100Z on chromosome 5q13 (33). These proteins exhibit cell type-, tissue- or cell cycle-specific expression patterns and unique complex structures that allow them to perform different functions in intracellular and extracellular environments (16). For example, intracellular S100A4 is associated with apoptosis, migration and maintenance of stem cell competence. Extracellular S100A4 activates different processes by inducing the expression and secretion of pro-inflammatory cytokines, growth factors and MMPs and stimulating pro-inflammatory pathways (19,34,35).

Most S100 proteins exist as heterodimers or antiparallel homodimers (36). The majority of these proteins bind to two homologous or heterologous target proteins in a Ca^{2+}-dependent manner (36). Certain members bind in a Ca^{2+}-independent manner, such as S100A1 and/or S100B, which interact with aldolase A, glycogen phosphorylase and tubulin in a Ca2-independent manner (36). Once stimulated by 5-HT_{1A} receptor agonists, glutamate, adenosine and lysophosphatydic acid in the cytoplasm, S100 proteins are activated, causing an instantaneous increase in the intracellular free Ca^{2+} concentration, thereby acting as Ca^{2+} sensors (36). S100 proteins undergo conformational changes upon Ca^{2+} binding, which exposes hydrophobic regions that bind to the target protein (36).

Most S100 proteins are involved not only in cell proliferation, differentiation, motility and apoptosis, but also in physiological processes such as the assembly-disassembly state of cytoskeletal components, phagocytosis, expression and activity of transcription factors, redox balance, Ca^{2+} release from Ca^{2+} stores, ion channel activity, protein degradation and immune cell responses, which function in a Ca^{2+}-dependent manner (16,47). Certain S100 proteins are constitutively secreted as extracellular signals by specific types of cell, including passive release from damaged or dying cells or immune cells during inflammatory events (16,48,49). For example, during acute or chronic local inflammation, myeloid cells actively secrete S100A8/A9 (49). Moreover, several extracellular S100 proteins serve as damage-associated molecular patterns and activate a series of membrane receptors, including pattern recognition and Toll-like receptor-4, G-protein coupled receptors, as well as receptor for advanced glycation end products (RAGE) (16,21,48-50). Under normal and pathological conditions, extracellular S100 proteins affect the activity of numerous types of cell, including neurons, astrocytes, cardiomyocytes, microglia, adipocytes, epithelial and smooth muscle cells and skeletal muscle fibroblasts, which suggests that S100 serves an important role in inflammation (16,21,32,49,51-55). In addition, S100 protein has been associated with fibrosis in multiple organs, such as the kidney (56), lung (57), skin (58) and liver (59) and may serve as a biomarker of fibrosis. It has also been reported that S100 protein expression is elevated in tumors, including such as breast (60), lung (61), colorectal (62) and pancreatic cancer (63), as well as HCC (28). Therefore, S100 proteins serve a key role in tumorigenesis and cancer progression and can also be used as potential markers and therapeutic targets for various types of tumors, such as osteosarcoma and lung, colorectal and breast cancer (64-68).

3. S100 protein in liver disease

As aforementioned, the S100 protein family is involved in numerous physiological processes. Previous studies have reported that S100 proteins serve an important role in the onset and progression of disease, including fibrosis, inflammatory disease and tumor (21,56-58,60-63). Similarly, S100 protein exerts a key role in liver disease (Table II), especially liver fibrosis and HCC, and may be a biomarker and therapeutic target for these diseases (28,59,69). Thus, the S100 protein family may facilitate the diagnosis and treatment of liver disease in the future.

Liver fibrosis. There are >100 million patients with liver fibrosis worldwide; this disease seriously affects patient health (70). Liver fibrosis involves abnormal proliferation of connective tissue in the liver and is caused by various factors, including viral and autoimmune hepatitis, NAFLD and ALD (71). The pathological mechanism of liver fibrosis is complex and is primarily driven by inflammation and immune regulation mechanisms (72,73). Previous studies identified the activation of hepatic stellate cells (HSCs) and excessive deposition of extracellular matrix (ECM) components as the two main processes leading to the development of liver fibrosis (72,74). In liver fibrosis, fibroblasts are primarily derived from activated (a)HSCs (75). The physiological role of quiescent (q)HSCs is storage of vitamin A in the liver (76). When external factors (such as viral infection, alcohol or drugs) cause liver damage, qHSC are activated by inflammatory mediators and differentiate into myofibroblasts (75). Subsequently, ECM proteins and MMPs secreted by aHSC begin to remodel tissue in the liver (77,78). Activated fibroblasts are the primary source of ECM following liver injury. Thus, activation and proliferation of myofibroblasts causes

Table I. Characteristics and expression of S100 proteins.

