Comparative Hepatology

BioMed Central

Review

Open Access

Evolving concepts of liver fibrogenesis provide new diagnostic and therapeutic options

Olav A Gressner, Ralf Weiskirchen and Axel M Gressner*

Address: Institute of Clinical Chemistry and Pathobiochemistry, RWTH-University Hospital, Aachen, Germany

Email: Olav A Gressner - ogressner@ukaachen.de; Ralf Weiskirchen - rweiskirchen@ukaachen.de; Axel M Gressner* - gressner@rwth-aachen.de

* Corresponding author

Published: 30 July 2007

Comparative Hepatology 2007, **6**:7 doi:10.1186/1476-5926-6-7

Received: 30 May 2007

Accepted: 30 July 2007

This article is available from: http://www.comparative-hepatology.com/content/6/1/7

Abstract

Despite intensive studies, the clinical opportunities for patients with fibrosing liver diseases have not improved. This will be changed by increasing knowledge of new pathogenetic mechanisms, which complement the "canonical principle" of fibrogenesis. The latter is based on the activation of hepatic stellate cells and their transdifferentiation to myofibroblasts induced by hepatocellular injury and consecutive inflammatory mediators such as TGF-β. Stellate cells express a broad spectrum of matrix components. New mechanisms indicate that the heterogeneous pool of (myo-)fibroblasts can be supplemented by epithelial-mesenchymal transition (EMT) from cholangiocytes and potentially also from hepatocytes to fibroblasts, by influx of bone marrow-derived fibrocytes in the damaged liver tissue and by differentiation of a subgroup of monocytes to fibroblasts after homing in the damaged tissue. These processes are regulated by the cytokines TGF-β and BMP-7, chemokines, colony-stimulating factors, metalloproteinases and numerous trapping proteins. They offer innovative diagnostic and therapeutic options. As an example, modulation of TGF-β/BMP-7 ratio changes the rate of EMT, and so the simultaneous determination of these parameters and of connective tissue growth factor (CTGF) in serum might provide information on fibrogenic activity. The extension of pathogenetic concepts of fibrosis will provide new therapeutic possibilities of interference with the fibrogenic mechanism in liver and other organs.

Introduction

Experimental and clinical studies of the past twenty years or so provide a detailed knowledge of structure and composition of extracellular matrix (ECM) in normal and fibrotic liver tissue [1,2], of the cellular origin of the various matrix components [3], of the cytokine- and growth factor-regulated stimulation of ECM synthesis (fibrogenesis) and regulation of matrix degradation (fibrolysis) [4-6], of several genetic conditions predisposing for fibrogenesis [7,8], and of multiple, experimentally successful therapeutic approaches [9]. However, up to now the clinical benefit derived from basic research in the context of translational medicine is scarce with regard to an effective, harmless and site-directed antifibrotic therapy and approved non-invasive diagnostic measures of the activity of fibrogenesis ("grading") and/or of the extent of the fibrotic organ transition ("staging") using serum parameters [10]. The failure of clinical success boosts current research on fibrosis and fibrogenesis not only of the liver, but also of the lung, kidney, pancreas, heart, skin, bone marrow, and other organs with the result that during the last four to five years very important new insights into the pathogenesis of fibrosis and of related diagnostic and therapeutic options have been made [11]. Evolving patho-

genetic concepts supplement the so called "canonical principle" of liver fibrogenesis, which has been worked out in detail during the last twenty years and which is based, in principle, on the activation of hepatic stellate cells (HSC).

The "canonical principle" of liver fibrogenesis

Fibrosis is characterized by a severalfold increase of the extracellular matrix that comprises collagens, structural glycoproteins, sulphated proteoglycans and hyaluronan, by a histological redistribution with preferred initial matrix deposition in the subendothelial space of Disse leading to the formation of an incomplete subendothelial basement membrane creating additional diffusion barriers between hepatocytes and the liver sinusoid ("capillarization of sinusoids"), and by changes in the microstructure of collagens (*e.g.*, degree of hydroxylation of prolin and lysin), glycoproteins (variations of the carbohydrate structure) and proteoglycans (changes of the degree of sulfation of the glycosaminoglycan side chains) (Fig. 1). It is known for a long time that the increase of ECM in the parenchyma is not a passive process caused by condensation of pre-existing septa of connective tissue due to necrotic and apoptotic collapse of the parenchyma, instead, it is an active biosynthetic process, which is attributed to stimulated matrix production in portal or peribiliary fibroblasts and, in particular, in contractile myofibroblasts (MFB) localized initially in the subendothelial space of Disse. The development of MFB is the result of a multi-step sequence, which originates from liver cell necrosis induced by various noxious agents (toxic, immunologic) [12,13] (Fig. 2). As a consequence, HSC, formerly called vitamin A-storing cells, fat-storing cells, arachnocytes, and Ito-cells [14,15], and localized in the immediate vicinity of hepatocytes are activated (Fig. 3). HSC are liver pericytes, which embrace with thorn-like microprojections the endothelial cell layer of the sinusoids providing physical contact not only to sinusoidal endothelial cells, but also with the cell body to the hepatocytes [16]. HSC constitute about 1/3 of the non-parenchymal cell population (Kupffer cells, endothelial cells, HSC) and about 15% of total liver resident cells including hepatocytes. The "hepatic stellate cell index", *i. e.*, the number of HSCs per 1000 hepatocytes was estimated to be 109 in the healthy rat liver [17]. The spindle-like cell body of HSC contains multiple triglyceride-rich vacuoles, in which vitamin A metabolites (retinoids) are dissolved and stored [18]. About 85% of the vitamin A of the liver is found in HSC. Additional functions of these cells were recently discovered: they seem to play a role as antigen presenting cells (APC) [19-21], as $CD133^+$ progenitor-cells with the ability to differentiate to progenitor endothelial cells and hepatocytes suggesting important roles in liver regeneration and repair [22], they are involved in endocytosis of apoptotic parenchymal cells [23,24], in secretion of apolipoproteins, matrix metalloproteinases (MMPs), respective MMP-inhibitors (TIMPs) [25,26] and growth factors [3], in the support of liver regeneration through promotion of hepatocyte proliferation involving the neurotrophin receptor p75 [27], in regulation of angiogenesis and vascular remodelling through secretion of angiogenic factors [28], and in hemodynamic functions since activated HSC contract under stimulation by thromboxan, prostaglandin F2, angiotensin II, vasopressin, and endothelin-1 leading to sinusoidal constriction [29-32]. Some of these functions, however, are not expressed in the quiescent status of HSC, but are symptoms of their activation triggered by inflammatory mediators in consequence of liver cell damage. The activation of HSC leads to the expression of α-smooth-muscle actin and a loss of fat vacuoles combined with a decrease of retinoids, but increases their contractility and strongly their capacity to express and secrete a broad spectrum of matrix components [3]. The activation process includes proliferation and phenotypic transdifferentiation of HSC to MFB, but both processes are not causally related. In the "canonical principle" of fibrogenesis HSC-derived MFB have the core competency not only for matrix synthesis, but also for the expression and secretion of numerous pro- and anti-inflammatory cytokines and growth factors (Fig. 4). They have a highly synthetic phenotype characterized by a hypertrophic rough endoplasmic reticulum containing ribosomes necessary for the synthesis of export proteins. The mechanism of fibrogenic activation and transdifferentiation of HSC to MFB can be summarized in a three-step cascade model [33], which is initialized by the pre-inflammatory phase due to direct paracrine activation of HSC by necrotic (apoptotic?) hepatocytes with release of activating cytokines supplemented by a loss of mito-inhibitory cell surface heparan sulfate [34-38]. The growth promoting activity of hepatocytes, partially due to IGF-1 and respective IGF-binding proteins [13], is released from damaged cells and parallels the elevation of lactate dehydrogenase and aspartate aminotransferase as known leakage enzymes of hepatocytes [39]. In the following inflammatory phase, the pre-activated HSC are further stimulated in a paracrine mode by invaded leucocytes and thrombocytes [40], but also by activated Kupffer cells [36,41-44], sinusoidal endothelial cells and hepatocytes [13,34,37] to transdifferentiate to MFB. The consecutive postinflammatory phase is characterized by the secretion of stimulating cytokines from MFB and interacting matrix components. Some of these cytokines can stimulate in an autocrine way MFB and in a paracrine mode resting HSC. Thus, the postinflammatory phase contributes significantly to the perpetuation of the fibrogenic process, even after elimination or reduction of the pre-inflammatory and inflammatory phases. Activation and transdifferentiation of HSC is the result of extensive interactions with liver-resident and non-resident cells (Fig. 5). Most rele-

