Minireview *Highlight article*

Endothelin-1 in portal hypertension: The intricate role of hepatic stellate cells

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Impact statement

Portal hypertension is pathologically defined as increase of portal venous pressure, mainly due to chronic liver diseases such as fibrosis and cirrhosis. In fibrotic liver, activated hepatic stellate cells increase their contraction in response to endothelin-1 (ET-1) via autocrine and paracrine stimulation from liver sinusoidal endothelial cells and injured hepatocytes. Clinical studies are limited with ET receptor antagonists in cirrhotic patients with portal hypertension. Hence, studies are needed to find molecules that block ET-1 synthesis. Accumulation of extracellular matrix proteins in the perisinusoidal space, tissue contraction, and alteration in blood flow are prominent during portal hypertension. Therefore, novel matrix modulators should be tested experimentally as well as in clinical studies. Specifically, tumor necrosis factor-α, transforming growth factor-β1, Wnt, Notch, rho-associated protein kinase 1 signaling antagonists, and peroxisome proliferator-activated receptor α and γ, interferon-γ and sirtuin 1 agonists should be tested elaborately against cirrhosis patients with portal hypertension.

Abstract

Portal hypertension is one of the most important cirrhosis-associated complications of chronic liver disease, leading to significant morbidity and mortality. After chronic liver injury, hepatic stellate cells reside in the perisinusoidal space activted and acquire a myofibroblast-like phenotype. The activated hepatic stellate cells act as both sources as well as the target for a potent vasoconstrictor endothelin-1. Activation of hepatic stellate cells plays a vital role in the onset of cirrhosis by way of increased extracellular matrix production and the enhanced contractile response to vasoactive mediators such as endothelin-1. In fibrotic/cirrhotic liver, activated hepatic stellate cells produce endothelin-1 leading to an imbalance between pro and antifibrotic factors responsible for enormous extracellular matrix synthesis. Thus, extracellular matrix deposition in the perisinusoidal space further augments liver stiffness and elevates the vascular tone and portal hypertension. Portal hypertension is a complex process modulated by several cell types like hepatic stellate cells, liver sinusoidal endothelial cells, Kupffer cells, injured hepatocytes, immune cells, and biliary epithelial cells. Therefore, targeting a single cell type may not be useful for regression of cirrhosis and portal hypertension. Nevertheless, numerous findings indicate that functionally liver sinusoidal endothelial cells and hepatic stellate cells closely regulate the sinusoidal blood flow via synthesis of several vasoactive molecules including endothelin-1, and hence targeting these cells with novel pharmacological agents may offer promising results.

Keywords: Endothelin, hepatic stellate cells, portal hypertension, cirrhosis, hepatic fibrosis

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Introduction

Portal hypertension develops as a consequence of increased intrahepatic vascular resistance often caused by chronic liver disease (CLD) that leads to structural distortion by fibrosis, microvascular thrombosis, dysfunction of liver sinusoidal endothelial cells (LSECs), and activation of hepatic stellate cell (HSC).[1–3] The resistance to blood flow through the liver is extremely low with pressure gradients between the portal venous inflow and a normal hepatic venous pressure gradient (HVPG) is 3–5 mmHg.[4] But in cirrhosis conditions, the HVPG increased to >5 mmHg. Clinically significant portal hypertension is defined as HVPG of >10 mmHg.[5] Gastroesophageal varices, variceal hemorrhage, ascites, hepatic encephalopathy, and hepatorenal syndrome are common and direct consequences of clinically significant portal hypertension, which lead to significant morbidity and mortality.[6,7] The increased intrahepatic resistance in cirrhosis and changes in perisinusoidal space are mediated by the transdifferentiation of

quiescent HSCs into myofibroblasts-like phenotype and accumulation of extracellular matrix (ECM), formation of regenerative nodules, and vasoconstriction in the intrahepatic circulation because of decreased production of vasodilators and increased production of vasoconstrictors from LSECs, HSCs, and Kupffer cells.[5,8] Hepatic stellate cells are one of the important intrahepatic components of portal hypertension pathophysiology. During CLD, HSCs regulate a variety of signaling molecules including endothelin, responsible for fibrosis/cirrhosis and portal hypertension. Therefore, the aim of this review is to discuss the intricate role of HSCs in endothelin signaling in portal hypertension.

