Molecular Biology Reports (2021) 48:2803–2815
https://doi.org/10.1007/s11033-021-06269-1

REVIEW

Liver sinusoidal endothelial cells are implicated in multiple fibrotic mechanisms

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Received: 25 September 2020 / Accepted: 5 March 2021 / Published online: 17 March 2021

Abstract

Chronic liver diseases are attributed to liver injury. Development of fibrosis from chronic liver diseases is a dynamic process that involves multiple molecular and cellular processes. As the first to be impacted by injury, liver sinusoidal endothelial cells (LSECs) are involved in the pathogenesis of liver diseases caused by a variety of etiologies. Moreover, capillarization of LSECs has been recognized as an important event in the development of chronic liver diseases and fibrosis. Studies have reported that various cytokines (such as vascular endothelial growth factor, transforming growth factor-β), and pathways (such as hedgehog, and Notch), as well as epigenetic and metabolic factors are involved in the development of LSEC-mediated liver fibrosis. This review describes the complexity and plasticity of LSECs in fibrotic liver diseases from several perspectives, including the cross-talk between LSECs and other intra-hepatic cells. Moreover, it summarizes the mechanisms of several kinds of LSECs-targeting anti-fibrosis chemicals, and provides a theoretical basis for future studies.

Keywords Liver sinusoidal endothelial cells · Capillarization · Liver fibrosis · Mechanisms · Drug treatment

Introduction

Liver sinusoidal endothelial cells (LSECs) encompass approximately 50% of hepatic non-parenchymal cells, and constitute the wall of the hepatic sinusoid. LSECs exhibit unique biological and functional characteristics, and are phenotypically different from vascular endothelial cells. They have small pores (fenestrae), while lack diaphragm and basal lamina, therefore, they are more permeable. By adjusting the diameter and number of fenestrae, LSECs regulate the bidirectional transport of macromolecules and solutes between hepatic cells and the perisinusoidal space (Disse space). Regarding their ultrastructural characteristics, LSECs have numerous bristle-coated micropinocytotic vesicles and lysosome-like vacuoles in the perikaryon, indicating a special endocytotic activity. In addition, LSECs membranes have high affinity endocytosis receptors, including scavenger receptors (SR-A, SR-B and SR-H), Fc gamma receptor IIb2 (FcγR IIb2, also known as CD32b) and mannose receptors (MRs). Through endocytic vesicles and receptor-mediated endocytosis, LSECs can eliminate antigens, cell debris and immune complexes [1, 2]. In addition, they can regulate inflammation, leukocyte recruitment and immune responses to pathogens, thereby maintaining liver homeostasis and normal immune tolerance [3].

Chronic liver injury can be caused by various factors, including viral infections, alcohol intake, cholestasis and abnormal fat accumulation. Uncontrolled chronic liver disease can progress to fibrosis and even cirrhosis, which enhances the risk of decompensation and hepatocellular carcinoma (HCC). Through a variety of mechanisms, LSECs are involved in various stages of liver fibrosis. Therefore, it is important to elucidate on the roles of LSECs in chronic liver diseases, fibrosis and cirrhosis. After hepatic injury, LSECs rapidly lose their highly specialized phenotype and become capillaried. Capillarization refers to the disappearance of fenestrae (defenestration) and formation of continuous basement membranes, which transforms LSECs into

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ordinary non-specific or microvascular endothelial cells. These alterations impair the specific functions of LSECs, leading to impaired filtration and endocytosis. Moreover, capillarized LSECs interact with other hepatic cells to mediate the adhesion of immune cells and to modulate inflammatory responses in chronic liver diseases. They also activate parenchymal and non-parenchymal cells, particularly hepatic stellate cells (HSCs), leading to accumulation of the extracellular matrix (ECM). This process can be partially modeled in vitro by culturing LSECs on plastic dishes in serum-containing medium, a process referred to as differentiation [4]. Figure 1 depicts a basic overview about comparing LSEC morphology and function in health versus fibrosis. In summary, capillarization of LSECs is a key step in the development of chronic liver diseases; thus maintaining normal LSECs phenotypes and functions can inhibit liver fibrosis and the development of cirrhosis.

Cytokines and pathways involved in the regulation of LSEC capillarization

Vascular endothelial growth factor (VEGF)

VEGF, derived from either hepatocytes (HCs) or HSCs, has been shown to be closely associated with LSEC phenotypes and functions, both in vivo and in vitro [5]. VEGF regulates LSEC capillarization through both NO-dependent and NO-independent pathways. In this process, after binding to its receptors (VEGFR), VEGF stimulates the release of endogenous nitric oxide synthase (eNOS)-induced NO in LSECs. Then, NO participates in the conversion of guanosine triphosphate (GTP) to 3′,5′-cyclic guanosine monophosphate (cGMP) as well as in the activation of protein kinase G (PKG) through the soluble guanylate cyclase (sGC) thereby, phosphorylating target proteins. Activation of the eNOS-NO-sGC pathway enhances LSEC differentiation and it inhibits capillarization [5]. However, NO-independent pathways have not been clearly elucidated. Luo et al. [6] reported that VEGF suppresses the caveolin-1 (CAV-1) autophagy on cell membranes, which maintains the fenestrae of LSECs. However, the role of VEGF in liver fibrosis is complex, because it is associated with the angiogenesis of liver sinusoids. Studies have documented the associations between angiogenesis and the progression of fibrosis, with the involvement of VEGF in this process [7]. Notably, Kantari-Mimoun et al. [8] documented that myeloid cells, particularly macrophages, are constantly recruited to the fibrotic liver. Myeloid cell-derived VEGF up-regulates the expression of matrix metalloproteases while down-regulating the expression of tissue inhibitor of metalloproteases in LSECs. These processes promote collagen degradation, which resolves liver fibrosis.

