Sinusoidal communication in chronic liver disease

Albert Gibert-Ramos[1,2], María Andrés-Rozas[1], Raül Pastó[1], Pablo Alfaro-Retamero[1], Sergi Guixé-Muntet[1,2*], Jordi Gracia-Sancho[1,2,3*]

[1] Liver Vascular Biology Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic de Barcelona, Barcelona, Spain.

[2] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain.

[3] Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

* co-senior authors

Corresponding author: Jordi Gracia-Sancho, MSc, PhD, FAASLD. IDIBAPS Biomedical Research Institute – Hospital Clínic de Barcelona. Rosselló 149, 08036, Barcelona, Spain. email: jgracia@recerca.clinic.cat. ORCID: 0000-0001-7736-4089

Abbreviations: angiopoietin 2, Ang2; alpha-smooth muscle actin, α-SMA; bile duct ligation, BDL; Bone morphogenetic protein 9 , BMP-9; caspase-1, CASP-1; chronic liver disease, CLD; connective tissue growth factor, CCN2; collagen type I alpha 1, Col1α1; C-X-C Motif Chemokine Ligand 1, CXCL1; C-X-C motif chemokine receptor, CXCR; cyclic guanosine monophosphate, cGMP; damage-associated molecular pattern, DAMP; delta like canonical notch ligand, DLL4; E2F transcription factor 3, E2F3; endothelial nitric oxide synthase, eNOS; endothelin 1, ET-1; extracellular matrix, ECM; extracellular vesicle, EV; focal adhesion kinase 1, FAK; fibroblast growth factor receptor 1, FGFR1; growth differentiation factor 15, GDF15; heparin-binding EGF-like growth factor, HB-EGF; hepatic stellate cells, HSC; hepatocellular carcinoma, HCC; Hepatocyte growth factor, HGF; high mobility group box 1, HMGB1; human amniotic membrane-derived epithelial stem cells; human amniotic membrane-derived mesenchymal stromal stem cells, hAMSC; hedgehog interacting protein, HHIP; human umbilical cord-derived MSCs, UC-MSC; induced pluripotent stem cells, iPSC; krüppel-like factor 2, KLF2; kupffer cells, KC; leukocyte cell derived chemotaxin 2, LECT2; liver sinusoidal endothelial cells, LSEC; lysyl oxidase, LOX; lymphatic vessel endothelial hyaluronan receptor 1, LYVE-1; MAPK activated protein kinase 2, MK2; metabolic dysfunction-associated steatotic liver disease, MASLD; macrophage inflammatory protein-1 alpha, MIP-1α; monocyte chemoattractant protein-1, MCP-1; nitric oxide, NO; non-parenchymal cell, NPC; nuclear factor-kappa B, NF-κB; obeticholic acid, OCA; platelet-derived growth factor, PDGF; peroxisome proliferator activated receptor gamma, PPARγ; quantum dots, QD; reactive nitrogen species, RNS; reactive oxygen species, ROS; R-spondin 3, RSPO3; SH2 domain-containing protein tyrosine phosphatase 2, SHP2; SK1 phosphate receptor 2, S1PR2; Sphingosine 1-phosphate receptor 2, S1PR2; sphingosine kinase-1, SK1; suppressor of cytokine signaling 3, SOCS3; thioacetamide, TAA; tissue inhibitor of metalloproteinase 1, TIMP1; transforming growth factor beta-1, TFG-β; tumor necrosis factor-alpha, TNF-α; vascular endothelial growth factor, VEGF; vascular endothelial growth factor receptor, VEGFR; wingless and Int-1 (Wnt), Wnt family member 2, WNT2.

Abstract

The liver sinusoid, mainly composed of liver sinusoidal endothelial cells, hepatic macrophages and hepatic stellate cells, shapes the hepatic vasculature and is key maintaining liver homeostasis and function. During chronic liver disease (CLD), the function of sinusoidal cells is impaired, being directly involved in the progression of liver fibrosis, cirrhosis, and main clinical complications including portal hypertension and hepatocellular carcinoma. In addition to their roles in liver diseases pathobiology, sinusoidal cells' paracrine communication or cross-talk is being studied as a mechanism of disease but also as a remarkable target for treatment. The aim of this review is to gather current knowledge of intercellular signalling in the hepatic sinusoid during the progression of liver disease. We summarise studies developed in pre-clinical models of CLD, specially emphasizing those pathways characterized in human-based clinically relevant models. Finally, we describe pharmacological treatments targeting sinusoidal communication as promising options to treat CLD and its clinical complications.

Keywords

Hepatic sinusoid, chronic liver disease, liver sinusoidal endothelial cells, hepatic stellate cells, Kupffer cells.

1- The hepatic sinusoid in health

The liver is the first organ to receive the nutrient-rich blood from the intestine. Before distributing it to other organs, the liver plays a pivotal role regulating its clearance and metabolic composition, thereby maintaining body homeostasis. In addition to its metabolic functions, the liver produces essential plasma proteins, such as albumin, transferrin, and lipoproteins, into the bloodstream and actively participates in the immune response(1).

Most of these processes take place in the liver sinusoids, a unique type of microvascular beds. The sinusoids receive a mixture of blood from the portal vein (nutrient-rich) and the hepatic artery (oxygen-rich); and deliver it to the central veins, that drain it to the vena cava and the systemic circulation(2). In the sinusoids, the different hepatic cells interact with each other, establishing an efficient signalling network that maintains liver function and homeostasis(3).

Liver sinusoidal endothelial cells (LSECs) surround these specialized blood vessels and are characterized by a thin cytoplasm, the presence of numerous fenestrae and lack of a basal membrane, allowing oxygen, nutrients and other small molecules to diffuse to the space of Disse and reach hepatocytes and hepatic stellate cells (HSCs)(4). LSECs also clear colloids and macromolecules from the blood circulation, contribute to the maintenance of the liver immunological tolerance and regulate the vascular tone of the sinusoids, secreting vasoconstrictive and vasodilatory mediators, such as endothelin 1 (ET-1) or nitric oxide (NO)(4). In order to maintain their healthy phenotype, LSECs require stimulation by paracrine molecules produced by hepatocytes and HSCs, such as vascular endothelial growth factor (VEGF)(5,6) or bone morphogenetic protein 9 (BMP-9)(7).

HSCs are mesenchymal cells located in the space of Disse, that wrap the sinusoids and are in close contact with LSECs and hepatocytes. HSCs store up to an 80% of total vitamin A in the body, and act as liver pericytes(8). HSCs maintain a “quiescent” non-proliferative state in healthy livers; however, the maintenance or loss of their quiescence is influenced by several extracellular signals. Healthy LSECs paracrinally maintain HSCs quiescence through different factors, such as NO(9) and heparin-binding EGF-like growth factor (HB-EGF)(10). Quiescent HSCs can also promote their quiescence autocrinally secreting microvesicles that contain the transcription factor Twist1 or the microRNA-214(11).

Kupffer cells (KCs) are the resident macrophages of the liver sinusoids that remain in the lumen and have a scavenger function, clearing cellular debris and metabolic waste. These cells also mediate the antimicrobial defence, promote immunological tolerance and, in conjunction with LSECs and hepatocytes, have an important role in both iron and cholesterol homeostasis (12). Importantly, KCs produce vasoprotective mediators, such as carbon monoxide(13), as a result of haemoglobin degradation. Interestingly, paracrine signalling from hepatocytes, LSECs and HSCs is crucial to differentiate monocytes into new KCs(14,15). Indeed, LSECs promote KCs differentiation and phenotype maintenance through the secretion of transforming growth factor beta-1 (TFG-β) proprotein family ligands and delta like canonical notch ligand (DLL4)(15).

Hepatocytes, the most abundant hepatic cell type, are polarised polyhedral cells that form the liver parenchyma. Their basal plasma membranes have microvilli that extend into the space of Disse, increasing the surface area for exchanging substances with the plasma(16). Hepatocytes perform the majority of the liver’s metabolic and synthetic functions, including carbohydrate, lipid and protein metabolism, detoxification and plasma protein secretion(17). Interestingly, many of these functions are regulated by their interactions with non-parenchymal cells (NPCs). In fact, hepatocytes not only present a more physiological phenotype in co-culture with NPCs(18), but also human hepatocytes engrafted in mice hosts show a better metabolic profile when engrafted in conjunction with NPCs(19).

