Review

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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.

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.

Keywords: Endothelial cell; Pericyte; Sinusoid; Pressure; Resistance; Therapy.
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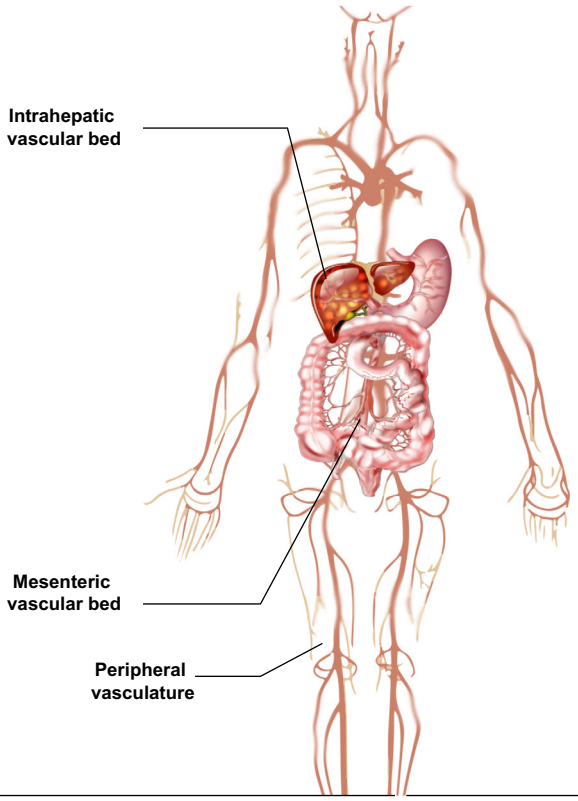
Abbreviations: No, nitric oxide; eNOS, endothelial nitric oxide synthase; VEGF, vascular endothelial growth factor; LSECs, liver sinusoidal endothelial cells; HSCs, hepatic stellate cells; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; PlGF, placental growth factor; KLF2, kruppel-like factor-2 (KLF2); VEGFR, VEGF receptor; PDGF, platelet derived growth factor; SMC, smooth muscle cells.



Molecular biology	Physiology
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	• Reduced resistance • 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 hypo-oxygenation • 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 synthesis 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 vein. 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.

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].

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 [5–8]. 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

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 isoform 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 an endothelialopathy in liver disease [5,18]. This has a number of important effects on portal hypertension, including that a reduction in intrahepatic NO appears to be a critical component of the intense vasoconstrictive nature of the injured liver [19].

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 [39]. 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.