Protein	Alias	Chromosome localization	Molecular weight, kDa	Expression	(Refs.)
S100A1	S100-α	1q21	10.40	Cardiomyocytes, skeletal muscle fibers, certain neuronal cells	(32)
S100A3	S100E	1q21	~12.00	Hair root cells, certain astrocytomas	(16)
S100A4	Fsp1, metastasin, pEL-98, 18A2, p9Ka, CAPL, calvasculin	1q21	12.00	Fibroblasts, macrophages, tumor cells	(37-39)
S100A6	Calcyclin	1q21	10.18	Fibroblasts, epithelial cells	(40)
S100A9	MRP14	1q21	13.20	Myeloid and cancer cells, tumor stroma	(41)
S100A10	Annexin 2 light chain, P11	1q21	11.21	Ubiquitously expressed in tissue, especially in kidney, lung, and intestine	(42)
S100A11	S100C, calgizzarin	1q21	13.00	Ubiquitous expression in various tissues	(43)
S100A12	Calgranulin C, MRP6, EN-RAGE	1q21	10.40	Neutrophils, macrophages, smooth muscle cells and lung	(16,44,45)
S100B	S-100β	21q22	10.40	Astrocytes, adipocytes, melanocytes, lymphocytes, chondrocytes and skeletal muscle, dendritic and certain neuronal cells	(46)

Fsp1, fibroblast-specific protein 1; MRP, myeloid-related protein; EN-RAGE, extracellular newly identified receptor for advanced glycation end products.

liver fibrosis (75,77). The onset and progression of liver fibrosis are associated with activation and proliferation of HSCs; therefore, a key target for the treatment of liver fibrosis is the inactivation of HSCs and apoptosis (79,80). Chen *et al* (59) reported that S100A4 is upregulated in liver tissue during the progression of liver fibrosis and that the degree of liver fibrosis decreases after blocking S100A4 expression *in vivo*. They also found that S100A4 promotes HSC activation via upregulation of c-Myb (Fig. 1). Chen *et al* (59) analyzed S100A4 levels in patients with cirrhosis and identified that serum S100A4 levels were significantly elevated. The same results were reported in a study by Louka and Ramzy (69), in which expression levels of S100A4 in liver tissue were positively correlated with the degree of liver fibrosis.

In addition to inactivation of HSCs, another important step to reverse fibrosis is degradation of the ECM (81). MMPs comprise a series of enzymes with different substrate affinities for matrix components and are the most important effector for the degradation of ECM (81). The interstitial collagenase MMP-13 is a highly specific protease derived from stellate cells (82). MMP-13 serves a key role in liver fibrosis by mediating the initial inflammatory response in the liver and accelerating the formation of cholestatic liver fibrosis (83). Previous studies have shown that S100A4 is directly involved in the transcription of the MMP-13 gene (84) and in human chondrocytes, extracellular S100A4 protein directly increases expression levels of MMP-13 by interacting with RAGE (85). Similarly, in liver fibrosis, MMP-13 expression levels are positively correlated with upregulation of S100A4 (69). Thus, it should be further investigated whether S100A4 also binds to RAGE in liver fibrosis, thereby promoting the expression of MMP-13.

Similar to S100A4, S100A6 facilitates liver fibrosis by promoting HSC proliferation, primarily by binding to RAGE and inducing ERK activation (86). S100B also serves a role in liver disease; previous studies have reported that S100B expression is decreased in the early stages of liver fibrosis and chronic liver disease and its expression levels during the course of chronic liver disease do not significantly change (87,88).

Viral hepatitis. Viral hepatitis is a major global public health problem affecting hundreds of millions of individuals and is associated with significant morbidity and mortality, with an estimated 257 million people worldwide living with HBV and 71 million with HCV in 2017 (89). Global mortality from chronic HBV and HCV infection is rising, with >1.4 million deaths/year (90). The liver stores vitamin A (retinol) and produces large amounts of all-trans retinoic acid (RA) (91). Previous studies have revealed that RA serves an important role in liver regeneration, fibrosis and tumor formation (92,93). Moreover, it has been shown that RA inhibits dendritic cell function via an S100A4-mediated mechanism, leading to downregulation of T-cell responses and ultimately decreasing hepatitis-induced viral liver injury (91). A recent study by Yan *et al* (94) compared liver biopsy results and serum S100A4 levels in patients with chronic hepatitis B (CHB; n=175); serum S100A4 levels were higher in CHB cases with significant fibrosis compared with those in CHB cases without fibrosis. Furthermore, S100A9 expression is increased during HBV infection and can be used as a marker to distinguish the severity of liver necrotizing inflammation (95). In addition, S100A12 reflects the level of oxidative stress and inflammation in HBV-associated acute and chronic liver failure and

Table II. S100 proteins in liver disease.

