Changing the pathogenetic roadmap of liver fibrosis? Where did it start; where will it go?
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Key words
diagnostic options, epithelial–mesenchymal transition, hepatic stellate cells, liver fibrogenesis, liver fibrosis, therapeutic options.

Accepted for publication 28 November 2007.
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Abstract
The pathophysiology of liver injury has attracted the interest of experimentalists and clinicians over many centuries. With the discovery of liver-specific pericytes – formerly called fat-storing cells, Ito-cells, lipocytes, and currently designated as hepatic stellate cells (HSC) – the insight into the cellular and molecular pathobiology of liver fibrosis has evolved and the pivotal role of HSC as a precursor cell-type for extracellular matrix–producing myofibroblasts has been established. Although activation and transdifferentiation of HSC to myofibroblasts is still regarded as the pathogenetic key mechanism of fibrogenesis, recent studies point to a prominent heterogeneity of the origin of myofibroblasts. Currently, the generation of matrix-synthesizing fibroblasts by epithelial–mesenchymal transition, by influx of bone marrow–derived fibrocytes into damaged liver tissue, and by differentiation of circulating monocytes to fibroblasts after homing in the injured liver are discussed as important complementary mechanisms to enlarge the pool of (myo-)fibroblasts in the fibrosing liver. Among the molecular mediators, transforming growth factor-beta (TGF-β) plays a central role, which is controlled by the bone-morphogenetic protein (BMP)-7, an important antagonist of TGF-β action. The newly discovered pathways supplement the linear concept of HSC activation to myofibroblasts, point to fibrosis as a systemic response involving extrahepatic organs and reactions, add further evidence to a more or less uniform concept of organ fibrosis in general (e.g. liver, lung, kidney), and offer innovative approaches for the development of non-invasive biomarkers and antifibrotic trials.

Where did it start?
More than 2000 years ago Erasistratos, the protagonist of the Alexandrian School of Medicine, recognized granular induration of the liver as cause of dropsy (ascites). Since then medical interest in the pathophysiology of hardening of the liver has continued, culminating in R.T.H. Laënnec’s 1819 description of this condition as an independent disease, which he designated as ‘cirrhosis’, in R. Carswell’s 1838 statement of fibrosis as a hallmark of cirrhosis, in J. Müller’s 1843 observation that chronic inflammation leads to hypertrophy of interlobular connective tissue, and in R. Virchow’s 1858 finding that the increase of connective tissue has a cellular source. In 1872 W. Legg recognized liver cell necrosis as the primary event for neof ormation of the extracellular matrix (ECM) as being an essential part of a repair process. Indeed, in 1954 W.S. Hartroft suggested passive mechanisms for the development of liver fibrosis based on reticular collapse of necrotic parenchyma, followed by condensation of pre-existing fibrous stroma to septa.1 However, in 1978 a working group of pathologists recommended definitions and nomenclature for cirrhosis and fibrosis, respectively.2 Fibrosis was defined ‘as the presence of excess collagen due to new fiber formation’. Fibrosis was emphasized as an essential criterion of cirrhosis supplemented by ‘conversion of normal liver architecture into structurally abnormal nodules’.2 Although this definition of fibrosis was still imperfect, because it neglected all important non-collageneous components of the ECM, it recognized active biosynthesis of ECM as the main pathogenetic pathway instead of collapse of the pre-existing reticulin framework. This stimulated further work into the pathogenesis of fibrosis and the identification of cell types engaged in ECM-production. The studies of McGee and Patrick3 and Kent et al.4–6 were among the first that linked vitamin A–storing perisinusoidal lipocytes with collagen synthesis. The first observation of this cell type in 1869 by F. Boll7 was followed by a detailed description in 1876 by C.W. von Kupffer using the gold-chloride staining procedure.8 In 1952 T. Ito discovered a cell type he designated as ‘fat storing cell’,9 which in 1971 K. Wake10 showed to be identical with the stellate cell discovered previously by von Kupffer (for a review of the history of perisinusoidal cells in the liver see K. Atermann)11 (Table 1). In the past a plethora of names was given to the pericytes located in the subendothelial space of Disse and surrounding the sinusoidal endothelial cell layer, e.g. fat storing cells, vitamin A–storing cells, lipocytes, perisinusoidal cells, Ito-cells, arachnocytes, and others, until in 1996 the term ‘hepatic stellate cells’ (HSC) was suggested by 98 investigators in this field and consequently accepted in the scientific community.12 The study on structure, function, and pathophysiology of HSC13,16 was
made possible by the pioneering work of the group of D. Knook in the Netherlands who in 1982 reported methods for isolation and culture of this cell type from rat livers.17,18 They combined the sequential pronase–collagenase perfusion technique of the role of HSC in the pathogenesis of liver fibrosis and synthesis of matrix components,29 of cytokine- and growth factor–regulated stimulation of ECM synthesis (fibrogenesis) and regulation of matrix degradation (fibrolysis).30–32 of several genetic conditions predisposing to fibrogenic reactions,33,34 and of multiple, experimentally successful therapeutic approaches.35