The various sinusoidal hepatic cell types engage in continuous crosstalk, which is necessary for maintaining liver function and for rapidly and coordinatedly responding to any detected stress or alteration in the physiological environment (Figure 1). This communication is performed through direct contact between cells or through different paracrine effectors, including soluble signalling molecules, proteins deposited in the extracellular matrix and extracellular vesicles (EVs). EVs can transport not only signalling proteins, but also lipids, metabolites and RNA, including non-coding RNAs such as miRNAs(20,21).

These paracrine signals may act as morphogen gradients and, together with the oxygen gradient, seem to maintain the liver metabolic zonation (22). For instance, the Wingless and Int-1 (Wnt)/β-catenin signalling pathway, which also plays a key role in liver development and liver regeneration, has recently proven to be a master regulator of hepatocyte liver zonation in mice (23), and the Hedgehog signalling pathway has also been suggested to play a similar role (24). In this regard, LSECs and HSCs in healthy livers are important sources of secreted factors such as Wnt family member 2 (WNT2), R-spondin 3 (RSPO3) or Hedgehog interacting protein (HHIP), that control these two pathways (24–26). Hepatocytes can also modulate the metabolism of neighbouring cells via the intracellular calcium signalling system, which affects both internal processes and adjacent hepatocytes, regulating the activity of the intracellular enzymes glycogen synthase and glycogen phosphorylase(27), and with the release of extracellular nucleotides, mainly ATP and UTP, to the sinusoidal space(28). Cytokines can also regulate hepatocyte metabolism (29–31). As examples, IL-6 and IL-13 inhibit the transcription of gluconeogenic genes acting through STAT-3 (29,30), while TGFβ inhibits the transcription of enzymes involved in cholesterol biosynthesis and accumulation (31).

Other paracrine signals seem to coordinate cells that sense biomechanical changes or stimuli with the effector cells that respond to them. For instance, an increase in blood shear stress further promotes the expression of the vasoprotective transcription factor Krüppel-like factor 2 (KLF2) in LSECs. This transcription factor modulates the endothelial nitric oxide synthase (eNOS) pathway, increasing the production of the vasodilator NO, mediating HSCs relaxation via cyclic guanosine monophosphate (cGMP) formation. As a result, the sinusoids vasodilate, compensating the increase in blood shear stress and maintaining homeostasis of the sinusoidal blood flow(32–35). Another example of the importance of sinusoidal communication is the regulation of hepcidin, a hormone that limits iron entry into the bloodstream(36). Hepcidin is mainly produced by hepatocytes, but is partially regulated by LSECs, which sense the changes in iron levels and secrete signals, such as BMP ligands, that induce hepcidin production(36). Although KCs also modulate hepcidin transcription, the precise mechanism needs further study(37).

Paracrine signalling in healthy livers is also necessary to confer immunological tolerance to the organ. Anti-inflammatory cytokines such as IL-10 from LSECs and KCs play a key role in generating and maintaining the tolerogenic microenvironment(38). Furthermore, LSECs present antigens to naïve CD4+ and CD8+ T cells and induce their differentiation into a regulatory and tolerogenic phenotype(39,40).

2- The hepatic sinusoid during the initiation of liver disease

Liver disease accounts for 4% of all deaths worldwide annually(41) and can result from different causes, including excessive alcohol consumption, metabolic disorders, viral infections, and genetic factors, among others(42). The healthy liver is primarily quiescent in terms of cellular proliferation. However, upon injury, cells rapidly enter the cell cycle, facilitating tissue repair and restoring homeostasis. During this wound healing process, both parenchymal and non-parenchymal cells respond in a coordinated manner(43,44) (Figure 2).

Initially, following liver injury, damaged hepatocytes undergo apoptosis or necrosis, releasing endogenous molecules, namely damage-associated molecular patterns (DAMPs), reactive oxygen species (ROS) and proinflammatory EVs(12,45). This process activates the inflammatory response, which in turn triggers the activation of resident KCs, the first-line responders upon liver injury, as well as the migration of infiltrated monocytes, macrophages, and dendritic cells to the damage site (44,46–48). Activated KCs actively participate in liver injury resolution through phagocytosis, danger signal recognition, cytokine release, antigen processing, and immune response orchestration (49). This role is accomplished through the release of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), IL-6, and IL-12(50); chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1α) (51); and profibrotic cytokines such as TGFβ and Platelet-derived growth factor (PDGF)(50). Also, activated KCs express the enzyme inducible nitric oxide synthase (iNOS), inducing excessive NO production, that reacts with ROS to form reactive nitrogen species (RNS) that prompt HSCs activation(52). Furthermore, TNF-α secreted by KCs activates nuclear factor-kappa B (NF-κB) in hepatocytes, promoting C-X-C Motif Chemokine Ligand 1 (CXCL1) expression, which induces neutrophil clearance of death cells(53). Inflammatory signals can also activate inflammasomes in KCs, which activates caspase-1 (CASP-1) and initiates the maturation of proinflammatory cytokines IL-1β and IL-18. Interestingly, both cytokines, IL-1β and IL-18, directly contribute to the activation of HSCs, inducing collagen deposition in the extracellular matrix (ECM) (54,55).

Also, during the first stages of liver damage, high mobility group box 1 (HMGB1) is secreted passively by dying hepatocytes as a DAMP; and actively by the subsequent arrival and activation of infiltrating macrophages, which signal neighbouring cells and enhance the inflammatory response (56). HMGB1 may also act as a nuclear DNA binding factor that in presence of oxidative stress relocates from the nucleus to the cytoplasm, inducing autophagy (56,57). In this regard, induction of autophagy by DAMPS may have a positive or deleterious effect in different situations or cell types (58). Particularly, autophagy generally functions as a protective mechanism, reducing damaged mitochondria, protein aggregates, oxidative stress levels and, subsequently, apoptosis. In the initial stages of liver fibrosis, autophagy would prevent fibrogenesis paracrinally, by supressing IL-1α/β secretion in KCs and preventing the recruitment of other inflammatory cells (59). In LSECs, autophagy induction would also maintain endothelial phenotype by conferring protection from oxidative stress through a KLF2-mediated mechanism (58,60), also suggesting a paracrine antifibrotic effect. Nonetheless, autophagy has a profibrogenic participation when acting on HSCs directly, as it aids the cells in consuming their lipid storage and obtain the necessary energy for their activation (58). This duality of autophagy in the initiation of liver diseases highlights the importance of considering cell communication in the hepatic sinusoid when designing therapeutic strategies for liver diseases.

In response to liver injury, LSECs also lose their differentiated phenotype in a process termed capillarization that is characterized by the loss of fenestrae and acquisition of a basement membrane which impedes an appropriate oxygenation of hepatocytes (61). Liver damage also causes the loss of

the cells' dilatory capacity, releasing high levels of vasoconstrictors such as COX-1-derived prostanoids and diminished NO production, leading to an increase in the vascular tone and portal pressure(62). Both factors, reduced endothelial porosity and limited microcirculatory perfusion, further aggravate and perpetuate hepatocyte mortality, which may progress to liver failure. Furthermore, studies on hepatectomized mice show that LSECs are required for hepatocyte proliferation through the secretion of angiopoietin 2 (Ang2), hepatocyte growth factor (HGF) and Wnt2; processes being ruled by VEGF receptor 1 (VEGFR1) and VEGFR2 activation(63–65). LSECs isolated from mice post-hepatectomy exhibited a notable expression pattern of Ang2. Immediately after surgery, Ang2 levels decreased, leading to reduced TGFβ1 production and thereby facilitating hepatocyte proliferation. Subsequently, during the angiogenic phase, Ang2 levels were restored, which promoted LSECs proliferation by autocrinally increasing VEGFR2 levels(63). After acute liver injury, LSECs upregulate C-X-C Motif Chemokine Receptor (CXCR)7 and CXCR4, which also induce liver regeneration through the transcription factor Id1, and the consequent secretion of HGF and Wnt2(66).

In addition, and contrary to the protective interactions observed in healthy status, capillarized LSECs actively contribute to the deregulation of neighbouring cells through the release of paracrine factors(61,67). In fact, capillarized LSEC no longer maintain HSCs quiescence due to the decrease in NO production and HB-EGF secretion(9,10), but actively induce their activation through the secretion of PDGF, TGF-β, fibroblast growth factor receptor 1 (FGFR1) and fibronectin(68); while decreasing the expression of the transcription factor KLF2, which acts as a vasoprotective molecule(35). During the initial stages of fibrosis, the lncRNA *Airn* maintains LSECs differentiated through the activation of the KLF2-eNOS-sGC pathway, prompting Wnt2a and HGF secretion, maintaining HSCs quiescence and inducing hepatocyte proliferation(69). However, in advanced fibrotic livers, the upregulation of this lncRNA is lost(69).

