

Contribution of Myfibroblasts of Different Origins to Liver Fibrosis

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Abstract The most common cause of liver failure is cirrhosis, due to progressive liver fibrosis and other architectural changes in the liver. Fibrosis occurs after liver injury or stress and results directly from an imbalance between the processes of extracellular matrix synthesis (fibrogenesis) and degradation (fibrolysis). Although research studies have identified several promising targets at the molecular level, current therapies to prevent and treat hepatic fibrosis in patients have only shown limited success. It is well established that liver myofibroblasts (MFs) are the primary effector cells responsible for the extensive extracellular matrix accumulation and scar formation observed during hepatic fibrosis, in both clinical and experimental settings. Thus, as the major fibrogenic cells implicated in wound healing and tissue repair response, liver MFs could represent excellent targets for antifibrotic therapies. Still, the exact nature and identities of liver MFs precursors have yet to be resolved, and their relative contribution to hepatic fibrosis to be determined. The goal of this review is to examine the relative importance of liver MF precursors in the pathogenesis of liver fibrosis.

Keywords Myofibroblasts · Hepatic stellate cells · Portal fibroblasts · Bone marrow · Liver mesothelial cells · Pathobiology

Introduction

Liver fibrosis is the common outcome of numerous chronic liver diseases with distinct etiologies, such as exposure to chemicals (e.g., alcohol liver disease, chronic drug toxicity), metabolic derangements (e.g., non-alcoholic steatohepatitis, Wilson disease, hemochromatosis), infectious diseases (e.g., viral hepatitis, schistosomiasis), and autoimmunity (e.g., primary biliary cirrhosis, autoimmune hepatitis) [1]. Liver fibrosis, via evolution to cirrhosis, is a pathophysiological condition that is associated with hepatic failure and portal hypertension. Notably, deposition of extracellular matrix fibers in the form of scar formation is the hallmark of liver fibrosis and represents an important determinant of disease progression [2]. Myofibroblasts (MFs) are the major effector cells during the development of liver fibrosis [1, 3, 4]. These multifunctional cells exhibit specific features: (1) high proliferation rate, through response to growth factors; (2) fibrogenicity, through production and release of connective tissue proteins in the extracellular environment and secretion of proteases and their natural inhibitors involved in matrix remodeling; (3) contractility, through the expression of intracellular contractile filaments and enhanced responsiveness to vasoactive molecules; (4) and immuno-modulatory properties, through the secretion and signal transduction of potent inflammatory cytokines, chemoattractants, growth factors and other bioactive mediators. Essentially absent from the healthy liver, MFs rapidly accumulate in the injured liver, following differentiation from distinct cellular sources of both intra- and extrahepatic origins [5]. Until now, hepatic stellate cells (HSCs), portal fibroblasts (PFs), and mesothelial cells (MCs) have been identified as resident hepatic cell populations contributing to the generation of MFs during liver fibrosis. Interestingly, it has been suggested,

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although not without controversy, that liver epithelial cells (e.g., hepatocytes, cholangiocytes, and their progenitors) could similarly act as potential intrahepatic precursors that give rise to MFs through the so-called epithelial-to-mesenchymal transition process during liver fibrosis. Moreover, extrahepatic bone marrow-derived fibrocytes and mesenchymal stem cells have been more recently shown to migrate and differentiate into hepatic MFs during liver fibrosis. Here, we review some aspects of the current knowledge regarding the distinct liver MFs precursors and evaluate their contribution to the liver fibrosis.

Major Cellular Sources of Myofibroblasts During Liver Fibrosis

Hepatic Stellate Cells

Hepatic stellate cells are vitamin A-rich, lipid-storing, spindle-shaped perisinusoidal cells located in the space of Disse, in the normal liver [6–8]. Quiescent HSCs express a variety of neuronal cell markers, such as glial fibrillary acidic protein (GFAP) [9, 10], synaptophysin [11], and neurotrophin receptor p75NTR [12], desmin intermediate filaments [10], all three transforming growth factor- β (TGF- β) receptors T β RI, -II, -III [13], cellular retinol-binding protein-1 (CRBP-1) [14], CD105/endoglin [15] and CD146 molecules [16]. At steady state, resting HSCs are known to mediate metabolic (e.g., retinoid homeostasis) and immune (e.g., antigen presentation and phagocytosis) functions [13, 17]. In contrast, in the fibrosing liver, HSCs undergo a process of “activation” and differentiate into MFs, while migrating to tissue lesion sites [3, 5, 8, 13]. This activation process is largely triggered by damage to parenchymal cells (hepatocytes), enhanced by numerous stimuli of a biological (e.g., cytokine, endotoxin, cell apoptotic bodies) and/or environmental (e.g., matrix rigidity, tissue oxygen deprivation) nature, and involves concomitant morphological and functional changes in MFs [8, 18]. Liver MFs originating from activated HSCs are established as the dominant fibrogenic cell type during non-biliary liver fibrosis, in both experimental (chronic carbon tetrachloride/CCl₄ and/or dimethylnitrosamine intoxication murine models) and clinical (human liver fibrosis, cirrhosis) settings [8, 19]. The typical HSC-derived MFs exhibit high proliferative capacity, upregulate the expression of mesenchymal markers, such as type 1 collagen and fibronectin extracellular matrix (ECM) proteins [20], α -smooth muscle actin (α -SMA) intracellular contractile filaments, desmin and vimentin intermediate filaments [20, 21], of matrix-metalloproteinases (MMP)-2, -9 and -14 [19, 22], tissue inhibitor of metalloproteinase (TIMP)-1 [19, 22], of collagen cross-linking lysyl oxidase

