

Liver sinusoidal endothelial cells as potential drivers of liver fibrosis (Review)

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Abstract. Liver fibrosis due to viral or metabolic chronic liver diseases is a major challenge of global health. It is a critical pre-stage condition of severe hepatopathy, characterized by excessive accumulation of extracellular matrix components and ongoing chronic inflammation. To date, early prevention of liver fibrosis remains challenging. As the most abundant non-parenchymal hepatic cell population, liver sinusoidal endothelial cells (LSECs) are stabilizers that maintain the intrahepatic environment. Notably, LSECs dysfunction appears to be implicated in the progression of liver fibrosis via numerous mechanisms. Following sustained liver injury, they lose their fenestrae (cytoplasmic pores) and change their crosstalk with other cellular interactions in the hepatic blood environment. LSEC-targeted therapy has shown promising effects on fibrosis resolution, opening up new opportunities for anti-fibrotic therapy. In light of this, the present study summarized changes in LSECs during liver fibrosis and their interactions with hepatic milieu, as well as possible therapeutic approaches that specially target LSECs.

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1. Introduction

As it has long been linked to severe morbidity and mortality, liver fibrosis is one of the most significant contributors to the worldwide disease burden (1). The liver, the most important detoxification organ, maintains a stable metabolic balance within the body. After exposure to toxic metabolites, viruses and alcohol that interfere with the normal physiological functions, the liver develops drug-induced liver injury, viral hepatitis and alcoholic liver disease (2), eventually progressing to fibrosis. In addition, nonalcoholic fatty liver disease (NAFLD) is a continuum of liver diseases, starting from simple steatosis (NAFL) and gradually progressing to nonalcoholic steatohepatitis (NASH), which can progress to liver fibrosis and cirrhosis. As NAFLD is a disease caused by excessive fat accumulation in the liver closely related to metabolic dysfunction, which excludes alcohol and other diseases, a recent consensus among international experts has proposed NAFLD as a change of nomenclature for metabolic dysfunction-associated fatty liver disease (3). Fibrosis is a risk factor for cirrhosis and hepatocellular carcinoma (4). In addition, liver fibrosis can result in chronic portal hypertension, a common clinical complication (5).

Liver fibrosis is characterized by excessive deposition of extracellular matrix (ECM) and its reduced degradation. The activation of hepatic stellate cells (HSCs) is a well-known event in liver fibrosis. HSCs are primarily activated by stimulated Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) via paracrine production of pro-inflammatory and pro-fibrotic cytokines such as TGF- β , connective tissue growth factor and platelet-derived growth factor (PDGF) and then transform into resilient myofibroblast which produces ECM to form fibrous scars around the damaged area (6). If liver injury is acute or self-limiting, the liver returns to normal due to the specific destruction of collagen and ECM by matrix metalloproteinases (MMPs). Otherwise, persistent injury can cause chronic inflammation and ECM deposition, eventually

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leading to replacement of parenchymal cells by scar tissues and ultimately cirrhosis. How to balance the synthesis and degradation of ECM is still challenging. Although regression of liver fibrosis in patients with chronic liver disease has been recognized for decades, there are still no FDA-approved anti-fibrosis drugs. Although the exact definition of fibrotic regression has not been properly established (7), the success of antiviral therapy has given us hope to elucidate the cellular and molecular mechanisms and develop therapeutic agents that mimic the endogenous capabilities of the liver (8).

Dysfunction of LSECs allows initiation and progression of liver fibrosis and is responsible for its clinical complication, portal hypertension. LSECs create a bridge between hepatocytes and hepatic sinusoids, acting as protective gatekeepers of the microvascular environment (9). Although HSC activation is the primary event in liver fibrosis, it is still important and necessary to seek other new directions to reverse the condition.

The present review summarized the structural and functional alterations of LSECs and their crosstalk with hepatic microenvironment during liver fibrosis and highlighted the role of LSECs in portal hypertension. Several novel therapeutic strategies targeting LSECs are also reviewed.