Where is it now?

Experimental studies over the last 20 years or so have provided a detailed knowledge on structure and composition of ECM in normal and fibrotic liver tissue,27,28 of the cellular origin of the various matrix components,29 of cytokine- and growth factor–regulated stimulation of ECM synthesis (fibrogenesis) and regulation of matrix degradation (fibrolysis).30–32 of several genetic conditions predisposing to fibrogenic reactions,33,34 and of multiple, experimentally successful therapeutic approaches.35 Accordingly, fibrosis of the liver is now characterized (i) by an up to 10-fold increase of ECM that comprises several types of collagens, structural glycoproteins, sulfated proteoglycans (glycosaminoglycans), and hyaluronan; (ii) by a histological redistribution with preferred initial matrix deposition in the perivenular zone 3 of the acinus along 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 (‘capilariation of sinusoids’),36 (iii) by changes to the ECM-profile; and (iv) by changes to the fine structure of collagens (e.g. degree of hydroxylation of proline and lysine), glycoproteins (variations of the carbohydrate structure), and proteoglycans (changes of the degree of sulfation of the glycosaminoglycan side chains) combined with certain splice variants of ECM molecules37 (Fig. 1).

The development of fibrosis 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.37 HSC are liver pericytes, which display a dendritic morphology.16 As liver-resident stellate cells, they are members of a diffuse stellate cell system in the body with cells in various tissues having a similar phenotype and cytoskeletal architecture.26 In the liver, HSC embrace with thorn-like microprojections of 20–39 μm length 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.38 They constitute about one-third of the non-parenchymal cell population (Kupffer cells, endothelial cells, HSC), 1.4% of liver volume, and about 15% of total liver resident cells including hepatocytes. The ‘hepatic stellate cell index’, i.e. the number of HSC per 1000 hepatocytes was estimated to be 109 in the healthy rat liver.39 The spindle-like cell body of HSC of about 700 μm3 contains multiple triglyceride-rich vacuoles, in which vitamin A metabolites (retinoids) are dissolved and stored.40 About 85% of the vitamin A of the liver is found in HSC. Additional functions of these cells have been recently discovered: (i) their role as antigen presenting cells (APC);41–43 (ii) their role as CD133+ progenitor cells with the ability to differentiate to progenitor endothelial cells and hepatocytes suggesting important roles in liver regeneration and repair;44 (iii) their involvement in endocytosis of apoptotic parenchymal cells;45,46 (iv) secretion of matrix metalloproteinases (MMPs), the respective MMP-inhibitors (TIMPs),47,48 and growth factors49 indicating a role both in matrix remodelling and degradation (fibrolysis); (v) support of liver regeneration through promotion of hepatocyte proliferation involving the neurotrophin receptor p75,49 (vi) regulation of angiogenesis and vascular remodelling through secretion of angiogenic factors such as VEGF, endothelin-1, IGF-II, neurotrophins, and erythropoietin;50 and (vii) hemodynamic functions as evidenced by activated HSC contraction stimulated with thromboxane, prostaglandin F2, angiotensin II, vasopressin, and endothelin-1 leading to sinusoidal constriction.51–55 Some of these functions, however, are not expressed in the quiescent state of HSC, but are triggered following their activation. The generation of MFB from HSC follows a multistep sequence, which is initiated by liver cell necrosis induced by toxic and immunologic agents and mechanisms55,56 (Fig. 2). As a consequence, HSC,10,16 which are localized in the immediate vicinity of hepatocytes, are activated (Fig. 3). Activation of HSC leads to the expression of α-smooth-muscle actin,