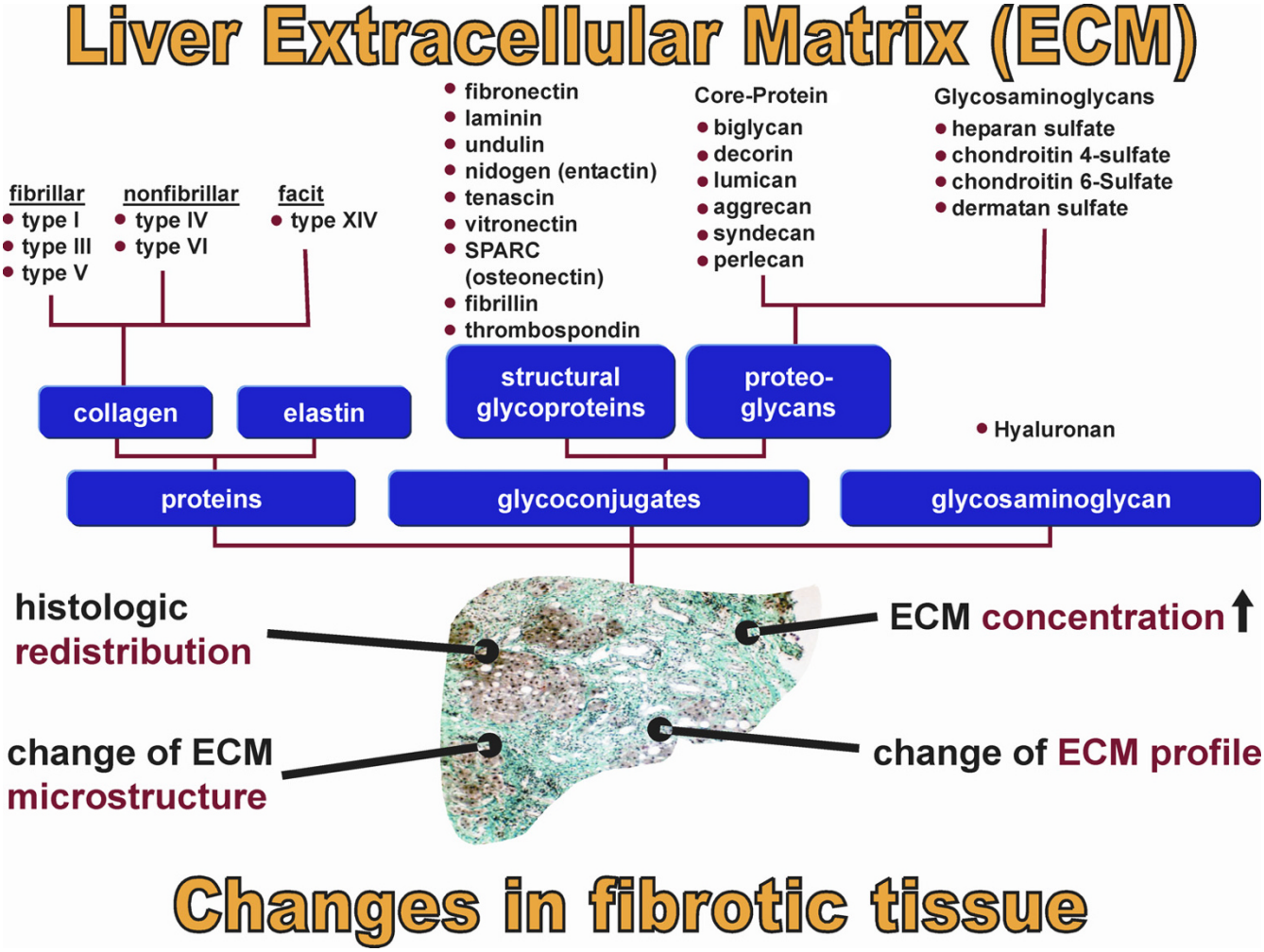

Figure 1
Matrix elements and fibrotic changes. Major components of the extracellular matrix (connective tissue) of the liver and the four most important changes in the fibrotic matrix.

vant cellular mediators are reactive oxygen species (hydroxyl radicals, oxygen radicals, superoxide anions, hydrogen peroxide) produced by activated Kupffer cells [41,45], the stimulated NAD(P)H oxidase activity of HSC [46] phagocytosing apoptotic bodies [24], the cytochrome P4502E1 (CYP2E1) pathway of ethanol-metabolizing hepatocytes [47], and leucocytes [48]. In addition, acetaldehyde of ethanol-exposed hepatocytes [49-52] and tissue hypoxia [53] promote the activation of HSC. Among the peptide mediators transforming growth factor (TGF)-β turned out to be the fibrogenic master cytokine [54-56]. Additional cytokines and growth factors involved in fibrogenesis are platelet-derived growth factor B and D (PDGF-B and PDGF-D), endothelin-1, several fibroblast growth factors (FGFs), insulin-like growth factor I, tumor necrosis factor (TNF)-α, adipocytokines (leptin, adiponectin), and others, which are partly bound as "crinopectins" [57] to the extracellular matrix [58]. The matrix serves as a sponge for several of these growth factors fixed in a covalent or non-covalent manner to fibronectin, proteoglycans and collagens. TGF-β, which is secreted in a high molecular (large) latent form (Fig. 6) by HSC/MFB, sinusoidal endothelial cells, and Kupffer cells and released by destructed thrombocytes and hepatocytes [59,60] initiates not only the activation of HSC to MFB, but also enhances matrix gene expression, decreases their degradation by down-modulation of matrix metalloproteinases and up-regulation of specific inhibitors (tissue inhibitors of metalloproteinases, TIMPs), induces apoptosis of hepatocytes [61,62], and inhibits (together with

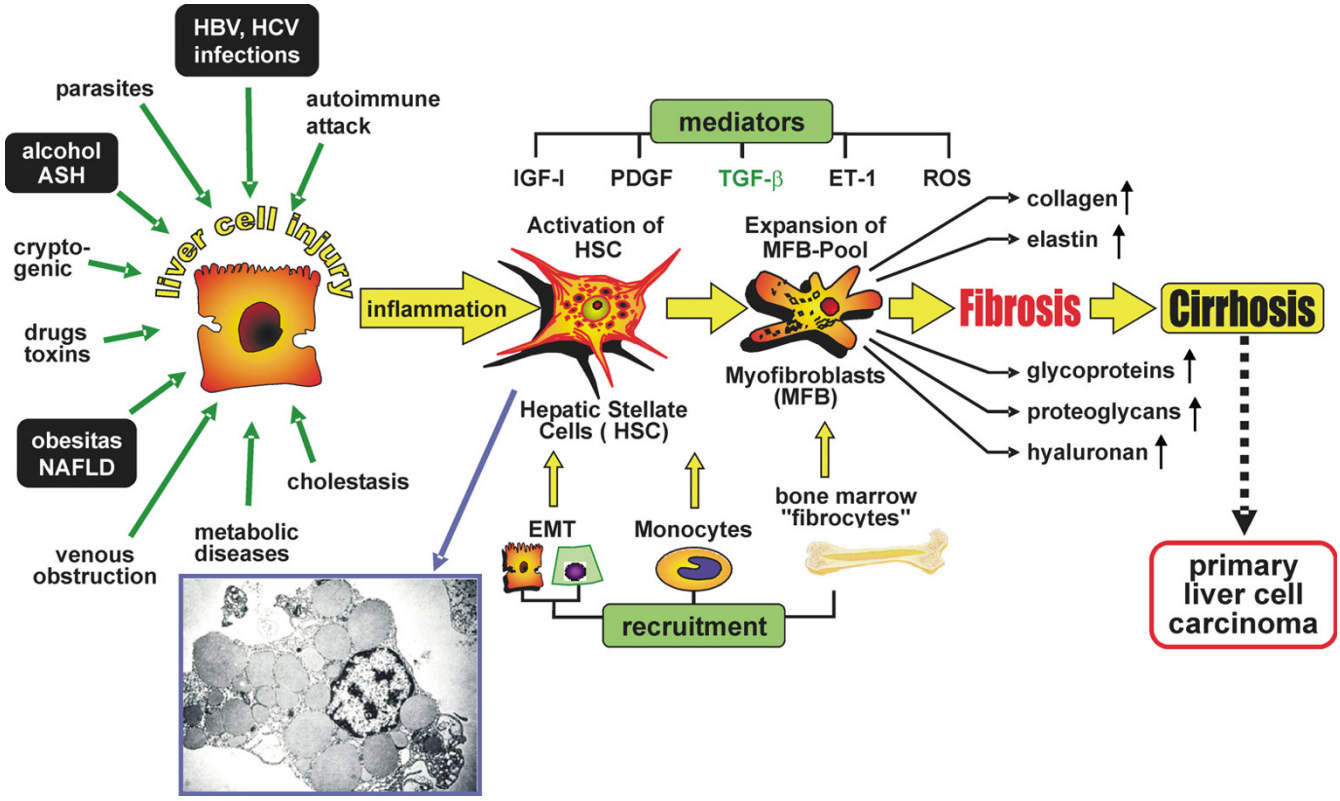

Figure 2

Formal pathogenesis of liver fibrosis (fibrogenesis). The "canonical principle" of fibrogenesis starts with necrosis or apoptosis of hepatocytes and inflammation-connected activation of hepatic stellate cells (HSC triggering), their transdifferentiation to myofibroblasts with enhanced expression and secretion of extracellular matrix and matrix deposition (fibrosis). The latter is a precondition for cirrhosis. New pathogenetic mechanisms concern the influx of bone marrow-derived cells (fibrocytes) and of circulating monocytes and their TGF-β driven differentiation to fibroblasts in the damaged liver tissue. A further new mechanism is epithelial-mesenchymal transition (EMT) of bile duct epithelial cells and potentially of hepatocytes. All three complementary mechanisms enlarge the pool of matrix-synthesizing (myo-)fibroblasts in the damaged liver. The most important fibrogenic mediators are transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), endothelin-1 (ET-1), and reactive oxygen species (ROS including hydroxyl radicals, superoxid anions). Abbreviations: ASH – alcoholic steatohepatitis; NAFLD – non-alcoholic fatty liver disease. Inset shows an electron micrograph of HSC with numerous lipid droplets indenting the nucleus.

activin A) liver cell proliferation [63,64]. Extracellular activation of latent TGF-β by proteases, oxygen radicals, thrombospondin type I, and $\alpha_v\beta_1$, $\alpha_1\beta_6$ integrins is an important step in the regulation of TGF-β bioavailability [65]. Antagonism of TGF-β [66] or inhibition of its intracellular Smad-signaling cascade by specific inhibitors [67] leads to a significant retardation of HSC activation and thus to a sustained antifibrotic effect. Interestingly, TGF-β response and signalling are modulated during transdifferentiation of HSC to MFB leading to their partial TGF-β insensitivity [68]. This observation suggests a role of TGF-β in the initiation of HSC activation *in vivo* but not a TGF-β requirement for the entire transdifferentiation process [69]. The activation of HSC to MFB in the chronically inflamed liver is partially mimicked by primary cultures of HSC, if these cells are plated on plastic surfaces instead of extracellular matrices with no possibility of integrin anchorage [70]. The model was previously suggested as a valuable tool for studying the role of HSC in chronic liver disease [71]. Accordingly, this cell culture system is quite extensively used for testing of potentially antifibrotic drugs, *e.g.*, PPAR-γ agonists [72], trichostatin A, pirfenidone, halofuginone, scavengers of reactive oxygen species (α-tocopherol, resveratrol, quercetin, curcumine), protease inhibitors, and others. However, a comparison of the gene expression profiles of HSC activated *in vivo* by bile-duct ligation or CCl_4-injury with that of culture activated HSC could establish major differences [73]. Thus, culture activation does not properly reflect genetic reprogramming of disease-driven HSC activation. Factors in the microenvironment such as Kupffer cells and lipopolysaccharides were identified to be relevant for the observed