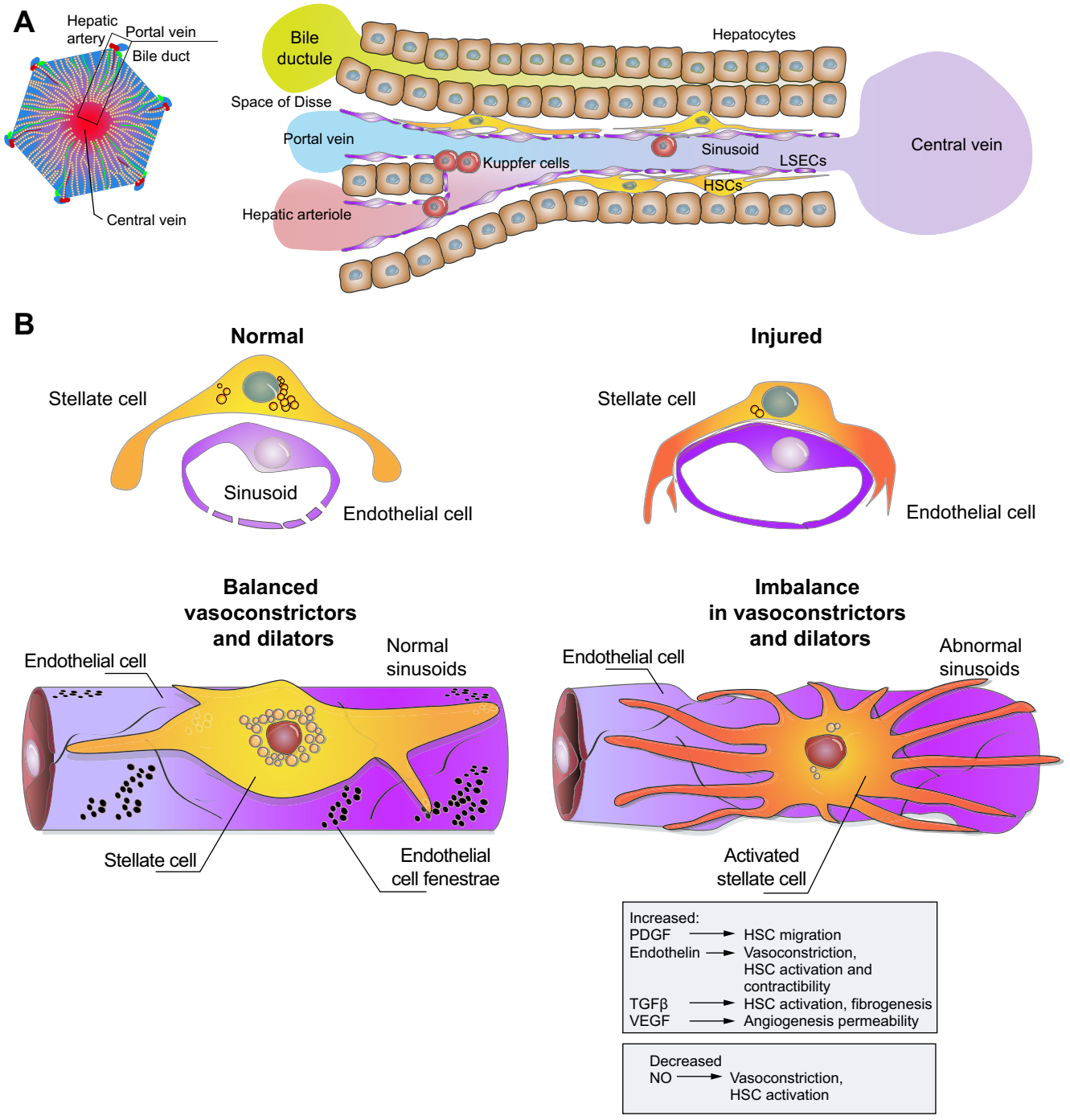


Fig. 2. Changes in the hepatic sinusoid in response to liver injury. (A) shows a simplified version of a portal tract (left), central vein (right), and hepatic sinusoidal morphology with associated cell types within the sinusoid. (B) shows the sinusoid in response to liver injury; the upper panel depicts a cross sectional image, while the lower panel shows a 3-dimensional view. In the normal state (left), sinusoidal endothelial cells produce NO supporting a low resistance state. In response to liver injury, extensive changes occur in the sinusoid. Liver injury (right) leads to both morphological as well as molecular abnormalities in sinusoidal endothelial cells and stellate cells. For example, endothelial cells become defenestrated, develop a defect in NO production, and concurrently increase production of other proteins such as endothelin and fibronectin that can contribute to stellate cell activation. Upon their activation, stellate cells develop an enhanced contractile phenotype and produce increased extracellular matrix. A number of paracrine and autocrine interactions occur between sinusoidal endothelial cells and stellate cells with some of the putative molecules and their associated functions shown (gray box). HSC, hepatic stellate cells; LSEC, liver sinusoidal endothelial cell.

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-exiting 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 fenestrae. 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.

