Vascular pathobiology in chronic liver disease and cirrhosis – Current status and future directions

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Summary

Chronic liver disease is associated with remarkable alterations in the intra- and extrahepatic vasculature. Because of these changes, the fields of liver vasculature and portal hypertension have recently become closely integrated within the broader vascular biology discipline. As developments in vascular biology have evolved, a deeper understanding of vascular processes has led to a better understanding of the mechanisms of the dynamic vascular changes associated with portal hypertension and chronic liver disease. In this context, hepatic vascular cells, such as sinusoidal endothelial cells and pericyte-like hepatic stellate cells, are closely associated with one another, where they have paracrine and autocrine effects on each other and themselves. These cells play important roles in the pathogenesis of liver fibrosis/cirrhosis and portal hypertension. Further, a variety of signaling pathways have recently come to light. These include growth factor pathways involving cytokines such as transforming growth factor β, platelet derived growth factor, and others as well as a variety of vasoactive peptides and other molecules. An early and consistent feature of liver injury is the development of an increase in intra-hepatic resistance; this is associated with changes in hepatic vascular cells and their signaling pathway that cause portal hypertension. A critical concept is that this process aggregates signals to the extrahepatic circulation, causing derangement in this system’s cells and signaling pathways, which ultimately leads to the collateral vessel formation and arterial vasodilation in the splanchnic and systemic circulation, which by virtue of the hydraulic derivation of Ohm’s law (pressure = resistance × flow), worsens portal hypertension. This review provides a detailed review of the current status and future direction of the basic biology of portal hypertension with a focus on the physiology, pathophysiology, and signaling of cells within the liver, as well as those in the mesenteric vascular circulation. Translational implications of recent research and the future directions that it points to are also highlighted.

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Introduction

Understanding of the basic elements of general and liver specific vascular biology has grown substantially over the last decade. This work has led to exciting new developments in the field of portal hypertension. This review aims to put these advances into context.

Vascular beds are diverse, each with their own specific functional attributes. Notwithstanding, a number of themes have emerged. In patients with chronic liver disease, the peripheral vasculature, the mesenteric vascular bed, and the intrahepatic microcirculatory unit have received attention (Fig. 1). A recurrent theme is that while the cells and molecules in each vascular structure exhibit many similarities, variability in signaling pathways lead to unique functional attributes in each. For example, in the sinusoid and liver, vasoconstriction and increased resistance to blood flow is prominent. In contrast, in the mesenteric vasculature, vasodilation is prominent. The combination of increased resistance in the liver and increased flow to the portal vein from the mesenteric circulation results in increased portal pressure as indicated by the hydraulic equivalent of Ohm’s Law (pressure = flow × resistance). Additionally, it is important to recognise that factors other than the vascular bed itself may be important in the development of changes in resistance or flow. For example, within the liver, it is likely that fibrosis, particularly in advanced states is critically important in the increased resistance, typical of portal hypertension.
Intrahepatic vascular bed
- Increased gene and peptide expression of vasoconstrictors (ET-1, Ang II)
- Reduced eNOS phosphorylation, activity and NO production
- Increased resistance
- Increased pressure
- Reduced portal venous blood flow

Mesenteric vascular bed
- Reduced gene and peptide expression of vasoconstrictors (ET-1, Ang II)
- Increased eNOS phosphorylation, activity and NO production
- Decreased Rho kinase activity
- Increased expression of angiogenic factors (VEGF, PDGF, PlGF)
- Arterial vasodilation
- Arterial wall thinning
- Decreased response to vasoconstrictors
- Increased portal venous blood flow

Peripheral vasculature
- Increased eNOS phosphorylation, activity and NO production
- Decreased arterial response to vasoconstrictors
- Autonomic dysfunction
- Increased cardiac output
- Increased blood flow

Pulmonary vasculature (typical, not "portopulmonary changes")
- Increased ET-1 gene and peptide production
- Increased ET-B receptor expression
- Increased eNOS phosphorylation, activity and NO production
- Pulmonary small vessel dilation
- Extensive shunting, with left sided hypoxemia
- Increased cardiac output