In conclusion, the role of VEGF in LSECs to the development of fibrosis is multifaceted, and it is thought to be a "double-edged sword". VEGF from different sources play multiple roles through several pathways.

Transforming growth factor-β (TGF-β) superfamily

Members of the TGF-β superfamily play important roles in liver fibrosis. This family has 33 members, including TGF-βs, activins and bone morphogenetic proteins (BMPs). TGF-β is secreted as part of a large latent complex that is then stored in the ECM, remaining inert under physiological conditions. Activation of latent TGF-β, a precursor of most fibrogenic TGF-β cytokines, is localized to sites where it is released [9]. This process is at least partially induced through integral interactions between α_v integrins, ECM, and the actin cytoskeleton. Briefly, under cellular contraction-induced mechanical force, α_v integrins attached to the actin cytoskeleton bind the latent complexes attached to

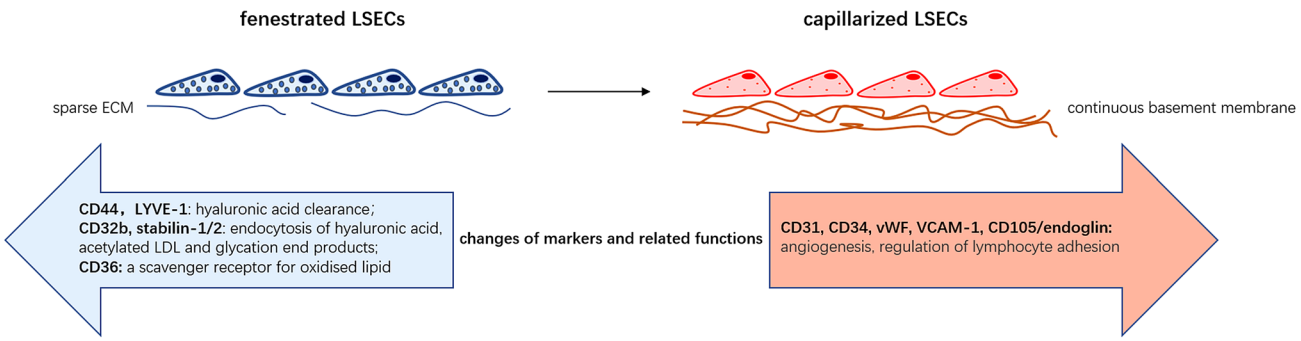

Fig. 1 Morphological and functional changes between fenestrated and capillarized LSECs. During capillarization, the number and diameter of LSEC fenestration decrease, a continuous basement membrane is formed. At the same time, markers of LSECs also change. Makers in the blue arrow is highly expressed in fenestrated LESCs, while markers in the pink arrow is highly expressed in capillarized LSECs. The corresponding functions also change accordingly. *LYVE-1* lymphatic vessel endothelial hyaluronic acid receptor 1, *VCAM-1* vascular cell adhesion molecule-1, *vWF* von Willebrand Factor, *ECM* extracellular matrix

ECM, thereby activating TGF-β, which binds its receptor [9, 10]. Moreover, LSEC-derived latent TGF-β is activated on HSCs membrane through plasma kallikrein, which is also released by LSECs during the process of fibrosis-related pathological angiogenesis [11]. Even though partially latent TGF-β is secreted by LSECs, it functions in HSCs. Activation of latent TGF-β promotes HSC transformation to myofibroblast and regulates remodeling of the extracellular matrix by stimulating the synthesis of ECM proteins, such as collagen and fibronectin [12]. Endothelial interstitial differentiation (EndMT) is involved in the development of some fibrotic diseases [13]. EndMT, a dynamic process in which endothelial cells evolve into myofibroblasts, is characterized by the loss of cell–cell junctions and endothelial markers, while acquiring mesenchymal phenotypes. Ribera et al. [14]. reported that a small subpopulation of LSECs in their study exhibited the EndMT process in a liver fibrosis mouse model and cirrhosis patients. TGF-β1 is one of the three isoforms of TGF-β proteins, it is a crucial molecular promoter of LSEC EndMT. BMP7, another member of the TGF-β superfamily, has been shown to antagonize EndMT in vivo [14]. However, the proportion of these transformed cells in endothelial cells is very low (about 4%) [14]. This study provides descriptive evidences that rely on co-localization of cell markers by IHC in cirrhotic patients, which is not a proof that EndMT occurs in humans. Therefore, the role of EndMT in liver fibrosis should be elucidated. Specific treatments of EndMT may have little effects on liver fibrosis. Additionally, HSCs-produced BMP9 occurs in blood as a BMP9-BMP10 dimer [15, 16], and plays an important role in maintaining endothelial cell quiescence through activin receptor-like kinase (ALK) [17]. In LSECs from BMP9-knockout mice, the number of fenestrae was shown to be significantly suppressed. Consistently, the expression of GATA-binding protein 4 (GATA4), a specific transcription regulator that maintains LSECs differentiation, were significantly inhibited. Endothelial differentiation markers, including lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE-1) and stabilin-1/2 were also shown to be down-regulated. Plasmalemmal vesicle-associated protein (PLVAP), is the main integral protein that is correlated with fenestrae. Addition of BMP9 to the LSECs medium maintains fenestrae and elevates the expression of GATA4 and PLVAP in LSECs. However, one in vitro study involving human LSECs reported dissimilar findings, in which co-stimulation of LPS and BMP9 inhibited the expression of stabilin-1 and LYVE-1 in LSECs [18]. Based on these findings, the overall impact of BMP9 on LSEC capillarization has not been fully elucidated, and should be studied further.

