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Review

# Cellular and molecular mechanisms in liver fibrogenesis

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ABSTRACT

Liver fibrogenesis is a dynamic and highly integrated molecular, tissue and cellular process, potentially reversible, that drives the progression of chronic liver diseases (CLD) towards liver cirrhosis and hepatic failure. Hepatic myofibroblasts (MFs), the pro-fibrogenic effector cells, originate mainly from activation of hepatic stellate cells and portal fibroblasts being characterized by a proliferative and survival attitude. MFs also contract in response to vasoactive agents, sustain angiogenesis and recruit and modulate activity of cells of innate or adaptive immunity. Chronic activation of wound healing and oxidative stress as well as derangement of epithelial–mesenchymal interactions are "major" pro-fibrogenic mechanisms, whatever the etiology. However, literature has outlined a complex network of pro-fibrogenic factors and mediators proposed to modulate CLD progression, with some of them being at present highly debated in the field, including the role of epithelial to mesenchymal transition and Hedgehog signaling pathways. Hypoxia and angiogenesis as well as inflammasomes are recently emerged as ubiquitous pro-inflammatory and pro-fibrogenic determinants whereas adipokines are mostly involved in CLD related to metabolic disturbances (metabolic syndrome and/or obesity and type 2 diabetes). Finally, autophagy as well as natural killer and natural killer-T cells have been recently proposed to significantly affect fibrogenic CLD progression.



## Liver fibrogenesis: a driving force for the progression of chronic liver diseases

### *Definitions, introductory remarks and the overall relevance of fibrogenesis*

Chronic liver diseases (CLD)[1] of clinical relevance are typically characterized by persisting parenchymal (i.e., hepatocyte) injury that can be induced by a number of well defined etiological agents or conditions. On a worldwide perspective the following CLD etiologies are the most relevant: (i) chronic infection by hepatotropic viruses (hepatitis B and C viruses only); (ii) chronic exposure to toxins or drugs (with excess alcohol consumption being predominant in western countries); (iii) chronic exposure to altered metabolic conditions; (iv) persisting autoimmune injury. Persistent liver injury can result in chronic activation of inflammatory and wound healing

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[1] *Abbreviations used:* ALD, alcoholic liver disease; AFP, alpha-fetoprotein; α-SMA, α-smooth-muscle actin; AMPK, AMP-activated protein kinase; APCs, antigen presenting cells; ASH, alcoholic steato-hepatitis; Atg, autophagy-related gene; bFGF, basic fibroblast growth factor; Bcl2, B-cell lymphoma/leukemia-2; BMP-7, bone morphogenetic protein-7; $CCl_4$, carbon tetrachloride; CDAA, choline-devoid and aminoacid-refined; CLD, chronic liver diseases; CK-19, cytokeratin 19; CREB, cAMP response element binding protein; CTGF, connective tissue growth factor; DAG, diacylglycerol; DALYs, disability adjusted life years; DAMPS, damage-associated molecular patterns; DC, dendritic cells; Dhh, Desert hedgehog; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; FasL, Fas ligand; FIP200, focal adhesion kinase family-interacting protein of 200 kD; FSP-1, fibroblast-specific protein 1; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; Gli, glioblastoma family of transcription factors; GPCRs, G-protein coupled seven-transmembrane receptors; GSK-3β, glycogen synthase kinase-3β; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; Hh, hedgehog; Hhip, Hh interacting proteins; HIF, hypoxia inducible factor; HMGB1, high-mobility group box 1; HPCs, hepatic progenitor cells; HNE, 4-hydroxy-2,3-nonenal; HO-1, heme oxygenase 1; HSC, hepatic stellate cells; HSC–MFs, activated, myofibroblast-like, hepatic stellate cells; HSP, sheat shock proteins; 5-HT, serotonin or 5 hydroxy-triptamine; 5-HTR, serotonin receptor; HVPG, hepatic vein pressure gradient; Ihh, Indian hedgehog; IL, interleukin; IL-1R1, IL-1 receptor type 1; IF/MFs, interface myofibroblasts; IFNγ, interferon-γ; IRE1α, inositol requiring protein 1α; JNK1/2, isoforms 1 and 2 of c-Jun-NH2-kinases; LC3, light chain 3; MAPK, mitogen-activated protein kinase; MCP1, monocyte chemoattractant protein 1 or CCL2; MFs, myofibroblasts; MMP, metalloprotease; MET, mesenchymal to epithelial transition; MS, metabolic syndrome; MSC, mesenchymal stem cells; mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NF-kB, nuclear factor kB; NGF, nerve growth factor, NK, natural killer; NKT, natural killer T; NLR, NOD-like receptor; NLRP3, NOD-like receptor family, pyrin domain containing 3; OLT, ortothopic liver transplantation; PAMPs, pathogen-associated molecular patterns; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol

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response that, in parallel or in association with other pathogenic mechanisms, including at least oxidative stress and the derangement of interactions between epithelial and mesenchymal cells, then sustain liver fibrogenesis, the process that represents a major driving force for liver fibrosis (that is, the net tissue result of fibrogenesis) [1–5].

Accordingly, liver fibrogenesis can be defined as a dynamic and highly integrated molecular, tissue and cellular process that during the course of a CLD leads to a progressive excess accumulation of extracellular matrix (ECM) components (i.e., liver fibrosis) in an attempt to limit the consequences of chronic parenchymal injury [1–3]. Liver fibrogenesis, irrespective of the etiology, is believed to be critical for the progression of any form of chronic liver disease (CLD) and persisting fibrogenesis is widely recognized as the major driving force eventually leading to liver cirrhosis and hepatic failure [1–3,6]. Along these lines, cirrhosis is currently defined as an advanced stage of CLD, characterized by the formation of regenerative nodules of parenchyma surrounded and separated by fibrotic septa, and associated with significant changes in organ vascular architecture, development of portal hypertension and related complications, including variceal bleeding, hepatic encephalopathy, ascites and hepatorenal syndrome [1–6].

In an attempt to describe the relevance of fibrogenesis, according to the concepts nicely outlined in a recent authoritative review on this specific topic [6], one may refer to a general scheme for CLD progression that include at least four stages which intimately related to major pathophysiological events.

The first stage, whatever the etiology, is dominated by the interrelated sequence of persisting chronic parenchymal injury (leading to chronic necrosis and/or apoptosis), chronic inflammatory response and chronic activation of fibrogenesis, which is the driving force for excess deposition of ECM component (i.e., fibrosis). When trying to synthetically describe this first stage (see Fig. 1), a number of concepts should be taken in mind [1–6]: (i) perpetuation of hepatic injury, a typical hallmark of CLD progression, depends not only from chronic exposure to the specific etiology but also results from chronic injury itself and chronic inflammatory response, through a number of final mediators (with a prevailing role of reactive oxygen species or ROS); (ii) chronic activation of inflammatory response and recruitment/activation of cells involved in either innate or acquired immunity can progressively result in what one may define pro-fibrogenic environment, in which synthesis and release of growth factors, cytokines, chemokines, ROS and other mediators will from one side impair significantly hyperplasia/regeneration of hepatic tissue and on the other side will favor chronic activation of wound healing and fibrogenesis; (iii) the pro-fibrogenic environment will, in turn, lead to persistent activation of MF-like cells and then increased deposition of ECM components which is paralleled by altered/inefficient remodeling; (iv) emerging evidence suggests a major role for hypoxia and angiogenesis in sustaining and, likely, driving fibrogenesis as well as vascular changes that become more and more relevant during CLD progression; (v) liver fibrosis in this first stage is potentially reversible, as shown by both experimental and clinical studies; fibrosis reversion may depend on the removal of exposure to the specific etiology or to effective therapy.

