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TOPIC HIGHLIGHT

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Targeting collagen expression in alcoholic liver disease

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Abstract

Alcoholic liver disease (ALD) is a leading cause of liver disease and liver-related deaths globally, particularly in developed nations. Liver fibrosis is a consequence of ALD and other chronic liver insults, which can progress to cirrhosis and hepatocellular carcinoma if left untreated. Liver fibrosis is characterized by accumulation of excess extracellular matrix components, including type I collagen, which disrupts liver microcirculation and leads to injury. To date, there is no therapy for the treatment of liver fibrosis; thus treatments that either prevent the accumulation of type I collagen or hasten its degradation are desirable. The focus of this review is to examine the regulation of type I collagen in fibrogenic cells of the liver and to discuss current advances in therapeutics to eliminate excessive collagen deposition.

Key words: Type I collagen; Fibrosis; Extracellular matrix; Hepatic stellate cell; Alcohol; Antioxidants; Endoplasmic reticulum chaperones; Matrix metalloproteinase; microRNA

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INTRODUCTION

Liver fibrosis is an exacerbation of the generic wound-healing process of the liver and is defined by excess synthesis and deposition of extracellular matrix (ECM) components, of which type I collagen predominates[1]. Accumulation of ECM in the sub-endothelial space of Disse can disrupt liver microcirculation, leading to damage and death of parenchymal cells[2]. Liver fibrosis is a common sequela for a variety of insults, such as viral infection, industrial solvent exposure, autoimmunity, cholestasis, inborn errors of metabolism, and ethanol abuse.

In a setting of chronic fibrogenic stimulus, myofibroblast-like cells produce large quantities of ECM components. Extensive investigation has revealed several cell types that are potential myofibroblast precursors, including the hepatic stellate cell (HSC). HSCs are star-shaped, vitamin A-storing cells residing within the subendothelial space of Disse[3]. In fibrosis HSCs lose their vitamin A stores, transdifferentiate to a proliferative, myofibroblast-like cell, and produce or secrete excess ECM components[4]. Though considered the predominant source of ECM in hepatic fibrosis, other cell populations have been identified as sources of collagen and other ECM components. Portal fibroblasts and bone marrow-derived myofibroblast precursors have also been implicated as sources of ECM in fibrosis. Evidence also suggests that hepatocytes may undergo epithelial to mesenchymal transition to produce ECM, at least *in vitro*[5-7].

Globally, viral hepatitis is the leading risk factor for hepatic fibrosis; however, in highly developed nations chronic, high ethanol consumption is the principal risk factor for developing fibrosis. Nonalcoholic steatohepatitis (NASH) has also been identified as a growing cause of fibrosis[8]. Untreated, liver fibrosis is a major contributor of morbidity and mortality, as unresolved fibrosis may progress to cirrhosis and result in organ failure or progression to hepatocellular carcinoma (HCC). Despite increased understanding of fibrogenesis, there remains a dearth of effective anti-fibrotic treatments. This review will focus on type I collagen expression in fibrosis in alcoholic liver disease (ALD) and therapeutic strategies to limit or reverse its accumulation.

REGULATION OF TYPE I COLLAGEN

Excess ECM deposition in liver fibrosis can largely be attributed to members of three families of proteins - collagens, in particular types Ⅰ, Ⅲ and Ⅳ; proteoglycans, such as fibronectin, laminin, and hyaluronic acid; and glycoproteins, including heparin, chondroitin sulfates, and biglycan. Although multiple ECM components are dramatically upregulated in hepatic fibrosis, type Ⅰ collagen is the most abundant protein in the body and has been extensively characterized, making it an attractive target for the development of anti-fibrotic therapies.

Collagens are synthesized as a triple helix from three polypeptide α chains composed of continuous Glycine (Gly)-X-Y peptide repeats[9]. Glycine is essential in the first position as its side chain is the only one small enough to fit within the center of the coiled-coil triple helix[10]. Proline is frequently found in the X position and hydroxyproline in the Y position[11]. These amino acids limit rotation of the triple helical structure and their placement on the surface facilitates self-assembly and polymerization of collagen molecules through charge-charge and hydrophobic interactions[9].

