Angiocrine signaling in the hepatic sinusoids in health and disease

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Kostallari E, Shah VH. Angiocrine signaling in the hepatic sinusoids in health and disease. Am J Physiol Gastrointest Liver Physiol 311: G246–G251, 2016. First published June 10, 2016; doi:10.1152/ajpgi.00118.2016.—The capillary network irrigating the liver is important not only for nutrient and oxygen delivery, but also for the signals distributed to other hepatic cell types necessary to maintain liver homeostasis. During development, endothelial cells are a key component in liver zonation. In adulthood, they maintain hepatic stellate cells and hepatocytes in quiescence. Their importance in pathobiology is highlighted in liver regeneration and chronic liver diseases, where they coordinate paracrine cell behavior. During regeneration, liver sinusoidal endothelial cells induce hepatocyte proliferation and angiogenesis. During fibrogenesis, they undergo morphological and functional changes, which are reflected by their role in hepatic stellate cell activation, inflammation, and distorted sinusoidal structure. Therapeutic strategies to target angiocrine signaling are in progress but are in the early stages. Here, we offer a short synthesis of recent studies on angiocrine signaling in liver homeostasis, regeneration, and fibrogenesis.

endothelial cell; angiocrine signaling; liver regeneration; fibrosis; angiogenesis

THE LIVER IS A HIGHLY VASCULARIZED organ with a unique anatomic structure. It receives 75% of its blood from the portal vein and 25% from the hepatic artery. The terminal portal veins give rise to septal branches that drain blood into sinusoids. The hepatic artery terminates in axial branches parallel to the portal veins, which merge into sinusoids. Finally, sinusoids conduct the blood to the central veins. These sinusoids consist of unique fenestrated endothelial cells that lack a basement membrane, termed liver sinusoidal endothelial cells (LSECs) (1).

While blood vessels are important entities for organ nutrition, recently their angiocrine signaling, a term describing endothelial cell-released paracrine molecule delivery to microenvironment cells, has been highlighted. These molecules include growth factors and trophogens, adhesion molecules, and chemokines, which can be involved in many processes such as tissue repair (6). Here, we provide a synthesis of recent studies pertaining to angiocrine signaling in hepatic health and disease.

Angiocrine Signaling in Hepatic Health

Liver endothelial cells are important from hepatic prenatal development until adulthood. A subtype of these endothelial cells play a central role in the zonation of the liver through their angiocrine signaling to adjacent hepatocytes, which allows effective execution of multiple hepatic functions (42). Indeed, a recent study reported that central vein, but not portal vein, endothelial cell-derived Rspondin3 (Rspo3) is implicated in hepatic development by regulating the Wnt/β-catenin pathway. Furthermore, mice lacking Rspo3 showed a downregulation of liver metabolic function, demonstrated by a decrease in direct (conjugated) bilirubin (42). During adulthood, Rspo3 is important for maintaining liver zonation (42). In line with this zonation concept, Wang et al. (56) showed that, during adulthood, central vein endothelial cells form a vascular niche for surrounding Axin2 hepatocytes. These endothelial cells constitute a local source for Wnt (Wnt2 and Wnt9b), which induces hepatocyte self-renewal (56). These studies show that central vein endothelial cells drive liver zonation and hepatocyte self-renewal through angiocrine signals. Future studies warrant attention to parallel portal vein endothelial cell angiocrine signals and their role in hepatic development and adulthood.

LSECs, which line the smaller sinusoidal vessels, have a distinct structure and function (59). They represent ~2.8% of the total liver volume and 15–20% of the total number of liver cells and contribute to 45% of the total pinocytic vesicle mass in the liver (3), reflecting their high endocytic activity. LSECs also differ from other endothelial cell types by their phenotype. They present a VEGFR3+/VEGFR2+/VE-cadherin+/factor VIII+/CD31+/CD34− phenotype (15). They lack a basement membrane (50); Geraud et al. did detect expression of tight junction proteins in LSECs (18). Ultrastructurally, LSECs present with ~50- to 150-nm-diameter fenestrations, which play an important role in the transfer of macromolecules between the blood and the space of Disse (the space between endothelial cells and hepatocytes) (1, 50). These unique characteristics have made LSECs attractive in the study of hepatic health and disease.