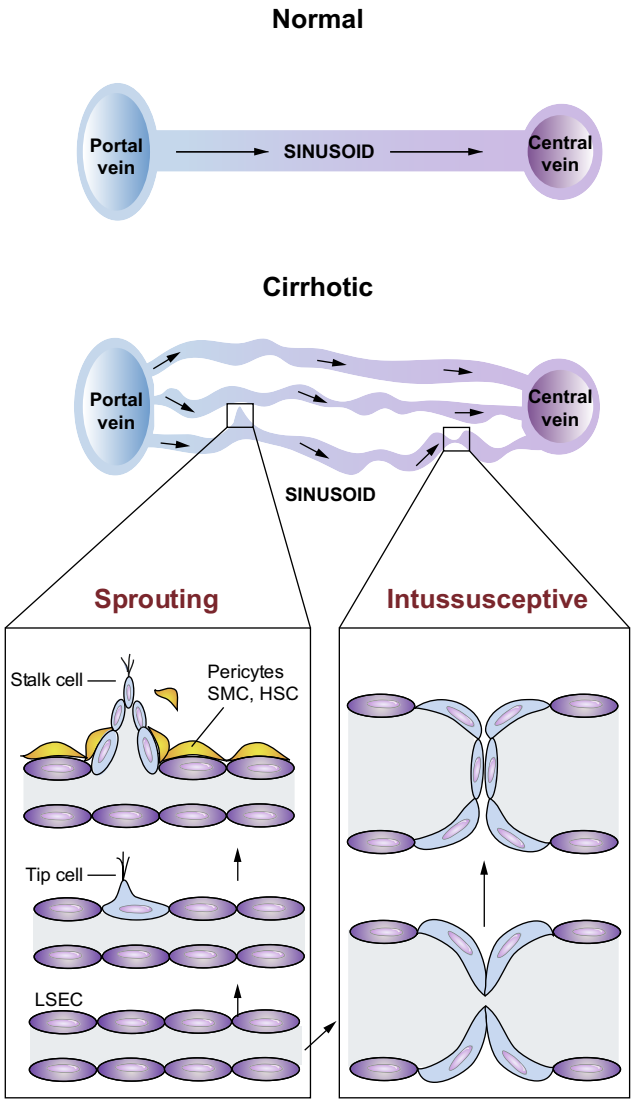


Fig. 3. Sprouting and intussusceptive angiogenesis in cirrhosis and portal hypertension. Normal architecture of sinusoidal vasculature is shown in left panel with normal flow from portal venules, through sinusoidal microcirculation, to central veins. The sinusoidal vasculatures in the cirrhotic livers (right panel) are altered significantly, with increased number of sinusoidal vessels (angiogenesis) of various diameters and flow pattern. This is a simplified diagram of the sinusoidal microcirculation in liver cirrhosis. In cirrhosis, however, the relationship between the portal and central veins is not maintained because of fibrous septa and regenerative nodules, which disrupt the normal vascular architecture of portal venules, sinusoid and central vein. In sprouting angiogenesis, it is proposed that an endothelial 'tip cell' leads the vessel sprouts at the forefront and a trailing endothelial 'stalk cell' elongates behind the tip cell of a growing branch of vessels. Intussusceptive angiogenesis involves splitting vessels by the formation of translumenal pillars. Both forms of angiogenesis require the recruitment of pericytes or smooth muscle cells (SMCs). In cirrhotic livers, activated hepatic stellate cells (HSCs) are thought to serve as a role of pericytes and SMCs.

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

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 cell 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 splanchnic and systemic circulations (Fig. 4).

Smooth muscle cell hypocontractility

Concomitant with vasodilation, splanchnic 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 splanchnic 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