When deposition of ECM components becomes significant and fibrotic septa and strictly related vascular changes start to modify significantly the overall structure of liver parenchyma, portal hypertension and related pathophysiological events start to ensue and turn CLD progression into the stage of cirrhosis. Indeed, apart from the histopathological diagnosis of cirrhosis, at least from a clinical point of view, one should not consider cirrhosis as an end point. Rather, it has been suggested the need to define at least two distinct stages of cirrhosis [8]: (i) a stage of compensated cirrhosis or cirrhosis without overt clinical manifestations, with hepatic vein pressure gradient (HVPG) still within a range of 5–10 mmHg; (ii) a stage of decompensated cirrhosis or cirrhosis with clinical manifestations (HVPG values > 10–12 mmHg).

*The clinical impact of fibrogenic progression*

If persistent liver fibrogenesis may be envisaged as a major driving force for the progression of CLD towards cirrhosis, liver failure and hepatocellular carcinoma (HCC), CLD fibrogenic progression has then a very significant clinical impact which is best described by the following facts [1,6–9].

1. Epidemiological data indicate that approximately 180 millions of patients worldwide are affected by a form of CLD, with HCV chronic infection becoming predominant in western countries, followed by and/or associated with chronic alcohol abuse. Chronic HBV and HCV infections are also predominant in Asia and Africa. 25–30% of these patients are expected to progress to cirrhosis. In addition, the epidemic of obesity and diabetes will accelerate progression of CLDs and is itself a cause of cirrhosis in the context of evolution of non-alcoholic steatohepatitis (NASH).
2. According to the 2010 Global Burden of Disease study [7], more than one million deaths (representing approx. 2.0% of all deaths) and 31,027,000 disability adjusted life years (DALYs, that is 1.2% of all DALYs) were due to liver cirrhosis. Alcohol-related liver cirrhosis alone was responsible for 493,000 deaths and 14,544,000 DALYs.
3. Among diseases of the gastro-intestinal tract, cirrhosis represents the most common non-neoplastic cause of death in Europe and USA and overall represents the 7th most common cause of death in western countries.
4. HCC, a very aggressive malignant cancer that represents the 5th most common cancer and the 3rd most common cause of cancer mortality worldwide, almost invariably develops on a cirrhotic background, although initial reports from NASH patients are suggesting that HCC may also develop in a fibrotic liver [8,9]. The annual rate of HCC development in cirrhotic patients has been estimated to vary, depending on etiology, from 2–3% to 7–8% patients.
5. Epidemiologists predict a peak for end-stage CLDs and HCC in the next decade [6,7], in parallel with a shortage of donor organs for ortothopic liver transplantation (OLT), which is currently the only effective treatment option for patients with cirrhosis.
6. Progression of a CLD towards cirrhosis has been estimated to take at least 10–15 years and sometimes to require even 30 or more years, but it may be also extremely rapid in particular clinical settings, such as in children affected by biliary atresia, in patients with HCV recurrence after OLT, or in HCV–HIV co-infected patients [1,6–9]. CLD progression is still then difficult

3-kinase; PKC, protein kinase C; PMFs, portal myofibroblasts PIIINP, N-terminal peptide of procollagen type III; PBC, primary biliary cirrhosis; PPAR-γ, peroxisome proliferator-activated receptor-γ; PRRs, pattern recognition receptors; PSC, primary sclerosing cholangitis; PS/MFs, portal/septal myofibroblasts; PKA, protein kinase A; Ptc, Patched; RA, retinoic acid; RAE1, retinoic acid inducible gene 1; ROS, reactive oxygen species; SEC, sinusoidal endothelial cells; SERT, specific serotonin transporter; Shh, Sonic hedgehog; Smo, smoothened receptor; TGFβ1, transforming growth factor β1; TGFß2, transforming growth factor β2; THP2, tryptophan-hydroxylase 2; Tie2, angiopoietin I receptor; TIMPs, tissue inhibitor of metalloproteases; TLR, toll-like receptor; TNFα, tumor necrosis factor α; TNFβ, tumor necrosis factor β; TRAIL, TNF-related apoptosis-inducing ligand ULC, Unc-51-like kinase; UPR, unfolded protein response; Vps34, vacuolar protein sorting 34; VEGF, vascular endothelial growth factor; VEGFR-2, VEGF receptor type 2; VHL, von Hippel–Lindau protein; XBP1, X-box binding protein 1.