cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

# Angiogenesis and Fibrogenesis in Chronic Liver Diseases

Claudia Bocca, Erica Novo, Antonella Miglietta, and Maurizio Parola

*Unit of Experimental Medicine and Clinical Pathology, Department of Clinical and Biological Sciences, School of Medicine, University of Torino, Torino, Italy*

**SUMMARY**

Pathologic angiogenesis is intrinsically associated with the fibrogenic progression of chronic liver diseases. Hypoxia, hypoxia-inducible factors, and other signals and mediators released by various cells of the liver drive and modulate the critical profibrogenic and proangiogenic role of hepatic myofibroblasts.

**Pathologic angiogenesis appears to be intrinsically associated with the fibrogenic progression of chronic liver diseases, which eventually leads to the development of cirrhosis and related complications, including hepatocellular carcinoma. Several laboratories have suggested that this association is relevant for chronic liver disease progression, with angiogenesis proposed to sustain fibrogenesis. This minireview offers a synthesis of relevant findings and opinions that have emerged in the last few years relating liver angiogenesis to fibrogenesis. We discuss liver angiogenesis in normal and pathophysiologic conditions with a focus on the role of hypoxia and hypoxia-inducible factors and assess the evidence supporting a clear relationship between angiogenesis and fibrogenesis. A section is dedicated to the critical interactions between liver sinusoidal endothelial cells and either quiescent hepatic stellate cells or myofibroblast-like stellate cells. Finally, we introduce the unusual, dual (profibrogenic and proangiogenic) role of hepatic myofibroblasts and emerging evidence supporting a role for specific mediators like vasohibin and microparticles and microvesicles. *(Cell Mol Gastroenterol Hepatol 2015;1:477–488; http://dx.doi.org/10.1016/j.jcmgh.2015.06.011)***

*Keywords:* Hypoxia; Liver Angiogenesis; Liver Fibrogenesis; Myofibroblasts.

## Angiogenesis and Liver Fibrogenesis

Fibrogenic progression of chronic liver diseases (CLDs), eventually leading to the development of liver cirrhosis and related complications including hepatocellular carcinoma (HCC), is intimately associated with pathologic angiogenesis and sinusoidal remodeling.[1–6] This is not surprising because angiogenesis is a major feature of any wound healing response and chronic activation of wound healing is a general mechanism involved in the progression of CLDs.[7–10] Some researchers, including the authors of this review, go further[2–4,9–15] in suggesting additionally that 1) hypoxia (the most obvious stimulus for angiogenesis, commonly detected in progressive CLDs[1–6,16]), hypoxia-inducible factors (HIFs), and angiogenesis may have a major role in sustaining and potentially driving liver fibrogenesis; 2) hepatic myofibroblasts (MFs), regardless of origin, are critical cells in governing and modulating the interactions between inflammation, angiogenesis, and fibrogenesis; 3) liver angiogenesis has a role in the genesis of portal hypertension and related complications in advanced stages of CLDs; and 4) microparticles/microvesicles released by either fat-laden hepatocytes or portal MFs have an emerging role in mediating angiogenesis and vascular remodeling. This review offers a synthesis of the most relevant recent data and opinions regarding the close relationship between liver angiogenesis and fibrogenesis. Established concepts about mechanisms of liver angiogenesis, liver fibrogenesis, and CLD progression will not be addressed. Moreover, in this review, the relationship between angiogenesis and portal hypertension and related complications are not discussed; readers interested in this specific topic should refer to a recent authoritative review.[13]

## Angiogenesis in the Liver: General Considerations

Liver angiogenesis occurs in both physiologic (ie, liver regeneration) and pathologic conditions, including ischemia, progressive CLDs, hepatocellular carcinoma, and metastatic liver cancer.[1–5] Angiogenesis in the liver is similar to angiogenesis in other tissues and organs; however, as suggested by several groups,[1–5,10–12] pathologic angiogenesis occurring during the progression of CLDs can be significantly affected by liver-specific events, interactions between different hepatic cell populations, and the involvement of atypical proangiogenic mediators.

***Abbreviations used in this paper:*** **Akt, protein kinase B; Ang-1, angiopoietin-1; ANGPTL3, angiopoietin-like-3 peptide; CCL2, chemokine ligand 2; CCR, chemokine receptor; CLD, chronic liver disease; eNOS, endothelial nitric oxide synthase; ET-1, endothelin 1; HCC, hepatocellular carcinoma; Hh, Hedgehog; HIF, hypoxia-inducible factor; HSC, hepatic stellate cell; HSC/MFs, myofibroblast-like cells from activated hepatic stellate cells; LSEC, liver sinusoidal endothelial cell; MF, myofibroblast; MP, microparticle; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NO, nitric oxide; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; α-SMA, α-smooth muscle actin; VEGF, vascular endothelial growth factor; VEGF-R2, vascular endothelial growth factor receptor type 2.**

**Most current article**



2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2015.06.011

From a general point of view, the pattern of fibrosis that predominates in a specific CLD is relevant to angiogenesis. Although pathologic liver angiogenesis has been described in all CLDs irrespective of etiology, it is much more prominent under conditions of bridging or postnecrotic fibrosis (eg, in chronic viral infection or, to a lesser extent, in autoimmune diseases) than in conditions characterized by pericellular or perisinusoidal fibrosis (as in non-alcoholic fatty liver disease or alcoholic liver disease) or by biliary fibrosis.[3,9,10] This suggests an inverse correlation between angiogenesis and the potential for fibrosis reversibility, which is more evident in conditions characterized by pericellular or perisinusoidal fibrosis and biliary fibrosis than in those associated with bridging fibrosis.[9] This may be related to the unique tissue localization, phenotypic profile, and functional role of hepatic stellate cells (HSCs).

