Best Practice & Research Clinical Gastroenterology 25 (2011) 305–317

Contents lists available at ScienceDirect

Best Practice & Research Clinical Gastroenterology

10

# Anti-fibrogenic strategies and the regression of fibrosis

Tatiana Kisseleva, M.D., PhD, Assistant Research Scientist,
David A. Brenner, M.D., Vice Chancellor for Health Sciences,
Dean School of Medicine, Professor *

*Department of Medicine, University of California, San Diego, La Jolla, CA, USA*

*Keywords:*
Activated myofibroblasts
Hepatic stellate cells
Portal fibroblasts
Fibrocytes
Epithelial-to-mesenchymal transition
Anti-fibrotic therapy

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-β1 and EGF, inhibitors of rennin angiotensin system, and blockers of TLR4 signalling. 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.



## 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

* Corresponding author. 1318 Biomedical Sciences Building, 9500 Gilman Drive, La Jolla, CA 92093-0602, USA. Tel.: +1 858 534 1501; fax: +1 858 822 0084.
*E-mail address:* dbrenner@ucsd.edu (D.A. Brenner).

1521-6918/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bpg.2011.02.011

the ECM constituting the fibrous scar. Myofibroblasts express a-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-β1, the major fibrogenic cytokine; (3) increased 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 anti-fibrotic therapy. The origin of fibrogenic cells (myofibroblasts) has been intensively discussed and studied, and several sources of myofibroblasts have been identified [2–5] (see Fig. 1). In the fibrotic liver, hepatic stellate cells (HSCs) have been reported to contribute>80% of the collagen producing cells [1]. Therefore, HSCs are currently considered to be the major, but not the only, source of myofibroblasts in the injured liver [6]. Hepatic myofibroblasts may also originate from portal fibroblasts, bone marrow (BM)-derived mesenchymal cells and fibrocytes [7]. 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

