



Review Article

Mechanisms of Epigenetic Regulation in the Fibrogenic Activation of Hepatic Stellate Cells in Non-alcoholic Fatty Liver Disease



Dmitry Victorovich Garbuzenko^{*}

Department of Faculty Surgery, South Ural State Medical University, Chelyabinsk, Russia

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is an extremely prevalent disease, and the presence and severity of liver fibrosis are considered one of the most important factors determining its prognosis. Hepatic stellate cells (HSCs) are essential in hepatic fibrogenesis associated with NAFLD. A number of factors underlying NAFLD pathogenesis may promote HSCs activation, leading to the development of profibrotic and proinflammatory signs. In addition, for the fibrogenic transdifferentiation of quiescent HSCs, alterations in multiple genes are necessary, where epigenetic regulation plays a defining role. Epigenetic regulation induces changes in gene activity without altering the coding sequence, and these changes are stably inherited after the factor causing the alteration has disappeared. Epigenetic modifications comprise several regulatory mechanisms, including DNA methylation, covalent histone modification, chromatin remodeling, and non-coding RNAs. Since the mechanisms underlying epigenetic regulation of HSCs fibrogenic activation are reversible and dynamic, molecular targeted therapies aimed at correcting these mechanisms provide promising prospects for novel therapeutic approaches for treating liver fibrosis associated with NAFLD.

Keywords: Hepatic stellate cells; Activation; Epigenetic regulation; Nonalcoholic fatty liver disease; Liver fibrosis.

Abbreviations: AKT, protein kinase B; APTR, Alu-mediated p21 transcriptional regulator; ASH1, Absent, small, or homeotic discs 1; circRNAs, circular RNAs; CpG, 5'-Cyto-sine-phosphate-Guanine-3'; CTGF, connective tissue growth factor; DNMTs, DNA methyltransferases; ERK, extracellular-signal-regulated kinase; EZH2, Enhancer of Zeste Homolog 2; GAS5, growth arrest-specific transcript 5; H3K27, histone H3 lysine 27; H3K4, histone H3 lysine 4; H3K9, histone H3 lysine 9; HDACs, histone deacetylases; HIF, hypoxia-inducible factor; HOTAIR, HOX transcript anti-sense intergenic RNA; HSCs, hepatic stellate cells; IFT80, Intraflagellar Transport 80; JAK/STAT, janus kinase/signal transducer and activator of transcription; JMJD1A, Jumonji Domain-Containing 1A; JNK, c-Jun NH2-terminal kinase; KDM4D, lysine demethylase 4D; LF, liver fibrosis; Inc-LFAR1, liver fibrosis-associated lncRNA 1; lncRNAs, long ncRNAs; MALAT1, metastasis-associated lung adenocarcino-ma transcript 1; MeCP2, methyl-CpG-binding protein 2; miRNAs, microRNAs; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ncRNAs, non-coding RNAs; NEAT1, nuclear enriched abundant transcript 1; NF, Nuclear Factor; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX4, NADPH oxidase 4; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; PVT1, plasmacytoma variant translocation 1; RSF1, Repressor splicing factor 1; SOX9, SRY-box transcription factor 9; TGF, transforming growth factor; TLR4, Toll-like receptor 4; TUBD1, Tubulin Delta 1; TUG1, Taurine Upregulated Gene 1; VEGF, vascular endothelial growth factor.

***Correspondence to:** Dmitry Victorovich Garbuzenko, Department of Faculty Surgery, South Ural State Medical University, 64 Vorovskogo Str., Chelyabinsk 454092, Russia. ORCID: <https://orcid.org/0000-0001-9809-8015>; Tel: +7-909-745-98-26, Fax: +8-351-2687772, E-mail: garb@inbox.ru

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Introduction

Non-alcoholic fatty liver disease (NAFLD) occupies a leading position among liver diseases worldwide. The prevalence of NAFLD increased from 25.26% between 1990 and 2006 to 38.00% from 2016 to 2019.¹ The total mortality associated with NAFLD across all causes is 0.17%.²

NAFLD is defined as a condition where a minimum of 5% of hepatocytes accumulate fat, excluding excessive alcohol consumption. It presents as simple steatosis or nonalcoholic fatty liver without liver fibrosis (LF) and nonalcoholic steatohepatitis (NASH), which in addition to steatosis, is characterized by lobular inflammation, hepatocyte ballooning, and various stages of LF.³ LF, an undesirable occasion in NAFLD, can progress to cirrhosis. Most complications of liver cirrhosis are primarily due to liver failure and portal hypertension, resulting in an unfavorable outcome.⁴

Inflammation and cell death are the main triggers of hepatic fibrogenesis in NAFLD, which are regulated by cellular cooperatives consisting of various resident and non-resident cells. Among these, the most important is the hepatocyte-macrophage-hepatic stellate cells (HSCs) network.⁵ Thus, HSCs are directly involved in hepatic fibrogenesis in NAFLD. These cells remain quiescent under physiological conditions and actively participate in the regulation of retinoid homeostasis.⁶ Several factors, such as lipid metabolites, free cholesterol buildup, oxidized low-density lipoprotein, palmitic acid, lipopolysaccharide, immune cell-associated