### Table 1 Discovery of hepatic stellate cells (compiled from review of K. Atermann11)

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1869</td>
<td>F. Boll</td>
<td>Star-shaped cells with cytoplasmic granules in the network surrounding liver cells, black staining with osmium</td>
<td>7</td>
</tr>
<tr>
<td>1876</td>
<td>C.W. von Kupffer</td>
<td>Stellate-shaped cells in the perivascular tissue, gold-chloride staining, later (1899) misinterpreted as endothelial cells capable of erythropathocytosis</td>
<td>8</td>
</tr>
<tr>
<td>1923</td>
<td>K.W. Zimmermann</td>
<td>Capillary attached, dendritic, perisinusoidal cells, introduction of the name ‘pericytes’</td>
<td>9</td>
</tr>
<tr>
<td>1952</td>
<td>T. Ito</td>
<td>Fat-storing cells (‘shibo-chozo-saibo’) of the liver (later on named ‘Ito-cells’)</td>
<td>10</td>
</tr>
<tr>
<td>1966</td>
<td>S. Bronfenmajer</td>
<td>Lipocytes in human liver</td>
<td>11</td>
</tr>
<tr>
<td>1971</td>
<td>K. Wake</td>
<td>Describes the identity of stellate cells of C. von Kupffer (1876) with the fat-storing cells of T. Ito (1952)</td>
<td>12</td>
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desmin, and gelsolin, and a concomitant decrease of glial fibrillary acidic protein (GFAP) and a loss of the volume density of lipid droplets <20% combined with a decrease of retinoids, but increased contractility and expression and secretion of a broad spectrum of matrix components.29 The activation process includes proliferation and phenotypic transdifferentiation of HSC to MFB, but both processes are not causally related. This mechanism, designated as the ‘canonical principle’ of fibrogenesis, is believed to be the central pathogenetic event in the development of fibrosis. The 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). MFB have features of a highly active biosynthetic phenotype characterized by a hypertrophic rough endoplasmic reticulum containing membrane-bound 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,57 which is initiated by the pre-inflammatory phase due to direct paracrine activation of HSC by necrotic (apoptotic?) hepatocytes with release of activating cytokines supplemented by loss of mito-inhibitory cell surface heparan sulfate of hepatocytes.58–62 The growth promoting activity of hepatocytes, partially due to IGF-1 and respective IGF-binding proteins,56 is released from damaged cells and parallels the elevation of LDH and AST as known leakage enzymes of hepatocytes.63 In the following inflammatory phase, the preactivated HSC are further stimulated in a paracrine mode by invaded leukocytes and thrombocytes,64 by activated Kupffer cells,60,65–68 sinusoidal endothelial cells, and hepatocytes56,58,61 to transdifferentiate to MFB. The consecutive post-inflammatory phase is characterized by the secretion of fibrogenic cytokines from MFB and interacting matrix components. Some of these cytokines can stimulate MFB in an autocrine way and HSC in a paracrine fashion. Thus, the post-inflammatory phase may contribute to the perpetuation of the fibrogenic process, even after elimination or reduction of the pre-inflammatory and inflammatory phases. Activation and transdifferentiation of HSC are the result of extensive interactions with liver-resident and non-resident cells (Fig. 5). Most relevant mediators are reactive oxygen species (hydroxyl radicals, oxygen radicals, superoxide anions, hydrogen peroxide) produced by activated Kupffer cells65,66 and leukocytes, the stimulated NAD(P)H oxidase activity of HSC,70 which phagocytose apoptotic bodies,66 the cytochrome P4502E1 (CYP2E1) pathway of ethanol-metabolizing hepatocytes,71 and subsets of leukocytes.72 In addition, acetaldehyde of ethanol-exposed hepatocytes73–76 and tissue hypoxia77 promote the activation of HSC.78 Among the peptide mediators, TGF-β is the profibrogenic master cytokine.79–81 Additional cytokines and growth factors involved in fibrogenesis are PDGF-B and PDGF-D, endothelin-1, several fibroblast growth factors (FGFs), insulin-like growth factor I, tumor necrosis factor-alpha (TNF-α), adipocytokines (leptin, adiponectin), and others, which are partly bound as ‘crinopectins’82 to the extracellular matrix.83 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 