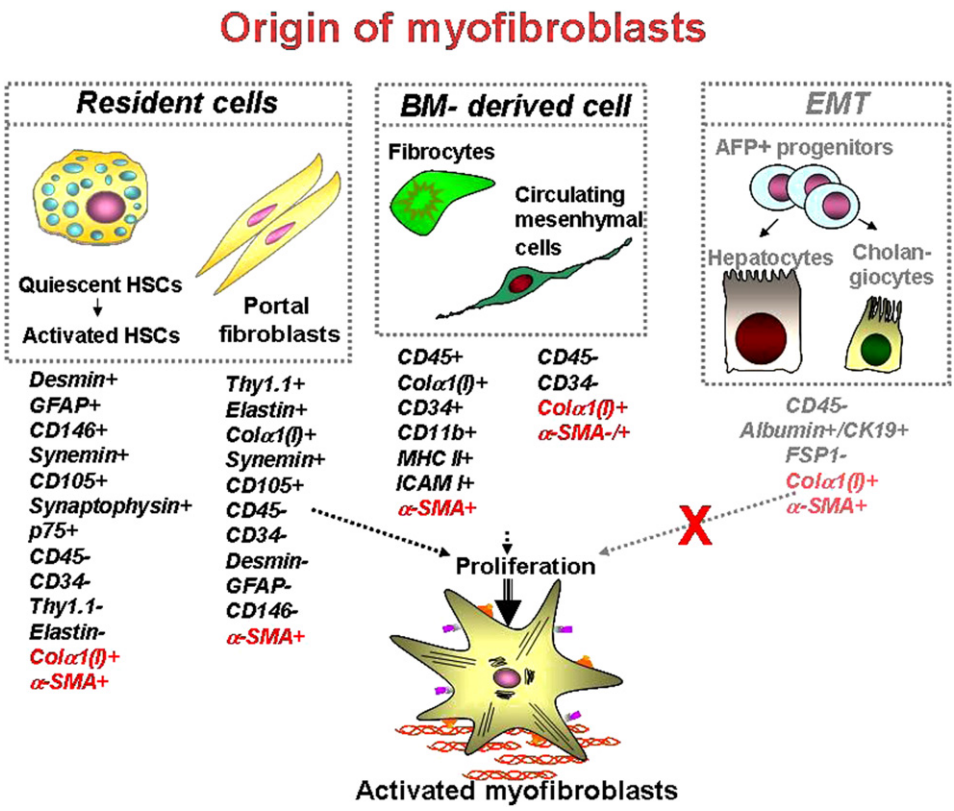


**Fig. 1.** Three sources of myofibroblasts have been proposed in fibrotic liver: resident cells (Hepatic stellate cells and portal fibroblasts); BM-derived cells (fibrocytes and mesenchymal cells), and cells originated by epithelial-to-mesenchymal transition (EMT or EndMT) and. Hence, recent studies suggested that EMT does not significantly contribute to liver fibrosis. Modified from [8].

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 junctions [13]. Myofibroblasts are implicated in wound healing and fibroproliferative disorders [14–16]. Studies of fibrogenesis conducted in many organs strongly suggest that myofibroblasts are the primary source of ECM [8]. In response to fibrogenic stimuli, such as TGF-β1, 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 fibres) [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 HSCs [1].

*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, synaptophysin [1], and nerve growth factor receptor p75 [19,20], Desmin, CD146, secrete HGF, and store vitamin A [21]. 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 $I^+$ $\alpha$-$SMA^+$ myofibroblasts. During development HSCs are derived from the translocation of submesothelial mesenchymal cells from the liver capsule [24].

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 fibrosis [25]. 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 fibrogenesis [26].

*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 HSCs [30]) and deposit collagen (e.g. type I) around portal tracts [31]. Portal fibroblasts are distinct from HSCs in that they express elastin and Thy-1.1 (a glycophosphatidylinositol-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 haematopoietic 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-β1, 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 $CCl_4$) [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 $CCl_4$ 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 mice [40].

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 $CCl_4$) 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 lungs [44] and liver [43], 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 haematopoietic stem cells, MSCs are more radioresistant [47], reside mostly in BM stroma, do not express haematopoietic markers and can be isolated as $Lin^-CD45^-CD31^-CD34^-133^-Sca\text{-}1^+$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 haematopoietic-derived fibrocytes [41].

*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 phenotype [51]. 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 $CCl_4$ models of liver fibrosis in mice. Moreover, genetic labelling 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 cardiac [11] and renal [12] fibrosis. EndMT is identified by expression of myofibroblast genes [51] in endothelial cells that are expressing or have a 'history' of expressing PECAM-1/CD31, Tie-1 [11], 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 haematopoietic 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 aetiology 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-β1 plays a critical role in activation of myofibroblasts. Although inhibitors of TGF-β1 are effective in short-term animal models [58–62], they are not suitable for long term therapy because of the significant role of TGF-β1 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 Fig. 2). RAS is regulated by a series of subsequent enzymatic reactions: angiotensinogen (AGT) from the liver is proteolitically cleaved by rennin to form angiotensin I (Ang I), which, in turn, is processed into angiotensisn 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 $AT_1$ receptor by angiotensin II results in proliferation of HSCs and extracellular matrix deposition.