In normal tissues, collagens are secreted into the ECM and help maintain the integrity of tissue by interacting with cell surfaces, with other ECM components, and with growth and differentiation factors[12]. Type Ⅰ collagen is an important component in the wound-healing process and is found in large quantities in scar tissue associated with a variety of pathological conditions[13,14]. In the liver, chronic damage stimulates activation of HSCs and other myofibroblast precursors, resulting in a phenotypic change towards excessive production and secretion of ECM products, particularly type Ⅰ collagen.

Transcriptional regulation of procollagens

Synthesis of type Ⅰ collagen is initiated by expression of the *col1a1* and *col1a2* genes, giving rise to α1(Ⅰ) and α2(Ⅰ) procollagen mRNAs, respectively. Levels of these gene products can be regulated at both the transcriptional and post-transcriptional level. Despite being located on different chromosomes, expression of these two genes are coordinately regulated in a tissue-specific manner giving rise to α1(Ⅰ) and α2(Ⅰ) procollagen mRNA products in a 2:1 ratio, respectively[15]. Numerous regulatory elements have been identified in the promoter and first intron of *col1a1* and *col1a2* that regulate expression of procollagen mRNA messages through interactions with transcription factors.

In ALD, ethanol consumption results in mediators that influence the expression of type Ⅰ collagen. Metabolism of ethanol by alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1) generates acetaldehyde and reactive oxygen species (ROS)[16]. Acetaldehyde treatment of HSCs increases binding of a kruppel-like transcription factor (KLF), basic transcription element binding protein (BTEB) to a region between -1484 and -1476 in the *col1a1* promoter in a *c-jun* N-terminal kinase (JNK)-dependent manner, enhancing α1(Ⅰ) procollagen mRNA levels[17,18]. Other KLFs, such as Sp1 and KLF6, have also been shown to upregulate transcription of procollagen mRNAs[19].

Transforming growth factor-β (TGF-β) has been described as the most potent fibrogenic cytokine for HSCs and is thus a common target for anti-fibrotic therapy[1]. TGF-β expression is upregulated in fibrosis and is secreted by HSCs and other cell types, such as Kupffer cells. Stimulation with TGF-β activates Smad signaling, which can upregulate procollagen expression *via* formation of a heterotrimeric complex of Smad2, 3, and 4, where Smad7 is inhibitory. TGF-β can also stimulate enhanced procollagen expression through the generation of intracellular H_2O_2 and subsequent activation of p38-mitogen activated protein kinase[1].

Nuclear factor κB (NFκB) is commonly associated with liver fibrosis, including ALD. Although NFκB is not required for the activation of HSCs, an increase in the p65/p50 heterodimer, with concomitant decrease in the NFκB inhibitory protein, IκBα, promotes survival of activated HSCs[20,21]. Overexpression of NFκB in activated HSCs, however, has been shown to inhibit α1(Ⅰ) and α2(Ⅰ) procollagen mRNA expression in culture-activated HSCs[22,23]. It is unclear, however, whether this observation occurs *in vivo*.

Post-transcriptional regulation of procollagen mRNAs

Upon activation of HSCs and other myofibroblast precursors, there is a > 50-fold increase in α1(Ⅰ) procollagen mRNA levels, with a concomitant increase in message half-life from 1.5 to 24 h[24]. Although fibrogenic stimuli, such as chronic ethanol consumption, increase the transcription rate of procollagen mRNAs, the major contributing factors associated with this increase are post-transcriptional. The α1(Ⅰ) procollagen mRNA possesses regulatory elements within both the 3' and 5' untranslated regions (UTRs), which influence the stability and translation of the message.