HSCs, which in physiologic conditions synthesize extracellular matrix components in the space of Disse, store retinoids and possibly contract in response to vasoactive mediators to modulate sinusoidal blood flux, are also liver-specific pericytes. During the progression of CLDs, HSCs are the most relevant myofibroblast precursors and profibrogenic hepatic cells.[2,17–21] HSCs, particularly in their activated and MF-like phenotype (HSC/MFs), modulate angiogenesis in a way that, as we will describe, relates to the role attributed to them as microcapillary pericytes. Hepatic MFs, a heterogenous population of profibrogenic, highly proliferative, and contractile cells, can also originate from portal MFs and bone marrow-derived stem cells recruited into the injured liver, and these may also play a role in angiogenesis.[17–22]

The relevance of intense cross-talk between hepatic cell populations in pathologic angiogenesis is strongly supported by the knowledge that major proangiogenic mediators such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), are produced and released by several liver cell types involved in CLD progression, including hypoxic hepatocytes and hypoxia-responsive macrophages and MFs.[1–3,9–12] The role of liver-specific proangiogenic mediators such as angiopoietin-like-3 peptide (ANGPTL3)[23] is controversial. ANGPTL3, which belongs to a family of mediators described as playing major roles in the trafficking and metabolism of lipids, was reported to induce haptotactic endothelial cell adhesion and migration, possibly by binding to $\alpha_v\beta_3$ integrin.[23] However, no further studies have been published on the role of this mediator in either physiologic or pathologic liver angiogenesis. As we will discuss, a more interesting role is attributed to the antiangiogenic protein vasohibin, which may have a dual role in inhibiting not only angiogenesis but also fibrogenesis, likely by deactivating HSCs.[24]

## Hypoxia, Pathologic Angiogenesis, and Liver Fibrogenesis: Interconnected Events

Angiogenesis is best defined as a dynamic, hypoxia-stimulated, and growth factor-dependent process leading to the formation of new blood vessels from preexisting ones.[25,26] Hypoxia and HIFs are critical in sustaining the fibrogenic progression of CLDs, representing a persistent driving force able to directly affect the behavior of hepatic cell populations, including profibrogenic and proangiogenic MFs.[1–5,10–13,16]

Detection of hypoxic areas is a common finding at any stage of CLD, increasing progressively from early injury to the development of cirrhosis, with hypoxia and HIFs serving throughout as proangiogenic stimuli in the overall, etiology-independent scenario of chronic wound healing. CLD progression itself is a major contributor to hypoxia due to the formation of regenerative nodules of parenchyma surrounded by evolving fibrotic septa and vascular remodeling that, along with progressive capillarization of the sinusoids, leads to an impairment of oxygen diffusion. A vicious circle between fibrosis and pathologic angiogenesis is likely to occur[10,12,27] in which parenchymal hypoxia, through the action of HIFs, up-regulates expression of wound healing-related factors and mediators that should facilitate liver repair and revascularization. However, pathologic angiogenesis can be inefficient due to the immaturity and permeability of VEGF-induced neovessels and, as a result, may be unable to correct liver hypoxia. Pathologic angiogenesis and hypoxia may act synergistically in disrupting normal tissue repair, thereby promoting the development of liver fibrosis.[27]

The connection between pathologic angiogenesis and fibrogenesis in progressive CLDs is strongly suggested by their parallel development in all major human forms of CLD and animal models of CLDs, with several laboratories describing high numbers of endothelial cells and microvascular structures within fibrotic septae and in expanded portal areas.[1–5,10–13,16,28–30] The response to hypoxia and VEGF (the major proangiogenic target gene) expression can be seen in liver sinusoidal endothelial cells (LSECs) and in hepatocytes[1,3,4,10,28] and HSC/MFs of developing septa and at the borders of more mature and larger fibrotic septa.[29,30]

Studies performed in HIF-1$\alpha$ liver conditional knockout mice provided the first definitive in vivo evidence for hypoxia-dependent induction of fibrogenesis.[31] However, the most convincing evidence relating angiogenesis and fibrogenesis came through in vivo studies indicating that experimental antiangiogenic therapy was highly effective in significantly reducing fibrogenic progression. As summarized in Table 1, whatever the specific molecule or tool employed, experimental antiangiogenic therapy always resulted in a significant reduction not only of angiogenesis but also of the inflammatory infiltrate, the number of $\alpha$-smooth muscle actin ($\alpha$-SMA)-positive MFs and the extent of fibrosis.[32–42] In some studies, experimental antiangiogenic therapy also resulted in a significant reduction of portal pressure and collateral vessel formation, as reported with sorafenib in cirrhotic animals[34,43] and with different molecules and tools in portal hypertensive animals.[9,24,44–49]

To our knowledge, only two studies have been published that represent exceptions to this general finding. The first one was published in 2009 by Patsenker et al,[50] who reported that pharmacologic inhibition of integrin $\alpha_v\beta_3$ by cilengitide, although able to suppress liver angiogenesis,