Fig. 1. Heterogeneity of vascular beds in portal hypertension. Shown are prominent vascular beds and their associated pathophysiology. The intrahepatic vascular bed is typified by an increase in resistance to flow. Liver injury leads to abnormal endothelial function, with a reduction in the production of vasodilators by sinusoidal endothelial cells such as nitric oxide (NO); concomitantly, there is an increase in the production of vasoconstrictors such as endothelin-1 (ET1) and angiotensin II (Ang II) by other cells in the sinusoid (see also Fig. 2). The mesenteric vascular bed is characterised by vasodilation by reduced resistance caused by upregulation of vasodilators such as NO, leading to increased flow to the portal veins. The net result of this physiology is an increase in intrahepatic resistance and portal blood flow from the splanchnic circulation, leading to increased portal pressure and portal hypertension. In peripheral vascular beds, increased eNOS activity and NO production typically leads to reduced resistance, low systemic pressure, and a hyperdynamic state typical of patients with cirrhosis. Abnormalities also exist in other vascular beds such as the pulmonary, brain, renal, and likely even others. Of note, increased intrahepatic resistance is only for the portal system, and the resistance of the hepatic artery is decreased. Therefore, due to the hepatic arterial buffer response, the total blood flow into the liver may be the same in cirrhosis [130], ET-1, endothelin-1; Ang II, angiotensin II; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; PlGF, placental growth factor.
A key signature of most forms of liver injury/disease is the loss of many of these unique phenotypes. Additionally, it should be emphasised that typical LSEC features are lost in culture, making in vitro study of LSEC phenotypes challenging. It is well appreciated that liver fibrosis is associated with alterations in the diameter and number of fenestrae [3]. A recent innovative structural analysis [4] showed that fenestrae formation could be regulated by membrane lipid rafts, which are cholesterol and sphingolipid rich domains that serve as a platform for many membrane proteins such as caveolin. Interestingly, fenestrae distribution appears to be inversely related to lipid raft regions.

Recent work has begun to untangle the molecular signaling pathways that lead from cell injury to abnormalities in fenestration. For example, exposure of LSECs to vascular endothelial growth factor (VEGF) treatment increases fenestrae formation by decreasing the abundance of lipid raft regions, which may explain an essential role of VEGF for the maintenance of fenestrae observed by others (see below). Furthermore, decreased fenestrae formation may be linked to increased caveolin-1 levels observed in LSEC after liver fibrosis [5]. Further work to better understand the pathways leading from injury and fibrosis to fenestral abnormality is expected.

Regulation of the LSEC phenotype. A variety of molecular signaling pathways regulate the LSEC phenotype; these are both soluble factors and mechanical forces. Among the soluble factors, growth factors appear to be the most prominent. As referred to above, VEGF appears to be the most critical molecule in the modulation of the size and number of LSEC fenestrae [3–8]. Removal of VEGF from the cell culture medium results in loss of fenestrae, which can be restored by resupply of VEGF [6]. Similarly, disruption of VEGF signaling by a conditional deletion of Vegfr1 in mice led to loss of fenestrae, while restitution of VEGFR1 led to re-fenestration [8]. A number of growth factors other than VEGF also regulate the LSEC phenotype, with most of these being activators of receptor tyrosine kinases and include angiopoietins, ephrins, and fibroblast growth factors [9,10].

The LSEC phenotype is also regulated by biomechanical forces such as shear stress. The most prominent effect of shear stress appears to be in the modulation of endothelial nitric oxide synthase (eNOS) activity in LSECs, thereby regulating flow and vascular tone in the sinusoids [11]. Exposure of cultured LSECs to varying degrees of flow leads to different degrees of eNOS activation and NO release [11]. Similarly, isolated perfused rat livers increased NO release as a result of shear stress [11].

LSEC-mediated paracrine regulation. Not only do exogenous factors play an important role in the regulation of the LSEC phenotype, but recent evidence indicates that LSECs themselves play an important role in the function of neighbouring cells and, therefore, the microenvironment. For example, LSECs produce angiocrine growth factors and regulate liver regeneration and fibrosis. Wnt2 and hepatocyte growth factor (HGF) induced by LSECs promote hepatocyte proliferation [12]. It has also been reported that bone marrow-derived LSEC progenitor cells are important for liver regeneration because of the large portion of HGF they induce [13]. Interestingly, however, LSECs isolated from biliary cirrhotic mice exhibit enhanced pro-fibrotic growth factors and cytokines, such as transforming growth factor (TGF)-β, bone morphogenetic protein 2 (BMP2) and platelet derived growth factor (PDGF)-C, with decreased anti-fibrotic

