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Hepatic fibrosis

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Abstract

Purpose of review—This review will summarize the most significant work that contributed to the understanding of liver fibrosis progression and resolution, which in turn has yielded new areas of therapeutic targeting.

Recent findings—Liver fibrosis is the result of an imbalance between production and dissolution of extracellular matrix. Stellate cells, portal myofibroblasts, and bone marrow derived cells converge in a complex interaction with hepatocytes and immune cells to provoke scarring in response to liver injury. Uncovering the specific effects of growth factors on these cells, defining the interaction of different cell population during liver fibrosis and characterizing the genetic determinants of fibrosis progression will enable the discovery of new therapeutic approaches.

Summary—The outcome of improved understanding of liver fibrosis process, especially the regulation and activation of stellate cells, is reflected in the development of new therapeutic strategies, which are validated in animal models.

Keywords

genetic polymorphism; hepatic stellate cells; liver fibrosis; PDGF; portal fibroblast; TGF- β

Introduction

Hepatic fibrosis represents the wound healing response to liver injury from a wide variety of etiologies. Cirrhosis is the most advanced stage of fibrosis, connoting more than fibrosis alone, but rather distortion of the liver parenchyma associated with septae and nodule formation, altered blood flow and the potential development of liver failure. The past year has seen remarkable progress in the field of hepatic fibrosis in a range of areas, including the expansion of potential cellular sources of extracellular matrix (ECM), the intimate crosstalk with immune and inflammatory cell subsets, pathways regulating fibrosis regression, genetic determinants of fibrosis risk, models of fibrosis and new therapeutic directions, among others.

Emerging cellular sources of hepatic fibrosis

Hepatic stellate cells (HSCs) have dominated studies exploring mechanisms of liver fibrosis over the last two decades. HSCs are resident vitamin A-storing cells in the perisinusoidal space of Disse between the sinusoidal endothelium and hepatocytes. Following hepatic injury, HSCs become activated, proliferate and produce extracellular matrix (ECM) [1]. Both the characterization of HSCs and their behavior in hepatic injury have been well characterized, and excellent models exist for their analysis *in vivo* and *in culture* [2].

Additional cellular sources of ECM have emerged from more recent studies. As well as HSCs, portal myofibroblasts and bone marrow-derived cells contribute to fibrosis, in addition to the growing potential of epithelial mesenchymal transition (EMT). Portal myofibroblasts, whereas similar to HSCs, have unique characteristics that include a distinct response to transforming growth factor β (TGF β) and a prominent role in responding to matrix stiffness [3,4].

There is growing evidence that bone marrow derived cells are recruited in the progression and regression of liver fibrosis [5,6]. During fibrosis regression after CCl₄, bone marrow-derived cells migrate into fibrotic liver, some of which express MMP-13 and MMP-9. In addition, G-CSF and HGF treatment significantly enhance migration of bone marrow cells into fibrotic liver and over-expression of HGF together with G-CSF, synergistically stimulate MMP-9 expression in liver, which is followed by an accelerated resolution of fibrotic bands [5,6].

Finally, EMT is emerging as yet another source of injury-associated mesenchymal cells, derived either from resident hepatocytes or biliary epithelial cells [7,8^{*},9,10]. Signaling pathways underlying this intriguing process are being clarified, with particular emphasis on hedgehog and its receptors [8^{*}], as this pathway also plays a similar role in other tissues. Two different groups reported that both murine and human hepatocytes might undergo EMT. During EMT cellular signaling, hepatocytes from cirrhotic liver switch from a MAPK-independent to a MAPK-dependent cell survival pathway [9]. In a recent study, experimental expression of Smad7 in hepatocytes of mice attenuated TGF- β signaling and EMT, resulting in less liver damage and reduced collagen accumulation [10]. Cell specific ablation of TGF- β signaling in hepatocytes may be sufficient to blunt the fibrogenic response. Another report focused on EMT in chronic hepatitis C virus infection [11], which has indicated that hepatocytes affected by chronic inflammation undergo transition from the tumor-suppressive pSmad3C pathway, which is characteristic of mature epithelial cells, to the JNK/pSmad3L pathway, which accelerates liver fibrosis and increases the risk of cancer.

The extent to which pathways other than activation of resident HSCs contribute to fibrogenesis in liver is unknown, but it remains likely that HSC activation is still the dominant pathway. Future studies will need to define not only the quantitative contribution of non-HSC sources of ECM, but also whether their importance varies with both the etiology as well as the chronicity of injury.

