Therapeutic targets in liver fibrosis

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Fallowfield JA. Therapeutic targets in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 300: G709–G715, 2011. First published January 13, 2011; doi:10.1152/ajpgi.00451.2010.—Detailed analysis of the cellular and molecular mechanisms that mediate liver fibrosis has provided a framework for therapeutic approaches to prevent, slow down, or even reverse fibrosis and cirrhosis. A pivotal event in the development of liver fibrosis is the activation of quiescent hepatic stellate cells (HSCs) to scar-forming myofibroblast-like cells. Consequently, HSCs and the factors that regulate HSC activation, proliferation, and function represent important antifibrotic targets. Drugs currently licensed in the US and Europe for other indications target HSC-related components of the fibrotic cascade. Their deployment in the near future looks likely. Ultimately, treatment strategies for liver fibrosis may vary on an individual basis according to etiology, risk of fibrosis progression, and the prevailing pathogenic milieu, meaning that a multitargeted approach could be required. The field continues to develop rapidly and starts to identify exciting potential targets in proof-of-concept preclinical studies. Despite this, no antifibrotics are currently licensed for use in humans. With epidemiological predictions for the future prevalence of viral, obesity-related, and alcohol-related cirrhosis painting an increasingly gloomy picture, and a shortfall in donors for liver transplantation, the clinical urgency for new therapies is high. There is growing interest from stakeholders keen to exploit the market potential for antifibrotics. However, the design of future trials for agents in the developmental pipeline will depend on strategies that enable equal patient stratification, techniques to reliably monitor changes in fibrosis over time, and the definition of clinically meaningful end points.

antifibrotic therapy; hepatic stellate cell

MECHANISMS AND MEDIATORS OF LIVER FIBROGENESIS AND FIBROSIS REGRESSION

As our understanding of the complex biological processes that regulate liver scarring increases, points of intervention in the fibrotic cascade are relentlessly uncovered. Key mechanisms and mediators are outlined below, to highlight emerging therapeutic targets (Fig. 1).

Liver Fibrogenesis and the Role of the Hepatic Stellate Cell-Myofibroblast

Liver fibrogenesis is orchestrated by a heterogeneous population of profibrogenic myofibroblasts (MFBs), the majority originating from hepatic stellate cells (HSCs), following a process termed “activation.” Other sources of fibrogenic cells have recently been implicated, including portal fibroblasts and cells derived from the bone marrow (including fibrocytes) (7). A more controversial phenomenon is epithelial-to-mesenchymal transition, whereby epithelial cells (hepatocytes or cholangiocytes) undergo transdifferentiation to MFBs driven by bone morphogenetic protein 7 and the hedgehog pathway, respectively, as well as a series of proinflammatory mediators. The relative contribution of these diverse precursors to fibrogenesis and the timing of their recruitment are unclear but may vary by disease etiology. In normal liver, quiescent HSCs store vitamin A in lipid droplets, but in response to liver injury they undergo characteristic morphological and functional changes to MFB-like cells. The activation process is initiated by many factors including reactive oxygen species, Toll-like receptor 4 (TLR4) ligands (lipopolysaccharide), ingestion of apoptotic bodies, and paracrine stimuli from neighboring cells including Kupffer cells, hepatocytes, and sinusoidal endothelial cells. Perpetuation of the HSC-MFB phenotype is sustained by autocrine and paracrine mediators including profibrogenic growth factors and cytokines [especially transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor (PDGF)], angiotensin II, leptin, cannabinoid receptor CB1 signaling, and extracellular matrix (ECM) stiffness. Activated cholangiocytes are a prominent source of profibrogenic growth factors and cytokines, which act on HSC-MFBs. In addition, they express the αvβ6 integrin that binds to and activates TGF-β1. Disease-specific fibrogenic mechanisms have also been elucidated, such as direct stimulation of HSCs by hepatitis C virus (HCV) and elevated levels of leptin and increased leptin signaling in nonalcoholic steatohepatitis (NASH). The pathway of HSC activation and perpetuation can also be checked, and endogenous mechanisms have been identified that include inhibitory mediators (e.g., interferon-γ, adiponectin) and receptors [e.g., peroxisome proliferator-activated receptor (PPAR)-γ, farnesoid X receptor (FXR), and CB2 receptor]. The activated HSC-MFB phenotype is characterized by the loss of lipid content, enhanced proliferation and migration, α-smooth muscle actin (α-SMA) expression, produc-
tion of excessive scar proteins (mostly type 1 collagen), and contractile and immune capability. The mechanisms of gene regulation in HSC-MFBs are also increasingly defined. Activated HSC-MFBs constitutively express the transcription factor NF-κB, via a critical phosphorylation event at a specific serine residue (Ser536) on the RelA subunit (15). Increased NF-κB activity promotes the survival of HSCs by inhibiting apoptosis, through enhanced transcription of antiapoptotic proteins and autocrine regulation of angiotensin II.