Disease	S100 protein	Description	(Refs.)
LF	S100A4	• Highly expressed in liver tissue. • Increased levels in serum. • Promotes HSC activation by upregulating c-Myb. • Biomarker and therapeutic target.	(59,69) (86)
	S100A6	• Highly expressed in liver tissue. • Induces activation of ERK by binding to RAGE to promote HSC activation.	
	S100B	• Decreased expression in liver tissue.	(87)
	S100A4	• Increased levels in serum. • Combination of serum S100A4 and liver stiffness assay improve the accuracy of diagnosis of severe fibrosis.	(94)
VH	S100A9	• Increased levels in serum. • Biomarker of severity of liver necrotizing inflammation.	(95)
	S100A12	• Increased levels in serum. • Elevated levels suggest higher oxidative stress and inflammation. • Predicts prognosis of patients with hepatitis B virus-associated liver failure.	(96)
	S100A4	• Highly expressed in mouse model liver tissue.	(100)
NAFLD	S100A9	• Highly expressed in rat model liver tissue. • Increased levels in rat serum. • Potential biomarker of liver and metabolic progress.	(101)
	S100A11	• Highly expressed in liver tissue. • Promotes fatty degeneration of the liver via the S100A11/histone deacetylase 6/FOXO1 axis. • Potential therapeutic target and key feature of the transition from steatosis to NASH/fibrosis.	(102-104)
ALD	S100A4	• Highly expressed in mouse model liver tissue. • Activates the STAT3 pathway to promote occurrence of early alcoholic hepatitis. • Inhibits lipid accumulation.	(112)
	S100A1	• Highly expressed in liver tissue. • Downregulates LATS1 and YAP phosphorylation, allowing YAP to enter the nucleus, thereby regulating the Hippo pathway to produce oncogenic effects. • Biomarker and therapeutic target.	 (118)
	S100A3	• Highly expressed in liver tissue. • Activation is associated with occurrence and invasiveness of HCC.	 (119)
	S100A4	• Highly expressed in liver tissue. • High expression is negatively correlated with differentiation, invasion, recurrence and overall survival of HCC. • Promotes migration and invasion by activating and promoting NF-κB p65 transport into the nucleus, thereby upregulating MMP-9. • Upregulates miR-155 expression, downregulates SOCS1 expression and activates STAT3 signaling, thereby upregulating MMP-9 to promote migration and invasion. • In the presence of collagen I, it activates β-catenin signaling by synergizing with RAGE, thus affecting the stemness of cancer cells. • Therapeutic target and biomarker.	(28,30,120-122,134)
HCC	S100A6	• Highly expressed in liver tissue. • Downregulates E-cadherin expression on the cell membrane and promotes nuclear accumulation of β-catenin in cells, thereby promoting proliferation and migration of HCC cells. • Promotes proliferation and migration of HCC cells by promoting p53 ubiquitin-dependent proteasomal degradation and downregulating p21 expression.	(135-139)

Table II. Continued.

Disease	S100 protein	Description	(Refs.)
		• Potential diagnostic marker and therapeutic target.	(140-145)
	S100A9	• Highly expressed in liver tissue • Increased levels in serum • Secretion is upregulated by TAMs • Interaction with RAGE upregulates ERK1/2 and p38MAPK, thereby activating the MAPK pathway and promoting proliferation and invasion of HCC cells. • Increased serum S100A9 levels are associated with poor prognosis	
	S100A10	• Highly expressed in liver cancer cells. • Potential therapeutic target.	(146,147)
	S100A11	• Highly expressed in liver tissue. • Secreted by cancer cells. • High expression indicates a poor prognosis. • Potential therapeutic target.	(104,150)
	S100A12	• Primarily expressed in myeloid immune cells. • Independent prognostic factor following radical surgical resection of HCC. • High expression associated with poor overall and disease-free survival. • Decreased expression may predict clinical efficacy of immunotherapy for HCC. • Potential target for immunotherapy.	(152)

HSC, hepatic stellate cell; RAGE, receptor for advanced glycation end products; miR, microRNA; LATS1, large tumor suppressor kinase 1; YAP, Yes-associated protein; SOCS1, suppressor of cytokine signaling 1; TAMS, tumor-associated macrophages; HCC, hepatocellular carcinoma; ALD, alcoholic liver disease; NAFLD, non-alcoholic fatty liver disease; LF, liver fibrosis; VH, viral hepatitis.