Activated HSCs gradually reduce their vitamin A storage capacity, and acquire a proliferative, pro-contractile, proinflammatory and profibrogenic phenotype, characterized by the release of IL-6 and TGFβ, along with a large number of collagen fibers and extracellular molecules within the liver parenchyma (70). Activated HSCs synthesize α-smooth muscle actin (αSMA) and migrate and proliferate to the sites of tissue injury to repair it, secreting collagens type I and III, vimentin, osteopontin, lysyl oxidases (LOX) and tissue inhibitor of metalloproteinase 1 (TIMP1) (71). Thus, HSCs are considered the main source of ECM components in the liver(72).

While transient inflammation is crucial for liver reparation and overall homeostasis, persistent injury alters the liver microarchitecture by interactions between cells and their niches, contributing to the progression of the disease(4). Thus, fibrosis is very dynamic and reversible at early stages but it may become irreversible with severe or chronic injury, potentially leading to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (73).

3- The hepatic sinusoid in chronic liver disease

During sustained liver damage, LSECs lose their characteristic fenestrae, stablish a basement membrane, and acquire a vasoconstrictor, proinflammatory and prothrombotic phenotype, with an altered pattern of adhesion molecules, such as ICAM-1, stabilin-1 or VAP1, that attract immune cells into the liver, followed by the acquisition of angiogenic properties(4,67,74). These alterations, coupled with intercellular communications within the liver sinusoid, will prompt LSECs to induce changes on the other hepatic cell types, directly contributing to liver disease development(4). Parallelly, KCs become chronically activated due to continuous binding of gut-derived bacterial compounds, such as LPS, or other proinflammatory factors secreted by different sinusoidal cell types, which leads to the secretion of many cytokines and proinflammatory factors that promote HSCs activation and the recruitment of leukocytes, perpetuating liver inflammation(12). Activated HSCs acquire a proliferative, profibrogenic, contractile and proinflammatory phenotype through their differentiation into myofibroblasts, being the main contributors to the accumulation of collagen and ECM components in the liver, thus, causing fibrosis(75,76). On top of this, hepatocyte death generates apoptotic bodies, that in turn, are captured by HSCs and KCs, which further prompts their activated phenotype(77). Particularly, the activation of KCs results in the secretion of death ligands, such as TNFα, that further stimulate hepatocyte apoptosis in a self-amplifying regulatory loop(77) (Figure 3).

Crosstalk between sinusoidal cells in cirrhosis

Communication through soluble factors

Liver damage and fibrosis induces the upregulation of TGFβ, which drives the overexpression of CD147 on the plasmatic membrane of hepatocytes and LSECs. This, in turn, triggers the upregulation of VEGF-A and VEGFR-2, respectively, via the PI3K/Akt pathway(78). This pathway is thought to be implicated in fibrosis progression through angiogenic signalling and the proliferation and tube formation of LSECs(78). In fact, in the hypoxic conditions that occur during cirrhosis, hepatocytes also secrete VEGF, further promoting LSECs proliferation(79). An increase in Leukocyte Cell Derived Chemotaxin 2 (LECT2) is also observed in hepatocytes and endothelial cells during liver cirrhosis, which promotes LSECs capillarization, further stimulating fibrogenesis(80).

The sinusoidal communication between LSECs and HSCs is critical during cirrhosis progression, with HSCs being primarily responsible for fibrotic accumulation. Principally, paracrine secretion of VEGF is necessary to maintain LSECs healthy phenotype and fenestrae(6). Moreover, VEGF signalling is required for NO induction(9), which also has a particular autocrine regulatory loop on LSECs. Inhibition of eNOS activity induces LSECs capillarization and HSCs activation, thus, maintenance of LSECs phenotype requires both a VEGF-stimulated-NO-dependent and independent pathway(6). Furthermore, Hedgehog ligands, which can be secreted by LSECs themselves, HSCs or hepatocytes, among other liver cell types, are also key inducers of LSECs capillarization(81). The significance of communication between LSECs and HSCs is highlighted by the fact that the restoration of LSECs phenotype is capable of reverting HSCs activation, halting fibrosis progression and facilitating regression of mild fibrosis (6,9). On the contrary, capillarized LSECs are incapable of reversing HSCs activation(9), which shows the importance of healthy LSECs maintenance and the treatments to reverse their dedifferentiation.

Sphingosine 1-phosphate receptor 2 (S1PR2) has recently been identified as a receptor that in preclinical models of CCl_4 and bile duct ligation (BDL)-induced liver fibrosis is upregulated in LSECs, inducing *Tgfβ* gene expression when activated, thus, promoting HSCs activation, proliferation and

migration, which leads to liver fibrosis(82). Similarly, the activation of S1PR1 and S1PR2, has been directly linked to induction of angiogenesis in human and mouse livers(83). Particularly, S1P, the ligand of SPR, induces the expression of Ang1 in HSCs(83). Further *in vivo* and *in vitro* studies in immortalized LSECs have shown that SK1, the enzyme that produces S1P, is an endothelial cell-derived exosome protein, and that these exosomes induce HSCs activation, demonstrated through increased cell migration and activation of AKT(84). In fact, SK1 was observed to be upregulated in cirrhotic livers from human patients and in preclinical models of cirrhosis, and the inhibition of S1PR2 protected against CCl_4-induced liver fibrosis in mice(84). Moreover, during the hypoxia that typically occurs during fibrosis, LSECs upregulate DLL4, a key inducer of capillarization, that is also implicated in HSCs activation through ET-1(85). At advanced stages of liver fibrosis, BMP9, expressed mainly by LSECs and HSCs is increased, inducing HSCs activation through SMAD/Id1 pathway(86).

Importantly, the complete secretome derived from primary LSECs cultured *in vitro* has the capacity to modulate the phenotype of the other cell types. For instance, HSC exhibit activation when exposed to the secretome from LSECs originating from cirrhotic rats, while KCs polarize to a proinflammatory phenotype(87).

Communication with the extracellular matrix

The liver relies extensively on the ECM for maintaining both its structural integrity and its function. Comprising a complex network of proteins, glycoproteins, and polysaccharides, the ECM serves as a dynamic scaffold, capable of orchestrating cell-cell communication, tissue architecture, and biochemical signalling in the hepatic sinusoid(88). In healthy conditions, ECM is being continuously remodelled, maintaining the balance between synthesis, secretion, degradation, and reorganization of its components (89). In contrast, during the wound healing that accompanies chronic liver injury, this equilibrium is disrupted by an excessive synthesis of matrix components and an inhibition of the degradative processes, leading to a distortion of the hepatic architecture (71,90). Moreover, this process is accompanied by an increase of inflammatory molecules such as MCP-1, PDGF, macrophage colony-stimulating factor (MCSF) and HMGB1; and an increase in the synthesis and deposition of type I and type III collagen, the most abundant components of the ECM (72,88). These processes collectively disrupt cell function and contribute to an increased liver stiffness, which influences cellular crosstalk, by perpetuating liver cell activation and disease progression (68,91,92).

For instance, the ECM component fibronectin, that can be found in the soluble form mainly secreted by hepatocytes, or in cellular form, produced by many cell types but particularly the endothelium, has also been shown in a preclinical model of cirrhosis to stimulate HSC activation. More specifically, in HSC, fibronectin induces the production of ET-1, which autocrinally activates HSCs contraction and alpha-smooth muscle actin (α-SMA) synthesis(93). Moreover, fibronectin can also be released by HSCs in an LPS-TLR4 dependent manner, activating liver endothelial cell angiogenesis through tubulogenesis and migration(94). Dysfunctional LSECs also contribute to generate ECM by releasing laminins, nidogen/entactin, proteoglycans, and the non-fibrillar collagens, like collagen type IV (72,95).

On the other hand, the composition of the ECM has also been shown to influence LSECs phenotype. Even though LSECs lose their fenestrae and the expression of CD32b *in vitro*, collagen I, III, and mixtures of I, IV and fibronectin showed positive effects on maintaining LSECs differentiation(96).