2. Physiological characteristics of LSECs

Compared to other endothelial cells, LSECs are morphologically and functionally specialized. LSECs are the most abundant non-parenchymal cells in the liver and form the sinusoidal wall (10). LSECs represent a gatekeeper between hepatocytes and hepatic sinusoids during liver injury. They are highly specialized as nondiaphragmatic fenestrae (cytoplasmic pores) and lack a basal membrane, facilitating high-efficient material exchange from the bloodstream into the space of Disse and regulate hepatic stellate cell activation status (11). The fenestrae are 100-150 nm in size and are organized in clusters termed sieve plates (12). Their size and number varies across liver lobules with larger but fewer fenestrae per sieve plate in the periportal region and smaller but more numerous fenestrae per sieve plate in the centrilobular region (13,14), which may be related to the need for oxygen exchange along the lobules. Notably, heterogeneities between periportal and centrilobular LSECs exist in morphology (15), antigen expression (16) and lectin affinity (17). Di Martino *et al* (18) established an approach of stimulated emission depletion (STED) microscopy to visualize and analyze LSEC fenestrae and revealed that cytoskeleton, especially actin, is closely associated with fenestrae formation. Herrnberger *et al* (19) found that plasma-membrane vesicle-associated protein (PLVAP) is required for the formation of endothelial diaphragms and endothelial fenestrae and opening fenestrae in LSECs are critical for the passage of lipoproteins.

In addition to their unique structure, LSECs contain various receptors to perform multiple functions. A total of three endocytosis receptors have been identified on LSECs, collagen- α -chain/mannose receptor, hyaluronan/scavenger receptor and Fc γ IIb2 receptor (20). Among them, mannose receptor is the main candidate for endocytosis of denatured collagen on LSECs and functions as MMPs, making it a promising target in the study of hepatic fibrosis (21). Through the combined effect of endocytic vesicles and receptor-mediated

endocytosis, LSECs can eliminate antigens, cell debris and immune complexes. SR-H/stabillin-1 and SR-H/stabillin2, the main scavenger receptors of LSECs, mediate endocytosis of polyanionic molecules, including oxidized low-density lipoproteins, hyaluronan, chondroitin sulfate, formaldehyde treated serum albumin, procollagen type I and III N-terminal peptides and advanced glycation end products (22). Fc γ IIb2 receptor on LSECs mediates vascular immunity mainly through cleaning circulating IgG immune complexes (23). Under homeostatic conditions, LSECs are not merely endocytic fenestrated endothelial cells, they also exhibit vasodilatory, anti-inflammatory, anti-thrombotic and anti-fibrotic phenotypes, which are important for orchestrating liver microenvironment response and regulating intrahepatic vascular tone and immune cell function (11).

3. Changes of LSECs in liver fibrosis

Capillarization. Maintenance of the highly specialized phenotype of LSECs is crucial for liver homeostasis. During liver fibrosis and cirrhosis, loss of LSEC phenotype and functions amplify liver damage by undergoing a process called 'capillarization', which is clarified by the loss of fenestrae (cytoplasmic pores) and the manifestation of a basement membrane, leading to LSEC dysfunction (20).

Su *et al* (24) mapped the spatial distribution of heterogeneous liver endothelial cells in normal and cirrhotic mouse liver and identified zone-specific transcriptomic changes of LSECs associated with liver cirrhosis using small conditional RNA-seq technology, finding that zone 3 LSECs are more susceptible to capillarization and that CD34 is more useful than conventional CD31 as a marker of capillarization in LSECs. In 2008, Deleve *et al* (25) employed cell co-culture to demonstrate that capillarized LSECs are unable to maintain HSC quiescence due to loss of VEGF-stimulated release of nitric oxide (NO), while differentiated LSECs can prevent HSC activation and promote reversion of activated HSCs to quiescent HSCs. This study highlighted the critical role of LSECs in regulating HSC status. In addition, capillarization of LSECs occurs in the early stage of NASH. An *in vivo* study showed that loss of the unique fenestrated phenotype is an initial step for fibrogenesis, preceding the activation of HSCs and onset of fibrosis in alcoholic liver injury (26), suggesting that signals from LSECs may be one of the earliest triggers of HSC activation.

Regulation of the LSEC phenotype. The exact mechanisms regulating the loss of fenestrae have not been fully elucidated, but several molecules and pathways have been identified. Maintenance of the fenestrated LSEC phenotype requires VEGF, which is derived from hepatocytes and HSCs. VEGF is a key regulator for the LSEC phenotype (27). It has been established from various *in vitro* and *in vivo* studies that VEGF operates in two pathways, the NO-dependent and NO-independent (28). In the NO-dependent pathway, endothelial nitric oxide synthase (eNOS)-NO-cyclic guanosine monophosphate signaling is critical for regulating the formation of fenestrae in LSECs (29). Endothelial Notch activation leads to LSEC dedifferentiation and promotes liver fibrogenesis through eNOS-soluble guanylate cyclase

Table I. Involved regulators for the LSEC phenotype.