is secreted in a high molecular (large) latent form (Fig. 6) by HSC/MFB, sinusoidal endothelial cells, and Kupffer cells, and released by thrombocytes and hepatocytes.84,85 It initiates not only the transdifferentiation of HSC to MFB, but also enhances matrix gene expression, decreases their degradation by down-regulation of MMPs and up-regulation of their specific inhibitors (i.e. TIMPs), induces apoptosis of hepatocytes,86–89 and inhibits (together with activin A) liver cell proliferation.90,91 Extracellular activation of latent TGF-β by proteases, oxygen radicals, thrombospondin type I, and αvβ1, αvβ8, αvβ1 integrins is an important step in the regulation of TGF-β bioavailability.92 Antagonism of TGF-β93 or inhibition of its intracellular Smad-signaling cascade by specific inhibitors94 results in a significant retardation or even inhibition of HSC activation and, thus, to a
sustained antifibrotic effect. Interestingly, TGF-β response and signaling are modulated during transdifferentiation of HSC to MFB leading to partial TGF-β insensitivity of MFB. This observation suggests that TGF-β plays a role in the initiation of HSC activation in vivo but TGF-β seems to be less important for the entire transdifferentiation process. Current research is focused on transcriptional control of HSC activation. A growing number of transcriptional mediators are implicated and epigenetic mechanisms (histone acetylation, promoter methylation) are recognized as major determinants of the activation process. 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 ECMs allowing no integrin anchorage. The model was previously suggested as a valuable tool for studying the role of HSC in chronic liver disease, e.g. PPAR-γ agonists, trichostatin A, pirfenidone, halofuginone, scavengers of reactive oxygen species (α-tocopherol, resveratrol, quercetin, curcumin), protease inhibitors, cytokines (hepatocyte growth factor (HGF), IL-10, and interferon-γ) and antagonists to receptors of endothelin, cannabinoid receptor CB1, and angiotensin. However, a comparison of the gene expression profiles of HSC activated in vivo by bile-duct ligation or CCl4-injury with that of culture-activated HSC established major differences. Thus, culture activation does not properly reflect genetic reprogramming of disease-driven HSC activation. Due to morphological and functional intralobular (zonal) heterogeneity of HSC, the processes of activation and transdifferentiation in situ are topographically different, which is also dependent on the different zonal vulnerability of hepatocytes. Accordingly, hepatocytes around the central vein (perivenous acinus zone 3) are most sensitive and fibrogenesis, i.e. in alcoholic liver injury starts there first. The heterogeneity of HSC or MFB is not confined to their topographic localization, but can also result from their different origins. Morphological and functional criteria and the response to growth factors point to different sources of MFB. As an example, HSC express the neural marker GFAP, vascular cell adhesion molecule 1, and the cytoskeleton protein desmin, which are almost absent in MFB. MFB, however, almost exclusively synthesize the matrix protein fibrillin-2. The ETM protein reelin, which is present in quiescent and activated HSC but not detectable in myofibroblasts was suggested as an additional marker to differentiate of HSC from other liver myofibroblasts. 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 α1 [I], respectively) differentially indicating functional heterogeneity. 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 and point to various sources of MFB beside transdifferentiating HSC.
Where will it go?

The ‘canonical principle’ of fibrogenesis based on generation of liver-resident MFB by cytokine-driven transdifferentiation of HSC is still thought to be the key pathogenetic mechanism of liver fibrosis, but studies of fibrosis in the kidney, lung, skin, and liver (see above) reveal a considerable heterogeneity of activated fibroblasts. These observations suggest a number of supplementary origins of MFB in fibrogenesis. The understanding of these mechanisms is essential for future development of innovative biomarkers and therapeutic approaches of fibrogenesis.