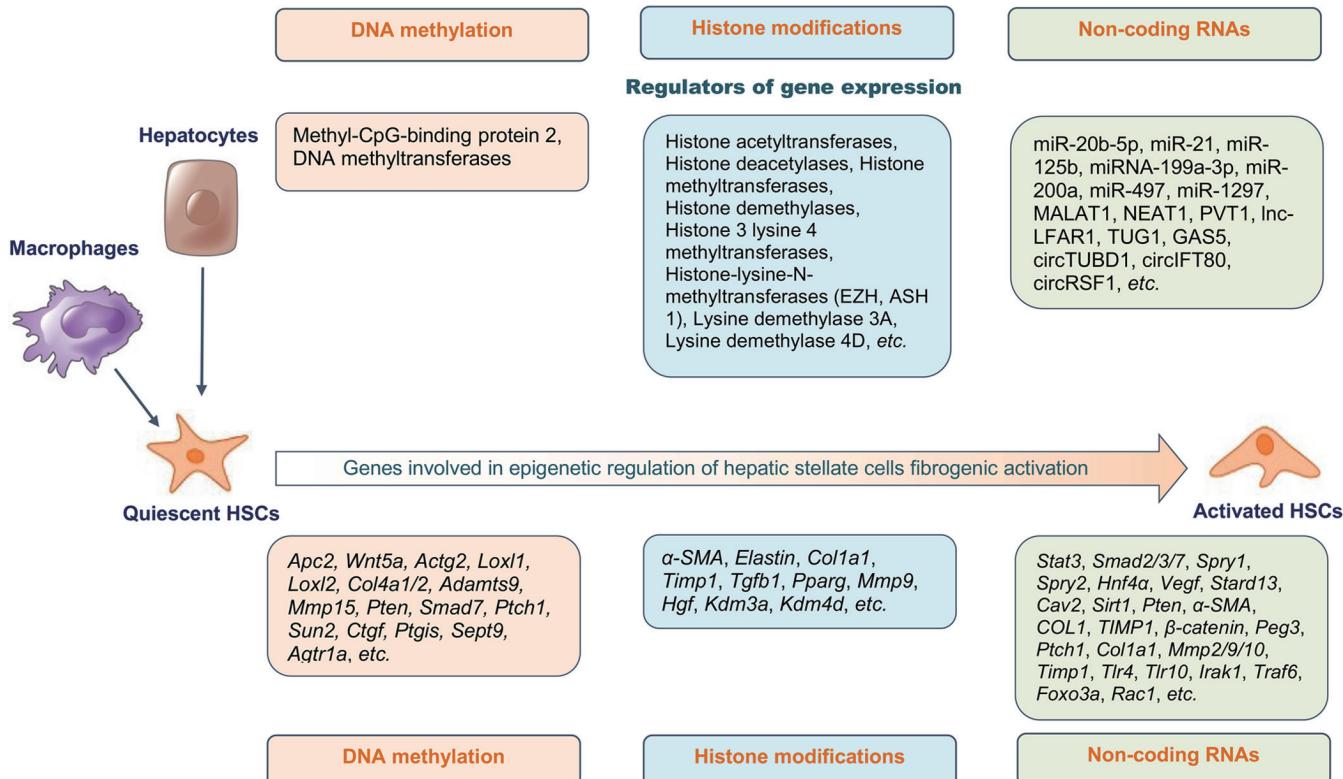


Fig. 1. The epigenetic regulation of fibrogenic activation of hepatic stellate cells (HSCs). ASH1, Absent, small, or homeotic discs 1; CpG, 5'-Cyto-sine-phosphate-Guanine-3' EZH, Enhancer of Zeste Homolog; GASS5, growth arrest-specific transcript 5; IFT80, Intraflagellar Transport 80; Inc-LFAR1, liver fibrosis-associated IncRNA 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; NEAT1, nuclear enriched abundant transcript 1; PVT1, plasmacytoma variant translocation 1; RSF1 Repressor splicing factor 1; TUBD1, Tubulin Delta 1; TUG1, Taurine Upregulated Gene 1.

profibrotic molecules and growth factors, induce HSCs activation in NAFLD with the acquisition of profibrotic and proinflammatory properties.⁷ For the fibrogenic transdifferentiation of quiescent HSCs, a change in the expression of multiple genes is required, where epigenetic regulation plays a defining role (Fig. 1).⁸

During hepatic fibrogenesis, HSCs undergo the initiation phase of activation, transitioning into the perpetuation phase of activation (Fig. 2).⁹ The activated HSCs excessively secrete type I and III collagen, fibronectin, and other extracellular matrix proteins. They also release a large number of tissue inhibitors of matrix metalloproteinases to prevent collagen destruction by matrix metalloproteinases. Among the paracrine and autocrine factors released by activated HSCs, the most potent profibrotic cytokine is the transforming growth factor (TGF)- β , in particular TGF- β 1. Low expression of peroxisome proliferator-activated receptor (PPAR) γ also promotes hepatic fibrogenesis. Osteopontin, connective tissue growth factor (CTGF) and basic fibroblast growth factor are other cytokines contributing to extracellular matrix synthesis by HSCs. In addition, oxidative stress promotes HSCs activation, type I collagen synthesis, and is essential in hepatic fibrogenesis.¹⁰

This review discusses the mechanisms underlying the epigenetic regulation of HSCs fibrogenic activation in NAFLD. Epigenetic regulation induces alterations in gene activity without modifying its coding sequence, which remains stable even after the factor causing this alteration disappears. Epigenetic modifications comprise several regulatory mechanisms, such as DNA methylation, covalent histone modification, chromatin remodeling, and non-coding RNAs (ncRNAs).¹¹

DNA methylation

DNA methylation is the most extensively studied mechanism of epigenetic regulation, during which a methyl group is added to the cytosine bases of DNA. The methyl group of methylcytosine is positioned within the large groove of the DNA helix, interacting with numerous DNA-binding proteins. Studies have demonstrated that changes in DNA methylation status within genes' promoter regions affect their transcriptional levels. Currently, two mechanisms of gene repression during methylation have been described. In the first instance, specific proteins bind to methylated CpG dinucleotides, subsequently recruiting proteins involved in chromatin remodeling, thereby rendering the region transcriptionally inactive. With an alternative mechanism, methylation prevents DNA binding to regulatory proteins necessary for gene expression. While DNA methylation is commonly observed on CpG islands, it is also possible to modify the methyl status of individual Cytosine-Guanine dinucleotides.¹² Methyl-CpG-binding protein 2 (MeCP2) is one of the primary regulators of HSCs activation. MeCP2 controls the gene expression during HSCs transdifferentiation, which is important for DNA replication and repair. It was reported that phosphorylation of MeCP2 leads to HSCs proliferation and hepatic fibrogenesis.¹³

DNA methyltransferases (DNMTs) promote DNA methylation by regulating gene expression.¹⁴ The DNMTs family mainly comprises DNMT1, DNMT3A, DNMT3B and DNMT3L. Notably, DNMT3L lacks inherent enzymatic activity unlike other DNMTs.¹⁵ DNMT1 is involved in maintaining DNA methylation dur-

