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Anti-fibrogenic Strategies and the regression of fibrosis

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Abstract

Liver fibrosis is an outcome of many chronic diseases, and often results in cirrhosis, liver failure, and portal hypertension. Liver transplantation is the only treatment available for patients with advanced stage of fibrosis. Therefore, alternative methods are required to develop new strategies for anti-fibrotic therapy. Available treatments are designed to substitute for liver transplantation or bridge the patients, they include inhibitors of fibrogenic cytokines such as TGF- β 1 and EGF, inhibitors of rennin angiotensin system, and blockers of TLR4 signaling. Development of liver fibrosis is orchestrated by many cell types. However, activated myofibroblasts remain the primary target for anti-fibrotic therapy. Hepatic stellate cells and portal fibroblasts are considered to play a major role in development of liver fibrosis. Here we discuss the origin of activated myofibroblasts and different aspects of their activation, differentiation and potential inactivation during regression of liver fibrosis.

Keywords

Activated myofibroblasts; Hepatic stellate cells; Portal fibroblasts; Fibrocytes; Epithelial-to-mesenchymal transition; Antifibrotic therapy

Introduction

Hepatic fibrosis is an outcome of many chronic liver diseases, including hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease and non-alcoholic steatohepatitis (NASH) ¹. Hepatic fibrosis consists of a fibrous scar that is constituted by many extracellular matrix proteins (ECMs) including type I collagen. In all clinical and experimental liver fibrosis, myofibroblasts are the source of the ECM constituting the fibrous scar. Myofibroblasts express α -smooth muscle actin (α -SMA) and type I collagen and are only found in the injured, but not the normal, liver. Thus, activation and proliferation of hepatic myofibroblasts is a key mechanism in development of liver cirrhosis.

Several injury-triggered subsequent events were identified to be critical for pathogenesis of liver fibrosis and its resolution. They include: 1) immediate damage to the epithelial/endothelial barrier; 2) release of TGF- β 1, the major fibrogenic cytokine; 3) increased

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intestinal permeability; 4) recruitment of inflammatory cells; 5) induction of reactive oxygen species (ROS); 6) activation of collagen producing cells; 7) matrix-induced activation of myofibroblasts.

Myofibroblasts are the primary target of anti-fibrotic therapy

Liver myofibroblasts represent a primary target for antifibrotic therapy. The origin of fibrogenic cells (myofibroblasts) has been intensively discussed and studied, and several sources of myofibroblasts have been identified²⁻⁵ (see Figure 1). In the fibrotic liver, hepatic stellate cells (HSCs) have been reported to contribute > 80% of the collagen producing cells¹. Therefore, HSCs are currently considered to be the major, but not the only, source of myofibroblasts in the injured liver⁶. Hepatic myofibroblasts may also originate from portal fibroblasts, bone marrow (BM)-derived mesenchymal cells and fibrocytes⁷. Two other mechanisms were recently implicated in fibrogenesis are the epithelial-to-mesenchymal transition (EMT), when epithelial cells acquire features of mesenchymal cells and may give rise to fully differentiated myofibroblasts^{9, 10}, and endothelial-to-mesenchymal transition (EndMT), when endothelial cells undergo a similar phenotypic change^{11, 12}.

Definition of myofibroblasts

Myofibroblasts are characterized by a stellate shape, expression of abundant pericellular matrix and fibrotic genes (α -smooth muscle actin (α -SMA), non-muscle myosin, fibronectin, vimentin.)¹³. Ultrastructurally, myofibroblasts are defined by prominent rough endoplasmic reticulum (rER), a Golgi apparatus producing collagen, peripheral myofilaments, fibronexus (no lamina) and gap junctions¹³. Myofibroblasts are implicated in wound healing and fibroproliferative disorders¹⁴⁻¹⁶. Studies of fibrogenesis conducted in many organs strongly suggest that myofibroblasts are the primary source of ECM⁸. In response to fibrogenic stimuli, such as TGF- β 1, myofibroblasts in all tissues express α -SMA, secrete ECM (fibronectin, collagen type I and III), obtain high contractility and change phenotype (production of the stress fibers)⁶. Classical myofibroblasts differentiate from a mesenchymal lineage and, therefore, lack expression of lymphoid markers such as CD45 or CD34. Sustained injury may trigger differentiation of myofibroblasts from several cellular sources, including HSCs¹.

