Acta Pharmaceutica Sinica B 2024;14(3):1009−1029

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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REVIEW

# Epigenetic modification in liver fibrosis: Promising therapeutic direction with significant challenges ahead

**Runping Liu[a,†], Yajing Li[b,†], Qi Zheng[a], Mingning Ding[b], Huiping Zhou[c,*], Xiaojiaoyang Li[b,*]**

[a]*School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 102400, China*
[b]*School of Life Sciences, Beijing University of Chinese Medicine, Beijing 102400, China*
[c]*Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 22460, USA*

Received 1 July 2023; received in revised form 18 August 2023; accepted 13 September 2023

**KEY WORDS**

Liver fibrosis;
Epigenetics regulation;
DNA methylation;
Histone acetylation;
Histone methylation;
mRNA methylation;
Non-coding RNA;
Drug development

**Abstract** Liver fibrosis, characterized by scar tissue formation, can ultimately result in liver failure. It's a major cause of morbidity and mortality globally, often associated with chronic liver diseases like hepatitis or alcoholic and non-alcoholic fatty liver diseases. However, current treatment options are limited, highlighting the urgent need for the development of new therapies. As a reversible regulatory mechanism, epigenetic modification is implicated in many biological processes, including liver fibrosis. Exploring the epigenetic mechanisms involved in liver fibrosis could provide valuable insights into developing new treatments for chronic liver diseases, although the current evidence is still controversial. This review provides a comprehensive summary of the regulatory mechanisms and critical targets of epigenetic modifications, including DNA methylation, histone modification, and RNA modification, in liver fibrotic diseases. The potential cooperation of different epigenetic modifications in promoting fibrogenesis was also highlighted. Finally, available agonists or inhibitors regulating these epigenetic mechanisms and their potential application in preventing liver fibrosis were discussed. In summary, elucidating specific druggable epigenetic targets and developing more selective and specific candidate medicines may represent a promising approach with bright prospects for the treatment of chronic liver diseases.



*Corresponding authors.
E-mail addresses: xiaojiaoyang.li@bucm.edu.cn (Xiaojiaoyang Li), huiping.zhou@vcuheath.org (Huiping Zhou).
†These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2023.10.023

## 1. Epigenetics: A potential target regulating liver fibrosis

Epigenetics refers to the study of changes in gene expression or cellular phenotype that occur without alterations to the underlying DNA sequence. It encompasses different molecular mechanisms that can modify the activity of genome. These modifications can regulate the accessibility of DNA to transcriptional machinery and, therefore can have significant effects on gene expression[1]. The role and mechanisms of epigenetics in disease biology are currently understood to operate at multiple levels, including DNA methylation, histone modifications, chromatin remodeling, and regulation by non-coding RNA (ncRNA), leading to variable expression of critical pathogenesis-related gene sets (Fig. 1). Hence, epigenetics has significant implications for understanding human diseases and has been studied as a promising target for therapeutic interventions.

Liver fibrosis, characterized by the progressive deposition of extracellular matrix (ECM) and activation of hepatic stellate cells (HSCs). Given the increasing prevalence of liver diseases and the lack of treatment options, it remains a significant challenge to address liver fibrosis[2]. Liver fibrosis often occurs and starts the cicatrization process following environmental insults, but fundamentally, it is considered as an imbalance between the production and degradation of ECM[3]. Under the stimulation of external damage signals in the initial inflammation phase of fibrogenesis, hepatocytes undergo injury and subsequently trigger myofibroblasts to generate excess ECM. Meanwhile, myofibroblasts, epithelial and endothelial cells also produce matrix metalloproteinases (MMPs), which contribute to ECM degradation. In the subsequent remodeling phase, $\alpha$-smooth muscle actin ($\alpha$-SMA)-positive myofibroblasts continually release cytokines to achieve self-activation, leading to uncontrolled ECM deposition and scar formation[4].

With genetic factors hardly explaining the pathogenesis of liver fibrosis and suffering the steady rise of cases over decades, increasing evidence has focused on the molecular mechanisms of extensive epigenetics remodeling in the profibrotic phenotype of various liver cells. Taking the above opinions into consideration, in the current review, we emphasize the interaction between epigenetic modification and liver fibrotic diseases and evaluate the potential of abnormal epigenetics as early diagnostic markers and therapeutic targets for liver fibrosis.

## 2. The modulation of DNA methylation in liver fibrosis

DNA methylation plays a crucial role in regulating gene expression by influencing the accessibility of DNA by transcription factors. It is catalyzed by DNA methyltransferases (DNMTs), which is responsible for 5-methylcytosine (5-mC) formation in the cytosine-guanosine dinucleotide (CpG) sites of the genome. Depending on the locations, DNA methylation manipulates gene silence and activation by recruiting certain proteins or regulating the binding of transcription factors to target genes[5]. Specifically, methylation in the transcription start point or promoters results in gene silence, while the gene-body methylation is associated with gene expression and alternative splicing. As major methylation inducer, DNMTs, including DNMT1, DNMT3a, DNMT3b and DNMT3L, are responsible for transferring methyl group from *S*-adenosyl-L-methionine (SAM) to the 5-position of DNA cytosine. Among DNMTs, DNMT1 mediated maintenance methylation while