Pathways of stellate cell activation mediated by soluble stimuli

Transforming growth factor β (TGF- β) and platelet-derived growth factor (PDGF) have traditionally been considered as the key fibrogenic and proliferative stimuli to HSCs, respectively. However, newer players and pathways are gaining attention. For example, connective tissue growth factor (CTGF) is an important fibrogenic mediator downstream of the TGF- β pathway. CTGF expression is induced by TGF- β in hepatocytes of carbon tetrachloride (CCl₄)-treated mice [12]. In response to TGF- β , the activin receptor-like kinase 5/Smad3 pathway induces the expression of CTGF, whereas activin receptor-like kinase 1 activation attenuates it. The intracellular TGF- β antagonist Smad7 completely blocks CTGF induction. In contrast to hepatocytes, a recent study did not find any significant interaction between TGF- β and CTGF in HSCs [13]. Moreover, whereas endothelin-1 has no effect on hepatocytes, it stimulates CTGF/CCN2 expression in HSCs, indicating that different pathways regulate CTGF in different cell types within the liver.

An important connection has been uncovered between innate immune responses mediated by the toll-like receptor 4 (TLR4) and TGF- β driven fibrogenesis. Seki *et al.* [14] reported that TLR4 stimulation by its main ligand, lipopolysaccharide (LPS), leads to increased activity of TGF- β 1, thereby stimulating production of hepatic scar. Among a large number of genes induced by LPS in quiescent HSCs, the TGF- β pseudoreceptor Bambi was most intriguing.

LPS-induces Bambi down-regulation (that is, leading to less inhibition of TGF- β) via the MyD88-NF κ B pathway, resulting in TGF- β amplification of fibrogenic signaling. Taken together, these data point to a new pathway contributing to chronic liver injury, in which bacterial products may activate TLR4 and alter fibrosis progression [15]. Indeed, different TLR4 polymorphisms have more recently been associated with variable rates of fibrosis progression among individuals infected with HCV [16], which may be attributable to altered HSC responses conferred by these different TLR4 sequences [17].

As noted earlier, PDGF is a major cytokine implicated in HSC proliferation. Two newly discovered PDGF isoforms, PDGF-C and PDGF-D have recently been linked to both fibrogenesis and liver cancer. In a bile duct ligation model of liver fibrosis, PDGF-D is significantly upregulated, increasing proliferation of culture-activated HSCs and expression of ECM proteins [18]. Similarly, a transgenic mouse model overexpressing PDGF-C in liver leads first to fibrosis then to hepatocellular carcinoma [19].

Activation of HSCs in response to liver injury is associated with a gradual replacement of the basement membrane-like ECM milieu in the space of Disse by collagenrich fibers. Currently, we do not know how HSCs retain their quiescence in normal liver, or how this homeostatic balance is disrupted to provoke the activation of HSC. It has been proposed that ECM breakdown initiated by MMPs contributes to HSC activation [20]. Dual stimulation of HSCs by IL-1 and type I collagen provokes ECM degradation and HSC activation. These studies have defined a cascade of MMP activation (MMP-14>MMP-13>MMP-9) and a positive feedback loop of MMP-9 more than MMP-13 mediated by HSCs. This study emphasizes the importance of microenvironment and its interaction with cytokine signaling in regulating HSC responses.

Intracellular events and gene regulation in hepatic stellate cells

Transcriptional events driving HSC activation downstream of cytokine signaling are increasingly being revealed. For example, PDGF or insulin induced phosphorylation of the forkhead transcription factor gene FoxO1, leads to translocation from the nucleus to the cytosol mediated by the PI3K/AKT pathway in HSC [21]. Moreover, constitutively active FoxO1 inhibits proliferation and transdifferentiation of HSC. This intriguing finding provides a new direct link between hyperinsulinemia and fibrosis, which may explain in part the significant fibrosis and HSC activation underlying many cases of nonalcoholic steatohepatitis associated with the current obesity epidemic.

Amphiregulin is an epidermal growth factor receptor (EGFR) ligand specifically induced upon liver injury. Following CCl₄ induced liver fibrosis, amphiregulin^{-/-} mice have markedly diminished fibrosis compared with the wild type controls [22]. In culture, amphiregulin stimulates cell proliferation and promotes survival of fibrogenic cell, which represents a new role of the EGFR system in hepatic fibrogenesis.

