Liver fibrosis: from mechanisms to treatment

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The field of hepatic fibrogenesis has grown tremendously due to major progress in defining pathogenic mechanisms, combined with the recognition that fibrosis represents a common pathway of chronic injury responsive to therapy. This article reviews some key basic and translational advances in understanding fibrosis and outlines the considerations for antifibrotic therapies.

Pathogenesis of hepatic fibrosis

Typically, hepatic injury leads to initiation of fibrogenesis; this injury is multi-factorial and often disease-specific. Stimuli may include hepatocyte necrosis, apoptosis, inflammatory cell infiltration, and ECM alterations. Both parenchymal and nonparenchymal cells participate in the response to injury, which relies on a convergence of cytokines and other extracellular signals, including reactive oxygen species. These stimuli provoke a fibrogenic response, resulting over time in an overall net accumulation of extracellular matrix proteins within the liver due to an imbalance between the deposition and degradation of ECM constituents.

The fibrogenic process results from proliferation and accumulation of myofibroblastic cells (MFBs) arising from different cell populations including local sources (e.g., hepatic stellate cells and portal mesenchymal cells) as well as from outside the liver (e.g., bone marrow and circulating fibrocytes). It is unknown, however, if the relative contributions of each source are the same in all forms of liver injury, as they may differ based on the underlying etiology and region(s) of injury within the liver. In other tissues, epithelial-mesenchymal cell transition (EMT) is also increasingly appreciated as a source of tissue myofibroblasts [1, 2], particularly in kidney and lung. In liver, the contribution of EMT to the total mesenchymal cell population may vary with the disease etiology and stage. No endogenous marker(s) have been identified that enable investigators to discriminate the source of myofibroblasts with certainty in an injured liver, but there is increasing reliance on use of genetic models in which lineage tracing can “mark” cells to define their origin(s), for example from bone marrow [3-5].

Myofibroblasts have several characteristic properties, not only in liver, but also in other tissues that display a wound-healing response. Their most classical feature is expression of smooth muscle α-actin (α-SMA), a contractile filament that in liver is a marker of fibrogenic cells [6], and may predict fibrosis progression [7]. Myofibroblasts also synthesize an array of ECM components, metalloproteinases and their inhibitors, and release a range of cytokines and chemokines [8].

Hepatic stellate cells

In normal liver, HSCs are resident perisinusoidal cells that store vitamin A. The isolation and analysis of HSCs has been a major reason why our understanding of hepatic fibrosis has accelerated over the past two decades [9-11]. These studies have led to the conclusion that HSCs are the major fibrogenic cell type in injured liver [12-14].

Other fibrogenic cells

As noted above, there are other sources of ECM-producing fibroblasts in injured liver besides those derived from activated HSCs. Different subpopulations of mesenchymal cells may be recruited depending on the main site of injury (e.g., cholestatic vs parenchymal injury) within the liver lobule [15, 16]. For example, there is a prominent accumulation of portal myofibroblasts (devoid of lipid droplets, desmin negative) compared to HSCs (desmin positive) in ischemic and biliary fibrosis [17].

Some fibrogenic cells in liver may also have a hematopoietic origin. However, it is unknown what proportion of myofibroblasts/fibrocytes are derived from bone marrow or circulating fibrocytes, whether myofibroblasts of these origins transition through a stellate cell phenotype, and what happens to activated myofibroblasts from various sources when liver injury resolves.

Although the conversion of hepatocytes to fibroblasts through epithelial mesenchymal transition (EMT) has not yet been quantified, colocalization of α-SMA and cytokeratin-19 in bile duct epithelium has been observed in fibrotic liver induced by bile duct ligation (BDL), suggesting the possibility of biliary epithelial to myofibroblast transition after BDL [18].

Cellular and molecular features of stellate cell activation

The fundamental features of stellate cell activation appear to be similar regardless of the initial cause of injury, although increasingly, disease-specific mechanisms of stellate cell activation have begun to emerge as well, especially for HCV infection [19, 20] and non-alcoholic steatohepatitis [21, 22]. Conceptually, activation occurs in two phases, initiation and perpetuation, followed by resolution if liver injury is abrogated. Initiation refers to the earliest events that render cells responsive to cytokines. It is mediated primarily by paracrine stimuli (oxidative stress, apoptotic fragments and cytokines) from injured neighboring liver cells and...
infiltrating inflammatory cells. Perpetuation connotes those responses to cytokines that collectively enhance scar formation (see below). Resolution refers to the fate of activated stellate cells when the primary insult is withdrawn or attenuated (reviewed in [8, 23]).