Compared to control rats, it was reported that bone marrow (BM)-derived endothelial progenitor cells (EPCs) were significantly recruited and migrated to the livers of thioacetamide (TAA)-induced fibrotic rats, implying the positive correlation between engraftment and fibrosis. However, EPCs did not upregulate ALK1 and TGF-β signaling, but mediated by noninhibited ALK5 which up-regulates thrombospondin. Up-regulation of thrombospondin effectively down-regulates NO production, and inhibits the maturation of LSEC progenitors [19]. In addition, an in vitro study showed that BM-derived EPCs isolated from patients with cirrhosis can stimulate profuse angiogenesis when co-cultured with rat resident LSECs [20], which presents a new perspective on the role of LSECs dedifferentiation in liver fibrosis.

Hedgehog (Hh)

The hedgehog signaling pathway is a complex system composed of ligands, receptors and transcription factors. The binding of Hh ligands to their transmembrane receptors (Patched, Ptc), activates Smoothened (Smo) to act as a co-receptor-like molecule in initiating the intracellular Hh-induced signaling. Hh signaling is important in the regulation of vasculogenesis. After chronic liver injury, its activation regulates the functions of several types of liver cells, including LSECs, cholangiocytes, HCs, HSCs, myofibroblastic hepatic stellate cells (MF-HSCs), and some immune cells [21]. Specifically, Pereira et al. [22] reported that, compared to healthy people, macrophages in schistosomiasis patients over-express Hh ligands. In response, Hh-responsive LSECs up-regulate the expression levels of Hh target genes, and the intensity of this response is positively correlated with fibrosis stage. In addition, in a BDL mice model, cholestasis enhanced the secretion of Hh-rich microparticles by cholangiocytes and myofibroblastic hepatic stellate cells. As a result, Ptc is up-regulated in LSECs, while the expression levels of CD31, and iNOS also increase [23]. Xie et al. [24] cultured freshly isolated primary LSECs to further evaluate the roles of Hh in the cellular phenotypic changes. They found that Hh induces LSEC capillarization and formation of vascular tubes in vitro. Hh-related gene expression in culture-induced capillaried LSECs was found to have increased. Moreover, Hh agonists further promoted LSEC capillarization, a process that was further shown to be suppressed in Smo-knocked out LSECs. Consistently, LSEC capillarization was found to be suppressed in chronic and acute liver injury mice models treated with a Hh signaling antagonist [24]. In general, activation of the Hh pathway in LSECs promotes the cirrhosis-related sinusoidal remodeling process.

Notch

Notch signaling is an evolutionarily highly conserved pathway in vertebrates. It encompasses four transmembrane receptors (Notch1-4), and five canonical Delta-like ligands

(DLL), 1 3, and 4 and Jagged (JAG) 1 and 2. The notch pathway is involved in multiple cellular physiological processes including angiogenesis and vascular remodeling [25]. Duan et al. [26] reported that endothelial Notch activation induced LSEC capillarization and deranged liver homeostasis in mice. This process enhances eNOS-sGC pathway dependent CCL_4-induced liver fibrosis. Consistently, activated Notch pathways in LSECs have also been reported in liver cirrhosis patients [26]. Regarding Notch ligands, DLL4 is primarily expressed in endothelial cells. Shen et al. [27] found that DLL4 was highly expressed in LSECs and kupffer cells (KCs) of HBV-associated active cirrhosis patients, when compared to those without cirrhosis. They reported that intraperitoneal injection of recombinant DLL4 in mice suppressed liver inflammation by inhibiting CCL2, thereby alleviating CCL_4-induced liver injury. However, their study did not establish the role of LSEC-specific DLL4 during fibrosis, and the positive correlation between DLL4 and liver injury may be attributed to other notch-responsive cells such as KCs. Chen et al. [28] found that the expression levels of DLL4 in LSECs were up-regulated in both cirrhotic patients and in CCL_4-induced fibrotic mice. Moreover, DLL4 and basement membrane proteins were found to be elevated in culture-induced dedifferentiated murine primary LSECs. Generation of basement membrane proteins was inhibited after DLL4 knockout. The same effect on basement membrane proteins was also shown to be caused by the overexpression, or deletion of DLL4 in an immortalized LSEC cell line (TMNK-1). This DLL4-induced upregulation of basement membrane proteins may be associated with ECM accumulation, which is correlated with liver fibrosis.

Mechanotransduction

Mechanical forces are determined by physical properties of the extracellular matrix, as well as by the pressure of the circulating blood flow. This force is detected and transmitted by mechanosensors on the plasma membrane, thereby affecting cellular functions. This process is called mechanotransduction.

Shear stress

Shear stress is a key factor in the regulation of the endothelial phenotypes, including those of LSECs. Mechanosensors, such as integrins, G protein receptors, tyrosine kinase receptors, and ion channels, are localized on endothelial cells, and respond to changes in wall flow shear stress [29]. KLF2 is an important transcription factor that is involved in the maintenance of endothelial cell quiescence. In addition, it exhibits vasoprotective effects under shear stress. Under healthy conditions, laminar flow-induced shear stress activates the transcription of myocyte enhancing factor 5 (MEF5), which subsequently induces a highly flow-induced factor, extracellular signal regulated kinase 5 (ERK5) [30]. Myocyte enhancing factor 2 (MEF2) is a characteristic target of EKR5 that can bind the KLF2 promoter [31]. Therefore, shear stress activates KLF2 expression through mechanosensory complexes on endothelial membranes. KLF2 expression was shown to be upregulated in the liver of cirrhotic rat models, especially in hepatic endothelial cells [32]. Elevated expression levels of KLF2 induces the expression of eNOS to mediate vasodilatation, which compensates for the dysregulated microcirculation and portal pressure. Marrone et al. [33] reported that overexpression of KLF2 in LSECs reduces endothelial dysfunction and inhibits the activation of HSCs co-cultured with LSECs. KLF2 inhibits the proliferation, migration, and angiogenesis of LSECs through the protein kinases 1/2 (ERK1/2) signaling pathway [34]. Endogenous KLF2 upregulation is not sufficient to maintain the normal phenotype and function of LSECs under pathological sinusoidal blood flow, neither is it sufficient to resist the progression of liver fibrosis. Animal model studies have shown that high perfusion pressure in the portal vein fuses with and enlarges the LSEC fenestrae leading to an abnormal transport of chylomicron to the liver parenchyma [35]. In addition, Hilscher et al. [36] mechanically stretched primary mice LSECs to mimic the pulsatile forces induced by congestion. This treatment activates integrins and piezo channels on LSEC membranes, thereby promoting the expression and secretion of CXCL1 from LSECs, culminating in the formation of neutrophil extracellular traps and microthrombi pivotal. This process is involved in pressure adjustment in the sinusoidal lumen and portal hypertension.