Hepatic stellate cells

Hepatic stellate cells (also called as Ito cells, perisinusoidal cells, fat-storing cells, lipocytes) are liver-specific mesenchymal cells that have features of resident fibroblasts and pericytes, residing in the perisinusoidal space or space of Disse between hepatocytes and LSECs.[9–12] HSCs account for 5 to 10% of total resident cells in the normal human liver.[13] Under normal physiological conditions, HSCs exhibit a quiescent phenotype (qHSC) with lipid-rich granules and are responsible for vitamin A storage and diverse roles like regulation of epithelial cell fate, immune modulation, tissue health, and matrix degradation, etc.[14,15] In the injured liver, qHSCs undergo sequential morphological and physiological changes to maintain the pathophysiological status of the injured liver.[16] (Figure 1). Firstly, in response to liver injury, qHSCs undergo transdifferentiation and acquire proliferative, contractile, and myofibroblast (MFBs)-like phenotype, a process termed as *initiation*. Secondly, the activated MFBs-like phenotypes secrete several pro-fibrotic cytokines and chemokines to maintain the pathophysiological status of the injured liver, and is termed as *perpetuation*.[8,17] For instance, in perpetuation process, the activated HSCs (aHSCs or MFBs) express α-smooth muscle actin (α-SMA) and respond to endothelin-1, transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), Krüppel-like factor 6 (KLF6), matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinases (TIMPs), and several other signaling molecules from injured hepatocytes, LSECs, and Kupffer cells. This process leads to HSCs contraction, migration, proliferation, synthesis, and accumulation of a variety of ECM proteins including fibril forming collagens 1α1, and 3α1 in the perisinusoidal space that causes portal hypertension and metabolic hindrance.[16,18,19] Regardless of etiology, aHSCs are recognized as one of the major contributors for fibrosis/cirrhosis. In injured liver, aHSCs are known to express/respond to endothelin-1 by an autocrine and paracrine fashion.[20]Endothelin-1 induces HSCs contractility and thereby triggers the pathological chain of events like ECM accumulation, metabolic hindrance, and portal hypertension. Increase in ECM deposition further elevates the vascular tone and augments liver stiffness.[8]

Endothelin-1: Synthesis, receptors, and function

Endothelin-1 (ET-1), a 21 amino-acid bioactive peptide first identified by Yanagisawa *et al.*, in 1988.[21]ET-1, is a potent

