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AT-A-GLANCE ARTICLE

Portal myofibroblasts connect angiogenesis and fibrosis in liver

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Abstract Liver fibrogenesis is a dynamic process including quantitative and qualitative changes of the extracellular matrix, of which the most prominent is the deposition of type I collagen. These changes progressively disrupt normal liver architecture and result in cirrhosis formation. In the fibrotic liver, as in all other fibrotic tissues, the extracellular matrix is produced by cells usually characterized by the de novo expression of alpha-smooth muscle actin and known as myofibroblasts. Portal myofibroblasts (PMFs) appear to be critical in pathological angiogenesis, which constantly occurs in advanced liver fibrosis. Whereas the association between angiogenesis and fibrosis during the progression of liver diseases remains to be elucidated, we suggest that collagen-type-XV-alpha1-producing PMFs could provide an important link both by stabilizing newly formed vessels and by forming a scaffold for the deposition of interstitial collagen.

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Abbreviations

α-SMA	Alpha-smooth muscle actin
BDL	Bile duct ligation
COL15A1	Collagen type XV alpha1
COX-2	Cyclooxygenase-2
HSC	Hepatic stellate cell
HSC-MF	Hepatic-stellate-cell-derived myofibroblast
MCD	Methionine-choline-deficient
MMP	Matrix metalloproteinase
NAFLD	Non-alcoholic fatty liver disease
PDGF-BB	Platelet-derived growth factor-BB
PDGFR-β	Platelet-derived growth factor receptor-beta
PlGF	Placental growth factor
PMF	Portal myofibroblast
TAA	Thioacetamide
TGF-β	Transforming growth factor-beta
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

Introduction

The incidence of chronic liver diseases keeps growing worldwide, largely because of the epidemic of metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). To a great extent, the prognosis of chronic liver diseases is determined by the development of liver fibrosis. However, no anti-fibrotic drug that would prevent the progression of liver fibrosis towards cirrhosis is yet available and a better understanding of fibrogenesis in the liver is still needed. Liver fibrogenesis is a dynamic process including quantitative and qualitative

changes of the extracellular matrix, of which the most prominent is the deposition of type I collagen. These changes progressively disrupt normal liver architecture and result in cirrhosis formation. In the fibrotic liver, as in all other fibrotic tissues, the extracellular matrix is produced by cells that are usually characterized by the de novo expression of alpha-smooth muscle actin (α-SMA) and that are known as myofibroblasts.