Type	Molecule	Mechanism(s)	(Refs.)
Fenestration factors	VEGF	eNOS-NO-cGMP signaling pathway	(29)
	KLF2	Upregulates eNOS expression	(36)
	lncRNA Airn	through KLF2-eNOS-sGC pathway and interacts with EZH2	(34)
	GATA4	Prevents a pathogenic switch in angiocrine signaling	(35)
	Zeb2	Preserves liver angioarchitecture and protects against liver fibrosis	(37)
	BMP9	Promotes GATA4 and PLVAP expression	(39)
	HB-EGF	Interferes in the nitric oxide pathway	(38)
Defenestration factors	Notch signaling	Binds to DLL4 and induces LSEC dedifferentiation through eNOS-sGC signaling	(30,31)
	Hh signaling	Induces formation of vascular tubes by binding to Ptc and activating Smo	(32)
	miR-511-3p	Activates Hh signaling via targeting Ptc1	(33)
	endothelial JAM-A	Triggers Hh signaling and loss of VEGFR1/2	(10)

eNOS, endothelial nitric oxide synthase; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; KLF2, Krüppel-like factor 2; lncRNA, long noncoding RNA; sGC, soluble guanylate cyclase; EZH2, enhancer of zeste homolog 2; Zeb2, zinc-finger E-box-binding homeobox2; PLVAP, plasmalemma vesicle-associated protein; HB-EGF, heparin-binding EGF-like growth factor; DLL4, Delta-like ligand 4; LSEC, liver sinusoidal endothelial cell; Hh, Hedgehog; Ptc, Patched; Smo, Smoothened; miR, microRNA; JAM-A, junctional adhesion molecule A; Ptc1, Patched homolog 1.

(sGC) signaling. Regarding Notch ligands, Delta-like ligand 4 (DLL4) is predominantly expressed in endothelial cells under hypoxic conditions during liver fibrosis (30). It is reported that DLL4 overexpression accelerated defenestration of LSECs and production of basement membrane in both human fibrotic livers and carbon tetrachloride (CCl₄)-induced mouse livers (31). In addition to Notch signaling, the Hedgehog (Hh) pathway also induces LSEC capillarization and formation of vascular tubes *in vitro* by Hh ligands binding to their transmembrane receptors [Patched (Ptc)] to activate Smoothened (32). Besides, miR-511-3p was found to positively regulate Hh signaling by targeting Ptc1 in hepatic sinusoidal obstruction syndrome (33). Long noncoding RNA (lncRNA) Airn was identified to interact with enhancer of zeste homolog 2 to maintain LSEC differentiation through Krüppel-like factor 2 (KLF2)-eNOS-sGC pathway, thus indirectly inhibiting HSC activation (34). Studies have found that several transcription factors in liver endothelial cells also play key roles in regulating the characteristics and function of LSECs. Genetic endothelial GATA4 deletion leads to liver fibrosis and hepatopathy by GATA4-MYC-PDGFB axis (35). In 2015, transcriptional factor KLF2 was found to upregulate eNOS expression, which is essential for maintaining a functional endothelial phenotype and also explains the molecular mechanisms of statins (36). In 2022, de Haan *et al* (37) found that LSEC-enriched zinc-finger E-box-binding homeobox2 (Zeb2) can preserve the liver angioarchitecture and prevent liver fibrosis.

On the other hand, capillarization is thought to be the repair of damaged LSECs by bone marrow endothelial progenitors that engraft but fail to fully mature through cell autonomous pathways that inhibit the NO-dependent pathway. Maretti-Mira *et al* (38) identified heparin-binding EGF-like growth factor (HB-EGF) as a signal to maintain HSCs quiescence while the immature LSECs from bone marrow were

unable to shed HB-EGF from the cytosolic membrane. These data demonstrate that capillarization is an early event in the fibrotic process and is a perfect target for the treatment of liver fibrosis. The number of fenestrae was shown to be significantly decreased in LSECs from BMP9-knockout mice and addition of BMP9 to the LSECs culture recovers fenestrae and elevates the expression of GATA4 and PLVAP, suggesting that BMP9 is a key paracrine regulator of LSEC fenestration (39). Conversely, Haristi *et al* found that high BMP9 in combination with LPS stimulation induced the expression of certain capillarization markers in LSEC (40). Desroches-Castan *et al* (41) found that the role of BMP9 in LSEC differentiation depends on genetic background of C57BL/6, BALB/c and 129/Ola mice. Further studies may warrant the exact roles of BMP9 in LSEC capillarization.