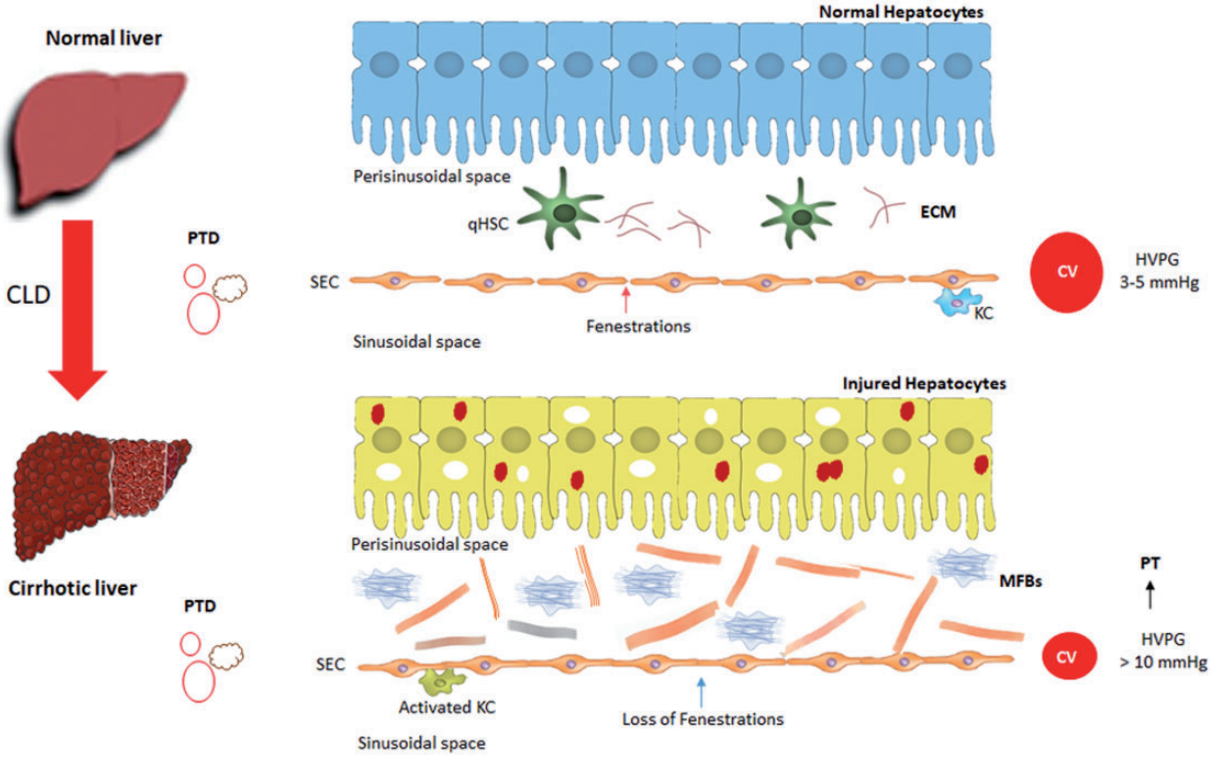

Figure 1. Portal hypertension-associated changes in the perisinusoidal space of cirrhotic liver. qHSC-quiescent hepatic stellate cells; aHSC-activated hepatic stellate cells; ECM: extracellular matrix; SEC: sinusoidal endothelial cells; PTD: portal triad; CV: central vein; PT: portal hypertension; HVPG: hepatic venous pressure gradient; CLD: chronic liver diseases; KC: Kupffer cells. (A color version of this figure is available in the online journal.)

endogenous vasoconstrictor peptide, which induces contraction, proliferation, and collagen synthesis of aHSCs and is a potent mediator of portal hypertension.[22] In normal conditions, hormones, other vascular mediators, and blood flow conditions seem to modulate precursor ET-1 synthesis in endothelial cells (including LSECs). The prepro-ET-1 is a biological precursor of ET-1 and is converted into active ET-1 via two steps. Firstly, cleavage of two basic amino acids by a furin-like enzyme results in the formation of big-ET-1. Secondly, the big ET-1 is further processed by cleavage of a Trp-Val bond by endothelin-converting enzyme-1 (ECE-1), a membrane-bound, phosphoramidon-sensitive metalloproteinase, into biologically active ET-1.[22,23] Endothelin has three subtypes such as ET-1, -2, and -3. All ET subtypes act through G protein-coupled receptors (GPCR) such as ET_A and ET_B.[22,24] However, ET-1 has high affinity and mediates long-lasting vasoconstriction effect mainly via the ET_A receptor. ET_A and ET_B receptors are present on HSCs and hepatocytes, while ET_B receptors are present on LSECs and Kupffer cells.[25] The ET_B receptor has two subtypes such as ET_{B1} and ET_{B2}. ET_{B1} receptor is responsible for the induction of endothelial cell nitric oxide synthetase resulting in nitric oxide (NO) release and vasodilation,[25] while ET_{B2} receptors are identified in HSCs and are responsible for vasoconstriction.[22] Increased plasma ET-1 and ET-3 levels were observed in patients with CLD and portal hypertension.[26–28] The role of ET-2 is not reported in CLD conditions. In liver microenvironment, ET-1 synthesized by LSECs acts on several cell types nearby such as hepatocytes, HSCs, and Kupffer cells via paracrine fashion. Kupffer cells induce thromboxane A2 (TXA2)-mediated ET-1 synthesis.[29] Particularly, ET-1 stimulates the contraction of qHSCs and myofibroblasts, which contribute to portal hypertension in the cirrhotic liver.[30]

Hepatic stellate cells in endothelin-1-associated portal hypertension

In the perisinusoidal space, HSCs function as liver-specific pericytes surrounding LSECs, and thus their contractility is controlled by the exposure to LSECs-derived vasoconstrictor like ET-1. Other vasoconstrictors like angiotensin II (Ang-II), TXA_2, carbon monoxide, and vasodilators like NO also control the diameter of sinusoids, fenestrae and regulate the hepatic microcirculation.[31]Endothelin-1 synthesized by LSEC, acts on HSCs in a paracrine fashion. After the liver injury, aHSCs are responsible for increased ET-1 synthesis, which contributes to significant vasoconstriction and portal hypertension.[30] Experimental studies have shown that aHSCs but not qHSCs are responsible for ET-1 synthesis.[22,32] However, in a study, endotoxin (lipopolysaccharide, LPS) treatment significantly increased ET-1 synthesis and its receptor activation in qHSCs and a non-significant ET-1 increase were also observed in aHSCs after LPS treatment.[33] The preproET-1 mRNA expression was reported to highly increase during cell culture-induced HSCs activation.[32] The ECE is responsible for the conversion of big ET-1 to the mature peptide.[22] The increased activity of ECE in aHSC is directly correlated with the up-regulation of ET-1. Upon chronic liver injury, hepatocytes secrete TGF-β, tumor necrosis factor-alpha (TNF-α), lipopolysaccharides, platelet-activating factor (PAF), and several other pro-inflammatory markers which, in turn, stimulate LSECs to secrete ET-1. ET-1 has prominent effects on key cellular effectors, including HSCs in an autocrine fashion.[20] (Figure 2). Chronic liver injury induce ET-1 from LSECs to act on HSCs via ET_A receptor and induces the secretion of profibrogenic cytokine, i.e. TGF-β1 and ECM-related markers such as collagen 1α1, collagen 3α1, TIMP 1, 2, and MMPs.[34] Regardless of etiology, ET-1 is implicated in HSCs activation and portal hypertension. For instance, high fat/methionine-choline-deficient diet and hyperleptinemia-induced NASH cirrhotic rats enhanced hepatic vasoconstrictive response to ET-1 and aggravated hepatic microcirculatory dysfunction which led to increased intrahepatic resistance and portal hypertension.[35]