Heterogenous ribonucleoprotein particles (hnRNPs) are a family of RNA-binding proteins that have a variety of functions, including prevention of mRNA folding, transporting, association with the splicing apparatus, and mRNA stability. αCP is an hnRNP that has demonstrated

binding to the 3' UTR of several messages, including α-globin[25]. αCP binds to a C-rich segment of the 3' UTR of α1(I) procollagen mRNA located downstream of the stop codon, stabilizing the message and preventing degradation[24,26]. Though expressed in both quiescent and activated HSCs, αCP only has binding activity in activated HSCs. Furthermore, the cellular localization of αCP varies in HSCs, with αCP localized in the nucleus of quiescent HSCs and in both the nucleus and cytoplasm of activated HSCs, suggesting a yet to be identified post-translational event regulating the localization of αCP[27].

A well-conserved 5' stem-loop structure has been described in the message of collagen mRNAs, including α1(I) procollagen mRNA, comprising the translation initiation codon[28,29]. Mutation of this 5' stem-loop structure revealed improperly assembled procollagen I, as demonstrated by pepsin-sensitivity and diminished intermolecular disulfide bond formation[30]. Recent work has revealed La ribonucleoprotein domain family member 6 (LARP6) as a sequence-specific 5' stem-loop binding protein of α1(I) procollagen mRNAs[30]. Reporter experiments in the same study revealed that deletion of the 5' stem-loop or LARP6 resulted in diffuse accumulation of the reporter throughout the endoplasmic reticulum (ER), in contrast to the focal areas of translation associated with proper assembly of procollagens[31]. Further investigations by the same group demonstrated that LARP6 interacts with non-muscle myosins, and disruption of this interaction results in increased intracellular degradation of procollagen polypeptides and a preference towards α1(I) homotrimers[32]. Emergence of LARP6 as a collagen-specific regulator of translation may present a new therapeutic target for modulating excessive collagen synthesis in fibrogenic conditions like alcoholic fibrosis and cirrhosis.

Post-translational modifications of procollagen polypeptides

Most proteins begin folding at the N-terminus prior to completion of translation and translocation. For type I collagen, however, folding is initiated at the C-terminus following co-translational translocation into the ER[33]. Several proteins play an important role in facilitating the proper folding and trafficking of α chains into triple helix procollagen molecules. The 78-kDa glucose-regulated protein (Grp78) recognizes hydrophobic residues on polypeptide chains to help maintain solubility and may also bind the C-propeptide[34]. Protein disulfide isomerase (PDI) also plays a role in triple helix formation by catalyzing disulfide bonds between C-propeptide domains of the three α chains[35,36]. PDI also acts as a β-subunit for prolyl 4-hydroxylase (P4H) by keeping the catalytic α-subunits in a soluble state[37]. Further stabilization of the triple helix is accomplished through hydroxylation of select proline residues (typically in the Y position) by the P4H enzyme, which in turn facilitates hydrogen bonding and the formation of water bridges within and between collagen chains[38,39]. A 47-kDa heat shock protein (Hsp47) is a collagen-specific chaperone that also plays an important role in collagen trafficking[40]. Although the exact role of Hsp47 has not been clearly defined, studies utilizing Hsp47-/- mice showed they are embryonically lethal at day 11.5[41]. After procollagens traverse the Golgi apparatus and are secreted into the extracellular space, the C- and N-prodomains are cleaved by C- and N-peptidases, respectively[42]. This process decreases the concentration required for fibril formation and results in the self-assembly of collagens into fibrils[43].

THERAPEUTIC TARGETING OF TYPE I COLLAGEN

Removal (or suppression) of the underlying pathology is considered the most effective way to reverse liver fibrosis; however, removal of the causative agent is not always feasible. In ALD, patients often fail to comply with abstention programs and many patients do not respond well to casual treatments, or present with advanced fibrosis and/or cirrhosis. Thus, there is a need to identify and develop anti-fibrotic agents that can retard, or even reverse, liver fibrosis. To date there is no well-regarded or frequently used anti-fibrotic therapy in clinical practice.

Therapeutic strategies for established liver fibrosis can target type I collagen accumulation by employing one or more strategies: (1) decrease the secretion of type I collagen by disrupting either its transcription or assembly; or (2) stimulating fibrinolysis of type I collagen that has accumulated extracellularly. Therapies to reduce the pool of fibrogenic myofibroblasts are also a therapeutic strategy; however, these approaches are beyond the scope of the current review.