Hepatic Stellate Cells (HSCs)

HSCs are perisinusoidal cells that normally reside in the space of Disse and contain numerous retinoid and lipid droplets^{17, 18}. Under physiological conditions, HSCs reside in the space of Disse and exhibit a quiescent phenotype. HSCs express neural markers, such as GFAP, synemin, synaptophysin¹, and nerve growth factor receptor p75^{19, 20}, Desmin, CD146, secrete HGF, and store vitamin A²¹. HSCs are also implicated in phagocytosis and antigen presentation^{22, 23}. In response to injury, quiescent HSCs down-regulate vitamin A expression, acquire contractility and activate into collagen type I⁺ α -SMA⁺ myofibroblasts. During development HSCs are derived from the translocation of submesothelial mesenchymal cells from the liver capsule²⁴.

If HSCs are the primary source of myofibroblasts in certain fibrotic liver diseases, then drugs can be targeted specifically to HSCs at higher doses to avoid toxicity to other cells. Vitamin A-coupled liposomes have been used to carry an anti-fibrosis drug in experimental models of liver fibrosis²⁵. The concept is that HSCs will take up the vitamin A liposome containing the drug by receptor-mediated uptake bound to retinol binding protein. Other strategies have used synthetic peptide ligands targeted the PDGF receptor, which is unique to HSCs in the liver. Most recently, mannose 6-phosphate modified human serum albumin

(M6PHSA) which binds to the mannose-6-phosphate/insulin growth factor type II receptor (M6P/IGII-R), has been used to target activated HSCs during experimental liver fibrogenesis²⁶.

Portal fibroblasts

Portal fibroblasts are spindle shaped cells of mesenchymal origin that are present in the portal tracts. Under normal conditions, they participate in physiological ECM turnover^{6, 27–29} and lack expression of α -SMA. Induced mostly by cholestatic liver injury, portal fibroblasts proliferate (though much slower than HSCs³⁰) and deposit collagen (e.g. type I) around portal tracts³¹. Portal fibroblasts are distinct from HSCs in that they express elastin and Thy-1.1 (a glycoposphatidylinositol-linked glycoprotein of the outer membrane leaflet described in fibroblasts of several organs)^{32, 33}, do not store retinoids, and do not express desmin or neural markers. Portal fibroblasts lack expression of Desmin, GFAP, CD146 or Vitamin A droplets. Their exact contribution to cholestatic liver injury or hepatic fibrosis of different etiologies is not well understood.

Fibrocytes

Fibrocytes originate from hematopoietic stem cells, and are defined as spindle shaped “CD45 and collagen type I (Col⁺) expressing leukocytes that mediate tissue repair and are capable of antigen presentation to naive T cells”³⁴. Due to their ability to differentiate into myofibroblasts, fibrocytes are implicated in the fibrogenesis of skin, lungs, kidneys, and liver^{7, 35, 36}. In addition to collagen Type I, fibronectin and vimentin, fibrocytes express CD45, CD34, MHCII, CD11b, Gr-1, and secrete growth factors (TGF- β 1, MCP-1) that promote deposition of ECM^{37, 38}. Upon injury or stress, fibrocytes proliferate and migrate to the injured organ^{34, 37}. The number of recruited fibrocytes varies from 25% (lung fibrosis)^{7, 39} to 5% (liver fibrosis, e.g. BDL and CCl₄)² of the collagen expressing cells, suggesting that the magnitude of fibrocyte differentiation into myofibroblasts depends on the organ and the type of injury. Although low number of fibrocytes was detected in response to BDL and CCl₄ models of liver injury, this may not extend to some genetic defects causing hepatic fibrosis in mice. Thus, Abcb4 deficiency in mice results in a significant flux of fibrocytes to the liver, which may contribute to the severe fibrosis in these mice⁴⁰.