**Key Points**

- Significant alterations in the intra- and extrahepatic vasculature are prominent in chronic liver diseases
- Hepatic vascular cells, such as sinusoidal endothelial cells and pericyte-like hepatic stellate cells, have paracrine and autocrine effects on each other and themselves, and play important roles in the pathogenesis of liver fibrosis/cirrhosis and portal hypertension
- An important feature of liver injury is development of an increase in intra-hepatic resistance, which is associated with alterations in hepatic vascular cells and their signaling pathway that causes portal hypertension
- Portal hypertension leads to the collateral vessel formation and arterial vasodilation in the splanchnic and systemic circulation, which worsens portal hypertension
- This review summarizes the current state and future directions in hepatic vascular biology and portal hypertension - with a focus on the physiology, pathophysiology of cells and molecules within the liver, as well as those in the mesenteric vascular circulation. Translational implications of current research are highlighted

**Intrahepatic vascular pathophysiology**

The intrahepatic microvascular unit is made up of several discrete units, including portal venules, hepatic arterioles, sinusoids, central venules, and lymphatics. The cellular elements in these structures include endothelial cells, smooth muscle cells, and in the sinusoid, pericyte-like hepatic stellate cells. It is important to recognise that these cells are intimately associated with one another, where they have paracrine and autocrine effects on each other and themselves. The canonical example of course is the paracrine effect of nitric oxide (NO), released by sinusoidal endothelial cells on smooth muscle cells and on stellate cells.

**Hepatic cells in intrahepatic vascular physiology and pathophysiology**

**Liver sinusoidal endothelial cells (LSEC)**

The majority of hepatic endothelial cells reside within the hepatic sinusoids; these cells, known as liver sinusoidal endothelial cells (LSECs), have therefore garnered great attention. The LSEC phenotype is uniquely different, not only from endothelial cells in other portions of the liver, but also from endothelial cells in other organs [1]. Perhaps the most unique feature of the LSEC phenotype is fenestration; fenestrae are organised in typical sieve plates [2]. While the function of fenestrae is controversial, it appears that they help facilitate the transport of macromolecules from the sinusoidal lumen, across the cell, into the space of Disse, providing access of these molecules to hepatocytes. Additionally, in vivo, LSECs lack a typical basement membrane, further facilitating macromolecular transport [1,3].
factors such as follistatin and apelin [14]. Furthermore, LSECs may release vesicles, including “microvesicles” (also referred to as “microparticles”) and exosomes; these structures appear to contain signaling molecules that regulate other cell types in a paracrine fashion [15]. Our understanding of both structures is at a nascent state but increasing information indicates a role in paracrine signaling. Interestingly, recent studies indicate that growth factor stimulation of endothelial cells may stimulate release of these “signaling vesicles.” One such growth factor may be the fibroblast growth factor (FGF). While less studied than VEGF in the hepatic microcirculation, FGF signaling through its cognate receptor FGFR is important for LSEC stimulatory signaling and release of paracrine molecules [9]. These features are pertinent not only in physiologic conditions but also in pathophysiologic situations, such as cirrhosis and portal hypertension as discussed below.

LSECs also appear to be an important source of certain types of extracellular matrix. For example, LSECs produce the cellular isofrom of fibronectin in response to injury [16]. Fibronectin in turn has potent paracrine effects on hepatic stellate cells (HSCs) [16], stimulating their activation early in the injury process. Additionally, fibronectin appears to stimulate HSC synthesis of endothelin-1 (ET-1), which in turn has paracrine effects on HSCs [17].

LSEC phenotype in disease. During liver injury, the LSEC phenotype changes dramatically [1]. One of the most remarkable phenotypic changes is “capillarisation”, characterised by loss of fenestrae and abnormal deposition of a basement membrane matrix on the abluminal surface of LSECs [1]. In addition to these anatomical changes, a number of biochemical changes also occur in the LSEC phenotype. For example, it is now well established that eNOS activity is diminished in LSECs after liver injury, consistent with a nitric oxide synthase phenotype. For example, it is now well established that eNOS activity is diminished in LSECs after liver injury, consistent with a nitric oxide synthase phenotype. It appears that there may be interplay between the smooth muscle and fibrogenic pathways, as the latter being a major component of the activation process.