Antioxidants

Liver fibrosis caused by ALD has a well-established link with oxidative stress. Metabolism of ethanol by ADH and CYP2E1 generate ROS and acetaldehyde, leading to a variety of cellular defects including depletion of reduced glutathione (GSH), the main intracellular antioxidant, lipid peroxidation, acetaldehyde-protein adducts, and proteasome inhibition[44-46]. Oxidative stress from Kupffer cell activation or from damaged hepatocytes can promote HSC activation and procollagen mRNA expression. These findings led investigators to evaluate a variety of antioxidants as a way to limit production of type I collagen and other matrix components, with mixed results.

S-adenosyl-L-methionine (SAMe) is the principal biological methyl donor and is a precursor to GSH, thus it has received interest as a potential treatment for liver diseases. Liver fibrosis has been shown to be attenuated by SAMe administration in several animal models of fibrosis, including a rat model of ALD[47-49]. *In vitro* experiments reported that SAMe inhibits both basal and TGF-β-stimulated type I collagen expression in activated HSCs[50,51]. Additionally, studies by our group indicate that SAMe can enhance polyubiquitination of type I collagen, possibly suggesting a novel mechanism to prevent secretion of collagen (Thompson

et al 2011 DOI:10.1111/j.1478-3231.2011.02512.x). SAMe supplementation prevented oxidative stress and lipid peroxidation in ethanol- and ethanol plus LPS-fed animals as evidenced by normal GSH:oxidized glutathione (GSSG) ratio and diminished levels of 4-hydroxynonenal, respectively[47]. Despite attractive results *in vitro* and with animal models, SAMe has shown mixed results in modulating liver disease in human trials. A comprehensive review by Rambaldi *et al* revealed no clear benefit by SAMe in most trials analyzed; however, a well-designed study by Mato *et al* reported that SAMe administration could delay the need for hepatic transplantation in alcoholic cirrhotics[52,53]. However, the combination of SAMe with other antioxidants, such as dilinoleoylphosphatidylcholine (DLPC), has attenuated liver injury in a NASH model and *in vitro* studies reported decreased collagen and TIMP-1 expression in HSCs[54-56].

Turmeric has been used for centuries in Indian Ayurvedic medicine to treat a variety of ailments. Curcumin is a polyphenolic compound and the principal curcuminoid in turmeric. Curcumin in part owes its antioxidant properties to stimulation of nuclear factor erythroid-2-related factor 2 (nrf2), a transcription factor that binds several intracellular oxidant genes and enhances their transcription, including genes associated with production of glutathione[57,58]. In rodent models of cirrhosis, curcumin is reported to be protective; however, differences were noted depending on the model used. Curcumin can prevent thioacetamide (TAA)-induced cirrhosis, but no effect by curcumin was seen on established cirrhosis[59]. On the other hand, using bile duct-ligation (BDL) and carbon tetrachloride (CCl_4) models of established cirrhosis, curcumin improved liver histology and diminished collagen accumulation[59]. Studies utilizing curcumin on activated stellate cells revealed enhanced expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), disruption of TGF-β signaling, and diminution of collagen expression[60,61]. Additionally, curcumin has been demonstrated to improve alcohol-induced liver injury through prevention of oxidative stress and inflammation *via* downregulation of NF-κB[62-64].

Another antioxidant that has received attention as a potential therapy for hepatic fibrosis is resveratrol, a phytoalexin (a class of antibiotics produced in plants) naturally found in grapes and commercially in red wine. Resveratrol exhibits anti-inflammatory properties, anti-oxidant effects, and modulates metabolism of lipids[65]. In a CCl_4-mediated model of fibrosis, resveratrol prevented fibrosis with concomitant inhibition of NF-κB translocation and attenuation of TGF-β production[66]. Inhibition of NF-κB by resveratrol was also reported *in vitro* along with a decrease in pro-inflammatory cytokine production[67,68]. In a rat model of alcoholic liver injury, resveratrol blocked increased oxidative stress, as measured by malondialdehyde, through upregulation of superoxide dismutase, glutathione peroxidase, and catalase[69]. Resveratrol has also been shown to alleviate alcohol-induced fatty liver disease in mice through promotion of sirtuin 1 and AMP-activated kinase[70]. There is, however, a lack of human studies examining the efficacy of resveratrol as a treatment for liver disease.