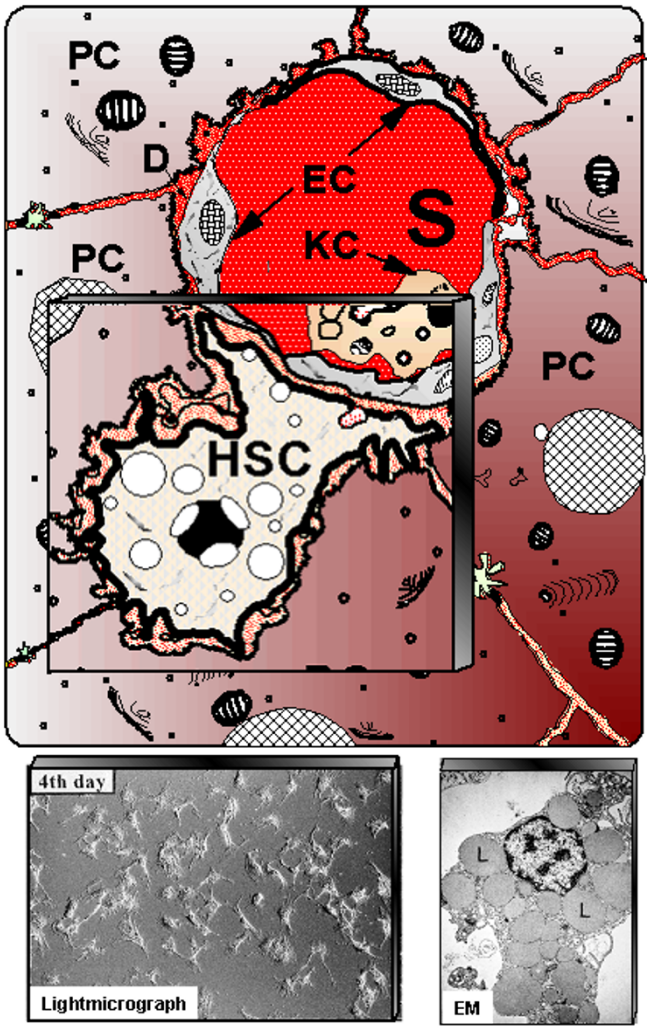

Figure 3
Schematic presentation of hepatic stellate cells (HSC) located in the vicinity of adjacent hepatocytes (PC) beneath the sinusoidal endothelial cells (EC). S – liver sinusoids; KC – Kupffer cells. Down left shows cultured HSC at light-microscopy, whereas at down right electron microscopy (EM) illustrates numerous fat vacuoles (L) in a HSC, in which retinoids are stored.

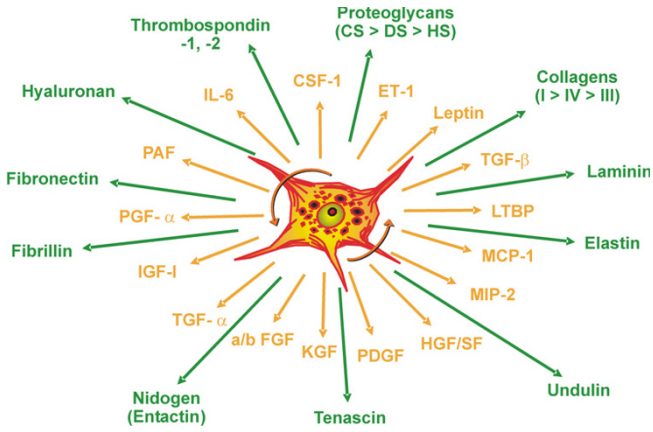

Figure 4
Compilation of the most important components of extracellular matrix and of mediators synthesized by activated hepatic stellate cells (HSC). Abbreviations: CF – colony-stimulating factor; ET – endothelin; HGF – hepatocyte growth factor; IGF – insulin-like growth factor; KGF – keratinocyte growth factor; LTBP – latent TGF-β binding protein; MCP – monocyte chemotactic peptide; MIP – macrophage inflammatory protein; PAF – platelet activating factor; PDGF – platelet-derived growth factor; PGF – prostaglandin F; SF – scatter factor; TGF – transforming growth factor.

differences [73]. Due to morphological and functional intralobular (zonal) heterogeneity of HSC [74-76], the processes of activation and transdifferentiation *in situ* are slightly different, which is also dependent on the different zonal vulnerability of hepatocytes. Accordingly, perivenous hepatocytes around the central vein (acinus zone 3) are the most sensitive and fibrogenesis, *e. g.*, in alcoholic liver injury, starts here first [77]. The heterogeneity of HSC or MFB is not confined to their topographic localization, but can also result from their origin, in particular since morphological and functional criteria and the response to growth factors point to different sources of origin of MFB [78]. As an example, HSC express the cytoskeleton proteins glial fibrillary acidic protein and desmin, which are absent in MFB and the matrix protein reelin. MFB, however, almost exclusively synthesize the matrix protein fibulin [79,80]. Using a dual reporter gene transgenic mouse model of secondary biliary fibrosis (bile duct ligation) it could be shown that peribiliary, parenchymal and vascular fibrogenic cells expressed both transgenes (α-smooth muscle actin and collagen α_1 (I), respectively) differentially indicating functional heterogeneity [81]. Taken together, there is considerable uncertainty on the relation between HSC and MFB suggesting several distinct myofibroblast-like cell types. Their composition and functional role might be dependent on the nature of the underlying disorder [82].

Contribution of bone marrow-derived cells to hepatic stellate cells, myofibroblasts, and fibroblasts in fibrotic liver tissue

Several studies have pointed to the bone marrow as a source of immature, multipotent cells in various organs. Bone marrow cells have the capacity to differentiate to hepatocytes, cholangiocytes, sinusoidal endothelial cells, and Kupffer cells, if the adequate micro-environment of the liver is present [83,84]. This phenomenon is of great importance for regenerative medicine (*e.g.*, bone marrow stem cell therapy). It was recently extended for HSC and (myo-)fibroblasts under experimental and clinical condi-

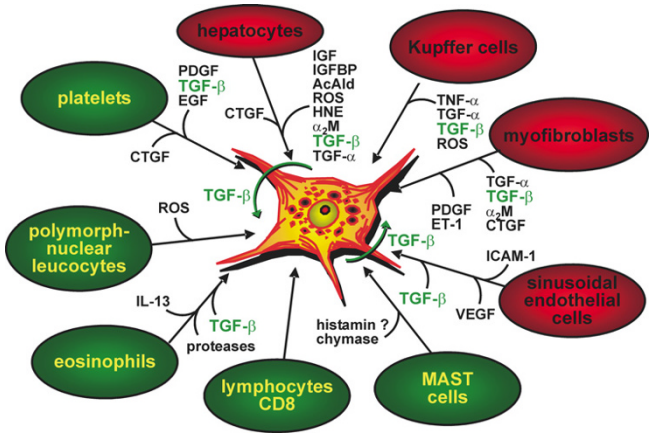

Figure 5

Cellular interactions. Synopsis of cellular interactions of resident liver cells (red) and immigrated inflammatory cells (green) with hepatic stellate cells in the process of activation and transdifferentiation to myofibroblasts. The most important paracrine mediators are given.

tions. By transplantation of genetically tagged bone marrow or of male bone marrow (Y-chromosome) to female mice, it was demonstrated that up to 30% of HSC in the liver originate from the bone marrow and acquire the MFB phenotype under injurious conditions [85]. Another study indicates that up to 68% of HSC and 70% of MFB in CCl_4-cirrhotic mice liver derive from the bone marrow [86]. Even in human liver fibrosis a significant contribution of bone marrow cells to the population of MFB was proven, but it is presently unclear which type of specific bone marrow cells or mesenchymal stem cells is relevant for the generation of hepatic (myo-) fibroblasts [87]. Another experimental study shows that myelogenic fibrocytes are present in the liver, which can be differentiated by TGF-β to collagen-producing MFB [88]. They are a subpopulation of circulating leucocytes, which display a unique surface phenotype with $CD45^+$ (haematopoietic origin), $CD34^+$ (progenitor cell), and type I collagen$^+$ (capability of matrix synthesis) [89], and exhibit potent immuno-stimulatory activities [90]. Fibrocytes represent a systemic source of contractile MFB in various fibrotic lesions, such as lung, keloids, scleroderma, and fibrotic changes of the kidney [91]. The mobilization of bone marrow cells and their recruitment into the damaged tissue is a general mechanism of tissue fibrosis and wound healing [92], which is most likely regulated by colony-stimulating factors (CSF), such as granulocyte-CSF (G-CSF) [93]. This mediator together with chemokines regulate the migration of bone marrow cells to sites of tissue injury, but also the efflux from the bone marrow into the circulation [90]. Activated HSC probably play an important role since these cells secrete a broad spectrum of inflammatory mediators (chemokines, M-CSF, SCF, PAF) and leukocyte adhesion molecules (ICAM-1, VCAM-1, NCAM) required for recruitment, activation, and maturation of blood-born cells at the site of injury [94]. The homing of myelogenic cells in the damaged liver was claimed to also have a positive effect on the resolution of liver fibrosis, since these cells express matrix metalloproteinases, which augment the degradation of fibrotic extracellular matrix [93].

Contribution of peripheral blood cells to (myo-)fibroblasts of the liver

Recent studies indicate a highly developed multi-differentiation potential of a subgroup of circulating blood monocytes, which can be recruited quickly for tissue repair processes [95]. In addition, the content of circulating myelogenic stem cells in the blood is suggested to be important for regenerative mechanisms in consequence of ischemic and degenerative diseases (*i.e.*, myocardial infarction). Investigations over the last years have proven that peripheral blood monocytes can be differentiated *in vitro* to hepatocyte-like cells if they are exposed with macrophage-colony stimulating factor (M-CSF) and specific interleukins (monocyte-derived neo-hepatocytes) [96,97]. Although for liver fibrogenesis not yet proven, subgroups of monocytes can differentiate into fibroblast-like cells (fibrocytes) after entering the damaged tissue. There they participate in fibrotic processes, *e.g.*, of the lung and kidney. The differentiation is positively influenced by G-CSF, M-CSF, monocyte chemotactic peptide 1 (MCP-1), and other chemokines and haematopoietic growth and differentiation factors, which are expressed and secreted by activated HSC [28,98-100] and other liver cell types [101]. It is of interest that very recently an inhibitory effect of the acute-phase protein serum amyloid P (SAP) on the process of differentiation of monocytes to fibrocytes could be established [102] and, consequently, a preventive effect of SAP-injections on the development of bleomycin-induced lung fibrosis was found [103]. C-reactive protein (CRP) failed to show an inhibitory effect on the differentiation of monocytes to fibrocytes. Since SAP is synthesized in hepatocytes, severe liver injury might facilitate the monocyte-fibrocyte differentiation process due to reduction of the inhibitory SAP. Although this mechanism is presently somewhat speculative for the liver, circulating monocytes might nonetheless be a pool for immediate repair processes of liver damage. Beside special monocytes as source of fibroblasts in the fibrotic liver, circulating stem cells have to be considered, which are $CD34^+$ and $CXCR4^+$ (a chemokine receptor) [95]. G-CSF and the stromal derived factor (SDF)-1 are probably the most important regulators of stem cell mobilisation from bone-marrow and their integration into the damaged tissue followed by differentiation to fibroblasts and other cells.