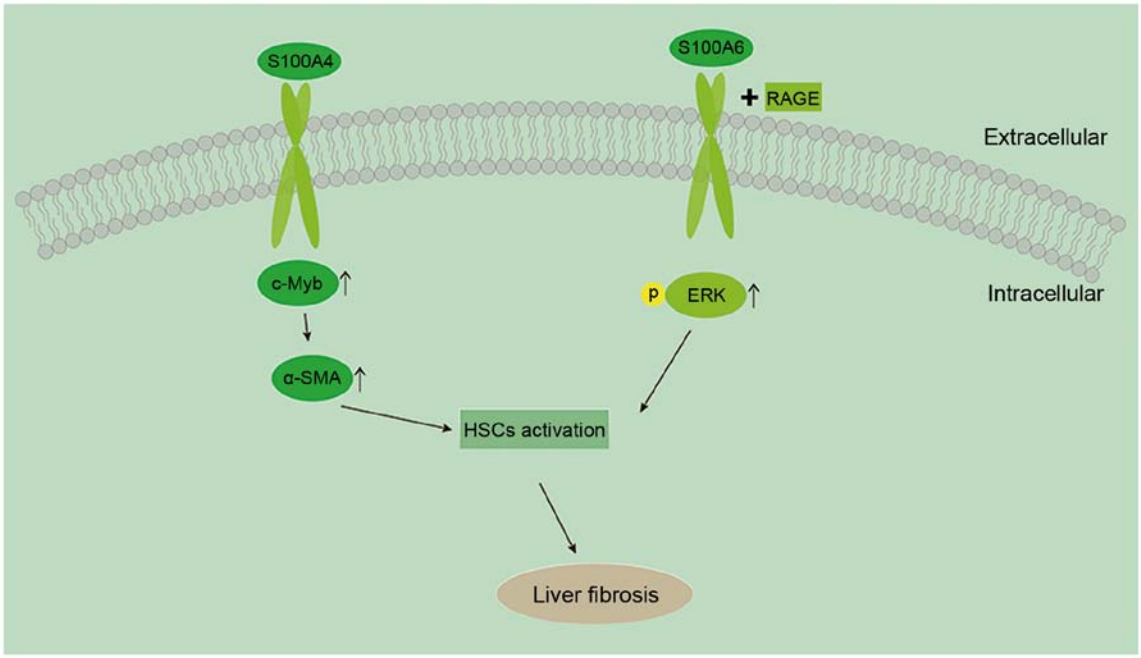

Figure 1. S100 proteins in liver fibrosis. S100A4 promotes HSC activation via the upregulation of c-Myb. S100A6 induces ERK activation by binding to RAGE and promotes HSC activation. HSC, hepatic stellate cell; RAGE, receptor for advanced glycation end products; SMA, smooth muscle actin.

its elevated expression may be an important marker of poor prognosis (96).

NAFLD. NAFLD is the most common chronic liver disease worldwide. A meta-analysis of studies from 1989 to 2015 reported that the global prevalence of NAFLD was ~25% (15), ranging from 13% in Africa to 42% in Southeast Asia (15,97). Its histological features include non-alcoholic steatohepatitis (NASH) and simple steatosis and it is characterized by fat accumulation, swelling of hepatocytes and the development of

