Liver Fibrosis: Which Mechanisms Matter?

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HEPATIC FIBROSIS

Historically, fibrosis was defined by a World Health Organization expert group in 1978 “as the presence of excess collagen due to new fiber formation.”¹ In the respective recommendation, hepatic fibrosis was classified as a component of many forms of liver injury rather than a disease by itself.¹ This specification majorly takes into account a (histo)-pathological assessment, in which hepatic fibrosis was hypothesized to be a “passive” process that results from the collapse of damaged, preexisting stroma that disintegrates and condensates into septa.² Fibrosis is the common consequence of chronic liver injury due to various etiologies, subsequently leading from injury to inflammation to liver

scarring (Fig. 1). However, during recent decades of intensive experimental research it became evident that fibrogenesis is a complex wound-healing process that requires the interaction of several cell types that become triggered by a broad spectrum of cytokines, chemokines, and nonpeptide mediators including reactive oxygen species, lipid mediators, and hormones.³ Progressive fibrosis is linked to architectural changes of the liver with increased stiffness favoring portal hypertension, it may advance to end-stage cirrhosis, and it provides a microenvironment that predisposes to liver cancer.⁴ Consequently, the presence of liver fibrosis in biopsy samples, but no other histological feature, is the strongest predictor of liver-related complications and mortality in patients with nonalcoholic fatty liver.⁵

Abbreviations: ASK, apoptosis signal-regulation kinase; ECM, extracellular matrix; FGF, fibroblast growth factor; FXR, farnesoid X receptor; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HGF, hepatocyte growth factor; HSC, hepatic stellate cell; IL, interleukin; IFN, interferon; LOXL2, lysyl oxidase-like 2; MFB, myofibroblast; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NASH, nonalcoholic steatohepatitis; NK, natural kill; NKT, natural kill T; OCA, obeticholic acid; PDGF, platelet-derived growth factor; PPAR, peroxisome proliferator-activated receptor; PSC, primary sclerosing cholangitis; ROS, reactive oxygen species; TGF- β , transforming growth factor- β ; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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Potential conflict of interest: F.T. is an investigator in clinical trials supported by Tobira, Genfit, and Intercept; in addition, he has received research funding from Noxxon and Tobira. R.W. cooperates with Silence Therapeutics.

Received 17 May 2016; accepted 4 August 2016

Contract grant sponsor: German Research Foundation; contract grant number: SFB/TRR57.

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FIG 1 Pathogenic sequence of chronic liver disease progression. Different types of injury to hepatocytes trigger inflammatory responses. These events activate HSCs to transdifferentiate into matrix-producing MFB, resulting in liver fibrosis. Liver fibrosis is decisive, because its progression will lead to liver cirrhosis and eventually hepatocellular carcinoma (HCC), whereas its regression can result in full resolution of injury.

FIG 2 Activation of HSCs to MFBs. Liver-resident cell types and infiltrating immune cells release inflammatory and profibrogenic mediators promoting the transdifferentiation of quiescent HSC into collagen-producing MFB. Abbreviations: FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IL, interleukin; IFN, interferon; MPO, myeloperoxidase; NK, natural kill; NKT, natural kill T; ROS, reactive oxygen species; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

FIG 3 Liver fibrosis regression. Upon cessation of the chronic injury, macrophages and other inflammatory cells acquire a restorative and anti-inflammatory phenotype. MFB become deactivated by cell death, senescence, or reversion to quiescent HSC. Restorative macrophages degrade ECM by matrix metalloproteinases (MMPs). Abbreviations: IL, interleukin; NK, natural kill; TNF, tumor necrosis factor.

FIBROGENIC LIVER CELL TYPES

The pathogenic key event in hepatic fibrosis is the activation and transdifferentiation of quiescent hepatic stellate cells (HSC) into fibrogenic myofibroblasts (MFBs) that

increasingly express α -smooth muscle actin as a characteristic cytoskeletal marker and a large variety of proteins forming the connective tissue (e.g., collagens and glycosaminoglycans). This process is primarily triggered by a

TABLE 1. SELECTIVE INNOVATIVE ANTIFIBROTIC THERAPIES CURRENTLY IN CLINICAL DEVELOPMENT

Drug	Mechanism (proposed)	Sponsor	Main Indication*	Phase [†]
OCA	FXR agonist	Intercept Pharmaceuticals	NASH	III
Px-104	FXR agonist	Gilead/Phenex Pharmaceuticals	NASH	II
GFT505/Elafibranor	PPAR α/δ agonist	Genfit	NASH	III
Cenicriviroc	Chemokine receptor CCR2/CCR5 antagonist	Tobira Therapeutics	NASH, PSC	II
GR-MD-02	Galectin inhibitor	Galectin Therapeutics	NASH, NASH-cirrhosis	II
Emricasan	Caspase protease inhibitor	Conatus Pharmaceuticals	Cirrhosis, NASH, HCV	II
GS-4997	ASK 1 inhibitor	Gilead	NASH	II
Simtuzumab (GS-6624)	Monoclonal antibody against LOXL2	Gilead	NASH, HCV, PSC	II
Liraglutide	GLP-1 analogue	Novo Nordisk A/S	NASH	II

Abbreviations: GLP-1, glucagon-like peptide 1; HCV, hepatitis C virus; PSC, primary sclerosing cholangitis.

*The main indications tested in clinical trials are listed.