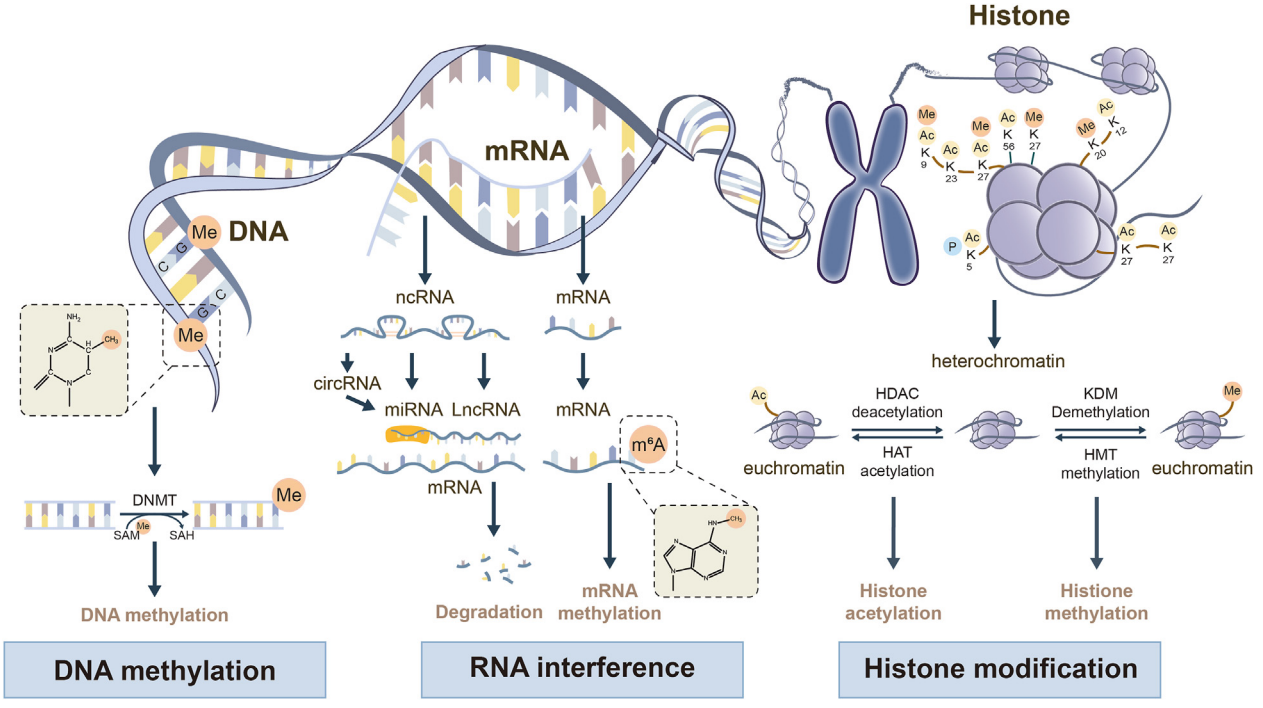


**Figure 1** The feature of epigenetic regulation. Epigenetic regulation involves DNA methylation, RNA modification, histone methylation and histone acetylation. DNMT family members mediate DNA methylation, which suppresses gene transcription by adding a methyl group to the 5′ position of cytosine to form 5-methylcytosine at cytosines within CpG dinucleotides. RNA modification, which includes mRNA and ncRNA, regulates gene expression by influencing the output and stability of genes in the nucleus. Histone methylation, catalyzed by HMTs and HDMTs, is involved in epigenetic remodeling in liver fibrosis by positively or negatively regulating transcriptional activation. Histone acetylation, regulated by HATs and HDACs, has also been found to be involved in liver diseases.

DNMT3a and DNMT3b are responsible for the *de novo* methylation[6]. DNMT3L does not have catalytic activity, but it enhances the DNA methylation activity of DNMT3A and DNMT3B by direct interaction with them. While a dynamic balance between methylation and demethylation was formed during the long-term transcriptional regulation. 5-mC formed in methylation can be oxidized by ten-eleven translocation (TET) proteins, including TET1/2/3. This results in the formation of 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), 5-carboxycytosine (5-caC) and 5-hydroxymethyluracil (5-hmU), initiating demethylation program that eliminates the methyl group from cytosine, ultimately reversing transcriptional suppression. Thus, the dynamic and reversible nature of DNA methylation also highlights the vital roles of DNA demethylation mediated by TET family. Emerging studies have identified that the DNA methylation pattern, including methylation and hydroxymethylation-mediated demethylation, is involved in HSC transdifferentiation during liver fibrotic diseases (Fig. 2). For instance, HSC transdifferentiation was accompanied by high densities of 5-mC and global reduction in 5-hmC, which was confirmed by comparing the numbers of 5-mC and 5hmC sites in activated-HSC and quiescent-HSC[7]. Meanwhile, when DNMT3a and DNMT3b were downregulated by siRNA, DNA methylation was reduced, and HSC activation was subsequently inhibited[7,8].