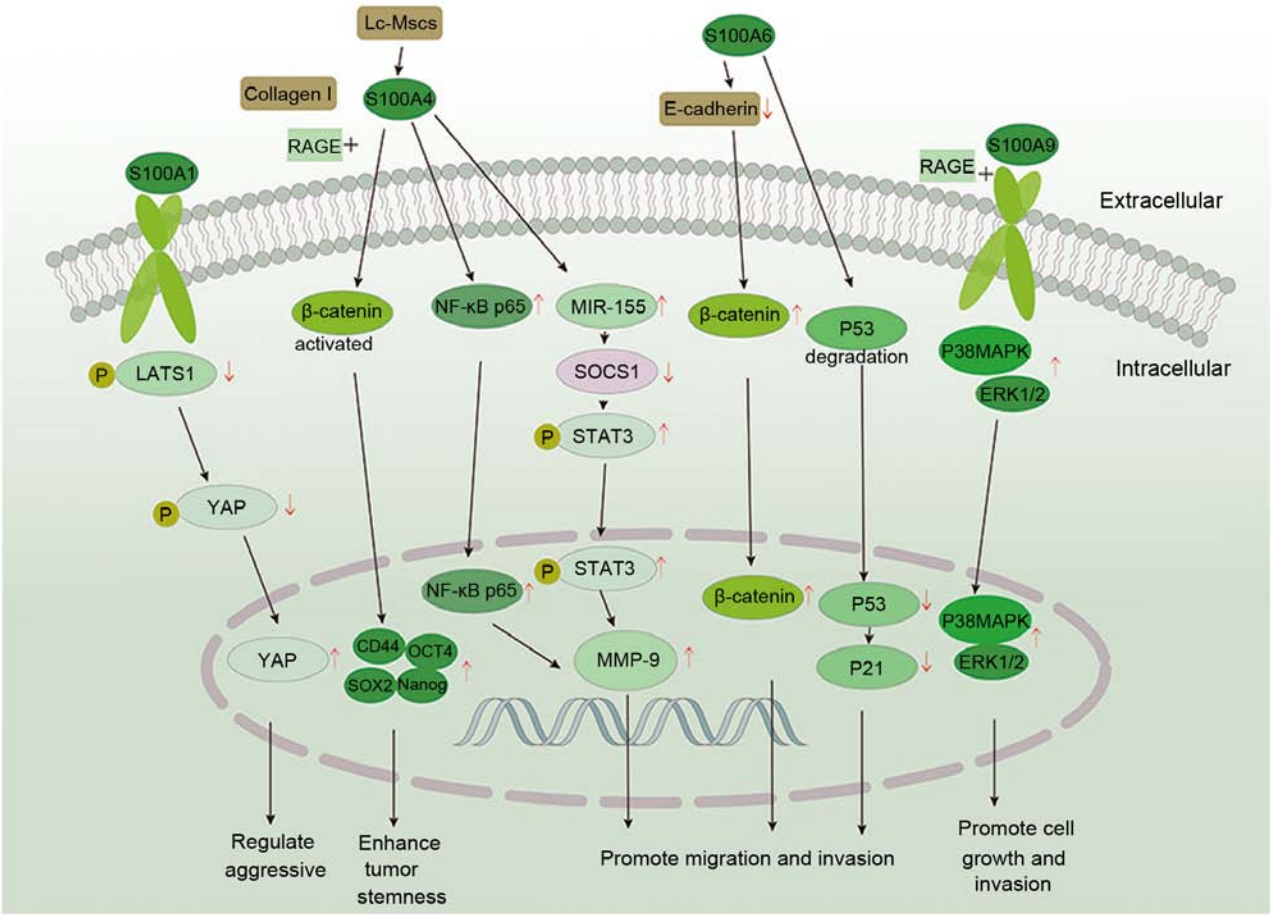

Figure 2. S100 proteins in HCC. S100A1 regulates the Hippo signaling pathway to produce oncogenic effects by downregulating phosphorylation of LATS1, thereby inducing YAP phosphorylation, which allows YAP to enter the nucleus. S100A4 promotes cell migration and invasion by activating and promoting NF-κB p65 entry into the nucleus, upregulating miR-155 and downregulating SOCS1 expression levels, activating STAT3 signaling, thereby upregulating MMP-9. In the presence of collagen I, S100A4 affect the stemness of cancer cells by synergizing with RAGE and upregulating β-catenin expression. S100A6 promotes the proliferation and migration of HCC cells by downregulating E-cadherin expression on cell membranes, promoting nuclear accumulation of β-catenin in cells and regulating the expression of p21 by promoting p53 ubiquitin-dependent proteasome degradation. S100A9 promotes the proliferation and invasion of HCC cells by interacting with RAGE to upregulate ERK1/2 and p38MAPK, thereby activating the MAPK pathway. RAGE, receptor for advanced glycation end products; miR, microRNA; LATS1, large tumor suppressor kinase 1; YAP, Yes-associated protein; SOCS1, suppressor of cytokine signaling 1; HCC, hepatocellular carcinoma.

inflammation and/or fibrosis (98). Furthermore, NAFLD may ultimately progress to liver cirrhosis and HCC (99). Recently, Zhang *et al* (100) established a mouse model of NAFLD and found that S100A4 expression was increased in NAFLD, whereas decreased inflammation and liver fibrosis were observed in S100A4 knockout mice. In a NAFLD rat model, serum S100A9 levels are positively correlated with the degree of hepatic steatosis, lobular inflammation and NAFLD activity score, indicating that these may be a potential biomarker of liver and metabolic progress (101). Zhang *et al* (102) established a tree shrew model of NAFLD and identified that overexpression of S100A11 promoted FOXO1-mediated autophagy and adipogenesis via the S100A11/histone deacetylase 6/FOXO1 axis, thereby promoting hepatic steatosis and providing a potential therapeutic target for NAFLD. However, further experiments are required to identify the role of autophagy and lipogenesis in the pathogenesis of NAFLD (103). Another study reported that S100A11 promotes liver inflammation and fibrosis in a mouse model of NAFLD and that upregulation of S100A11 may be a key feature in the transition from steatosis to NASH/fibrosis in mice (104). However, current studies on the role of the S100 family in NAFLD primarily use cellular and animal models and further analysis of clinical samples is required.