In the healthy liver, LSECs send angiocrine signals to surrounding cells such as hepatic stellate cells (HSCs) and hepatocytes (Fig. 1, left). These signals maintain HSC quiescence (10, 60). In turn, HSCs control the contraction of the endothelial tubes and the blood flow through their processes (4). Nitric oxide (NO) secretion is controlled by endothelial cell NO synthase (48). Endothelial NO synthase is regulated through multiple mechanisms, including shear stress (26, 49). In healthy liver, the balance between vasoconstrictors [endothelin (ET)-1 or thromboxane A2] and vasodilators (NO or PGI2) is shifted toward vasodilatation with a low amount of ET-1 and a high amount of NO secreted by LSECs, maintaining sinusoids in a low-resistance state (31, 43). Furthermore, LSECs produce small amounts of hepatocyte growth factor (HGF), which does not stimulate hepatocyte proliferation but maintains hepatocyte homeostasis (4). LSECs can also produce Wnt2 and Wnt9b, which induce hepatocyte self-renewal (56). Taken together, these and other parallel studies with other molecular models suggest that the LSECs are a crucial component of liver homeostasis through angiocrine signaling (Fig. 1, left). In response, cells targeted by angiocrine signaling reciprocally maintain the LSEC phenotype by possible direct contacts (23) and/or paracrine interactions. Precise paracrine
mediators remain to be elucidated. Xie and colleagues recently reported that NO donors do not maintain HSC quiescence (60).

**Angiocrine Signaling in Liver Regeneration**

During liver injury and the ensuing regeneration response, LSECs undergo changes in their porosity (55) and gene expression that lead to changes in angiocrine signaling. There are two phases of liver regeneration: the early phase (phase I), characterized by massive hepatocyte proliferation, and the angiogenic phase (phase II), during which new blood vessels are generated for liver reconstitution (41). During phase I (Fig. 1, middle), LSECs secrete HGF to induce hepatocyte proliferation (4, 31, 41) through multiple mechanisms including HGF-induced phosphorylation of hepatocyte cyclin-dependent kinase 2 (36). During phase II, hepatocytes secrete VEGF, which promotes angiogenesis, which in turn drives angiocrine signaling (41). Mechanisms that coordinate phases I and II are an area of active investigation.

In the experimental regeneration model of partial hepatectomy (PHx), the angiogenic molecule angiopoietin-2 (Angpt2) is upregulated in LSECs (19) (Fig. 1, middle). Using an Angpt2-deficient mouse, Hu et al. (19) observed enhanced hepatocyte proliferation during phase I and reduced proliferation of nonparenchymal cells during phase II. In vitro studies showed that downregulation of Angpt2 reduced TGFβ1 expression in endothelial cells, which led to increased hepatocyte proliferation. Thus the early decrease in Angpt2 after PHx allows hepatocyte proliferation by removing the TGFβ1 “brake.” During phase II, Angpt2 upregulates VEGF receptor type 2 (VEGFR2) through autocrine stimulation of the Angpt2 receptor Tie2, which leads to LSEC proliferation. These studies suggest that LSECs not only respond to secreted signals, but they also generate signals to directly modulate liver regeneration (19). Interestingly, HSCs also express high levels of HGF after PHx, perhaps to overcome the antiproliferative effect of TGFβ1 (41, 63). However, the synchrony between HSCs and LSECs in this process is not fully defined.

A recent study suggests that an important contributor to liver regeneration is the platelet (28). Indeed, LSECs interact with platelets following PHx and allow platelets to translocate into the space of Disse to make contact with hepatocytes. Lesurtel et al. suggested that inhibition of platelet function impairs their proliferation effect on hepatocytes (28, 34) (Fig. 1, middle). More studies are necessary to understand if LSECs attract platelets to initiate the regenerative process and the role of other hepatic cell types to mediate this process.

Another interesting point is the recruitment of bone marrow-derived progenitor cells (BM-PCs) in the damaged liver. Recent studies show that LSEC-derived stromal cell-derived factor (SDF)-1 drives BM-PC traffic into the liver (12, 33). Moreover, one of these studies shows that these BM-PCs are LSEC progenitors expressing the chemokine receptor CXCR7 (12), which is known to enhance liver regeneration (14). Resident LSECs express mostly the chemokine receptor CXCR4 (12), which may drive fibrogenesis (14). These studies support the hypothesis that different LSEC subpopulations may play distinct roles.