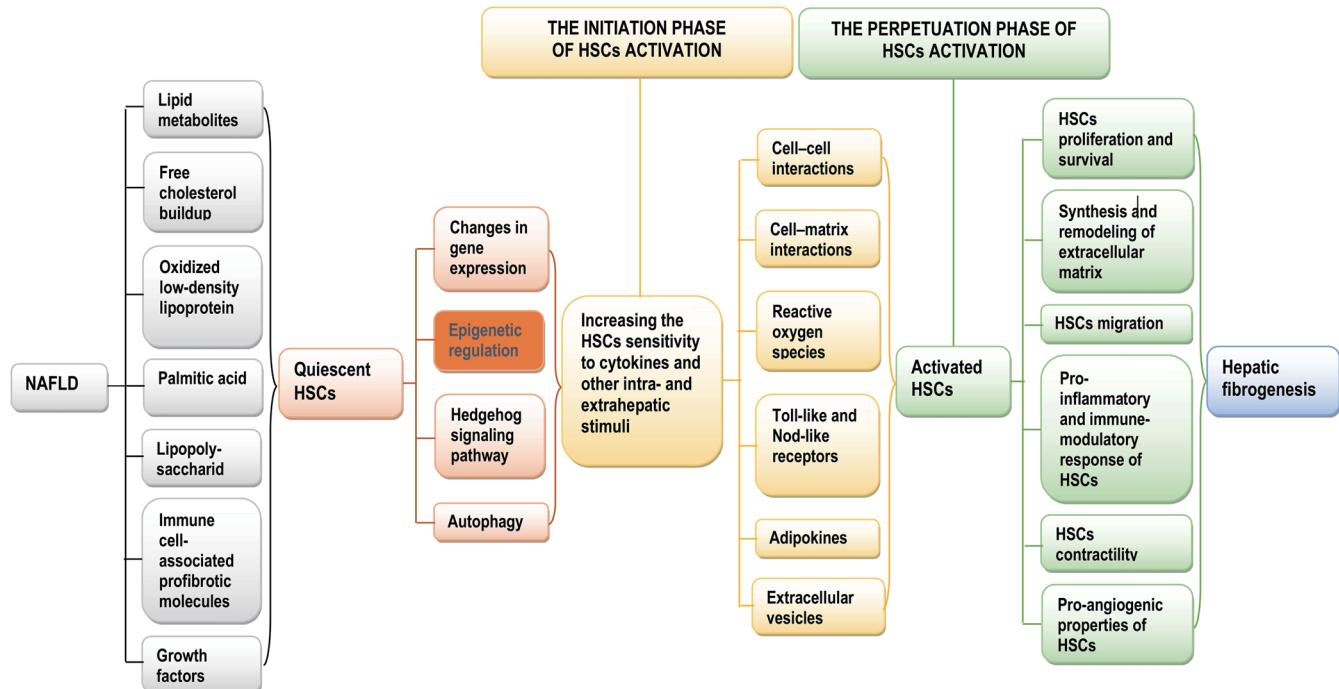


Fig. 2. The pathophysiological mechanisms of fibrogenic activation of hepatic stellate cells (HSCs) in non-alcoholic fatty liver disease (NAFLD).

ing cell division or regeneration, while DNMT3A and DNMT3B are involved in *de novo* methylation in the absence of cell division. Ten-eleven translocation family of methylcytosine dioxygenases carries out DNA demethylation by oxidizing methylcytosine to hydroxymethylcytosine, which is a key step in cytosine restoration.¹⁶ LF is accompanied with an increase in DNMTs activity, and a reduction in the activity of ten-eleven translocation methylcytosine dioxygenases, which leads to activation (or elongation) of transcription.¹⁷

DNA methylation is essential in NAFLD,¹⁸ particularly in the transition from nonalcoholic fatty liver to NASH.¹⁹ It was shown that hypermethylation at the *MT-ND6* gene is associated with the histological severity of NAFLD, and the ratio of methylated *MT-ND6* DNA to unmethylated DNA consistently correlates with the NAFLD activity scale.²⁰ In a study by Zeybel *et al.*,²¹ NAFLD patients without LF had stronger methylation of genes involved in hepatic fibrogenesis, whereas in NAFLD patients with LF, antifibrotic genes were involved in methylation, particularly *PPARA* and *PPARD*. *PPARG* was found to negatively affect HSCs activation and hepatic fibrogenesis.²² Differences in DNA methylation within the promoter region of the *PPARG* gene in circulating cell-free DNA enabled the stratification of NAFLD patients based on the severity of LF.²³ Further studies have indicated a significant correlation between cell-free DNA plasma concentration and non-invasive markers of NAFLD activity and severity.²⁴

DNA methylation is also involved in fibrogenic transdifferentiation of quiescent HSCs. Mann *et al.*²⁵ were the first to demonstrate that suppression of DNA methylation interferes with fibrogenic transdifferentiation of primary rat HSCs activated in culture. Subsequent research revealed total hypomethylation of DNA during the transdifferentiation or activation of HSCs.²⁶ Significant alterations in the DNA methylation profile were revealed at the beginning of HSCs activation *in vitro*. HSCs activation leads to a complete cessation of DNA methylation. However, at

CpG-rich sites, there was gene-specific hyper- and hypomethylation of DNA, resulting in changes in gene expression in activated HSCs.²⁷ An established association exists between gene-specific hyper- and hypomethylation of DNA at promoter and CpG-rich sites, with changed gene expression in activated HSCs.²⁸ *Ptch1* gene hypermethylation was found to correlate with the preservation of HSCs activation and hepatic fibrogenesis.²⁹ Silencing of the *Sun2* gene via DNA hypermethylation was accompanied by HSCs activation and hepatic fibrogenesis *in vitro*.³⁰ *Pten* gene hypermethylation influenced by DNMT1 led to the disappearance of its expression, thereby activating the PI3K/Akt and ERK signaling pathways, and contributing to HSCs activation.³¹ *Ctgf* gene promoter methylation in HSCs led to hepatic fibrogenesis.³² DNA methylation of *Ptgis* (prostaglandin I2 synthase) gene enhanced HSCs activation and hepatic fibrogenesis.³³ DNA methylation of *Sept9* (septin 9) gene mediated by DNMT3a enhanced HSCs activation and hepatic fibrogenesis.³⁴ Methylation of angiotensin II type I receptor (AT1aR) gene (*Agtr1a*) is potentially associated with NASH-related LF and HSCs activation.³⁵

Genes involved in DNA methylation during HSCs fibrogenic activation are presented in Table 1.²⁷⁻³⁶

Histone modifications and chromatin remodeling

Posttranslational histone modifications include acetylation, methylation, phosphorylation, ubiquitination and sumoylation. These modifications target specific residues, such as lysine, arginine, serine and threonine. Depending on the timing, type, location, and sequence, posttranslational histone modifications can either enhance or weaken gene expression, suggesting the presence of a histone code. Specific histone codes affect chromatin density and DNA availability for transcription factors, ultimately regulating gene expression.³⁷

Table 1. Genes involved in DNA methylation during hepatic stellate cells fibrogenic activation

| First author, year, ref. | Target genes | Expression | Regulatory mechanism |
|-----------------------------------|--------------------------------------|----------------------|-------------------------------|
| Götze, 2015 ²⁷ | <i>Apc2</i> | Increased expression | Promoter-DNA hypomethylation |
| El Taghdouini, 2015 ²⁸ | <i>Actg2, Loxl1, Loxl2, Col4a1/2</i> | Increased expression | Promoter-DNA hypomethylation |
| | <i>Adamts9, Mmp15</i> | Decreased expression | Promoter DNA-hypermethylation |
| Yang, 2013 ²⁹ | <i>Ptch1</i> | Decreased expression | Promoter DNA-hypermethylation |
| Chen, 2018 ³⁰ | <i>Sun2</i> | Decreased expression | Promoter DNA-hypermethylation |
| Bian, 2012 ³¹ | <i>Pten</i> | Decreased expression | Promoter DNA-hypermethylation |
| Bian, 2014 ³² | <i>Smad7</i> | Decreased expression | Promoter DNA-hypermethylation |
| Shi, 2016 ³³ | <i>Ctgf</i> | Decreased expression | Promoter DNA-hypermethylation |
| Pan, 2018 ³⁴ | <i>Ptgis</i> | Decreased expression | Promoter DNA-hypermethylation |
| Wu, 2017 ³⁵ | <i>Sept9</i> | Decreased expression | Promoter DNA-hypermethylation |
| Asada, 2016 ³⁶ | <i>Agtr1a</i> | Increased expression | Promoter DNA-hypermethylation |

