HHS Public Access

Author manuscript

Clin Res Hepatol Gastroenterol. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Clin Res Hepatol Gastroenterol. 2015 September ; 39(0 1): S60–S63. doi:10.1016/j.clinre.2015.06.015.

Reversibility of Liver Fibrosis*

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Abstract

Liver fibrosis is a serious health problem worldwide, which can be induced by a wide spectrum of chronic liver injuries. However, until today, there is no effective therapy available for liver fibrosis except the removal of underlying etiology or liver transplantation. Recent studies indicate that liver fibrosis is reversible when the causative agent (s) is removed. Understanding of mechanisms of liver fibrosis regression will lead to the identification of new therapeutic targets for liver fibrosis. This review summarizes recent research progress on mechanisms of reversibility of liver fibrosis.

While most of the research has been focused on HSCs/myofibroblasts and inflammatory pathways, the crosstalk between different organs, various cell types and multiple signaling pathways should not be overlooked. Future studies that lead to fully understanding of the crosstalk between different cell types and the molecular mechanism underlying the reversibility of liver fibrosis will definitely give rise to new therapeutic strategies to treat liver fibrosis.

Keywords

liver fibrosis; myofibroblasts; inactivation

Pathogenesis of Liver Fibrosis

Liver fibrosis is a significant health problem, which can ultimately lead to end stage cirrhosis and hepatocellular carcinoma. A wide spectrum of chronic liver injuries, including viral hepatitis, cholestatic liver diseases, alcohol abuse, non-alcoholic steatohepatitis, and nonalcoholic fatty liver disease, can cause chronic hepatic inflammation and deregulated wound healing process in the liver, which give rise to fibrosis [1]. Liver fibrosis is characterized by excessive extracellular matrix (ECM) deposition and fibrous scar formation. The destruction of the normal liver architecture by fibrous scar and the loss of

*Note de titre : This article is part of the special issue “Alcohol, Virus and Steatosis evolving to cancer” featuring the conference papers of the 10th International Symposium organized by the Brazilian Society of Hepatology in São Paulo, Brazil, September 30th-October 1st, 2015.

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Conflict of interests: none to declare

hepatocytes can prevent the liver from its physiological functions and in the end, result in liver failure [1,2].

As the major source of ECM, activation and proliferation of myofibroblasts are essential in fibrogenesis [2]. These ECM producing myofibroblasts are only found in the injured liver, but not under normal physiological conditions [1,2]. The major source of myofibroblasts is activated hepatic stellate cells (aHSCs). Depletion of HSCs has been shown to significantly attenuate liver fibrosis and liver injury in both CCl_4 (carbon tetrachloride)- and BDL (bile duct ligation)-induced mouse liver fibrosis [3]. While HSCs are important in fibrogenesis, they are not the only source of hepatic myofibroblasts. Both portal fibroblasts and bone marrow derived collagen producing cells can transdifferentiate into myofibroblasts and the origins of myofibroblasts in liver fibrosis caused by various etiologies can be different [4]. For instance, in CCl_4-induced mouse liver fibrosis, HSCs are the major source of myofibroblasts. However, in BDL-induced liver fibrosis, more than 70% of myofibroblasts are originated from portal fibroblasts after 5 days of injury. With the progression of cholestatic liver injury, HSCs become activated and eventually become the largest contributor to the myofibroblast population [5]. In healthy liver, HSCs are localized in the space of Disse, where they display a quiescent phenotype (qHSCs). qHSCs store vitamin A in lipid droplets and are the major sites of vitamin A storage in the body [6]. qHSCs express neural markers like glial fibrillar acidic protein (GFAP), synemin, synaptophysin, and nerve growth factor receptor p75 [1,7]. In response to fibrogenic stimuli, like increased levels of transforming growth factor beta (TGFβ) and platelet-derived growth factor (PDGF), HSCs activate into myofibroblasts and migrate to the site of injury, where they express fibrogenic genes like vimentin, collagen α1 (I) (Col1a1) and α smooth muscle actin (α-SMA) [8]. The transition of HSCs into myofibroblasts is characterized by down-regulated expression of lipogenic genes (like peroxisome proliferator-activated receptor gamma (PPARγ)), decreased vitamin A storage, and up-regulated expression of fibrogenic genes, such as Col1a1 and α-SMA. The vitamin A stored in HSCs has been suggested to be hydrolyzed to fuel HSCs activation [9]. However, HSCs that lack vatimin A storage also preserved the capacity to activate into myofibrobalsts, indicating that vitamin A metabolism is not necessary for HSC activation [10]. Another important feature of HSC transition is the activation of cell growth cycle, which leads to the proliferation of HSCs and increased numbers of myofibroblasts/aHSCs that produce ECMs in the liver [11].