Extracellular matrix (ECM)

Studies have shown that HSCs are not the only ones involved in the formation of extracellular matrix (ECM) as LSECs also play an important role in the excessive deposition of ECM in fibrotic liver by secreting collagen type I, type IV, and fibronectin, which are important units of the ECM [37, 38]. In the early stages of liver fibrosis, LSECs-dependent angiogenesis promotes the condensation of collagen fibrils. Remodeled collagen-mediated mechanotransduction activates HSCs, thereby increasing the stiffness of ECM [4]. In effect, stiff ECM plays an important role in regulating the LSEC phenotype. Juin and colleagues [39] found that a harder ECM matrix increases the number of LSECs-formed podosomes. Podosomes are actin-rich structures that are involved in the migration, proteolysis, and cytoskeletal remodeling of LSECs. Consistently, in vitro studies revealed that the harder the ECM, the earlier the cells lose their fenestrae [40]. In summary, LSECs fenestrae is directly

involved in ECM accumulation, which is maintained by complex matrix components.

Metabolic regulation of LSECs

Autophagy

Liver fibrosis refers to the scarring of liver tissues, a process that is mediated by integral participation of liver cells, including HSCs, KCs and LSECs, and HCs. Autophagy is central to fibrotic progression, however, it performs different, or even opposite functions in specific cell types. Autophagy in HCs and macrophages promotes anti-fibrosis [41]. However, it enhances the activation of HSCs, which exacerbates fibrotic liver disease [42].

In the early stages of liver injury, autophagy is rapidly up-regulated to maintain the LSECs phenotype and hepatic homeostasis [43]. However, the protective functions of autophagy decrease as the damage continues [43]. Accordingly, dedifferentiation of LSECs and development of liver fibrosis continues [43]. Autophagy inhibits fibrosis by increasing NO bioavailability and through the excretion of reactive oxygen species (ROS) in LSECs [43]. Moreover, it up-regulates transcription factor KLF2, which has a protective role in endothelial cells [44]. A recent study [41] reported that endothelial autophagic defects enhances susceptibility to liver damage, liver cell apoptosis and perisinusoidal fibrosis in mice fed on a high-fat diet and treated with CCL_4. In an immortalized LSEC line, deficiency in autophagy enhanced inflammation, EndMT, and apoptosis. In general, up-regulation of autophagy confers protective effects on LSECs, in both early acute injury and advanced fibrosis.

Notably, due to different cell types and disease stages, autophagy has a context-dependent impact on liver fibrosis, which complicates autophagy-targeted therapy for fibrosis. Selective enhancement of autophagy in LSECs in the early stages of liver disease may prevent fibrotic progression.

Lipids

Non-alcoholic fatty liver disease (NAFLD) can develop from a simple degeneration of fatty acids in the liver to cirrhosis, one of the main causes of chronic liver disease. Sinusoidal endothelial cell dysfunction is an early event in the development of NASH [45]. In vitro, excessive lipid exposure led to a decrease in the diameter and number of fenestrae. In addition, NO bioavailability in LSECs was suppressed after exposure to ox-LDL and free fatty acids (FFA) [46]. Using a nutrition-induced NAFLD model, Miyao et al. [45] revealed that sinusoidal capillarization occurs in the early stages of NAFLD, and it can be partially reversed by discontinuing the high-fat diet. Consistently, Cogger et al. [47] used mice models to demonstrate that fenestrae frequency and overall porosity are negatively correlated with dietary fat intake and FFA levels.

Adipocytokines

Adipocytokines are involved in the pathogenesis of obesity and NAFLD-induced liver fibrosis. Among them, adiponectin has a strong anti-fibrosis effect, whereas leptin exhibits a pro-fibrosis effect. Leptin promotes fibrosis by up-regulating TGF levels in LSECs and KCs, as well as by promoting angiogenesis [46]. Using a toxin-induced NASH rodent model, Pourhoseini et al. [48] found that leptin impairs functional activities of nitric oxide synthase 3 (NOS3) through miR-21. Since NOS3 activation is important for increased NO bioavailability, miR-21 induces LSEC capillarization. However, the functionality of NOS3 can be restored by deleting leptin or its receptors.

Ethanol

In vitro and in vivo, chronic stimulation of LSECs using ethanol was shown to lead to early defenestration and formation of a basement membrane. Over time it may prevent fluid and macromolecular exchange as well as immune cell migration, which eventually promotes tissue fibrosis [49, 50]. Due to the ability of LSECs to clear hyaluronic acid (HA), circulating HA levels are a hallmark of LSECs function. Chronic alcoholic exposure upregulates circulating HA levels. Deaciuc et al. [51] reported that elevated circulating HA levels are due to apoptosis, which impairs LSECs functions, and that this process is preceded by alcoholic hepatocyte injury. In LSEC-specific STAT3-knockout mice, it was confirmed that during chronic alcohol consumption, STAT3 in endothelial cells inhibit LSEC apoptosis and hepatic inflammation, thereby reducing alcoholic liver injury [52]. Consistently, alcohol exacerbates acetaminophen-induced LSECs injury, which occurs prior to liver parenchymal injury [53]. In general, alcohol-induced LSEC injury is the initial step in chronic alcoholic liver damage, and restoring LSEC functions may alleviate alcoholic cirrhosis.