Acetylation and methylation of histones are well-investigated post-translational modifications. Two enzyme families regulate histone acetylation: histone acetyltransferases, which attach an acyl group and thus open access to transcription factors to genes, and histone deacetylases (HDACs), which detach it and, accordingly, close access to genes.³⁸ An imbalance in the activity of these enzymes affects gene expression in NAFLD, which contributes to impaired liver metabolism.³⁹ p300 histone acetyltransferase, a transcription regulator, is involved in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent inflammatory pathways. Its participation in NAFLD pathogenesis involves activating glycolytic and lipogenic genes through histone acetylation.⁴⁰ In addition, p300 histone acetyltransferase induces TGF-β1-stimulated HSCs activation by both canonical (histone acetylation) and non-canonical (cytoplasm-to-nucleus shuttle for SMAD2/3 and TAZ) mechanisms.⁴¹ Eighteen HDACs are categorized into four classes based on structure and mechanism of action. The class I HDACs include HDAC 1, -2, -3 and -8. The class II HDACs are subdivided into class IIa (HDAC4, -5, -7, -9) and class IIb (HDAC 6, -10). The class III HDACs form a unique group known as sirtuins (SIRT1-7) and possess distinctive properties, thus considered separately from the “classic” HDACs. The class IV HDACs are represented by a single protein -HDAC 11.⁴² During HSCs transdifferentiation, changes in the expression of these HDACs may occur, mainly due to temporal differences in HDACs gene expression. In a study by Mannaerts *et al.*,⁴³ HDAC 1, -2 were detected in quiescent HSCs, with their protein expression decreasing in activated HSCs. In contrast, HDAC 3 maintained constant levels, while HDAC 8 was induced upon HSCs transdifferentiation. No significant changes in HDAC1, -2, -3 expression were observed during HSCs transdifferentiation, while HDAC4 expression significantly increased.⁴⁴ HDAC4 contributes to HSCs activation and promotes hepatic fibrogenesis by differentially regulating the expression of multiple microRNAs (miRNAs), thereby simultaneously amplifying a number of pro-fibrotic pathways.⁴⁵ Class IIa HDACs are overexpressed in activated HSCs.⁴⁶ The class III HDACs (sirtuins) are involved in NAFLD pathogenesis due to their involvement in lipid metabolism, oxidative stress and insulin resistance.⁴⁷

Histone H3 lysine 27 (H3K27) is an extensively studied histone modification with a complex biological role. The H3K27 acetylation is essential in hepatic fibrogenesis by elevating the expression of *Colla1* and *Colla2* genes through the engagement of epidermal

growth factor receptor and TGF-β signaling pathways.⁴⁸ Histone H4 is one of the main histone proteins participating in the chromatin structure in eukaryotic cells. The acetylation of histone H4 at the antifibrogenic factor *Pparg* gene was impaired in rats with a model of CCl4-induced liver cirrhosis.⁴⁹

Histone methylation/demethylation also plays an important regulatory role in NAFLD. Methylation of lysine and arginine residues of histone N-terminal tails induces chromatin silencing and transcription inactivation. However, in specific scenarios, histone methylation can activate gene transcription and initiate chromatin remodeling.⁵⁰ Histone methylation is regulated by the action of proteins with opposite properties-histone methyltransferases and histone demethylases.⁵¹ Similar to the HDACs, these enzymes can regulate gene expression during the fibrogenic transdifferentiation of quiescent HSCs, and participate in hepatic fibrogenesis. Histone H3-lysine 36 methyltransferase absent, small, or homeotic discs 1 (ASH1) binds to regulatory regions of genes like smooth muscle α-actin (*α-SMA*), type I collagen [α 1(I) collagen] (*Col1a1*), *Timp1* and *Tgb1* during HSCs activation. ASH1 depletion contributes to the decrease in fibrogenic gene expression.⁵² H3K27 methyltransferase enhancer of zeste homolog 2 (EZH2) is well represented in activated HSCs, and its expression is directly associated with LF severity. It is one of the primary epigenetic regulators of HSCs fibrogenic activation.⁵³ EZH2 upregulation in activated HSCs is associated with H3K27 methylation in the exons of the *Pparg* gene, resulting in reduced transcription.⁵⁴ The histone lysine demethylase 3A activates HSCs, promoting hepatic fibrogenesis through H3K9me2 demethylation at *Pparg* promoter, thereby positively regulating its expression.⁵⁵ Moreover, the upregulation of *Kdm4d* in activated HSCs induces hepatic fibrogenesis by influencing the signaling pathway of Toll-like receptor 4 in the CCl4-treated mouse model, while *Kdm4d* knockdown decelerates LF progression.⁵⁶

Genes involved in histone modifications during HSCs fibrogenic activation are presented in Table 2.^{44,52,55-59}

Crosstalk between DNA methylation and histone modifications

Chromatin remodeling coordinates gene expression under the control of epigenetic modifications. The crosstalk between DNA methylation and post-translational histone modifications is a functional regulatory mechanism affecting the structure of chromatin. DNA modifications provide binding sites for most transcriptional

Table 2. Genes involved in histone modifications during hepatic stellate cells fibrogenic activation

| First author, year, ref. | Target genes | Expression | Regulatory mechanism |
|--------------------------------|-----------------------------|----------------------|---|
| Qin, 2010 ⁴⁴ | <i>Mmp9</i> | Decreased expression | HDAC4-mediated histone deacetylation |
| Perugorria, 2012 ⁵² | α -SMA | Increased expression | p300 histone acetyltransferases-dependent transcription |
| | <i>Col1a1, Timp1, Tgfb1</i> | Increased expression | ASH1-mediated H3K4 methylation |
| | <i>Pparg</i> | Decreased expression | G9a-mediated H3K9 methylation |
| Jiang, 2015 ⁵⁵ | <i>Kdm3a</i> | Increased expression | JMJD1A-mediated H3K9 demethylation |
| Dong, 2019 ⁵⁶ | <i>Kdm4d</i> | Increased expression | KDM4D-mediated H3K9 demethylation |
| Dou, 2018 ⁵⁷ | α -SMA | Increased expression | p300 histone acetyltransferases-dependent transcription |
| Page, 2015 ⁵⁸ | <i>Elastin</i> | Increased expression | Mixed lineage leukemia 1-mediated H3K4 methylation |
| Pannem, 2014 ⁵⁹ | <i>Hgf</i> | Decreased expression | HDAC7-mediated histone deacetylation |