Liver myofibroblasts

Myofibroblasts form heterogeneous populations of cells, with different possible origins. Current evidence indicates that, in the liver, myofibroblasts are mainly derived from hepatic stellate cells (HSCs; Mederacke, et al. 2013). However, cells that are distinct from HSCs and located in the portal tract can also give rise to myofibroblasts that we now globally refer to as portal myofibroblasts (PMFs; Lemoinne et al. 2015; Lua et al. 2016). PMFs were first described in the setting of biliary-type liver fibrosis such as that induced by bile duct ligation (BDL) in rats or mice (Kinnman et al. 2003). PMFs outnumber hepatic-stellate-cell-derived myofibroblasts (HSC-MFs) at the onset of biliary-type liver injury (Beaussier et al. 2007) contributing by more than 70 % to liver myofibroblasts at 5 days after BDL (Iwaisako et al. 2014). Simultaneously, in sinusoids, HSCs undergo phenotypic changes including the overexpression of desmin and of platelet-derived growth factor receptor-beta (PDGFR-β) and increased DNA synthesis but yet without fully converting into myofibroblasts at this stage (Beaussier et al. 2007). Studies of PMFs in culture have demonstrated that they require transforming growth factor-beta (TGF-β) and that they are also dependent on mechanical tension for myofibroblastic differentiation (Li et al. 2007), a typical feature of myofibroblasts (Eyden 2008). In vivo, the emergence and expansion of PMFs can be triggered by interactions with ductular epithelial cells, which proliferate after bile duct injury in the so-called ductular reaction. Thus, a marked increase in the expression of αvβ6 integrin on the surface of ductular epithelial cells is induced, in mice, by acute bile duct obstruction, which directly triggers the periductal accumulation of myofibroblasts and fibrosis, through the activation of TGF-β (Wang et al. 2007). In human liver tissues, the expression of αvβ6 in ductular epithelial cells has also been found to be increased in acute but not chronic, biliary-type injury (Wang et al. 2007). Reactive ductules also express profibrogenic factors such as TGF-β itself or platelet-derived growth factor-BB (PDGF-BB), which also stimulates PMF expansion (Kinnman et al. 2003). Nevertheless, the biology of PMFs remains poorly known, mainly because of the lack of markers that would allow investigators to authenticate and distinguish them from HSC-MFs in the injured liver. In a majority of studies, HSC-MFs have been identified on the unique basis of their expression of α-SMA, one of the many markers that they have in common with PMFs. Therefore, the contribution of PMFs might have been underestimated and some of their properties improperly allocated to HSC-MFs. By comparing the transcriptome of PMFs and HSC-MFs, we have identified a marker of PMFs, almost not expressed in HSC-MFs or in any other liver cell type, i.e., collagen type XV alpha1 (COL15A1; Lemoinne et al. 2015) and this result has subsequently been confirmed by another group (Lua et al. 2016). We have shown that, both in animal models and in patients with chronic liver diseases, a marked increase in the hepatic expression of COL15A1 occurs at the time of progression to advanced liver fibrosis (Lemoinne et al. 2015). This is the case not only in biliary-type liver fibrosis (i.e., bile-duct-ligated rats and patients with primary biliary cholangitis) but also in post-necrotic liver fibrosis (i.e., carbon-tetrachloride-treated rats and patients with NAFLD), suggesting that PMFs or at least sub-populations of PMFs are involved in the progression of all types of liver fibrosis. In addition to α-SMA expression, myofibroblasts are characterized by ultrastructural features including a prominent rough endoplasmic reticulum, a Golgi apparatus producing collagen secretory granules and peripheral myofilaments (Eyden 2008). Yet another highly characteristic trait is a cell-to-matrix junction that consists in an aligned myofilament bundle and a fibronectin fibril contacting one another through a point at the cell surface termed a fibronexus (Eyden 2008). Of particular interest in this respect, we have found, in our previous comparative analyses, that PMFs compared with HSC-MFs express fibronectin at higher levels and virtually no desmin, both features belonging to the definition of myofibroblasts (Eyden 2008). Therefore, PMFs fulfil more criteria that define myofibroblasts than do HSC-MFs (Eyden 2008).

Liver angiogenesis

Angiogenesis is a dynamic process leading to the formation of news vessels from pre-existing vessels. In all tissues, angiogenesis is determined by two main pathways, i.e., hypoxia and inflammation and comprises the following steps: (1) sprouting and budding of endothelial cells; (2) degradation of the extracellular matrix by proteinases and migration of endothelial cells; (3) endothelial cell proliferation, tube formation and branching; and (4) vessel maintenance, maturation and stabilization (Elpek 2015). Vascular endothelial growth factor (VEGF) signaling has been implicated in virtually all steps of angiogenesis, whereas the recruitment of mural pericyte-like cells is required in the last step for nascent vessels to mature and stabilize. As is now well established, liver disease progression is accompanied by angiogenesis. The first demonstration was provided more than thirty years ago by Rappaport et al. (1983) who showed that human cirrhotic

livers contained more vessels than healthy livers and that cirrhotic nodules were surrounded by a dense vascular plexus. Liver tissue hypoxia occurs at early stages of liver injury and increases with disease progression as a result of the structural and functional changes in the liver angioarchitecture (Corpechot et al. 2002). During liver fibrogenesis, fibrillar type I collagen progressively replaces type IV collagen in the perisinusoidal space of Disse; this replacement, together with the loss of endothelial fenestrations, causes sinusoidal capillarization. Therefore, fibrosis by itself can contribute to the development of hypoxia and, thereby, can promote angiogenesis. Vascular remodeling leading to the capillarization of the sinusoids and the generation of intrahepatic shunts characterizes hepatic angiogenesis. Such changes in angioarchitecture cause a decrease in hepatocyte perfusion that aggravates hypoxia. Liver angiogenesis is also stimulated by inflammation. The chemokine-dependent accumulation of monocyte-derived macrophages is an important mechanism of hepatic inflammation and fibrogenesis in human liver diseases and experimental mouse models. The chemokine receptor CCR2 and its ligand CCL2 (MCP-1) promote the accumulation of monocyte-derived macrophages releasing pro-inflammatory and pro-angiogenic cytokines in the liver. Infiltrating CCL2-dependent inflammatory monocytes also provide pro-angiogenic signals via the production of VEGF-A and matrix metalloproteinase-9 (MMP-9; Ehling et al. 2014). Three-dimensional micromorphological analyses in mouse models of carbon-tetrachloride- or BDL-induced liver injury have demonstrated that macrophage-dependent angiogenesis during chronic liver injury is largely confined to portal veins and that the pharmacological inhibition of CCL2-mediated inflammatory monocyte infiltration primarily reduces angiogenic vessel sprouting in the portal vein (Ehling et al. 2014). Therefore, infiltrating bone-marrow-derived inflammatory monocytes possibly mediate the induction of hepatic angiogenesis by effects that are primarily attributable to changes in the portal vein system.