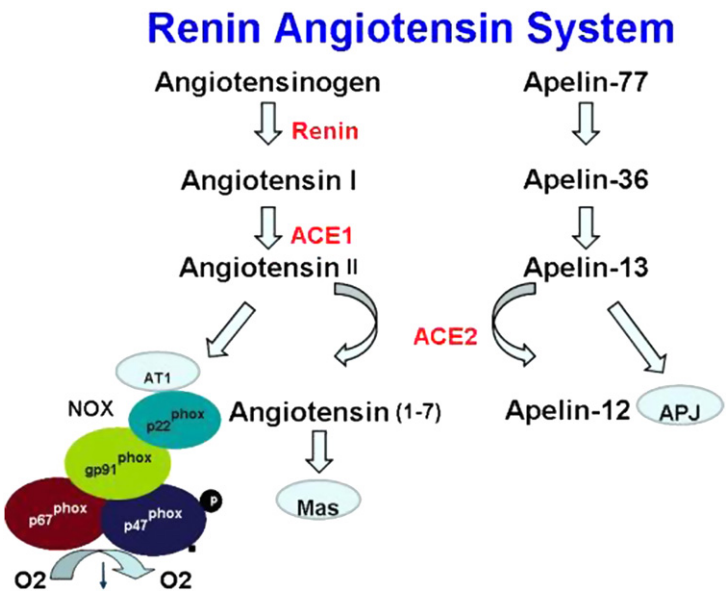


**Fig. 2.** The Renin Angiotensin pathway. The entire pathway is expressed in the fibrotic liver. ACE1 generates the fibrogenic Angiotensin II, which in turn binds to its receptor AT1 to activate NADPH oxidase (NOX). ACE2 has anti-fibrotic effects and degrades angiotensin II and apelin-12.

Angiotensin II also plays an important role in ROS formation by activating NADPH oxidase in HSCs [66]. In concordance, several experimental models of liver fibrosis in rodents have demonstrated that prolonged administration of angiotensin II directly causes HSC activation [67]. Mice lacking $AT_{1a}$ receptors are protected from liver fibrosis.

This makes RAS an attractive target for anti-fibrotic therapy. Angiotensin-converting enzyme (ACE1) and angiotensin type 1 ($AT_1$) receptors are upregulated in fibrotic livers, and can be successfully blocked by already widely used ACE inhibitors or $AT_1$ receptor antagonists. ACE inhibitors block angiotensin II production, while $AT_1$ receptor antagonists prevent Ang II binding to $AT_1$ receptors. Disruption of RAS pathway by RAS inhibitors have been shown to be effective to attenuate liver fibrosis [68] 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-β1 and increased intestinal permeability. Gut sterilization with antibiotics attenuates liver fibrosis, whereas pathogen free animals are resistant to liver fibrosis [71]. Bacteria and LPS signal via Toll-like receptor pathway. Consistently, mice with deficiencies in CD14, LPS binding protein (LBP), or TLR4 have impaired TLR signalling and are not susceptible to liver fibrosis [72]. Emerging observations indicating that the TLR4 signalling 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 angiopoeitin 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 mice [73]. 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 donor [75]. This experimental procedure has been successfully used in patients to correct certain metabolic disorders [75]. Another experimental procedure involves transplantation of haematopoietic bone marrow (BM) progenitor cells or adipose-tissue derived mesenchymal cells [76]. Transplantation of foetal hepatocytes has also been considered as an alternative treatment [77].

Liver cell transplantation (LCT) has been attempted in patients with acute liver failure, chronic liver disease with end-stage cirrhosis, and children with metabolic disease [75]. 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 haemodynamic 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 aetiology, metabolic disorders could be divided in two groups, inherited clotting factor deficiencies and metabolic deficiencies [75]. 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 spleen [83]. On average, only 30% of hepatocytes survive transplantation. Therefore, successful engraftment often depends on the number of infused hepatocytes [83]. Infusion of hepatocytes into the portal vein causes transient portal hypertension, and must be combined with pharmacological disruption of endothelial integrity required for hepatocyte extravazation [84]. 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 hepatocytes [84]. 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 LCT [87]. 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 haematopoietic progenitors in mice and rats provided the basis for clinical trials. To date, 11 clinical trials with BM-derived cells have been conducted [77]. 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 coagulopathy [88]. Similar to that, autologous infusion of $CD34^+$ blood cells, or even concentrated monocytes, improved biochemical parameters and stimulated liver regeneration [89]. While transplantation of haematopoietic 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 haematopoietic 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 steatohepatitis [90].

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 then 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 $CCl_4$-liver injury [43]. Taken together, both haematopoietic 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 $CCl_4$ [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 (TGF-β1) 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 (glyotoxin, 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 metalloproteinasesis [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 remodelling is limited in cirrhosis by formation of non-reducible cross-linked collagen and an ECM rich with elastin fibres 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 apoptosis [107] and senescence [115] during the regression of liver fibrosis is well documented, its quantitative contribution is unknown [114]. 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 $\alpha$-$SMA^+$ $Col^+$ 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 culture [116]. 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 ECM [119], and plastic activated HSCs reverse their phenotype and down-regulate fibroblast markers if transferred to basement membrane-like ECM [119]. 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 death [107], 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.