Growing evidence established that transcriptional regulation played a pivotal role in liver fibrosis[9]. Proliferator-activated receptor-$\gamma$ (PPAR$\gamma$) is one of the attractive targets and its transcriptional suppression was closely related the transactivation of HSCs and liver fibrosis[10]. Indeed, numerous attempts have been made to evaluate the clinical efficacies of PPAR$\gamma$ agonists, including troglitazone[11], rosiglitazone[12] and pioglitazone[13], for treating NASH and liver fibrosis. Caldwell and his colleagues showed that short-term troglitazone therapy led to histological improvements and discontinued the progression of fibrosis in two NASH subjects. Mechanistically, quiescent HSCs contain lipid droplets responsible for hepatic vitamin A storage, maintaining liver homeostasis under physiological conditions. As a master negative-regulator of HSC activation, PPAR$\gamma$ binds to the receptor of retinoic acid, a vitamin A metabolite, and activates the expression of several lipid biosynthesis-related genes, including sterol regulatory element binding protein 1 (*Srebp1*), *Seipin* and *Lpin*, by recognizing DNA sequence element peroxisome proliferator response element (PPRE). Upon fibrogenesis, PPAR$\gamma$ undergoes transcriptional suppression, resulting in the impaired ability to induce certain genes. As a result, there is a loss of lipid droplets and transdifferentiation of myofibroblasts in activated HSCs, which are responsible for the accumulation of ECM, leading to fibrosis[14]. Several studies further demonstrated that PPAR$\gamma$ could directly regulate fibrotic classical signaling transforming growth factor-$\beta$ (TGF-$\beta$)/Smad pathway to alleviate the generation of profibrotic myofibroblasts[15]. PPRE mutation could also promote HSC differentiation by abolishing the recruitment of PPAR$\gamma$ to the MAT2A promoter and subsequently increasing the expression cell prefoliation-related target methionine adenosyltransferase 2A (MAT2A)[16]. Indeed, plentiful CpG islands have been identified in the promoter, 5′UTR and gene body of PPAR$\gamma$, suggesting a higher possibility of epigenetic modification. Thus, transcriptional suppression of PPAR$\gamma$ may be closely related to epigenetic regulation during fibrogenesis. Human NASH liver biopsies, animal studies and *in vitro* investigations have substantiated this hypothesis and provided evidence that the promoter region of PPAR$\gamma$ undergoes methylation remodeling, transforming to a hypermethylated pattern with increasing fibrosis severity. Zeybel and his colleagues[17] identified CpG sites in human PPAR$\gamma$ promoter and further demonstrated that hypermethylation was positively correlated with liver fibrosis progression in male patients between the age of 46−65 with biopsy-proven NAFLD. Under myofibroblast phenotypic transformation, methyl CpG binding protein 2 (MeCP2), with high affinity to methylated DNA, maintained the hypermethylation (one with 109 CpG and the other with 21 CpG) of PPAR$\gamma$ promoter region and thus facilitated PPAR$\gamma$ suppression and liver fibrosis progression[18]. Recently, Moran-Salvador et al.[19] established MeCP2 deletion and MeCP2 S80A mutated mouse model and further verified that the phosphorylation of MeCP2 at S80 site is required for HSC activation and collagen expression in the $CCl_4$ mouse model. Additionally, MeCP2 upregulated myofibroblast DNA replication-related genes, including *Cdc7*, *Has2* and *Dna2*, and thus enhanced active HSC proliferation and hepatic fibrogenesis. Despite multiple studies demonstrating a clear positive correlation between PPAR$\gamma$ methylation and liver fibrosis from bench to bed, research on PPAR$\gamma$ methylation is limited by the need for access to liver biopsy to measure methylation at the PPAR$\gamma$ promoter. Thus, several groups focused on identifying plasma PPAR$\gamma$ methylation markers to establish potential non-invasive biomarkers for stratification of liver fibrosis. Hlady et al.[20] found hypermethylated PPAR$\gamma$ DNA in the plasma *via* genome-wide cell-free 5-mC landscape analysis and identified a set of differentially methylated CpGs (cg04645914, cg06215569, cg23663760, cg13781744 and cg07610777) in cell free PPAR$\gamma$ DNA by comparing plasma from cirrhosis patients and healthy controls. Notably, these high performing sites and differential CpG methylation events were also observed in different liver tissues, which distinguished cirrhosis and hepatocellular carcinoma patients from normal liver. Therefore, CpGs in cell free PPAR$\gamma$ DNA have the potential to differentiate non-cirrhosis from cirrhosis patients and may be used for the clinical non-invasive screen[20]. In addition to the diagnosis of liver diseases, differential plasma DNA methylation of PPAR$\gamma$ may be utilized as a non-invasive method to stratify the severity of liver fibrosis in NAFLD patients. Indeed, liver DNA methylation of PPAR$\gamma$ has been validated as a means to stratify patients in terms of fibrosis severity. A clinal study including 26 NAFLD patients provided further validation by demonstrating that sequence-specific quantification of methylation densities at PPAR$\gamma$ DNA in plasma correlated with the progression of fibrosis. This study also showed that hypermethylation degree at 2 positions, named CpG1 and CpG2, in PPAR$\gamma$ was similar between plasma and hepatocyte-rich liver tissue. Patients with mild live fibrosis exhibited lower levels of PPAR$\gamma$ methylation in plasma (CpG1:63%, CpG2:51%), while patients with severe fibrosis showed higher levels (CpG1:86%, CpG2:65%). Indeed, hepatocyte injury, which is a characteristic feature of NAFLD, may result in substantial leakage of cell-free DNA into the circulation. This suggests that liver contributes significantly to the pool of DNA present in plasma[21]. Taken together, DNA methylation patterns in plasma may potentially originate from injured hepatocytes and serve as a diagnostic marker for liver fibrosis. Paradoxically, another study reported that the promoter undergoes demethylation, leading to the accumulation of hepatic lipids in high-fat diet (HFD)-induced NAFLD mice, which in turn aggravates fibrosis. Specifically, this study showed that HFD increased PPAR$\gamma$ expression by decreasing cytosine methylation levels in the PPAR$\gamma$ promoter and thus activated its target genes, such as very low-density lipoprotein (VLDLR) and cluster differentiating 36 (CD36). While this study