Interestingly, the substrate rigidity has also been shown to induce significant alterations in LSECs and HSCs phenotype, highlighting the importance of ECM mechanical properties in liver homeostasis and disease. For instance, human LSECs lose their fenestrae and increase CD31 protein expression, a

known marker of LSEC capillarization at high substrate rigidities(97,98). This effect was reported to be induced through the activation of focal adhesion kinase signalling, which prompted actin remodeling(98). Similarly, primary rat HSCs get activated as the stiffness of their substrate increases(99). The authors emphasized that this activation depended predominantly on the substrate's physical characteristics, rather than the chemical properties(99). Hepatocytes, LSECs and HSCs isolated from cirrhotic rats, cultured in polyacrylamide gels with different degrees of stiffness, showed an amelioration on their phenotype when cultured in low stiffness, with LSECs decreasing the expression of the capillarization marker *Lamb1* and increasing the number of fenestrae, and HSCs showing a lower degree of activation(91). Similar results were obtained in HSCs isolated from healthy rats, where on stiff substrate, HSCs acquired an activated phenotype characterized by lipid droplet loss and higher α-SMA protein levels(100). All these studies suggest that not only molecules in the ECM, but also its mechanical properties influence the phenotype of sinusoidal cells.

Extracellular vesicles in sinusoidal communication

EVs are comprised of cell-derived lipid bilayer membranes that encapsulate various molecules(101). We can classify them in exosomes, microvesicles or apoptotic bodies depending on whether they originate in the cytoplasm from the endosome, are created directly from the plasma membrane, or produced during apoptosis(101). In the sinusoid, hepatocytes, HSCs and LSECs have all been shown to secrete EVs(102). Interestingly, a high percentage of the genes that are altered during cirrhosis in human livers have been shown to be involved in EVs biogenesis, a fact that was also observed in LSECs during the progression of CLD in a preclinical model, suggesting the key role of EVs communication in liver disease(87).

EVs have been found to be key in sinusoidal communication during advanced CLD (ACLD) in different aetiologies. For instance, in a preclinical model of metabolic dysfunction-associated steatotic liver disease (MASLD), the concentration of EVs increased both in the liver and circulating blood, compared to the healthy group, and particularly, these EVs contained proteins involved in hepatocyte death, inflammation and pathological angiogenesis(103). Other clinical and preclinical studies have shown that the EVs secreted by these lipotoxic hepatocytes contain inhibitors of peroxisome proliferator activated receptor gamma (PPARγ), such as the miR128-3p, that can activate HSCs when internalized(104), and other miRNAs such as miR1297 and miR27a, all of them inducers of HSCs activation and proliferation(105,106). In MASH, the EVs secreted by lipotoxic hepatocytes carry mitochondrial DNA that signals nearby macrophages inflammatory pathways and angiogenesis(107–109); while in *vitro* studies have shown that exosomes from palmitic acid-treated primary mice hepatocytes induced the activation of HSCs(110).

Cirrhotic patients show an increase in microparticles derived from hepatocytes in the plasma, which, together with microparticles derived from other cell types, have been shown to disrupt the vasoconstrictor capacity of endothelial cells in the systemic circulation, contributing to the arterial vasodilation associated with portal hypertension(111). More in detail, the uptake of these microparticles by endothelial cells, leads to the formation of vasodilatory prostaglandins and hyporeactivity to vasoconstrictors, in a COX-1 phosphatidylserine- and phospholipase A2-dependent way(111). On the inverse, intrahepatically, COX-1-derived prostanoids synthetised by LSECs of cirrhotic rats induce vasoconstriction of the sinusoids(112). Microvesicles are also being studied in LSECs and HSCs communication. For instance, portal myofibroblasts have been shown to release microparticles packed with VEGFA, which promote an increase in LSECs proangiogenetic activity(113). On the other hand, in disease, LSECs release exosomes rich in sphingosine kinase-1 (SK1), that adhere

to HSCs through a fibronectin mediated mechanism, and activate HSCs, inducing their migration(84). However, the role of microvesicles on liver fibrosis is not clear, with some studies showing their role inducing a proinflammatory response, and others showing the contrary(114). In patients with ACLD linked to ethanol consumption, the quantity of exosomes secreted by hepatocytes is also increased, and these are packed with mitochondrial double strand RNA, that activate KCs' TLR3 receptors, inducing the secretion of IL-1β and further contributing to liver inflammation(115). Also in alcohol-related liver disease, activated HSCs secrete EVs that induce the activation of quiescent HSCs, by increasing their expression of VEGF. These same EVs activate glycolytic pathways, not only in HSCs, but also in the other non-parenchymal sinusoidal cells: KCs and LSECs, being partially responsible for a metabolic switch in the sinusoidal cell types(116). *In vitro,* human hepatocytes infected by HCV secrete exosomes carrying miR19a, that are internalized by HSCs, inhibiting the suppressor of cytokine signaling 3 (SOCS3), which in turn activated the TGFb1 signalling pathway, thus, activating HSCs and the expression of profibrotic genes(117).

Activated HSCs secrete exosomes with different content than quiescent HSCs(118). For instance, EVs from quiescent HSCs can contain Twist1 and miR-214, inhibitors of connective tissue growth factor (CCN2), a key gene in activated HSC that regulates the expression of α-SMA and collagen; however, in liver fibrosis, the levels of this microRNA is diminished, thus, CCN2 is increased (11,119). On the other hand, exosomes from activated HSCs can induce macrophage migration and activation, a mechanism though to be induced by ectodysplasin-A mRNA included in the exosomes(118), or contain profibrogenic CCN2 protein and mRNA; promoters of HSCs activation, that are captured by other quiescent or activated HSCs(120).

Despite significant advances in our understanding of exosomes in the hepatic sinusoid communication, further research is necessary to fully unlock their potential. Recent studies have demonstrated that EVs secreted by hepatocytes, particularly their molecular content, hold promise as biomarkers. These specific molecular signatures can accurately stratify patients based on the severity of cirrhosis(121). Thus, future studies should focus on exosomes, and their content, as biomarkers for both the diagnosis and prognosis of ACLD patients; and new therapies targeting exosomes should be explored for treating ACLD.

New insights on sinusoidal communication in cirrhosis

Using CellChat, an open source R package(122), we reanalized previously published human liver scRNA-seq data(123) to ultimately predict sinusoidal intercellular communications in chronic liver disease. As shown in Figure 4, sinusoidal cell-cell communication greatly increases in cirrhosis, with a higher number of ligand-receptor pairs between the different sinusoidal cell types. Moreover, the number of ligand-receptor interactions increases between most of the main cell types of the liver sinusoid (LSECs, HSCs, KCs and hepatocytes), which correlates with the numerous changes that occur during CLD development in the hepatic sinusoid. Figure 4C shows changes in the top significant communication pathways between control and cirrhotic livers. These new analyses highlight the key interactions between sinusoidal cells reviewed in the present paper in the human liver, both in physiological and pathological conditions. Interestingly, some of these communication pathways seem exclusive to either health or the diseased stage. For instance, in a healthy microenvironment, our analysis indicates the activation of the neurotrophic receptor tyrosine kinase 2 (NTRK2) (Netrin and NT pathways) in HSCs either by endothelial or mesenchymal ligands. Consistently, this receptor has recently been associated to antifibrotic properties in animal models (124). Similarly, our analysis predicts that the PROS1 ligand signals from endothelial cells to either mesenchymal cells or

macrophages through the AXL receptor, which could have anti-inflammatory properties as recently described in human biopsies (125). On the other hand, our predicted ligand-receptor pathways which are exclusively found in cirrhosis mostly involve receptors for cell-ECM or cell-cell attachment, including integrins, cadherins and occludins (FN1, THY1, CDH, ANGPT, CADM, OCLN, GAP pathways). In this regard, an increased cellular attachment has been extensively described in response to elevated liver stiffness due to cirrhosis as a mechanism of mechanosensing (126). Altogether, these analyses support the use of recent single-cell sequencing data for hypothesis generation and data validation for the discovery of new markers and targets of sinusoidal communication in liver diseases.

Sinusoidal crosstalk in hepatocellular carcinoma

Although the role of NPCs and their communication during HCC is not well understood, it has recently been reported that HSCs directly contribute to the growth of the tumour by expressing growth differentiation factor 15 (GDF15) through an autophagy dependent-manner(127). On the other hand, LSECs lose specific phenotype markers such as stabilin-1, stabilin-2, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) and CD32, while acquiring a sprouting angiogenic phenotype(128). During MASH, LSECs have been shown to express FABP4, which is found downregulated in healthy conditions. This increased expression of FABP4 responds to increased levels of glucose, insulin and VEGFA, and hypoxia. Moreover, in these same conditions, endothelial cells secrete FAB4 contained in vesicles that can be absorbed by HepG2 cells, inducing pro-oncogenic effects such as increased viability, proliferation and migration(129). Although further study is needed, these results suggest that HSCs and LSECs might have a key role in the progression of HCC, inducing the transformation of hepatocytes into a pro-tumoral phenotype and actively participating on its growth.