Originating in the BM, fibrocytes comprise a small subset (0.1%) of mononuclear cells, which in response to injury or stress, proliferate and migrate to the injured organ from the blood stream^{34, 37}. Under physiological conditions fibrocytes do not egress the BM, and only egress the BM in response to injury. Interestingly, in addition to the damaged liver, both cholestatic and toxic liver injury (BDL and CCl₄) triggers migration of fibrocytes to lymphoid organs (spleen, and lymphoid patches in the intestine)⁴¹, suggesting that the function of fibrocyte-like cells may not be limited to ECM deposition.

BM mesenchymal progenitors

Myofibroblasts can also arise from BM-derived mesenchymal progenitors^{42, 43}, and were shown to populate fibrotic lungs⁴⁴ and liver⁴³, and contribute to fibrosis by differentiating into tissue myofibroblasts^{8, 17, 43}. By fractionating the BM stem cell compartment, hepatic BM-derived myofibroblast-like cells were demonstrated to originate from mesenchymal stem cells (MSCs)^{43, 45}. MSCs are defined as self-renewable, multipotent progenitor cells with the capacity to differentiate into lineage specific cells that form bone, cartilage, fat, tendon and muscle^{45, 46}. Unlike hematopoietic stem cells, MSCs are more radioresistant⁴⁷, reside mostly in BM stroma, do not express hematopoietic markers and can be isolated as Lin[−]CD45[−]CD31[−]CD34[−]133[−]Sca-1⁺Vitamin A[−]CD146⁺ cells^{45, 48, 49}. Whether circulating mesenchymal progenitors significantly contribute to ECM deposition in the course of liver

fibrosis is unknown, but they most likely represent a population, distinct from hematopoietic-derived fibrocytes⁴¹.

Contribution of EMT and EndMT to hepatic fibrosis

Another mechanism implicated in fibrogenesis is EMT^{9, 10}, a process in which fully differentiated epithelial cells undergo phenotypic transition to fully differentiated mesenchymal cells (fibroblasts or myofibroblasts)⁵⁰. During EMT, epithelial cells detach from the epithelial layer, lose their polarity, down-regulate epithelial markers (e.g., cytokeratin-19 (K19), CK-7, E-cadherin) and tight junction proteins (zonula occludens-1, ZO-1), increase their motility, and obtain a (myo)fibroblast phenotype⁵¹. Epithelial cells transitioning into (myo)fibroblasts are reported to express fibroblast specific protein-1 (FSP1), which is used as a universal marker of EMT in fibrogenesis and cancer^{51, 52}. However, an activated monocytic cell population expresses FSP1 in the injured liver, so FSP1 is not a valid marker of EMT in liver fibrosis. EMT of hepatocytes and cholangiocytes has been reported in patients and in mice with liver fibrosis^{53–56}. However, only minimal or no contribution of hepatocytes or cholangiocytes to myofibroblasts was detected *in vivo*^{11, 57} using BDL or CCl₄ models of liver fibrosis in mice. Moreover, genetic labeling of hepatocytes, cholangiocytes and their progenitors (Oval cells) has been recently tested using alpha-fetoprotein (AFP)-Cre mice in response to multiple liver injuries (Dr. R.G. Wells, in press). In concordance with previous studies, no EMT-derived myofibroblasts were detected in these mice.

Endothelial cells may also transition to mesenchymal cells (EndMT), giving rise to (myo)fibroblasts in response to fibrogenic injury. EndMT has been reported to contribute to cardiac¹¹ and renal¹² fibrosis. EndMT is identified by expression of myofibroblast genes⁵¹ in endothelial cells that are expressing or have a “history” of expressing PECAM-1/CD31, Tie-1¹¹, Tie-2 and CD34^{2, 12}. A difficulty in interpreting these studies is that it is now recognized that Tie-2 is not a specific marker for endothelial cells in that it is also expressed in BM derived hematopoietic cells.

Thus, many studies have demonstrated a lack of EMT in the liver and other organs in experimental murine models using genetic fate mapping. However, due to differences in etiology and duration between liver fibrosis in patients and experimental models in mice, EMT of hepatocytes, cholangiocytes and their progenitors (Oval cells) has not been fully addressed in patients.