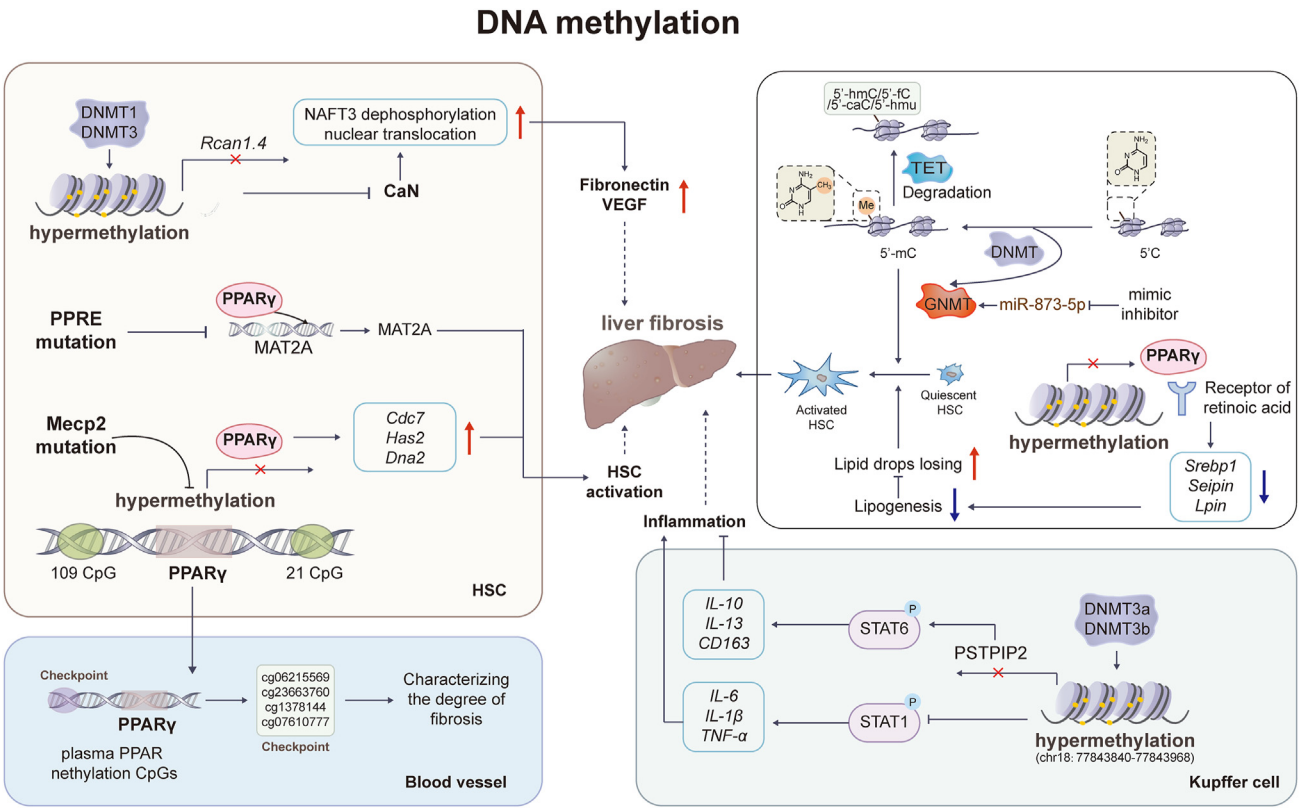


**Figure 2** The modulation of DNA methylation in liver fibrosis. Advances have been made in the understanding of the roles of DNA methylation in liver fibrosis. It has been observed that a repressive chromatin structure in exons can lead to gene transcription suppression through the addition of a methyl group at the 5′ position of cytosine. This process is mediated by the DNMT and GNMT family. Several genes, including PPARγ, RCAN1.4, and PSTPIP2, and methylated binding protein Mecp2 have been found to experience DNA methylation, and their transcriptional suppression has been shown to be closely related to the transactivation of HSCs and liver fibrosis.

is inconsistent with mainstream understanding, it still highlights the importance of PPARγ methylation in the progression of liver diseases[22].