Age

The phenotype, and functions of LSECs change with age. LSECs exhibit fewer fenestrae, more basal lamina deposition, and less endocytosis during aging, a process referred to as pseudocapillarization. In this process, the expression of eNOS and sGC, as well as NO bioavailability in aging LSECs is suppressed, resulting in decreased liver blood flow [54]. Aging LSECs are in an inflammatory status, and elevated intercellular inflammatory molecule 1 expression

mediates a substantial increase in leukocyte adhesion, further reducing the sinusoidal blood flow [55]. Age-associated plastic roles and peculiar characteristics of LSECs have a special significance in elderly liver injury patients.

Epigenetic changes in LSECs during liver fibrosis

lncRNAs and miRNAs

Changes of several lncRNAs or miRNAs are involved in LSEC injury during fibrosis as shown in Table 1. miR-199 was found to be involved in alcohol-induced improvement of endothelin-1 (ET-1) and HIF-α in rat LSECs and in human endothelial cells. However, miR-155 was shown to induce the expression of ET-1 and HIF-α in rat LSECs. Elevated ET-1 and HIF-α levels are associated with microcirculatory failure and portal hypertension in liver fibrosis [56]. In addition, in patients and mice with liver cirrhosis, the expression levels of miR-322 in mice and its human rodent homolog miR-424 were found to be elevated. miR-322/424 promotes cirrhosis-related angiogenesis by targeting the Cullin-2/HIF-1α pathway [57]. Transcriptional changes in some non-coding RNAs (lncRNA) regulate the LSEC phenotype. H19, a lncRNA located on human chromosome 11p15.5, regulates microvascular formation [58] as well as tumors development [59]. Zhu et al. [60] reported that H19 negatively regulates miRNA-148b-3p, while NADPH oxidases (NOX4) were negatively regulated by miR-148b-3p. In patients with cirrhosis or in human LSECs under hypoxia conditions, H19 exacerbates hypoxic stress of LSECs through indirect positive regulation of endothelial NOX4 and negative regulation of eNOS/NO signals. Moreover, lncRNA taurine-upregulated gene 1 (TUG1) is another lncRNA associated with LSEC-related liver fibrosis. TUG1, a 7.6-kb gene located on chromosome 22q12.2, is involved in several biological processes (e.g., cell proliferation, apoptosis, EMT) and disorders (e.g., liver fibrosis, tumors including HCC) [61]. Zhang et al. [62] reported that TUG1 is up-regulated, while miR-142-3p is down-regulated in patients with cirrhosis. They treated LSECs with LPS or starvation to partially simulate a fibrotic microenvironment. After LPS or starvation exposure upregulates TUG1 and competitively inhibits miR-142-3p. As a result, the expression of autophagy-related gene ATG5, a miR-142-3p-targeted gene, is upregulated, which induces autophagy-mediated EndMT in LSECs.

DNA methylation

Brahma-related gene 1 (BRG1) is a chromatin remodeling protein that participates in hepatic fibrosis by regulating transcription in endothelial cells. Shao et al. [63] reported that a lack of endothelial-specific BRG1 alleviates TAA-induced liver fibrosis in mice. Their study confirmed that deleting endothelial BRG1 is accompanied by the up-regulation of eNOS activity and NO bioavailability, which together resolves fibrosis. Further analysis showed that Sp1 recruits BRG1 to the CAV-1 promoter, in which BRG1 interacts and regulates the H3K4 trimethyltransferase MLL1. This cascade of events activates CAV-1 transcription, and further inhibits eNOS activity. Therefore, BRG1-induced DNA methylation regulates endothelial functions and plays an anti-fibrosis role.

Histone modification

Lysine residue acetylation, catalyzed by histone acetyltransferase (HAT), is one of the most common forms of histone modification. Conversly, histone deacetylase (HDAC) catalyzes deacetylation. Silent information regulator 1 (SIRT1) is an NAD^+-depended HDAC. Down-regulation of SIRT1

Table 1 miRNAs/lncRNAs involved in LSECs

miRNAs/lncRNAs	Research objects	Changes of miRNAs/lncRNAs in chronic liver injuries	Roles in diseases
miR-21	LSECs in rodent models of NASH	↑	Induces the capillarization of LSECs in NASH
miR-199/155	Primary LSECs in alcohol-exposured rats; ethanol-treated human dermal microvascular endothelial cell line	↓	Induces the release of chemokines by KCs and the contraction of HSCs in ethanol-induced liver injury
miR-322/424	LSECs in patients with cirrhosis/CCL_4-induced mouse liver fibrosis/CCl_4 and dimethylnitrosamine-induced liver fibrosis; HHECs cutured in vitro	↓	Induces proliferation, migration, and tube formation of LSECs in patients and rodents with cirrhosis
H19/miR-148b	LSECs in patients with cirrhosis; HHECs cultured under hypoxia conditions	H19↓, miR-148b↑	Induces the capillarization of LSECs in patients or hypoxic HHECs
TUG1/miR-142-3p	Primary HHECs treated by LPS and starvation; cirrhosis patients	TUG1↑, miR-142-3p ↓	Induced aotuphagy-related EndMT in LSECs

expression causes the contraction and capillarization of LSECs, thereby increasing oxidative stress and ultimately activating of HSCs [64].

Receptor-mediated regulation of LSECs

Nuclear receptors

Farnesoid X receptor (FXR), known as bile acid receptor, is an essential factor in bile and lipid metabolism. FXR deficiency aggravates liver inflammation and fibrosis. Obeticholic acid (OCA), a steroidal FXR agonist, reversed the impaired vasodilation in TAA and BDL-treated rats by up-regulating eNOS expression in endothelial cells [65]. Verbeke et al. reported that OCA inhibits the expression of MCP-1 in LPS or TGF-α treated LSECs. MCP-1 suppresses chemotactic and fibrogenic potential of HSCs [66]. In addition, the non-steroidal FXR agonist (PX20606), was shown to elevate eNOS expression and NO production in human LSECs and rat tissues, thereby ameliorating portal hypertension by reducing vascular remodeling, sinusoidal dysfunction, and liver fibrosis in CCL_4-treated rats [67].