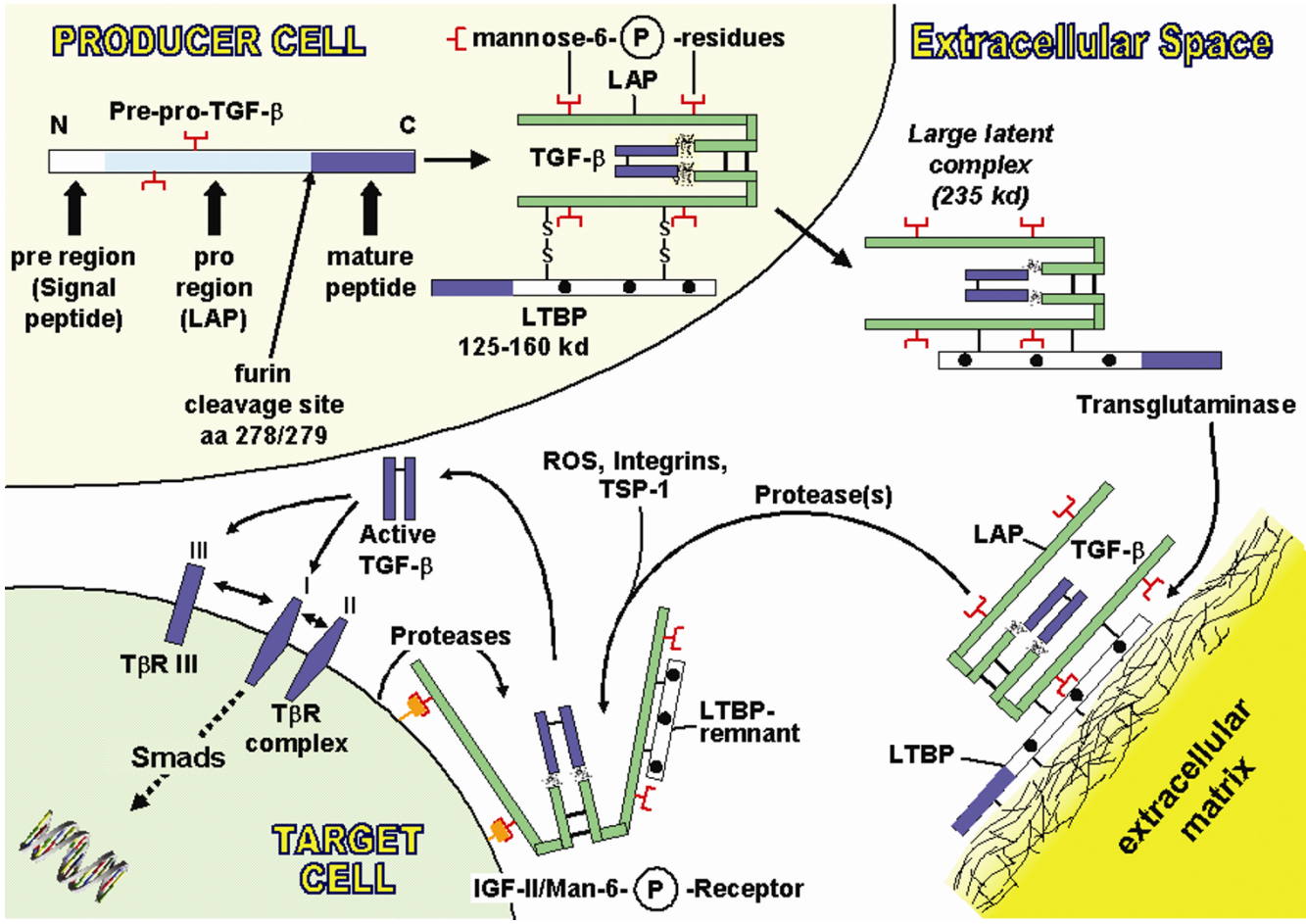

Figure 6

Extracellular matrix and TGF-β. Schematic presentation of intracellular TGF-β synthesis, secretion and extracellular immobilization via transglutaminase-dependent fixation of the large latent TGF-β binding protein (LTBP) to extracellular matrix, release by proteases and activation of the latent TGF-β complex by reactive oxygen species (ROS), specific integrins, thrombospondin-1 (TSP-1) or proteases with release of the active TGF-β homodimer, which binds to TGF-β receptors (TβR) III, II, and I to initiate the intracellular signalling cascade of Smad phosphorylation. Regulation of TGF-β occurs at the transcriptional level and, most importantly, by extracellular activation. LAP – latency associated peptide.

Epithelial-mesenchymal transition (EMT)

Beside activation and transdifferentiation of HSC, a cell type, which is developmentally most likely derived from the *septum transversum* mesenchyme, from endoderm or from the mesothelial liver capsule [104], an increasing number of experimental studies points to an additional mechanism for the enlargement of the resident (local) pool of fibroblasts during the fibrotic reaction of the damaged organs, *e.g.*, in kidney and lung [105]. This process, termed epithelial-mesenchymal transition (EMT), is well known in the context of embryonic development, but is now discussed as an important mechanism in the generation of fibroblasts during fibrogenesis in adult tissues [106] (Fig. 7). It was proven that in fibrotic kidney disease tubulus epithelial cells can transdifferentiate to fibroblasts expressing the fibroblast-specific protein 1 (FSP-1), also known as S100A4 calcium-binding protein, and are able to express collagens [106]. Similarly, alveolar epithelial cells of the lung are subject to EMT and also cardial endothelial cells can switch to fibroblasts under conditions of damage (mesenchymal-mesenchymal transition). It is estimated that in the kidney about 66% of fibroblasts are the result of EMT, in the heart the number climbs to about 20% (R. Kalluri, personal communication). *In* vitro and *in* vivo observations made in blood vessels following sustained inflammation support a hypothesis that endothelial cell transformation to myofibroblast-like cells may explain the increase of matrix proteins and of MFB pathognomonic of fibrotic diseases [107]. Very recent studies have also discussed EMT in liver fibrogenesis, after a transition of albumin-positive hepatocytes to FSP-1 positive and albumin-negative fibroblasts was shown. Pre-

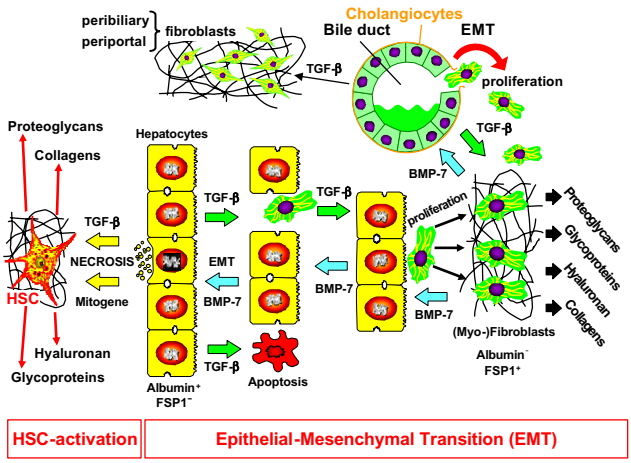

Figure 7
Up-to-date mechanisms of fibrogenesis. HSC activation, EMT, influx of fibrocytes, and differentiation of peripheral monocytes to fibroblasts at sites of injury. (Explanation is in the text).

liminary studies claim that about 40% of hepatic fibroblasts derive from hepatocytes, but these data need further confirmation (R. Kalluri, personal communication). A very recent report provides evidence for EMT of mature mouse hepatocytes *in vitro* and of the mouse hepatocyte cell line AML12 [108]. The EMT-state was indicated by strong up-regulation of α_1(I) collagen mRNA expression and type I collagen deposition. Thus, hepatocytes are capable of EMT changes and type I collagen synthesis. A further source of EMT are cholangiocytes (bile duct epithelial cells). In primary biliary cirrhosis (PBC) it was proven that bile duct epithelial cells express FSP-1 (S100A4) and vimentin as early markers of fibroblasts [109]. The bidirectional consequence of EMT of cholangiocytes are ductopenia (reduction of bile ducts) and enlargement of the pool of portal fibroblasts, which significantly contributes to portal fibrosis. *In vitro* studies with cultured human cholangiocytes have confirmed the clinical observations described. Thus, EMT proves to be a general pathogenetic principle of chronic cholestatic liver diseases [110]. In addition, activation and proliferation of portal/periportal mesenchymal cells to peribiliary MFB, which are stimulated in a paracrine manner by bile duct epithelial cells via TGF-β, PDGF-BB and endothelin-1 [111] turned out to be an important pathogenetic mechanism of portal fibrosis and septa formation in cholestatic liver diseases. Indeed, only a minority of ECM-producing MFB in obstructive cholestatic injuries are derived from HSC [112,113]. This also underlines the heterogeneous origin of MFB in fibrogenesis and emphasizes the importance of the underlying fibrogenic liver disease [82].