4- Therapies targeting hepatic sinusoid communication in chronic liver disease

Understanding the molecules and mechanisms involved in the development of CLD, including cell–cell crosstalk and signalling pathways, could reveal potential targets for clinical treatment. In this section, we summarize the current clinical and preclinical evidence to tackle CLD from a sinusoidal crosstalk perspective (see also Table 1).

Extracellular matrix/stiffness crosstalk

As commented above, communication through the ECM is crucial in both fibrosis and cirrhosis. High stiffness of the ECM promotes HSCs activation through diverse mechanisms including a E2F transcription factor 3 (E2F3)-dependent one, which activates fibroblast growth factor (FGF), contributing to tumour development and metastasis by activation of PI3K/AKT and MEK/ERK pathways. Interestingly, E2F3 knockdown mice subjected to CCl_4 treatment showed a reduction of expression in HSC activation markers, α-SMA, collagen type I alpha 1 (Col1α1) and FGF2, in comparation to wildtype mice, emerging as a potential target for HCC treatment(130). Moreover, it is known that increased matrix stiffness causes LSEC defenestration via focal adhesion kinase 1 (FAK), leading to the activation of p38-MAPK Activated Protein Kinase 2 (MK2), and the inhibition of either FAK or p38-MK2 effectively restored fenestrae in LSECs from fibrotic livers(98). Targeting cross-linking components of the ECM, such as with simtuzumab, a humanized anti-LOXL2 antibody, was proposed for the treatment of liver disease(131). However, it proved unsuccessful in phase 2b clinical trials(132).

Restoration of LSECs phenotype

Capillarized LSECs constitute a pivotal factor in the initiation and progression of liver diseases. Accordingly, the restoration of LSECs phenotype is essential for vascular permeability, hepatocyte functionality, HSCs activation and macrophages recruitment and polarization, and therefore, is a crucial therapeutic target. The inhibition of Hedgehog signalling, a pathway that increases during chronic liver injury, has been reported to protect LSECs against capillarization in cirrhotic mice(81). Furthermore, tofogliflozin, an antidiabetic drug (133), has shown beneficial effects in intrahepatic vascular resistance as well as a reduction in liver fibrosis and hepatic inflammation. More specifically, tofogliflozin restores LSECs phenotype by increasing CAV-1 expression, a marker of fenestration and NO production, while reducing ET-1 expression; altogether suppressing HSCs activation by inhibiting profibrogenic and proliferative activities via the NO/sGC/cGMP pathway (134).

Although statins are not currently indicated for liver disease treatment, recent reports have increasingly shown their favourable effects in reducing portal pressure, inhibiting fibrogenesis, and improving liver sinusoidal endothelial function and hepatic microvascular dysfunction (135). For instance, simvastatin ameliorates HSCs and LSECs phenotype which, paracrinally, ameliorates the dysfunctional sinusoidal milieu in cirrhotic rats (34,35), while atorvastatin inhibits non-canonical Hedgehog pathway and angiogenesis in CCl_4 and BDL-induced fibrotic rats (136).

Autophagy modulation

As previously mentioned, the transcription factor KLF2 plays a protective role in the sinusoidal endothelium by promoting the activation of diverse vasoprotective pathways inducing autophagy. In LSECs, KLF2 expression can be upregulated by simvastatin and resveratrol treatments, facilitating the autophagic flux as shown in a preclinical study, thereby conferring vasoprotective effects to the liver(60). Furthermore, KLF2-mediated endothelial protection extends paracrinally to the other hepatic cell types.

On the other hand, during fibrosis development, autophagy in HSCs contributes to their activation (58). Consequently, inhibitors of autophagy specifically targeting HSCs may represent a novel therapeutic approach to promote fibrosis regression. Preclinical studies have supported this hypothesis, demonstrating improvement in different parameters during chronic liver disease. For instance, doxazosin, which blocks autophagic flux through activation of the PI3K/Akt/mTOR pathway, has been shown to mitigate liver fibrosis in CCl_4-induced cirrhotic mice(137). Another approach involves HMGB1 inhibitors such as jaceosidin acid, which has been shown to suppress fibrogenesis and inflammation in thioacetamide (TAA)-induced cirrhotic mice(138). *In vitro* studies on the human hepatic stellate cell line LX2 have demonstrated that carvedilol treatment decreases α-SMA expression in a dose-dependent manner, indicating HSCs deactivation associated with autophagy suppression (139). On the contrary, autophagy induction through the inhibition of mTOR by rapamycin, in primary human HSCs, showed a decrease in PDGF-mediated exosome release. In addition, inhibition of mTOR pathway through deletion of SH2 domain-containing protein tyrosine phosphatase 2 (SHP2) in mice exhibited a decrease in liver fibrosis and α-SMA and collagen type I expression, showing that inhibiting the SHP2-mTOR signalling could potentially serve as a new target for treating liver fibrosis (140).

On the other hand, autophagy has been suggested to prevent tumorigenesis in patients with a high risk of developing HCC (58). In preclinical studies, drugs such as sorafenib or bortezomib, which inhibit the PI3K/Akt pathway, have shown an activation of autophagic flux in tumoral cells inducing cell death, angiogenesis and a reduction of tumour size ***(141–143)***. Conversely, other studies have associated autophagy induction with higher tumorigenesis, tumour proliferation, migration, and drug resistance in patients with advanced HCC. Thus, combining autophagy inhibitors with chemotherapy drugs has shown improved therapeutic effects both in vivo and in vitro ***(144)***. For these reasons, a deeper understanding of autophagy during the different stages of liver disease is required.

Extracellular vesicles

Crosstalk between liver cells also involves EVs, which regulate the phenotype of neighbouring cells depending on their content. EVs have emerged as promising targets for attenuating diseases or serving as drug delivery nanovectors due to their lower toxicity and minimal reactivity with the immune system. After liver injury, cells release EVs to regulate hepatic response(145). For instance, as commented above, SK1 is overexpressed in exosomes derived from serum samples of cirrhotic patients, showing a negative effect on HSCs. Interestingly, the treatment with SK1 phosphate receptor 2 (S1PR2) inhibitor protected mice from BDL-induced or CCl_4-induced liver fibrosis(84). Emricasan is an irreversible pan-caspase inhibitor, which improves portal hypertension, fibrosis and liver function in decompensated cirrhotic rats by improving hepatocyte, HSCs, LSECs and macrophages phenotype. Interestingly, in vitro experiments with primary cirrhotic liver cells observed that the beneficial effects were mediated by hepatocyte-derived EVs, and not by a direct effect on HSCs, LSECs and KCs(146). However, these effects were not confirmed in a phase II clinical trial in patients with MASH cirrhosis and portal hypertension(147).

miRNAs are also involved in liver disease and rule the expression of different pathways involved in sinusoidal crosstalk. For this reason, their modulation is being considered as promising therapy(148). For instance, the miR-690, which is normally secreted by KCs, plays a role in inhibiting lipogenesis in hepatocytes, exerting anti-inflammatory effects in other KCs and inhibiting profibrotic signalling in HSCs. When miR-690 was injected to mice in a preclinical model of MASH, it demonstrated beneficial effects on hepatocytes and HSCs by inhibiting fibrosis and inflammation, indicated by a reduction in collagens, α-SMA, Timp1, Tgfβ, Ccl2, Il1β, and TNFα; as well as steatosis (149). Additionally, miR-411-

5p, which is downregulated in the serum of MASH patients and secreted by M2-macrophages, is known to paracrinally inhibit HSC activation via CAMSAP1. This miRNA was shown to reduce the expression of α-SMA, COL1a1 and COL3a1 in LX2 cells (150) .