Molecular signaling associated with endothelin synthesis and HSCs contraction

At a molecular level, several signaling pathways tightly regulate ET-1 synthesis and function. The transcriptional regulation of preproET-1 synthesis is well established in HSCs. Upon profibrogenic stress, ET-1 acts on qHSCs via its ET_A receptor and phosphorylates c-Jun N-terminal kinase (JNK) intracellularly. The phosphorylated JNK subsequently activates and phosphorylates its downstream effectors, i.e. Smad 3 and c-jun. The phosphorylated Smad 3 translocates to the nucleus to induce the activation of Smad3/4 present in the Smad3 binding site at the preproET-1 promoter. Similarly, phosphorylated c-jun translocates to the nucleus to induce the activation of c-fos/c-jun in the activator protein-1 (AP-1) binding site at the preproET-1 promoter. These signaling events trigger the preproET-1 at a transcription level which subsequently converts into active ET-1.[32] Thus, after liver injury, aHSCs and LSECs secrete more preproET-1 and convert to active ET-1. In a study, TGF-β1, a potent profibrogenic cytokine was shown to induce ET-1 secretion mediated by the activation of rho-associated protein kinase 1 (ROCK 1) in human HSCs.[36] Fibronectin plays an important role in the development of hepatic fibrosis.[37] Accumulation of fibronectin along with the different type of fibril forming collagens in the perisinusoidal space increases intrahepatic resistance.[8,38] Fibronectin was shown to induce ERK-mediated ET-1 synthesis in aHSCs. Briefly, fibronectin promotes activation of Src via their α5β1 and αVβ3 integrin receptors. The activated Src directly phosphorylates Shc present in Shc/Grb2/Sos complex. The phosphorylated Shc, in turn, activates ERK singling pathway, which increases the preproET-1 expression at the transcription level. Thus, increased ET-1 activates α-SMA expression in aHSCs and induces their contraction.[39]

Interestingly, unlike the hepatocytes, HSCs are not highly equipped with intracellular antioxidant defense, and hence they are highly susceptible to oxidative and pro-inflammatory mediators' attack.[40] The reactive oxygen species (ROS) play a significant role in the