Liver X receptors (LXRs), including LXRα and LXRβ, are members of the ligand-excited transcription factor family. LXRs agonists were shown resolve CCL_4-induced liver fibrosis in mice. However, LXRs gene knockdown impairs this protective effect. The roles of LXRs in inhibiting the Hh signaling pathway and the subsequent capillarization of LSECs have been reported [68]. In hepatitis C virus infected human liver tissues, Baiocchini et al. [69] reported that LSECs capillarization was only observed in a few patients with stage S2 fibrosis. Lack of capillarization may be associated with indirect LXRs-up-regulation by HCV cores and NS5A proteins.

Peroxisome proliferation-activated receptors (PPARs) are nuclear receptors of the steroid hormone receptors superfamily. PPARα, PPARβ and PPARγ are all expressed in endothelial cells. During cirrhosis, PPARα is significantly up-regulated in LSECs, and PPARs agonists can reverses liver fibrosis by preventing vasoconstriction, thereby, inhibiting angiogenesis as well as reducing undesirable inflammatory responses [70, 71].

These nuclear receptors negatively modulate LSECs-related angiogenesis and fibrosis progression.

G protein-coupled receptors

Sphingosine-1-phosphate receptor-1 ($S1P_1$) is widely expressed in endothelial cells. Its ligand is commonly bound to a high-density lipoprotein (HDL) to form a HDL-S1P complex, which regulates lipid transport. The binding of $S1P_1$ with its ligand was shown to maintains the normal phenotype and functions of LSECs after partial hepatectomy in mice, thereby inhibiting fibrosis [72].

TGR5 is a G protein-coupled cell surface receptor that responds to activation by bile acids and multiple steroid hormones in LSECs, KCs and cholangiocytes. Activation of TGR5 in LSECs upregulates eNOS expression of and NO production [73], and further reduced the expression and secretion of ET-1 in primary murine LSECs cultured in vitro [74].

GPR182, a novel marker for LSEC differentiation, is potentially an LSEC-specific orphan G-protein coupled receptor. Expression of GPR182 in LSECs during liver fibrosis and cirrhosis is suppressed [75]. In addition, GPR14, a urotensin II receptor, is mainly found in LSECs and KCs. It is highly expressed in patients with portal hypertension and cirrhosis. However, its role in cirrhosis is not well understood, even though it is thought to be associated with pro-vasoconstriction [76]. Several nuclear receptors and G protein-coupled receptors involved in fibrosis-related LSEC injury are shown in Table 2. In addition, above signaling molecules and pathways is depicted in Fig. 2.

Table 2 Nuclear receptors and G protein-coupled receptors in fibrosis-related LSEC injury

	Receptors	Roles in fibrosis-related LSEC injury
Nuclear receptors	FXR	Increase eNOS expression and NO production in LSECs
	LXRs	Inhibit the Hh pathway in LSECs
	PPARs	Ameliorate the angiogenic and proinflammatory phenotypes of LSECs
G protein-coupled receptors	$S1P_1$	Maintain the normal phenotype and function of LSECs, induce endothelial regeneration without causing fibrosis
	TGR5	Increase eNOS expression and NO production, inhibit ET-1 expression and secretion in LSECs
	GPR182	Maintain the special phenotype and function of differentiated LSECs
	GPR14	The exact mechanism is not clear, may be involved in LSEC-related vasoconstriction and portal hypertension