In addition to PPARγ, another protein called Calcineurin (CaN), a calcium/calmodulin-activated serine/threonine phosphatase, has been shown to play a role in TGF-$\beta$-induced ECM accumulation. CaN can be activated by TGF-$\beta$ in a time and dose-dependent manner[23]. The most studied substrates of CaN are the family of nuclear factors of activated T-cells (NFATs). CaN induces dephosphorylation and nuclear translocation of NFAT3 and subsequently leads to the overexpression of fibrosis-related genes, including Fibronectin (Fn) and vascular endothelial growth factor (*Vegf*). Interestingly, a member of the regulator of calcineurin (RCAN) family, RCAN1.4 has been shown to improve liver fibrosis by acting as an endogenous inhibitor of CaN[24]. Specifically, RCAN1.4 was significantly suppressed at transcription level in both $CCl_4$-induced mouse model and TGF-$\beta$-activated HSCs due to the presence of two hypermethylation sites in its promoter region. In more detail, the hypermethylation of RCAN1.4 was mediated by DNMT1 and DNMT3, which was confirmed by DNMT inhibitor 5-aza-2′-deoxycytidine (5-AzaC) and DNMTs-siRNA *in vitro*. Furthermore, Pan and his colleagues[25] developed a liver-specific RCAN1.4 overexpression mouse model by injecting RCAN1.4 plasmid specifically into to the liver tissue. In this study, the authors confirmed that the induction of RCAN1.4 had a beneficial effect on liver fibrosis by inhibiting CaN-NFAT3 signaling.

Persistence of chronic inflammation often caused by activated inflammatory immune cells such as macrophages is commonly associated with the progression of liver fibrosis. M2 macrophage-derived cytokines, including IL-4, IL-10 and IL-13, are related to epigenetic modifications that contribute to fibrotic processes. Proline-serine-threonine-interacting protein 2 (PSTPIP2), mainly expressed in Kupffer cells, has been found to be significantly decreased in the $CCl_4$-induced mouse model of liver fibrosis and LPS-treated RAW264.7 cell line. Mechanically, DNMT3a and DNMT3b promote the hypermethylation of PSTPIP2 (chr18:77843840−77843968) in the 5′-UTR region and subsequently repress PSTPIP2 expression. This association was confirmed by representation bisulfite sequencing (RRBS) in primary macrophages derived from $CCl_4$-induced mice. Further investigation revealed that liver-specific PSTPIP2 overexpression alleviated inflammation and liver fibrosis by regulating the expression and secretion of cytokines including *IL-6*, *IL-1β* and *IL-10*, which is relied on the suppression of signal transducer and activator of transcription 1 (STAT1) phosphorylation. On the other hand, accompanied with STAT6 phosphorylation, the overexpression of PSTPIP2 upregulated the mRNA levels of *IL-10*, *IL-13* and *CD163*[26]. These findings highlight the complex role of PSTPIP2 in modulating cytokine expression and suggest its potential as a therapeutic target for liver inflammation and fibrosis.

In addition to DNMTs, another important enzyme involved in liver methylation processes is glycine *N*-methyltransferase (GNMT). GNMT is the most abundant SAM-dependent

methyltransferase in the liver and hepatocytes. Interestingly, GNMT act epigenetic regulatory elements by competing with DNMT to remodel transmethylation flux. Recently, the inverse correlation between hepatic GNMT and miR-873-5p was identified in cirrhotic patients, BDL mice and primary mouse hepatocytes. Fernandez-Ramos et al.[27] established that in BDL and the *Mdr2*-deficient mouse models, anti-miR-873-5p therapy ameliorates hepatocyte apoptosis, cholangiocyte proliferation and liver fibrosis by recovering GNMT expression and steering SAMe flux afar DNA.

## 3. Histone modifications in liver fibrosis

The transcriptional or replication machinery must arrive at the specific genomic region in order to function, which requires the DNA to be accessible. DNA is tightly organized into regulated structures by octameric protein complex, forming nucleosomes core particle. Each of these contains two of each core histones (H2A, H2B, H3 and H4) with 145−147 base pairs of DNAs. The N- and C-terminal histone tails are enriched with basic amino acid residues, such as lysine and arginine, which endow nucleosomes with unique properties and thus influence gene transcription and epigenetic states[28]. Histones modifications, including methylation and acetylation have the ability to influence the compaction of chromatin as well as the accessibility of DNA to transcription factors and other regulatory proteins[29]. The possible outcomes of different histone modifications in liver fibrosis are summarized in Table 1. Notably, histone lactylation and phosphorylation were both recently identified and are differentially involved in the mRNA expression of genes[30,31]. However, whether these novel histone modifications contribute to hepatic fibrogenesis is not elusive, yet worth to be investigated in the future.