In addition, cell-specific junctional adhesion molecule A (JAM-A) is reported to exert crucial functions in hepatic fibrogenesis (10). Loss of endothelial JAM-A induces LSEC capillarization and HSC activation by triggering Hh signaling and loss of VEGFR1/2 with no alterations in myeloid recruitment (10). Elevated portal blood lipopolysaccharide (LPS) due to leaky gut induces LSEC capillarization in mice and *in vitro*, while antimicrobial treatment lowers portal blood LPS concentration and inhibits LSEC capillarization (42). Overall, the signaling pathways that regulate LSEC fenestration varies under physiological and pathological conditions and the mechanisms involved by various factors are summarized in Table I.

4. Changes in crosstalk of LSECs in hepatic microenvironment

Increasing evidence has indicated that fibroblast-like cells in liver fibrosis are mainly derived from activated hepatic stellate cells (aHSCs). Therefore, HSC activation is considered a pivotal event leading to fibrosis. HSC activation is fine-tuned

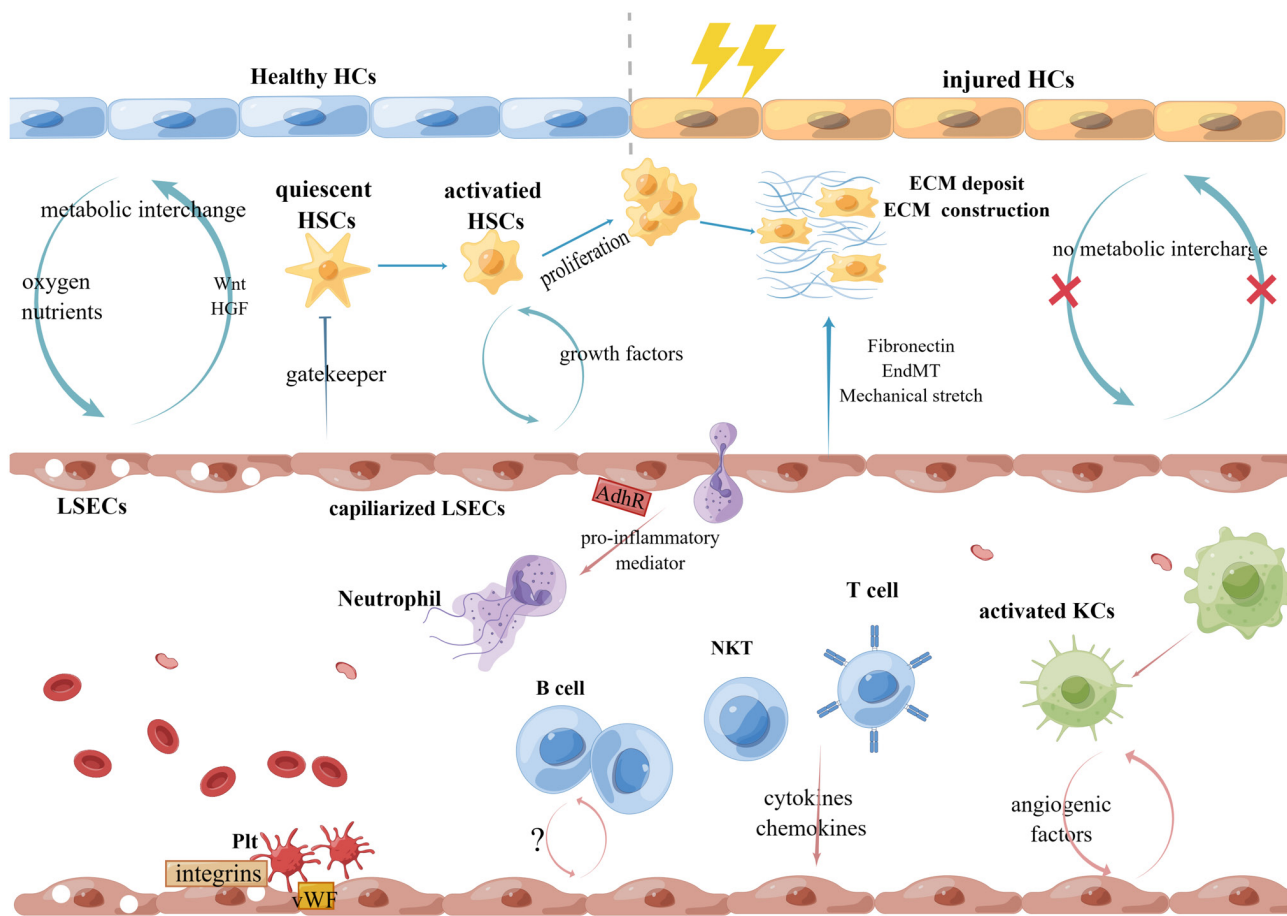


Figure 1. The crosstalk between LSECs and hepatic microenvironment in liver fibrosis (using Figdraw). LSECs, liver sinusoidal endothelial cells; HCs, hepatocytes; HSCs, hepatic stellate cells; ECM, extracellular matrix; HGF, hepatocyte growth factor; EndMT, endothelial-mesenchymal transition; AdhR, adhesion receptor; NKT, natural killer T cells; KCs, Kupffer cells; Plt, platelet; vWF, Von Willebrand factor.

by the angiocrine signaling from LSECs and is also tightly modulated by dynamic interactions between LSECs and other cell types in the liver. Understanding these crosstalk is expected to improve liver disease therapy (Fig. 1).

LSECs and HSCs. HSCs are the main source of ECM and thus have been the central to the development of liver fibrosis. The interaction between LSECs and HSCs forms a positive feedback loop. The starting point of the loop is VEGF. As described above, VEGF from hepatocytes and HSCs maintains the phenotype of fenestrated LSECs and, in turn, NO from VEGF-stimulated LSECs prevents HSC activation. Therefore, VEGF