[†]Phase II denotes controlled trials to evaluate efficacy and safety in patients; phase III denotes the testing of a drug on patients to assess efficacy, effectiveness, and safety in a large cohort before approval by the regulatory affairs.

FIG 4 New approaches to antifibrotic therapies. A variety of small molecules with either agonistic or antagonistic activities on key pathways for fibrosis progression are currently being tested in early-phase clinical trials (see also Table 1, the structure of selected drugs is exemplarily shown). Moreover, some large peptides that require parenteral administration (subcutaneous or intravenous) such as the glucagon-like peptide 1 (GLP-1) agonists or the anti-LOXL2 antibody are under clinical evaluation.

multitude of profibrogenic and promitogenic mediators that are released from stressed or injured liver cells (hepatocytes, Kupffer cells, and sinusoidal endothelial cells) and from infiltrating immune cells (Fig. 2). Among these factors, transforming growth factor-β (TGF-β) and members of the platelet-derived growth factor (PDGF) protein family are the most effective profibrogenic mediators.³ Animal models of hepatic fibrosis confirmed that HSC are the major source of MFB *in vivo*, although portal MFB may contribute especially to cholestatic fibrosis.⁶ Upon cessation of injury, MFB can revert their phenotype to either a quiescent HSC or to a senescent or apoptotic cell that is removed by immune cells.⁷ More recently, the conversion of MFB to functional hepatocytes by introducing a set of transcription factors via gene

therapy was shown in animal models, opening potential new avenues for therapeutic modification of fibrogenic cells in liver fibrosis.⁸

IMPACT OF INFILTRATING CELLS

Liver injury is associated with inflammatory cell infiltration that has dual functions during the injury and recovery phases of inflammatory scarring. Circulating monocytes entering the liver from the bloodstream may develop into infiltrating macrophages that exhibit a proinflammatory phenotype, which perpetuates chronic injury and promotes fibrosis. However, depending on signals from the microenvironment, these cells can adopt a restorative phenotype that is involved in the

degradation of excessive extracellular matrix (ECM) and induction of HSC apoptosis⁹ (Fig. 3). The bipartite activities of macrophages were shown in conditional macrophage ablation experiments in mice demonstrating that macrophage depletion during the injury phase was associated with a dramatic loss of MFB and matrix components, whereas the depletion during the recovery phase attenuated matrix degradation.¹⁰

EXPERIMENTAL MODELS

Most of the experimental liver fibrosis research is conducted in HSC cell lines (like the LX-2 cells), isolated primary HSC (from mouse, human, or rat liver), and numerous surgical, genetic, toxic, and dietary animal models that mimic different types of fibrosis observed in humans.¹¹ All of these models are extremely helpful to understand general pathogenic pathways of fibrogenesis, and a large number of studies have shown that most of the findings can be translated to human disease.¹²

RELEVANCE FOR DIAGNOSING HEPATIC FIBROSIS

Understanding basic mechanisms of fibrogenesis has improved the current methods of diagnosing fibrosis. Although this has been possible only by liver biopsy in the past, a large array of noninvasive serum biomarkers and physical techniques such as transient elastography (FibroScan) or magnetic resonance elastography are now being implemented into clinical algorithms.¹³ However, all these methods (including liver biopsy) have limitations, especially in the large group of patients with nonalcoholic fatty liver disease.¹⁴

REVERSAL AND THERAPY OF HEPATIC FIBROSIS

Liver fibrosis and its final stage, that is, cirrhosis, were considered irreversible in the past. However, the resolution of hepatic fibrosis regularly occurs after removal of the fibrogenic stimuli in human patients and in animal models.¹⁵ This prompted intense research on the mechanisms, resulting in several innovative therapies under evaluation for the treatment of liver fibrosis (Fig. 4, Table 1). Most antifibrotic agents are currently tested in patients with nonalcoholic fatty liver diseases and have thus oftentimes additional metabolic effects (Table 1).

Therefore, it is currently unclear whether the results expected from the ongoing trials can be extrapolated to other chronic liver diseases. Several agonists that target either the farnesoid X receptor (FXR) such as obeticholic acid (OCA) or the nuclear receptors peroxisome proliferator-activated receptors (PPARs) α/δ such as Elafibranor are currently being tested in clinical studies. These FXR and PPAR α/δ agonists augment beneficial metabolic pathways in hepatocytes. New inhibitors against apoptosis (such as the caspase inhibitor emricasan) or apoptosis-related proteins (such as apoptosis signal-regulation kinase [ASK] 1 inhibitors) aim at reducing hepatocyte cell death. A different strategy is the inhibition of inflammatory monocyte infiltration into the liver by the dual chemokine receptor CCR2/CCR5 antagonist cenicriviroc. In a first phase 2b trial, cenicriviroc significantly improved fibrosis stage in patients with nonalcoholic steatohepatitis (NASH) already after 1 year of oral and well-tolerated therapy. Polysaccharide galectin inhibitors (GR-MD-02) may reduce TGF- β -driven HSC activation and inflammation-associated chemoattraction.¹⁶ Other strategies target enzymes that are essential to the biogenesis of connective tissue components, such as inhibiting the matrix cross-linking enzyme lysyl oxidase-like 2 (LOXL2) by the antibody simtuzumab. However, no general antifibrotic therapy is currently available in clinical practice, leaving treatment of the underlying disease and ultimately liver transplantation as the main therapeutic options for advanced liver fibrosis.

ACKNOWLEDGMENT

The authors thank Sabine Weiskirchen for preparing the figures.

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