ASH1, Absent, small, or homeotic discs 1; H3K4, histone H3 lysine 4; H3K9, histone H3 lysine 9; HDAC, histone deacetylase; JMJD1A, Jumonji Domain-Containing 1A; KDM4D, lysine demethylase 4D.

regulatory proteins involved in gene expression.⁶⁰

MECP2, a protein with a specific affinity for methylated DNA, plays a crucial role in regulating transcription and chromatin organization.⁶¹ It is essential for the coordinated epigenetic regulation of HSCs transdifferentiation and hepatic fibrogenesis. Through the histone-lysine-N-methyltransferases EZH2 and ASH1, MeCP2 can regulate the fibrogenic transdifferentiation of quiescent HSCs. While EZH2 is recruited to the *Pparg* gene, contributing to HSCs fibrogenic activation, ASH1 is simultaneously recruited to genes such as α -SMA, *Col1a1*, *Timp1* and *Tgfb1*, inducing an active transcriptional state.⁵⁴ MeCP2 has a favorable effect on ASH1 expression, which makes it possible to identify ASH1 as an important component within the transcriptional activator of the MeCP2 epigenetic relay pathway.⁵² Other epigenetic complexes are also involved in crosstalk during HSCs transdifferentiation. In particular, the interaction between the histone H3 lysine 9 (H3K9) methyltransferase G9a and DNMT1 may suppress *Pparg* gene expression during TGF- β -mediated HSCs activation.⁶² Thus, the crosstalk among DNA methylation, histone modifications, and epigenetic enzymes contributes to both stimulation and suppression of transcription, depending on specific molecular interactions in addition to the promoters of target genes.

Non-coding RNAs

The epigenetic landscape in LF is also influenced by numerous non-coding RNAs (ncRNAs). They have a profibrotic effect by regulating genes in various signaling pathways involved in HSCs activation.⁶³ ncRNAs are a group of transcripts that do not undergo protein translation. They participate in the regulation of gene expression, including chromatin remodeling, transcriptional and post-transcriptional processes, and gene methylation. ncRNAs are classified into small ncRNAs and long ncRNAs (lncRNAs), depending on the length of the nucleotide chain. Small ncRNAs demonstrate considerable heterogeneity and comprise various types, including microRNAs (miRNAs), transfer RNAs, small nucleolar RNAs, short interfering RNAs, ribosomal RNAs, and small RNAs interacting with PIWI proteins. The most extensively studied ncRNAs are representatives of the miRNAs and long ncRNA (lncRNA) families.⁶⁴

miRNAs are short, highly conserved single-stranded endogenous ncRNAs, with a length of 18 to 24 nucleotides. They are transcribed by RNA polymerase II and III, generating precursors that undergo

cleavage to form mature miRNAs.⁶⁵ miRNAs act as gene repressors by binding to a complementary site of the 3'- or 5'-untranslated region of the target mRNAs, leading to mRNA degradation or inhibition of its translation into a protein.⁶⁶ lncRNAs are a large group of ncRNAs, containing more than 200 nucleotides, with limited or no ability to encode proteins. Circular RNAs (circRNAs) represent a form of competitive endogenous single-stranded ncRNAs with a closed structure, which participate in the regulation of transcriptional and post-transcriptional gene expression. CircRNAs can serve as RNA-binding proteins, miRNA sponges, and participate in the regulation of transcription, translation, and splicing processes.⁶³ A large number of ncRNAs are found in the liver, exhibiting altered expression profiles in various diseases, including NAFLD. Moreover, the expression patterns of ncRNAs may vary among different morphological forms of NAFLD.⁶⁷

MicroRNAs

miRNAs carry out post-transcriptional regulation by controlling the gene expression of other RNAs, particularly mRNAs, either by promoting mRNA degradation or suppressing their translation. miRNAs are the primary regulators influencing the expression of numerous coding and non-coding genes.⁶⁸ Currently, accumulating evidence indicates that dysregulation in miRNAs gene expression is associated with pathological processes in different morphological forms of NAFLD.⁶⁹ miRNAs are involved in insulin resistance, adipocyte differentiation, lipid and glucose metabolism, as well as immune response. The disruption of these mechanisms in NAFLD leads to changes in miRNA expression, contributing to disease progression.⁷⁰ Extensive evidence supports the involvement of miRNAs in hepatic fibrogenesis, highlighting the isolation of both profibrotic and antifibrotic liver miRNAs.⁷¹ Besides, specific miRNAs have been identified for their modulatory effects on HSCs activation in hepatic fibrogenesis.⁷² Notably, in this pathophysiological situation, some miRNAs can be upregulated, while other miRNAs are downregulated.⁷³ In addition, integrative gene expression and miRNA profiling have been reported in quiescent human HSCs.⁷⁴

miR-21

There is a lot of evidence indicating the involvement of miR-21 in NAFLD pathogenesis.⁷⁵ miR-21 deficiency diminishes the expression of genes affecting lipogenesis and cell cycle transition via the p53 pathway.⁷⁶ Hepatic miR-21 overexpression has been

observed in both NASH patients and mice with NASH caused by a diet deficient in methionine and choline. A decreased hepatic level of miR-21 can restore PPAR α expression, contributing to a favorable outcome in NASH.⁷⁷ Simultaneously, PPAR α inhibition caused by miR-21 contributes to the development of NASH by inducing liver inflammation, fibrosis and steatosis.⁷⁸ miR-21 up-regulation can cause hepatic fibrogenesis in NASH through the inhibition of SMAD7.⁷⁹ TGF- β promotes miR-21 overexpression, which acquires profibrotic properties by suppressing the TGF- β inhibitory SMAD7 protein.⁸⁰ miR-21 can activate HSCs by binding to specific transcripts, particularly *PDCD4*, *SMAD7* and *PTEN*.⁸¹ Additionally, miR-21 interacting with 3'-UTR *Spry2* and *Hnf4a* leads to enhanced ERK1 signaling in HSCs.⁸²

miR-103-3p

miR-103-3p plays a crucial role in NAFLD pathogenesis.⁸³ Hepatic miR-103-3p overexpression was observed in mouse models of NAFLD. miR-103-3p inhibition improved pathophysiological disorders and liver morphology in NAFLD.⁸³ miR-103-3p expression correlated with histological activity of steatosis and LF severity in patients with NAFLD.⁸⁴ miR-103-3p can promote the activation and proliferation of HSCs by suppressing Kruppel-like factor 4 suppression.⁸⁵