**Practice points**

- Development of liver fibrosis is closely associated with up-regulation of fibrogenic cytokines, such as TGF-β1, increased intestinal permeability and neovascularization.
- Inhibitors of TGF-β1 are a potential short-term treatment in patients to attenuate liver fibrosis.
- Inhibition of LPS signalling via TLR4 may be a target of choice for prolonged treatment of patients with liver fibrosis.
- The close association between liver fibrosis and neovascularization may enable treatment of fibrosis by inhibiting the inappropriate neovascularization.

**Research agenda**

- The origin and composition of myofibroblasts in different fibrotic liver diseases requires better characterization.
- The ability of fibrogenic myofibroblasts to revert their phenotype should be carefully investigated.
- New pathways of anti-fibrotic therapy should be designed to (1)induce apoptosis of activated myofibroblasts, or (2) inactivate their phenotype from fibrogenic collagen producing cells into quiescent cells.

## 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 death [79], 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.

## Conflict of interest

No conflict of interest has been declared by the authors.

## References

[1] Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209–18.
[2] Kisseleva T, Brenner DA. Hepatic stellate cells and the reversal of fibrosis. J Gastroenterol Hepatol 2006;21(Suppl. 3): S84–7.

[3] Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003;112: 1776–84.
[4] Gomperts BN, Strieter RM. Fibrocytes in lung disease. J Leukoc Biol 2007;82:449–56.
[5] Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, et al. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. J Immunol 2007;178:5288–95.
*[6] Parola M, Marra F, Pinzani M. Myofibroblast - like cells and liver fibrogenesis: Emerging concepts in a rapidly moving scenario. Mol Aspects Med 2008;29:58–66.
[7] Kisseleva T, Brenner DA. Fibrogenesis of parenchymal organs. Proc Am Thorac Soc 2008;5:338–42.
*[8] Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med (Maywood) 2008;233:109–22.
[9] Kalluri R. EMT: when epithelial cells decide to become mesenchymal-like cells. J Clin Invest 2009;119:1417–9.
[10] Choi SS, Diehl AM. Epithelial-to-mesenchymal transitions in the liver. Hepatology; 2009.
[11] Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med 2007;13:952–61.
[12] Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M, Kalluri R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. J Am Soc Nephrol 2008;19:2282–7.
[13] Eyden B. The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. J Cell Mol Med 2008;12:22–37.
[14] Majno G, Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR. Contraction of granulation tissue in vitro: similarity to smooth muscle. Science 1971;173:548–50.
[15] Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 1971;27:549–50.
[16] Schurch W, Seemayer TA, Gabbiani G. The myofibroblast: a quarter century after its discovery. Am J Surg Pathol 1998; 22:141–7.
*[17] Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. J Clin Invest 2007;117:539–48.
[18] Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 2001;21:311–35.
