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Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches

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ABSTRACT

The pathogenesis of hepatic fibrosis involves significant deposition of fibrilar collagen and other extracellular matrix proteins. It is a rather dynamic process of wound healing in response to a variety of persistent liver injury caused by factors such as ethanol intake, viral infection, drugs, toxins, cholestasis, and metabolic disorders. Liver fibrosis distorts the hepatic architecture, decreases the number of endothelial cell fenestrations and causes portal hypertension. Key events are the activation and transformation of quiescent hepatic stellate cells into myofibroblast-like cells with the subsequent up-regulation of proteins such as α-smooth muscle actin, interstitial collagens, matrix metalloproteinases, tissue inhibitor of metalloproteinases, and proteoglycans. Oxidative stress is a major contributing factor to the onset of liver fibrosis and it is typically associated with a decrease in the antioxidant defense. Currently, there is no effective therapy for advanced liver fibrosis. In its early stages, liver fibrosis is reversible upon cessation of the causative agent. In this review, we discuss some aspects on the etiology of liver fibrosis, the cells involved, the molecular pathogenesis, and the current therapeutic approaches.

1. Introduction

Fibrosis is the wound healing response to a variety of acute and/or chronic stimuli, including to name a few, ethanol, viral infection, drugs and toxins, cholestasis, and metabolic disease [1,2]. Hepatic fibrosis develops due to an increase in fibrillar collagen synthesis and deposition along with insufficient remodeling [3,4]. Fibrosis is associated with a number of pathological and biochemical changes leading to structural and metabolic abnormalities, as well as with increased hepatic scarring [5,6]. The progression of liver fibrosis leads to cirrhosis, a condition characterized by distortion of the normal architecture, septae and nodule formation, altered blood flow, portal hypertension, hepatocellular carcinoma, and ultimately liver failure [7].

2. Etiology of hepatic fibrosis

Most chronic liver diseases are associated with fibrosis and are characterized by parenchymal damage and inflammation. Alcohol abuse, chronic viral hepatitis (HBV and HCV), obesity, autoimmune hepatitis, parasitic diseases (i.e. schistosomiasis), metabolic disorders (hemochromatosis and Wilson's disease), biliary disease, persistent exposure to toxins and chemicals, and drug-induced chronic liver diseases are the most common causes of hepatic fibrosis.

2.1. Alcohol

Alcohol consumption is a predominant etiological factor in the pathogenesis of chronic liver diseases worldwide, resulting in fatty liver, alcoholic hepatitis, fibrosis/cirrhosis, and hepatocellular carcinoma [8]. Acetaldehyde, the product of alcohol metabolism via alcohol dehydrogenase, increases the secretion of transforming growth factor β1 (TGFβ1) and induces TGFβ type II receptor expression in hepatic stellate cells (HSC), the key collagen-producing cell within the liver [9]. Both, ethanol and acetaldehyde induce the *COL1A2* promoter and up-regulate collagen I protein expression [10]. In cultured human HSC, acetaldehyde up-regulates *COL1A1* mRNA expression via distinct mechanisms in the early and late responses [11]. Acetaldehyde-induced fibrogenesis involves a complex signaling pathway, which differs from that mediated by TGFβ1 in the early time points to up-regulate *COL1A2* gene expression [11].

TGFβ1 is a critical factor in the progression of alcoholic liver disease (ALD) in patients with steatosis and steatohepatitis [12]. Acetaldehyde does not alter the Smad3 and Smad4 protein concentration; however, it selectively induces phosphorylation of Smad3 but not of Smad2 [13]. Weng et al. [12] identified a significant

Abbreviations: ALD, alcoholic liver disease; AP1, activator protein 1; α-SMA, α-smooth muscle actin; CCl_4, carbon tetrachloride; CYP2E1, cytochrome P450 2E1; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSC, hepatic stellate cells; JNK, c-*jun* N-terminal kinase; MCP1, monocyte chemoattractant protein 1; miRNA, micro RNA; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa B; PDGF, platelet-derived growth factor; RAGE, advanced glycation-end products; ROS, reactive oxygen species; TGFβ1, transforming growth factor β1; TIMP1, tissue inhibitor of metalloproteinase 1.