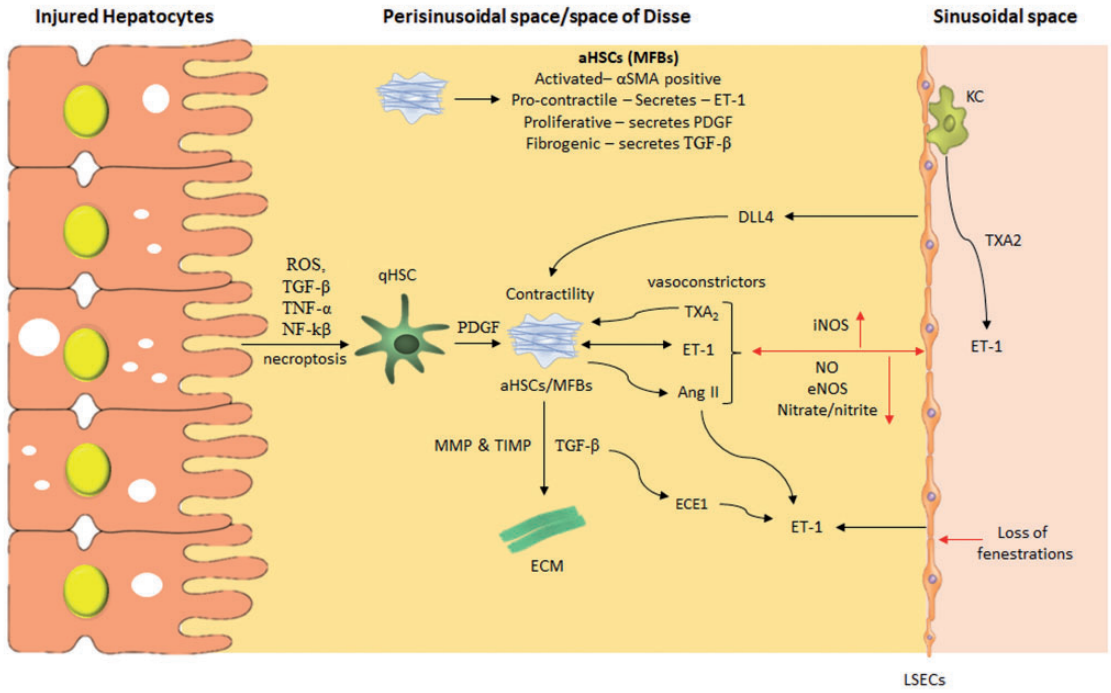

Figure 2. Portal hypertension-mediated changes in the perisinusoidal space of the liver. ROS: reactive oxygen species; TNF-α: tumor necrosis factor alpha; qHSC: quiescent hepatic stellate cells; MFBs: myofibroblasts or activated HSCs; PDGF: platelet-derived growth factor; ET-1: endothelin 1; TGF-β: transforming growth factor-β; MMPs: matrix metalloproteinases; TIMPs: tissue inhibitors of metalloproteinases; ECM: extracellular matrix; LSECs: liver sinusoidal endothelial cells; ECE1: endothelial converting enzyme 1; KC: Kupffer cells; Ang-II: angiotensin-II; DLL4: delta-like Ligand 4; TXA_2: thromboxane A_2; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase. (A color version of this figure is available in the online journal.)

activation HSCs in the injured liver.[41] Unsurprisingly, liver inflammation results in the liberation of several pro-inflammatory molecules from injured hepatocytes that directly activate HSCs.[42] For instance, TNF-α, a common proinflammatory cytokine secreted by injured hepatocytes promotes ET-1 production by activating the inhibitor of kappa B kinase (IKKα, β, and γ) complex via TNF receptor-associated factor 1, which, in turn, directly induces the phosphorylation of the extracellular-signal-regulated kinase (ERK) and JNK. Subsequently, the phosphorylated JNK translocates to the nucleus to activate c-Jun present in the preproET-1 promoter and triggers preproET-1 mRNA and protein expression.[43]

In a study, Ang-II was reported to induce ET-1 expression via Ang-II type 1 receptor by the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway in human HSCs. This study suggests that ET-1/ET_A receptor axis can promote the Ang-II-mediated transdifferentiation of qHSCs into MFBs.[44] The GPCR-mediated signaling, via ET-1 and Ang II, increases HSCs contraction, migration, and fibrogenic potential. Regulator of G-protein signaling-5 (RGS5), an inhibitor of vasoactive GPCR agonists functions to control GPCR-mediated contraction in smooth muscle cells. In the context of HSCs, RGS5 controls GPCR signaling in aHSCs of CCl_4-induced fibrotic mice liver, while RGS5 knockdown enhances ET-1-mediated signaling in aHSCs *in vitro*.[45] The G protein-coupled bile acid receptor TGR5 (Gpbar1) is expressed in aHSCs, LSECs, and Kupffer cells.[46,47] The TGR5 receptor activation reduces hepatic vascular resistance through several mechanisms, both in LSECs and in HSCs. A recent finding showed negative effect of Gpbar1 on ET-1 signaling in HSCs.[47] Activation of Notch signaling pathway is implicated in many liver diseases and is also responsible for HSCs activation in the injured liver.[48,49]Delta-like ligand 4 (DLL4), a ligand of the Notch signaling pathway, is predominantly expressed in LSECs and it is one of the factors responsible for the maintenance of liver sinusoidal homeostasis.[50,51] Higher levels of DLL4 were detected in LSECs of CCl_4-induced fibrotic liver in mice and in fibrotic human liver.[52,53] The overexpression of DLL4 caused the defenestration of LSECs and the accumulation of ECM in the injured liver. Overexpression of DLL4 stimulates the aHSCs to induce ET-1 synthesis which is further responsible for pathological hepatic sinusoidal remodeling and this study also suggests that DLL4/Notch signaling could activate ET-1 synthesis via LSECs and aHSCs in the fibrotic liver.[53]

The farnesoid X receptor (FXR) is a ligand-activated transcriptional factor.[54] Hepatic stellate cells express FXR and it negatively regulates their phenotype transdifferentiation and TGF-β-mediated collagen synthesis.[55,56] In a study, treatment with GW4064, a synthetic FXR agonist inhibited the qHSCs transdifferentiation by an inhibition of the upregulation of ET-1 expression. The FXR agonist treatment also reduced contractile response to ET-1 as compared to untreated HSCs.[55] Apelin, a peptide, is overexpressed in HSCs of rat and human cirrhotic livers and studies have also found high levels of this peptide in the serum of cirrhotic patients.[57,58] Apelin promotes hepatic fibrosis/cirrhosis through ERK signaling in LX-2 cells.[59] In a study, apelin has been reported to mediate fibrogenic effects predominantly via ET-1 activation. Both ET-1 and