Progress in developing therapies for liver fibrosis

Several molecules have been successfully identified as targets for anti-fibrotic therapy. TGF- β 1 plays a critical role in activation of myofibroblasts. Although inhibitors of TGF- β 1 are effective in short-term animal models^{58–62}, they are not suitable for long term therapy because of the significant role of TGF- β 1 in homeostasis and repair. Hepatocyte growth factor (HGF) is a pleiotropic cytokine produced by hepatic stellate cells and implicated in liver regeneration and fibrosis. Similarly, treatment with inhibitors of HGF produces anti-fibrogenic effects, but also increases the risk of tumorigenesis in mice^{63–65}.

Inhibition of renin angiotensin system

The renin angiotensin pathway in hepatic stellate cells induces reactive oxygen species and accelerates hepatic fibrosis. The renin angiotensin system (RAS) regulates the systemic arterial blood pressure, but, in response to sustained liver injury, locally accelerates inflammation, tissue repair and fibrogenesis by production of angiotensin II (Ang II), a vasoconstricting agonist implicated in pathogenesis of liver fibrosis (see Figure 2). RAS is regulated by a series of subsequent enzymatic reactions: angiotensinogen (AGT) from the

liver is proteolytically cleaved by rennin to form angiotensin I (Ang I), which, in turn, is processed into angiotensin II (Ang II) by angiotensin converting enzyme (ACE). Ang II binds either to AT₁ or AT₂ plasma membrane receptor to mediate its biological activity.

Fibrogenic actions of Ang II are mostly mediated by angiotensin receptor AT₁. Stimulation of AT₁ receptor by angiotensin II results in proliferation of HSCs and extracellular matrix deposition. Angiotensin II also plays an important role in ROS formation by activating NADPH oxidase in HSCs⁶⁶. In concordance, several experimental models of liver fibrosis in rodents have demonstrated that prolonged administration of angiotensin II directly causes HSC activation⁶⁷. Mice lacking AT_{1a} receptors are protected from liver fibrosis.

This makes RAS an attractive target for antifibrotic therapy. Angiotensin-converting enzyme (ACE1) and angiotensin type 1 (AT₁) receptors are upregulated in fibrotic livers, and can be successfully blocked by already widely used ACE inhibitors or AT₁ receptor antagonists. ACE inhibitors block angiotensin II production, while AT₁ receptor antagonists prevent Ang II binding to AT₁ receptors. Disruption of RAS pathway by RAS inhibitors have been shown to be effective to attenuate liver fibrosis⁶⁸ and are suitable for the long term treatment. On the other hand, ACE2 degrades the active angiotensin II to block fibrogenesis. Mice lacking ACE2 have increased liver fibrosis, and recombinant ACE2 inhibits murine models of liver fibrosis^{69, 70}.

Inhibition of TLR4 signalling and increased intestinal permeability

Development of liver fibrosis is associated with elevated levels of TGF- β 1 and increased intestinal permeability. Gut sterilization with antibiotics attenuates liver fibrosis, whereas pathogen free animals are resistant to liver fibrosis⁷¹. Bacteria and LPS signal via Toll-like receptor pathway. Consistently, mice with deficiencies in CD14, LPS binding protein (LBP), or TLR4 have impaired TLR signaling and are not susceptible to liver fibrosis⁷². Emerging observations indicating that the TLR4 signaling pathway plays an important role in the pathogenesis of liver fibrosis. Therefore, administration of TLR4 antagonists, LPS neutralizing agents or prolonged antibiotic intake are of major consideration for clinical trials of patients with liver fibrosis.

Inhibition of angiogenesis

Development of liver fibrosis is closely associated with angiogenesis and neovascularization. Extensive neovascularization is usually observed around dense scar tissue in cirrhotic liver, and is proposed to be mediated by elevated levels of vascular endothelial growth factor (VEGF) and angiopoietin 1 and 2 in the course of hepatic fibrosis. The mechanism of how angiogenesis promotes liver fibrosis remains unknown. However, administration of VEGF-receptor 1 and 2 neutralizing antibodies results in significant inhibition of liver fibrosis in mice⁷³. Inhibition of angiogenesis by several drugs has been also shown to be effective in suppression of liver fibrosis. Thus, semisynthetic analogue of fumagillin (TNP-470)⁷⁴, exhibit anti-angiogenic properties and is currently used in clinical trials to prevent the progression of hepatic. The tyrosine kinase inhibitor Sunitinib (SU11248) also attenuates experimental models of liver fibrosis in rats.⁷⁴ In addition to other effects, inhibition of angiogenesis may also prevent progression of liver fibrosis into hepatocellular carcinoma.