(LOX) protein [23], of purinergic CD39L1/NTPDase2 [24, 25] and glycosylphosphatidylinositol (GPI)-anchored CD73/ecto-5'-ectonucleotidase ecto-enzymes [26, 27], of platelet-derived growth factor (PDGF) β -receptor subunit (PDGF β R) [8], and of the reactive oxygen species scavenger cytoglobin [28, 29]. HSC-derived MFs participate in the regulation of critical inflammatory processes, not only by secreting, but also by transducing signals from key fibrogenic cytokines, chemokines and growth factors, in autocrine and paracrine fashions [3, 8, 30]. Indeed, critical functions of HSC-derived MFs are modulated by a plethora of multipotent stimuli, including PDGF-BB (e.g., proliferation, chemotaxis), vascular endothelial growth factor A (e.g., proliferation, chemotaxis), angiotensin II (AT-II, e.g., proliferation, chemotaxis, contraction), monocyte chemoattractant protein-1 (MCP-1, e.g., chemotaxis), endothelin-1 (ET-1, e.g., proliferation, contraction) and TGF- β (e.g., proliferation), among others [3, 8, 30]. HSC-derived MFs consequently play a significant role in scar formation and portal hypertension, owing to their matrix-producing/remodeling properties [31], contractile phenotype [21] and responsiveness to vasoactive molecules, such as ET-1 and AT-II [31–33]. Moreover, HSC-derived MFs have been shown lately to support extramedullary hematopoiesis functions, acting as intrahepatic “feeder” cells, i.e., liver resident mesenchymal stem cells [16]. Interestingly, fully-activated HSC-derived MFs do not necessarily exhibit all the above-described features during the development of liver disease, suggesting that MFs are heterogeneous and/or asynchronous in terms of activation [34]. This newly appreciated heterogeneity adds degrees of complexity when assessing their roles as mediators of liver fibrosis.

Portal Fibroblasts

Portal fibroblasts are spindle-shaped peribiliary mesenchymal cells located in the connective tissue surrounding portal tracts, in the normal liver [18, 35]. Quiescent PFs express cell markers including elastin intracellular contractile filaments [25], GPI-anchored CD90/Thy1.1 molecule [36], CD39L1/NTPDase2 ecto-enzyme [24, 37], all three TGF- β receptors T β RI, -II, -III [38], and interleukin-6 [35]. Unlike HSCs, PFs do not store retinoids, and lack expression of α -SMA, desmin and GFAP filaments, as well as of markers CD146 and CRBP-1 proteins [14, 39]. In resting state, the main functions attributed to PFs involve ECM turnover [40] and regulation of bile duct epithelium (BDE) cell mass, through constitutive NTPDase2 expression and modulation of peribiliary nucleotide-dependent mitogenic signals [37]. In the fibrosing liver, akin to HSCs, PFs undergo myofibroblastic activation in response to tissue injury. Proliferation of fibrogenic PF-derived MFs primarily occurs in liver diseases associated with

cholestasis and/or ductular reaction, in which the portal area is considered the initial injury site [10, 40, 41]. Hence, in a cholestatic liver injury setting, activated PFs sensing cellular damage would act as “first responders” to initiate the wound healing and tissue repair response [42]. Liver MFs originating from activated PFs likely predominate in experimental liver fibrosis due to bile duct injury, as in bile duct ligation (BDL) murine models, and human cholangiopathies, such as primary biliary cirrhosis, primary sclerosing cholangitis, and cystic fibrosis hepatopathy [35]; however, these cells are probably expanded in all forms of fibrotic liver injury [43]. The typical PF-derived MFs exhibit high proliferative capacity [28•, 38], express de novo both α -SMA intracellular contractile fibers [28•, 44], and TE-7 fibroblast marker [35], upregulate the expression of type 1 collagen [27, 28•, 44], fibronectin [27, 45], and fibulin-2 ECM components [27, 45], of LOX protein [23], and of CD73 ecto-enzyme [26, 27], while specifically down-regulating expression of NTPDase2 ecto-enzyme [25]. In functional terms, little is known about PF-derived MFs functions during liver fibrosis. Besides their established role in scar formation through ECM deposition [18, 35], PF-derived MFs may mediate bile ductular reaction in vivo, as these cells have been shown to promote unchecked BDE proliferation in vitro, through down-regulation of NTPDase2 expression and subsequent activation of mitogenic ATP-binding P2Y receptors [37]. It is likely that PF-derived MFs are a key component of the “ductular reaction”, although this hypothesis has not been fully tested.

Phenotypical Transition to Mesenchymal Cell Type

Epithelial to Mesenchymal Transition

Epithelial-to-mesenchymal transition (EMT) is a well-established process by which fully-differentiated polarized cells of epithelial origin undergo phenotypic transition to fully-differentiated, non-polarized, highly-motile myo/fibroblasts upon organ injury [46]. This cellular conversion process is accompanied by important gene expression changes, i.e., down-regulation of epithelial markers, such as intermediate cytokeratin filaments, cell adhesion E-cadherin molecules and tight junction *zona occludens* 1 (ZO-1) proteins, and up-regulation of mesenchymal markers, such as α -SMA intracellular contractile fibers, vimentin intermediate filaments and fibronectin ECM proteins [47]. In functional terms, given that EMT contributes to fibrogenesis in other tissues, such as kidney, heart and lungs [48], EMT has eventually been considered and proposed as an additional cellular source of matrix-producing fibroblasts and MFs during liver fibrosis, based on in vitro studies and clinical

observations [49–52]. However, recent genetic fate mapping studies have challenged that notion [53]. For instance, it was demonstrated that the fibroblast specific protein-1 (FSP-1)/S1004 is not a universal “EMT-derived” fibroblast marker and actually identifies a CD45⁺, CD11b⁺, CD11c⁺, F4/80⁺ myelomonocytic cell population expanding during experimental liver fibrosis in reporter mice [54]. Moreover, genetic labeling of hepatocytes (Albumin-Cre \times ROSA26-stop- β Gal \times Col1 α 1-GFP reporter mice, CCl₄ model) [55••], cholangiocytes (cytokeratin 19-CreERT \times ROSA26-stop-YFP mice, CCl₄ and BDL models) [56••] and their bipotential epithelial progenitors (Alfp-Cre mice \times ROSA26-stop-YFP mice, CCl₄ and BDL models) [57••] did not show any evidence of EMT upon experimental liver fibrosis. Hence, while the prospect of EMT occurrence in human liver diseases is not totally excluded [58, 59], these studies clearly question the existence and functional importance of this process in the pathogenesis of liver fibrosis in experimental settings [5, 39]. At present, one must view the pathophysiological role of such cells in liver fibrosis as an open question, which is nevertheless highly relevant and exciting. On the other hand, EMT-derived liver MFs may be of particular importance in the desmoplastic reaction observed in hepatobiliary cancers [60].

Mesothelial-to-Mesenchymal Transition

Recently, the newly-described process of mesothelial-to-mesenchymal transition (MMT) has been shown to contribute to the progression of liver fibrosis [61••]. Mesothelial cells are peripheral epithelial cells covering the capsule of Glisson, in the normal liver [62]. Quiescent MCs harbor an intermediate phenotype by expressing mesothelial markers, such as CD200/OX-2 molecule, podoplanin glycoprotein, Wilms tumor 1 (Wt1) protein and neuronal glycoprotein M6-a (Gpm6a), epithelial markers, such as keratin 8 intermediate filaments, gap junction connexin 43 protein and ZO-1 tight junction protein, and mesenchymal markers, such as vimentin intermediate filaments [61••]. The role of liver MCs at homeostasis is, however, poorly understood. The hepatic localization of MCs would suggest their involvement in the regulation of solutes and fluids transport/movement, leucocyte migration, and antigen presentation at the liver/peritoneum interface [63]. Following experimental liver injury (CCl₄ and BDL mouse models), hepatic MCs have been also shown to undergo a process of “activation”, in which they transition to a cell type expressing mesenchymal markers, such as α -SMA intracellular contractile filaments, desmin intermediate filaments and/or type 1 collagen that are generally observed in HSC-derived MFs [61••]. Moreover, this particular cell activation phenomenon could be recapitulated in vitro in isolated MCs, following culture-activation. Taken together,

these observations strongly suggest that hepatic MCs represent a novel endogenous source of fibrogenic MFs during liver fibrosis. However, further studies are needed to evaluate the physiological relevance of MMT in the context of liver fibrosis. Again, this is an exciting, but preliminary, area of liver disease research.