### 3.1. *The modulation of histone methylation in liver fibrosis*

Histone methylation is dynamically mediated by several histone methyl transferase (HMT) and histone demethylase (HDMT), which orchestrates gene expression in liver fibrosis by depositing and removing methylation marks, respectively (Fig. 3). The lysine residues of histones could be mono-, di- and trimethylated by adding one, two or three methyl groups respectively to act as active or repressive marks of gene expression[32]. Numerous studies have identified that methylation at different lysine sites play antipodal effects on transcription by influencing the binding of proteins to modified histones. The methylated histone 3 lysine 9 (H3K9), H3K27 and H4K20 function as "silencer" proteins and are associated with gene silencing, while methylated H3K4, H3K36, and H3K79 function as "activator" proteins to activate the transcription of implicated genes.

#### 3.1.1. *Histone methylation suppresses gene transcription as 'Silencer'*

Chromatin is organized as the euchromatin and heterochromatin in eukaryotes. Euchromatin is less condensed and allows transcription factors to access the DNA and initiate transcription. Euchromatin is characterized by a more open structure and is enriched in specific histone modifications associated with gene activation. However, heterochromatin is a tightly packed form of chromatin and is associated with gene silencing and a more repressive state of transcription. Methylation of H3K27, H3K9 and H4K20 is generally associated with packed heterochromatin and gene repression.

TGF-$\beta$, a master profibrogenic cytokine, triggers HSC activation and ECM deposition and thus plays a critical role in hepatic fibrogenesis. In these processes, TGF-$\beta$ upregulated the expression of enhancer of zeste homolog 2 (EZH2) a polycomb repressive complex (PRC) component that specifically mediated the trimethylation of H3K27, and subsequently promoted H3K27me3 at the promoter of several downstream genes. Reversing H3K27me3 by EZH2 inhibitor GSK-503 or siRNA effectively attenuated TGF-$\beta$-stimulated HSC activation by inhibiting fibrotic gene transcription, including *Fn*, *Col1a1* and *Acta2* in $CCl_4$-and BDL-mouse model[33]. Bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI) is regarded as the pseudo receptor of TGF-$\beta$ due to the similar extracellular ligand binding domain structure as TGF$\beta$R, yet lacks an intercellular serine/threonine adaptor. BAMBI has been confirmed as a negative regulator of the TGF-$\beta$ pathway and a potential anti-fibrosis target. EZH2 inhibitor 3-deazaneplanocin A (DZNep) promoted the expression of BAMBI, IL-10 and cell cycle regulators, including Cdkn1a, Gadd45a and Gadd45b, and thus led to TGF-$\beta$/Smads suppression and anti-inflammatory response by removing H3K27me3 signatures associated with these genes in TGF-$\beta$-induced HSCs[34]. Furthermore, by enhancing the transmembrane transport of TGF-$\beta$, the scaffold protein GIPC (also known as synectin) epigenetically upregulated Insulin-like growth factor binding protein 3 (IGFBP3) is predominantly expressed in HSCs, and promoted HSC activation and migration. Chromatin immunoprecipitation showed that GIPC significantly decreased H3K27me3 modification in *Igfbp3* transcription start site region (chr7:45959379−45960659 and chr7: 45959718−45961110) and promoter region (chr7:

**Table 1** Summary of histone modification in liver fibrosis.

| Histone modification | Consequence | Target | Cell type/animal model |
|---|---|---|---|
| Methylation | Inhibits transcription | | |
| H3K27me3 | | TGF-$\beta$/IGFBP3 | HSC |
| H3K9me2/3 | | TLR4/NF-$\kappa$B/PPAR$\gamma$ | HSC |
| H4K20me3 | | Bax | HSC |
| Methylation | Promotes transcription | | |
| H3K4me1/3 | | NLRP3/Bivalent domains in PPAR$\gamma$ | Hepatocyte/HSC |
| H3K36me3 | | Bivalent domains with H3K27me3 | HSC |
| H3K79me3 | | Cul4 with Clr4 | Hepatocyte |
| Acetylation | Promotes transcription | | |
| H3K27ac | | CCL2 | LSEC |
| H3K9ac | | Fibronectin and Serpine1 | Cholangiocytes |