Silibinin is an active flavinoligand derived from milk thistle, which has been used for several millennia as a treatment for a variety of liver disorders. Silibinin has demonstrated anti-proliferative, anti-fibrogenic, and anti-cancer properties with *in vivo* animal models and can inhibit TGF-β-induced collagen secretion in a human HSC cell line[71,72]. In addition to direct antioxidant properties, silibinin can inhibit CYP2E1 expression in the setting of chronic alcohol consumption, suggesting decreased ROS production in alcoholics[73]. Despite encouraging results in animal studies, enthusiasm for its use in humans to treat chronic liver disorders is limited by questionable success in clinical trials. A review of 13 randomized clinical trials revealed silibinin had no effect on mortality, liver histology, or liver-related complications, but there was a significant decline in liver-related mortality; however, others found no decrease in liver-related mortality in trials that were deemed to be of high quality[74].

MicroRNAs

MicroRNAs (miRNA) belong to a class of small non-coding RNAs involved with post-transcriptional regulation of gene expression, termed RNA interference (RNAi). These sequences are typically 18-25 nucleotides and are generated by processing of full-length primary transcript miRNAs, termed pri-miRNA, through enzymatic cleavage by RNase Ⅲ Drosha, generating pre-miRNAs. Subsequent transport to the cytosol permits additional processing by dicer to produce double-stranded miRNAs. One strand is loaded into the silencing complex and translation is disrupted by imperfect binding of the miRNA and elements within the 3'-UTR of target transcripts[75].

miRNAs have demonstrated roles in most biological events, including proliferation, differentiation, cell-fate determination, apoptosis, and signal transduction. Disregulation of miRNAs has been implicated in a number of disease states, including cancer and fibrogenesis in a number of solid organs including liver[76]. Comparisons of miRNA expression between quiescent and activated HSCs revealed several miRNAs that may be involved in liver fibrosis and are thus attractive candidates for targeting. Expression of miR-27a and miR-27b were shown to increase during activation of rat HSCs. Inhibition of these miRNAs reverted activated HSCs back to a quiescent state that was associated with an increase in retinyl ester storage and decreased proliferation[77]. Studies by Guo *et al*[78] suggested that miR-15b and miR-16 reduce Bcl-2 and increase caspase signaling, promoting apoptosis of activated HSCs. Overexpression of miR-150 and miR-194 in human LX-2 cells (a human activated HSC line) resulted in decreased expression of α-SMA and type I collagen, possibly through inhibition of c-myb and rac 1[79].

MiRNAs that specifically target collagen production have also been identified in a variety of tissues, including liver. Accumulating evidence implicates the miR-29-

family in the regulation of type I collagen expression in several disease states[80,81]. Examination of miR-29-family members in two models of liver fibrosis revealed downregulation of miR-29a, b, and c with associated increases in type I collagen expression. Cell-specific expression from isolated primary liver cells revealed high expression of miR-29b in HSCs, which was lost upon culture-activation[82]. *In vitro* experiments aimed to determine a mechanism revealed that TGF-β treatment downregulated miR-29b expression with a concomitant increase in type I collagen expression. Another striking observation was that miR-29a serum levels are downregulated in human fibrotic patients compared to healthy patients; the degree of fibrosis and cirrhosis correlated with the extent of miR-29a suppression, suggesting that miR-29a may be a novel serum marker of liver fibrosis in humans[82].

Small interfering RNA therapy

Small interfering RNAs (siRNA), like miRNAs, are a class of double-stranded RNA molecules 20-25 nucleotides in length that participate in the RNAi pathway and have received considerable attention as a therapeutic strategy for a variety of conditions. Several barriers exist to effective siRNA therapies in hepatic fibrosis, including targeting delivery to the intended liver cell(s) to avoid systemic consequences and overcoming the physical barriers that occur in fibrosis that limit exchange, including the loss of endothelial cell fenestration and accumulation of ECM components in the space of Disse.