[19] Sachs BD, Baillie GS, McCall JR, Passino MA, Schachtrup C, Wallace DA, et al. p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. J Cell Biol 2007;177:1119–32.
[20] Kendall TJ, Hennedige S, Aucott RL, Hartland SN, Vernon MA, Benyon RC, et al. p75 Neurotrophin receptor signaling regulates hepatic myofibroblast proliferation and apoptosis in recovery from rodent liver fibrosis. Hepatology 2009; 49:901–10.
[21] Senoo H, Kojima N, Sato M. Vitamin a-storing cells (stellate cells). Vitam Horm 2007;75:131–59.
[22] Bataller R, Schwabe RF, Choi YH, Yang L, Paik YH, Lindquist J, et al. NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. J Clin Invest 2003;112:1383–94.
[23] Winau F, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, et al. Ito cells are liver-resident antigen-presenting cells for activating T cell responses. Immunity 2007;26:117–29.
[24] Asahina K, Tsai SY, Li P, Ishii M, Maxson Jr RE, Sucov HM, et al. Mesenchymal origin of hepatic stellate cells, submesothelial cells, and perivascular mesenchymal cells during mouse liver development. Hepatology 2009;49: 998–1011.
[25] Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, et al. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol 2008;26:431–42.
[26] Moreno M, Gonzalo T, Kok RJ, Sancho-Bru P, van Beuge M, Swart J, et al. Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats. Hepatology 2010;51:942–52.
[27] Tuchweber B, Desmouliere A, Bochaton-Piallat ML, Rubbia-Brandt L, Gabbiani G. Proliferation and phenotypic modulation of portal fibroblasts in the early stages of cholestatic fibrosis in the rat. Lab Invest 1996;74:265–78.
[28] Guyot C, Lepreux S, Combe C, Doudnikoff E, Bioulac-Sage P, Balabaud C, et al. Hepatic fibrosis and cirrhosis: the (myo) fibroblastic cell subpopulations involved. Int J Biochem Cell Biol 2006;38:135–51.
[29] Kinnman N, Francoz C, Barbu V, Wendum D, Rey C, Hultcrantz R, et al. The myofibroblastic conversion of peribiliary fibrogenic cells distinct from hepatic stellate cells is stimulated by platelet-derived growth factor during liver fibrogenesis. Lab Invest 2003;83:163–73.
*[30] Wells RG, Kruglov E, Dranoff JA. Autocrine release of TGF-beta by portal fibroblasts regulates cell growth. FEBS Lett 2004;559:107–10.
[31] Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, et al. Rat liver myofibroblasts and hepatic stellate cells: different cell populations of the fibroblast lineage with fibrogenic potential. Gastroenterology 1999;117:1205–21.
[32] Dudas J, Mansuroglu T, Batusic D, Saile B, Ramadori G. Thy-1 is an in vivo and in vitro marker of liver myofibroblasts. Cell Tissue Res 2007;329:503–14.
[33] Yovchev MI, Zhang J, Neufeld DS, Grozdanov PN, Dabeva MD. Thymus cell antigen-1-expressing cells in the oval cell compartment. Hepatology 2009;50:601–11.
[34] Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1994;1:71–81.
[35] Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. J Immunol 2001;166:7556–62.
[36] Strieter RM, Gomperts BN, Keane MP. The role of CXC chemokines in pulmonary fibrosis. J Clin Invest 2007;117: 549–56.
[37] Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: collagen-secreting cells of the peripheral blood. Int J Biochem Cell Biol 2004;36:598–606.
[38] Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab Invest 2007;87:858–70.