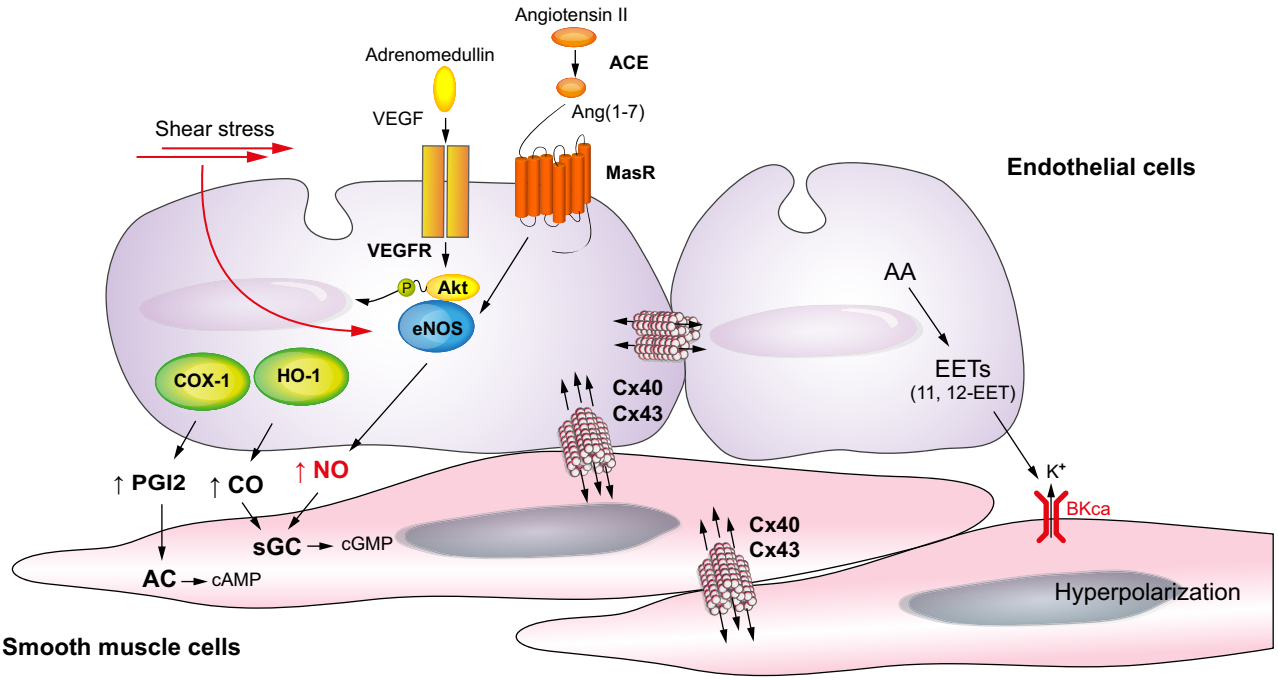


Fig. 4. Mechanisms of increased vasodilation in arteries of the splanchnic circulation in cirrhosis with portal hypertension. In the splanchnic arterial circulation, agonists such as vascular endothelial growth factor (VEGF) and Ang(1-7) or physical stimuli such as shear stress activate endothelial nitric 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 cirrhosis, resulting in an increase in eNOS activity and NO production. Besides NO, carbon monoxide (CO) produced by hemeoxygenase-1 (HO-1) causes vasodilation by activating soluble guanylate cyclase (sGC) to generate cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells. Prostacyclin (PGI_2) is synthesised by cyclooxygenase (COX) and relaxes smooth muscle cells by stimulating adenylate cyclase (AC) to generate cyclic adenosine monophosphate (cAMP). Arachidonic acid (AA) metabolites [epoxyeicosatrienoic acids (EETs)] cause hyperpolarisation/relaxation, acting through voltage-gated potassium channel (BKCa) and gap junction [in particular connexins (Cx) 40 and 43].

produce various angiogenic factors, such as VEGF [81–83] and placental growth factor (PlGF) [84], which stimulate angiogenesis and promote the formation of portosystemic collaterals. Using a vascular corrosion technique, it was demonstrated that collaterals are formed in the splanchnic circulation in portal hypertensive mice by both sprouting and intussusceptive angiogenesis [85].

Collaterals develop to decompress the portal system. However, portal pressure remains elevated because of increased splanchnic blood flow resulting from splanchnic vasodilation. Additionally, these collaterals cause serious complications such as variceal bleeding and hepatic encephalopathy [65]. To what extent collaterals develop through the opening of pre-existing vessels, sprouting and intussusceptive angiogenesis remains an active area of investigation.