miR-125b

The role of miR-125b in NAFLD pathogenesis has not been definitively established. High upregulation of miR-125b was detected in animal models of NAFLD. miR-125b induces an inflammatory response in NAFLD via the NF- κ B signaling pathway by directly targeting tumor necrosis factor- α -induced protein 3.⁸⁶ miR-125b-2 knockout in mice increased high-fat diet-induced insulin resistance and fat accumulation.⁸⁷ miR-125b upregulation in HSCs was demonstrated in hepatic fibrogenesis, which promoted α -SMA and type I collagen expression and HSCs contractility.⁸⁸

miRNA-181a

miR-181a is involved in lipidosis and promotes lipid peroxidation in NAFLD. miR-181a can also influence NAFLD pathogenesis and progression by inhibiting SIRT1 expression.⁸⁹ Besides, miR-181a might act as a positive regulator of TGF- β -induced LF.⁹⁰

miR-200a

miR-200a-3p promotes the development of liver steatosis induced by free fatty acid *in vitro*.⁷² Upregulation of miR-200a is associated with hepatic fibrogenesis in NAFLD.⁹¹ It is also established that miR-200a is a key regulator in HSCs fibrogenic activation, particularly via the SIRT1/Notch1 signaling pathway.⁹²

miR-221/222

Hepatic miR-221/222 overexpression was detected in NASH, which contributed to the progression of liver inflammation, fibrosis and steatosis.⁹³ In addition, the expression levels of miR-221/222 increased with LF progression and correlated with the mRNA expression levels of COL1A1 and α -SMA.⁹⁴ miR-221/222 promotes LF by activating profibrotic signaling pathways of TGF- β and NF- κ B.⁹⁵ miR-221 also regulates some targets involved in hepatic fibrogenesis, including E-cadherin, cyclin-dependent kinase inhibitors, cytokine signaling 1, PTEN, and Bcl-2 modifying factor.⁹⁶

Long non-coding RNAs

lncRNAs participate in many complex cellular processes, such as cell growth and differentiation, cell cycle control, maintenance

of cellular structure integrity, intracellular transport, apoptosis and cell death. They are also involved in basic processes, including transcription, splicing, translation and epigenetic regulation. lncRNAs exert their effects at both the transcriptional and post-transcriptional levels. At the transcriptional level, lncRNAs recruit transcription factors or epigenetic modification complexes. At the post-transcriptional level, lncRNAs can regulate mRNA translation, and modulate alternative splicing and mRNA degradation.⁹⁷ Although information regarding the role of lncRNAs in NAFLD pathogenesis is limited, recent studies have indicated their involvement in all stages of NAFLD, including the development of LF and cirrhosis. Based on their influence mechanisms on NAFLD, lncRNAs are categorized into those inducing only liver steatosis, those involved in both steatosis and inflammation and/or LF, and those exclusively impacting hepatic fibrogenesis.⁹⁸ Furthermore, lncRNAs can also regulate HSCs fibrogenic activation.⁹⁹

Metastasis-associated lung adenocarcinoma transcript 1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is located on human chromosome 11q13.1 and is involved in various physiological processes, including alternative splicing, epigenetic modification of gene expression, synapse formation and myogenesis.¹⁰⁰ MALAT1 is also involved in insulin resistance and liver steatosis due to the overexpression of SREBP-1c and target genes in liver.¹⁰¹ Moreover, MALAT1 induces hepatic lipogenesis in NAFLD by modulating the miR-206/ARNT axis.¹⁰² MALAT1 overexpression has been observed in activated HSCs, suggesting a potential role in hepatic fibrogenesis through mechanisms associated with the inflammatory chemokine CXCL5.¹⁰³ Simultaneously, MALAT1 suppression led to a decrease in HSCs activation, α -SMA and COL1A1 levels, and inhibited collagen accumulation in liver tissue.¹⁰⁴ Exosomal MALAT1 derived from liver cells has also been shown to participate in HSCs activation via miR-26b in the arsenite-induced LF mouse model.¹⁰⁵ In addition, the MALAT1/miR-181a-5p axis contributes to HSCs activation by transmitting TLR4/NF- κ B signals, thereby regulating collagen production and promoting LF *in vitro*.¹⁰⁶ These findings indicate that MALAT1 can induce hepatic lipogenesis, cause hepatic inflammation, and also promote hepatic fibrogenesis by directly affecting HSCs.

Nuclear enriched abundant transcript 1

In recent years, the role of nuclear enriched abundant transcript 1 (NEAT1) in various liver diseases has garnered increasing attention.¹⁰⁷ NEAT1 overexpression has been observed in animal models of NAFLD, while its suppression beneficially effects NAFLD through the mTOR/S6K1 signaling pathway.¹⁰⁸ NEAT1 promoted liver steatosis by enhancing estrogen receptor alpha-mediated aquaporin-7 expression upon treatment with 17 β -estradiol (E2) and oleic acids in HepG2 cells.¹⁰⁹ NEAT1 contributed to NAFLD by targeting miR-146a-5p, which in turn regulates ROCK1, and additionally influences the AMPK/SREBP pathway.¹¹⁰ NEAT1 was involved in hepatic fibrogenesis and inflammatory response in NAFLD by influencing the miR-506/GLI3 axis.¹¹¹ NEAT1 also activated HSCs by influencing the miR-129-5p/PEG3 axis in mouse model of NASH.¹¹² Inhibition of NEAT1 expression improved experimental LF, while its stimulation led to HSCs activation by influencing the miR-122-KLF6 axis, thereby contributing to their proliferation and collagen production.¹¹³ The involvement of the NEAT1/miR-29b/Atg9a regulatory axis in autophagy and HSCs activation was discovered in an adenovirus-mediated IGFBP1-induced LF mouse model.¹¹⁴ NEAT1 overexpression was also detected in fibrous liver

tissues of CCl4-treated mice. Knockdown of NEAT1 attenuated LF and inhibited HSCs activation by sponging miR-148a-3p and miR-22-3p, thereby regulating Cyth3 expression.¹¹⁵

Plasmacytoma variant translocation 1

Plasmacytoma variant translocation 1 (PVT1) is a lncRNA related to cell invasion, proliferation, and metastasis.¹¹⁶ PVT1 is abundantly expressed in adipose tissue in a mouse model of obesity compared to the non-obese mice.¹¹⁷ In addition, increased PVT1 level has been observed in NAFLD patients.¹¹⁸ Increased PVT1 expression was also found in activated HSCs and fibrous liver tissues, whereas its suppression reduced collagen deposition. PVT1 promotes HSCs activation through the Hedgehog (Hh) signaling pathway and stimulates epithelial-mesenchymal transition.¹¹⁹