Hepatocyte growth factor (HGF) is the most powerful hepatotrophic factor identified to date. HGF also suppresses TGF- β expression and promotes the complete resolution of fibrosis in rodent models of cirrhosis. A recent study has implicated a novel molecular mechanism by which HGF represses TGF- β -stimulated profibrogenic signaling [23^{**}]. Using immunoprecipitation and mass spectrometry, the investigators identified galectin-7 as a key factor interacting with phosphorylated Smad3. HGF treatment enhances the interaction between those two proteins, and accelerates their nuclear export. Functional assays indicate that galectin-7 represses TGF- β -stimulated COL1A2 and plasminogen-activatorinhibitor-1 (PAI-1) transcription.

ATP-binding cassette (ABC) transporters are involved in the hepatobiliary transport of metabolites, phospholipids, cholesterol and bile acids. The survival and possible expansion of progenitor cells during liver injury has been attributed to high expression of these ABC transporters. Two subclasses of ABC transporters, multidrug resistance protein (Mdr)-type and multidrug resistance-associated protein (Mrp) type transporters have been characterized during activation of HSCs [24]. Activated HSCs isolated from CCl₄ induced fibrotic rat liver express high levels of Mrp1 and levels of Mrp3, Mrp4, Mdr1a and Mdr1b similar with hepatocytes. During HSC activation, Mrp1 increases, whereas the Mrp inhibitor leads to HSC necrosis. Thus, the Mrp transporter might provide a survival role for HSCs under conditions prevailing in the chronically injured liver. In addition, inhibiting Mrp1 function in chronic liver disease might be used therapeutically to provoke death in activated HSCs without affecting hepatocyte survival.

Hepatic stellate cell interactions with other liver cells and immune cells

Characterizing the interaction of HSCs with parenchymal and nonparenchymal liver cells is a research priority, yet has been largely overlooked until recently. Moreover, chronic liver injury is associated with varying degrees of hepatocyte apoptosis, yet its relationship to HSC activation is unknown. A recent study has established that apoptotic hepatocytes can upregulate TGF- β 1 and collagen 1 mRNA in a human HSC line (LX-2) and in primary mouse HSCs via the Toll-like receptor 9 (TLR-9) [25]. In addition, apoptotic hepatocyte DNA also inhibits PDGF-mediated HSC chemotaxis through TLR-9, which blocks the PDGF signaling molecule inositol 1,4,5-triphosphate and reduces cytosolic Ca²⁺. These findings support the hypothesis that DNA from apoptotic hepatocytes is a biologically active molecule in HSC activation mediated by TLR-9. In addition, apoptotic hepatocyte DNA provides a critical stop signal to localize activated HSCs to sites of matrix remodeling.

Natural killer (NK) cells have been implicated in directing HSC turnover by induction of HSC apoptosis [26] via the actions of TRAIL (tumor-necrosis factor-related apoptosis-inducing ligand). In the bile duct ligated mouse model, TRAIL mRNA is increased, which is also associated with increased hepatocyte apoptosis [27]. Thus, TRAIL on NK cells induces apoptosis of hepatocytes, and the apoptotic bodies are phagocytosed by hepatic stellate cells (HSC) or Kupffer cells, leading to increased TGF- β and enhanced fibrogenesis.

Lymphocytes may also directly contribute to activation of HSCs, independent of cytokine release. Using a coculture system, CD8+ and CD4+ T cells from peripheral blood lymphocytes (PBL) of HBV/HCV-infected patients with advanced fibrosis could be phagocytosed by HSCs and provoke cellular activation [28*]. This response is mediated by the interaction of ICAM and integrins. In addition, Rac1 and Cdc42 pathways are involved downstream. These data expand our understanding of HSCs cross-talk with specific lymphocyte subsets in chronic liver diseases.

Pathways of fibrosis associated with hepatic viral infection and ethanol

There is ample evidence that the HBV X protein (HBx) contributes to viral replication and tumor development; however, little is known about its possible role in the development of liver fibrosis. In human primary and rat HSCs, hepatitis B virus X protein (HBx) expression in hepatocytes leads to paracrine activation and proliferation of HSCs [29].

Superinfection of hepatitis delta virus (HDV) in chronic HBV patients accelerates progression of fibrosis. HDV RNA encodes only a single protein, the hepatitis delta antigen (HDAg). HDAg has two isoforms, a small HDAg (SHDAg) and a large HDAg (LHDAg), which contains an additional 19 amino acids at its C terminus. A recent study indicates that LHDAg, not the SHDAg, potentiates TGF- β - and c-Jun- induced signal activation, and the isoprenylation of

LDHAg plays a major role in signaling [30]. LHDAG synergistically activates hepatitis B virus X protein-mediated TGF- β and AP-1 signaling. In addition, LHDAG enhances the level of TGF- β -induced plasminogen activator inhibitor-1 (PAI-1). Collectively, these data indicate that LHDAG can induce liver fibrosis through the regulation of TGF- β -mediated signal transduction.

A quantitative proteomic study on HCV-infected human liver tissue from 15 patients at different stages of fibrosis [31], reported that several proteins associated with fatty acid beta-oxidation were downregulated in livers with advanced fibrosis. Additional downregulated proteins included mitochondrial proteins of the oxidative phosphorylation system and those responding to oxidative stress and reactive oxygen species. In contrast, the majority of endoplasmic reticulum and cytosolic proteins were upregulated. The findings contribute to an emerging association between HCV pathogenesis and both oxidative stress and hepatic mitochondrial dysfunction. In a more recent proteomics study of HCV infected liver, a series of potential biomarkers has emerged that may prove useful in estimating the severity of fibrosis using serum markers [32].

HIV-HCV coinfecting patients have increased liver disease progression compared with patients with HCV infection alone. However, the mechanisms by which HIV infection increases the risk of liver disease are unknown. In human cohorts in whom HCV and HIV outcomes were carefully evaluated [33], HIV-related CD4+ lymphocyte depletion was strongly associated with microbial translocation and progression to cirrhosis. These data suggest that microbial translocation may accelerate liver fibrosis progression but it is unclear whether microbial translocation causes fibrosis progression or merely results from it. Data reviewed above (see Pathways of Stellate cell Activation Mediated by Soluble Stimuli) implicating LPS signaling in HSC fibrogenesis represents a possible mechanism wherein bacterial products may be causative, however.

Alcohol accelerates progression of liver fibrosis through several mechanisms. In an animal model [34], chronic ethanol consumption stimulated oxidative stress and induced TGF- β 1, associated with decreased NK-cytotoxicity to HSCs and inhibition of the antifibrotic effects of IFN- γ , collectively resulting in acceleration of liver fibrosis.

Genetic determinants of hepatic fibrosis progression

Increasing evidence indicates that host polymorphisms (i.e., genetic factors) can regulate fibrosis progression. Kruppel-like factor 6 (KLF6) belongs to the Kruppel-like family transcription factors and contributes to hepatic fibrosis progression, hepatocyte proliferation and differentiation. A role of KLF6 in progression of nonalcoholic fatty liver disease (NAFLD) progression has been suggested by a recent study in which wild type (wt) KLF6 expression is increased in advanced stages of NAFLD. Interestingly, KLF6-IVS1-27G/A, a KLF6 SNP located within the first intron of the gene, was found to abrogate the wt-KLF6 induced transcriptional increase in α SMA and type 1 collagen mRNA, and was associated with reduced fibrosis in NAFLD [35]. This SNP favors the generation of dominant negative alternative splice forms that antagonize wt KLF6 [36]. Furthermore, there was preferential transmission of the wt allele to children who will progress to advance fibrosis secondary to NAFLD. These findings provide the first clinical evidence for the role of wt-KLF6 and its splice variants in NAFLD and liver fibrosis.

In a similar type of genetic risk assessment, Wasmuth *et al.* [37] conducted a systematic study examining the impact of CX3CR1 SNPs on HCV-induced liver fibrosis. There was an association of the CX3CR1 gene variant V249I with the severity of liver fibrosis. CX3CR1 is expressed on HSCs, and fractalkine stimulation of these cells could significantly downregulate TIMP-1 mRNA expression in primary HSCs. On the basis of these and additional genetic data,

the investigators hypothesized that impaired binding of fractalkine to CX3CR1 carrying the V249I SNP could lead to reduced repression of TIMP-1 expression, thereby promoting more profibrogenic activity than the wild-type CX3CR1.