Reversibility of Liver Fibrosis

Liver fibrosis has been shown to be reversible after the removal of causative agent(s) in both patient and experimental fibrosis models induced by CCl_4, alcohol and BDL [1,12,13]. The reversal of liver fibrosis is characterized by decreased inflammatory and fibrogenic cytokine levels, increased collagenase activity and the disappearance of myofibroblasts and fibrous scars. During the resolution of liver fibrosis, myofibroblasts have been shown to undergo senescence and apoptosis [8,12]. Activated HSC/myofibroblasts are susceptible to apoptosis and can undergo senescence and death receptor-mediated cell death caused by deprivation of fibrogenic cytokines [6]. Activation of death receptor-mediated pathways, increased expression of pro-apoptotic proteins, and decreased expression of pro-survival proteins have been suggested to contribute to myofibroblasts apoptosis [14]. In response to reduced

fibrogenic signals or antiviral drug therapy, HSCs increase expression of Fas receptor (Fas) or TNF receptor 1 (TNFR1) and their ligands and undergo a caspase8/caspase3-dependent apoptosis. Alternatively, overexpression of pro-apoptotic proteins such as p53, Bax and Bcl-2 leads to caspase-9-mediated programmed cell death [1]. Natural killer (NK) cells and liver-specific cells γδ T (NKT) are also involved in the resolution of liver fibrosis. Activated by interferon-γ (IFN-γ), they induce rapid killing of HSC [2,6,15]. Moreover, recent studies from our laboratory and subsequently others showed that besides senescence and apoptosis, myofibroblasts/aHSCs can also revert to an inactive phenotype during liver fibrosis regression [16,17]. The development of research tools like the promoters that drive transgenes selectively in HSCs for cell-specific gene expression/deletion has facilitated our understanding of the fate of HSCs. Using two different fate mapping of myofibroblasts/aHSCs (Col-a1(I)$^{Cre\text{-}YFP}$ and Vimentin-CreER) and single-cell polymerase chain reaction of HSCs from alcohol- and/or CCl_4-induced mouse liver fibrosis, approximately half of hepatic myofibroblasts have been proved to escape apoptosis after cessation of liver injury. These myofibroblasts returned to the space of Disse and reverted to an inactivated phenotype, which is similar to, but distinct from, the quiescent state [16,17]. This is in accordance with previous *in vitro* experiments, where aHSCs were shown to be capable to revert to a quiescent phenotype in cell culture [18]. Compared to aHSCs, in inactivated HSCs (iHSCs) the expression of fibrogenic genes (including Col1a1 and α-SMA) is decreased and the expression of some quiescence-associated genes like PPARγ is increased, to the level that is similar to qHSCs. However, some quiescent-associated genes such as GFAP, Adipor1, Adpf, and Dbp are not re-expressed in iHSCs, indicating the difference between qHSCs and iHSCs [16]. By comparing the global gene expression in HSCs depending on their stage of activation, several genes that are differentially expressed in qHSCs, aHSCs and iHSCs are identified and can be used to distinguish different HSCs. Moreover, compared to original qHSCs, iHSCs are more responsive to fibrogenic stimuli and can contribute to recurring liver fibrosis more effectively [16,19].