ALD. ALD often occurs in individuals who drink a significant amount alcohol over a long period of time. There were 1,256,900 deaths in 2016 due to cirrhosis and chronic liver disease (105). Among those, 334,900 (27%) were attributed to alcohol (105). The initial stage of ALD is alcoholic fatty liver (AFL), which is caused by alcohol and its metabolites (106). The primary effect of AFL on the liver includes synthesis and breakdown of fatty acids, which leads to hepatitis and fibrosis, and ultimately cirrhosis and HCC, in a subset of drinkers (those with viral hepatitis, diabetes or a history of smoking) (106-108). Studies have shown an increased risk of alcoholic hepatitis and cirrhosis among individuals drinking >40 g/day (109-111). A previous study reported that ethanol-fed mice exhibit higher levels of S100A4 in the liver, whereas S100A4-knockout mice have decreased liver inflammation, and that S100A4 promotes early alcoholic hepatitis primarily by activating the STAT3 pathway (112). At

the same time, S100A4 inhibits lipid accumulation in chronic alcohol-induced fatty liver (112). To be best of our knowledge, no studies have analyzed clinical samples of ALD.

HCC. As one of the most common types of malignancy worldwide, recent statistics have shown that liver cancer is the sixth most commonly diagnosed cancer and is the fourth leading cause of cancer-associated mortality (113). There were 905,677 new cases of liver cancer and 830,180 deaths in 2020, which accounted for 4.7% of new cancer cases and 8.3% of deaths, respectively (114).The most common type of primary liver cancer is HCC, which accounts for 80-90% of cases and usually presents following chronic liver disease (113). Currently, the most effective treatments for HCC are surgery, liver transplantation, chemotherapy and targeted therapy, but the overall survival rate remains unsatisfactory (115). The highest 5-year survival rate in the world was 27.9% in Taiwan, China (116). Furthermore, for patients with recurrence or distant metastases, prognosis is particularly poor (117). Therefore, the treatment of HCC requires further research and investigation.

Guo *et al* (118) demonstrated that S100A1 is upregulated in HCC tissue (n=104) and tumor size, level of differentiation and survival are correlated with its upregulation. Increased S100A1 expression decreases large tumor suppressor kinase 1 phosphorylation, which leads to downregulation of Yes-associated protein (YAP) phosphorylation in the cytoplasm, allowing YAP to enter the nucleus and thus regulating the Hippo pathway to produce oncogenic effects (118) (Fig. 2). Moreover, Tao *et al* (119) analyzed HCC tissue (n=62) and identified increased expression levels of the S100A3 gene, the activation of which was associated with development and invasiveness of HCC.

S100A4 also serves a key role in the occurrence of HCC and is associated with the development, invasion and recurrence of HCC (28,30). A previous study analyzed HCC tissue (n=72) and revealed that S100A4 expression was significantly increased and negatively correlated with overall survival, which was associated with differentiation, invasion and recurrence of HCC (30). Similarly, Zhang *et al* (120) analyzed invasive (n=20) and non-invasive HCC (n=20) tissue; S100A4 was highly expressed in invasive HCC tissue and was positively correlated with the invasiveness of HCC. Moreover, it was suggested its underlying mechanism may involve activation and translocation of NF-κB p65 into the nucleus, thereby upregulating MMP-9 to promote cell migration and invasion. Zhai *et al* (121) reported that upregulation of S100A4 expression is associated with the aggressive and malignant phenotype of HCC, as determined by analyzing HCC tissue (n=113). A recent study showed that tumor size in HBV-HCC is associated with S100A4 expression; elevated expression of S100A4 promotes HCC cell proliferation and is associated with prognosis of patients with HCC (122).

The tumor microenvironment serves an important role in tumor progression (123,124). An important component of the tumor stroma is mesenchymal stem cells (MSCs), which serve a key role in tumor proliferation and metastasis (125,126). Previous studies have shown that certain microRNAs (miRs) act as typical oncogenes or tumor suppressor genes (127-129). miR-155 is upregulated in a mouse HCC model (130) and aberrant expression of miR-155 accelerates proliferation of HCC cells (131,132). Yan *et al* (28) reported that S100A4 is highly expressed in liver cancer-associated MSCs *in vivo* and that it promotes proliferation and metastasis of liver cancer cells by upregulating expression levels of miR-155 in these cells, subsequently downregulating suppressor of cytokine signaling 1 expression and activating STAT3 signaling to upregulate MMP-9.