Efforts to target TGF-β, the most potent pro-fibrotic cytokine, has led to the development of siRNAs that can inhibit TGF-β mRNA expression in a rat HSC cell line[83]. These siRNAs have been conjugated with galactosylated poly(ethylene glycol)(Gal-PEG) or mannose 6-phosphate poly(ethylene glycol)(M6P-PEG) and targeted to HCC (HepG2) and HSC (HSC-T6) cell lines, respectively[84]. M6P-PEG targets to HSCs *via* M6P/insulin-like growth factor-Ⅱ receptor-mediated endocytosis and Gal-PEG targets to hepatocytes *via* asialoglycoprotein receptor-mediated endocytosis[84]. Though specific targeting produced favorable results *in vitro* these strategies have yet to be validated *in vivo*.

A novel approach to deliver siRNAs against Hsp47 has recently been reported utilizing two models of liver fibrosis; CCl_4 and BDL, and a lethal model of dimethylnitrosamine (DMN)-induced cirrhosis. Investigators conjugated vitamin A to liposomes carrying siRNAs targeting gp46, a homolog of Hsp47, which rapidly resolved fibrosis and prolonged survival in DMN-induced cirrhosis[85]. Evaluation of radiolabeled vitamin A-coupled liposomes showed uptake predominantly in livers, demonstrating organ specificity. These data represent an exciting advance in siRNA-mediated treatment of fibrosis and demonstrate that targeting of collagen production, not just the underlying pathology, can be an effective anti-fibrotic strategy. However, further studies to assess the functional consequences of Hsp47 disruption need to be conducted, as the investigators revealed that Hsp47 repression stimulated collagenase activity[85].

Inhibitors of chaperone proteins

A novel approach to targeting type I collagen secretion is inhibiting the activity of one or more chaperone proteins associated with the numerous post-translational modifications procollagens undergo prior to secretion. As described in the previous section, one such approach utilized siRNA against Hsp47 to abolish fibrosis in two animal models[85]. However, systemic administration of inhibitors to collagen chaperones poses systemic risks, particularly in tissues with high normal expression of type I collagen, like skin and bone.

One such approach to prevent systemic consequences centered on the design of a pro-drug inhibitor of prolyl 4-hydroxylase, HOE 077, which would be converted to the active form by liver cytochrome P450 activity to pyridine-2,4-dicarboxylate (2,4-PDCA). Use of this drug attenuated liver fibrosis and collagen accumulation induced by CCl_4 administration[86]. However, *in vitro* mechanistic studies reported that HOE 077 prevented activation of HSCs as opposed to type I collagen production[87].

Hsp47 is an attractive target for the generation of small inhibitors, as type I collagen is the only reported target for this chaperone. Several inhibitors of Hsp47 have been reported; however, their efficacy in inhibiting collagen production by mediators of fibrosis, or in animal models, has yet to be demonstrated[88].

Matrix metalloproteinases

Regulation of the ECM is accomplished in part by a diverse family of calcium- and zinc-dependant endopeptidases called MMPs. There are 25 identified unique members of the MMPs, of which 24 are found in mammals, and they are capable of degrading a variety of matrix components. MMPs can be divided into five categories: interstitial collagenases, gelatinases, stromelysins, membrane-type collagenases, and a metalloelastase (MMP-12), although there is some overlap in function between these groups.

In liver, the main interstitial collagenases are MMP-1 in humans, and MMP-8 and MMP-13 in rodents. MMP-3 (stromelysin-1) is another interstitial collagenase expressed in liver; however, it exhibits weak proteolytic activity towards ECM components[89]. Liver fibrosis results from an imbalance between fibrinogenesis and fibrinolysis, with an increase in tissue inhibitors of metalloproteinases (TIMPs) primarily responsible for this imbalance. Four TIMPs have been identified (TIMP-1, TIMP-2, TIMP-3, and TIMP-4), each consisting of 184-194 amino acids. TIMPs inhibit MMPs by directly binding to the catalytic domain of MMPs in a 1:1 stoichiometric ratio. TIMPs have been shown to inhibit the activity of each MMP, though with varying efficiency[90]. Besides the well-established direct role of TIMPs as inhibitors of MMPs, TIMP-1 is capable of indirect inhibition of HSC apoptosis[91]. Furthermore, reduction of TIMP-1 levels is associated with increased hepatocyte proliferation *via* degradation of fibrotic ECM to permit hepatocyte expansion and liberation of ECM-bound hepatocyte growth factor[92]. Thus, therapeutic strategies to improve fibrinolysis can focus either on in-

creasing the pool of active MMPs or reducing the expression of TIMPs.