Besides the disappearance of myofibroblasts, another important component of liver fibrosis regression is the conversion of macrophages. Macrophages play dual roles through liver fibrosis progression and resolution. During the progression of fibrosis, injury induced inflammatory response triggers the recruitment of macrophages into the liver, where they produce cytokines and chemokines to induce the transition of HSCs into ECM producing myofibroblasts. CCL2, which can be secreted by Kupffer cells and HSCs, facilitates the recruitment of immature monocyte-derived Ly6C^{hi} macrophages into the liver [20]. Deletion of macrophages in CD11b-DTR transgenic mouse led to reduced scarring and fewer myofibroblasts in CCl_4-induced liver fibrosis, indicating the role of macrophages in promoting fibrosis [21]. However, during the recovery of liver fibrosis, macrophages change to a Ly6C^{low} phenotype and stop the production of fibrogenic and inflammatory factors; alternatively they secrete matrix metalloproteases (MMPs), like MMP9 and MMP12 [22]. MMPs are the major enzymes capable of ECM degradation [1]. They are secreted by many cell types, including macrophages, as pro-active enzymes and require post-translational modification for their function [6,23]. While the disappearance of myofibroblasts can decrease the production of ECM, increased collagenolic activity is another primary mechanism of fibrosis resolution. The conversion of macrophages and the production of MMPs help to

degrade and phagocytose existing ECM during regression of liver fibrosis. Accordingly, depletion of macrophages during liver fibrosis recovery led to failure of ECM degradation [21]. Additionally, myofibroblasts are the major source of tissue inhibitor of metalloproteinase (TIMP) production. The disappearance of myofibroblasts leads to reduced TIMPs levels and contributes to increased MMPs activities and the degradation of existing ECM [14].

Because reversibility of liver fibrosis depends on the collagenolic activity of ECM-degrading MMPs, sustained expression of TIMPs inhibits active MMP function. Moreover, lack of ECM degradation may be caused by tissue transglutaminase, which mediates cross-linking of ECM (which prevents different types of collagens from proteolytic cleavage) and prevents HSC apoptosis [6,12,24].

Conclusions and Future Prospective

Liver fibrosis is a serious health problem with an unmet need for effective therapy. The reversibility of liver fibrosis provides potential novel approaches to manage liver fibrosis. However, there are still many unanswered questions. The underlying mechanism of myofibroblast inactivation remains to be determined. The factors that determine the fate of myofibroblasts during liver fibrosis regression are still unknown. The switch between the two different phenotypes of macrophages is still hard to manipulate *in vivo*. Recent studies indicate that epigenetic regulation also affects the progression and resolution of liver fibrosis. Liver fibrosis is the consequence of a complex multicellular response to hepatic injury. Besides HSCs and macrophages, hepatocytes, sinusoidal endothelium cells, and infiltrating immune cells, among many other cells, also contribute to the progression and resolution of liver fibrosis [25]. Moreover, liver fibrosis can also be influenced by other organs like intestine, muscle and adipose tissues [26]. While most of the research has been focused on HSCs/myofibroblasts and inflammatory pathways, the crosstalk between different organs, various cell types and multiple signaling pathways should not be overlooked. Future studies that lead to fully understanding of the crosstalk between different cell types from different organs and the molecular mechanism underlying the reversibility of liver fibrosis will definitely give rise to new therapeutic strategies to treat liver fibrosis.

Acknowledgments

We thank Dr. David Brenner for his critical reading of this manuscript.

Grant support: Supported by the National Institutes of Health (DK099205, AI0777802 P50 AA011999).

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Abbreviations

ECM extracellular matrix

aHSCs	activated Hepatic Stellate Cells
CCl_4	carbon tetrachloride
BDL	bile duct ligation
qHSCs	quiescent Hepatic Stellate Cells
GFAP	glial fibrillar acidic protein
TGFβ	transforming growth factor beta
PDGF	platelet-derived growth factor
Col1a1	collagen α1(I)
α-SMA	α-smooth muscle actin
PPARγ	peroxisome proliferator-activated receptor gamma
Fas	Fas receptor
TNFR1	TNF receptor 1
IFN-γ	interferon-γ
iHSC	inactivated Hepatic Stellate Cells
MMPs	matrix metalloproteinases
TIMP	tissue inhibitor of metalloproteinase