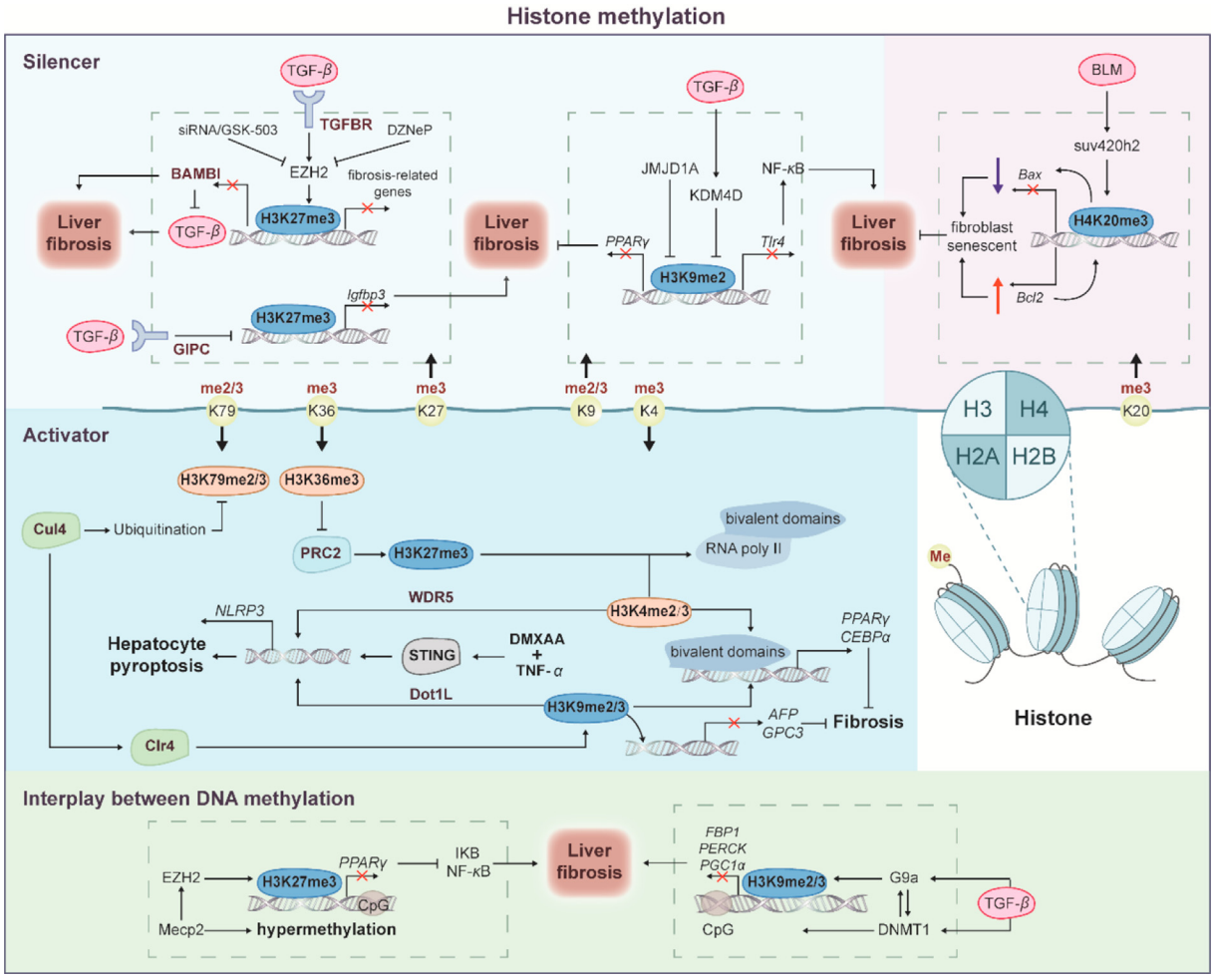


**Figure 3** The modulation of histone methylation in liver fibrosis. Mounting studies have shown correlations between histone methylation and liver fibrosis. Methylation at different lysine sites can have antipodal effects on transcription. As "Silencer" proteins, H3K9, H3K27, and H4K20 are involved in gene suppression, whereas H3K4, H3K36, and H3K79 function as "Activator" proteins that promote gene transcription. The "bivalent domains", formed by H3K4me3, H3K36me3, and H3K79me2/3, as well as the silent markers H3K27me3 and H3K9me2/3, keep gene transcription at a low level, preparing for rapid gene expression with RNA polymerase II loaded. Since chromatin structure is created by DNA wrapping around histones, histone methylation works in conjunction with DNA methylation through EZH2, G9a, and DNMT1.

45961121−45962023). Usm and his colleagues further established a global *Igfbp3* knockout mouse model, illustrating that *Igfbp3* absent reversed HSC activation and migration and inhibited liver fibrosis. Thus, by employing EZH2 inhibitors, such as GSK-503 and DZNep or siRNAs, the studies confirmed that H3K27me3 is involved in epigenetic regulation of TGF-$\beta$-related targets, suggesting the therapeutic potential of targeting H3K27me3 modification in liver fibrosis[35].

H3K9 methylations are also generally regarded as a crucial mark of heterochromatin formation and transcriptional silencing. G9a and Glp are required for H3K9 di-methylation and Suv39h enzymes, including Suv39h1 and Suv39h2, predominantly mediate H3K9 tri-methylation[36]. The transcriptional silencing of PPAR$\gamma$ during HSC activation is not only attributed to DNA methylation but can also be epigenetically repressed by H3K9me2 near the PPAR$\gamma$ gene promoter region. JMJD1A (Jumonji domain-containing protein 1A), a histone demethylase catalyzing the removal of H3K9me1 and H3K9me2, was abnormally decreased in TGF-$\beta$-treated HSCs and $CCl_4$-treated mice. Meanwhile, JMJD1A overexpression significantly decreased fibrosis-associated gene expression and reversed PPAR$\gamma$ transcription by removing the two methyl groups from H3K9[37]. Another histone demethylase catalyzing H3K9 di-, and tri-demethylation, lysine(K)-specific demethylase 4D (KDM4D) also gradually increased during HSC activation in $CCl_4$-, BDL and TAA-treated mice and primary HSCs. KDM4D knockdown stimulated the enrichment of H3K9me2 and H3K9me3 in the Toll-like receptor 4 (*Tlr4*) promoter and therefore promoted fibrosis regression *in vivo*. In detail, *Tlr4* was critically involved in pro-fibrogenic gene expression in HSCs and promoting liver fibrosis *via* triggering downstream nuclear factor kappa-B (NF-$\kappa$B), a master regulator for the proinflammatory mediators[38].