may have a dual role in fibrosis. It induces fibrosis-associated angiogenesis while recruiting monocytes mainly through the CXC chemokine (CXCL)9-MMP13 axis to control the secretion of multiple MMPs and tissue inhibitor of MMPs during fibrosis resolution (43). Therefore, as a key mediator of angiocrine signaling, the regulatory role of VEGF in liver diseases remains to be elucidated. Endothelin-1 from HSCs also plays a key role in the regulation of eNOS activation in LSECs (44). In addition, capillarized LSECs activate HSCs through secreting PDGF, TGF- β and lowering a transcription factor KLF2, which acts as a vasoprotective molecule of the liver endothelium, subsequently activating HSCs (45). Activated HSCs further act on quiescent HSCs and LSECs by autocrine TGF- β 1 (46). Thrombospondin-1 can be induced by

HSC activation, enhancing LSEC capillarization by blocking the NO-dependent pathway (47). In addition, LSECs stimulate HSC migration by producing CXCL12/stromal-derived factor-1. CXCL12 binds to CXC chemokine receptor (CXCR)4 on HSCs, thereby inducing HSC proliferation and collagen production perpetuating fibrosis, while CXCR7-expressing LSECs activated by CXCL12 initiate liver regeneration (48). Enhancement of intracellular adipocyte fatty acid-binding protein (A-FABP) potentiates the LSEC capillarization by inducing Hh signaling, leading to impairment of the gatekeeper function of LSECs on HSC activation. On the other hand, LSEC derived A-FABP releases and acts on HSCs in a paracrine manner to potentiate the transactivation of TGF- β 1 by activating JNK/c-Jun signaling. Elevated TGF- β 1 subsequently promotes the expression of ECM in both paracrine and autocrine manners. These findings define that A-FABP is a novel therapeutic target of liver fibrosis (49). The contribution of exosomes and their cargoes has been proposed as a new model of intercellular communication between LSECs and HSCs. LSEC-derived sphingosine kinase 1-containing exosomes regulate HSC signaling and migration through fibronectin-integrin-dependent exosome adherence and dynamin-dependent exosome internalization (50).