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correlation of Smad2 phosphorylation with the fibrosis stage and the inflammation score. In addition, an association between serum pro-collagen III N-pro-peptide and TGFβ1 has been reported in patients with ALD [14]. These results demonstrate a significant role for TGFβ1 as mediator of alcohol-induced liver fibrosis.

Hepatic alcohol metabolism generates reactive oxygen species (ROS) causing significant cell death [15]. Indeed, oxidative stress, likely by increasing mitochondrial permeability transition, promotes hepatocyte necrosis and/or apoptosis. Generation of ROS within hepatocytes may be a consequence of an altered metabolic state, as it occurs in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Alternatively, it could result from ethanol metabolism as in alcoholic steatohepatitis, with ROS being generated mainly by the mitochondrial electron transport chain, cytochrome P450 isoforms such as cytochrome P450 2E1 (CYP2E1), damaged mitochondria, xanthine oxidase, NADPH oxidase, and generation of lipid peroxidation-end products [16]. In addition, it is known that chronic alcohol consumption lowers glutathione levels; thus, contributing to liver injury [17]. ROS-derived mediators released by damaged neighboring cells can directly affect the HSC behavior. ROS up-regulate the expression of critical genes related to fibrogenesis including pro-collagen type I, monocyte chemoattractant protein 1 (MCP-1), and tissue inhibitor of metalloproteinase-1 (TIMP1), possibly via activation of a number of critical signal transduction pathways and transcription factors, including c-*jun* N-terminal kinases (JNKs), activator protein 1 (AP-1), and nuclear factor kappa B (NFκB) [18].

2.2. Chronic viral hepatitis

Chronic hepatitis B and C virus are the most common causes of liver disease worldwide, with an estimated 350 and 170 million of individuals with chronic infection, respectively [19]. In addition, these infections are the primary cause of hepatocellular carcinoma (HCC). In both cases, there is significant chronic liver injury with subsequent progression to advanced liver fibrosis and in many cases cirrhosis. While HBV can be integrated into the host genome leading to changes in genomic function or chromosomal instability, HCV cannot integrate into the host genome. Various HCV proteins, including the HCV core protein, the envelope and non-structural proteins present oncogenic properties. In HBV infection, antiviral therapy and vaccination decrease the risk of HCC. Current antiviral therapies for HCV such as ribavirin significantly reduce the risk of HCC.

2.3. Other causes of hepatic fibrosis

In addition to alcoholism and chronic viral hepatitis, other factors contributing to hepatic fibrosis are obesity and steatosis, which can lead to nonalcoholic fatty liver disease and to chronic steatohepatitis. Nonalcoholic fatty liver disease has also been reported in non-obese individuals in developing countries [20].

Autoimmune hepatitis, the anomalous presentation of human leukocyte antigen class II in hepatocytes, causes cell-mediated immune responses against the host liver, and may lead to liver fibrosis as well [21]. Parasitic infections like schistosomiasis, have been shown to trigger advanced liver fibrosis and portal hypertension [22].

Metabolic disorders such as hemochromatosis and Wilson's disease are typically accompanied by chronic hepatitis and fibrosis [23]. In hereditary hemochromatosis, the excessive absorption and accumulation of iron in tissues and organs including liver is related to mutations in the *HFE* (High-iron) gene [24]. Wilson's disease or hepatolenticular degeneration is a genetic disorder leading to copper accumulation in the liver and it is due to a mutation in the APTase (ATP7B) that transports copper [25].

Lastly, cholestasis due to bile duct obstruction, leads to chronic portal fibrosis and eventually cirrhosis. Moreover, chronic exposure to toxins or chemicals such as N-nitrosodimethylamine, carbon tetrachloride (CCl_4) or thioacetamide leads to severe hepatic fibrosis in experimental animal models [26–28]. Exposure to these chemicals in humans is rare and generally occurs in the industry during manufacture and in places where these chemicals are routinely used.