HOX transcript antisense intergenic RNA

HOX transcript antisense intergenic RNA (HOTAIR) is a well-studied lncRNA, known for its essential role in oncogenesis.¹²⁰ HOTAIR overexpression was found in animal models of NAFLD, whereas its suppression significantly reduced NAFLD progression via the miR-130b-3p/ROCK1 axis.¹²¹ The HOTAIR level was significantly increased in mice with CCl4-induced LF, in humans with LF, and in activated HSCs following stimulation with TGF-β1.¹²² It has been demonstrated that HOTAIR enhances PTEN methylation by suppressing miR-29b expression, which leads to HSCs activation and LF progression.¹²³

Alu-mediated transcriptional regulator

lncRNA Alu-mediated p21 transcriptional regulator (APTR) is involved in cell proliferation.¹²⁴ APTR was overexpressed in fibrous liver tissues and activated HSCs. Inhibition of APTR suppressed HSCs activation and reduced collagen accumulation in mice with CCl4-induced LF, and also abrogated TGF-β1-induced α-SMA upregulation in activated HSCs.¹²⁵

LF-associated lncRNA1

LF-associated lncRNA1 promotes hepatic fibrogenesis and HSCs activation by activating the TGF-β and Notch signaling pathways. Conversely, suppressing LF-associated lncRNA1 inhibits HSCs activation, reduces TGF-β-induced hepatocyte apoptosis, and improves LF.¹²⁶

Taurine upregulated gene 1

lncRNA taurine upregulated gene 1, also known as LIN00080 or TI-227H, regulates gene expression at the transcriptional and post-transcriptional levels by interacting with miRNAs or proteins. lncRNA TAG 1 expression was increased in fibrous liver tissues and activated HSCs, but not in injured hepatocytes. In addition, lncRNA taurine upregulated gene 1 induces the expression of profibrogenic genes such as α-SMA, COL1A1, TIMP1 and MMP2/9/10 by suppressing miR-29b, thereby accelerating LF progression.¹²⁷

Circular RNAs

CircRNAs include transcripts wherein the 5' and 3' ends of the molecule form a ring structure connected by a phosphodiester bond, thus rendering them resistant to exonuclease-mediated degradation. They originate from precursor RNAs through backsplicing.¹²⁸ CircRNAs are associated with the fundamental processes underlying the occurrence and progression of NAFLD, such as steatosis, autophagy, fibrosis, inflammation, and oxidative stress. Notably, studies on mice with CCl4-induced LF have predominantly

explored the association between circRNAs and HSCs fibrogenic activation.¹²⁹ It has been demonstrated that some circRNAs contribute to HSCs activation by upregulating the expressions of the fibrogenic marker gene in response to the various pro-fibrotic signaling pathways, like TGF-β, JAK/STAT, PI3K/Akt, Hedgehog (Hh), etc.¹³⁰

CircPWWP2A increases the expression level of follistatin-like receptor 1 and Toll-like receptor 4 by suppressing miR-203 and miR-223, respectively, thereby activating the TGF-β signaling pathway.¹³¹ *circUBE2K* overexpression promotes LF by sponging miR-149-5p/TGF-β2 axis, whereas its suppression disrupts TGF-β signaling and inhibits HSCs fibrogenic activation.¹³² *CircTBD1* acts as a miR-146a-5p sponge and promotes the production of proinflammatory cytokines in activated human HSCs line LX-2 via the Toll-like receptor 4 signaling pathway.¹³³ *CircIFT80* (CircBase ID: hsa_circ_0067835) is involved in LF pathogenesis by acting as a miR-155 sponge, promoting FOXO3a expression.¹³⁴ *CircRSF1* participates in LF pathogenesis by acting as a miR-146a-5p sponge. It promotes profibrotic and pro-inflammatory phenotypes of irradiated human HSCs line LX2 by modulating miR-146a-5p.¹³⁵ *CircARID1A* (CircBase identifier: hsa_circ_0008494) overexpression was detected in fibrous liver tissues and HSCs cytoplasm. A study by Li *et al.*¹³⁶ revealed the influence of the circ_0008494/miR-185 axis on the proliferation, migration, apoptosis of HSCs, as well as the activation of HSCs via the *Colla1* gene. *CircPALLD* (CircBase identifier: hsa_circ_0071410) is capable of activating HSCs and improving cell survivability by interacting with miR-9-5p targeting annexin A2.¹³⁷ *CircASPH* was upregulated in LF, and downregulation of circASPH suppressed HSCs activation and hepatic fibrogenesis via the circASPH/miR-139-5p/Notch1 axis.¹³⁸

ncRNAs that regulate genes in signaling pathways involved in histone modifications during HSCs fibrogenic activation are presented in Table 3.^{80,82,88,92,106,112,119,126,127,133-135,139-146}

Epigenetic mechanisms of HSCs fibrogenic activation as a new therapeutic target for LF

Current guidelines for the management of NAFLD recommend lifestyle changes, normalization of body weight, specific pharmacotherapy for LF, and treatment of metabolic syndrome-related diseases.¹⁴⁷ Epigenetic mechanisms underlying HSCs fibrogenic activation may be a new therapeutic target for LF.¹⁴⁸

DNA methylation inhibitors may ameliorate LF by upregulating the expression of genes, whose expression in HSCs decreases due to hypermethylation.¹⁴⁹ For example, curcumin reversed LF in a CCl4-treated mouse model and inhibited HSCs activation through downregulation of *Dnmt1*, α-SMA, and *Colla1*, and by demethylation of the key genes.¹⁵⁰

Various histone modifications present potential therapeutic targets for LF treatment.⁴⁸ In particular, chronic administration of valproic acid, a class I and IIa HDAC inhibitor, in a mouse model of CCl4-induced LF, enhanced histone H4 acetylation, resulting in reduced collagen deposition and inhibited HSCs activation. In addition, it has been suggested that valproic acid can prevent further progression of LF.⁴³ Suberoylanilide hydroxamic acid, another HDAC inhibitor, alleviated LF in CCl4-treated rat model by suppressing TGF-β1/Smad signaling pathway, which is a pivotal for HSCs activation.¹⁵¹ Suppression of H3K27 methyltransferase EZH2 by 3-Deazaneplanocin A weakens the TGF-β1/Smad signaling, inhibiting HSCs activation, reducing the accumulation of extracellular matrix in the liver and ultimately at-