The mechanism for the reduction in eNOS activity and NO synthesis after injury is tied to extensive post-translational dysregulation of eNOS. For example, it has been established that eNOS function is tied to a series of events that regulate the phosphorylation status of eNOS, including by interacting and/or binding to calmodulin, caveolin-1, HSP90, Akt, and a variety of other intracellular proteins [20,21]. In the liver increased expression of caveolin-1 in LSECs appears to be important in the reduced eNOS activity described [5]. More recent work suggests that a series of complex molecular events, involving molecules that regulate and/or dampen G protein coupled receptor signaling, potently modulate eNOS [22,23].

Reduced NO from LSECs may also play a role in progression of fibrosis. NO has been shown to maintain quiescence of hepatic stellate cells (HSCs) and reduced exposure of HSCs to NO may facilitate their activation [24,25].

As mentioned above, VEGF is important in the maintenance of LSEC fenestrae and may prevent LSECs from undergoing capillarisation [6–8]. The mechanism of this effect is currently unknown. However, there may be a role for VEGF in NO signaling in LSECs, and it is possible that VEGF’s downstream NO signaling plays an important role in the maintenance of LSEC fenestrae [26].

Neighbouring cells also appear to change the LSEC phenotype in disease. For example, in response to a treatment with saturated free fatty acids in vitro, which mimics lipid accumulation in steatosis, hepatocytes release microvesicles that have pro-angiogenic activity [27]. Microvesicles collected from conditioned media from these lipid-challenged hepatocytes enhanced migration and tube formation of endothelial cells in vitro. Although the effects of these hepatocyte-derived microvesicles on LSECs have not been clearly specified, these observations suggest that the hepatocyte-LSEC communication induces angiogenesis.

Pericytes, stellate cells, and myofibroblasts
By virtue of their anatomic position in the sinusoid (Fig. 2), stellate cells have also been coined liver specific pericytes. Pericytes are found throughout the body in small calibre blood vessels, typically capillaries [28]. They exhibit many features of smooth muscle cells and are believed to play a role in blood flow regulation. Recent work has suggested that their role may be more complex, and that they have more pleiotropic roles, in particular in wound healing; here they differentiate into cells with other functions [29,30]. For example, fate mapping experiments in several organs, including the liver, indicate that pericytes are the cellular precursors of myofibroblasts (see below) and transform in response to injury [29,30].

Current evidence indicates that during wound healing, stellate cells undergo a transformation process. This process, termed “activation”, involves a number of complex and integrated features. Characteristics of activation include morphologic and functional changes, such as loss of vitamin A, acquisition of stress (actin) bundles, development of a prominent rough endoplasmic reticulum, and production of increased quantities of extracellular matrix [31]. One of the most prominent features of stellate cell activation is an increase in the expression of smooth muscle α actin. Thus, these data point to stellate cells as liver specific myofibroblasts. Myofibroblasts appear to be key elements in the wounding response to injury in many tissues, producing abundant quantities of extracellular matrix, as well as smooth muscle proteins [32–36]. The functional role of myofibroblasts in the liver is most likely tied to fibrogenesis, and thus indirectly linked to portal hypertension, since it is believed that both perisinusoidal and bridging scar formation contributes to portal hypertension at the sinusoidal level, and perhaps at the whole liver level, respectively.

From a cell biological and molecular signaling pathway, the upregulation of smooth muscle α actin in the activation process is complex, and involves interplay between stellate and other cells, cytokines and other soluble elements, and the extracellular matrix itself. Having said that, there appear to be some parallels between the regulation of smooth muscle α actin and fibrogenesis, the latter being a major component of the activation process. For example, transforming growth factor beta-1 (TGF-β1) the most profibrogenic cytokine for stellate cells, may also regulate smooth muscle α actin gene expression [37]. Recent evidence indicates that smooth muscle α actin expression in stellate cells is controlled transcriptionally by canonical muscle specific transcription factors, including serum response factor [38]. Further, it appears that there may be interplay between the smooth muscle α actin cytoskeleton and various stellate cell functions, including cell motility and contractility [29]. These data indicate that smooth muscle α actin itself is not a static component of the cytoskeleton, but rather a dynamic regulator of stellate cell behaviour and function.
Sinusoidal vascular remodelling

Sinusoidal vascular remodelling involves a complex interplay of hepatic cells and appears to contribute to increased intrahepatic resistance. For example, when quiescent HSCs become activated and start to deposit extracellular matrix proteins this creates a basement membrane around the sinusoids. In turn vigorous “coverage” of the sinusoids with activated HSCs could contribute...
to increased intrahepatic resistance in cirrhotic livers [40]. Furthermore, with injury, reduced LSEC NO production appears to stimulate cell proliferation, upregulates various arterial surface markers and contributes to loss of LSEC fenestrations, resulting in de-differentiation and capillarisation of the hepatic microvascular bed [41]. These changes facilitate remodelling and constriction of the sinusoidal vasculature, which increases hepatic vascular resistance and is an early feature of intrahepatic portal hypertension.