Cancer stem cells have the ability to undergo self-renewal and differentiation (133). These cells are have the potential to form tumors and develop into cancer (133). In previous studies, S100A4 promoted the development of liver fibrosis (59,69). In a recent study, Li *et al* (134) established a mouse model of HCC involving significant liver fibrosis; S100A4-deficient mice were found to have decreased HCC nodules and unchanged hepatomas, as well as decreased expression levels of stem cell markers in liver fibrosis and HCC tissue. It has also been shown that S100A4 in the presence of collagen I promotes carcinogenesis by synergizing with RAGE and activating β-catenin signaling, thereby increasing the stemness of cancer cells (134).

A 2002 study reported that S100A6 expression is upregulated in 10% (n=20) of patients with HCC and its expression is significantly lower compared with that in cholangiocarcinoma (135). Hua *et al* (136) showed that the difference in S100A6 expression between HCC (n=51) and normal liver tissue (n=10) was >10-fold and S100A6 expression was upregulated in 31.4% of HCC samples. The different results of these two studies may be due to different sample sources and sizes, thus additional clinical samples are required for further investigation. At the cellular level, S100A6 is highly expressed in HepG2 cells (137). S100A6 is highly expressed in 36.2% (n=47) of HCC tissue samples (138). Following silencing of S100A6 in HepG2 cells, cell proliferation is inhibited; following restoration S100A6 expression, the proliferation and motility of HepG2.2.15 cells was restored (138). The proposed mechanism is that S100A6 expression results in downregulation of E-cadherin on the plasma membrane and promotes nuclear accumulation of β-catenin in cells, which ultimately leads to proliferation and invasion of liver cancer cells (138). A recent study also reported that the expression levels of S100A6 are higher in HCC (n=6) compared with adjacent liver tissue and that overexpression of S100A6 in HepG2 cells promotes p53 ubiquitin-dependent proteasomal degradation, which regulates expression of p21 and ultimately promotes proliferation and migration of HCC cells (139).

S100A9 was demonstrated to be upregulated in HCC as early as 2000; it is also associated with poorly differentiated HCC (140). Studies have reported that S100A9 is upregulated in mouse HCC models and liver cancer cells (141,142). Wu *et al* (142) confirmed that S100A9 is highly expressed in HepG2 cells (primarily in the cytoplasm) and promotes proliferation and invasion of liver cancer cells by upregulating ERK1/2 and p38, thereby activating the MAPK pathway. These functions of S100A9 were primarily generated via interaction with RAGE (143). In a study of S100A9 and HBV-associated HCC, S100A9 served a key role in HBV-encoded X protein-induced HCC growth and metastasis, while TNM stage and liver metastasis status of HBV-associated HCC were associated with serum S100A9 expression (144).

Moreover, a recent study showed that S100A9 secretion is upregulated by tumor-associated macrophages, which subsequently enhances stem cell-like properties of liver cancer cells to promote tumor development (145).

In a previous study, S100A10 was observed to be highly expressed in liver cancer cell lines (146). The same results were reported in a recent study by Zhao *et al* (147). However, these studies were limited to *in vitro* experiments, and further *in vivo* experiments and analysis of clinical samples are required. EGFR belongs to the family of growth factor receptor tyrosine kinases and serves an important role in the survival, proliferation and motility of tumor cells (148). The type III EGFR deletion mutant (EGFRvIII) is the most common EGFR mutant (149). A previous study demonstrated that S100A11 expression is increased in 68.6% of HCC tissue samples (n=51) and promotes HCC cell invasion and migration primarily via the EGFRvIII/-STAT3 pathway (150). Recent studies have also shown that S100A11 is secreted by cancer cells and is a marker of liver cell dedifferentiation and also promotes cell proliferation and migration (104,151). Furthermore, increased expression of S100A11 is associated with poor prognosis in high-grade HCC cases (104). By analyzing HCC tissue (n=130), Cai *et al* (152) reported that S100A12 is expressed only in the cytoplasm of stromal cells. In addition, high expression of S100A12 in patients with HCC is an independent prognostic factor for overall and progression-free survival (152).