LSECs and hepatocytes. Loss of fenestration of LSECs protects the liver from ongoing damage by limiting toxins to

specific areas (19). However, the formation of a continuous basement membrane lining the sinusoids disrupts the bidirectional exchange of oxygen and nutrients between hepatocytes and sinusoids (51), as well as the lipoprotein secretion of hepatocytes and the de novo lipogenesis in hepatocytes which aggravates hepatic steatosis in NASH (52). LSECs can also secrete Wnt and hepatocyte growth factor (HGF), which together act as key hepatocyte mitogens with a potent liver regenerative capacity that is readily switched to a pro-fibrotic phenotype, in which the Erk1/2/Akt axis in LSECs acts as a switch (53). Growth factors from LSECs, such as bone morphogenetic protein (BMP)2, BMP6 and TGF β 1 act on hepatocytes and HSCs to control systemic iron homeostasis and fibrotic processes in a paracrine manner, respectively (54). Notch activation can alter the angiocrine profile of LSECs to compromise hepatocyte proliferation and liver regeneration through downregulating critical hepatocyte mitogens, including Wnt2a, Wnt9b and HGF (31). A recent study found that lncRNA Airn, a nuclear localized and highly unstable in the form of non-splicing 108 kb ncRNA from LSECs, promoted hepatocyte proliferation by increasing paracrine secretion of Wnt2a and HGF from LSECs (34). lncRNA Airn is inherent and might be a serum biomarker for liver fibrogenesis. Indeed, after acute injury, endothelial cells determine if the liver undergoes regeneration or fibrosis by angiocrine signaling (55). LSECs and hepatocytes also communicate with each other through the VEGF-A/VEGFR-2 signaling which is regarding to angiogenesis. Yan *et al* (56) investigated the biological roles of cluster of differentiation (CD)147 in a CCl₄-induced liver fibrosis mouse model and found that CD147 promoted liver fibrosis progression via VEGF-A/VEGFR-2 signaling, mediating crosstalk between hepatocytes and LSECs. A combination of leukocyte derived chemokine2 expressed by hepatocytes and Tiel produced by LSECs can promote LSECs capillarization and reduce portal angiogenesis (57).

LSECs and macrophages. Hepatic macrophages are important to the pathogenesis of chronic liver injury. Activated KCs can secrete a wide variety of proinflammatory activating HSCs. KCs and infiltrating macrophages can usually be divided into two subtypes, including M1 macrophages, which produce proinflammatory cytokines such as TNF- α , IL-1b, CCL2 and CCL5, and M2 macrophages which secrete a distinct set of mediators including IL-13, IL-10, IL-4 and TGF- β (58,59). Imbalance of M1/M2 ratio may be the key to the progression of NASH to liver fibrosis (52). Pro-inflammatory KCs may also produce cytokines and chemokines which increase the expression of adhesion molecules by LSECs, leading to leukocyte infiltration and activation (60). In a murine model of acute liver injury caused by overdose of acetaminophen, You *et al* (61) found that liver resident macrophages and infiltrating macrophages express an array of angiogenic factors and induce LSEC proliferation and migration to repair liver blood vessels, while in NASH, the capillarization of LSECs is necessary for activating KCs in that LSECs injury appeared during the simple steatosis phase and preceded the appearance of activate KCs and HSCs (62). Ford *et al* (63) investigated the effects of KCs on human liver sinusoidal endothelial cells (hLSECs) phenotype on soft materials, which indicate that TNF- α secreted by KCs undermines the effects of a soft

matrix that is representative of healthy tissues and leads to loss of LSEC fenestrae. The relationship between LSECs and macrophages in liver fibrosis needs further investigation.

LSECs and lymphocytes. LSECs belong to nonprofessional antigen presenting cells, plasmacytoid DCs and are primary immunologic mediators underlying hepatic tolerance. They express major histocompatibility complex (MHC) class I, MHC class II and the co-stimulatory molecules CD80, CD86 and CD40 (11). The LSECs mediate hepatic immune tolerance toward self or foreign antigens by expression of anti-inflammatory mediators under physiological conditions, but they gain pro-inflammatory functions upon viral infection (64). LSECs release cytokines via toll-like receptors and contribute to viral infection of the liver, such as hepatitis B or hepatitis C virus (65). The physiologic consequences of higher antigen-presenting cell-related immunogenicity is associated with elevated intrahepatic inflammatory milieu in fibrosis. These LSECs gain enhanced capacity to capture antigens and induce the immunogenic T cell to sensitize endogenous cytotoxic T lymphocytes (66). During liver fibrosis, Th1 and Th2 cells recruit and adhere to liver sinusoids using α 4 β 1-integrin and vascular adhesion protein-1 (VAP-1), respectively (67). However, the specific mechanisms by which T cells and LSECs crosstalk regulate sinusoidal capillarization remain largely unknown. Emerging evidences suggested B-cell activation is involved in NAFLD (68), while crosstalk between LSECs and B-cell remains to be elucidated. In addition, chemokine receptor CXCR6 on LSECs and its ligand CXCL16 on natural killer T cells (NKTs) control NKT cell migration (69).