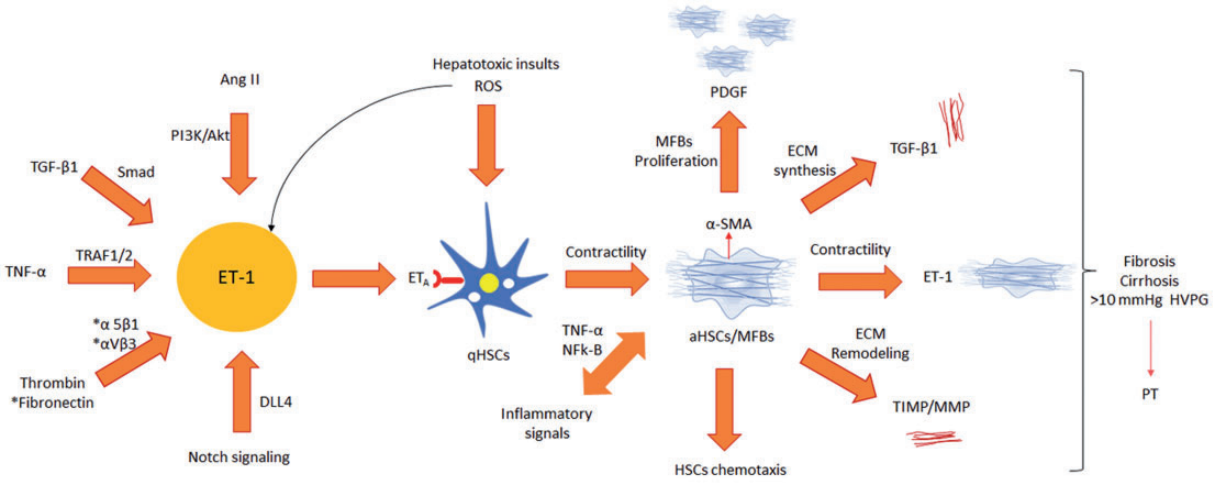

Figure 3. Enothelin-1 synthesis-associated signaling and its impact on HSCs. qHSC-quiescent hepatic stellate cells; aHSC: activated hepatic stellate cells; MFBs: myofibroblasts; ET-1: entdothelin-1; ET_A: endothelin receptor A; Ang II: angiotensin II; TGF-β1: transforming growth factor-β1; TNF-α: tumor necrosis factor-α; TRAF1/2: TNF receptor-associated factor 1/2; DLL4: DLL4 delta-like canonical Notch ligand 4; ROS: reactive oxygen species; α-SMA: α-smooth muscle actin; PDGF: platelet-derived growth factor; TIMP: tissue inhibitor metalloproteinase-1; MMP: matrix metalloproteinase; NFkB: nuclear factor kB; ECM: extracellular matrix; PT: portal hypertension; HVPG: hepatic venous pressure gradient. (A color version of this figure is available in the online journal.)

Ang II enhanced apelin expression in HSCs *in vitro*.[60]ET-1 receptor antagonist (RO 48–5695) treatment reduced the apelin synthesis, collagen-I, and PDGF-β in fibrotic rats.[60]Endothelin-1 synthesis-associated signaling and their impact on HSCs are depicted in Figure 3.

Modulators of ET-1 signaling and portal hypertension

There are two types of ET-1 receptor antagonists such as BQ-123 and BQ-788 and they act on ET_A and ET_B receptors, respectively, as identified so far. Several studies have tried these ET-antagonists in experimental and human portal hypertension. Interestingly, inhibition of ET signaling using ET receptor antagonists reduces the hepatic fibrogenic response in experimental models.[61,62] When BQ-123 and BQ-788 were continuously infused at the rate of 10 nmol/min for 10 min into normal rats, only BQ-123, an ET_A receptor antagonist has produced a gradual fall in portal pressure and also caused a marked dilatation of the sinusoidal endothelium fenestrae as compared to BQ-788 indicating the potential role of ET_A in portal hypertension.[63] The tissue mRNAs levels of ECE-1a and –b, ET-1, and ET_A and ET_B receptors expressions were significantly upregulated in bile duct ligated (BDL) and CCl_4-induced fibrotic liver. This study suggested that ECE-1a and -b isoforms are responsible for the ET-1 generation during biliary and hepatic fibrogenesis and portal hypertension.[22] High concentration of plasma ET-1 was also reported in cirrhotic rats and intraportal infusion of ET receptor antagonists such as BQ-123, BQ-788, and bosentan administration resulted in decreased portal venous pressure in these rats.[64] In an experimental study, BQ-123 infusion in CCl_4-induced cirrhotic rats markedly reduced the portal pressure by causing pores in sinusoidal endothelial fenestrae.[65] A randomized double-blind study demonstrated the limited role of ET_A and ET_B antagonists in HVPG patients.[66] The release of ET-1 was increased after HSCs are challenged with hypoxanthine/xanthine oxidase, a ROS-generating system *in vitro* and ET_B receptors were significantly expressed than ET_A in oxidative stress conditions. Consistently, the ET_B receptor antagonist, BQ788 significantly inhibited ET-1 expression in oxidative stress condition.[67] This study also indicates ET_B is responsible for the activation of ET-1 in oxidative stress condition.