Angiogenesis

Angiogenesis, the process of new blood vessel formation from pre-existing vascular beds, takes place in two distinctive manners, namely through sprouting from the existing vasculature or splitting of the existing vasculature. In sprouting angiogenesis, angiogenic growth factors, through activation of endothelial cells, facilitate the degradation of the basement membrane in pre-existing blood vessels, which allows endothelial cells, pericytes and smooth muscle cells to detach and migrate towards angiogenic stimuli (Fig. 3). Endothelial cells then proliferate and form solid sprouts connecting neighbouring sprouts or blood vessels. Endothelial cells finally stop proliferating and bind to each other, to the pericytes and to the basement membrane, forming a new blood vessel [42,43]. Sprouting angiogenesis appears to involve a complex interplay between numerous signalling pathways such as Notch and Notch ligands, vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFRs), semaphorins, and netrins [44], while signaling pathways regulating intussusceptive angiogenesis are less well studied but include Notch, Notch ligands, Tek/Tie-2, mTOR, ephrins and Eph receptors [45].

Intussusceptive angiogenesis, also known as splitting angiogenesis, was discovered relatively recent as an alternative process [46]. In intussusceptive angiogenesis, the two opposing walls of a capillary extend towards each other and form an intraluminal pillar. The cellular junctions of opposing endothelial cells are reorganised, which facilitates further growth of the pillar and finally results in splitting of the capillary into two new vessels [47]. Intussusceptive angiogenesis relies less on endothelial cell proliferation and generates blood vessels more rapidly [44,48]. Therefore, intussusceptive angiogenesis is particularly important in embryonic development where pre-existing blood vessels are limited to create new vessels [49].

Both forms of angiogenesis, sprouting and intussusceptive, appear to be important in normal liver physiology and in pathophysiologic states, including liver organogenesis [50,51], liver regeneration [12,52], chronic liver diseases with fibrosis [53], nodular regenerative hyperplasia [45], hepatocarcinogenesis [54], and tumour angiogenesis [45].

Angiogenesis in the intrahepatic circulation

In portal hypertension, angiogenesis plays a crucial role in both intra and extra hepatic circulations. In the intrahepatic circulation, for example, it is reported that conditional Notch 1 knockout mice develop intussusceptive angiogenesis, nodular regenerative hyperplasia and portal hypertension. LSECs from these mice show reduced endothelial fenestrations. These observations indicate that Notch1 in LSEC is required for fenestration of LSECs and the loss of Notch 1 results in pathological intussusceptive angiogenesis and the development of nodular regenerative hyperplasia and portal hypertension [45]. Irregular flow patterns generated as a result of intussusceptive angiogenesis might contribute to an increase in resistance within the intrahepatic circulation, leading to portal hypertension.

Furthermore, angiogenesis occurring after liver injury appears to increase the vascular volume in response to inflammation and hypoxia induced in the fibrogenic process [55]. Histological
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analyses of cirrhotic livers indicate an increased number of vessels in the fibrotic septa and surrounding regenerative nodules [56]. This observation has led to the hypothesis that activated HSCs and/or other myofibroblasts such as portal myofibroblasts promote angiogenesis in liver cirrhosis. In fact, activated HSCs are known to enhance activation of LSECs by releasing angiogenic factors, such as angiopoietins [10,40,57] and VEGF [58].

Mesenteric vascular pathophysiology

In portal hypertension, increased portal blood inflow from the splanchnic circulation augments portal pressure and thereby contributes to the maintenance and exacerbation of portal hypertension. Arterial vasodilation in the splanchnic circulation plays a critical role in increasing the blood flow to the portal vein. To ameliorate portal hypertension, therefore, blocking arterial vasodilation in the splanchnic circulation is necessary. Further, blocking the development of collaterals could be useful for decreasing the incidence of portosystemic encephalopathy and variceal bleeding.