LSECs and leucocytes. Inflammation is an essential part involved in the process of liver fibrosis. Hepatitis and NAFLD, especially NASH, have recently received increasing attention (70). LSECs interact with portal circulation via expression of surface receptors and release of paracrine factors, such as intercellular adhesion molecule, vascular cell adhesion molecule-1, VAP-1, chemokines and cytokines (71), recruiting the circulating leucocytes to enter the liver parenchyma. Exploring these inflammatory mediators may help to discover new therapeutic targets for the treatment of liver fibrosis. Gola *et al* (72) considered LSECs as a microbiome sensor, sustaining commensal-induced MyD88-dependent signaling to regulate the composition of the peri-cellular matrix involved in chemokine gradient formation. *In vitro* and *in vivo* findings demonstrate that specific focal adhesion proteins recruited by phosphofructokinase can parlay LSEC mechanotransduction into stiffness-induced angiocrine signaling and mediate neutrophil infiltration and promote liver fibrosis (73). By contrast, Brozat *et al* found that LSEC-derived JAM-A had no effect on cell migration from sinusoids or adhesion to LSECs (10), suggesting a complex role of leukocyte-endothelial cell interactions in promoting liver fibrosis.

LSECs and platelets. At present, an increasing number of individuals realize that the platelets are no longer bystanders in liver diseases and anti-platelet therapy has been proven effective in treating chronic liver injury (74,75). *In vitro* studies show that platelet adhesion is partly mediated by integrin (glycoprotein IIb/IIIa and α v β 3) with human hepatic sinusoidal endothelial

cells (76), leading to platelet and endothelial activation and leukocyte recruitment. On the other hand, in wild-type mice after chronic CCl₄ challenge plasma Von Willebrand factor levels are increased (77), which could be associated with the interaction between platelets and LSECs. However, it is not clear whether platelet adhesion is associated with capillarization. Platelets have well appreciated roles in physiological and pathological processes including inflammation, tissue repair, angiogenesis and tumor growth (78). A few studies have focused on the release of platelet granule content or platelet RNA transfer to activated HSCs (79,80), but the influence of platelets on LSECs needs more research. Although platelets have been shown to simulate proliferation of cultured hepatocytes (81), this is a long way to elucidating the role of modulators of platelets in chronic liver diseases.

LSECs and ECM. While most researchers focused on intercellular crosstalk of LSECs, Brougham-Cook *et al* (82) redirected attention to identify the unique phenotypes that LSECs exhibit due to ECM composition, stiffness and soluble factor alterations in fibrotic models by a high-throughput cellular microarray. LSECs play a crucial role in hepatic fibrogenesis by participating in ECM deposition in the form of collagen and fibronectin synthesis and regulating ECM metabolism. Extra domain A from injured LSECs promotes stellate cell motility and parenchymal liver fibrosis (83). Liu *et al* (84) used the principles of mechanical mechanics to explain capillarization of LSECs, which might contribute to early-stage collagen contraction, then collagen fibers function as mechanical transducer to activate HSCs. Notably, researchers found that capillarized LSECs undergo a partial endothelial-mesenchymal transition characterized by increased ECM production without activating cell mobility, leading to perisinusoidal ECM deposition preferentially in liver sinusoids but not septal/portal scars (85) (Fig. 1)

5. Dysfunction of LSECs and portal hypertension

Portal hypertension, defined as increased pressure in the portal vein, develops as a consequence of increased intrahepatic vascular resistance and is the initial step towards complications of chronic liver disease (86). It can cause ascites and gastroesophageal varices, as well as hepatic encephalopathy due to portosystemic shunting, hepatorenal syndrome and hypersplenism (87). To date, a number of researchers have considered that the effect of increased intrahepatic vascular resistance in liver fibrosis is closely related to LSECs, ultimately leading to portal hypertension and its complications (88-90). Decreased NO levels and capillarization can increase vascular resistance (91). In addition, endothelial autophagy (92) and aging (93) are also involved in the occurrence of portal hypertension and liver fibrosis.