Accumulation of ECM in liver fibrosis results from increased synthesis and decreased degradation of the constitutive components. Studies of human and rodent liver indicate that matrix metalloproteinases (MMPs) with proteolytic activity against several scar constituents are expressed even in end-stage cirrhosis. However, these enzymes are held in check by concurrent secretion of their potent specific inhibitors (tissue inhibitors of metalloproteinases, TIMPs) by HSC-MFBs. Furthermore, TIMP-1 may also promote the survival of fibrogenic HSC-MFBs by inhibiting their clearance by apoptosis.

Mechanisms Mediating Regression of Liver Fibrosis

Studies in animal disease models have shown that, after the insult that induced fibrosis is withdrawn, TIMP levels fall dramatically, MMP activity increases, and scar is degraded (7). In parallel, HSC-MFBs are lost from the receding hepatic scar by apoptosis or through senescence, or possibly they revert to a more quiescent cellular phenotype. In reversal of biliary fibrosis, phagocytosis of apoptotic cholangiocytes by macrophages leads to upregulation of MMP expression (18). Indeed, hepatic macrophages have been identified as important regulators of matrix remodeling, with recent studies demonstrating a capacity for injury-inducing or repair-promoting roles in liver fibrosis (4). Data derived from animal models and explanted human liver have demonstrated that as advanced fibrosis resolves, the micronodules typical of “active” cirrhosis are remodeled and eventually coalesce into macronodules. These pathological findings are consistent with recent clinical data showing that increased septal thickness and smaller nodule size are associated with portal hypertension and poorer clinical outcomes (13).

Factors Affecting the Progression and Regression of Liver Fibrosis

The rate of progression of liver fibrosis can vary considerably, and in specific clinical situations (e.g., recurrent HCV infection after liver transplantation) progression of fibrosis is particularly rapid. Additional risk factors such as viral genotype, alcohol consumption, a high body mass index, or concurrent immunosuppressive therapy may also accelerate progression. Conversely, there is reasonable evidence that coffee consumption might exert a protective effect on fibrosis pro-
gression and hepatocellular carcinoma (HCC) risk. Genetic factors are also likely to influence remodeling of liver fibrosis and single-nucleotide polymorphisms (SNPs) in candidate genes with relevance to fibrosis risk have been identified (e.g., TGF-β1, TNF-α, IL-10, angiotensinogen, CCR5, MCP-1, DD5). A seven-gene signature (cirrhosis risk score) predicts fibrosis progression in patients with chronic HCV (6). Validation of these genetic determinants will equalize stratiﬁcation of patients in antifibrotic trials and shorten timelines if patients at high risk of progression can be selected.

Studies have demonstrated that liver ﬁbrosis varies in reversibility according to the duration of injury, the degree of angiogenesis, and the composition, spatial distribution, and cellularity of scar. Areas of ﬁbrosis that are not susceptible to degradation in animal models or human cirrhosis are relatively acellular and rich in elastin and highly cross-linked collagen, suggesting that ECM cross-linking might represent a “point of no return” in ﬁbrosis (7). The degree to which partial or complete ﬁbrosis regression restores normal portal blood ﬂow is uncertain, as the vascular distortion, shunting, and angiogenesis, and the composition, spatial distribution, and cellularity of scar.