**Angiocrine Signaling in Liver Fibrosis**

Despite the regenerative potential of the liver, chronic damage leads to liver fibrosis, cirrhosis, and, ultimately, liver failure. The signals responsible for reparative liver regeneration as opposed to counterproductive fibrotic regenerative responses are not well defined. Recently, Ding et al. suggested that the decision between liver regeneration and liver fibrosis is dependent on the balance between the SDF-1 receptors CXCR4 and CXCR7 (14, 41). Indeed, upregulation of CXCR7 in LSECs led to increased endothelial inhibitor of DNA binding 1 (Id1) expression, which is important for hepatocyte proliferation and liver regeneration. Deletion of CXCR7 re-
Perspectives

In cells, such as HSCs and hepatocytes (Fig. 1), signaling during CLD.

An investigation is necessary to fully elucidate the role of Notch, which could mediate signals leading to liver fibrosis (52). More markers (13). Another study suggests that Notch signaling loss of fenestrae and upregulation of LSEC capillarization et al. show that the deletion of Notch1 in LSECs induces the testing pathway that maintains LSEC phenotype is Notch. Dill et al. show that the deletion of Notch1 in LSECs induces the loss of fenestrae and upregulation of LSEC capillarization markers (13). Another study suggests that Notch signaling could mediate signals leading to liver fibrosis (52). More investigation is necessary to fully elucidate the role of Notch signaling during CLD.

LSEC dedifferentiation can affect the behavior of surrounding cells, such as HSCs and hepatocytes (Fig. 1, right). First, dedifferentiated LSECs increase the release of ET-1 and decrease NO secretion. LSEC capillarization leads to HSC activation, which contributes to fibrogenesis and portal hypertension (14, 26, 31, 43). In turn, activated HSCs/myofibroblasts can secrete VEGF, which may interact with LSEC VEGFR and contribute to fibrosis-associated angiogenesis (11, 44). VEGFR1 and/or VEGFR2 activation increases the release of LSEC-derived HGF, which is known to stimulate and sustain hepatocyte proliferation (4, 15, 27, 31). The cascade of cross talk between LSECs, HSCs, and hepatocytes is crucial for liver fibrosis progression.

It has been reported that portal pressure can be regulated by relaxin, which is naturally secreted by activated LSECs and HSCs. In vitro studies showed that relaxin reduces HSC contraction (52). Furthermore, relaxin administration reduced portal pressure in rats pretreated with CCl4 and increased intrahepatic NO levels (16). In line with this, a recent study affirms that adenoviral delivery of relaxin in the liver attenuates portal pressure in rats pretreated with CCl4 and increased intrahepatic NO levels (16). In line with this, a recent study affirms that adenoviral delivery of relaxin in the liver attenuates endotoxemia (32). Interestingly, TLR4 is implicated during liver fibrogenesis, since the suppression of its pathway through dextra-1 activation is protective against fibrosis (46) and TLR4 regulates fibrogenesis and angiogenesis in liver injury (20, 47). Probably, activation of sinusoidal TLR4 could initiate neutrophil adhesion during CLD, which could participate in fibrosis-associated angiogenesis. In several diseases, besides cell adhesion and infiltration, neutrophils can also expel their chromatin to form local snares called NETs (neutrophil extracellular traps), which are implicated in thrombogenesis, autoimmunity, and cancer progression such as liver metastasis (51, 54). Moreover, NETs have been found to be closely apposed to apoptotic endothelial cells (39). The role of NETs in angiocrine signaling and fibrogenesis requires further investigation.

Fibrosis can lead to hepatocellular carcinoma (HCC), where angiogenesis and inflammation are important components. TNFα is a proinflammatory cytokine secreted in HCC (21). In vitro experiments show that, in response to TNFα, activated LSECs upregulate CXCL9 and CXCL10 expression (37). These chemoattractants participate in CD4+ T cell recruitment during inflammation (37). Moreover, in vivo studies show that LSECs secrete CCL3, which contributes to HCC growth by binding to its receptor CC chemokine receptor type 1 (CCR1) (30). These studies show that LSECs can contribute to HCC growth by delivering oxygen and nutrients not only through the traditional “angiogenesis” paradigm, but also through specific angiocrine signaling.