The molecular inducers of EMT are TGF-β [106], epidermal growth factor (EGF), insulin-like growth factor (IGF)-II, and fibroblast growth factor (FGF)-2, which promote the genetic and phenotypic programming of epithelial cells to mesenchymal cells (fibroblasts). The prototype of the most powerful inducer of EMT is TGF-β. The inducing function of TGF-β for the above described mesenchymal transition of mouse hepatocytes was shown by activation of Smad2/3 phosphorylation, inhibition by Smad4 silencing using siRNA and induction of the snail transcription factor [108]. Interestingly, TGF-β induces EMT only of those hepatocytes resisting to the pro-apoptotic effects of this cytokine [114,115]. The subpopulation of surviving hepatocytes exhibits an overexpression of Snail by TGF-β conferring resistance to programmed cell death [116]. Several additional pathways are involved in the generation of apoptosis resistance, *e.g.*, proteinkinase A [114] and epidermal growth factor (EGF)/TGF-α [115]. Thus, EMT of hepatocytes is dependent on the balance between apoptotic and survival mechanisms. The process of EMT also requires the action of metalloproteinases and a TGF-β dependent down-regulation of E-cadherin both contributing to the release of epithelial cells from cell-cell and cell-basement membrane binding (Fig. 7). The most important molecular counterpart is the bone morphogenetic protein (BMP)-7, also belonging to the TGF-β superfamily. BMP-7 not only inhibits EMT, but can even induce a mesenchymal-epithelial transition (reverse EMT = MET) [117]. It has anti-apoptotic properties, anti-inflammatory and proliferation-stimulating effects [118]. BMP-7 inhibits TGF-β signalling via Smads [119], which transduce the effect of the latter cytokine from its receptor, a serine/threonine kinase, to the Smad-binding element (SBE) of respective target genes in the nucleus [120]. In addition, several trapping proteins such as the small proteoglycans decorin and biglycan, latency associated peptide (LAP), BAMBI (BMP and activin membrane-bound inhibitor), KCP (kielin-chordin-like protein), gremlin, and α_2-macroglobulin change the balance between TGF-β and BMP-7 in favour of an anti-EMT effect due to binding a neutralization of TGF-β [121]. Similarly, the important downstream-modulator protein connective tissue growth factor (CTGF/CCN2) [122], which is expressed in hepatocytes, HSC, portal fibroblasts, and cholangiocytes [123,124] changes the functional TGF-β/BMP-7 ratio [125]. CTGF is over-expressed in experimental and human liver cirrhosis [126-128], which is mediated mainly by TGF-β, but also by endothelin-1, TNF-α, vascular endothelial growth factor (VEGF), nitrogen oxide (NO), prostaglandin E2, thrombin, high glucose, and hypoxia [129]. CTGF inhibits BMP, but activates TGF-β signalling by modulation of the receptor-binding of these ligands [123]. This is supported by very recent data, which show prominent antifibrotic effects of reduction of CTGF by siRNA [130,131]. Thus, depletion of CTGF greatly attenuates the develop-

Table 1: Therapeutic and diagnostic options based on newly identified pathogenetic mechanisms of liver fibrosis

Parameter	Pathobiochemical basis	Potential serum markers of fibrosis	Therapeutic approach
TGF-β	Fibrogenic master cytokine, up-regulation in fibrotic liver; inducer of epithelial-mesenchymal transition (EMT)	Elevation by up-regulation in the fibrotic liver, release from necrotic hepatocytes and reduced hepatic clearance	Inhibition of TGF-β, blockade of intracellular signalling
BMP-7	TGF-β antagonist: anti-apoptotic; anti-inflammatory; anti-EMT	Elevation in serum, indicator of slow fibrosis?	BMP-7 or BMP-7 peptide fragments antagonize TGF-β, antifibrotic effect, stimulation of liver regeneration
TGF-β/BMP-7 Ratio	Determines epithelial-mesenchymal transition (EMT) and profibrogenic action of TGF-β	Potentially of prognostic significance for estimation of the progression rate of fibrosis (rapid versus slow fibrosis)	Modulation of the ratio by addition of recombinant BMP-7 has an antifibrotic effect
CTGF	Down-stream modulator protein of TGF-β, influences functional TGF-β/BMP-7 ratio by elevation of TGF-β and decrease of BMP-7 action	Elevation under conditions of active fibrogenesis, decrease with advancing cirrhosis and in the terminal stage without fibrogenic activity	Inhibition of CTGF expression by siRNAs or blocking with humanized monoclonal anti-CTGF antibodies (FG-3019, FibroGen); has a strong antifibrotic effect
Fibrocytes	Bone marrow-derived progenitor cells of fibroblasts increase the pool of fibroblasts in the fibrotic liver	Flow-cytometric detection of $CD34^+$, $CD45^+$, and collagen-I^+ cells in peripheral blood or buffy coat leucocytes; potential indicator of increased influx into the damaged liver tissue	Hormonal modulation of release of fibrocytes from bone marrow and integration into the liver?
G-CSF	Recruitment of bone marrow-derived cells in the circulation and stimulation of their homing in the fibrotic liver tissue	Elevated concentrations, relation to fibrogenesis not yet established	G-CSF triggered haematopoietic stem cells or G-CSF itself accelerates healing of experimental liver damage and improves the survival rate

ment of experimental liver fibrosis. Taken together, both EMT, but also MET, in special conditions even MMT (mesenchymal-mesenchymal transition, *e.g.*, vascular endothelial cells to fibroblasts), and the fine tuning of the bioactive TGF-β/BMP-7 ratio and of their adaptor- and trapping proteins offer multiple regulatory possibilities of influencing fibrogenesis. These mechanisms are known in some detail for the kidney [132], but need more experimental proof for the liver, in particular with regard to its quantitative contribution to fibrogenesis.

Options for diagnostic and therapy

Newly recognized pathogenetic mechanisms of fibrosis described above provide several innovative options for therapy of liver fibrogenesis and non-invasive diagnostic strategies (Table 1). The determination of the TGF-β/BMP-7 ratio in serum or plasma is potentially promising, since this ratio might reflect the process of EMT and, thus, at least partially the rate of progression of fibrosis. A decrease of this ratio might indicate those patients with slow progression (slow fibroser), an increase a fast progression (rapid fibroser). However, some precautions have to be considered. The cytokine ratio in the circulation might be not an accurate reflection of their activity at the immediate environment of epithelial cells and fibroblasts, respectively, and major fractions of these cytokines might be in a biologically latent form. Thus, the protein ratio does not necessarily mimic the diagnostically important activity ratio of these mediators.

The determination of CTGF in serum or plasma is suggested as a further innovative parameter of fibrogenesis, since this modulator protein is strongly up-regulated in the fibrotic liver, synthesized and secreted by parenchymal and non-parenchymal cells [124] and since the action of the profibrogenic TGF-β is stimulated but that of the antifibrogenic BMP-7 is inhibited [123]. Preliminary studies point to significantly enhanced concentrations of CTGF in blood of patients with active liver fibrogenesis [133] in contrast to advanced cirrhosis with low activity of active fibrogenesis, which is reflected by a relative decrease of serum CTGF.

The flowcytometric detection of circulating fibrocytes in blood or in buffy coat leucocytes by using $CD34^+$, $CD45^+$, and collagen I positivities as identifying markers might be a way for evaluation of their diagnostic potential. Alternatively, these antigens might be detected by amplifying their mRNA using a quantitative PCR approach. In addition, a re-evaluation of the high concentrations of G-CSF, GM-CSF, and M-CSF in serum of cirrhotic patients published previously [134] as mobilizers of bone marrow cells and fibrocytes and of their integration into the damaged liver tissue [135] might be a promising task. It should be analyzed whether a systemic elevation of the haematopoietic growth factors correlates with the activity of liver fibrogenesis.

Numerous publications discuss anti-fibrotic therapeutic strategies by inhibition of TGF-β [9,67,136-138], but the systemic application of inhibitors and consequently an overall and ubiquitous reduction of TGF-β activity will most likely have severe side effects, *i.e.*, on tumor development and progression, auto-immunopathy and degenerative diseases [139]. Therefore, the therapeutic application of recombinant human BMP-7 or functionally active BMP-7 fragments might be advantageous since BMP-7 inhibits experimental fibrosis in rats [140], stimulates liver regeneration [118], and inhibits TGF-β-driven parenchymal cell apoptosis due to its antagonism of TGF-β. Experimental trials with thioacetamide-induced rat liver fibrosis point to successful antifibrotic results [140]. Similarly, extensive studies with experimental kidney diseases prove that BMP-7 can induce MET and, thus, has regenerative and antifibrotic effects [141]. Presently, it is not known whether the positive CTGF-inhibitory experiments for suppression of experimental fibrosis [130,131] can be translated into clinical practice, but studies – in which CTGF activity is reduced by systemic application of a humanized, monoclonal, blocking antibody (F-3019), which neutralizes and accelerates the clearance of this protein [142] – are in progress and point to successful preliminary results. Pathophysiologically, the inhibitors of CTGF should have fibro-suppressive effects since the TGF-β/BMP-7 ratio is switched in favour of BMP-7. This was recently shown by inhibition of CTGF expression [130,131]. In conclusion, further intensive studies are required to translate the positive results of cell culture studies and of animal experiments into clinical application. The new pathogenetic insights justify strong optimism since the spectrum of potential approaches to interfere with the fibrogenic pathway are greatly broadened.

Conclusion

The above described changing view on the pathogenetic mechanisms of liver fibrosis clearly suggests that one has to reconsider the exclusive role of HSC in the development of fibrosis. Although some of the newly proposed fibrogenic mechanisms have to be consolidated by additional experimental evidence in vitro and in situ, they indicate the presence of distinct subpopulations of myofibroblasts/fibroblasts in fibrosing liver, of which HSC-derived fibrogenic cells are only one of several sources. Most important, the composition of (myo-)fibroblasts may vary with the etiology of fibrosis, *e.g.*, primary biliary cirrhosis might activate a pathogenetic pathway different from alcoholic fibrosis. These facts point to the important notion that results obtained with various models of experimental fibrogenesis cannot be generalized because different classes of (myo-)fibroblasts are generated by diverse pathways. Furthermore, HSC-activation in culture cannot be regarded any longer as the almost dogmatic paradigm of the liver fibrogenic mechanism as it was in the past. Since now detailed information on the molecular cascades of intracellular fibrogenic signaling is available, we have learned that several of them are modulated cell-type specifically. Therefore, it is conceivable that distinct subpopulations of fibroblasts and their transient precursor cell types respond differently to major fibrogenic cytokines, *e.g.*, TGF-β. If this is the case, the complexity of the fibrogenic mechanisms will increase strongly in the future and the experimental conditions have to be described in detail. Taken together, studies on fibrogenesis in the liver (and other organs as well) are now pushed forward a lot, hopefully resulting in new impulses for therapy and diagnosis.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

All the authors contributed equally to this work. All authors read and approved the final manuscript.

References

1. Schuppan D, Gressner AM: **Function and metabolism of collagens and other extracellular matrix proteins.** In *Oxford Textbook of Clinical Hepatology Volume 2.15*. 2nd edition. Edited by: Bircher J, Benhamou JP, McIntyre N, Rizzetto M and Rodés J. Oxford, Oxford Medical Publications; 1999:381-407.
2. Schuppan D, Ruehl M, Somasundaram R, Hahn EG: **Matrix as a modulator of hepatic fibrogenesis.** *Semin Liver Dis* 2001, **21:**351-72.
3. Gressner AM, Weiskirchen R: **Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets.** *J Cell Mol Med* 2006, **10:**76-99.
4. de Caestecker MP, Piek E, Roberts AB: **Role of transforming growth factor-beta signaling in cancer.** *J Natl Cancer Inst* 2000, **92:**1388-1402.
5. Bataller R, Brenner DA: **Liver fibrosis.** *J Clin Invest* 2005, **115:**209-18.
6. Tsukada S, Parsons CJ, Rippe RA: **Mechanisms of liver fibrosis.** *Clin Chim Acta* 2006, **364:**33-60.
7. Bataller R, North KE, Brenner DA: **Genetic polymorphisms and the progression of liver fibrosis: A critical appraisal.** *Hepatology* 2003, **37:**493-503.
8. Hillebrandt S, Wasmuth HE, Weiskirchen R, Hellerbrand C, Keppeler H, Werth A, Schirin-Sokhan R, Wilkens G, Geier A, Lorenzen J, Kohl J, Gressner AM, Matern S, Lammert F: **Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans.** *Nat Genet* 2005, **37:**835-43.
9. Rockey DC: **Antifibrotic therapy in chronic liver disease.** *Clin Gastroenterol Hepatol* 2005, **3:**95-107.
10. Gressner OA, Weiskirchen R, Gressner AM: **Biomarkers of liver fibrosis: Clinical translation of molecular pathogenesis or based on liver-dependent malfunction tests.** *Clin Chim Acta* 2007, **381:**107-13.
11. Friedman SL, Rockey DC, Bissell DM: **Hepatic fibrosis 2006: Report of the third AASLD Single Topic Conference.** *Hepatology* 2007, **45:**242-49.
12. Gressner AM: **Transdifferentiation of hepatic stellate cells (Ito cells) to myofibroblasts: A key event in hepatic fibrogenesis.** *Kidney Int* 1996, **54:**S39-S45.
13. Gressner AM, Lahme B, Brenzel A: **Molecular dissection of the mitogenic effect of hepatocytes on cultured hepatic stellate cells.** *Hepatology* 1995, **22:**1507-18.
14. Wake K: **Sternzellen in the liver: Perisinusoidal cells with special reference to storage of vitamin A.** *Amer J Anat* 1971, **132:**429-62.
15. Geerts A: **History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells.** *Semin Liver Dis* 2001, **21:**311-35.

16. Wake K: **Hepatic stellate cells: Three-dimensional structure, localization, heterogeneity and development.** *Proc Jpn Acad* 2006, **82:**155-64.
17. Marcos R, Rocha E, Henrique RMF, Monteiro RAF: **A New Approach to an Unbiased Estimate of the Hepatic Stellate Cell Index in the Rat Liver: An Example in Healthy Conditions.** *J Histochem Cytochem* 2003, **51:**1101-04.
18. Blomhoff R, Wake K: **Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis.** *Faseb J* 1991, **5:**271-77.
19. Winau F, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, Modlin RL, Liblau RS, Gressner AM, Kaufmann SHE: **Ito cells are liver-resident antigen-presenting cells for activating T cell responses.** *Immunity* 2007, **26:**117-29.
20. Maubach G, Lim MCC, Kumar S, Zhuo L: **Expression and upregulation of cathepsin S and other early molecules required for antigen presentation in activated hepatic stellate cells upon IFN-[gamma] treatment.** *Biochim Biophys Acta-Mol Cell Res* 2007, **1773:**219-31.
21. Vinas O, Bataller R, Sancho-Bru P, Gines P, Berenguer C, Enrich C, Nicolas JM, Ercilla G, Gallart T, Vives J, Arroyo V, Rodes J: **Human hepatic stellate cells show features of antigen-presenting cells and stimulate lymphocyte proliferation.** *Hepatology* 2003, **38:**919-29.
22. Kordes C, Sawitza I, Muller-Marbach A, Ale-Agha N, Keitel V, Klonowski-Stumpe H, Haussinger D: **CD133+ hepatic stellate cells are progenitor cells.** *Biochem Biophys Res Commun* 2007, **352:**410-17.
23. Canbay A, Taimr P, Torok N, Higuchi H, Friedman S, Gores GJ: **Apoptotic body engulfment by a human stellate cell line is profibrogenic.** *Lab Invest* 2003, **83:**655-63.
24. Zhan SS, Jiang JX, Wu J, Halsted C, Friedman SL, Zern MA, Torok NJ: **Phagocytosis of apoptotic bodies by hepatic stellate cells induces NADPH oxidase and is associated with liver fibrosis in vivo.** *Hepatology* 2006, **43:**435-43.
25. Benyon RC, Iredale JP, Goddard S, Winwood PJ, Arthur MJP: **Expression of tissue inhibitor of metalloproteinases 1 and 2 is increased in fibrotic human liver.** *Gastroenterology* 1996, **110:**821-31.
26. Benyon RC, Arthur MJP: **Extracellular matrix degradation and the role of hepatic stellate cells.** *Semin Liver Dis* 2001, **21:**373-84.
27. Passino MA, Adams RA, Sikorski SL, Akassoglou K: **Regulation of Hepatic Stellate Cell Differentiation by the Neurotrophin Receptor p75NTR.** *Science* 2007, **315:**1853-56.
28. Lee JS, Semela D, Iredale J, Shah VH: **Sinusoidal remodeling and angiogenesis: A new function for the liver-specific pericytes?** *Hepatology* 2007, **45:**817-25.
29. Bataller R, Gines P, Nicolas JM, Gorbig MN, Garciaramallo E, Gasull X, Bosch J, Arroyo V, Rodes J: **Angiotensin II induces contraction and proliferation of human hepatic stellate cells.** *Gastroenterology* 2000, **118:**1149-56.
30. Bataller R, Nicolas JM, Gines P, Esteve A, Gorbig MN, Garciaramallo E, Pinzani M, Ros J, Jimenez W, Thomas AP, Arroyo V, Rodes J: **Arginine vasopressin induces contraction and stimulates growth of cultured human hepatic stellate cells.** *Gastroenterology* 1997, **113:**615-24.
31. Rockey D: **Hepatic blood flow regulation by stellate cells in normal and injured liver.** *Semin Liver Dis* 2001, **21:**337-49.
32. Reynaert H, Thompson MG, Thomas T, Geerts A: **Hepatic stellate cells: role in microcirculation and pathophysiology of portal hypertension.** *Gut* 2002, **50:**571-81.
33. Gressner AM, Gao C: **A cascade-mechanism of fat storing cell activation forms the basis of the fibrogenic reaction of the liver.** *Verh Dtsch Ges Path* 1995, **79:**1-14.
34. Gressner AM, Lotfi S, Gressner G, Lahme B: **Identification and partial characterization of a hepatocyte-derived factor promoting proliferation of cultured fat storing cells (parasinusoidal lipocytes).** *Hepatology* 1992, **16:**1250-66.
35. Gressner AM, Lahme B: **Inhibitory actions of hepatocyte plasma membranes on proliferation, protein- and proteoglycan synthesis of cultured rat fat storing cells.** In *Cells of the Hepatic Sinusoid* Edited by: Wisse E, Knook DL and McCuskey RS. Vol.3; 1991:237-241.
36. Gressner AM, Lotfi S, Gressner G, Haltner E, Kropf J: **Synergism between hepatocytes and Kupffer cells in the activation of fat storing cells (perisinusoidal lipocytes).** *J Hepatol* 1993, **19:**117-32.
37. Gressner AM, Lahme B: **Treatment of rat hepatocytes with transforming growth factor-beta1 increases their mitogenic activity for cultured fat storing cells.** *Inter Hepatol Commun* 1995, **4:**94-101.
38. Roth S, Michel K, Gressner AM: **(Latent) transforming growth factor-beta in liver parenchymal cells, its injury-dependent release and paracrine effects on hepatic stellate cells.** *Hepatology* 1998, **27:**1003-12.
39. Hoffmann C, Lahme B, Brenzel A, Gressner AM: **The relation between hepatocellular damage and activation of fat storing cell proliferation in vitro.** *Inter Hepatol Commun* 1994, **2:**29-36.
40. Bachem MG, Melchior R, Gressner AM: **The role of thrombocytes in liver fibrogenesis: Effects of platelet lysate and thrombocyte-derived growth factors on the mitogenic activity and glycosaminoglycan synthesis of cultured rat liver fat storing cells.** *J Clin Chem Clin Biochem* 1989, **27:**555-65.
41. Decker K: **Biologically active products of stimulated liver macrophages (Kupffer cells).** *Eur J Biochem* 1990, **192:**245-61.
42. Friedman SL, Arthur MJP: **Kupffer cell mediated proliferation of lipocytes occurs via induction of receptors for platelet-derived growth factor - studies in rats and humans.** *Hepatology* 1989, **10:**632-632.
43. Gressner AM, Haarmann R: **Regulation of hyaluronate synthesis in rat liver fat storing cell cultures by Kupffer cells.** *J Hepatol* 1988, **7:**310-18.
44. Gressner AM, Zerbe O: **Kupffer cell-mediated induction of synthesis and secretion of proteoglycans by rat liver fat storing cells in culture.** *J Hepatol* 1987, **5:**299-310.
45. Nieto N: **Oxidative-stress and IL-6 mediate the fibrogenic effects of rodent Kupffer cells in stellate cells.** *Hepatology* 2006, **44:**1487-1501.
46. Adachi T, Togashi H, Suzuki A, Kasai S, Ito J, Sugahara K, Kawata S: **NAD(P)H oxidase plays a crucial role in PDGF-induced proliferation of hepatic stellate cells.** *Hepatology* 2005, **41:**1272-81.
47. Baroni GS, D'Ambrosio L, Ferretti G, Casini A, di Sario A, Salzano R, Ridolfi F, Saccomanno S, Jezequel AM, Benedetti A: **Fibrogenic effect of oxidative stress on rat hepatic stellate cells.** *Hepatology* 1998, **27:**720-26.
48. Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, Foschi M, Caligiuri A, Pinzani M, Surrenti C: **Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: role of nitric oxide.** *Hepatology* 1997, **25:**361-67.
49. Casini A, Cunningham M, Rojkind M, Lieber CS: **Acetaldehyde increases procollagen type I and fibronectin gene transcription in cultured rat fat storing cells through a protein synthesis-dependent mechanism.** *Hepatology* 1991, **13:**758-65.
50. Casini A, Galli G, Salzano R, Ceni E, Franceschelli F, Rotella CM, Surrenti C: **Acetaldehyde induces c-fos and c-jun proto-oncogenes in fat-storing cell cultures through protein kinase C activation.** *Alcohol Alcohol* 1994, **29(3):**303-314.
51. Anania FA, Womack L, Potter JJ, Mezey E: **Acetaldehyde enhances murine alpha(2)(I) collagen promoter activity by Ca2+-independent protein kinase C activation in cultured rat hepatic stellate cells.** *Alcohol Clin Exp Res* 1999, **23:**279-84.
52. Svegliati-Baroni G, Ridolfi F, di Sario A, Saccomanno S, Bendia E, Benedetti A, Greenwel P: **Intracellular signaling pathways involved in acetaldehyde- induced collagen and fibronectin gene expression in human hepatic stellate cells.** *Hepatology* 2001, **33:**1130-40.
53. Corpechot C, Barbu V, Wendum D, Kinnman N, Rey C, Poupon R, Housset C, Rosmorduc O: **Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis.** *Hepatology* 2002, **35:**1010-21.
54. Gressner AM, Weiskirchen R, Breitkopf K, Dooley S: **Roles of TGF-b in hepatic fibrosis.** *Front Biosci* 2002, **7:**D793-D807.
55. Gabele E, Brenner DA, Rippe RA: **Liver fibrosis: Signals leading to the amplification of the fibrogenic hepatic stellate cell.** *Front Biosci* 2003, **8:**D69-D77.
56. Inagaki Y, Okazaki I: **Emerging insights into Transforming growth factor {beta} Smad signal in hepatic fibrogenesis.** *Gut* 2007, **56:**284-92.
57. Feige JJ, Baird A: **Crinopexy: extracellular regulation of growth factor action.** *Kidney Int* 1995, **47, Suppl. 49:**S15-S18.
58. Pinzani M, Marra F: **Cytokine receptors and signaling in hepatic stellate cells.** *Semin Liver Dis* 2001, **21:**397-416.
59. Bissell DM, Wang SS, Jarnagin WR, Roll FJ: **Cell-specific expression of transforming growth factor-beta in rat liver - Evidence for autocrine regulation of hepatocyte proliferation.** *J Clin Invest* 1995, **96:**447-55.
60. Marra F, Bonewald LF, Parksnyder S, Park IS, Woodruff KA, Abboud HE: **Characterization and regulation of the latent transforming growth factor-beta complex secreted by vascular pericytes.** *J Cell Physiol* 1996, **166:**537-46.
61. Gressner AM, Polzar B, Lahme B, Mannherz HG: **Induction of rat liver parenchymal cell apoptosis by hepatic myofibroblasts**