CLINICAL REQUIREMENT FOR ANTIFIBROTIC APPROACH

Current antifibrotic therapy is anchored in treatment of the underlying cause. This can be highly effective (e.g., viral eradication in chronic hepatitis B virus or HCV), leading to regression of ﬁbrosis and improvement in liver function, even in some patients with histological cirrhosis. For patients in whom disease-speciﬁc treatment is unsuccessful or not possible, therapies that directly target the ﬁbrotic cascade could theoretically slow, halt, or regress liver ﬁbrosis to precirrhotic stages and prevent the development of liver failure or HCC and/or reduce portal hypertension and its complications. A review of current (Table 1) and recently completed antifibrotic trials suggests that repositioned drugs such as angiotensin II type 1 receptor (AT1R) blockers and thiazolidinediones are poised to enter the clinical arena. Other candidates have reached proof of concept in animal models (e.g., TIMP-1 inhibitors, anti-TGF-β strategies), but possible toxicity and/or off target effects in humans mean that further preclinical development will be required.

ANTIFIBROTIC TARGETS IN LIVER FIBROSIS

For the purposes of this mini-review, the spotlight is focused on therapies that are either on the cusp of clinical use or are leading preclinical candidates (see Ref. 19 for a recent comprehensive review).

Reduction of Inflammation/Tissue Injury (Hepatoprotectants)

Liver ﬁbrosis is invariably preceded by inﬂammation and oxidative stress. Indeed, persistent inﬂammation from a sustained hepatic insult perpetuates the ﬁbrogenic HSC phenotype and progression to cirrhosis. Consequently, a number of agents that attenuate or neutralize upstream inﬂammatory responses, and thus HSC activation, have been studied in vitro and in vivo. Some complementary medicinal agents, including silymarin (milk thistle), curcumin (turmeric), resveratrol (red wine), and coffee have an antioxidant effect, are generally safe, and are widely consumed, although controlled trials in humans are scarce. Vitamin E was evaluated in a recent large-scale trial in NASH (without diabetes) and, although no antifibrotic effect was demonstrated, histological liver injury was attenuated in 43% of patients (22). Promising results have been observed using hepatocyte growth factor (HGF) as a hepatoprotective agent. HGF is a hepatocyte mitogen but also modulates HSC proliferation, collagen synthesis, and TGF-β expression. Administration of HGF by gene therapy or as a recombinant protein prevents the progression of experimental liver ﬁbrosis. There are theoretical concerns regarding the stimulation of hepatic overgrowth and potential risk of oncogenesis, although a small-molecule HGF mimetic (Refanalin) is now in development.

Apoptotic cell death generates apoptotic bodies, which are phagocytosed by tissue macrophages (Kupffer cells) and to a lesser degree also by HSCs through interaction with the phos-
phatidylserine receptor. In Kupffer cells, engulfment of apoptotic bodies leads to expression of death ligands [TNF-α, TNF-related apoptosis-inducing ligand (TRAIL), and Fas ligand] capable of inducing death receptor-mediated apoptosis in hepatocytes, thus further amplifying liver inflammation and fibrosis. Phagocytosis by activated HSCs induces TGF-β and collagen-1 synthesis. Thus extensive apoptotic cell death triggers HSC activation either directly or indirectly through Kupffer cells. Caspase inhibitors have shown promise in animal models of fibrosis and NASH (e.g., VX-166) (29). However, although a pan-caspase inhibitor reduced serum transaminases in a short trial in patients with HCV without affecting viral replication, other early-phase human studies have been less successful. Clinical development has been halted for two drugs (IDN-6556 and GS-9450) with significant safety concerns emerging.