Attempts to enhance the expression of fibrinolytic MMPs have been carried out in various animal models of fibrosis. Infection with an adenovirus carrying human MMP-1 gene attenuated fibrosis with concomitant decrease in α-SMA positive cells in a rat TAA model[93]. An adenoviral delivery strategy was also utilized to stimulate human MMP-8 expression to abrogate fibrosis in rats treated with CCl_4 or subjected to BDL[94].

A similar approach was taken to inhibit expression of TIMP-1 in HSCs, utilizing an siRNA against TIMP-1 packaged in an adeno-associated virus (AAV) vector. AAV vectors have the ability to infect dividing and non-dividing cells, to incorporate into the genome at a specific site (AAVS1) in human chromosome 19 for sustained expression, and are non-immunogenic[95]. TIMP-1 expression was suppressed for > 12 wk, suggesting AVV-delivered siRNA against TIMP-1 has the potential for long-term efficacy. The study also reported concomitant increases in MMP-13 expression, the rodent equivalent to MMP-1 in humans; however, the investigators did not assess MMP-13 activity by zymography and have not investigated the efficacy of this system *in vivo*[96].

MMPs and their inhibitors clearly play an important role in the development, progression, and resolution of hepatic fibrosis. However, the context of MMP and TIMP expression must be considered when developing therapeutic strategies to target their activity. In early stages of hepatic fibrosis, MMPs appear to play a deleterious role, whereas in the resolution of fibrosis, MMP activity is critical to reduce the scar and achieve restoration of the normal liver architecture. Additional work to evaluate their effectiveness in treating hepatic fibrosis should be conducted.

CONCLUSION

Liver fibrosis is a complex disease that represents a common pathology for a variety of liver insults, including ALD. Sustained fibrogenesis can be linked to an exacerbation of the wound-healing process and results in the accumulation of ECM products, which can impair oxygen and nutrient delivery, stimulate proliferation of fibrogenic cells and result in injury. To date, there are no established therapies for liver fibrosis outside removal of the causative agent. Type I collagen is the most abundant component of the extracellular scar in liver fibrosis and is an attractive target for anti-fibrotic therapies.

Therapies to limit pro-fibrotic mediators, such antioxidants to scavenge ROS, have produced promising results *in vitro* and with animal models of fibrosis; however, antioxidants have failed to consistently reduce fibrosis in human trials. This discrepancy is not understood, and therefore, attention should be made to developing new therapeutics. Regardless of the etiology, it is widely accepted that other factors (e.g. genetic and environmental) contribute to the development and progression of fibrosis, thus a therapeutic target directed towards the culprit of fibrosis (collagen) might be a more successful and comprehensive therapy. Attempts to reduce accumulated type I collagen through MMP-mediated fibrinolysis has generated attractive results in animal models; however, their efficacy has yet to be tested in human trials. Additionally, further work to refine targeting and delivery of MMP-based therapies needs to be performed, as non-specific delivery of MMPs could have unintended consequences in other collagen-rich tissues.

Recent work in targeted delivery of siRNAs against the collagen-specific chaperone Hsp47 represents exciting proof-of-concept therapy of tissue-directed suppression of type I collagen, potentially reducing deleterious effects of systemic type I collagen inhibition. Despite this promising finding, additional studies to determine the efficacy and safety of this approach in humans need to be conducted. Continued investigation into the molecular mechanisms of type I collagen production and secretion in fibrogenic mediators should be performed to produce new targets for anti-fibrotic therapy.

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