Angiogenesis and fibrosis progression are closely correlated in experimental liver injury and human liver disease. Angiogenesis and fibrogenesis are also triggered by similar pathways in response to hypoxia and inflammation, so that a potential causal relationship between them has been difficult to establish. In addition, dichotomous effects of angiogenesis on fibrosis have now been reported in various tissues. The administration of VEGF, while stimulating angiogenesis, has been shown to reduce renal fibrosis and to stabilize renal function in the remnant kidney model of progressive renal failure (Kang et al. 2001). Proangiogenic activity induced in an adipocyte-specific VEGF-A overexpression model has been shown to suppress fibrosis, inflammation and insulin resistance during the early phase of high-fat-diet-induced adipose tissue expansion. Conversely, VEGF-A-VEGFR2 blockade has an aggravating effect in this context, whereas in *ob/ob* mice with pre-existing adipose tissue dysfunction, the anti-angiogenic action of VEGF-A-VEGFR2 blockade causes an improvement in metabolism and a decrease in inflammatory factors (Sun et al. 2012). Many studies in animal models of liver injury have shown that the inhibition of angiogenesis results in a decrease in liver fibrosis (Table 1). However, the pathways targeted in these studies might also have promoted fibrosis directly. In addition, a few studies have led to different conclusions. Thus, pharmacological inhibition of the vitronectin receptor integrin alphavbeta3, which stimulates endothelial cell proliferation and HSC activation, while suppressing hepatic angiogenesis, aggravates liver fibrosis induced by BDL or thioacetamide (TAA) (Patsenker et al. 2009). Pharmacological inhibition of the chemokine CCL2 reduces monocyte infiltration and angiogenesis but not fibrosis progression in mouse models of carbon tetrachloride or BDL-induced liver injury (Ehling et al. 2014). Dichotomous effects of angiogenesis have also been reported in a model of fibrosis resolution generated by cholecystojejunostomy, which restores bile flow after BDL (L. Yang et al. 2014). In this model, VEGF-induced angiogenesis promotes fibrogenesis after BDL but is also required for fibrosis resolution after cholecystojejunostomy. Accordingly, VEGF-neutralizing antibodies not only prevent the development of fibrosis but also disrupt hepatic tissue repair and fibrosis resolution (L. Yang et al. 2014).

Contribution of PMFs to liver angiogenesis

HSC-MFs and PMFs probably both contribute to liver angiogenesis, although at different stages. HSCs in their quiescent state act as pericytes that regulate the functions of sinusoidal endothelial cells. Following myofibroblastic differentiation, they acquire a proangiogenic phenotype and secrete proangiogenic factors such as angiopoietin-1 (Semela et al. 2008; Thabut et al. 2011). At an early stage of liver injury, HSC-MFs promote an enhanced coverage of sinusoids and angiogenesis in areas of active fibrogenesis at the leading edge of developing fibrotic septa (Novo et al. 2007). At later stages, endothelial cell proliferation is correlated with the expansion of PMFs suggesting a role for PMF in liver angiogenesis (Lemoinne et al. 2015). Further evidence that this is the case has been provided by the immunostaining of human cirrhotic livers showing that COL15A1-positive PMFs display a perivascular distribution and outline vascular capillaries within large fibrotic septa. Using a cell model of PMFs that we have previously described in detail, we have demonstrated that PMFs are able to enhance angiogenesis in vitro and in vivo by various mechanisms including the formation of direct intercellular junctions with endothelial cells and the production of VEGF-A-containing microparticles (Lemoinne et al. 2015). Compared with HSC-MFs, PMFs largely overexpress COL15A1 and also COL18A1, which are the α1 chains of collagen XV and collagen XVIII, respectively. Both collagens