**Prevention of HSC Activation and/or Proliferation**

**PPAR-γ ligands.** Activation of HSCs is associated with low-level expression of PPAR-γ. Upregulation of PPAR-γ or addition of PPAR-γ ligands reverses the characteristic biochemical and morphological features of HSC activation, highlighting the importance of PPAR-γ in the maintenance of the quiescent HSC phenotype and identifying this axis as a potential therapeutic target. Several PPAR-γ ligands (“glitazones”) have been studied in experimental models. An important observation using pioglitazone was that both the nature of liver injury and the severity of fibrosis at the time of treatment initiation had a major influence on the therapeutic effect of this drug. In cholestatic fibrosis pioglitazone was ineffective, whereas in toxic (CCL4) or metabolic (choline-deficient diet) models pioglitazone only slowed fibrosis progression early in the disease course (8). This has clinical resonance, since farglitazar was recently shown to have no significant effect on α-SMA or collagen expression in a large cohort of patients with chronic HCV and moderate fibrosis at entry (10). Glitazones have an insulin-sensitizing effect and continue to be evaluated in patients with NASH (Table 1), although their future role is uncertain following recent reports of excess cardiovascular events and weight gain.

A phase II study of an FXR agonist (the semisynthetic bile acid derivative INT-747) has recently been undertaken in patients with Type 2 diabetes and presumed nonalcoholic fatty liver disease to assess effects on insulin resistance and liver biochemistry. The antifibrotic potential of stimulating FXR is unproven and the recent observation that there is no FXR expression in mouse or human HSCs and MFBs in liver fibrosis suggests that these cells are not direct therapeutic targets for FXR ligands (5).

**HMG-CoA reductase inhibitors (“statins”).** Statins are widely prescribed in human disease and are generally safe. Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase by statins inhibits HSC proliferation in vitro and has shown beneficial effects on portal hypertension and on angiotensin II-induced inflammation in liver fibrosis models. More recently, early treatment with atorvastatin after bile duct ligation in rats attenuated activation of HSCs and subsequent collagen deposition (28). However, collagen content was unaffected if atorvastatin was initiated once fibrosis was established, although HSC turnover and profibrotic cytokine expression were reduced. The observation that statins may enhance the antifibrotic effect of AT1R antagonists has obvious implications for the treatment of NASH/metabolic syndrome, although this synergy has yet to be tested in clinical trials.

**Reducing Fibrogenesis**

**Angiotensin system inhibitors.** The renin-angiotensin system has been implicated in liver fibrogenesis and portal hypertension. The key downstream effector is angiotensin II, and both angiotensin-converting enzyme inhibitors and AT1R antagonists (“sartans”) can retard fibrosis in animal models. Moreover, liver inflammation and fibrosis induced by CCL4 and bile duct ligation are attenuated in AT1R knockout mice (30). Long-term administration of the AT1R antagonist losartan in patients with chronic HCV slowed fibrosis progression and downregulated expression of NAPDH oxidase and profibrogenic genes (e.g., collagen-1, MMP-2) (2). Telmisartan could be the AT1R antagonist of choice since it shows the greatest affinity for the AT1R, has favorable pharmacokinetic and pharmacodynamic properties, and also acts as a partial agonist of PPAR-γ. Further randomized controlled trials of sartans are underway in HCV and NASH (Table 1), and clinical application seems inevitable. Coupling losartan to an HSC-specific carrier in rat models of liver fibrosis was also antifibrotic (11), and this strategy is appealing because it could circumvent unwanted systemic effects (e.g., hypotension). The recently described angiotensin 1–7/mas receptor axis has emerged as an additional potential antifibrotic target.

**Targeting the TGF-β pathway.** TGF-β plays a central role in the pathogenesis of liver fibrosis. Consequently, reducing TGF-β synthesis or inhibiting components of its complex signaling pathway represent important therapeutic targets. Various strategies have been developed to block TGF-β effects, including the use of TGF-β neutralizing antibodies, soluble TGF-β decoy receptors, small interfering RNA (siRNA), and blocking oligonucleotides. Despite showing antifibrotic effects in preclinical models, none of these approaches is ready for clinical application. Furthermore, since TGF-β receptors are expressed on essentially all cell types, an inhibitor could trigger autoimmune diseases or cellular dedifferentiation, unless it was selectively targeted to activated HSCs/MFBs.