[39] Strieter RM, Keeley EC, Hughes MA, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. J Leukoc Biol; 2009.
[40] Roderfeld M, Rath T, Voswinckel R, Dierkes C, Dietrich H, Zahner D, et al. Bone marrow transplantation demonstrates medullar origin of CD34+ fibrocytes and ameliorates hepatic fibrosis in Abcb4-/- mice. Hepatology 51: 267–276.
*[41] Kisseleva T, Uchinami H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, et al. Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. J Hepatol 2006;45:429–38.
[42] Forbes SJ, Russo FP, Rey V, Burra P, Rugge M, Wright NA, et al. A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. Gastroenterology 2004;126:955–63.
[43] Russo FP, Alison MR, Bigger BW, Amofah E, Florou A, Amin F, et al. The bone marrow functionally contributes to liver fibrosis. Gastroenterology 2006;130:1807–21.
[44] Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. J Clin Invest 2004;113:243–52.
[45] Kallis YN, Forbes SJ. The bone marrow and liver fibrosis: friend or foe? Gastroenterology 2009;137:1218–21.
[46] Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. FASEB J 2004;18:980–2.
[47] Bartsch K, Al-Ali H, Reinhardt A, Franke C, Hudecek M, Kamprad M, et al. Mesenchymal stem cells remain host-derived independent of the source of the stem-cell graft and conditioning regimen used. Transplantation 2009;87:217–21.
[48] Short BJ, Brouard N, Simmons PJ. Prospective isolation of mesenchymal stem cells from mouse compact bone. Methods Mol Biol 2009;482:259–68.
[49] Simmons PJ, Przepiorka D, Thomas ED, Torok-Storb B. Host origin of marrow stromal cells following allogeneic bone marrow transplantation. Nature 1987;328:429–32.
[50] Zavadil J, Bitzer M, Liang D, Yang YC, Massimi A, Kneitz S, et al. Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. Proc Natl Acad Sci U S A 2001;98:6686–91.
[51] Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest 2009;119:1429–37.
[52] Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 2002;110:341–50.
[53] Nitta T, Kim JS, Mohuczy D, Behrns KE. Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling pathways. Hepatology 2008;48:909–19.
[54] Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, et al. Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. J Biol Chem 2007;282:23337–47.
[55] Omenetti A, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, et al. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. J Clin Invest 2008;118:3331–42.
[56] Syn WK, Jung Y, Omenetti A, Abdelmalek M, Guy CD, Yang L, et al. Hedgehog-Mediated epithelial-to-mesenchymal transition and fibrogenic repair in nonalcoholic fatty liver disease. Gastroenterology; 2009.
[57] Seki E, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, et al. CCR2 promotes hepatic fibrosis in mice. Hepatology 2009;50:185–97.
[58] Subeq YM, Ke CY, Lin NT, Lee CJ, Chiu YH, Hsu BG. Valsartan decreases TGF-beta1 production and protects against chlorhexidine digluconate-induced liver peritoneal fibrosis in rats. Cytokine 2011;53:223–30.
[59] He H, Mennone A, Boyer JL, Cai SY. Combination of retinoic acid and ursodeoxycholic acid attenuates liver injury in bile duct-ligated rats and human hepatic cells. Hepatology 2011;53(2):548–57.
[60] Szuster-Ciesielska A, Plewka K, Daniluk J, Kandefer-Szerszen M. Betulin and betulinic acid attenuate ethanol-induced liver stellate cell activation by inhibiting reactive oxygen species (ROS), cytokine (TNF-alpha, TGF-beta) production and by influencing intracellular signaling. Toxicology 2011;280(3):152–63.
[61] Liu Y, Wang Z, Kwong SQ, Lui EL, Friedman SL, Li FR, et al. Inhibition of PDGF, TGF-beta and Abl signaling and reduction of liver fibrosis by the small molecule Bcr-Abl tyrosine kinase antagonist Nilotinib. J Hepatol.
[62] Giannelli G, Mazzocca A, Fransvea E, Lahn M, Antonaci S. Inhibiting TGF-beta signaling in hepatocellular carcinoma. Biochim Biophys Acta; 2010.
[63] Baek JY, Morris SM, Campbell J, Fausto N, Yeh MM, Grady WM. TGF-beta inactivation and TGF-alpha overexpression cooperate in an in vivo mouse model to induce hepatocellular carcinoma that recapitulates molecular features of human liver cancer. Int J Cancer 2010;127:1060–71.
[64] Trobridge P, Knoblaugh S, Washington MK, Munoz NM, Tsuchiya KD, Rojas A, et al. TGF-beta receptor inactivation and mutant Kras induce intestinal neoplasms in mice via a beta-catenin-independent pathway. Gastroenterology 2009; 136:1680–8. e7.
[65] Arteaga CL, Dugger TC, Hurd SD. The multifunctional role of transforming growth factor (TGF)-beta s on mammary epithelial cell biology. Breast Cancer Res Treat 1996;38:49–56.
[66] De Minicis S, Brenner DA. NOX in liver fibrosis. Arch Biochem Biophys 2007;462:266–72.
[67] Yang L, Bataller R, Dulyx J, Coffman TM, Gines P, Rippe RA, et al. Attenuated hepatic inflammation and fibrosis in angiotensin type 1a receptor deficient mice. J Hepatol 2005;43:317–23.
[68] Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, et al. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. Hepatology 2004;40:1222–5.
[69] Molteni A, Heffelfinger S, Moulder JE, Uhal B, Castellani WJ. Potential deployment of angiotensin I converting enzyme inhibitors and of angiotensin II type 1 and type 2 receptor blockers in cancer chemotherapy. Anticancer Agents Med Chem 2006;6:451–60.
[70] Schuppan D, Popov Y. Rationale and targets for antifibrotic therapies. Gastroenterol Clin Biol 2009;33:949–57.
*[71] Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13:1324–32.
[72] Isayama F, Hines IN, Kremer M, Milton RJ, Byrd CL, Perry AW, et al. LPS signaling enhances hepatic fibrogenesis caused by experimental cholestasis in mice. Am J Physiol Gastrointest Liver Physiol; 2006.
[73] Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, et al. Vascular endothelial growth factor and receptor interaction is a prerequisite for murine hepatic fibrogenesis. Gut 2003;52:1347–54.