To explore the role of keratin gene polymorphisms on liver fibrosis, CCl₄ or thioacetamide was administered to transgenic mice expressing the human keratin 18 (K18) R89C SNP [38]. Compared with control animals, CCl₄ led to similar liver fibrosis but increased injury in K18 R89C mice. In contrast, thioacetamide caused more severe liver injury and fibrosis in K18 R89C mice, compared with wild type and nontransgenic mice, leading to increased mRNA expression for collagen, TIMP-1 MMP-2 and MMP-13. Analysis of nontransgenic mice indicated that thioacetamide and CCl₄ provoke dramatically different responses of cytoskeletal and chaperone proteins to injury.

Hemochromatosis due to mutations in the HFE gene is the most common genetic liver disease, and thus correlations of specific sequences of the gene to outcomes have important clinical relevance. In a recent study, the presence of C282Y+/- mutations in the HFE gene was associated with advanced hepatic fibrosis among caucasian patients with NASH, which has been attributed to increased oxidative stress in the liver due to increased iron deposition [39].

Mechanisms regulating fibrosis resolution

Degradation of collagens by matrix metalloproteinases (MMPs) has been investigated for many years; however, clarification of how collagen fragments are metabolized following MMP degradation is needed. A recent study indicates that collagen α -chains in rat liver are transported to the circulation and removed rapidly via receptor-mediated uptake by the mannose receptor on liver sinusoidal endothelial cells (LSECs) [40].

In order to define targets of antifibrotic therapies, increasing attention has been focused on mechanisms underlying clearance of activated HSCs as fibrosis regresses, in hope of exploiting these pathways therapeutically. In that context, several studies have recently illuminated this area of inquiry.

Nitric oxide is an important mediator implicated in the pathogenesis of liver cirrhosis, largely by its effects on portal hemodynamics. However, role of nitric oxide/inducible nitric oxide synthase (iNOS) – the enzyme that generates nitric oxide – on liver fibrosis progression is unclear. In a mouse model, iNOS deficiency has a protective effect on hepatic fibrosis induced by CCl₄ associated with increased apoptosis and, therefore, reduced numbers of activated stellate cells [41].

A related paper has suggested that senescence of activated HSCs, in addition to apoptosis, may be an important mechanism of cellular clearance during fibrosis resolution [42^{••}]. This pathway is well characterized in other tissues, and thus it represents an appealing target for antifibrotic drug development. What is unclear, however, is whether senescence is truly distinct from apoptosis in these models [43]; regardless, the findings are very exciting.

Models of hepatic fibrosis

Subtle differences between models of experimental liver fibrosis underscore the importance of studying mechanisms in more than one model to ensure that findings are generalizable and relevant to human disease [44]. In the support of this point, gene expression profiles of HSC activation were compared between activation in culture and *in vivo* in two different animal models [45]. In bile duct ligation (BDL) and CCl₄ models activated HSC displayed an almost identical pattern of upregulated and downregulated genes, whereas gene expression in culture-activated HSC overlapped only partially with BDL and CCl₄-activated HSCs. In addition,

coculture of HSCs with Kupffer cells or following LPS treatment led to a closer approximation of culture-induced changes with those *in vivo*.

New therapeutic directions in hepatic fibrosis

Newer models of fibrosis are also being developed that are proving valuable in uncovering mechanisms of fibrosis and defining new therapies. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) pathways are crucial to angiogenesis, a process that contributes significantly to the pathogenesis of portal hypertension. Inhibition of VEGF and/or PDGF signaling attenuates the hyperdynamic splanchnic circulation and portosystemic collateralization in rats with established portal hypertension [46]. Moreover, dual inhibition of VEGF and PDGF signaling significantly reduced splanchnic neovascularization (that is, CD31 and VEGFR-2 expression) and pericyte coverage of neovessels (that is, alpha-smooth muscle actin and PDGFR- β expression) leading to marked decreases in portal pressure, superior mesenteric artery blood flow, and increases superior mesenteric artery resistance. Portosystemic collateralization was reduced as well. Importantly, these findings also suggest that an extended antiangiogenic strategy [46] may be a novel approach to the treatment of portal hypertension.

In contrast to studies of regression, cirrhosis progression is associated with a significant enhancement of vascular density and expression in cirrhotic livers of vascular endothelial growth factor-A, angiopoietin-1, angiopoietin-2, and placental growth factor [47]. Treatment with a small molecule receptor tyrosine kinase inhibitor SU11248, which blocks VEGF, significantly decreases hepatic vascular density, inflammatory infiltration, alpha-SMA abundance, collagen expression, and portal pressure. These results suggest that multitargeted therapies against angiogenesis, inflammation, and fibrosis merit consideration in the treatment of cirrhosis.