3. Cell types involved in the pathogenesis of hepatic fibrosis

3.1. Hepatic stellate cells

Several cell types are involved in the pathogenesis of hepatic fibrosis. HSC reside in the *space of Disse* between hepatocytes and sinusoidal endothelial cells [29]. Quiescent HSC are characterized by significant expression of desmin and vitamin A storage. Following liver injury, HSC lose their vitamin A content, increase the expression of α-smooth muscle actin (α-SMA), acquire a myofibroblast-like phenotype losing their typical star-shape, become proliferative, motile, pro-fibrogenic, contractile, and show abundant rough endoplasmic reticulum [30].

Many factors have been identified to contribute to HSC activation. Damage to hepatocytes and Kupffer cell activation are still considered the primary effectors driving HSC activation [31,32]. Mediators released from damaged hepatocytes, such as lipid peroxidation products, intermediate metabolites of drugs or hepatotoxins, acetaldehyde, and 1-hydroxyethyl radical from alcohol metabolism as well as ROS (hydrogen peroxide, superoxide radical, and others) are strong inducers of HSC activation.

Activated Kupffer cells release ROS and cytokines that are crucial for HSC activation as well [32]. They are a major source of TGFβ and platelet-derived growth factor (PDGF), two potent profibrogenic cytokines that traditionally have been considered key fibrogenic and proliferative stimuli to HSC, respectively [33]. In addition, the Kupffer cell phagocytic activity generates large amounts of ROS that could further activate HSC and induce their fibrogenic potential.

We have previously demonstrated that cytochrome P450 2E1-dependent generation of ROS is critical for increased collagen I protein synthesis in co-cultures of hepatocytes and HSC [31]. Furthermore, addition of ethanol and arachidonic acid synergized to activate Kupffer cells and modulated the fibrogenic response by a mechanism involving TNFα, reduced glutathione and TGFβ [34].

It has been also demonstrated that *in vivo* ablation of TNFα, TLR4, CD14, and lipopolysaccharide-binding protein protects from the fibrogenic response [35]. Despite the close association of inflammation and fibrosis, little is known on the crosstalk between these two key events and the intracellular signal transduction pathways activated. For example, TLR4 is activated by lipopolysaccharide in Kupffer cells leading to NFκB and IRF3 activation, and the subsequent transcriptional activation of pro-inflammatory mediators such as TNFα and IFNγ. Moreover, TNFα activates the NFκB signaling pathway in hepatocytes, which is key for their survival [8,36]. However, there is no crosstalk with the TGFβ pathway that results in the activation of Smad3 and Smad4 and the associated induction of TGFβ-responsive genes.

3.2. Portal fibroblasts

The portal connective tissue in healthy liver is surrounded by quiescent portal fibroblasts, which constitute a second population of liver cells implicated in portal fibrosis [37]. Derived from small

portal vessels, they express markers distinct from HSC (e.g. elastin) [38]. Proliferation of biliary cells is often accompanied by proliferation of portal fibroblasts, which form onion-like configurations around biliary structures and acquire a myofibroblast phenotype, and are thus implied in the early deposition of extracellular matrix (ECM) in portal zones [39]. It is generally believed that substantial signaling from biliary epithelial cells leads to portal fibroblast activation, although the key factors remain to be identified.

3.3. Bone marrow-derived mesenchymal stem cells

Several studies have indicated that bone marrow derived mesenchymal stem cells could be a source of multi-lineage cells for various organs. They have the capacity to differentiate into hepatocytes, biliary epithelial cells, sinusoidal endothelial cells and even Kupffer cells in the presence of a suitable hepatic microenvironment [40,41]. There is growing evidence to suggest that bone marrow-derived stem cells are recruited during both progression and regression of liver fibrosis. During regression from CCl_4 induced hepatic fibrosis, bone marrow-derived mesenchymal stem cells migrate into the fibrotic liver, where they can express matrix metalloprotease-13 (MMP13) and MMP9 [42]. In addition, granulocyte colony-stimulating factor and hepatocyte growth factor treatment significantly enhance migration of bone marrow-derived cells into the fibrotic liver and accelerate the regression of liver fibrosis [43]. Over-expression of hepatocyte growth factor together with granulocyte colony-stimulating factor, synergistically stimulate MMP9 expression, which is followed by accelerated resolution of fibrotic scars [44]. A significant contribution of bone marrow-derived cells has been shown in human liver fibrosis, but it is unclear the specific type of mesenchymal stem cells [45].