Contribution of bone marrow–derived cells

In progressive renal fibrosis more than 30% of α-smooth muscle actin–positive interstitial myofibroblasts are derived from the bone marrow.
bone marrow.\textsuperscript{116} But also in the liver immature, multipotent bone marrow cells have the capacity to differentiate to hepatocytes, cholangiocytes, sinusoidal endothelial cells, and Kupffer cells, if the adequate microenvironment of the liver is present.\textsuperscript{117,118} This was recently extended to HSC and (myo-)fibroblasts under experimental and clinical conditions. By transplantation of genetically tagged bone marrow or of male bone marrow (Y-chromosome) to female mice, it was estimated that up to 30\% of HSC in the liver originate from the bone marrow and acquire the MFB phenotype under injurious conditions.\textsuperscript{119} Another study indicates that up to 68\% of HSC and 70\% of MFB in CCl\textsubscript{4}-cirrhotic mice liver are derived from the bone marrow.\textsuperscript{120} Even in human liver fibrosis, a significant contribution of bone marrow cells to the population of MFB has been proven, even though it is presently unclear which type of specific bone marrow cells or mesenchymal stem cells is relevant for the generation of hepatic (myo-)fibroblasts.\textsuperscript{121} Another experimental study has shown that myelogenic fibrocytes are present in the liver, which can be differentiated by TGF-\beta to collagen-producing MFB.\textsuperscript{122} They are a subpopulation of circulating leukocytes displaying a unique surface phenotype with positivity for CD45 (hematopoietic origin), CD34 (progenitor cell), and type I collagen (capability of matrix synthesis).\textsuperscript{123,124} They have potent immuno-stimulatory activities.\textsuperscript{125} Fibrocytes represent a systemic source of contractile MFB in various fibrotic lesions, such as lung, keloids, scleroderma, and fibrotic changes of the kidney.\textsuperscript{126,127} The mobilization of bone marrow cells and their recruitment into the damaged tissue is a central mechanism of tissue fibrosis and wound healing.\textsuperscript{128} which is most likely regulated by colony-stimulating factors (CSF), such as granulocyte-CSF (G-CSF),\textsuperscript{129} and chemokine receptors 4 (CXC4R), 2 (CCR2), and 7 (CCR7).\textsuperscript{125,130} Thus, activated HSC probably play an important role since they 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.\textsuperscript{131} 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 MMPs, which augment the degradation of fibrotic extracellular matrix.\textsuperscript{129} Furthermore, a striking relationship between increasing hepatic fibrosis and periportal ductular reaction has been demonstrated.\textsuperscript{132} The role of steatosis in chronic hepatitis C infection–related fibrosis is associated with an increase in both the number of cytokeratin-7-positive hepatic progenitor cells and the extent of the ductular reaction, providing a potential mechanism whereby steatosis contributes to the progression of portal fibrosis.\textsuperscript{132}

**Figure 6** Schematic presentation of the compartments of TGF-\beta synthesis, secretion, and extracellular immobilization via tissue transglutaminase-dependent fixation of the large latent TGF-\beta binding protein (LTBP) to extracellular matrix, release by proteases, and activation of the latent TGF-\beta complex by reactive oxygen species (ROS), specific integrins, thrombospondin-1 (TSP-1), or proteases with release of the active TGF-\beta homodimer, which binds to TGF-\beta receptors (T\betaR) III, II, and I to initiate the intracellular signaling cascade by Smad phosphorylation. Regulation of the bioactivity of TGF-\beta occurs at the transcriptional level and, most importantly, by extracellular activation. LAP, latency associated peptide; TIMP, tissue inhibitor of metalloproteinases.