Table 1 Effects of angiogenesis inhibition on liver fibrosis in experimental models (*CCl_4* carbon tetrachloride, *BDL* bile duct ligation, *Ab* antibody, *AdsTie2* adenovirus expressing the extracellular domain of Tie2, *PlGF* placental growth factor, *COX-2* cyclooxygenase-2, *MCD* methionine-choline-deficient, *TAA* thioacetamide, *TK* tyrosine kinase, *VEGFR* vascular endothelial growth factor receptor)

Reference	Animal model	Anti-angiogenic treatment	Assay (change in liver fibrosis)
Yoshiji et al. 2003	Mouse - CCl_4	Anti-VEGFR-1 Ab Anti-VEGFR-2 Ab	Hydroxyproline ↘
Tugues et al. 2007	Rat - CCl_4	Sunitinib (multiple TK inhibitor)	Masson trichrome staining ↘
Taura et al. 2008	Mouse - CCl_4, BDL	Blockade of angiopoietin signaling by AdsTie2	Hydroxyproline, Sirius red staining ↘
Mejias et al. 2009	Rat - BDL	Sorafenib (multiple TK inhibitor)	Sirius red staining ↘
Patsenker et al. 2009	Rat - BDL, TAA	Cilenglinide ($\alpha v\beta 3/\alpha v\beta 5$ integrin inhibitor)	Hydroxyproline, Sirius red staining ↗
Thabut et al. 2011	Rat - BDL	Sorafenib	Magnetic resonance elastography, Sirius red staining ↘
Van Steenkiste et al. 2011	Mouse - CCl_4	Anti-PlGF Ab	Sirius red staining ↘
Hennenberg et al. 2011	Rat - BDL	Sorafenib	Sirius red staining ↘
May et al. 2011	Mouse	Blockade of VEGF signalling by transgenic conditional induction of a VEGF decoy receptor	Goldner staining ↗
Sahin et al. 2012	Mouse - CCl_4	CxCl9 (angiostatic chemokine)	Hydroxyproline, Sirius red staining ↘
Huang et al. 2013	Rat - CCl_4	Bevacizumab (anti-VEGF Ab)	Hydroxyproline, Sirius red staining ↘
Gao et al. 2013	Rat - TAA	Celecoxib (COX-2 inhibitor)	Masson trichrome staining ↘
Y.Y. Yang et al. 2014	Rat - High-fat/MCD	Sorafenib, Brivanib (muliple TK inhibitors)	Hydroxyproline ↘
L. Yang et al. 2014	Mouse BDL BDL followed by cholecystojejunostomy	Anti-VEGF Ab	Hydroxyproline, Sirius red staining ↘↗
Ehling et al. 2014	Mouse - CCl_4, BDL	CCl2 pharmacological inhibitor	Sirius red staining →
Liu et al. 2015	Rat - dimethylnitrosamine	Sorafenib + gadolinium chloride	Hydroxyproline, Sirius red staining ↘
Yan et al. 2015	Mouse - CCl_4	Anti-CD147 Ab	Hydroxyproline, Sirius red staining ↘

belong to the superfamily of multiplexins and their C-terminal parts, endostatin and restin, respectively, are anti-angiogenic, which could provide a negative retrocontrol in PMF-induced angiogenesis. Collagen XV provides stability and resilience to mechanical forces in the skeletal muscle and microvessels. It is contained in the basement membrane of continuous capillaries and serves as a scaffold that anchors the basement membrane to interstitial collagen; loss of its expression results in collapsed capillaries in mice. It is absent from specialized capillaries such as fenestrated liver sinusoids. The normal liver contains almost no collagen XV, with the exception of trace amounts in the portal and periportal area. Collagen XV forms a proangiogenic matrix for endothelial cells and thereby might contribute to the angiogenic properties of PMFs. Increased expression of collagen XV has been reported in hepatocellular carcinogenesis in mice and humans. In human hepatocellular carcinoma, collagen XV has