[74] Tugues S, Fernandez-Varo G, Munoz-Luque J, Ros J, Arroyo V, Rodes J, et al. Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and portal pressure in cirrhotic rats. Hepatology 2007;46:1919–26.
*[75] Soto-Gutierrez A, Navarro-Alvarez N, Yagi H, Yarmush ML. Stem cells for liver repopulation. Curr Opin Organ Transplant 2009;14:667–73.
[76] Forbes SJ. Stem cell therapy for chronic liver disease–choosing the right tools for the job. Gut 2008;57:153–5.
[77] Fitzpatrick E, Mitry RR, Dhawan A. Human hepatocyte transplantation: state of the art. J Intern Med 2009;266:339–57.
[78] Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis 1999; 19:39–48.
[79] Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation 1994;58:951–2.
[80] Sterling RK, Fisher RA. Liver transplantation. Living donor, hepatocyte, and xenotransplantation. Clin Liver Dis 2001;5: 431–60 [vii].
[81] Quaglia A, Lehec SC, Hughes RD, Mitry RR, Knisely AS, Devereaux S, et al. Liver after hepatocyte transplantation for liver-based metabolic disorders in children. Cell Transplant 2008;17:1403–14.
[82] Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. J Inherit Metab Dis 2006;29:431–5.
[83] Wu YM, Joseph B, Berishvili E, Kumaran V, Gupta S. Hepatocyte transplantation and drug-induced perturbations in liver cell compartments. Hepatology 2008;47:279–87.
[84] Kumaran V, Joseph B, Benten D, Gupta S. Integrin and extracellular matrix interactions regulate engraftment of transplanted hepatocytes in the rat liver. Gastroenterology 2005;129:1643–53.
[85] Hughes RD, Mitry RR, Dhawan A. Hepatocyte transplantation for metabolic liver disease: UK experience. J R Soc Med 2005;98:341–5.
[86] Terry C, Mitry RR, Lehec SC, Muiesan P, Rela M, Heaton ND, et al. The effects of cryopreservation on human hepatocytes obtained from different sources of liver tissue. Cell Transplant 2005;14:585–94.
[87] Bumgardner GL, Orosz CG. Unusual patterns of alloimmunity evoked by allogeneic liver parenchymal cells. Immunol Rev 2000;174:260–79.
[88] am Esch 2nd JS, Knoefel WT, Klein M, Ghodsizad A, Fuerst G, Poll LW, et al. Portal application of autologous CD133+ bone marrow cells to the liver: a novel concept to support hepatic regeneration. Stem Cells 2005;23:463–70.
[89] Gordon MY, Levicar N, Pai M, Bachellier P, Dimarakis I, Al-Allaf F, et al. Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells 2006;24:1822–30.
[90] Gaia S, Smedile A, Omede P, Olivero A, Sanavio F, Balzola F, et al. Feasibility and safety of G-CSF administration to induce bone marrow-derived cells mobilization in patients with end stage liver disease. J Hepatol 2006;45:13–9.
[91] Mercer AE, Regan SL, Hirst CM, Graham EE, Antoine DJ, Benson CA, et al. Functional and toxicological consequences of metabolic bioactivation of methapyrilene via thiophene S-oxidation: induction of cell defence, apoptosis and hepatic necrosis. Toxicol Appl Pharmacol 2009;239:297–305.
[92] Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M, et al. Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure. J Gastroenterol Hepatol 2009;24:70–7.
[93] Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, et al. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology 2008;134:2111–21. 21 e1–3.
[94] Abdel Aziz MT, Atta HM, Mahfouz S, Fouad HH, Roshdy NK, Ahmed HH, et al. Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. Clin Biochem 2007;40:893–9.
[95] Sgodda M, Aurich H, Kleist S, Aurich I, Konig S, Dollinger MM, et al. Hepatocyte differentiation of mesenchymal stem cells from rat peritoneal adipose tissue in vitro and in vivo. Exp Cell Res 2007;313:2875–86.
[96] Yagi H, Parekkadan B, Suganuma K, Soto-Gutierrez A, Tompkins RG, Tilles AW, et al. Long-term superior performance of a stem cell/hepatocyte device for the treatment of acute liver failure. Tissue Eng Part A 2009;15:3377–88.
[97] van Poll D, Parekkadan B, Cho CH, Berthiaume F, Nahmias Y, Tilles AW, et al. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. Hepatology 2008;47:1634–43.
[98] Thorgeirsson SS, Grisham JW. Hematopoietic cells as hepatocyte stem cells: a critical review of the evidence. Hepatology 2006;43:2–8.
[99] Alison MR, Islam S, Lim S. Stem cells in liver regeneration, fibrosis and cancer: the good, the bad and the ugly. J Pathol 2009;217:282–98.
[100] Kharaziha P, Hellstrom PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. Eur J Gastroenterol Hepatol 2009;21:1199–205.
[101] Ramachandran P, Iredale JP. Reversibility of liver fibrosis. Ann Hepatol 2009;8:283–91.
*[102] Hammel P, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Flejou JF, et al. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. N Engl J Med 2001;344:418–23.
[103] Arthur MJ. Reversibility of liver fibrosis and cirrhosis following treatment for hepatitis C. Gastroenterology 2002;122: 1525–8.
[104] Kweon YO, Goodman ZD, Dienstag JL, Schiff ER, Brown NA, Burchardt E, et al. Decreasing fibrogenesis: an immunohistochemical study of paired liver biopsies following lamivudine therapy for chronic hepatitis B. J Hepatol 2001;35: 749–55.
[105] Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. Hepatology 2004;39:1647–54.
[106] Czaja AJ, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. J Hepatol 2004;40: 646–52.
*[107] Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998;102:538–49.