**Contribution of peripheral blood cells**

Recent studies indicate a highly developed multidifferentiation potential of a subgroup of circulating blood monocytes, which can be recruited quickly for tissue repair processes.\textsuperscript{133} In addition, the content of circulating myelogenic stem cells in the blood is suggested to be important for regenerative mechanisms as found in ischemic and degenerative diseases (i.e. myocardial infarction). In vitro investigations over recent years have shown that peripheral blood monocytes can differentiate in hepatocyte-like cells if they are exposed to macrophage-colony stimulating factor (M-CSF) and specific interleukins (monocyte-derived neurotropic)\textsuperscript{134,135} Although not yet proven for liver fibrogenesis, subgroups of monocytes may also differentiate into fibroblast-like cells (fibrocytes) after entering the damaged tissue. Here, 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 hematopoietic growth and differentiation factors, which are also expressed and secreted by activated HSC\textsuperscript{136–138} and other liver cell types.\textsuperscript{139} It is of interest that 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\textsuperscript{140} and, consequently, a preventive effect of SAP-injections on the development of bleomycin-induced lung fibrosis was reported.\textsuperscript{141} Since SAP is synthesized in hepatocytes, severe liver injury might facilitate the monocyte–fibrocyte differentiation process due to reduction of the inhibitory SAP. It should be emphasized that this mechanism is presently speculative for the liver, but circulating monocytes might be a pool for immediate repair processes of liver damage. However, beside special monocytes as source of fibroblasts in the fibrotic liver, also circulating stem cells should be considered, which are positive for CD34 and CXCR4 (a chemokine receptor).\textsuperscript{133} G-CSF and the stromal derived factor (SDF)-1 are probably the most important regulators of stem-cell mobilization from the bone marrow and their integration into the damaged tissue followed by differentiation to fibroblasts and other cells (see above).