Vasodilation in the mesenteric vasculature

Arterial vasodilation in the splanchnic and systemic circulations is an important feature of portal hypertension. Splanchnic arterial vasodilation increases the blood inflow to the portal system and exacerbates portal hypertension. Splanchnic arterial vasodilation is attributed to abnormal cell function in different layers of the vasculature, namely, endothelial cells, smooth muscle cells and the adventitial layer that contains neuronal termini. Because of the disparate regulation of the vascular tone in the intrahepatic and extrahepatic circulations (i.e., vasoconstriction in the intrahepatic circulation vs. vasodilation in the extrahepatic circulation), the organ/tissue specific modulation of the vasodilator molecules is of paramount importance.

Increased vasodilator molecules in endothelial cells

eNOS-derived NO is increased in the splanchnic and systemic circulation and plays a principal role in arterial vasodilation. Complex regulatory mechanisms of eNOS activation appear to be important in these pathological vasculature structures. For example, a recent study [59] described a new mechanism for the modulation of eNOS in cirrhosis, which involves the renin-angiotensin (Ang) system. The renin-Ang system plays a critical role in blood pressure control, body fluid and electrolyte homeostasis. Angiotensin II is a vasoconstrictor generated by the action of angiotensin-converting enzyme (ACE) and is further cleaved by ACE2 to generate a biologically active peptide, Ang-(1-7). Ang-(1-7) is however a vasodilator, which binds to the G-protein coupled receptor Mas (MasR) [60] and leads to eNOS activation and NO production in endothelial cells [61]. In an animal model of cirrhosis, expression of ACE2 and MasR in mesenteric arteries and Ang-(1-7) production in mesenteric arterial beds was increased in an ACE2 dependent manner [59]. Furthermore, Ang-(1-7)/MasR contributed to vasodilation in mesenteric arterial beds of cirrhotic rats, suggesting that NO might mediate this vasodilation.

Of note, NO may also contribute to the regulation of lymphatic flow by modulating smooth muscle cell contractility [62]. For example, mesenteric lymphatic vessels in cirrhotic rats were found to have increased endothelial eNOS expression and decreased smooth muscle cell coverage [63]; this diminished smooth muscle cell coverage was reversed by inhibition of eNOS. These and other data emphasise the importance of the lymphatic vascular system in liver diseases [64].

Besides NO, other vasodilator molecules, such as CO, prostacyclin (PGI2), adrenomedullin, endocannabinoids and endothelium-derived hyperpolarising factors (EDHF), also mediate arterial vasodilation. Some controversy surrounds the identity of EDHF in the hepatic system [65]. Candidate molecules include arachidonic acid metabolites (epoxyeicosatrienoic acid [EET]), the monovalent cation K+, components of gap junctions, and hydrogen peroxide. A recent study showed that in small resistance mesenteric arteries of cirrhotic rats, an arachidonic acid metabolite (11,12-EET) and gap junctions (in particular connexins 40 and 43) mediate increased vasodilation in the splanchnic circulation [66]. Collectively, the data suggest that multiple factors are involved in the excessive vasodilation, observed in the splanchic and systemic circulations (Fig. 4).

Smooth muscle cell hypocontractility

Concomitant with vasodilation, splanchic and systemic arteries exhibit decreased contractile response to vasoconstrictors. This is caused not only by increases in vasodilator molecules mentioned above, but also by impaired contractile RhoA/Rho-kinase signaling in smooth muscle cells (see [67] for further review) and sympathetic nerve regression in these arteries [68]. A variety of vasoconstrictor molecules are also decreased in smooth muscle cells in the arteries of the splanchic and systemic circulations; these include neuropeptide Y [68], urotensin II [69,70], angiotensin [71] and bradykinin [72,73]; this sets up impairment of contractility in the mesenteric vasculature in portal hypertension.

Arterial thinning

Vascular remodelling of the mesenteric vascular bed is another major event in portal hypertension. In a murine model of liver cirrhosis with portal hypertension, the thinning of arterial walls is observed in the splanchnic and systemic circulations [74,75]. Arterial walls consist of endothelial cells, smooth muscle cells and adventitia. The cellular and molecular mechanisms responsible for arterial thinning remain to be fully elucidated. One hypothesis is that increased apoptosis of smooth muscle cells in the mesenteric artery leads to thinning [76]. Through these structural changes as well as possible changes in the levels of proteins important for arterial integrity and function, arterial thinning may help to impair contractile responses of the arteries. Further, arterial thinning may contribute to increased permeability through structural and compositional changes in vessel junctions and thereby facilitate the development of ascites and oedema. Thus, arterial thinning that results from hemodynamic changes caused by portal hypertension may further help to sustain arterial vasodilation and worsen portal hypertension [65,77].