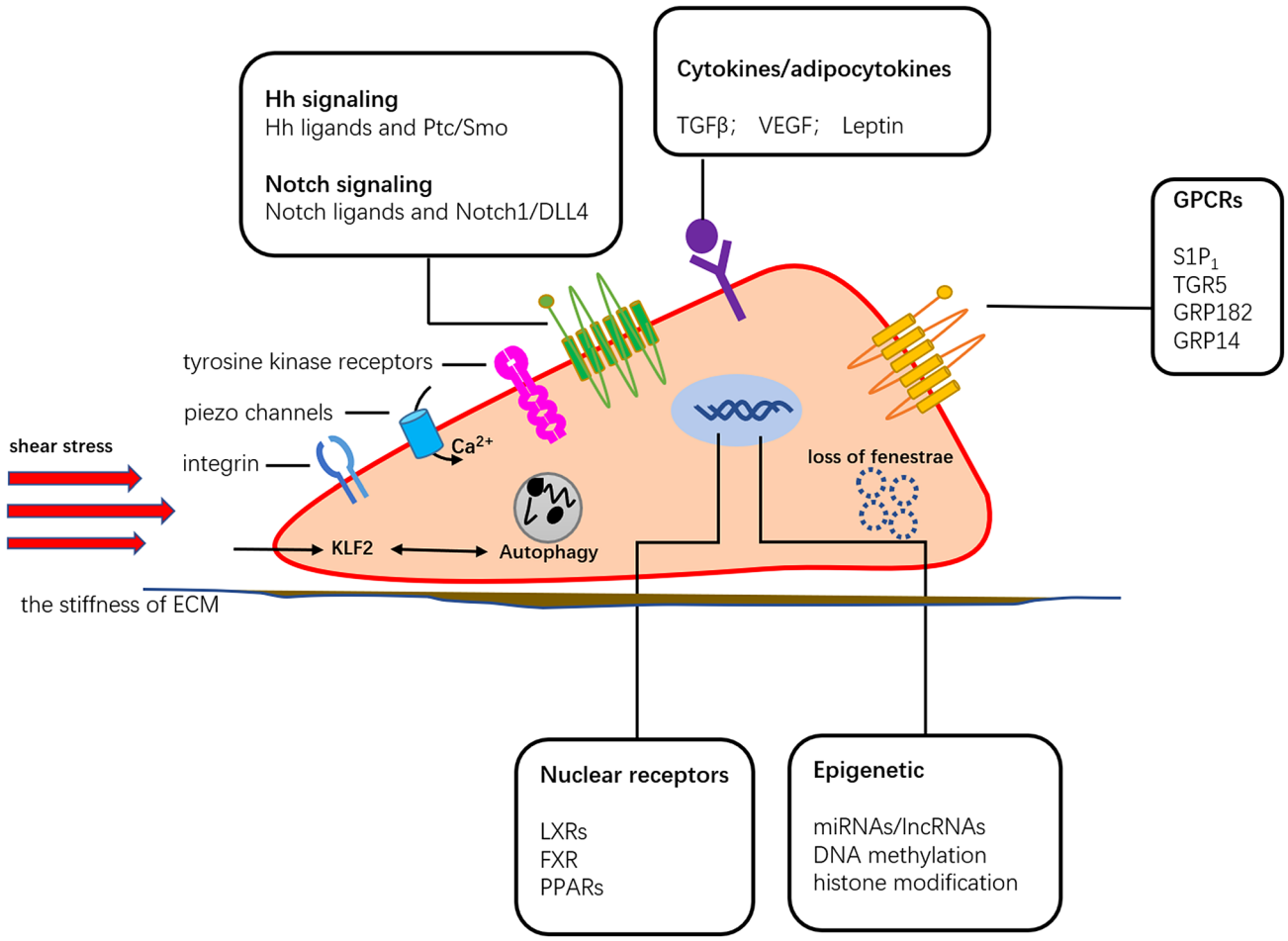

Fig. 2 Signalling molecules and pathways involved in LSECs capillarization. Key fibrogenic cytokines and adipocytokines contribute to capillarization of LSECs, include transforming growth factor-β (TGFβ), vascular endothelial growth factor (VEGF), and leptin. Hedgehog (Hh) ligand and its receptor (Patched, Ptc) and/or smoothened (Smo) promote LSECs capillarization. The Notch signaling pathway also induces capillarization of LSECs. G protein-coupled receptors expressed by LSECs can either negatively or positively affect LSECs capillarization. Autophagy maintains phenotype of LSECs. Nuclear receptors negatively modulate LSECs capillarization. Epigenetic signals including microRNAs/lncRNAs, DNA methylation and histone modification either induce or inhibit capillarization of LSECs. Stimulation of mechanotransduction, including upregulation of shear stress and stiffness of extracellular matrix (ECM), promotes LSECs capillarization. *DLL4* delta-like canonical notch ligand 4, *S1P1* sphingosine-1-phosphate receptor-1, *TGR5*, takeda G protein-coupled receptor 5, *GPR182* G protein-coupled receptor 182, *GPR14* G protein-coupled receptor 14, *KLF2* kruppel-like factor 2

Interactions between LSECs and other hepatic cells during liver injury

LSECs-HSCs

The extracellular matrix in fibrotic liver is mainly produced by activated HSCs, and is involved in the development of liver fibrosis. Differentiated LSECs maintain the nonproliferative quiescent phenotype of HSCs. Loss of the normal LSECs phenotype is permissive for the activation of HSCs in vivo [5]. Several cytokines involved in LSECs-HSCs crosstalk have been mentioned above. After capillarization, LSECs can secrete exosomes containing sphingosine kinase-1 (SK1) and sphingosine 1-phosphate, which activate HSCs [77]. Stromal cell-derived factor 1 (SDF-1), also known as CXCL12, is an LSEC and HSC derivative. HSCs express its downstream receptor, CXCR4. In chronic liver injury, SDF-1 binds to CXCR4, thereby activating HSCs, while also recruiting mesenchymal cells from the bone marrow that in effect, promotes liver fibrosis progression [78, 79]. CXCR7, expressed on LSECs, is another chemokine receptor for SDF-1. During chronic liver injury, SDF-1-CXCR7 interactions promote liver cell regeneration [80]. In addition, LSECs, HSCs and KCs can all secrete platelet derived growth factor (PDGF) [81–83], which, upon binding to its homologous receptor (PDGFR-β) on HSCs, induces the activation, proliferation and migration of HSCs and subsequent sinusoidal remodeling.

On the other hand, activated HSCs act on LSECs through VEGF and large amounts of ECM. This process leads to the defenestration of LSECs, thereby promoting fibrosis-related angiogenesis. Activation of HSCs can also induce the production of thrombospondin-1 (TSP-1), which enhances LSEC capillarization by blocking the NO-dependent pathway. In addition, multiple in vitro studies have shown that TSP-1 promotes dedifferentiation of LSECs through the Rho/Rho-kinase K pathway [84]. The cross-talk between LSECs and HSCs is depicted in Supplementary Fig. 1.

LSECs-hepatocytes (HCs)

LSECs secrete Wnt2a, Wnt9b and a small amounts of hepatocyte growth factor (HGF), which together, act as key hepatocyte mitogens that induce the self-regeneration of hepatocytes in the early stages of CCL_4/BDL-induced liver fibrosis [85].

In contrast, HCs secrete VEGF to regulate the LSECs phenotype [5]. In addition, HC-derived membrane-shed microparticles contents are high in cirrhosis patients, playing a regulated role in endothelial cells [86]. One study [35] showed that under adipotoxicity, HCs release VNN1-rich exosomes, which mediates the transformation into angiogenic phenotypes after uptaken by endothelial cells.

LSECs-liver macrophages

Through the cross-talk with liver macrophages, LSECs are involved in the regulation of liver immunity, among liver diseases. Liver macrophages include liver resident macrophages (KCs) and monocyte-derived macrophages (MoMFs), which play a dual role in the process of liver fibrosis. LSECs interact with both KCs and MoMFs.

