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Mini Review

Molecular and Epigenetic Mechanisms of Bidirectional Liver Fibrosis - ②

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ABSTRACT

Liver fibrosis is natural wound healing response to different etiologies of chronic liver insults leading to accumulation of Extra Cellular Matrix (ECM) due to imbalance between synthesis and degradation. Fibrogenesis is consequence of multicellular response; activation of Hepatic Stellate Cells (HSCs) and transdifferentiation into myofibroblasts are crucial for development of hepatic scar. Recent studies evidenced that liver fibrosis is potentially bidirectional regulated by complex cytokines, growth factors, genetic and epigenetic mechanisms (DNA methylation, histone modifications and miRNAs mediated gene silencing). Regression of liver fibrosis is due to increase in collagenolytic activity and increased Metalloproteinase (MMPs) activity with decreased expression and activity of Tissue Inhibitors of Metalloproteinases (TIMPs). Reversible epigenetic mechanisms, pro-fibrotic and anti-fibrotic miRNAs regulate progression and regression of liver fibrosis which initiates to discover diagnostic, prognostic and therapeutic should be comprehensively defined. Hence, in this review we made an attempt to understand molecular, genetic and epigenetic mechanisms of bidirectional liver fibrosis.

Keywords: Liver fibrosis; Hepatic regeneration; Cytokines; Epigenetics

ABBREVIATIONS

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GLDH: Glutamate Dehydrogenase; γ GT: Gamma Glutamyl Transferase; LDH: Lactate Dehydrogenase; CRP: C-Reactive Protein; α 2M: α 2 Macroglobulin; CTGF: Connective Tissue Growth Factor; PIINP: Procollagen III amino peptide; MMPs: Matrix Metallo Proteinases; TIMPs: Tissue Inhibitors of Metalloproteinases; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; ASH: Alcoholic Steatohepatitis; NAFLD: Non Alcoholic Fatty Liver Diseases; IGF-1: Insulin like Growth Factor 1; EMT: Epithelial Mesenchymal Transition; TGF- β : Transforming Growth Factor β ; PDGF: Platelet Derived Growth Factor; ET-1: Endothelin-1; ROS: Reactive Oxygen Species

INTRODUCTION

Liver, a vital organ performs several crucial functions; substrate metabolism, detoxification, protein and digestive enzyme synthesis and immune response for human survival [1]. Being highly vascular organ, it is continuously exposed to injury and damage by hepatotoxins *viz* viruses, drugs, alcohol, excess fat etc., leading to inflammation and fibrosis [2]. Liver fibrosis is natural wound healing response to chronic liver insults which involves deposition of Extra Cellular Matrix (ECM). Accumulated ECM destroys liver by forming fibrotic scar and subsequent nodular development ultimately leading to liver cirrhosis. Fibrotic liver contains three to ten times more ECM which in turn distorts liver parenchyma and vascular architecture and results in liver dysfunction. Hepatic Stellate Cells (HSCs) are ECM producing cells in fibrotic liver effective after activation and trans-differentiation into myofibroblasts which attains contractile, inflammatory and fibrogenic properties (Figure 1). HSCs activation results from interactions with damaged hepatocytes, Kupffer cells, disintegrated platelets and sub-fractions of leucocytes [3,4]. Progression of inflammatory and fibrogenic pathways are mediated by cytokines, genetic and epigenetic mechanisms. After chronic liver injury, fibrogenesis starts with necrosis or apoptosis of hepatocytes and inflammation connected activation of hepatic stellate cells, their trans-differentiation to myofibroblasts with enhanced expression and secretion of extracellular matrix and deposition which attains contractile, proinflammatory and fibrogenic property. In pathophysiology of liver fibrogenesis, Transforming Growth Factor- β (TGF- β), Platelet Derived Growth Factor (PDGF), endothelin-1 and Vascular Endothelial Growth Factor (VEGF) play a dominant role [4]. Genes regulating hepatocellular damage, inflammatory response to injury and Reactive Oxygen Species (ROS) generation regulates extent of hepatic damage, inflammation and ECM deposition [5].