Studies in experimental models of portal hypertension and cirrhosis have shown that portal systemic collaterals can be reduced by a variety of approaches, including inhibiting VEGF (with anti-VEGFR2) or a combination of anti-VEGF (rapamycin)/anti-platelet derived growth factor (PDGF) (gleevec), PlGF [84], apelin [86], sorafenib [87,88] and cannabinoid signaling [89]. However, the reduction of collateral vessels does not always result in a decrease in portal pressure to the normal level, since this reduction does not significantly change the portal blood flow [84,90].

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 (Table 1). Here, as examples, 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 [91]. It is also possible that statins may stimulate downstream signaling molecules that have beneficial effects in the liver. One mechanism of statins appears to be via selective effects on LSECs through stimulation of the expression of the KLF2 transcription factor [92,93]. KLF2 is highly expressed in vascular endothelial cells and protects the endothelium by upregulating the expression of a wide variety of vasoprotector genes [94,95], including eNOS [96]. Interestingly, LSECs overexpressing KLF-2 lead to HSC quiescence when these cells are co-cultured, suggesting that the statins' anti-fibrogenic effect through up-regulation of KLF-2 is LSEC mediated [93]. However, statins may also exert their anti-fibrotic effect via direct activity on HSCs. Early atorvastatin exposure in a rat model of hepatic fibrosis attenuated HSC activation and fibrosis [97]; additionally, atorvastatin induced senescence in hepatic myofibroblasts *in vitro* and *in vivo* [98].

It has also been documented that atorvastatin decreases portal pressure in cirrhotic rats by inhibiting Rho-kinase and by activating eNOS [91]. Rho-kinase contributes to increased intra-

Table 1. Target molecules and experimental approaches/drugs that are reported to decrease portal pressure. (See below-mentioned references for further information.)

Target molecules	Approaches/drugs
Intrahepatic circulation	
Nitric oxide (NO)/endothelial NO synthase (eNOS)	Statins [111], eNOS gene transfer [112], neuronal NOS gene transfer [113], Akt gene transfer [114], tetrahydrobiopterin (BH4) [115,116]
TXA2	COX-1 inhibitor (SC-560) [117], PGH2/TXA2 receptor blocker (SQ-29548) [118]
Superoxide radicals	Antioxidants: vitamin C [119], vitamin E [120], superoxide dismutase (SOD) gene transfer [121], SOD mimetic [121,122], N-acetylcysteine [123]
Hydrogen sulfide (H2S)	Sodium hydrogen sulfide [124]
Notch 1	*Notch 1* KO mice [79]
Nogo-B	*Nogo-B* KO mice [125]
Toll like receptor 4	*TLR4* mutant mice [126]
The farnesoid-X-receptor (FXR)	A selective FXR agonist, obeticholic acid (INT-747) [102]
Splanchnic and systemic circulation	
Cannabinoid receptor type 2 (CB2) receptor	CB2 receptor agonist (JWH-015) [89]
RhoA/Rho-kinase	Neuropeptide Y [68]
Peroxisome proliferator-activated receptor gamma (PPARγ)	PPARγ agonist (pioglitazone) [127]
Vascular endothelial growth factor (VEGF)	Anti-VEGFR2 monoclonal antibody (SU5416) [82,128], receptor tyrosine kinase inhibitors (sorafenib [87,88], sunitinib [129])
Placental growth factor (PIGF)	*PlGF* KO mice and anti-PIGF monoclonal antibody [84]
Apelin	Apelin antagonist (F13A) [86]

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 [8]. 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 understand 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 neovascularisation is a key pathological feature of patients with non-alcoholic 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 underlining 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 LSECs 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

D. Rockey has disclosed a research agreement with Actelion Pharmaceuticals Ltd. and consulting within the past year with Ono Pharmaceuticals Co. The other authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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