The perpetuation of stellate cell activation can be further subdivided into at least seven distinct events that can occur simultaneously: 1) proliferation, due to several mitogenic cytokines, including PDGF [24], FGF, thrombin [25] and VEGF; 2) chemotaxis and migration, is an equally important mechanism of stellate cell accumulation, which has been attributed to cytokines (e.g., PDGF, TGF-β1, ET-1) [26, 27] altered cell-matrix interactions, and Rho signaling; 3) fibrogenesis, which is largely driven by the cytokine TGFβ1 [28], whose activity is amplified by increased production, [29] increased activation of the latent form [30] and enhanced receptor expression [31]; 4) release of pro-inflammatory, profibrogenic and pro-mitogenic cytokines (in particular MCP-1), which increase the accumulation of inflammatory cells and stimulate ECM production via autocrine pathways, especially through the actions of TGFβ1 and PDGF; 5) contractility, which confers upon the cells the potential to constrict sinusoids and reduce blood flow. Actions of endothelin-1 are key components of this response, which likely contributes to increased portal pressure in patients with chronic liver disease [32]; 6) degradation of the liver’s normal matrix, thereby disrupting the delicate scaffolding required to preserve liver function; 7) loss of vitamin A droplets, whose functional role is not clear, but may involve altered retinoid receptor signaling.

Considerations for Developing Antifibrotic Therapies

In envisioning new treatments for hepatic fibrosis, realistic goals must be defined. Thus, the aim of therapy with an antifibrotic is to attenuate and not necessarily abrogate the stellate cell’s response to chronic injury, since it is only in the most advanced stages of fibrosis that morbid complications ensue. Thus, antifibrotic therapies may only need to downregulate and not eliminate the scar response to be effective in some patients.

What are the ideal qualities of an antifibrotic drug?

The ideal antifibrotic therapy should have several features: 1) easily and specifically delivered to the HSC. The liver provides an inherent “targeting” for orally absorbed agents, if its hepatic metabolism is efficient. Very low molecular weight, non-peptide molecules typically possess these qualities; 2) minimal toxicity to neighboring parenchymal and non-parenchymal hepatic cells, or to non-hepatic tissues; 3) effective in reversing advanced fibrosis and not just preventing new scar accumulation.

How long will patients with fibrosis require treatment with antifibotics?

The optimal duration of therapy for an antifibrotic drug is unknown. Since fibrosis is a chronic process, years of antifibrotic therapy might be anticipated, although such treatment might be intermittent. Moreover, studies now suggest that significant reversal of fibrosis may be accomplished in less than five years, so shorter treatment periods may be possible. This issue will need to be addressed through careful prospective clinical trials. Clearly, the amount of fibrosis at the onset of therapy will be an important determinant of treatment intensity and duration. Further, since resolution of the underlying cause of liver disease may lead to spontaneous improvement in fibrosis, we may anticipate antifibrotic agents being effectively used in conjunction with approaches such as antiviral agents in chronic viral hepatitis, or chelating agents in patients with hemochromatosis, for example.

Specific mediators of stellate cell activation provide potential targets for therapy. For putative agents, it is important to carefully distinguish a direct anti-fibrotic effect in experimental models from an indirect effect due to reduced liver injury. For example, agents that solely neutralize the toxicity of a hepatotoxin cannot be considered truly “antifibrotic”, even if they still have a rationale for use in patients with liver disease.

Once a potential antifibrotic is identified, analysis of its potential efficacy should proceed in a systematic and specific manner. First, in vitro studies can be performed in either freshly isolated rat or human stellate cell lines, and/or in immortalized lines. If in vitro analysis of a drug candidate appears promising, then in vivo studies can be performed in animal models. Experimental mouse and rat models of liver fibrosis have been well established and validated, and have many features of chronic liver disease similar to humans including the induction of matrix mRNAs and proteins. A large number of models are available in rodents and large animals, each of which has its unique strengths and weaknesses, including the relative site of injury (e.g., parenchymal vs cholestatic), mechanism (necrotic vs immunologic), or relative reversibility. Common fibrotic models include carbon tetrachloride, thioacetamide, bile duct occlusion, and heterologous serum.

A key consideration in designing in vivo experiments is whether the potential antifibrotic therapy should be instituted at the onset of injury, following establishment of injury and fibrosis, or after cirrhosis has been fully established. Instituting the antifibrotic therapy after fibrosis has been established most closely parallels the situation in patients with liver disease, in which fibrosis has been accumulating for many years.

Once an in vivo study to test a candidate antifibrotic has been completed, several analyses are possible, including serum assays of liver chemistries and assessment of morphology and matrix accumulation. It is anticipated that as more antifibrotic drugs require pre-clinical testing, methods for analysis will be increasingly standardized and optimized.

Conclusions

With emerging new technology and greatly refined methodologies, our knowledge about the natural history of liver fibrosis and its pathogenesis are continuously expanding. Several issues remain unanswered. These include how epigenetic mechanisms act as determinants of gene activation and repression in stellate cell biology and the origin(s) of ECM-producing myofibroblasts from sources other than HSCs. Future studies will need to characterize the different fibrogenic cell populations and their regulation in more detail, and to identify cell-specific markers and promoters to refine genetic models. Biomarkers and non-invasive tests that are sensitive, specific and respond quickly to changes in fibrogenic activity are urgently needed. Genetic determinants of fibrosis progression and regression are also active areas of inquiry. Collectively, current and future basic and translational advances will continue to improve the evaluation and treatment of patients with chronic liver disease.

REFERENCES