LSECs-KCs

KCs adhere to the sinusoidal endothelial layer, where they closely interact with LSECs. In an organotypic three-dimensional (3D) hepatic culture, KCs maintained the dedifferentiated phenotype of LSECs by down-regulating the FAK pathway [87]. However, in severe liver injuries, LSECs secrete proinflammatory mediators such as TNF-α、IL-6、IL-1 and CCL2, which activates KCs [88]. Capillarization of LSECs in the early stages of NASH is necessary for KCs activation [45]. In return, activated KCs impair fenestrae of LSECs while increase CD31 expression [89].

LSECs-MoMFs

During liver injury, LSECs promote the migration and adhesion of circulating monocytes through surface ICAM-1, VCAM-1, and vascular protein-1 (VAP-1), thereby mediating inflammation and fibrosis in the liver [88]. However, little is known about the pathophysiological roles of MoMFs toward LSECs, therefore, further studies are needed to discern the complex immune microenvironment in the liver and the interaction mechanisms between LSECs and liver macrophages.

Anti-fibrosis chemicals for LSECs

LSEC injury occurs at the onset of liver fibrosis and dysfunctions of LSECs lead to hepatic sinus microcirculation disorders, which affect the role of anti-fibrosis drugs in the liver. Drugs targeting LSECs have been shown to effectively ameliorate chronic liver disease or fibrosis in rodent models or in vitro. (as shown in Supplementary Table 1).

Increasing NO availability

Statins promote eNOS phosphorylation, thereby increasing NO bioavailability. This effect is achieved by inhibiting the RhoA/Rho-kinase signaling pathway, which down-regulates the expression and activity of eNOS. Statins can also increase Akt/protein kinase B activity, which phosphorylates and activates eNOS [90]. In addition, statins were shown to directly protect LSECs and inactivate HSCs in cirrhotic rats by up-regulating autophagy and the KLF2-related pathway [33]. Using simvastatin and atorvastatin to treat early-stage NASH rat without fibrosis, Bravo et al. [91] showed that statins can maintain the differentiated phenotype and significantly reverse the dysfunctional LSEC phenotype, thereby inducing activated HSCs to quiescent cells.

5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR) is an agonist of the adenosine 5′-monophosphate-activated protein kinase (AMPK). By activating the AMPK/eNOS pathway in LSECs, AICAR enhances NO synthesis in the liver without changing systemic hemodynamics, thereby ameliorating cell functions as well as reducing portal pressure. AICAR has also been shown to directly alleviate HSCs contraction in vitro [92]. Consistently, metformin improves the age-related pseudocapillarization of LSECs by activating the AMPK/eNOS pathway [93].

Angiogenesis

Hepatic angiogenesis is highly associated with liver fibrosis progression. Fibrosis-induced hypoxia and immature LSEC angiogenesis interact as both cause and effect. Some drugs target key angiogenic-related factors to restore the normal functions of injured LSECs. Oral administrations of vatalanib, a small molecule tyrosine kinase inhibitor, has been shown to be effective against all VEGF proteins. In CCL_4-induced mouse liver fibrosis, vatalanib reduced

LSECs capillarization and resolved fibrosis by regulating VEGF and TGF-β [94]. In addition, temsirolimus and everolimus are rapamycin analogues that can maintain the normal phenotype and functions of LSECs by inhibiting the PI3K-AKT-mTOR signaling, which also prevents fibrosis-related angiogenesis [95]. Vatalanib, temsirolimus and everolimus also inhibit the activation and proliferation of HSCs [95]. Therefore, besides LESCs, these drugs also alleviate liver fibrosis through HSCs.

Adrenergic receptor blocker

Carvedilol, an adrenergic receptor blocker, inhibits sympathetic activation by antagonizing β1-, β2- and α1-adrenoreceptors. Ling et al. [96] reported that carvedilol alleviates PH in rats by inhibiting neovascularization and reconstruction of the hepatic sinusoid in the liver. Separate in vitro studies in cultured human umbilical vein endothelial cells (HUVECs) showed that it can also impair fibronectin synthesis. Consistently, treatment of CCL_4-induced liver fibrosis with carvedilol showed that this drug inhibits LSECs capillarization [97]. In addition, it induces apoptosis but inhibits the activation of HSCs, which is another anti-fibrosis mechanism.

Miscellaneous

As a dual PPARα-PPARγ agonist, Aleglitazar (Ale) alleviates liver fibrosis by inhibiting inflammatory responses and neovascularization [70]. Similarly, the Pan-peroxisome proliferator-activated receptors (pan-PPAR) agonist (lanifibranor) protects LSECs in cirrhotic rats [71]. Moreover, the two chemicals potentially reduce portal pressure, while avoiding the side effects associated with one single PPAR agonist. Actin, a cytoskeleton component, has been implicated in the regulation of size and formation of fenestrae in LSECs. Actin-targeted chemicals such as cytochalasin D (cyto-D) and Latrunculin A, bind actin filaments and inhibit actin monomer polymerization, thereby promoting the formation of fenestrae in differentiated LSECs [98]. These chemicals regulate other hepatic cells, especially HSCs, through similar effects as in LSECs [70, 71, 99]

In general, clinical trials of drugs targeting LSECs, specifically for the treatment of chronic liver diseases or liver fibrosis, are limited. Since most drugs act on several types of hepatic cells, the therapeutic effects of most drugs may be multi-dimensional. In-depth investigations of LSEC targeting drugs and their interactions with other hepatic cells are needed.

Conclusions

Elucidation of the roles of LSECs have informed the diagnosis and treatment of chronic liver diseases or fibrosis. Cellular signal regulation and cellular interactions have been documented. Besides, a variety of anti-fibrosis chemicals targeted at LSECs have been developed, which lays a concrete foundation for resolving liver fibrosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06269-1.

Funding This manuscript is supported by no funding.

Declaration

Conflict of interest The authors have no conflicts of interest to disclose.

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