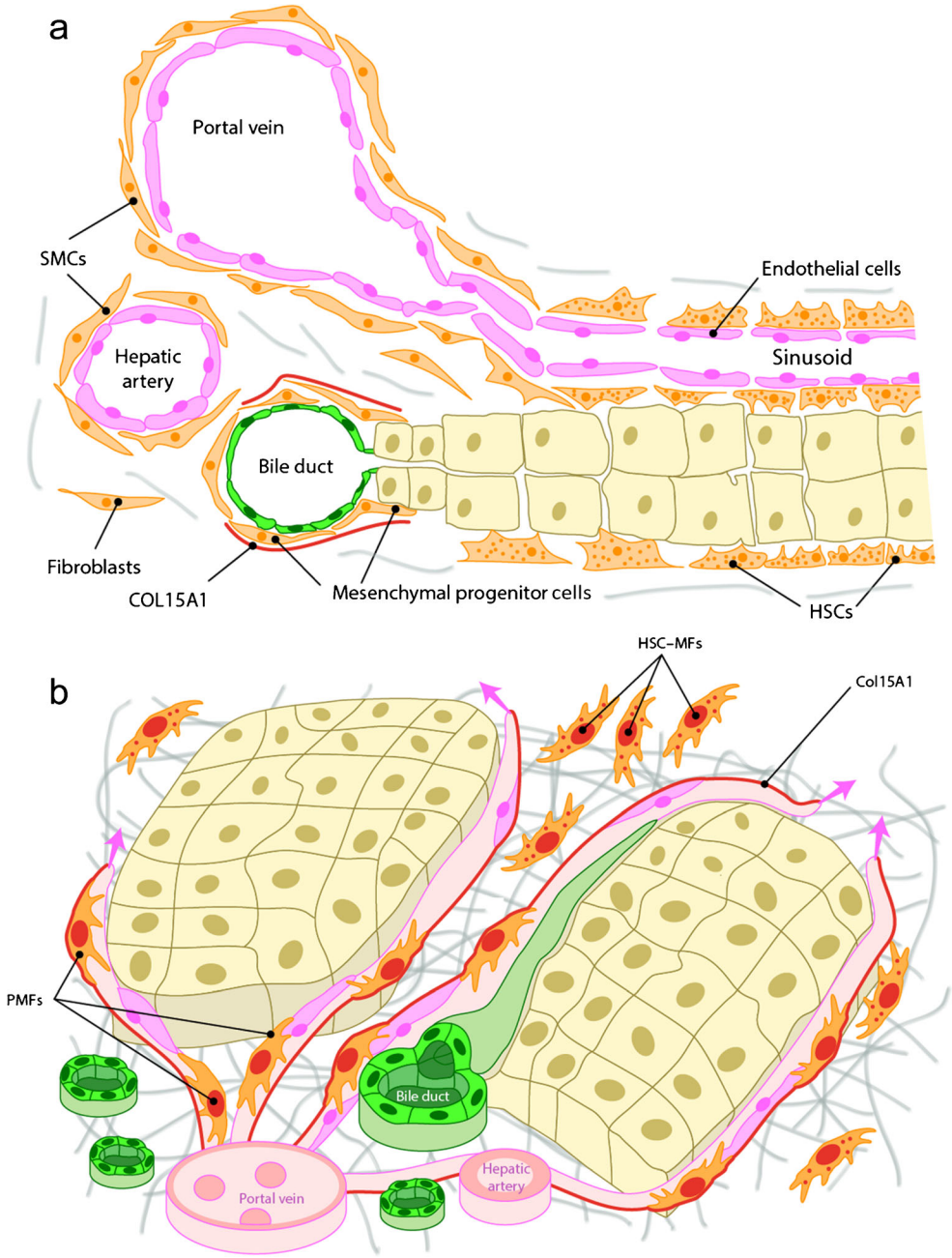

Fig. 1 Role of portal myofibroblasts in liver angiogenesis and fibrosis. **a** In normal liver, quiescent hepatic stellate cells (*HSCs*) form a continuum with portal mesenchymal cells, which include vascular smooth muscle cells (*SMCs*), portal fibroblasts and a few mesenchymal progenitor cells. The extracellular matrix is poorly abundant and contains trace amounts of collagen type XV alpha1 (*COL15A1*) along mesenchymal progenitors. **b** In advanced fibrosis, portal myofibroblasts (*PMFs*) proliferate and promote angiogenesis. COL15A1 expressed by PMFs provides a scaffold for interstitial collagen produced in excess mostly by hepatic stellate cell-derived myofibroblats (*HSC-MFs*)

been identified as a prominent histopathological component of intratumoral capillaries (Kimura et al. 2016).

Concluding remarks

In summary, PMFs appear to be critical in pathological angiogenesis, which constantly occurs in advanced liver fibrosis. Although the way that angiogenesis and fibrosis are linked to each other during the progression of liver diseases remains to be elucidated, we suggest that COL15A1-producing PMFs provide an important link both by stabilizing newly formed vessels and by forming a scaffold for the deposition of interstitial collagen (Fig. 1).

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