In recent years, stem cells have also been proposed as a novel treatment for liver diseases. For instance, Povero et al. treated activated human primary HSCs with EVs isolated from human and murine induced pluripotent stem cells (iPSCs), and showed a reduction in the expression of profibrogenic, proliferative and migration markers. Similar results were obtained when murine iPSC-derived EVs were injected in two murine models of fibrosis (CCl_4 and BDL) (151). In this regard, decompensated CCl_4-induced cirrhotic rats treated intraperitoneally with primary human amniotic membrane-derived mesenchymal stromal (hAMSCs) or epithelial (hAECs) stem cells isolated from human placentas presented a significant lower portal pressure and improved liver microcirculatory function, as well as decreased inflammation and oxidative stress compared to vehicle. These results were attributed to a protective paracrine signalling, which translated to a healthier LSECs phenotype, and overall HSCs deactivation(152). These results were validated *in vitro* with primary LSECs and HSCs, which were co-cultured with hAMSCs and hAECs. The results showed an improvement in LSECs phenotype, characterized by the upregulation of eNOS, stabilin 2, Vefgr1 and Klf2. Additionally, HSCs inactivation was observed, accompanied by changes in pathways related to extracellular matrix remodelling, hypertension pathophysiology, and inflammation, caused by a paracrine effect of stem cells (152). The beneficial effects of mesenchymal stromal cells (MSCs)-derived EVs have demonstrated a positive synergy when combined with other drugs possessing antifibrotic properties, such as obeticholic acid (OCA) or TC14102, in murine models of liver fibrosis (153,154). OCA, an FDA-approved FXR agonist for treating cholestatic liver disease, reduces HSCs activation and remodels the ECM, without inhibiting apoptosis (155). Enhanced responses in combating liver fibrosis have also been observed with the combination of OCA and apoptosis inhibitors (156). Additionally, TC4102, a CXCR7 agonist, inhibits HSC activation, reduces collagen deposition and attenuates the inflammatory response. CCl_4-induced cirrhotic mice treated with human umbilical cord-derived MSCs (UC-MSCs) pre-treated with TC14012 showed an enhanced anti-fibrotic and anti-inflammatory response compared to the group only treated with UC-MSCs. Underlying mechanisms included a decrease in CXCR7 expression in LSECs, which reduced α-SMA protein expression, fiber deposition and IL-1β positive cells in the liver (154) through crosstalk.

Nanomaterial-based drug delivery targeting

In recent years, polymer-drug conjugate micelles have emerged as a promising new treatment modality for CLD and HCC. Micelles offer several advantages over free drugs, including improved drug solubility, prolonged *in vivo* circulation, the ability to co-load different types of drugs, and selective distribution (157). In the context of liver diseases, a treatment involving micelles containing Hedgehog pathway inhibitors has shown promising results in mice with BDL-induced liver fibrosis. This treatment effectively restored LSECs fenestrae, thereby improving the exchange between blood and liver cells. Additionally, a paracrine effect via NO signalling was observed, leading to HSCs deactivation and a liver fibrosis attenuation (158).

Additionally, micelles loaded with chemotherapeutic drugs targeted to treat HCC, such as NO and paclitaxel, showed a tumour growth inhibitory effect in the Kunming mouse model, promoting cell death pathways (159). Another type of nanoparticle for drug delivery with specific cellular targeting, ultrasmall size, water-solubility, and outstanding biocompatibility are quantum dots (QDs) (160). These QDs can be conjugated with hyaluronic acid derivatives for the treatment of various chronic liver diseases (161). For instance, trichrome-tryptophan-sorbitol-QDs have demonstrated significant

tumour inhibition by inducing autophagy in *in vitro* and *in vivo* studies, with minimal systemic toxicity (162). Interestingly, metformin incorporated into QDs demonstrated enhanced pharmacokinetics and pharmacodynamics in the liver of aged mice, compared to free metformin(163). This novel approach is promising, as metformin treatment in cirrhotic rats has shown a decrease in fibrosis, portal pressure and hepatic vascular resistance(164), while in aged mice has shown a restoration in the number of fenestrations in LSECs(165), key in sinusoidal communication. This opens a potential new avenue for research in the treatment of cirrhosis.

5- Conclusions and future directions

Communication between sinusoidal cells, parenchymal cells and the ECM is essential for the proper function of the liver. Therefore, understanding the changes occurring in intrahepatic communication in the setting of CLDs is key for the discovery of new treatments and biomarkers.

However, the study of sinusoidal communication possesses complex challenges. The first one being that studying a specific communication pathway usually involves *in vitro* cross-talk studies. It is well known that traditional *in vitro* systems present several limitations, the main ones being the absence of physiologic biochemical and biomechanical stimulation. In this regard, recent research has proposed new *in vitro* culture systems that better recapitulate the sinusoidal microenvironment, such as dynamic flow chambers allowing co-culture of cells, gels for 3D culture, liver organoids and others (166). Although these techniques still present some limitations, a combination of them could improve our understanding of the complexity of cellular communication.

On a different path, the study of human biopsies is the gold standard for keeping relevant cellular phenotypes. However, unlike *in vitro* systems, the complexity of tissues usually impairs the ability to discern the sources or targets of cellular communication. Nevertheless, novel omics technologies such as single-cell / single-nuclei sequencing or spatial transcriptomics / proteomics / metabolomics are currently being used to deconvolute the information found in a tissue sample into more specific data on individual cell types and areas of the tissue. The combination of these techniques with bioinformatic analyses allows the study of possible communication patterns within a liver sample, as shown in our analysis in Figure 4.

Therefore, the tandem of single-cell omics with novel *in vitro* culture systems is currently revolutionizing the field of Hepatology, allowing for faster and more accurate discovery of biomarkers and targets of liver disease. We believe that these technologies, in combination with novel cell-type specific drug/miRNA delivery systems, will represent a big step towards precision medicine, allowing for a better characterization of patients and personalized treatments.

Methods

Analysis of human sinusoidal cell-cell interactions

Sinusoidal cell-cell interactions were analyzed in previously published scSeq RNA data(123). Briefly, the scSeq RNA data was processed as described in the original paper using the Seurat package for R(167). The Seurat object containing 4 main clusters (epithelia, including hepatocytes; endothelia, including LSEC; mesenchyma, including HSCs, and macrophages, including KCs) was then loaded into a CellChat object(122) in order to predict cell-cell interactions using the default package's functions.

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Schematic figures were created with biorender.com.

Author's contribution

A.G.-R., M.A.-R, R.P. searched data for the article. S.G.-M and P.A.-R. performed the analysis of human sinusoidal cell-cell interactions. A.G.-R., S.G.-M. and J.G.-S. coordinated the work. J.G.-S. conceived the work. All authors contributed substantially to discussion of the content. All authors wrote the article. All authors reviewed and/or edited the manuscript before submission.

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Table 1. Therapeutic strategies to treat CLD involving a paracrine mechanism in the sinusoid. BDL = bile duct ligation. CCl_4 = carbon tetrachloride.

Main mechanism	Drug	Mechanism of action	Administration method	Experimental model	Hemodynamics effects	Cellular effects	Reference
ECM/Stiffness	Simtuzumab	anti-LOXL2 antibody	Subcutaneous injection	Clinical trials	-	-	(131)
	PF-573228	FAK inhibition	Intraperitoneal injection	CCl_4-induced fibrotic mice	-	LSEC restoration	(98)
	OCA and IDN-6556	FXR activation pathway and apoptosis inhibitor	Oral gavage	CCl_4-induced fibrotic mice	-	↓α-SMA ↓ fibrosis ↓ Death cell	(156)
LSECs phenotype restoration	Tofogliflozin	SGLT2 inhibition	Oral gavage	CCl_4-induced cirrhotic rats	↓PP ↓ IHVR	Sinusoidal capillarization inhibition ↓VWF ↑ CAV-1 ↑ NO production ↓ ET-1 expression HSC deactivation (↓α-sma, Col1α1, Pdgfrβ,) ↓ Fibrosis ↓Inflammation	(134)
	Simvastatin	KLF2 induction	Oral gavage	CCl_4-induced cirrhotic rats	↓ IHVR	HSC deactivation (↓α-sma, pro-Col1, Des) ↓ Fibrosis	(35)
	Atorvastatin	Hg pathway inhibition	Oral gavage	CCl_4-induced and BDL fibrotic rats	↓ IHVR	-	(136)
Autophagy	Simvastatin	KLF2 inducer	Oral gavage	Healthy rats	-	Vasoprotective effects	(60)
	Carvedilol	Nonselective β-blocker	*In vitro*	LX2 cells	-	↓α-SMA	(139)