via transforming growth factor-beta. *Hepatology* 1996, **23:**571-81.
62. Gressner AM, Lahme B, Mannherz HG, Polzar B: **TGF-beta-mediated hepatocellular apoptosis by rat and human hepatoma cells and primary rat hepatocytes.** *J Hepatol* 1997, **26:**1079-92.
63. Kiso S, Kawata S, Tamura S, Ito N, Takaishi K, Shirai Y, Tsushima H, Matsuzawa Y: **Alteration in growth regulation of hepatocytes in primary culture obtained from cirrhotic rat: Poor response to transforming growth factor-beta 1 and interferons.** *Hepatology* 1994, **20:**1303-8.
64. Bissell DM, Roulot D, George J: **Transforming growth factor b and the liver.** *Hepatology* 2001, **34:**859-67.
65. Koli K, Saharinen J, Hyytiainen M, Penttinen C, Keski-Oja J: **Latency, activation, and binding proteins of TGF-beta.** *Microsc Res Technique* 2001, **52:**354-62.
66. Arias M, Lahme B, Van de Leur E, Gressner AM, Weiskirchen R: **Adenoviral delivery of an antisense RNA complementary to the 3' coding sequence of transforming growth factor-b1 inhibits fibrogenic activities of hepatic stellate cells.** *Cell Growth Differ* 2002, **13:**265-73.
67. De Gouville AC, Huet S: **Inhibition of ALK5 as a new approach to treat liver fibrotic diseases.** *Drug News Perspect* 2006, **19:**85-90.
68. Dooley S, Delvoux B, Lahme B, Mangasser-Stephan K, Gressner AM: **Modulation of transforming growth factor beta response and signaling during transdifferentiation of rat hepatic stellate cells to myofibroblasts.** *Hepatology* 2000, **31:**1094-106.
69. Hellerbrand C, Stefanovic B, Giordano F, Burchardt ER, Brenner DA: **The role of TGF beta 1 in initiating hepatic stellate cell activation in vivo.** *J Hepatol* 1999, **30:**77-87.
70. Iwamoto H, Sakai H, Nawata H: **Inhibition of integrin signaling with Arg-Gly-Asp motifs in rat hepatic stellate cells.** *J Hepatol* 1998, **29:**752-59.
71. Geerts A, Vrijsen R, Rauterberg J, Burt A, Schellinck P, Wisse E: **In vitro differentiation of fat storing cells parallels marked increase of collagen synthesis and secretion.** *J Hepatol* 1989, **9:**59-68.
72. Sun K, Wang Q, Huang XH: **PPAR gamma inhibits growth of rat hepatic stellate cells and TGF beta-induced connective tissue growth factor expression.** *Acta Pharmacol Sin* 2006, **27:**715-23.
73. De Minicis S, Seki E, Uchinami H, Kluwe J, Zhang Y, Brenner DA, Schwabe RF: **Gene expression profiles during hepatic stellate cell activation in culture and in vivo.** *Gastroenterology* 2007, **132:**1937-46.
74. Wake K, Sato T: **Intralobular heterogeneity of perisinusoidal stellate cells in porcine liver.** *Cell Tissue Res* 1993, **273:**227-37.
75. Ballardini G, Groff P, DeGiorgi LB, Schuppan D, Bianchi FB: **Ito cell heterogeneity: Desmin-negative Ito cells in normal rat liver.** *Hepatology* 1994, **19:**440-46.
76. Zou ZZ, Ekataksin W, Wake K: **Zonal and regional differences identified from precision mapping of vitamin A-storing lipid droplets of the hepatic stellate cells in pig liver: A novel concept of addressing the intralobular area of heterogeneity.** *Hepatology* 1998, **27:**1098-108.
77. Horn T, Junge J, Christoffersen P: **Early alcoholic liver injury. Activation of lipocytes in acinar zone 3 and correlation to degree of collagen formation in Disse space.** *J Hepatol* 1986, **3:**333-40.
78. Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G: **Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: Distinct roles of (Myo-)fibroblast subpopulations in hepatic tissue repair.** *Histochem Cell Biol* 1999, **112:**387-401.
79. Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, Ramadori G: **Rat liver myofibroblasts and hepatic stellate cells: Different cell populations of the fibroblast lineage with fibrogenic potential.** *Gastroenterology* 1999, **117:**1205-21.
80. Kobold D, Grundmann A, Piscaglia F, Eisenbach C, Neubauer K, Steffgen M, Ramadori G, Knittel T: **Expression of reelin in hepatic stellate cells and during hepatic tissue repair: a novel marker for the differentiation of HSC from other liver myofibroblasts.** *J Hepatol* 2002, **36:**607-13.
81. Magness ST, Bataller R, Yang L, Brenner DA: **A dual reporter gene transgenic mouse demonstrates heterogeneity in hepatic fibrogenic cell populations.** *Hepatology* 2004, **40:**1151-59.
82. Cassiman D, Roskams T: **Beauty is in the eye of the beholder: emerging concepts and pitfalls in hepatic stellate cell research.** *J Hepatol* 2002, **37:**527-35.
83. Gao Z, McAlister VC, Williams GM: **Repopulation of liver endothelium by bone-marrow-derived cells.** *The Lancet* 2001, **357:**932-33.
84. Fujii H, Hirose T, Oe S, Yasuchika K, Azuma H, Fujikawa T, Nagao M, Yamaoka Y: **Contribution of bone marrow cells to liver regeneration after partial hepatectomy in mice.** *J Hepatol* 2002, **36:**653-59.
85. Baba S, Fujii H, Hirose T, Yasuchika K, Azuma H, Hoppo T, Naito M, Machimoto T, Ikai I: **Commitment of bone marrow cells to hepatic stellate cells in mouse.** *J Hepatol* 2004, **40:**255-60.
86. Russo FP, Alison MR, Bigger BW, Amofah E, Florou A, Amin F, Bou-Gharios G, Jeffery R, Iredale JP, Forbes SJ: **The bone marrow functionally contributes to liver fibrosis.** *Gastroenterology* 2006, **130:**1807-21.
87. Forbes SJ, Russo FP, Rey V, Burra P, Rugge M, Wright NA, Alison MR: **A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis.** *Gastroenterology* 2004, **126:**955-63.
88. Kisseleva T, Uchinami H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, Brenner DA: **Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis.** *J Hepatol* 2006, **45:**429-38.
89. Quan TE, Cowper SE, Bucala R: **The role of circulating fibrocytes in fibrosis.** *Curr Rheumatol Rep* 2006, **8:**145-50.
90. 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.
91. 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.
92. Ishii G, Sangai T, Sugiyama K, Ito T, Hasebe T, Endoh Y, Magae J, Ochiai A: **In Vivo Characterization of Bone Marrow-Derived Fibroblasts Recruited into Fibrotic Lesions.** *Stem Cells* 2005, **23:**699-706.
93. Higashiyama R, Inagaki Y, Hong YY, Kushida M, Nakao S, Niioka M, Watanabe T, Okano H, Matsuzaki Y, Shiota G, Okazaki I: **Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice.** *Hepatology* 2007, **45:**213-22.
94. Marra F: **Hepatic stellate cells and the regulation of liver inflammation.** *J Hepatol* 1999, **31:**1120-30.
95. Romagnani P, Lasagni L, Romagnani S: **Peripheral blood as a source of stem cells for regenerative medicine.** *Exp Opin Biol Ther* 2006, **6:**193-202.
96. Ruhnke M, Ungefroren H, Nussler A, Martin F, Brulport M, Schormann W, Hengstler JG, Klapper W, Ulrichs K, Hutchinson JA, Soria B, Parwaresch RM, Heeckt P, Kremer B, Fandrich F: **Differentiation of in vitro-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells.** *Gastroenterology* 2005, **128:**1774-86.
97. Ruhnke M, Nussler AK, Ungefroren H, Hengstler JG, Kremer B, Hoeckh W, Gottwald T, Heeckt P, Fandrich F: **Human monocyte-derived neohepatocytes: A promising alternative to primary human hepatocytes for autologous cell therapy.** *Transplantation* 2005, **79:**1097-103.
98. Pinzani M, Abboud HE, Gesualdo L, Abboud SL: **Regulation of macrophage colony-stimulating factor in liver fat-storing cells by peptide growth factors.** *Am J Physiol* 1992, **262:**C876-81.
99. Marra F, Valente AJ, Pinzani M, Abboud HE: **Cultured human liver fat storing cells produce monocyte chemotactic protein-1 - Regulation by proinflammatory cytokines.** *J Clin Invest* 1993, **92:**1674-80.
100. Sprenger H, Kaufmann A, Garn H, Lahme B, Gemsa D, Gressner AM: **Induction of neutrophil attracting chemokines in transforming hepatic stellate cells.** *Gastroenterology* 1997, **113:**277-285.
101. Marra F, Defranco R, Grappone C, Milani S, Pastacaldi S, Pinzani M, Romanelli RG, Laffi G, Gentilini P: **Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: Correlation with monocyte infiltration.** *Am J Pathol* 1998, **152:**423-30.
102. Pilling D, Buckley CD, Salmon M, Gomer RH: **Inhibition of Fibrocyte Differentiation by Serum Amyloid P.** *J Immunol* 2003, **171:**5537-46.
103. Pilling D, Roife D, Wang M, Crawford JR, Travis EL, Gomer RH: **Inhibition of bleomycin-induced pulmonary fibrosis by serum amyloid P.** *Keystone Symposia on Molecular and Cellular Biology - Abstract Book* 2007:45 [Abstract].
104. Cassiman D, Barlow A, Vander Borght S, Libbrecht L, Pachnis V: **Hepatic stellate cells do not derive from the neural crest.** *J Hepatol* 2006, **44:**1098-104.
105. Lee JM, Dedhar S, Kalluri R, Thompson EW: **The epithelial-mesenchymal transition: new insights in signaling, development, and disease.** *J Cell Biol* 2006, **172:**973-81.
106. Kalluri R, Neilson EG: **Epithelial-mesenchymal transition and its implications for fibrosis.** *J Clin Invest* 2003, **112:**1776-84.