In addition to H3K27 and H3K9 methylation, H4K20 methylation was well-established as a repressive hallmark of histone modification contributing to fibrogenesis. Although the potential connection between H4K20 and liver fibrosis remains to be clarified, the figure of H4K20 mono- and trimethylation in pulmonary fibrosis and fibroblast senescence has attracted

attention. In idiopathic pulmonary fibrosis myofibroblasts, H4K20me1 and its methyltransferase SET8 were higher than those in normal lung fibroblasts[39]. Fibroblast senescence, a state of irreversible growth arrest, is associated with apoptosis, including pro-apoptotic gene *Bax* and anti-apoptotic gene *Bcl2*. Due to the high-expression of *Bcl2* and low level of *Bax*, senescent fibroblasts are more resistant to external injury. Chromatin immunoprecipitation indicated that H4K20me3 was enriched in the *Bax* promoter and decreased in the *Bcl2* promoter to induce senescent phenotypes in fibroblasts, ultimately favoring the abrogation of fibrogenesis[40].

#### 3.1.2. *Histone methylation promotes gene transcription as 'Activators'*

Except for inhibiting gene transcription, histone methylation could also be considered transcriptional-active marks of specific genes. The methylation at H3K4, H3K36 and H3K79 are known as active marks that are responsible for gene activation. H3K4me2 and H3K9me3 were demonstrated to be increased at the promoter regions of NOD-like receptor protein 3 (*NLRP3*) inflammasome, contributing stimulator of interferon genes (STING) overexpression-stimulated hepatocyte pyroptosis during liver fibrosis. Detailly, in response to STING agonist DMXAA plus TNF-$\alpha$, the H3K4-specific histone methyltransferase WDR5 and H3K79 methyltransferase DOT1L formed a transcription activator complex with interferon regulatory transcription factor 3 (IRF3) thus recruited IRF3 to NLRP3 promoter and activated NLRP3 inflammasome pathway, which was significantly abrogated by WDR5 and DOT1L inhibitors ICR-9429 and EPZ004777, respectively[41]. In addition to the direct roles of these histone modifications, with the remission of liver fibrosis, the interaction between 'Activator' in 'Silencer' and the 'bivalent domains' formed by these active marks and recognized silence markers, including H3H27me3 and H3K9me were detected[42]. These 'bivalent domains' kept genes expressed at low levels while stimulated and poised for rapid activation if required. In detail, RNA polymerase II (poly II) is loaded in 'bivalent domains' formed by H3K4me3 and H3K27me3, preparing for rapid gene activation in embryonic fibroblast cells. Genes destined for activation lose the suppressive H3K27me3 and expand the active H3K4me3 to the gene body, establishing bimodal peaks of H3K4me3 through poly II elongation, and *vice versa*[43]. Meanwhile, bivalent H3K4me3 and H3K9me3 chromatin domains maintained adipogenic master regulatory genes, including PPAR$\gamma$ and Cebp$\alpha$, low expression and stimulated poised for rapid activation when differentiation[44]. Meanwhile, H3K4me2 and H3K9me2 were reported to maintain a negative correlation. Depletion of LSD2, a specific H3K4me2 demethylase, increased H3K4me2 and thus decreased H3K9me2[45]. While H3K36me2/3 and H3K79me3 rarely co-exist with H3K27me3 and H3K9me2 and the mutually exclusive effects were applied by influencing enzyme activity. Chromatin immunoprecipitation sequencing demonstrated that H3K36me3 suppressed H3K27me3 in the promoter region *via* inhibiting activity of H3K27 methyltransferase complex PRC2[46]. H3K79me3 elimination, mediated by Cul4-mediated ubiquitination degradation, significantly increased methyltransferase Clr4-dependent H3K9me3 modification and turned off the expression of hepatocyte marker $\alpha$-fetoprotein (*AFP*) and glypican-3 (*GPC3*) in the liver[47]. Thus, the methylation of H3K4, H3K36 and H3K79 may become a potential regulatory target in liver fibrosis by interacting with H3K9me3 and H3K27me3 and DNA methylation.

Additionally, H3K79me2/3, H3K36me3 and H3K4me3 have been illustrated to play vital roles in other organ fibrosis, including renal fibrosis, pulmonary fibrosis and cardiac fibrosis. For example, H3K79 methyltransferase DOT1L increased the enrichments of H3K79me3 on the promoter of Jagged gene and thus enhanced Jagged expression, then stimulating Notch signaling and fibrosis response in Bleomycin-stimulated pulmonary fibrosis[48]. Furthermore, