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Review article

The characteristics of activated portal fibroblasts/myofibroblasts in liver fibrosis

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ABSTRACT

Liver fibrosis results from chronic injury of hepatocytes and activation of Collagen Type I producing myofibroblasts that produce fibrous scar in liver fibrosis. Myofibroblasts are not present in the normal liver but rapidly appear early in experimental and clinical liver injury. The origin of the myofibroblast in liver fibrosis is still unresolved. The possibilities include activation of liver resident cells including portal fibroblasts, hepatic stellate cells, mesenchymal progenitor cells, and fibrocytes recruited from the bone marrow. It is considered that hepatic stellate cells and portal fibroblasts are the major source of hepatic myofibroblasts. In fact, the origin of myofibroblasts differs significantly for chronic liver diseases of different etiologies, such as cholestatic liver disease or hepatotoxic liver disease. Depending on etiology of hepatic injury, the fibrogenic foci might initiate within the hepatic lobule as seen in chronic hepatitis, or primarily affect the portal areas as in most biliary diseases. It has been suggested that **activated portal fibroblasts/myofibroblasts work as "myofibroblasts for cholangiocytes"** while hepatic stellate cells work as "myofibroblast for hepatocytes". This review will focus on our current understanding of the activated portal fibroblasts/myofibroblasts in cholestatic liver fibrosis.

Contents

Abbreviations: Col, collagen α1(I); α-SMA, α-smooth muscle actin; BDL, bile duct ligation; CCl_4, carbon tetrachloride; qHSCs, quiescent Hepatic Stellate Cells; aHSCs, activated Hepatic Stellate Cells; aPFs/myofibroblasts, activated portal fibroblasts /myofibroblasts; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TGF-β1, Transforming growth factor-β1; GFP, green fluorescent protein

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1. Introduction

Liver fibrosis and cirrhosis are the common outcomes of chronic liver diseases. Liver cirrhosis is characterized by the deposition of extracellular matrix proteins, composed mostly of Collagen Type I, formation of fibrous scar, and loss of liver function. There is no curative therapy for advanced liver cirrhosis, often liver transplantation is the only treatment available for these patients. Dependent on the etiology, liver fibrosis is caused by cholestatic liver injury (obstruction of biliary tract) such as primary sclerosing cholangitis, primary biliary cholangitis, secondary biliary cirrhosis and biliary atresia, or hepatotoxic injury (such as hepatitis B virus infection, hepatitis C virus infection, alcoholic liver disease and non-alcoholic steatohepatitis (NASH)). Despite the differences in etiology, development of liver fibrosis is associated with several early events that play an important role in the pathogenesis of liver fibrosis, including: 1) damage to hepatic epithelial (hepatocytes and cholangiocytes) and endothelial cells; 2) release of transforming growth factor-β1 (TGF-β1), the major fibrogenic cytokine; 3) increase of intestinal permeability and endogenous bacterial products; 4) recruitment of inflammatory cells; 5) induction of reactive oxygen species; and 6) generation of extracellular matrix producing myofibroblasts, which are not present in the normal liver. Hence, myofibroblasts represent a primary target for antifibrotic therapy.

Immunophenotypically, myofibroblasts are characterized by expression of abundant pericellular matrix proteins (vimentin, α-smooth muscle actin (α-SMA), non-muscle myosin, fibronectin) (Eyden, 2008). Ultrastructurally, myofibroblasts are identified by a rough endoplasmic reticulum, a Golgi apparatus producing collagen, peripheral myofilaments, fibronexus (no lamina), and gap junctions (Eyden, 2008). Studies of fibrogenesis conducted in different organs implicated myofibroblasts in wound healing and fibroproliferative disorders (Gabbiani et al., 1971; Majno et al., 1971; Schurch et al., 1998), suggesting that myofibroblasts are the primary source of extracellular matrix. Several sources of myofibroblasts have been identified in the liver: liver resident cells (Hepatic stellate cells, HSCs, and portal fibroblasts, PFs); cells originated by epithelial-to-mesenchymal transition (EMT) and bone marrow-derived cells (fibrocytes and mesenchymal cells) (Iwaisako et al., 2014; Kisseleva et al., 2006; Nitta et al., 2008; Scholten et al., 2011). Fibrocytes were shown to contribute to 3–6% of collagen Type I expressing cells in fibrotic liver, suggesting that fibrocytes are not a significant source of extracellular matrix (Kisseleva et al., 2006). Furthermore, the contribution of EMT to liver fibrosis is still controversial. Recently, Lua et al. reported that proliferating cholangiocytes in response to bile duct ligation express collagen, which means EMT of cholangiocytes (Lua et al., 2016). But several cell fate mapping of hepatic epithelial progenitors, hepatocytes, and cholangiocytes failed to detect the presence of EMT-derived myofibroblasts in the livers following cholestatic or hepatotoxic liver injury (Chu et al., 2011; Scholten et al., 2010; Taura et al., 2010).

Despite the extensive studies, there is still an ongoing discussion regarding which cell types can give rise to the hepatic myofibroblasts in response to chronic liver injury. Still "the primary suspects", as the major source, are the resident mesenchymal cells of the liver (Iwaisako et al., 2014; Mederacke et al., 2013; Wells, 2014); Hepatic Stellate Cells (HSCs) which have been extensively studied and aPFs/myofibroblasts which are less well characterized due to the difficulties in isolation and culturing. HSCs and aPFs/myofibroblasts have been reported to comprise $>90\%$ of the collagen expressing cells (Iwaisako et al., 2014; Kisseleva et al., 2006), suggesting that they are the major source of collagen expressing cells in fibrotic liver. HSCs are generally accepted as major contributors to liver fibrosis that give rise to hepatic myofibroblasts in response to toxic liver injury. aPFs/myofibroblasts, on the other hand, have been implicated in pathogenesis of cholestatic liver fibrosis (Iwaisako et al., 2014). While experimental data validates that both HSCs and PFs can activate into myofibroblasts, the contribution of aPFs /myofibroblasts versus aHSCs to cholestatic liver fibrosis remains controversial and requires thorough examination, including lineage tracing experiments, identification and characterization of cell specific markers, and generation of new transgenic mice to study the functional properties of identified markers.

In the normal non-fibrotic liver, hepatic myofibroblasts become locally and transiently activated in response to bacterial infection. During wound healing, hepatic myofibroblasts apoptose upon completion of repair process (Iredale et al., 1998; Kisseleva et al., 2012). Therefore, pathogenic hepatic fibrosis could be viewed as a chronic state of hepatic myofibroblast activation and finding ways to terminate that activation or induce apoptosis in those aberrantly activated myofibroblasts may be the key to arresting hepatic fibrosis.

Recent studies provide potential experimental models of fibrosis reversal upon cessation of liver injury, or successful pharmacological treatment of underlying causative liver injury (Iredale et al., 1998). Experimental reversal of liver fibrosis has been closely associated with disappearance of hepatic myofibroblasts (Iredale, 2001; Iredale et al., 1998). The mechanism of hepatic myofibroblast disappearance during regression of liver fibrosis in these experiments has been suggested for aHSC-derived myofibroblasts, but remains unknown for myofibroblasts originated from aPFs /myofibroblasts. Thus, 50% aHSC-derived myofibroblasts undergo senescence (Schrader et al., 2009) and concomitant apoptosis (Iredale, 2001, 2007; Iredale et al., 1998) during regression of liver fibrosis. The cell-fate mapping-based studies have demonstrated that 50% of aHSCs survive during regression of liver fibrosis, and obtain "inactivated" phenotype (iHSCs). iHSCs downregulate myofibroblast-specific genes, such as Collagen Type I, α-SMA. Spp1, TIMP1, and others, and upregulate some of genes associated with quiescent phenotype in qHSCs, therefore reverting to a quiescent-like state (Kisseleva and Brenner, 2008; Troeger et al., 2012).