4. Perspectives of S100 family in clinical applications in liver disease

The treatment of liver disease is an area of ongoing research. When progressing to end-stage liver disease, patient survival rates remain low, although current treatments, such as surgical techniques and therapeutic regimens, have improved on previous approaches (153,154). The research and clinical treatment of liver disease is limited by the lack of sensitive markers of liver fibrosis, which also limits the development of anti-fibrotic drugs (155). Previous studies have suggested that S100A4 may be a potential marker and therapeutic target for liver fibrosis (59,69). A recent study by Yan *et al* (94) demonstrated that the combination of serum S100A4 and liver stiffness assay improved the accuracy of diagnosis of severe fibrosis in hepatitis B. Therefore, serum S100A4 levels may be used as a marker of liver fibrosis in patients with CHB. S100A9 may also be a potential marker of NAFLD and metabolic progression (101). Among other S100 proteins, S100A11 may provide a potential therapeutic target for NAFLD (102) and the upregulation of S100A11 may be amarker of the transition from steatosis to NASH/fibrosis (104).

The treatment of HCC is a hot topic of research. Treatment outcomes remain poor when progressing to end-stage HCC. In particular, the prognosis remains poor for patients with recurrence or distant metastasis (117). Among the S100 protein family, S100A1, S100A3, S100A4, S100A6, S100A9, S100A10, S100A11 and S100A12 are upregulated in HCC and may be potential therapeutic targets and/or markers for HCC (30,118-121,138,141,147,150,152). Among these, high expression of S100A1 can be used as a predictive marker for poor prognosis of HCC (118). Furthermore, S100A4 can be used as a therapeutic target and a useful indicator of tumor aggressiveness and prognosis (30). In addition, S100A6 can be used as an important marker for HCC (139), while S100A9 can be used as a marker of HCC metastasis (144). Huang *et al* (156) revealed that assessment of urinary S100A9 and granulocyte protein levels may be helpful in the diagnosis of early HCC. Moreover, S100A9 may be a potential target for the treatment of HCC (145). In a recent retrospective study, Meng *et al* (157) examined patients with HCC (n=379) who underwent radical resection and found that elevated serum S100A9 levels were associated with poor prognosis, suggesting that S100A9 may be a prognostic indicator following HCC resection. In HepG2 cells, S100A10 inhibits proliferation by upregulating miR-590-5p (146). At the cellular level, LINC00174 is be associated with the development of HCC and its downstream genes included S100A10 and miR-320 (147). Thus, S100A10 may be a therapeutic target and biomarker for HCC. Similarly, S100A11 and S100A12 may be potential targets and biomarkers for immunotherapy for HCC (104,150,152).

In the study of S100 protein as a therapeutic target, sodium cantharidinate inhibits expression of S100A3 in HepG2 cells and suppresses cell viability (119). Zhang *et al* (158) reported that decreased tumor incidence and growth following anti-miR-21 treatment in a mouse model may be associated with decreased fibrosis and high consumption of S100A4. Moreover, decreased expression of S100A4 following anti-miR-21 treatment suggests that anti-miR-21 may be effective in tumor-targeted therapy, but further studies are required to determine whether anti-miR-21 treatment is effective as an adjuvant therapy to drugs that kill tumor cells. A study by Jiao *et al* (159) using a mouse model of HCC treated with ganciclovir injection, reported a decrease in incidence of HCC and tumor size in model mice compared with controls, but this change was not statistically significant. Therefore, S100A4 as a target for treatment of HCC requires further investigation. At present, there are only therapeutic approaches targeting S100A3 and S100A4 (158,159) and additional experiments are needed to target S100 protein for the treatment of HCC.

5. Conclusion

In numerous types of liver disease, especially liver fibrosis and HCC, S100 protein is highly upregulated and promotes the development and progression of liver disease. Both S100A4 and S100A6 promote the development and progression of liver fibrosis by activating HSC and may be potential therapeutic targets and markers for liver fibrosis (59,86). S100A1, S 100A3, S100A4, S100A6 S100A9, S100A10, S100A11, and S100A12 are all upregulated during HCC and may be potential targets and/or markers of HCC (30, 118-121,138,141,147,150,152). However, the mechanisms of action remain unknown and further research is required. In mouse experiments, inhibition of S100A4 expression decreases tumorigenesis and development. Moreover, inhibition of S100A10 at the cellular level inhibits proliferation. In conclusion, S100 protein is a promising target and potential marker for liver disease treatment in future. However, drugs targeting S100 protein need to be examined further in animal experiments and clinical studies.

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Authors' contributions

SY and XY made substantial contributions to the conception and design of the study. JA, HJ, GW, HW and BT were involved in revising the manuscript critically for important intellectual content. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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