Epigenetic mechanisms [DNA methylation, histone modifications and noncoding micro RNA (miRNA)] have been shown to orchestrate many aspects of fibrogenesis of liver [6]. Recent studies have shown that liver fibrosis is dynamic and potentially bidirectional process. Treatment aimed at underlying cause especially at early stage of the disease can reverse fibrosis to normal liver architecture by spontaneous resolution of hepatic scar. Reasons for resolution may be due to increase in collagenolytic activity, increased Matrix Metalloproteinase (MMPs) activity and decreased expression of Tissue Inhibitors of Metalloproteinase (TIMPs). Cytokine mobilization of bone marrow derived stem cells restores neutrophil function and promotes hepatic regeneration [3]. The stage at which disease become irreversible is not well established but it is believed that irreversibility attains once septal neovascularisation happens and portal pressure increases significantly [7]. Hence, we have made an attempt in this review to understand the molecular and epigenetic mechanisms involved in bidirectional liver fibrogenesis.

PATHOPHYSIOLOGY OF BIDIRECTIONAL LIVER FIBROSIS

Natural wound healing response of liver for chronic liver injury results in the formation of hepatic scar leading to fibrosis of liver. After an acute injury, liver parenchymal cells regenerate and replace the necrotic and apoptotic cells. If hepatic injury persists, there will be failure in hepatic regeneration and substitution of hepatocytes with abundant ECM having contractile, inflammatory and fibrogenic properties [8]. Different types of cells (resident innate inflammatory cells, hepatocytes, liver sinusoidal endothelial cells and Kupffer cells) play a role in liver fibrogenesis. Activation of HSCs is a crucial step in inter-linked process of tissue injury and regeneration [9]. Quiescent HSCs present in space of Disse will be activated and trans-differentiate into myofibroblasts like cells which are responsible for ECM production and accumulation in injured liver [10]. Accumulation of ECM is due to increased synthesis and decreased degradation by over expression of TIMPs which inhibits MMPs [9]. Fibrotic liver contains three to ten times more ECM compared to normal liver includes collagen types, glycoproteins, proteoglycans and glycosaminoglycans [3]. Chief mitogen of HSCs activation is PDGF produced by Kupffer cells; macrophages are source of pro-fibrotic chemokines [11,12]. Activated HSCs activate immune response by secretion of cytokines, chemokines and interacting with immune cells. Complex network of cytokines (Table 1) modify activities of circulating immune cells, HSCs, hepatocytes, liver sinusoidal endothelial cells and Kupffer cells (Figure 2). Autocrine and paracrine secretions of cytokines activate and trans-differentiate HSCs into myofibroblasts [9]. Activated HSCs migrates to tissue repair site and secrete ECM; collagen synthesis is

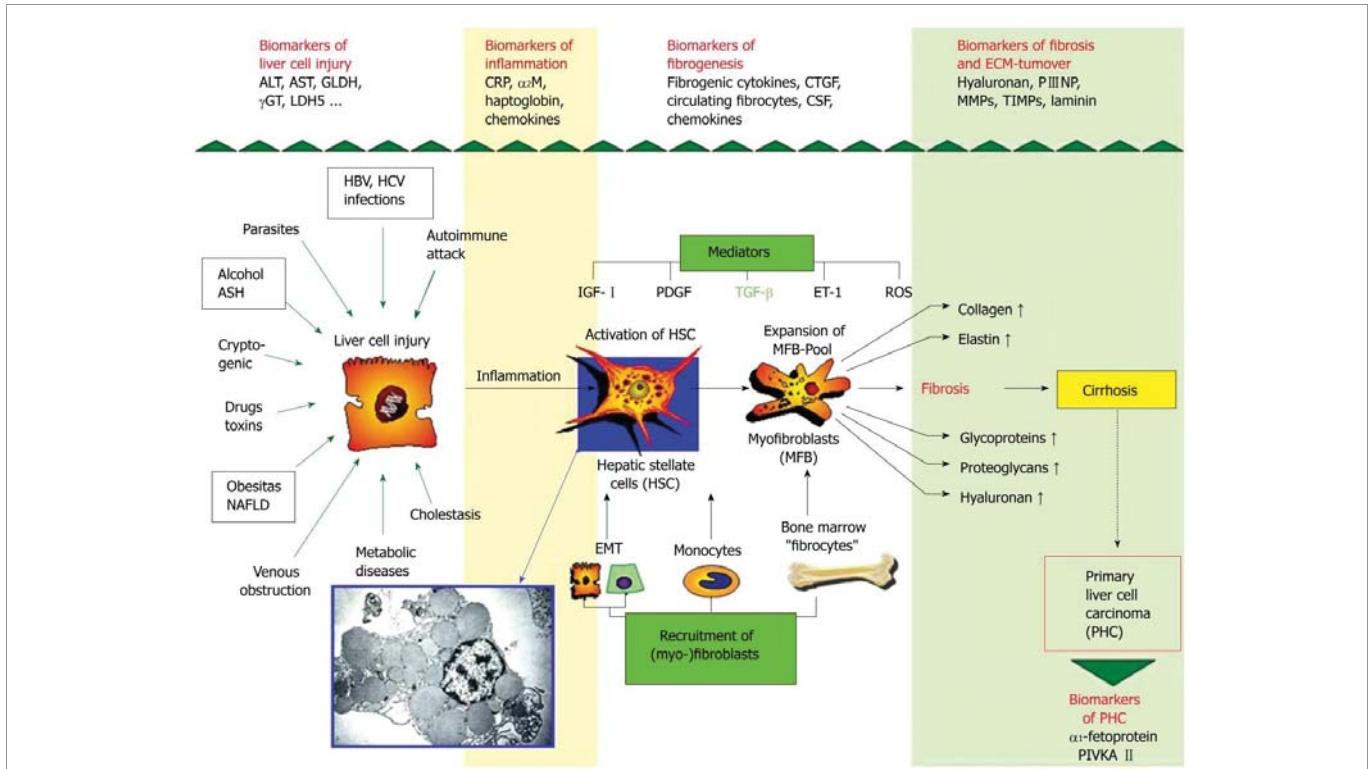


Figure 1: Pathophysiology of liver fibrosis [4]. Reproduced with permission from Gressner, et al.

Table 1: Role of cytokines involved in regulation of liver fibrosis [9].

Mediators	Mechanism of action
Growth Factors	
Platelet-derived growth factor (PDGF)	Stimulates activation and proliferation of HSCs. Upregulates expression of TIMP-1 Inhibits collagenase activity
Transforming growth factor-β (TGF-β)	Stimulates activation of HSCs. Upregulates type I collagen and α-SMA synthesis. Promotes MFs survival through activation of FAK. Inhibits DNA synthesis and induces hepatocytes apoptosis Upregulates expression of TIMPs
Interleukins	
Interleukin-1 (IL-1)	Activates HSCs and promotes HSCs survival through NF-κB. Promotes lipid accumulation in NAFLD Promotes type I collagen synthesis, Upregulates TIMP-1
Tumor necrosis factor-α (TNF-α)	Promotes activation of HSCs and reduces apoptosis of activated HSCs Upregulates TIMP-1, Stimulates hepatocyte apoptosis
Interleukin-17 (IL-17)	Upregulates type I collagen, TGF-β, and TNF-α through STAT3 pathway, Promotes activation of HSCs
Interleukin-10 (IL-10)	Inhibits HSCs activity. Inhibits expression of TIMP-1 and TGF-β
Interleukin-22 (IL-22)	Promotes HSCs senescence
Interleukin-6 (IL-6)	Attenuates hepatocytes apoptosis and induce regeneration of hepatocytes through NF-κB Induces insulin resistance
Interferon	
Interferon-α (IFN-α)	Has an anti-apoptotic effect on activated HSCs
Interferon-β (IFN-β)	Inactivates HSCs and decrease production of type I collagen and α-SMA through inhibition of PDGF and TGF-β
Interferon-γ (IFN-γ)	Inhibits activation of HSCs and reduce type I collagen deposition. Induces hepatic HSCs apoptosis and cell cycle arrest
Chemokine	
CCL2	Promotes migration of bone marrow-derived monocyte to liver. Activates HSCs. Induces insulin resistance in NAFLD
CXCL10	Promotes hepatocytes apoptosis. Inhibits NK cells-mediated HSCs inactivation. Stimulates T-cell chemotaxis to the liver
CXCL16	Promotes intrahepatic accumulation of NKT cells

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HSC: Hepatic Stellate Cell; NF-κB: Nuclear Factor-κB; NAFLD: Non-Alcoholic Fatty Liver Disease; TIMP: Tissue Inhibitors of Metalloproteinase; STAT: Signal Transducer and Activator of Transcription; SMA: Smooth Muscle Actin; NKT: Natural Killer T cell; FAK: Focal Adhesion Kinase; CXCL: Chemokine (C-X-C motif) Ligand; CCL: Chemokine (C-C motif) Ligand.

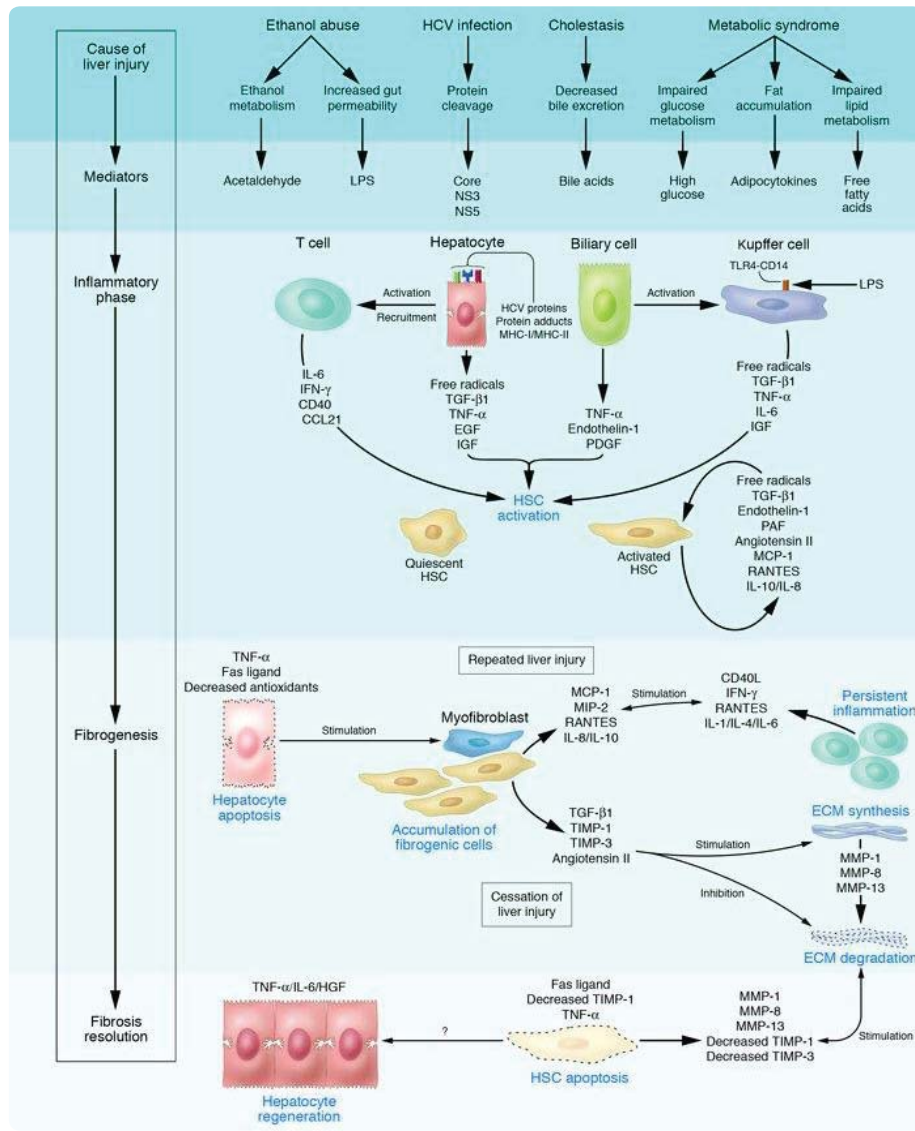


Figure 2: Cellular mechanisms of Liver fibrosis [5]. Reproduced with permission from Bataller, et al.

regulated by transcription and post-transcription. Collagen fibrils can be cross-linked by tissue transglutaminase and lysyl oxidase pathways which make collagen susceptible for collagenase activity [13]. Low density matrix is replaced by high density interstitial matrix which disturbs metabolic functions and impairs solute transport; altered cellular behavior is mediated by Integrins [11]. Damaged hepatocytes release ROS and fibrogenic mediators which stimulate inflammatory cells and fibrogenic action of activated HSCs. Activated HSCs stimulate lymphocytes by secreting inflammatory chemokines. It is a cyclic stimulation process of inflammatory and fibrogenic cells vice versa [5]. Damaged hepatocytes release inflammatory cytokines which activate Kupffer cells and stimulate the recruitment of activated T cells. This activates quiescent HSCs into fibrogenic myofibroblasts secrete cytokines. Due to chronic liver injury, activated HSCs express and deposits ECM leads fibrosis of liver. ECM degradation is inhibited by the actions of TIMPs. When etiology of liver fibrosis removed, there will be spontaneous resolution of fibrosis by apoptosis of activated HSCs and regeneration of hepatocytes. Accumulated collagen is degraded by increased activity of MMPs. Spontaneous resolution of

liver fibrosis is possible after successful treatment of causative agent and may take several years depending on cause and severity of the disease [5,12]. Characteristic features of liver fibrosis reversal are decreased inflammation and decreased fibrogenic cytokines, increased collagenase activity and disappearance of myofibroblast and fibrotic scar [14]. Regression of liver fibrosis consists of thinning of fibrous septa, regeneration of hepatocytes and recovery of acinal structure [15]. Reversal of liver fibrosis can be achieved by inhibition of HSCs activation, neutralization of proliferative, fibrogenic, contractile and proinflammatory response of HSCs, stimulation of HSCs apoptosis or senescence and degradation of scar matrix. Inhibition of HSCs activation and trans-differentiation into myofibroblasts can be attained by reducing oxidant stress [10]. Interferon-β (IFN-β) inactivates HSCs and decrease production of collagen I and a Smooth Muscle Actin (α-SMA) by inhibiting PDGF and TGF-β; Interferon-γ (IFN-γ) has inhibitory action on activation of HSCs [9]. Fibrillar collagens are degraded by interstitial MMPs (MMP-1, -8 and -13) which are released in pro-enzyme form and activated by cleavage of inhibitory N-terminal peptide by plasmin. Plasmin synthesis

in fibrotic liver is inhibited by synthesis of plasminogen activator inhibitor-1 expressed from activated HSCs [13]. During resolution of fibrosis, MMPs activity is increased due to decreased expression of TIMPs; monocyte/macrophage lineage expresses MMPs [16]. After removal of inflammation, macrophages are differentiated into Ly6c^{low} phenotype which produces MMP9 and MMP12 capable of matrix degradation [14]. Altered interactions between activated HSCs and ECM favor apoptosis [5,13]. Myofibroblast apoptosis is contributed by activation of death receptor mediated pathway, increased expression of pro-apoptotic proteins (p53, Bax and Bcl-2) and decreased expression of pro-survival proteins [14]. After successful removal of causative agent, HSCs undergo caspase-8/caspase-3 dependent apoptosis. Over expression of pro-apoptotic proteins leads to caspase-9 mediated programmed cell death. Over expression of CXCL9 by macrophages and VEGF expression accelerate fibrosis resolution by angiogenesis [16].

GENETICS OF BIDIRECTIONAL LIVER FIBROSIS

Genetics of liver fibrosis progression are highly complex and influenced by multiple factors. Hepatocellular apoptosis/ necrosis genes *viz* Bcl-xL, Fas influence the extent of hepatic damage and fibrinogenesis. Inflammatory genes *viz* IL-1 β, IL-6, IL-10, IFN-γ, SOCS-1 and osteopontin determines the fibrogenic response to injury. Genes regulating ROS generation (NADPH oxidase) regulate inflammation and ECM deposition [5]. Trans-differentiation of activated HSCs into myofibroblasts is mediated by down regulation of lipogenic genes like peroxisome proliferator-activated receptor gamma (PPARγ) and up-regulation of fibrogenic genes. Activated HSCs and myofibroblasts migrate to site of injury and express fibrogenic genes *viz* vimentin, collagen α1 (Colla1) and α-SMA stimulated by increased levels of PDGF and TGF-β [17,18]. Fibrogenic growth factors, vasoactive substances and adipokines are required for fibrogenesis (Table 2). In fibrosis resolution, expression of fibrogenic genes is decreased by inactivated HSCs in association with increased expression of genes like PPAR-γ. Genes *viz* GFAP, *Adipor1*, *Adpf* and *Dbp* are not expressed by inactive HSCs shows the difference between quiescent HSCs and inactive HSCs [19]. Gene polymorphisms play a major role in progression of liver fibrosis due to chronic liver diseases. In Alcoholic Liver Disease (ALD), polymorphisms of genes encoding alcohol metabolizing enzymes and proteins (alcohol dehydrogenase, aldehyde dehydrogenase and P450), genes encoding inflammatory mediators and antioxidants influence fibrogenesis [5]. In nonalcoholic steatohepatitis, genotypes of IL-10-1082G/ A and TNF-α 308G/ A express elevated levels of inflammatory cytokines [20]. In non-alcoholic fatty liver disease, *CDKN1A* variant rs762623 is related to development of liver fibrosis [21]. In primary biliary cholangitis, polymorphisms of genes encoding IL-1β, IL-1 and TNF-α are responsible for diseases progression. In Hepatitis C Virus (HCV) infection, polymorphisms of genes involved in immune response (transporter associated with antigen processing 2, specific HLA-II alleles), fibrogenic agonists (angiotensinogen and TGF-β) enhances fibrosis [5]

EPIGENETICS OF BIDIRECTIONAL LIVER FIBROSIS

Cellular composition and phenotype changes in chronic liver diseases are under the control of chromatin configuration of regulatory genes directed by epigenetic mechanisms [6]. Multifactorial causes

Table 2: Genetic and non-genetic factors of liver fibrosis [5].

Type of Liver Disease	Genes	Non-genetic factors
HCV	Hereditary hemochromatosis Angiotensinogen Transforming growth factor β1 Tumor necrosis factor α Apolipoprotein E Microsomal epoxide hydroxylase Monocyte chemotactic protein type 1 Monocyte chemotactic protein type 2 Factor V (Leiden)	Alcohol intake Co-infection with HBV/HIV Age at time of acute infection Diabetes mellitus
Alcohol-induced	Interleukin 10 Interleukin 1β Alcohol dehydrogenase Aldehyde dehydrogenase Cytochrome P450, family 2, subfamily e, polypeptide 1 Tumor necrosis factor α Cytotoxic T lymphocyte antigen type 4 Transporter-associated antigen-processing type 2 Manganese superoxide dismutase	Episodes of alcoholic hepatitis
NASH	Hereditary hemochromatosis gene Angiotensinogen Transforming growth factor β1	Age, Severity of obesity Diabetes mellitus Hypertriglyceridemia
PBC	Interleukin 1β Tumor necrosis factor α Apolipoprotein E	
Autoimmune hepatitis	Human leukocyte antigen type II haplotypes	Type II Autoimmune hepatitis No response to therapy

Modified from Bataller *et al*
HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; HIV: Human Immuno deficiency Virus; NASH: Non Alcoholic Steatohepatitis; PBC: Primary Biliary Cholangitis

influence the epigenetic mechanisms through SNPs [3]. Epigenetics are reversible changes in gene expression which are inherited through cell division without altering underlying DNA sequence; DNA methylation, post transcriptional modifications of amino acid tails of histones and non-coding RNA mediated gene silencing. These mechanisms organize many aspects of liver fibrosis by regulating chromatin structure, modifications and initiation of transcription that alters the accessibility of genes [22]. Diverse biological functions of liver are regulated by noncoding small microRNAs and play role in pathophysiology of liver [23]. Epigenetic mechanisms are determinants of gene expression during HSCs activation and deactivation by controlling transcription activity during fibrosis progression and regression. Unlike genetic mutations, epigenetic changes undergo reversion with the resolution of liver fibrosis and can be modulated pharmacologically [6,22].

DNA METHYLATION

Development of liver fibrosis is associated with aberrant DNA methylation patterns which lead to inappropriate gene expression. DNA methylation is regulated by DNA methyltransferases (DNMT1, DNMT3a and DNMT3b), hydroxymethylases which increases in fibrotic liver while hepatic expression of Ten Eleven Translocation (TET) demethylase is down regulated [6]. Hypermethylation of cell cycle genes (p15 and p16), tumor suppressor genes (RASSF1A and E-cadherin) and anti-fibrotic gene PPARγ is associated with liver fibrosis progression [22]. Transdifferentiation of HSCs expresses methyl-CpG-binding domain nuclear proteins (MeCP2, MBD1, MBD2 and MBD3) which are transcriptional repressors of epigenetic

silencing of PPAR γ gene. MeCP2 has positive regulation on expression of histone methyltransferase ASH1 which is required for expression of pro-fibrogenic genes collagen1, TIMP1 and TGF- β 1. In hepatic myofibroblasts, MeCP2 regulate gene expression by direct methyl-CpG-dependent transcription and indirect post-transcriptional mechanisms [6]. Transdifferentiation of HSCs to pro-fibrogenic myofibroblast phenotype is suppressed by DNMT inhibitor 5'-aza-deoxycytidine [24].

HISTONE MODIFICATIONS

Liver damaging agents dysregulate chromatin structure by epigenetic mechanisms which involve action of ROS on histone modification. In post-translational modifications of histone proteins, lysine methylation or acetylation regulates liver fibrosis by perspective activation of HSCs. In activated HSCs, histone methyltransferase (H3K4 methyltransferase), ASH1 is up-regulated during progression of fibrosis and binds to promoters and 5' end of α -SMA, collagen1, TIMP-1 and TGF- β 1 which results in transcriptional activation of gene expression. H3K27 methylation leads to repression of PPAR γ gene; H3K9 dimethylation results in repression of inhibitory protein I κ B α leads to up-regulation of transcription factor NF κ B which has an important role in liver fibrosis. HSCs transdifferentiation requires chromatin signature H3K27me3 by recruited PPAR γ gene. Lysine acetylation of histone proteins can up-regulate expression of collagen1, TIMP-1 and TGF- β 1. Histone acetylation can be inhibited which can reverse myofibroblast differentiation by Histone Acetylation (HDAC) inhibitors [6,22,25].

SMALL NON CODING RNAS (MIRNAS)

miRNAs are essential for cellular process by regulating mRNA transcripts and are involved in activation of HSCs and liver fibrosis through regulation of proliferation and apoptosis (Table 3). Liver homeostasis is regulated by miRNA-122 affects on various genes involved in metabolism. miRNA-155 involved in innate and adaptive immune response by targeting TNF and promotes liver inflammation; miRNA-146a is a negative regulator of Toll Like Receptor (TLR) signaling proinflammatory response. Hepatocyte proliferation is regulated by miRNA-21 gene mediated cell cycle and DNA synthesis. Cytokines and growth factors of liver fibrosis regulate expression of pro-fibrogenic and anti-fibrogenic miRNAs (Figure 3). Key factors of fibrogenesis viz Coll1 α 1, TGF- β receptor II, hepatocyte nuclear factor 4 α (HNF4 α) are regulated by miRNAs effect on mRNA 3'-UTR [6,22,23].

LONG NON-CODING RNAS (LNCRNAs) AND CIRCULAR RNAS (CIRC RNAs)

lncRNAs (exonic, intronic, overlapping and intergenic) effect gene expression by modulation of chromatin remodeling, control of gene transcription, post-transcriptional mRNA processing, protein function or localization and intracellular signaling; H19 and XIST were first identified lncRNAs for liver diseases [26]. lncRNA maternally expressed gene3 (lncRNA MEG3) located on chromosome 14q32.3 is a tumor suppressor gene which is down regulated due to TGF- β mediated methylation in disease progression of liver fibrosis [27]. Epigenetic regulation of MEG3 regulates fibrosis by inducing apoptosis by Bax/ Bcl-2 and cytoplasmic cytochrome C expressed p53 mediation. ECM synthesis will be reduced by over expression of MEG3 by suppressing cell proliferation [26]. LALR1 (human ortholog hLALR1) enhances hepatocyte proliferation through activation of Wnt/ β -catenin signaling and suppressing Axin1 [28]. Circular RNAs

Table 3: Role of miRNAs in liver diseases [23].

miRNAs	Functions
Mitochondrial miRNAs	
Rno miR-21, miR-130a, miR-130b, miR-140, miR-320, miR-494 and miR-671	Potential role in apoptosis, cell proliferation and Differentiation
miR-705, miR-494, miR-202-5p miR-451-7b, miR-26a, miR-122, miR805, miR-690, miR-155 and miR-134	Mitochondrial dysfunctions
pre-miR-302a, pre-let-7b, miR-365, miR-720, miR-133b, miR-1974, miR-24, miR-133a, miR-125a-5p and let-7 family members	Potential role in cell proliferation and differentiation
has-miR-494, miR-1275 and miR-1974	A possible role in regulating translation in mitochondria
miR-181c	Mitochondrial dysfunctions
Nuclear miRNAs	
miR-29b	Might regulate transcription or splicing of target Transcripts
miR-320	Transcriptional gene silencing
miR-671	Gene silencing of noncoding antisense transcripts
Circulating miRNAs	
Exosomal miRNAs	Cell-cell communication
Epstein-Barr virus-encoded miRNAs	
miR-126	Atherosclerosis
miR-150	Cell migration
miR-146a	Cell growth inhibition
miR-29a	HIV Tat and morphine-mediated neuronal dysfunction
miR-133b	Neurite outgrowth

Modified from Szabo, *et al.*

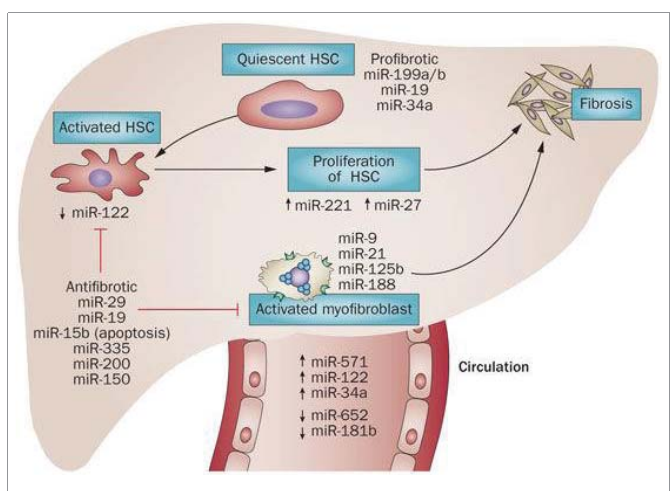


Figure 3: miRNA regulation of liver fibrosis [23]. Reproduced with permission from Szabo, et al.

(circRNAs) a class of endogenous RNAs regulate gene expression at transcriptional or post-transcriptional level by acting as miRNAs sponges [29]. In bidirectional liver fibrosis, has_circ-0004018 transcribed by *SMYD4* has lower expression in disease progression [30]. circHIPK3 derived from Exon2 of *HIPK3* gene is a modulator of cell proliferation and significantly up-regulated in liver cancer [29].

CONCLUSION

Natural wound healing response to chronic liver insults results in the formation of liver fibrosis which is mediated by complex network of cytokines, growth factors, genetic and epigenetic mechanisms. Recent studies have shown that liver fibrosis is potentially bidirectional. Molecular mechanisms for liver regression in humans need to be more comprehensively defined. At which point, liver fibrosis will become irreversible is not well established, early stages may give witness for reversibility. Even though reversible epigenetic mechanisms of liver fibrosis can be modulated pharmacologically, it needs extensive research to improve anti-fibrotic drug therapies.

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