Cellular therapy for cirrhosis

Therapies with the potential to substitute for liver transplantation or bridge the patient awaiting transplantation are required. Liver cell transplantation (LCT), an experimental procedure designed to reconstitute the liver mass with functional hepatocytes, is based on transplantation of isolated hepatocytes from a cadaver or from a liver portion from a living

donor⁷⁵. This experimental procedure has been successfully used in patients to correct certain metabolic disorders⁷⁵. Another experimental procedure involves transplantation of hematopoietic bone marrow (BM) progenitor cells or adipose-tissue derived mesenchymal cells⁷⁶. Transplantation of fetal hepatocytes has also been considered as an alternative treatment⁷⁷.

Liver cell transplantation (LCT) has been attempted in patients with acute liver failure, chronic liver disease with end-stage cirrhosis, and children with metabolic disease⁷⁵. To date, the best outcome of allogenic hepatocyte transplantation was reported for treatment of acute liver failure. In this case, allogenic infusion of hepatocytes is aimed to provide rapid metabolism of liver toxins and stabilization of hemodynamic parameters. Thus, in 20% of patients LCT of hepatocytes from cadaver livers resulted in recovery without solid organ transplant, and in 30% bridged patients to liver transplantation^{78–80}. Some progress has been made in correction of metabolic diseases. According to their etiology, metabolic disorders could be divided in two groups, inherited clotting factor deficiencies and metabolic deficiencies⁷⁵. Since metabolic deficiencies are often associated with the severe damage to hepatocytes, transplanted hepatocytes have a growth advantage over recipient hepatocytes. Under these conditions, donor hepatocytes have a selective pressure and LCT has been reported in patients to correct ornithine trans-carbamylase (OTC) deficiency, α -1-anti-trypsin deficiency, glycogen storage disease type Ia, infantile Refsum's disease, factor VII deficiency, bile salt export protein deficiency, and Crigler-Najjar syndrome type 1^{75, 81, 82}.

Purified hepatocytes are infused in patients via the portal vein or are injected into the spleen⁸³. On average, only 30% of hepatocytes survive transplantation. Therefore, successful engraftment often depends on the number of infused hepatocytes⁸³. Infusion of hepatocytes into the portal vein causes transient portal hypertension, and must be combined with pharmacological disruption of endothelial integrity required for hepatocyte extravasation⁸⁴. Hepatocytes do not tolerate cryopreservation well and lose expression of adhesion molecules, which play an important role in hepatocyte extravasation and engraftment between the host hepatocytes⁸⁴. In addition, cryopreservation further decreases the viability of hepatocytes by 30%^{85, 86}. Immunological rejection of hepatocytes requires prolonged immunosuppressive therapy in patients suitable for LCT⁸⁷. Identification and characterization of hepatocyte progenitor/stem cells and their differentiation into functionally mature liver cells is an evolving goal for the stem cell therapy.

The improvement of liver function following the transplantation of hematopoietic progenitors in mice and rats provided the basis for clinical trials. To date, 11 clinical trials with BM-derived cells have been conducted⁷⁷. Clinical studies with transplantation of autologous CD133⁺ BM cells in patients have been reported to stimulate liver regeneration, as demonstrated by a reduction of bilirubin, increased albumin, and improved coagulopathy⁸⁸. Similar to that, autologous infusion of CD34⁺ blood cells, or even concentrated monocytes, improved biochemical parameters and stimulated liver regeneration⁸⁹. While transplantation of hematopoietic progenitors appeared beneficial in patients, the mechanism of their action remains controversial and may not reflect the generation of BM-derived hepatocytes. Such improvement may result from release of cytokines and growth factors by transplanted hematopoietic cells, or occur due to infusion of scar-resorbing monocytes. In concordance with this observation, treatment with granulocyte-colony stimulating factor (G-CSF) was used to mobilize the BM cells and demonstrated a positive effect in patients with alcoholic steatohepatitis⁹⁰.