**Epithelial–mesenchymal transition (EMT)**

Beside activation and transdifferentiation of HSC, which developmentally derive from the *septum transversum* mesenchyme, from endoderm, from the mesothelial liver capsule\textsuperscript{142} or even from other embryonic origins,\textsuperscript{143} recent studies point to a potentially important mechanism for the enlargement of the resident pool of fibroblasts during the fibrotic reaction of the damaged organ, e.g. in kidney and lung.\textsuperscript{144} This process, designated as epithelial–mesenchymal transition (EMT), is well known in the context of embryonic development, but is now recognized as an important mechanism in the generation of fibroblasts during fibrogenesis in adult tissues\textsuperscript{145} (Fig. 7). It has been proven that in fibrotic kidney disease tubulus epithelial cells can transdifferentiate to fibroblasts expressing collagens and the fibroblast-specific protein 1 (FSP-1), also known as S100A4 calcium-binding protein.\textsuperscript{145} It is estimated that in kidney fibrosis about 40\% of all fibroblasts are the result of EMT.\textsuperscript{146} Similarly, alveolar epithelial cells of the lung are subject to EMT and even cardiac endothelial cells can switch to fibroblasts under conditions of damage (mesenchymal–mesenchymal transition). In *vitro* and *in vivo* observations made in blood vessels following sustained inflammation support the hypothesis that vascular endothelial cell transformation to myofibroblast-like cells may increase matrix proteins in fibrotic diseases.\textsuperscript{147} Recent studies further provide evidence for the importance of EMT in liver fibrogenesis, as evidenced by transition of albumin-positive hepatocytes to FSP-1 positive and albumin-negative fibroblasts.\textsuperscript{148} It is claimed that up to 45\% of hepatic fibroblasts are derived from hepatocytes, and up to 60\% of FSP-1-positive hepatocytes are colabeled with albumin indicating an intermediate transitional stage of EMT of hepatocytes.\textsuperscript{148} Another recent report has shown EMT of mature mouse hepatocytes *in vitro* and of the mouse hepatocyte cell line AML12.\textsuperscript{149} Here, the EMT-state was indicated by strong up-regulation of $\alpha_1$(I) collagen mRNA expression and type I collagen deposition. Thus, hepatocytes are capable of EMT changes and type I collagen synthesis and might be a source of a substantial population of (myo-)fibroblasts in fibrogenesis. A further target for EMT are cholangiocytes (bile duct epithelial cells). In primary biliary cirrhosis (PBC) it has been proven that bile duct epithelial cells express FSP-1 (S100A4) and vimentin as early markers of fibroblasts.\textsuperscript{150} The bidirectional consequence of EMT for cholangiocytes are ductopenia (reduction of bile ducts) and enlargement of the pool of portal fibroblasts, which significantly contribute to portal fibrosis. *In vitro* studies with cultured human cholangiocytes have confirmed the clinical observations described. Thus, EMT as a result of hepatocellular pluripotency proves to be a general pathogenetic principle of chronic cholestatic liver diseases.\textsuperscript{151} In addition, activation and proliferation of portal/perportal mesenchymal cells to peribiliary MFB, which are stimulated in a paracrine manner by bile duct epithelial cells via TGF-\(\beta\), PDGF-BB, and endothelin-\(1\) are important pathogenetic
mechanisms 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. This also underlines the heterogeneous origin of MFB in fibrogenesis and emphasizes the importance of the underlying fibrogenic liver disease. The molecular inducers of EMT are TGF-β, 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. Interestingly, TGF-β induces EMT only of those hepatocytes, which escape from the pro-apoptotic effects of this cytokine. The subpopulation of surviving hepatocytes exhibits a strong overexpression of snail by TGF-β conferring resistance to programmed cell death. Several additional pathways are involved in the generation of apoptosis resistance, e.g. protein kinase A and epidermal growth factor (EGF)/transforming growth factor-alpha (TGF-α). Thus, EMT of hepatocytes is dependent on the balance between apoptotic and survival mechanisms. The process of EMT requires the action of metalloproteinases and a TGF-β dependent snail-mediated downregulation 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, which belongs to the TGF-β superfamily. BMP-7 does not only inhibit EMT, but can even induce a mesenchymal–epithelial (reto)-transition (reverse EMT = MET). BMP-7 has been shown to inhibit TGF-β dependent EMT of hepatocytes and the progression of experimental fibrosis in mice. It has also antiapoptotic properties, anti-inflammatory, and proliferation-stimulating effects. BMP-7 inhibits TGF-β signaling via Smads, 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. 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 α2-macroglobulin change the balance between TGF-β and BMP-7 in favor of an anti-EMT effect by binding and neutralization of TGF-β. Similarly, the important downstream-modulator protein connective tissue growth factor (CTGF/CCN2), which is expressed in hepatocytes, HSC, portal fibroblasts, and cholangiocytes changes the functional TGF-β/BMP-7 ratio. CTGF is over-expressed in experimental and human liver cirrhosis, 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. CTGF inhibits BMP, but activates TGF-β signaling by modulation of the receptor-binding of these ligands. The prominent functional role of CTGF is supported by very recent data, which show sustained antifibrotic effects if CTGF expression is reduced by siRNA. Taken together, EMT, but also MET (mesenchymal–epithelial transition), and in special conditions, even MMT (mesenchymal–mesenchymal transition, e.g. of 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, but still need more experimental proof for the liver, in particular with regard to their quantitative contribution to fibrogenesis.

Conclusion

Despite intensive experimental studies, the clinical opportunities for patients with fibrosing liver diseases have not yet significantly improved. It is expected that increasing knowledge of new pathogenetic mechanisms, which complement the ‘canononical principle’ of fibrogenesis, will have a beneficial effect on the translation to clinical medicine. It is now evident that the heterogeneous pool of (myo-)fibroblasts originates from the EMT of cholangiocytes and most likely of hepatocytes, from the influx of bone marrow-derived fibrocytes into the damaged liver tissue and from differentiation of a subgroup of circulating monocytes to fibroblasts after homing in the damaged tissue. These processes offer innovative diagnostic and therapeutic options. As an example, modulation of the TGF-β/BMP-7 ratio changes the rate of EMT and by this progression of fibrosis. Over-expression of BMP-7 or application of recombined BMP-7 have a sustained antifibrotic effect. In addition, the determination of some of these parameters and of connective tissue growth factor (CTGF) in serum might provide information on fibrogenic activity. Thus, the pathogenetic road map of fibrosis has not (yet?) changed, but newly discovered backstreets now establish a much more complex network of interacting pathways radiating to systemic responses.

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