Extrahepatic collateral vessel formation

Porto-systemic collaterals (or shunts) develop through the opening of pre-existing vessels in response to an increase in portal pressure [78,79]. Changes in portal pressure are sensed first by the intestinal microcirculation and then by arteries of the splanchnic circulation [80]. It also appears that these vascular beds in turn
The development of novel therapies for patients with portal hypertension.

**Smooth muscle cells**

- Prostacyclin (PGI2) is synthesized by sGC to generate cyclic guanosine monophosphate (cGMP) and relaxes smooth muscle cells by stimulating adenylate cyclase (AC) to generate cyclic adenosine monophosphate (cAMP).
- Arachidonic acid (AA) is also involved in the synthesis of growth factors such as VEGF, PlGF, apelin, and VEGFR2.

**Endothelial cells**

- VEGF stimulates the formation of portosystemic collaterals.
- Shear stress induces the expression of KLF2, which promotes the formation of collaterals.

**Translational implications and future directions**

Much has been learned in the area of hepatic vascular biology in the last decade, and this new information holds great promise for the development of novel therapies for patients with portal hypertension. Based on an understanding of vascular pathology in chronic liver disease and portal hypertension, many potential therapies are evolving. Here, we review three specific potential biological systems and associated therapies.

**Statins and intrahepatic resistance**

The statin class of compounds has gained considerable interest in vascular biology and in portal hypertension because they appear to stimulate eNOS and activate endothelial NO production. Statins may also exert their anti-fibrogenic effect through up-regulation of KLF-2. In vivo and in vitro studies have shown that statins induce senescence in hepatic myofibroblasts.

**Fig. 4. Mechanisms of increased vasodilation in arteries of the splanchnic circulation in cirrhosis with portal hypertension.** In the splanchnic arterial circulation, agonists such as vascular endothelial growth factor (VEGF) and Ang(1-7) or physical stimuli such as shear stress activate endothelial nitric oxide (NO) synthase (eNOS). In cirrhosis, Ang(1-7) levels are significantly increased by upregulation of angiotensin-converting enzyme (ACE)-2. In addition, MasR, a receptor of Ang(1-7) is up-regulated in connexins (Cx) 40 and 43.
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Table 1. Target molecules and experimental approaches/drugs that are reported to decrease portal pressure. (See below-mentioned references for further information.)

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<th>Target molecules</th>
<th>Approaches/drugs</th>
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<td><strong>Intrahepatic circulation</strong></td>
<td>Statins [111], eNOS gene transfer [112], neuronal NOS gene transfer [113], Akt gene transfer [114], tetrahydrobiopterin (BH4) [115,116], COX-1 inhibitor (SC-560) [117], PGH2/TXA2 receptor blocker (SQ-29548) [118], Antioxidants: vitamin C [119], vitamin E [120], superoxide dismutase (SOD) gene transfer [121], SOD mimetic [121,122], N-acetylcysteine [123], Sodium hydrogen sulfide [124], Notch 1 KO mice [79], Notch 1 KO mice [125], Nogo-B KO mice [126], TLR4 mutant mice [126], A selective FXR agonist, obeticholic acid (INT-747) [102]</td>
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<tr>
<td><strong>Splanchnic and systemic circulation</strong></td>
<td>CB2 receptor agonist (JWH-015) [89], Neuropeptide Y [68], PPARγ agonist (pioglitazone) [127], Anti-VEGFR2 monoclonal antibody (SU5416) [82,128], receptor tyrosine kinase inhibitors (sorafenib [87,88], sunitinib [129]), PI GF KO mice and anti-PIGF monoclonal antibody [84], Apelin antagonist (F13A) [86]</td>
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hepatic resistance in cirrhosis, by mediating contraction of activated HSCs. Further, HSC-specific inhibition of Rho-kinase decreased intrahepatic resistance and lowered portal pressure in an experimental model [99].

Initial studies have indicated that statins can reduce portal pressure in cirrhotic patients and clinical trials are ongoing in patients with cirrhosis that are aimed at identifying a clinical niche for statins [100].