107. Karasek MA: **Does transformation of microvascular endothelial cells into myofibroblasts play a key role in the etiology and pathology of fibrotic disease?** *Med Hypotheses* 2007, **68:**650-55.
108. Kaimori A, Potter J, Kaimori J, Wang C, Mezey E, Koteish A: **TGF-beta 1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in-vitro.** *J Biol Chem* 2007:in press.
109. Robertson H, Kirby JA, Yip WW, Jones DEJ, Burt AD: **Biliary epithelial-mesenchymal transition in posttransplantation recurrence of primary biliary cirrhosis.** *Hepatology* 2007, **45:**977-81.
110. Rygiel KA, Robertson H, Burt AD, Jones DEJ, Kirby JA: **Demonstration of the transition of intrahepatic biliary epithelial cells to fibroblasts during chronic inflammatory liver diseases.** *J Hepatol* 2006, **44:**S241.
111. Kinnman N, Francoz C, Barbu W, Wendum D, Rey C, Hultcrantz R, Poupon R, Housset C: **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.
112. Beaussier M, Wendum D, Schiffer E, Dumont S, Rey C, Lienhart A, Housset C: **Prominent contribution of portal mesenchymal cells to liver fibrosis in ischemic and obstructive cholestatic injuries.** *Lab Invest* 2007, **87:**292-303.
113. Ramadori G, Saile B: **Portal tract fibrogenesis in the liver.** *Lab Invest* 2003, **84:**153-59.
114. Yang Y, Pan X, Lei W, Wang J, Shi J, Li F, Song J: **Regulation of transforming growth factor-{beta}1-induced apoptosis and epithelial-to-mesenchymal transition by protein kinase A and signal transducers and activators of transcription 3.** *Cancer Res* 2006, **66:**8617-24.
115. del Castillo G, Murillo MM, varez-Barrientos A, Bertran E, Fernandez M, Sanchez A, Fabregat I: **Autocrine production of TGF-[beta] confers resistance to apoptosis after an epithelial-mesenchymal transition process in hepatocytes: Role of EGF receptor ligands.** *Exp Cell Res* 2006, **312:**2860-71.
116. Vega S, Morales AV, Ocana OH, Valdes F, Fabregat I, Nieto MA: **Snail blocks the cell cycle and confers resistance to cell death.** *Gene Dev* 2004, **18:**1131-43.
117. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R: **BMP-7 counteracts TGF-beta 1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury.** *Nat Med* 2003, **9:**964-968.
118. Sugimoto H, Yang C, LeBleu VS, Soubasakos MA, Giraldo M, Zeisberg M, Kalluri R: **BMP-7 functions as a novel hormone to facilitate liver regeneration.** *Faseb J* 2007, **21:**256-64.
119. Wang S, Hirschberg R: **Bone morphogenetic protein-7 signals opposing transforming growth factor {beta} in mesangial cells.** *J Biol Chem* 2004, **279:**23200-6.
120. ten Dijke P, Hill CS: **New insights into TGF-beta-Smad signalling.** *Trends Biochem Sci* 2004, **29:**265-73.
121. Neilson EG: **Setting a trap for tissue fibrosis.** *Nat Med* 2005, **11:**373-74.
122. Varga J, Abraham D: **Systemic sclerosis: a prototypic multisystem fibrotic disorder.** *J Clin Invest* 2007, **117:**557-67.
123. Abreu JG, Ketpura NI, Reversade B, De Robertis EM: **Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta.** *Nat Cell Biol* 2002, **4:**599-604.
124. Gressner O, Lahme B, Demirci I, Gressner AM, Weiskirchen R: **Differential effects of TGF-beta on connective tissue growth factor (CTGF/CCN2) expression in hepatic stellate cells and hepatocytes.** *J Hepatol* 2007, **in press:**.
125. DeLeeuw AM, McCarthy SP, Geerts A, Knook DL: **Purified rat liver fat-storing cells in culture divide and contain collagen.** *Hepatology* 1984, **4:**392-403.
126. Rachfal AW, Brigstock DR: **Connective tissue growth factor (CTGF/CCN2) in hepatic fibrosis.** *Hepatol Res* 2003, **26:**1-9.
127. Abou-Shady M, Friess H, Zimmermann A, di Mola FF, Guo XZ, Baer HU, Buchler MW: **Connective tissue growth factor in human liver cirrhosis.** *Liver* 2000, **20:**296-304.
128. Paradis V, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, Ba N, Sobesky R, Ratziu V, Bedossa P: **Expression of connective tissue growth factor in experimental rat and human liver fibrosis.** *Hepatology* 1999, **30:**968-76.
129. Blom IE, Goldschmeding R, Leask A: **Gene regulation of connective tissue growth factor: new targets for antifibrotic therapy.** *Matrix Biol* 2002, **21:**473-82.
130. Li G, Xie Q, Shi Y, Li D, Zhang M, Jiang S, Zhou H, Lu H, Jin Y: **Inhibition of connective tissue growth factor by siRNA prevents liver fibrosis in rats.** *J Gene Med* 2006, **8:**889-900.
131. George J, Tsutsumi M: **siRNA-mediated knockdown of connective tissue growth factor prevents N-nitrosodimethylamine-induced hepatic fibrosis in rats.** *Gene Ther* 2007, **14:**790-803.
132. Zeisberg M: **Bone morphogenic protein-7 and the kidney: current concepts and open questions.** *Nephrol Dial Transplant* 2006, **21:**568-73.
133. Gressner AM, Yagmur E, Lahme B, Gressner O, Stanzel S: **Connective tissue growth factor in serum as a new candidate test for assessment of hepatic fibrosis.** *CLIN CHEM* 2006, **52:**1815-17.
134. Kubota A, Okamura S, Omori F, Shimoda K, Otsuka T, Ishibashi H, Niho Y: **High serum levels of granulocyte-macrophage colony-stimulating factor in patients with liver cirrhosis and granulocytopenia.** *Clin Lab Haematol* 1995, **17:**61-3.
135. Yannaki E, Athanasiou E, Xagorari A, Constantinou V, Batsis I, Kaloyannidis P, Proya E, Anagnostopoulos A, Fassas A: **G-CSF-primed hematopoietic stem cells or G-CSF per se accelerate recovery and improve survival after liver injury, predominantly by promoting endogenous repair programs.** *Exp Hematol* 2005, **33:**108-19.
136. Liu X, Hu H, Yin JQ: **Therapeutic strategies against TGF-beta signaling pathway in hepatic fibrosis.** *Liver Int* 2006, **26:**8-22.
137. Fallowfield JA, Iredale JP: **Targeted treatments for cirrhosis.** *Expert Opin Ther Targets* 2004, **8:**423-35.
138. Huang Y, Border WA, Noble NA: **Perspectives on blockade of TGF[beta] overexpression.** *Kidney Int* 2006, **69:**1713-14.
139. Gressner AM, Weiskirchen R: **The tightrope of therapeutic suppression of active transforming growth factor-beta: high enough to fall deeply?** *J Hepatol* 2003, **39:**856-59.
140. Kinoshita K, Iimuro Y, Otogawa K, Saika S, Inagaki Y, Nakajima Y, Kawada N, Fujimoto J, Friedman S, Ikeda K: **Adenovirus-mediated expression of BMP-7 suppresses the development of liver fibrosis in rats.** *Gut* 2007, **56:**706-14.
141. Zeisberg M, Shah AA, Kalluri R: **Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney.** *J Biol Chem* 2005, **280:**8094-100.
142. Liu DY, Jacob CT, Zhang W, Wong C, Oliver N, Guo G, Lin A, Bradham D, Coker G, Spong S, Stephenson R, Klaus S, Wang QJ, Langsetmo I: **Anti-CTGF (CCN2) human antibody therapy, FG-3019, prevents and reverses renal and cardiovascular pathologies in animal models of diabetic complications.** *Keystone Symposia on Molecular and Cellular Biology - Abstract Book* 2007:33 [Abstract].