Pirfenidone, an orally available pyridine derivative, inhibits TGF-β production and has been subjected to a small uncontrolled pilot study in HCV-related fibrosis, in which it improved liver inflammation and fibrosis in some patients after 12 mo of treatment (1).

Protein kinase inhibitors have been used in patients with neoplastic and chronic inflammatory diseases. Imatinib inhibits several tyrosine kinases (including c-Kit, PDGF receptor, and c-Abl) and has been shown to inhibit HSC proliferation and reduce fibrosis and portal hypertension in experimental models. However, many patients with liver fibrosis may be unable to tolerate imatinib treatment, which can lead to a wide spectrum of complications including edema and blood dyscrasias. Second generation inhibitors (e.g., nilotinib) have better side effect profiles and will need to be evaluated.

The integrin αvβ6, expressed on activated cholangiocytes in injured liver, is capable of activating matrix-bound latent TGF-β in the local microenvironment. Inactivation of αvβ6 with a nonpeptide inhibitor or blocking antibody inhibits

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cholangiocyte proliferation and collagen deposition in biliary and nonbiliary fibrosis models (17). If proven effective, this would be an attractive approach in humans, since blocking activity would be restricted to sites of active injury without compromising the more general homeostatic effects of TGF-β.

**Stimulating HSC-MFB Apoptosis**

Drugs that can stimulate apoptosis of activated HSC-MFBs could have an antifibrotic effect as the cellular source of scar matrix and TIMP-1 is depleted. Such a strategy might prove most effective in the setting of active fibrogenesis, in contrast to “burnt-out” inactive cirrhosis in which mature collagen scars tend to be relatively acellular and highly cross-linked. In a recent in vitro study, the pyrimidine synthesis inhibitor lefunomide was shown to enhance HSC apoptosis by upregulating TRAIL expression by Kupffer cells (25). Gliotoxin and sulfasalazine both exert their proapoptotic effect via NF-κB inhibition in HSCs and have been shown to accelerate spontaneous resolution of bridging fibrosis in animal models (3, 14, 15). Gliotoxin has toxic effects on other cell types, but coupling to a single-chain antibody against synaptophysin (which is expressed in activated HSCs) conferred selectivity and reduced fibrosis in a CCl₄ rat model (3). Sulfasalazine is cheap, off-patent, and has a good safety profile in other human fibroinflammatory disorders (e.g., inflammatory bowel disease); further studies are probably warranted.

Cannabinoid receptor signaling has been shown to play a key role in fibrogenesis, and CB2 receptor activation promoted HSC apoptosis and reduced fibrosis in cirrhotic rats (12) whereas CB1 receptor antagonism was also shown to be antifibrotic (26). Rimonabant, a selective CB1 full inverse agonist, was a promising agent not least in obesity-induced liver disease because of its anorectic effect. Unfortunately, it has recently been withdrawn from the European market because of severe psychiatric effects and suicides. Selective peripheral CB1 antagonists, without central effects, are already in development.

**Promoting ECM Degradation**

Although many drug candidates can inhibit fibrogenesis, it may be necessary to deploy other agents capable of stimulating degradation of accumulated scar, particularly in patients with advanced disease. This may be facilitated by manipulating the local balance between MMPs and their regulators. One difficulty is that ECM turnover occurs constantly throughout the body and any modulation by targeting MMPs, TIMPs, urokinase plasminogen activator, or other activators would need to be liver specific to avoid systemic adverse effects (e.g., tendon pain, cataracts). In addition, MMP activity can also result in the generation of epitopes that act as cell effectors or the release of sequestered growth factors from ECM. Proteolysis of adhesion molecules, growth factors, cytokines, chemokines, and receptors have all been documented, with potentially widespread effects on cell behavior, cell-cell communication, and tumor progression. In animal models, collagenolytic MMPs (e.g., MMP-1, MMP-8) or TIMP-1 scavengers (MMP-9 mutants) have been delivered systemically via adenoviral vectors and can stimulate ECM degradation (20, 23). Halofuginone, an oral semisynthetic plant alkaloid that stimulates expression of MMP-1, MMP-3, and MMP-13 (via NF-κB and p38 kinase), induced degradation of established fibrosis in a thioacetamide rat model (16). Recent studies using autologous cell therapies in animal models suggest that infusion of bone marrow-derived cells may lead to engraftment in the liver, upregulate of hepatic MMPs (e.g., MMP-9, MMP-13), and facilitate matrix degradation (21). Although precise mechanisms require further clarification, data are already emerging from small, uncontrolled studies in human cirrhosis that suggest cell therapies could have clinical utility (27).