**Obeticholic acid**

Obeticholic acid is a semisynthetic bile acid analogue and a potent selective farnesoid-X receptor agonist [101]. A recent study demonstrated that obeticholic acid decreased intrahepatic resistance and ameliorated portal hypertension in both thioacetamide (TAA) treated – and bile duct ligated rats, by increasing intrahepatic eNOS activity through down-regulation of Rho-kinase and through up-regulation of dimethylarginine dimethylaminohydrolase 2 (DDAH-2), respectively [102].

**VEGF**

A number of preclinical studies support the concept that inhibition of VEGF may have beneficial therapeutic effects in portal hypertension. Mechanisms by which VEGF inhibition may be beneficial include attenuation of mesenteric angiogenesis and portosystemic collaterals as well as reduction in intrahepatic vascular remodelling and fibrogenesis. Additional effects of VEGF inhibition on reduction in vascular permeability and ascites are also documented [103]. However, further studies are needed in humans and this is being pursued in an indirect manner through analysis of small molecule inhibitors of receptor tyrosine kinases such as sorafenib (with the understanding that these inhibitors target a multitude of receptor tyrosine kinases on different cell types) [10,104]. It should be pointed out that based on data with VEGF inhibition in the cancer arena, unanticipated effects of VEGF inhibition may be possible. Furthermore, some data indicate that VEGF itself may be important in hepatic tissue healing, sinusoidal normalisation, and regeneration. For example, VEGF may induce fibrosis regression through effects on macrophage infiltration and ensuing matrix degradation [105]. Further, in one study, re-establishment of LSEC fenestrae via restoration of VEGF function fully reversed portal hypertension and its secondary manifestations. Finally, VEGF facilitates the recruitment of bone marrow-derived LSEC progenitor cells during liver regeneration [106]. Thus, the role of VEGF in liver injury, fibrosis, and portal hypertension, as well as its role in the recovery from these processes will require further exploration.

**Future**

Here, we have reviewed current concepts in the area of intra- and extra-vascular pathophysiology in portal hypertension. A number of novel areas are on the horizon. For example, an attractive future area will likely include inter-organ relationships in the pathogenesis of portal hypertension in the context of vascular biology. An excellent example in portal hypertension will be the gut-liver axis. The importance of bacterial translocation from the gut to the portal circulation has been long recognised in the study of portal hypertension, but the molecular basis of this relationship has been little investigated. The vascular biology associated with the gut-liver axis, including an understanding of how TLR signaling to key cellular players is initiated, or how vascular permeability may be changed facilitating bacterial translocation, will be important.

An understanding of VEGF function in the liver and mesenteric circulation is just now coming into focus. VEGF has different isoforms and distinct VEGF receptors mediate a variety of...
signaling cascades in cell specific manners [107]. Therefore, a
detailed characterisation of VEGF induced pathways is needed to
determine the intra- and extra-hepatic neovascularisation in
portal hypertension. In addition, angiocrine growth factors in
addition to VEGF are likely to play a role in the neovascularisation
typical of portal hypertension and will be important.

An accumulating body of evidence suggests that neovascular-
isation is a key pathological feature of patients with non-alco-
holic steatohepatitis (NASH) [108]. In mouse models of NASH,
treatment with anti-mouse VEGFR2 antibody improved disrupted
liver vasculature, decreased inflammatory gene expressions, and
reduced steatosis to some extent, suggesting that VEGF is
involved in the pathogenesis of NASH [109]. However, the
mechanisms underlying hepatic neovascularisation in NASH
are largely unknown and are important for investigation.

Other unexplored, but yet important areas include the role of
the extracellular matrix components and their signaling in the
vascular cells such as integrins as well as that of non-coding RNAs
in portal hypertension. In particular, certain extracellular matrix
components may have an important role in vascular remodelling
associated with portal hypertension, as suggested by a recent
study emphasising the role of integrins in fibrosis in several
organs, including the liver [110].

Finally, available data suggest that the pathogenesis of portal
hypertension is linked to specific and targetable molecular
pathways. This suggests that approaches exist for manipulating
these pathways. For example, correcting the defect in eNOS
signaling in intrahepatic SECs is attractive. Alternatively,
abrogating the effect of the abundant vasoconstrictors in the
intrahepatic circulation, or approaching neoangiogenesis in the
mesenteric circulation would be therapeutically attractive.
Indeed, the prospects for new therapy in the area of vascular
biology of the liver are bright.

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Conflict of interest
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