Bone Marrow-Derived Fibrocytes and Stem Cells

Fibrocytes are circulating spindle-shaped leucocytes originating from the bone marrow that can differentiate into MFs in several organs including the liver [20, 43, 64]. Upon injury, fibrocytes proliferate and migrate to the lesion sites in the affected organs, to mediate wound healing and tissue repair response [20, 43, 64]. Fibrocytes express mesenchymal markers, such as type 1 collagen and fibronectin ECM constituents [64], vimentin intracellular filaments [64], lymphoid markers, such as CD34, CD45, MHC I and MHC II molecules, in addition to myeloid markers, such as CD11b and Gr-1/Ly-6G molecules, and secrete fibrogenic growth factors, such as TGF- β and MCP-1 [3, 20]. In experimental liver fibrosis (CCl₄ and BDL models), it has been reported that recruited fibrocytes could account for up to 5 % of hepatic collagen-producing cells, suggesting their functional involvement in the pathogenesis of liver fibrosis [65, 66]. In addition to fibrocytes, bone marrow-derived mesenchymal stem cells (BM-MSCs) have also been shown to differentiate into hepatic MFs [67]. BM-MSCs are multipotent stromal progenitors that express

CD146 and GPI-anchored Sca-1/Ly6A/E molecules, but no common hematopoietic markers, such as CD34, CD45, and CD133 molecules [5]. BM-MSC-derived MFs have been shown to contribute to the post-injury fibrogenesis process in the liver [68]. Although both bone marrow-derived fibrocytes and MSCs have been identified as extrahepatic precursors of functional (i.e., collagen-producing) liver MFs, the significance of their overall contribution to the pathogenesis of liver fibrosis remains to be determined.

Conclusion

Hepatic MFs are the dominant contributors in the process of wound healing and tissue repair following liver injury experimentally and clinically [3, 8, 19, 69]. It is now clear that hepatic MFs originate from various cellular sources that are endogenous (resident) and/or exogenous (infiltrating) to the liver (see Table 1). Activated HSCs, portal fibroblasts, and recently-described mesothelial cells are established as MFs precursors of intrahepatic origin [10, 24, 28, 61, 70], while bone marrow-derived fibrocytes and mesenchymal stem cells migrating to the liver, as precursors of extrahepatic origin [65, 67, 68, 71]. Activated HSCs and portal fibroblasts represent the *bona fide* sources of hepatic MFs in liver fibrosis, whereas the relative importance of liver MFs originating from bone marrow-derived cells, EMT, and MMT may vary greatly depending on disease etiology. The ongoing studies from a variety of research groups will hopefully provide novel answers to

Table 1 Distinct cellular sources of MFs

Origin/cell type	Precursor ^a	Mechanism	Evidences	Specific markers	References
Liver					
Hepatic stellate cells	Yes	Activation	Human fibrosis, experimental liver fibrosis (CCl ₄ , BDL)	Desmin, synaptophysin, GFAP, p75NTR, NTPDase2	[6, 7, 9–12, 24, 45, 70]
Portal fibroblasts	Yes	Activation	Human fibrosis, experimental liver fibrosis (BDL)	Elastin, IL-6, Thy-1, Fibulin-2, TE-7	[10, 24, 25, 36, 45, 70]
Hepatocytes	No	N/D	Experimental liver fibrosis (CCl ₄)	N/D	[56, 72]
Cholangiocytes	No	N/D	Experimental liver fibrosis (CCl ₄ , BDL)	N/D	[56, 72]
Mesothelial cells	Yes	Phenotypic transition (MMT)	Experimental liver fibrosis (CCl ₄)	Gpm6a, Wt1, Podoplanin, CD200	[61, 71]
Bone marrow					
Fibrocytes	Yes	Activation	Human fibrosis, experimental liver fibrosis (CCl ₄ , BDL)	CD45, CD11d, MHC class II	[64, 65, 68, 73]
Mesenchymal stem cells	Yes	Activation	Experimental liver fibrosis (CCl ₄)	N/D	[68, 71]

N/D non-determined

^a Precursor of cells identified as liver MFs via expression of α -SMA and/or type 1 collagen

these important questions, potentially providing new modalities to prevent and/or treat patients with liver fibrosis.

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Compliance with Ethics Guidelines This article does not contain any studies with human or animal subjects performed by any of the authors.

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