	Doxazosin	PI3K/Akt/mTOR pathway activation	*In vitro* and oral gavage	LX2 cells and CCl_4-induced cirrhotic rats	-	↓HSC proliferation↓α-SMA ↓COL1α1 ↑HSC apoptosis	(137)
	Rapamycin	mTOR inhibition	*In vitro*	Primary human HSCs	-	↓PDGF-related exosomes	(140)
	Jaceosidin	Modulation of HMGB1 / TLR4 signalling pathway	Oral gavage	TAA-induced fibrotic mice	-	↓α-SMA ↓COL1α1 ↓VGLL3 ↓IL1β	(138)
Extracellular vesicles interactions	Emricasan (IDN-6556)	Pan-caspase inhibitor	Oral gavage	CCl_4-induced cirrhotic rats	↓PP	↓α-SMA ↓DES ↓p-MOESIN/MOESIN ↓ Fibrosis ↓vWF ↑fenestrae porosity ↓inflammation	(146)
	Exosomes-loaded OCA	FXR activation pathway	Intraperitoneal injection	CCl_4-induced fibrotic mice	-	↓α-SMA ↓COL1 α1 ↓TGFβ ↓TIMP-1 ↓Fibrosis	(153)
	TC14012-treated UC-MSCs	CXCR7 agonist	Tail vein injection	CCl_4-induced fibrotic mice	-	↓α-SMA ↓IL1-β ↓Fibrosis	(154)
Drug delivery	MDB5 loaded micelles	Hg pathway inhibitor	Tail vein injection	BDL fibrotic mice	-	↓Collagen deposition ↓α-SMA LSEC capillarization prevention	(158)

	Micelles loaded with NO and PTX	Cell cycle arrest	Intraperitoneal injection	Liver tumour mice model	-	↑ Cell death ↓Tumour growth	(159)
	Trichrome-tryptophan-sorbitol QDs	autophagy induction via p53-AMPK pathway	Tail vein injection	Liver tumour mice model	-	↓Tumour growth	(162)

TAA = thioacetamide. ECM = extracellular matrix. OCA = obeticholic acid. LOXL2 = lysyl oxidase-like 2. FAK = focal adhesion kinase. FXR = farnesoid X receptor. SGLT2 = sodium glucose transporter 2. KLF2 = Krüppel-like factor 2. Hg = Hedgehog. CXCR7 = C-X-C chemokine receptor type 7. PTX = Paclitaxel. QDs = quantum dots. HSC = hepatic stellate cell. LSEC = liver sinusoidal endothelial cell. UC-MSCs = umbilical cord-derived mesenchymal stem cells. PP = portal pressure. IHVR = intrahepatic vascular resistance. α-SMA = α-smooth muscle actin. vWF = von Willebrand factor. TGFβ = transforming growth factor-β. CAV-1 = caveolin-1. NO = nitric oxide. ET-1 = endothelin-1. COL1α1 = collagen type-1 alpha 1. Pdgfrβ = platelet-derived growth factor receptor beta. DES = desmin. VGLL3 = vestigial like family member 3. IL1β = interleukin-1 beta. TIMP-1 = issue inhibitor of metalloproteinases 1.

Figure Legends

Figure 1. Liver sinusoidal crosstalk in health. In physiological conditions, the hepatic cells interact with each other maintaining liver homeostasis. The bone morphogenetic protein 9 (BMP-9) released by HSCs and the vascular endothelial growth factor (VEGF) secreted by HSCs and hepatocytes promote the maintenance of LSECs healthy phenotype. LSECs contribute to the differentiation and phenotype maintenance of KCs through delta-like protein 4 (DLL4) and the secretion of transforming growth factor-β (TGF-β) family ligands. Healthy LSECs also promote HSCs quiescence through different factors, such as nitric oxide (NO) and the secretion of the heparin binding epidermal growth factor-like growth factor (HB-EGF). An increase in mechanical shear stress in LSECs induces the expression of Krüppel-like factor 2 (KLF2), a vasoprotective transcription factor that modulates the endothelial nitric oxide synthase (eNOS) pathway, further increasing NO synthesis. HSCs can also promote their quiescence autocrinally secreting microvesicles that contain the transcription factor Twist1 or the microRNA-214 (miR-214), and as a result of haemoglobin degradation, KCs also produce vasoprotective mediators, such as carbon monoxide (CO). Communication in the liver sinusoids also coordinates the liver metabolic and synthetic functions. Together with the oxygen gradient, the Wnt/β-catenin and the Hedgehog signalling pathways are suggested to regulate hepatocyte liver zonation. Hepatocytes can also modulate their metabolism autocrinally via the intracellular calcium (Ca^{2+}) signalling system or the release of extracellular nucleotides, mainly ATP and UTP, to the sinusoidal space. Hepatocyte synthetic functions can be regulated by non-parenchymal cells. For instance, LSECs sense changes in iron levels and secrete signals, such as bone morphogenetic proteins (BMP) ligands, that induce hepcidin production, and KCs can also modulate hepcidin transcription. Finally, interleukin 10 (IL-10) secretion by LSECs and KCs and LSEC antigen presentation to naïve CD4+ and CD8+ T cells are key to confer immunological tolerance to the organ.

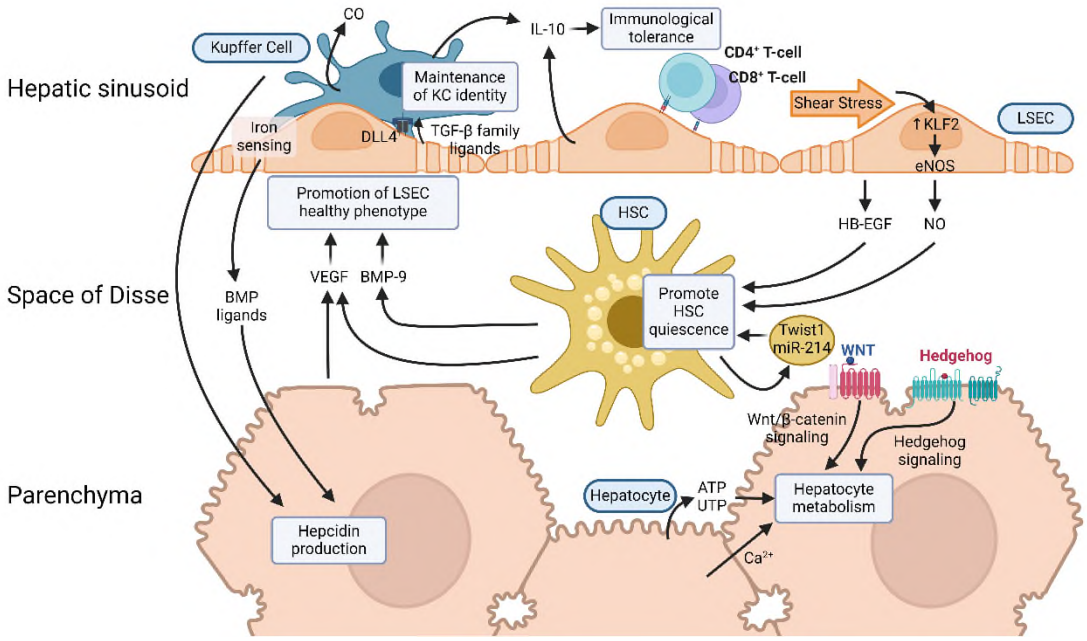

Figure 2. Crosstalk in liver sinusoids upon liver injury. Following liver injury, hepatocytes release damage-associated molecular patterns (DAMPs), reactive oxygen species (ROS), and proinflammatory signals that activate Kupffer cells (KCs). Activated KCs secrete various proinflammatory factors that orchestrate the immune response to resolve the liver injury. In parallel, liver damage induces the capillarization of liver sinusoidal endothelial cells (LSECs), which are responsible for activating hepatic stellate cells (HSCs) through the secretion of different paracrine signals, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), fibroblast growth factor receptor 1 (FGFR1), and fibronectin. At the initial stages of liver damage, the long non-coding RNA Airn maintains LSECs in a differentiated state through the activation of the KLF2-eNOS-sGC pathway, inhibiting the process of capillarization. This allows for the secretion of Wnt2a and hepatocyte growth factor (HGF), which maintain HSCs in a quiescent state and signal hepatocytes to regenerate. Moreover, the process of autophagy within the liver sinusoids protects hepatocytes from apoptosis by removing damaged mitochondria and protein aggregates, and by mitigating oxidative stress. Autophagy in KCs also prevents the activation of other immune cells and HSCs, thus protecting against fibrogenesis and chronic inflammation.