Mesenchymal stem cells serve as another attractive target for the liver stem cell therapy. Although infusion of MSCs often results in attenuation or improvement of liver disease, current studies have not provided definitive evidence that MSCs have a capability to

differentiate into functional hepatocytes *in vivo* ^{91–95}. In turn, these improvements could be attributed to the secretion of soluble factors by MSCs, rather than transdifferentiation into hepatocytes. In concordance with this notion, injection of MSC-derived conditioning media into a liver-assist device can prevent hepatocyte apoptosis and increase their proliferation ^{96, 97}. Moreover, recent studies have raised a safety question of MSCs transplantation, demonstrating that MSCs can give rise to fibrogenic myofibroblasts in mice in response to liver injury. BM-derived MSCs contributed to the development of liver fibrosis in chimeric mice that received bone marrow transplantation with the enriched BM mesenchymal fraction, and subjected to the CCl₄-liver injury ⁴³. Taken together, both hematopoietic and mesenchymal stem cells demonstrated a limited contribution to hepatocyte replenishment, but may stimulate liver function by providing soluble growth factors or cytokines ^{41, 98, 99}.

A few clinical trials have been performed in patients with the cirrhosis caused by hepatitis B, hepatitis C, alcohol, or cryptogenic. These patients were transplanted with autologous MSCs harvested from the iliac crest. The tested parameters (albumin, creatinine) demonstrated a modest but significant improvement without severe adverse effects, suggesting that MSCs injection might be useful for the treatment of end-stage liver disease with satisfactory tolerability ¹⁰⁰. In a different clinical trial, MSCs were used in patients with decompensated cirrhosis. All patients showed good tolerance and decreased Model for end-stage liver disease (MELD) scores, and improvement in albumin production and liver function after six month of follow up ¹⁰⁰.

Reversal of liver fibrosis

Mechanism of regression of liver fibrosis

Until recently, it was believed that hepatic fibrosis was irreversible ¹⁰¹. However, sequential liver biopsies have documented that removing the underlying etiological agent may reverse hepatic fibrosis in patients with secondary biliary fibrosis ¹⁰², Hepatitis C ¹⁰³, Hepatitis B ¹⁰⁴, NASH ¹⁰⁵, and autoimmune hepatitis ¹⁰⁶. Furthermore, in experimental models of CCl₄ ^{107, 108} and BDL ¹⁰⁹ induced liver fibrosis, removal of the etiological agent results in reversal of fibrosis ¹. Withdrawal of the etiological source of the chronic injury (e.g. HBV, HCV) ¹ results in decrease of pro-inflammatory and fibrogenic (TGFβ1) cytokines, decreased ECM production, increased collagenase activity ^{1, 2}, and the disappearance of activated myofibroblasts. The currently accepted mechanism for the elimination of activated myofibroblasts is that these cells rapidly decline due to apoptosis of activated HSCs ¹¹⁰. Several mechanisms are implicated in the apoptosis of activated HSCs: 1). Activation of death receptor-mediated pathways (Fas or TNFR-1 receptors) and caspases 8 and 3; 2) up-regulation of pro-apoptotic proteins (e.g. p53, Bax, caspase 9); and 3) decrease of pro-survival genes (e.g. Bcl-2) ¹¹¹. A population of liver associated natural killer (NK) cells and γδ T (NKT) cells stimulate apoptosis of activated HSCs. Drugs that induce apoptosis in activated HSCs (glytotoxin, sulfasalazine, IKK inhibitors, and anti-TIMP antibodies) cause liver fibrosis to regress ^{1, 2}. Increased collagenase activity is a primary pathway of fibrosis resolution. At this stage, activated macrophages/Kupffer cells secrete matrix metalloproteinases, e.g. MMP-13 interstitial collagenase, responsible for matrix degradation ^{5, 112}. Moreover, increased activity of collagen degrading enzymes correlates with decreased TIMPs, tissue inhibitors of matrix metalloproteinases ¹¹¹. Whether end-stage cirrhosis can reverse to a normal liver architecture remains controversial ^{17, 113}. However, significant improvement in hepatic structure and function provide evidence of regression of liver fibrosis ¹¹⁴. Perhaps ECM remodeling is limited in cirrhosis by formation of non-reducible cross-linked collagen and an ECM rich with elastin fibers preventing its degradation. This pathophysiological state may lead to a “point of no return” for liver fibrosis ^{17, 114}.