3.4. Hepatocytes and biliary epithelial cells

Epithelial-to-mesenchymal transition (EMT) is now emerging as a possible source of injury-associated mesenchymal cells, derived either from resident hepatocytes or from biliary epithelial cells [46,47], although its role in liver fibrosis is still controversial [48,49]. The main molecules inducing EMT are TGFβ, epidermal growth factor, insulin-like growth factor-II, and fibroblast growth factor-2 [50]. Hepatocytes that express albumin also express fibroblast-specific protein-1 in response to CCl_4 *in vivo* or to TGFβ1 *in vitro* [50]. Kaimori et al. [51] reported that hepatocytes express *COL1A1* in response to TGFβ1 *in vitro*, and that Smad signaling mediates EMT.

On the contrary, a recent study shows that hepatocytes isolated from mice chronically treated with CCl_4, neither express mesenchymal markers nor exhibit a phenotype clearly distinguishable from control hepatocytes [52]. In order to confirm this, Scholten et al. [53] studied EMT using the Cre-LoxP system to map the cell fate of $CK19^+$ cholangiocytes in CK19 (YFP) or fibroblast-specific protein-1 (FSP-1) (YFP) mice. Mice were bile duct ligated or subjected to CCl_4-induced liver injury and the livers were analyzed for expression of mesoderm and epithelium markers. The results demonstrated that EMT of cholangiocytes does not contribute to hepatic fibrosis in mice. Likewise, GFAP (Cre)-labeled HSC showed no co-expression of epithelial markers, providing no evidence for EMT in HSC in response to fibrogenic liver injury [53].

Biliary epithelial cells have been described to be involved in EMT in liver fibrogenesis. In primary biliary cirrhosis, it has been shown that cells of the bile duct express fibroblast-specific protein-1 and vimentin, early markers of fibroblasts [54]. A consequence of EMT in biliary epithelial cells is the amplification of the pool of portal fibroblasts, contributing significantly to portal fibrosis. *In vitro* studies with human biliary epithelial cells have confirmed these clinical observations [55]. Thus, EMT could be considered a mechanism participating in the pathogenesis of chronic cholestatic liver disease. However, additional research is necessary to validate this possibility.

3.5. Fibrocytes

Fibrocytes constitute a circulating bone marrow-derived $CD34^+$ cell subpopulation with fibroblast-like properties initially associated with tissue repair in subcutaneous wounds [56]. They comprise a fraction of about 1% of the circulating pool of leukocytes expressing markers of mesenchymal cells [57]. Subsequently, two studies have demonstrated the bone marrow origin of fibrogenic cell populations in the CCl_4 mouse model of fibrosis and in the bile duct ligation model of biliary hyperplasia [58,59]. Roderfeld et al. [60] hypothesized that bone marrow-derived circulating $CD34^+$ fibrocytes represent key mediators of liver fibrogenesis in the $Abcb4^{-/-}$ mice, which represent a highly reproducible, well-characterized non-surgical mouse model for cholangiopathy in humans.

4. Molecular pathogenesis of hepatic fibrosis

Fig. 1 summarizes key concepts involved in the molecular pathogenesis of liver fibrosis. A recent review by Hernandez-Gea et al. [61] provides more insight into the pathogenesis of hepatic fibrosis.

4.1. Cell–cell and cell–matrix interactions

Alterations in normal cell-cell and cell-matrix interactions play a significant role in the pathogenesis of hepatic fibrosis. When normal cell-cell and cell-matrix interactions are altered due to hepatocyte necrosis or invasion of inflammatory cells, new interactions are established that trigger a fibrogenic response. In the fibrotic liver, significant quantitative and qualitative changes occur in the composition of ECM in the periportal and perisinusoidal areas. During cirrhosis, the amount of fibrillar collagens and proteoglycans can be up to six times higher than in healthy livers [62,63]. The scar is typically composed of fibrillar collagen type I and III, proteoglycans, fibronectin and hyaluronic acid [64]. As a result, alteration in the physiological architecture of the liver occurs, particularly in the *space of Disse*, where the low electron-dense ECM is replaced by one rich in fibrillar collagens and fibronectin [61]. This leads to loss of endothelial cell fenestrations, impaired exchange of solutes among neighboring cells, altered hepatocyte function and subsequent non-parenchymal cell damage [61]. Previous work from our group has shown that co-culture of Kupffer cells or hepatocytes with HSC induces a more activated phenotype, greater proliferation rates and increased collagen synthesis in HSC compared to HSC cultured alone [31,32,65].

4.2. Oxidative stress

Chronic HBV infection and long-term consumption of alcohol induce cell damage through increased generation of ROS [66]. Indeed, oxidative stress, which favors mitochondrial permeability transition, is able to promote hepatocyte necrosis and/or apoptosis. In some clinically relevant conditions, generation of ROS within hepatocytes may represent an altered metabolic state as in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis, or significant ethanol metabolism as it occurs in alcoholic steatohepatitis. ROS are generated mainly via the mitochondrial electron transport chain or via activation of cytochrome P450 - mostly cytochrome P450 2E1-, NADPH oxidase, xanthine oxidase or via mitochondrial damage. The ROS generated can directly affect

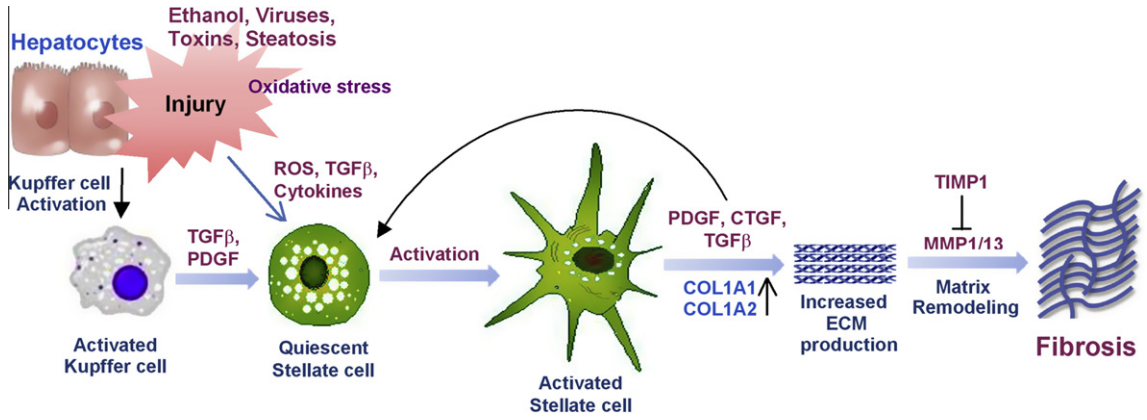

Fig. 1. Key concepts involved in the activation of hepatic stellate cells and pathogenesis of hepatic fibrosis.

the HSC and myofibroblasts behavior [32,67]. ROS up-regulate the expression of critical fibrosis-associated genes such as *COL1A1*, *COL1A2*, *MCP1*, and *TIMP1* via activation of signal transduction pathways and transcription factors, including JNK, activator protein-1, and NFκB [18]. ROS generation in HSC and myofibroblasts occurs in response to several known pro-fibrogenic mediators, including angiotensin II, PDGF, TGFβ, and leptin [68]. Overall, a decrease in the antioxidant defense such as GSH, catalase or SOD, in conjunction with enhanced lipid peroxidation leads to a pro-fibrogenic response by enhancing collagen I protein expression [69].

4.3. Role of MMPs and TIMPs

The ECM is a highly dynamic *milieu* subject to constant remodeling whereby synthesis of new components occurs with simultaneous degradation. Life-threatening pathological conditions arise when ECM remodeling becomes excessive or uncontrolled. Among the cells involved in hepatic ECM degradation are HSC, neutrophils, and macrophages. MMPs are the main enzymes responsible for ECM degradation and TIMPs have the ability to inhibit MMPs [70]. Therefore, regulation of the MMP-TIMP balance is crucial for efficient ECM remodeling. The MMP-TIMP ratio is tipped because of multiple pro-fibrogenic insults. Activated HSC not only synthesize and secrete ECM proteins such as collagens type I and type III, but also produce MMP1 [71] and MMP13 [72]. However, MMP1 and MMP13 expression decreases as HSC activation progresses, while the activity of other MMPs remains relatively constant, except for MMP2 and MMP9 [73]. The increase in MMP2 activity is associated with distortion of the normal lobular architecture, which further activates HSC [73]. Moreover, activated HSC up-regulate the expression and synthesis of TIMP1 and TIMP2 [74]. TIMP1 not only prevents the degradation of the rapidly increasing ECM by blocking MMPs, but also inhibits the apoptosis of activated HSC [75]. The net result is the deposition of mature collagen fibers within the *space of Disse* and thus scaring.