It is known that ET-1 causes smooth muscle cell contraction through phosphorylation of myosin and activation of the actin-myosin contractile apparatus in smooth muscle cells, and thereby leads to narrowing of vascular structures and an increase in resistance to blood flow.[68] *In vitro*, aHSCs showed increased expression of contractile regulatory proteins myosin light chain kinase (MLCK), ROCK2, and 17-kDa PKC-potentiated protein phosphatase 1 inhibitor protein during their activation.[69] Activated HSCs induce hepatic sinusoids constriction via ET-1 stimulation in the cirrhotic liver through both Ca^{2+}-dependent MLCK pathway and Ca^{2+}-sensitization mechanism.[69]ET-1-mediated HSCs contraction is mainly induced by the phosphorylation of myosin light chain, actin stress fiber assembly, and reorganization of myosin to stress fibers.[69]BQ-123 treatments inhibited ET-1 stimulated myosin phosphorylation and contractility in rat HSCs. Similarly, Y-27632 (a selective ATP-competitive inhibitor of ROCK) treatments also blocked ET-1-induced myosin phosphorylation and contractile force generation in rat HSCs.[70] Xu *et al.* evaluated the effect of salvianolic acid B (Sal B) on HSCs contractility and portal hypertension. In their *in vivo* study, Sal B administration reduced ET-1-associated portal pressure in dimethylnitrosamine-induced cirrhotic rats. *In vitro*, Sal B treatments reduced ET-1-induced HSCs contraction by inhibiting the activation of RhoA, ROCK II, and the downstream myosin phosphatase target subunit 1 phosphorylation at Thr(696).[71] Another study by the same group revealed that Sal B is capable to inhibit ET-1 and thereby reduces the contraction of primary rat HSCs through its suppressive effects on myosin light chain 2 phosphorylation in HSCs.[72]

Darusentan, a potent blocker of ET_A receptor induced hepatic sinusoidal vasodilation, decreased hepatic ischemia, endothelial injury, and improved liver repopulation after cell transplantation in rats. However, *in vitro* ET_A receptor blockade failed to improve the engraftment of subsequently transplanted hepatocytes.[73] Treatment with Octreotide, an analogue of somatostatin, caused downregulation of ET-1 and fibrosis markers in aHSCs in culture.[74] Silent information regulator (SIRT) plays an important role in many liver diseases including cirrhosis.[75] Resveratrol, a SIRT1 activator treatment in aHSCs and LSECs resulted in a significant downregulation of ET-1 expression along with other profibrogenic markers. This study suggests that SIRT1 activators can restore the LSECs function and inhibit HSCs activation.[76] However, the exact mechanism of SIRT 1 action on ET-1 downregualtion is not reported so far. Therefore, further studies are needed on these lines. Interestingly, HSCs-specific vitamin A-decorated nanoparticles with NO donor molecules (S-nitrosoglutathione) released NO specifically in liver cells. Further, HSCs-specific nano construction inhibited ET-1 synthesis, HSCs contraction, and attenuated hemodynamic disorders in BDL-mediated portal hypertension evidenced by decreased portal pressure (≈20%) and unchanging mean arterial pressure.[77]