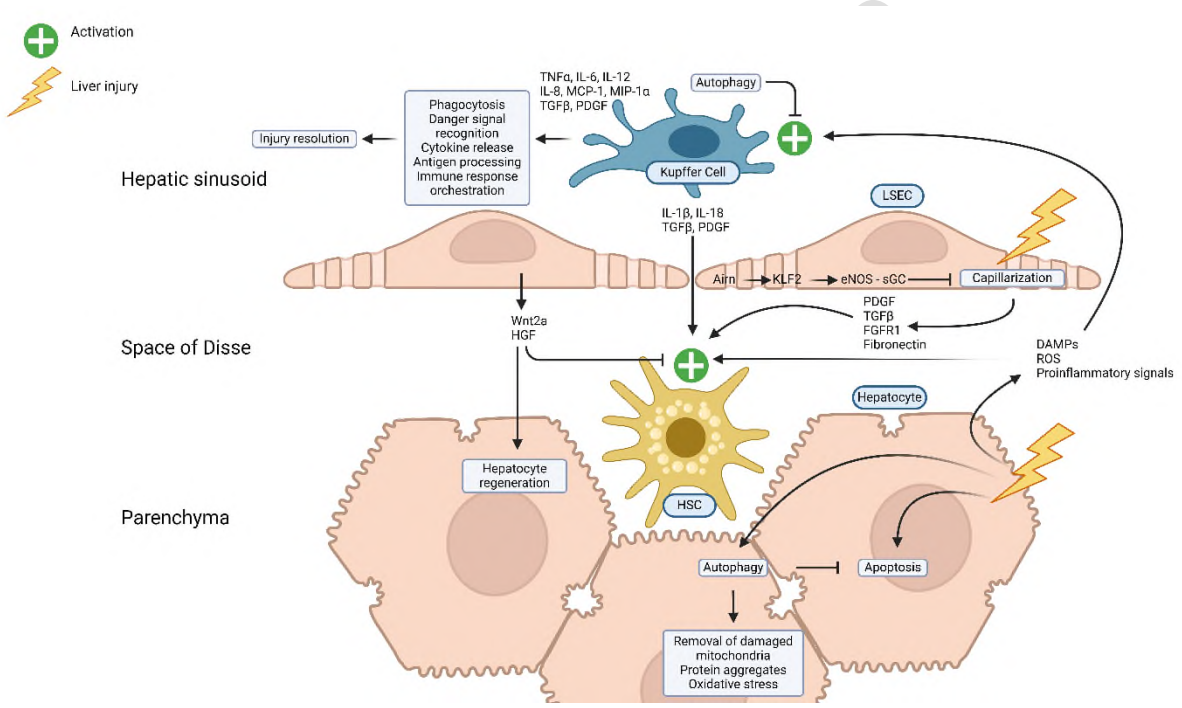

Figure 3. Liver sinusoidal crosstalk in chronic liver disease. Chronic liver disease (CLD) induces persistent hepatocyte damage, leading to the release of apoptotic bodies, damage-associated molecular patterns (DAMPs), reactive oxygen species (ROS), and proinflammatory extracellular vesicles. These factors collectively activate the inflammatory response. Kupffer cells (KCs) are particularly chronically activated by these factors, as well as by gut-derived compounds such as lipopolysaccharides (LPS). Hepatocyte damage also promotes the secretion of transforming growth factor-beta (TFGβ), which in turn induces liver sinusoidal endothelial cell (LSECs) angiogenesis and tube formation while activating the endothelial nitric oxide synthase (eNOS) pathway. However, due to endothelial damage, inflammation, and oxidative stress, nitric oxide (NO) inhibition of capillarization is arrested. Moreover, in the context of CLD, there is an increase in LECT2 in hepatocytes and endothelial cells, which promotes LSECs capillarization. This process is further influenced by Hedgehog ligands released by various sinusoidal cell types in response to cellular damage, NO signaling inhibition, and Delta-like 4 (DLL4) activation, all of which are key drivers of LSECs capillarization. Capillarized LSECs contribute to liver inflammation by recruiting immune cells via Stabilin-1, intercellular adhesion molecule 1 (ICAM-1), and vascular adhesion protein-1 (VAP-1) surface receptors. The capillarization of LSECs also leads to hepatic stellate cell (HSCs) activation. This activation is caused by decreased NO signaling and the secretion of sphingosine-1-phosphate (S1P) contained within exosomes, which also autocrinally induce endothelial TGFβ secretion, a critical activator of HSC. Activated HSCs synthesize excessive extracellular matrix (ECM) components, resulting in ECM accumulation and increased liver stiffness, which further promotes HSC activation and LSEC capillarization. Additionally, the loss of fenestrae during LSEC capillarization and the architectural distortion of the liver due to fibrosis leads to hypoxia. Hypoxia not only induces rapid endothelial growth through vascular endothelial growth factor (VEGF) but also further activates HSC via the DLL4-endothelial differentiation gene-1 (ET-1) pathway.

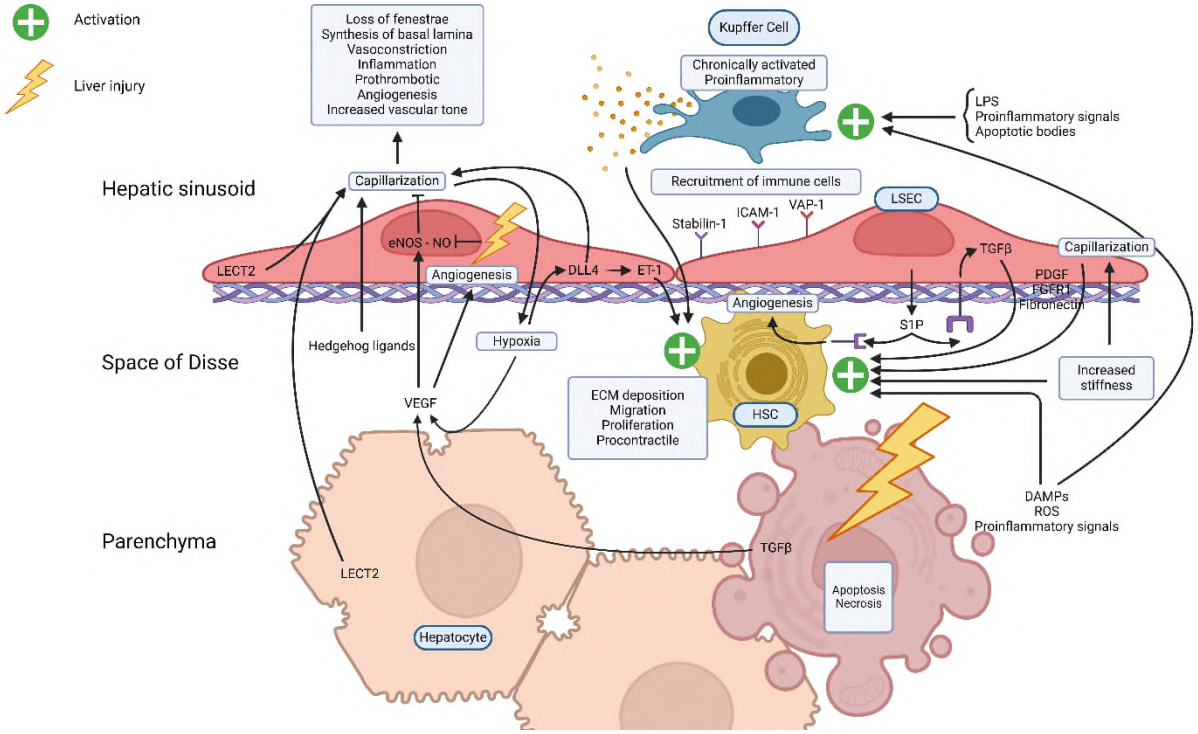

Figure 4. Liver sinusoidal intercellular communication in healthy and cirrhotic human livers. A) Number of ligand-receptor pairs predicted in cirrhotic vs healthy livers. B) Difference in ligand-receptor pairs by cell type. Red = increased in cirrhotic vs health; blue = decreased in cirrhotic vs health. C) Specific ligand-receptor pathways predicted in livers described in A. Pathways in white are overrepresented in healthy livers, while pathways in black are overrepresented in cirrhotic livers; *p<0.05. Epithelia (including hepatocytes), endothelial cells (including LSECs), mesenchyma (including HSCs) and macrophages (including KCs). Reanalysis of scRNA-seq data from Ramachandran et al. (2019) Nature.