As indicated above, scar formation is regulated by the balance between MMPs and TIMPs, which are induced by ROS and RNS in the CCl_4 model of liver fibrosis [76]. This was elucidated in a study using a mutant form of MMP9 that scavenged TIMP1 and inhibited CCl_4-induced fibrosis [77]. Thus, TIMP1 activity may be a crucial factor in the regression of fibrosis. Several factors can activate TIMP1, including leptin [78], angiotensin II [79], and sphingosine 1 phosphate [80]. The delineation of the signaling pathways elicited by these factors is important for successful inhibition of TIMP1 activity. Increased liver MMP activity either by recruitment of bone marrow-derived cells or by decreasing TIMP1 level may lead to regression of fibrosis [81].

5. Current therapies for hepatic fibrosis

Despite significant advances in understanding hepatic fibrosis and defining targets for therapy, there are limited anti-fibrotic drugs approved for clinical use in patients with advanced liver disease. Regression of established fibrosis can be accomplished in selected individuals with chronic liver diseases that have effective interferon therapies [82]. However, a large cohort of patients does not respond to conventional treatment and thus remain at risk for progression of fibrosis to cirrhosis.

Ideally, the anti-fibrotic therapy should be liver-specific, selective for targeting of the fibrogenic cascade, including inhibition of matrix deposition, collagen synthesis, modulation of HSC activation, enhancing matrix degradation, stimulating HSC death or apoptosis, and it should be well tolerated if administered for a prolonged period. Several compounds including colchicine, and malotilate, with potential antifibrotic activity, have been studied in human trials but found to be not very effective [83,84]. The ideal anti-fibrotic agent, which should be safe when used over a long period, liver-specific, non-hepatotoxic, for oral administration and inexpensive is not yet available.

Many agents such as malotilate, genistein, curcumin, and silymarin have been shown to be effective *in vitro* and in experimental animal models [84–86]. Although there are no perfect and effective anti-fibrogenic agents, the potential candidates include agents that can reduce inflammation and the immune response such as corticosteroids, colchicines and IL-10; agents that reduce the activation of ECM-producing cells as well as their pro-fibrogenic potential such as inhibitors of TGFβ1, INFα, and peroxisome proliferator activated receptor-γ agonists [87]; antioxidants such as vitamin E [88], phosphatidylcholine [89], and *S*-adenosyl-L-methionine [90]; N-acetylcysteine and 5-nitroso-N-acetylcysteine [91], which appears to be more efficient, agents capable of increase the degradation of ECM fibrillar such as MMPs and uroplasminogen activator [92] and interferons [93]. Combination therapy that works at different mechanistic levels would be more appropriate to block HSC activation and the pathogenesis of liver fibrosis.

6. Molecular therapy for hepatic fibrosis

Compared with other antisense strategies such as antisense oligonucleotides, ribozymes or DNAzymes, siRNA has been proved to be a potent knockdown of any given target gene with high sequence specificity [94]. Hence, siRNA would be a powerful strategy to treat liver fibrosis in the future. There are three ways to deliver siRNA: synthetic duplex, plasmid, and viral vectors. While viral vectors give high transduction efficiency, their immune reactions limit their application in therapeutics. On the other hand,

plasmid DNA complexes with cationic liposomes may not pass through sinusoidal gaps, because endothelial cells fenestrations are closed during liver fibrosis. In contrast, low molecular weight synthetic duplex siRNA is likely to pass through sinusoidal gaps in fibrotic livers, and thus they may be ideal candidates for treating liver fibrosis [95].