TNF-α reportedly induces ET-1 gene expression in human dermal microvascular endothelial cells.[78] A previous study has reported that peroxisome proliferator-activated receptor activators directly inhibit thrombin-induced ET-1.[79] In liver inflammation context, TNF-α activates HSCs in the injured liver and causes elevated imbalance of ET-1 MMPs/TIMPs.[80] Therefore, in a study, aleglitazar, a PPARα or PPARγ agonist has been tested experimentally. This study reported the increased systemic and hepatic TNF-α levels in cirrhotic rats. Further, *in vitro*, the hyper-expression of hepatic ET-1 in rat perfused livers along with TNF-α coincubation enhanced ET-1-induced primary rat HSCs contraction. The aleglitazar treatments induced the inhibition of ET-1 and TNF-α coincubation-mediated HSCs contraction. Aleglitazar treatments also reduced vasoconstrictor hyper-responsiveness, splanchnic vasodilatation, portal hypertension in BDL, and thioacetamide-induced cirrhotic rats.[81] Interferon-γ (IFN-γ), a Th1 cytokine produced by T cells caused significant downregulation of ET-1 precursor, i.e. preproET-1 mRNA expression and ET-1 peptide production. In HSCs, IFN-γ treatment resulted in decreased ET-1 expression via downregulation of JNK phosphorylation and its downstream targets c-Jun and Smad3 which downregulated the activation of c-fos/c-jun in the activator protein-1 (AP-1) binding site at the preproET-1 promoter.[32] Collectively, these studies showed promising results of TNF-α antagonists, PPARα, γ agonists, and IFN-γ against ET-1-induced hepatic cirrhosis and portal hypertension.

Therapeutic avenues and future directions

Undoubtedly, activation of HSCs results in fibrosis, cirrhosis leading to portal hypertension due to increased intrahepatic resistance. ET-1 exhibits an autocrine effect on HSCs and is involved in their activation and contractile response.[55,82] Clinically, very few studies were conducted with ET receptor antagonists in cirrhotic patients with portal hypertension. More studies specifically using ET_A receptor antagonists in portal hypertension conditions are warranted in the near future. In recent studies, the TGR5 receptor activation showed negative regulation of ET-1 signaling in HSCs, suggesting that TGR5 activators may contribute to a reduction in hepatic vascular resistance[47] and portal hypertension via modulation of ET-1 singling in cirrhotic liver. The DLL4 inhibitors and other Notch signaling modulators may also have a beneficial effect in portal hypertension through modulation of ET-1 synthesis by aHSCs as well as LSECs. There are several pro-inflammatory mediators like TNF-α and TGF-β1 which directly activates LSECs and HSCs to secrete ET-1. Therefore, their antagonists can reduce inflammation-mediated portal hypertension, though ET-1 is predominantly responsible for HSCs contraction and it is not the only factor for HSCs activation in the injured liver. There are several other molecules like Ang II, thrombin, VEGF, fibronectin, etc. which also acts on HSCs and modulates ET-1 level in cirrhotic liver. Once ET-1 is synthesized by LSEC, it acts on HSCs via paracrine signaling. Hence, it is suggested that more studies are warranted on molecules that block ET-1 synthesis rather than its receptor blockers. Finally, TNF-α, TGF-β1, Wnt, Notch, ROCK antagonists, and PPARα, γ, IFN-γ, SIRT1 agonists should be tested elaborately against portal hypertension. From a pathological point of view, portal hypertension is a direct consequence of increased hepatic vascular resistance due to both ECM accumulation and microvascular dysfunction. Therefore, novel matrix modulators should also be tested experimentally as well as in clinical studies. In a recent phase 2 randomized controlled trial, serelaxin has been tested against a small group of cirrhotic patients ($n = 17$), in which serelaxin infusion did not induce significant adverse effects and showed a neutral effect in patients with portal hypertension.[83] Indeed, portal hypertension occurs due to multifactorial conditions, and hence combinational therapy with novel vasodilators like serelaxin along with anti-inflammatory agents and matrix modulators may be effective and these combinations should be tested experimentally as well as against patients with portal hypertension.

Conclusions

The role of HSCs in the onset of cirrhosis and portal hypertension is complex. The activated HSCs secrete ET-1 and they also respond to ET-1 from LSECs due to their unique anatomic location. In the injured liver, aHSCs receive stress and inflammatory signals from both sides. Firstly, from the parenchymal domain, injured hepatocytes can release proinflammatory markers from one side. Secondly, from the sinusoidal domain, HSCs receive a paracrine signal via ET-1 from LSECs and Kupffer cells. After receiving these pleiotropic signals, qHSCs become contractile, acquire MFBs like transition, and synthesize an enormous amount of ECM, which subsequently causes cirrhosis and its associated complications like a hindrance in hepatic

metabolism, loss of fenestration of LSECs, increased hepatic vascular resistance, and portal hypertension. Thus, cirrhotic complications and portal hypertension are not a single entity and it is a complex process involving HSCs, LSECs, Kupffer cells, injured hepatocytes, immune cells, and biliary epithelial cells. Therefore, targeting a single cell type may not be useful to effectively regress cirrhosis and its associated complications like portal hypertension. Hence, targeting these cells with novel pharmacological agents' like matrix modulators may offer promising results.

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