**MONITORING FIBROSIS PROGRESSION/REGRESSION IN RESPONSE TO THERAPY**

In clinical practice, there is an obvious requirement for effective biomarkers to gauge disease severity and response to therapy (24). Liver biopsy is invasive, examines only 1/50,000th of the liver, and is compromised by sampling variability. Additionally, a change in fibrosis on biopsy (even when assessed quantitatively) is simply a notional surrogate for future clinical outcomes. We require noninvasive markers that are sensitive, specific, and responsive to changes in fibrogenic/fibrolytic activity. In patients with advanced cirrhosis, in which the capacity to remodel mature scar tissue may be limited, measurement of a functional parameter such as portal pressure (hepatic venous pressure gradient) might be more relevant. Serum marker panels to predict fibrosis and cirrhosis are being continuously refined and evaluated in large-scale studies, but the current inability to differentiate intermediate fibrosis stages precludes their use as surrogates in antifibrotic trials. Fibroscan (transient elastography), a bedside test to assess liver fibrosis by measuring liver stiffness, gives immediate results and has become popular with clinicians. It samples a 100-fold larger volume of the liver than biopsy (~1/500th), correlates well with historical stages of fibrosis, and compares favorably with other noninvasive tests. In the future, more than one modality (i.e., a combination of serum markers and imaging) may be used clinically. One major advantage of these noninvasive approaches over liver biopsy is the ability to repeat measurements at regular intervals and thereby give some idea of the speed of change in fibrosis development or reversal.

**TRANSLATIONAL BARRIERS**

Given the striking progress in understanding the biochemistry and cell biology of liver fibrosis, it is frustrating that so many novel early projects fail to translate to the market. The high costs associated with drug development and target validation are a considerable barrier, requiring well-resourced industrial partners who are “in it for the long haul.” Additionally, the regulatory bar has risen progressively, partly in response to societal demands for more certainty about drug efficacy and safety prior to marketing. This has shifted focus to drug repositioning as a means of fast tracking established drugs that target the fibrotic cascade (e.g., AT1R blockers, glitazones) into the clinical arena. Unfortunately, positive results observed in animal models have not always been replicated in clinical studies. There are plausible reasons for this, including differences in pharmacokinetics, dynamics of scarring, and degree of ECM cross-linking. Hidden phenotypes related to toxicity are also responsible for the high failure rate of drug candidates, although strategies are emerging to directly probe biochemical pathways and identify these unintended effects in...
intact, living cells (9). Targeted drug delivery to fibrogenic liver cells could potentially circumvent these off-target effects. The need for validated noninvasive biomarkers to accurately measure fibrogenic/fibrolytic activity over time and the lack of consensus on the optimal end point(s) for antifibrotic trials remain significant (but not insurmountable) challenges.

CONCLUSIONS

The anticipated burden of disease from chronic viral hepatitis, obesity- and alcohol-related liver fibrosis, and cirrhosis will mean that many patients in whom disease-specific treatment is not possible or ineffective will, at some stage, be candidates for antifibrotic therapies. In practical terms, the potential to achieve stasis or lack of progression of fibrosis in the face of continued liver injury would be clinically meaningful if liver function was preserved, complications of decompensated cirrhosis were reduced, or the need for liver transplantation was delayed or averted. Future antifibrotic strategies are likely to be customized on the basis of disease-specific features and host (genetic) determinants of fibrosis progression and treatment response. In the absence of a “magic bullet,” a multiagent approach targeting mechanistically distinct components of the fibrotic cascade could be synergistic and might permit the use of lower, less toxic doses of individual agents required for protracted therapy.

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