New insight into the role of lipid abnormalities associated with liver cirrhosis as well as a new therapeutic target was provided by a recent report [48]. In cirrhotic rats, recombinant HDL administration decreases the hepatic proinflammatory signals induced by LPS, restores hepatic eNOS activity, and lowers portal pressure. This suggests that the decrease in circulating HDL in cirrhosis may contribute to the excessive proinflammatory response and intrahepatic eNOS down-regulation.

Previous work indicated that fibrosis resolution in rat was accelerated by stimulating the apoptosis of myofibroblasts using the compound gliotoxin [49]. In order to more accurately target fibrogenic myofibroblasts, a gliotoxin-conjugated monoclonal single chain antibody has been developed that binds to the activated HSC membrane antigen synaptophysin (C1-3 ScAb-gliotoxin) in CCl₄-induced fibrosis [50]. With ongoing fibrogenesis, C1-3 Sc Ab-gliotoxin could effectively induce apoptosis of myofibroblasts and reduce fibrosis severity without affecting the Kupffer cell population that produces MMP-13, which contributes to degradation of scar matrix.

Another targeting approach has utilized liposomes coupled to vitamin A to specifically target activated stellate cells in delivering an antifibrotic siRNA [51*]. An elegant report using this approach was validated in three different models and raises the prospect of using this targeting mechanism not only for therapeutics but also diagnostic approaches [52].

The role of HSCs in retinoid metabolism has led to another possible therapeutic target. The storage of retinoids in the HSC is the result of the crosstalk between lecithin:retinol acyltransferases (LRAT) and diacylglycerol acyltransferase (DGAT). Inhibition of DGAT1 in liver recently had no effect on hepatic triglyceride content or liver necroinflammation but reduced HSC activation and liver fibrosis in mice with NASH [53]. HSC isolated from DGAT1

antisense oligonucleotide-treated rats had reduced DGAT1 expression and increased mRNA levels of LRAT and cellular retinol binding protein-1. During culture, they retained more vitamin A, had repressed collagen $\alpha 2$ (I) transcriptional activity, and expressed less collagen $\alpha 1$ (I) and $\alpha 2$ (I) mRNA.

Uno *et al.* [54] has investigated the antifibrotic effects of tranilast, an antifibrogenic agent that inhibits the action of TGF- β and is used clinically for fibrogenesis-associated skin disorders including hypertrophic scars and scleroderma. In a dietary animal model of NASH, obese diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and nondiabetic control Long-Evans Tokushima Otsuka (LETO) rats fed a methionine-deficient and choline-deficient diet, treatment with 2% tranilast (420 mg/kg/day) for 8 weeks prevented the development of hepatic fibrosis and the activation of HSCs, and downregulated the expression of genes for TGF- β and TGF- β -target molecules, including $\alpha 1$ procollagen and plasminogen activator-1. Tranilast ameliorated hepatic steatosis and upregulated the expression of genes involved in β -oxidation, such as peroxisome proliferator-activated receptor- α and carnitine O-palmitoyltransferase-1.

Because angiotensinogen II type I receptors are profibrogenic, a large number of studies have explored the impact of drugs that block these receptors, which are an especially appealing option because the drugs are widely used to treat hypertension. For example, olmesartan's effects on methionine-deficient and choline-deficient (MCD) diet-induced steatohepatitis were investigated in obese, diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and control Long-Evans Tokushima Otsuka (LETO) rats [55]. In both OLETF and LETO rats, olmesartan inhibited hepatic oxidative stress (4-hydroxy-2-nonenal-modified protein) and expression of NADPH oxidase. Olmesartan also inhibited hepatic fibrosis, HSCs, and expression of fibrogenic genes (transforming growth factor-beta, $\alpha 1$ [I] procollagen, plasminogen activator inhibitor-1) in both OLETF and LETO rats.

Conclusion

The past year has witnessed a remarkable number of advances in our understanding of hepatic fibrosis, setting the stage for continued progress in the development of antifibrotic therapies that will benefit patients with fibrosing liver diseases. Not only have these advances uncovered new sources and pathways of fibrogenesis, but also the impact of genetic polymorphisms has emerged as an important new variable regulating fibrosis progression risk and clinical outcomes. As these different areas of progress continue to converge, a more coherent and comprehensive hepatic fibrosis will emerge.

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