Table 3. ncRNAs regulating genes in signaling pathways involved in hepatic stellate cells fibrogenic activation

| First author, year, ref. | ncRNAs | Target genes | Signaling pathways |
|----------------------------|------------------------|--|--|
| Noetel, 2012 ⁸⁰ | miR-21 | <i>Smad2/3/7</i> | TGF- β /Smad |
| Zhao, 2014 ⁸² | miR-21 | <i>Spry2, Hnf4α</i> | ERK1 |
| You, 2018 ⁸⁸ | miR-125b | <i>Stard13</i> | RhoA/Mrtf-A/Srf |
| Yang, 2017 ⁹² | miR-200a | <i>Sirt1</i> | SIRT1/Notch1 |
| Wang, 2021 ¹⁰⁶ | MALAT1/miR-181a-5p | <i>α-SMA, COL1, TIMP1</i> | TLR4/NF- κ B |
| Zhang, 2021 ¹¹² | NEAT1/miR-129-5p | <i>Peg3</i> | NF- κ B |
| Zheng, 2016 ¹¹⁹ | PVT1/miR-152 | <i>Ptch1</i> | Hedgehog |
| Zhang, 2017 ¹²⁶ | lnc-LFAR1 | <i>Smad2/3</i> | TGF- β , Notch |
| Han, 2018 ¹²⁷ | TUG1/miR-29b | <i>α-SMA, Col1a1, Mmp2/9/10, Timp1</i> | NF- κ B, JAK/STAT |
| Niu, 2020 ¹³³ | circTUBD1/ miR-146a-5p | <i>Tlr4, Irak1, Traf6</i> | TLR4/NF- κ B |
| Zhu, 2018 ¹³⁴ | circIFT80/miR-155 | <i>Foxo3a</i> | AKT/FOXO3a |
| Chen, 2020 ¹³⁵ | circRSF1/miR-146a-5p | <i>Rac1</i> | NF- κ B, JNK |
| Lv, 2023 ¹³⁹ | miR-20b-5p | <i>Stat3</i> | STAT3 |
| Ning, 2017 ¹⁴⁰ | miR-21 | <i>Spry1, Smad7</i> | Spry1/ERK/NF- κ B, Smad7/Smad2/3/NOX4 |
| Sun, 2021 ¹⁴¹ | miR-21 | <i>Vegf</i> | HIF-1 α /VEGF |
| Yang, 2020 ¹⁴² | miR-199a-3p | <i>Cav2</i> | TGF- β /Smad |
| Zhou, 2021 ¹⁴³ | miR-497 | <i>Smad7</i> | TGF- β /Smad |
| Luo, 2021 ¹⁴⁴ | miR-1297 | <i>Pten</i> | PI3K/AKT |
| Wang, 2021 ¹⁴⁵ | NEAT1/miR-139-5p | <i>β-catenin</i> | β -catenin/SOX9/TGF- β 1 |
| Su, 2022 ¹⁴⁶ | GASS5/miR-433-3p | <i>TLR10</i> | NF- κ B |

AKT, protein kinase B; ERK, extracellular-signal-regulated kinase; GASS5, growth arrest-specific transcript 5; HIF, Hypoxia-Inducible Factor; HIF-1 α , hypoxia-induced factor-1 α ; IFT80, Intraflagellar Transport 80; JAK/STAT, Janus kinase/Signal Transducer and Activator of Transcription; JNK, c-Jun NH2-terminal kinase; lnc-LFAR1, liver fibrosis-associated lncRNA 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; miR, micro RNA; ncRNAs, non-coding RNAs; NEAT1, nuclear enriched abundant transcript 1; NF, Nuclear Factor; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX4, NADPH oxidase 4; PI3K, phosphoinositide 3-kinase; PVT1, plasmacytoma variant translocation 1; RSF1, Repressor splicing factor 1; SOX9, SRY-box transcription factor 9; TGF, transforming growth factor; TLR4, Toll-like receptor 4; TUBD1, Tubulin Delta 1; TUG1, Taurine Upregulated Gene 1; VEGF, vascular endothelial growth factor.

tenuating LF.⁵³

Antifibrotic therapy targeting ncRNAs involves various signaling pathways. It is based on the gene-suppressing effect of miRNAs and the sponging action of lncRNAs and circRNAs on miRNAs.⁶³ For example, treatment with human umbilical cord mesenchymal stem cells inhibited HSCs activation, protected hepatocytes, and improved LF by up-regulating miR-148-5p expression and attenuating the Notch signaling pathway.¹⁵² miR-150, a typical antifibrotic microRNA, suppresses HSCs activation and prevents collagen types I and IV synthesis by activating HSCs via attenuation of the TGF- β signaling pathway.¹⁵³ The synthetic miR-223 analog miR-223-3p considerably slowed the development of LF, inhibited HSCs and caspase-1 p10 activation, as well as NLRP3 inflammasome activation in a mouse model of NASH.¹⁵⁴ In addition, administration of miR-223 ameliorated LF in a CCl4-treated mouse model, and miR-223 overexpression downregulated the expression of *Gli2* and platelet-derived growth factor receptor α/β (*Pdgfra/β*) genes in HSCs, thus inhibiting HSCs activation and proliferation.¹⁵⁵ Exosomes derived from natural killer cells mitigated HSCs activation by miR-223 transfer.¹⁵⁶ miR-338-3p overexpression suppressed HSCs activation and proliferation via the miR-338-3p/CDK4 signaling pathway.¹⁵⁷ miR-690 directly inhibited HSCs fibrogenic activation, mitigated inflammation in recruited hepatic macrophages, and reduced *de novo* lipogenesis in hepatocytes in a mouse model of NASH.¹⁵⁸ In a CCl4-treated mouse model, 3D human embryonic stem cell exosomes enriched with miR-6766-3p

ameliorated LF by weakening activated HSCs via the TGF β RII-SMADS signaling pathway.¹⁵⁹ microRNA-23b/27b/24-1 overexpression through intravenous delivery of miR-23b/27b/24-1 lentivirus improved LF in a CCl4-treated mouse model. Increasing the miR-23b/27b/24-1 cluster level inhibited HSCs activation by directly targeting the mRNAs of five profibrotic genes, namely, *Tgfb2*, *Gremlin 1*, *LOX*, *Itga2* and *Itga5*.¹⁶⁰ Bone marrow mesenchymal stem cells inhibited LF by downregulating the lnc-BI-HAA1/rno-miR-667-5p signaling pathway in HSCs.¹⁶¹ CircDIDDO1 transfer mediated by exosomes isolated from mesenchymal stem cells inhibited HSCs activation via the miR-141-3p/PTEN/AKT signaling pathway.¹⁶²

Conclusions

The presence and severity of LF are considered crucial factors determining NAFLD prognosis. HSCs are the precursors for the majority of profibrogenic myofibroblasts, which produce extracellular matrix in NAFLD. A number of factors underlying NAFLD pathogenesis may promote HSCs activation. One of them is the epigenetic regulatory mechanism, including DNA methylation, covalent histone modification, chromatin remodeling, and ncRNAs. Since these mechanisms are reversible and dynamic, targeted molecular therapies that aim to correct them present promising avenues for novel therapeutic approaches in managing LF associated with NAFLD.

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Conflict of interest

There are no conflicts relevant to this work.

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