**Antiangiocrine Therapeutic Approaches**

Angiocrine signaling in hepatic disease is often driven by activated and angiogenic endothelial cells. Thus therapies targeting angiocrine signaling, including pathological angiogenesis, may be mechanisms to modulate these processes.

However, angiocrine signals may not always be angiogenic. An interesting example is vasohibin, a natural antiangiogenic molecule released by endothelial cells. Vasohibin-1 is expressed under the control of VEGF and represents an intrinsic
negative feedback on the angiogenic potential of endothelial cells (7, 45). Moreover, Coch et al. reported that overexpression of vasoactive1-1 led to a reduction of pathological angiogenesis and an attenuation of liver fibrogenesis partly mediated through inhibition of HSC activation (8). Nevertheless, a recent study showed that the downregulation of the endogenous vasoactive1-1 has no effect on early fibrosis after bile duct ligation (17). More studies are needed to elucidate the role of vasoactive1-1 and if it could be a target for future therapy.

Nanoparticles constitute a potentially attractive delivery vehicle to target angiocrine signaling in liver diseases. Recently, Lin et al. (29) developed and optimized poly(lactic-co-glycolic acid)-based nanoparticles loaded with sorafenib, an inhibitor of angiogenesis. The systemic administration of these nanoparticles led to their uptake by the liver, ameliorated liver fibrosis, and decreased pathological microvascular density (29). Other nanoparticles used as delivery vectors are cationic lipid nanoparticles. Jiménez Calvente et al. (22) reported that nanoparticles loaded with small interfering RNA to the procollagen-1 gene significantly ameliorated progression and accelerated regression of fibrosis. These particles are retained in the liver of fibrotic mice and accumulate mostly in nonparenchymal cells for prolonged periods (22). Another study affirmed that administration of cerium oxide nanoparticles to CCl4-treated rats protects against chronic liver injury by reducing liver steatosis and portal hypertension (38). Given the phagocytic activity of LSECs (2), it is possible that they may participate in the internalization of these nanoparticles and perhaps even distribute nanoparticle cargo to HSCs or other surrounding hepatic cells.

The VEGF pathway is essential for LSEC fenestration. By administrating a pharmacological activator of the VEGF-NO-soluble guanylate-cGMP-protein kinase G pathway during thiacetamide-induced liver fibrosis, Xie et al. showed restored LSEC fenestration in liver cirrhosis, which promotes the reversal of HSC activation to both quiescence and apoptosis (9, 60). In line with this study, VEGF is also essential for CCl4-induced liver fibrosis resolution through CXCL9 and matrix metalloproteinase 13. Indeed, administration of VEGF-neutralizing antibody impaired fibrosis resolution, while CXCL9 overexpression has the opposite effect (62). Thus, VEGF is an encouraging candidate for future therapies. However, VEGF also drives fibrosis-associated angiogenesis (61), making it a complex target dependent on context and temporal kinetics.

Conclusion

Here, we have identified liver endothelial cells, and in particular LSECs, as a “gatekeeper” of fibrotic and regenerative hepatic responses (Fig. 1) (9). LSECs can maintain HSCs and hepatocytes in quiescence; central vein endothelial cells can induce the self-renewal of a subtype of hepatocyte. After acute injury, endothelial cells determine if the liver undergoes regeneration or fibrosis by angiocrine signaling (14, 41). Hepatic regeneration begins by a massive hepatocyte proliferation, followed by angiogenesis. If the damage is chronic, hepatocyte proliferation is replaced by HSC proliferation and scar tissue formation. In both cases, LSECs play a crucial role by generating signals through direct contacts, soluble molecules, and/or extracellular vesicles.

The study of the hepatic vascular niche remains incomplete. More investigations are needed to uncover the cross talk between endothelial cells and other hepatic cell types, especially during liver disease. An interesting area could be the role of angiocrine signaling in biliary epithelial cells during liver damage and if it participates in epithelial-to-mesenchymal transition. Many therapeutic strategies are proposed and warrant further investigation (40). The ability to modulate angiocrine signaling to drive beneficial hepatocyte repair programs without neoplastic transformation and without HSC activation and exuberant matrix responses will be the focus for the coming years.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

E.K. prepared the figure; E.K. drafted the manuscript; V.H.S. edited and revised the manuscript; V.H.S. approved the final version of the manuscript.

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