Most of the targeted genes are those critical for HSC activation, proliferation, and/or collagen synthesis and deposition, which are usually markedly up-regulated during hepatic fibrogenesis, including CTGF, TGFβ1, PDGF, TIMPs, and plasminogen activator inhibitor-1 [96]. However, these molecules may also play a role in many physiological processes and their inhibition may lead to adverse side effects. Cheng et al. [95] have successfully designed and validated TGFβ1-specific siRNAs and then converted two potent siRNA sequences into shRNAs, which effectively silenced TGFβ1 gene expression in HSC cells. TGFβ1 gene silencing significantly reduced the production of collagen I, TIMP1 and inflammatory cytokines [95]. Their results suggested that silencing TGFβ1 by siRNA and shRNA may be an efficient and more specific approach for treating liver fibrosis [95]. Another study [97] showed that inhibiting the receptor for advanced glycation end products (RAGE) gene, which is involved in migration of activated HSC and myofibroblasts [98], by a specific RAGE siRNA expression vector, inhibited the expression of collagen I in CCl_4-induced rat liver fibrosis and dramatically reduced the levels of serum pro-collagen type III, hyaluronic acid and laminin. These results indicated that inhibition of RAGE had important anti-fibrogenic effects [98]. However, the mechanisms of the effects of RAGE on the accumulation of ECM in hepatic fibrosis need further investigation.

Using the RAGE specific siRNA strategy, they effectively inhibited RAGE gene expression in rat liver fibrosis and successfully prevented experimental liver fibrosis in rats [98]. The suppression of the up-regulated expression and activity of NFκB and HSC activation via inhibition of IκBα degradation, inhibition of ECM production and attenuation of liver injury may be possible strategies to prevent liver fibrosis by inhibiting RAGE. These findings strongly suggests that RAGE may be a new target for combating liver fibrosis and RAGE specific siRNA might be an effective candidate to prevent liver fibrosis.

Based on recent studies, micro RNAs (miRNAs) have gained significant interest as diagnostic biomarkers and as therapeutic targets. miRNAs belong to a class of small, non-coding RNA molecules that control protein expression at the post-transcriptional level [99]. Their mechanism of action involves imperfect binding to complementary sequences in the 3'-untranslated region of target mRNAs, leading to either cleavage of the mRNA [100] or suppression of protein translation [101]. In liver fibrosis, activation of HSC, which is regulated by multiple signal transduction pathways, is considered a key event; thus, proteins from the pathways involved could be important targets for miRNAs.

In order to understand the critical pathways of HSC activation, Guo et al. [102] performed comprehensive comparative bioinformatics analysis of microarrays of quiescent and activated HSC. Changes in miRNAs associated with HSC activation status revealed that 13 pathways were up-regulated and 22 pathways were down-regulated by miRNA. Lee and colleagues dissected a novel mechanism for cystogenesis involving miRNA [103]. They demonstrated that levels of the miRNA, miR15a are decreased in livers of patients with autosomal recessive and autosomal dominant polycystic kidney disease and congenital hepatic fibrosis as well as in the *PKC* rat model of autosomal recessive polycystic kidney disease. This results in increased expression of the cell-cycle regulator Cdc25A, which is a direct target of miR15a, and increased cellular proliferation and cystogenesis *in vitro*.

Roderburg et al. [104] studied the regulation of miRNAs in experimentally induced hepatic fibrosis by microarray. They found that three members of the miR-29-family are significantly down-regulated in livers of CCl_4-treated mice as well as in mice that underwent bile duct ligation [104]. Specifically, their data indicate that miR-29 mediates the regulation of liver fibrosis and it is part of a signaling nexus involving TGFβ- and NFκB-dependent down-regulation of miR-29 family members in HSC with the subsequent up-regulation of ECM genes [104]. Therefore, identifying potential miRNAs for the arrest of HSC activation and proliferation could lead to therapeutic intervention of hepatic fibrosis.

7. Conclusion

Understanding the mechanisms and the pathways involved in the pathogenesis of the fibrogenic response could provide novel therapeutic approaches for liver diseases in which fibrosis is a detrimental component. Identifying potential new therapeutic agents that target specific pathways involved in fibrosis is critical to overcome the barriers to progress in the field of liver fibrosis. Thus, prevention of hepatic fibrosis could help ameliorate the complications of cirrhosis, and improve the quality of life of many patients worldwide.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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