The role of myofibroblasts in reversal of liver fibrosis

HSCs are a major source of collagen producing cells in fibrotic liver. Although HSC death by apoptosis¹⁰⁷ and senescence¹¹⁵ during the regression of liver fibrosis is well documented, its quantitative contribution is unknown¹¹⁴. Theoretically, activated HSCs/myofibroblasts may transdifferentiate into another phenotype or revert to quiescent HSCs. Although the cellular population of quiescent HSCs are restored in mice recovering from fibrosis, the source of these quiescent HSCs is unknown. In concordance, studies in culture suggest that HSCs, at least in part, can reverse to a quiescent phenotype. Therefore, the disappearance of activated α -SMA⁺ Col⁺ activated HSCs/myofibroblasts in the course of fibrosis reversal may indicate that activated HSCs return to a quiescent state.

The quiescent phenotype of HSCs is associated with expression of lipogenic genes and storage of vitamin A in lipid droplets. Depletion of peroxisome proliferator-activated receptor gamma (PPAR- γ) constitutes a key molecular events for HSC activation, and ectopic expression of this nuclear receptor results in the phenotypic reversal of activated HSC to quiescent cells in culture¹¹⁶. The treatment of activated HSC with an adipocyte differentiation cocktail or over-expression of SREBP-1c results in up-regulation of adipogenic transcription factors and causes morphologic and biochemical reversal of activated HSC to quiescent cells^{117, 118}. In addition, quiescence of HSCs largely depends of the ECM environment, and matrix composition is important to maintain the quiescent phenotype of HSCs. Thus, spontaneous activation of quiescent HSCs is attenuated when cultured on basement membrane-like ECM¹¹⁹, and plastic activated HSCs reverse their phenotype and down-regulate fibroblast markers if transferred to basement membrane-like ECM¹¹⁹. Although this data suggests that activated HSCs can reverse to quiescent state, these findings have only been documented in cultured cells.

Inactivation of myofibroblasts during reversal of fibrosis opens new prospectives for therapy

Hepatic fibrosis is reversible in patients and in experimental models with decreased fibrous scar and disappearance of the myofibroblast population. However, the fate of the myofibroblasts is unknown. Although some myofibroblasts undergo cell death¹⁰⁷, an alternative untested hypothesis is that the myofibroblasts revert to their original quiescent phenotype or obtain a new phenotype. Understanding of the origin and biology of fibrogenic myofibroblasts will provide a new target for anti-fibrotic therapy.

Summary

Inactivation of myofibroblasts during reversal of fibrosis opens new prospects for therapy. Hepatic fibrosis is reversible in patients and in experimental models with decreased fibrous scar and disappearance of the myofibroblast population. However, the fate of the myofibroblasts is unknown. Although some myofibroblasts undergo cell death⁷⁹, an alternative untested hypothesis is that the myofibroblasts revert to their original quiescent phenotype or obtain a new phenotype. Understanding of the origin and biology of fibrogenic myofibroblasts will provide a new target for anti-fibrotic therapy.

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Figure 1.

Three sources of myofibroblasts have been proposed in fibrotic liver: resident cells (Hepatic stellate cells and portal fibroblasts); BM-derived cells (fibrocytes and mesenchymal cells), and cells originated by epithelial-to-mesenchymal transition (EMT or EndMT) and. Hence, recent studies suggested that EMT does not significantly contribute to liver fibrosis. Modified from ⁸.

Figure 2.

The Renin Angiotensin pathway. The entire pathway is expressed in the fibrotic liver. ACE1 generates the fibrogenic Angiotensin II, which in turn binds to its receptor AT1 to activate NADPH oxidase (NOX). ACE2 has anti-fibrotic effects and degrades angiotensin II and apelin-12.