Recently, an increasing number of studies have suggested that thrombosis is one of the key pathological factors mediating portal hypertension and anticoagulation therapy seems to be beneficial in the treatment of liver fibrosis and portal hypertension in rats, although it depends on the stage of cirrhosis (86,94). The current consensus is that most portosystemic collateral vessels are formed through angiogenesis, the formation of new blood vessels from pre-existing vasculature especially within fibrous scar (95). In addition, mechanical

stretch increases expression of CXCL1 in LSECs to recruit neutrophils, generate sinusoidal microthrombi and promote portal hypertension (96). Therefore, reversing LSEC dysregulation maybe an attractive strategy for portal hypertension.

6. LSEC targeting: Potential for therapy

It is well-known that statins have beneficial effects on dysfunctional sinusoidal cells by selectively NO bioavailability and inhibiting RhoA/Rho-kinase in the liver. This was confirmed in portal hypertension of rats with NASH (88). In addition, simvastatin administration for 15 days in aged cirrhotic rats improves the hepatic sinusoidal milieu, demonstrating its therapeutic potential in advanced chronic liver disease (93). Simvastatin restores the quiescence of aHSCs via stimulation of KLF2-NO signaling in LSECs (97). Treatment with the pan-peroxisome proliferator-activated receptor (pan-PPAR) agonist lanifibranor demonstrates phenotypic improvement in a rat model of cBDL as well as in human hepatocytes from patients with cirrhosis (98). Although further validation is needed in human trials, it paves a way to develop novel drugs that target both HSCs and LSECs to treat liver fibrosis.

The property of containing numerous receptors to endocytose soluble macromolecules and small particles (20) makes LSECs suitable for specific drug delivery. To date, several modified polymers targeting LSECs have been developed to delivery drugs. Hide *et al* (99) designed simvastatin-loaded polymeric micelles to treat chronic liver disease and showed promising results, thus suggesting that nanoparticles are a promising therapeutic approach. On the other hand, liver sinusoidal capillarization and ECM deposition are dual pathological barriers to drug delivery (20), Zhang *et al* (100) constructed an efficient nanodrug delivery system with LSEC-targeting and fenestrae-repairing nanoparticles (named HA-NPs/SMV) on the basis of the modification with hyaluronic acid.

Based on a specific route of drug delivery targeting LSECs, strategies that reverse endothelial dysfunction by inhibiting Notch signaling (30,101) and Hh signaling (102,103), downregulating VEGF-R2 (104), or suppressing KLF5-mediated LSEC angiogenesis (105), inhibiting hypoxia-inducible factor-1 α (HIF-1 α) (106) or enhancing endothelial barrier function through the cyclic adenosine monophosphate/Rac/cortactin pathway (107), can be applied to improve liver fibrosis. Besides special targeting of LSECs, there are other methods of liver fibrosis treatment that act via an indirect effect on LSECs, such as simultaneous consumption of LSECs and aHSCs (108), artificial control of the switch of the Erk1/2-Akt axis in LSECs to reverse LSECs from a proregenerative to a profibrotic phenotype (53), prevention of interstellar interaction of LSECs (109,110), selective enhancement of autophagy in LSEC in the early stages (111) and treatment of liver fibrosis with anti-angiogenic agents (112). Finally, a more detailed understanding of cytoskeletal structure and function of LSECs may help with the design of drugs that control fenestrae opening, which seems to be a simple and straightforward anti-liver fibrosis approach.

7. Conclusion and future perspectives

The present study reviewed the capillarization of LSECs and involved mechanisms and sinusoidal communication

mediated by LSECs in liver fibrosis. LSECs play a central role in the maintenance of HSC quiescence and modulating the crosstalk among various types of liver cells and dysfunction of LSEC characterized by loss of fenestration is widely considered to be an early event in the development of liver fibrosis. In recent years, a number of anti-fibrotic drug candidates have shown potent effects in experimental animal models; however, an urgent question remains that they have limited or no anti-fibrotic effects in clinical trials. At present, there are no approved drugs for liver fibrosis. The studies on LSECs suggest that protection of LSECs may be an effective strategy to prevent fibrosis initiation and progression. LSEC-targeted drugs or technologies will provide some novel strategies for the treatment of liver fibrosis in the future. Meanwhile, exploring the regulatory mechanisms of LSEC development and crosstalk with other liver cells will help understand the initiation progress of liver fibrosis and develop improved therapeutic targets.

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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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