*[108] Issa R, Zhou X, Constandinou CM, et al. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. Gastroenterology 2004;126:1795–808.
[109] Issa R, Williams E, Trim N, Kendall T, Arthur MJ, Reichen J, et al. Apoptosis of hepatic stellate cells: involvement in resolution of biliary fibrosis and regulation by soluble growth factors. Gut 2001;48:548–57.
[110] Phan SH. The myofibroblast in pulmonary fibrosis. Chest 2002;122:286S–9S.
[111] Iredale JP. Hepatic stellate cell behavior during resolution of liver injury. Semin Liver Dis 2001;21:427–36.
[112] Uchinami H, Seki E, Brenner DA, D'Armiento J. Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. Hepatology 2006;44:420–9.
[113] Friedman SL, Bansal MB. Reversal of hepatic fibrosis – fact or fantasy? Hepatology 2006;43:S82–8.
[114] Varga J, Brenner D, Phan SE. Fibrosis research methods and protocols. Totowa, New Jersey: Humana Press; 2005.
*[115] Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C, et al. Senescence of activated stellate cells limits liver fibrosis. Cell 2008;134:657–67.
[116] She H, Xiong S, Hazra S, Tsukamoto H. Adipogenic transcriptional regulation of hepatic stellate cells. J Biol Chem 2005; 280:4959–67.
[117] Tsukamoto H. Fat paradox in liver disease. Keio J Med 2005;54:190–2.
[118] Tsukamoto H. Adipogenic phenotype of hepatic stellate cells. Alcohol Clin Exp Res 2005;29:132S–3S.
[119] Gaca MD, Zhou X, Issa R, Kiriella K, Iredale JP, Benyon RC. Basement membrane-like matrix inhibits proliferation and collagen synthesis by activated rat hepatic stellate cells: evidence for matrix-dependent deactivation of stellate cells. Matrix Biol 2003;22:229–39.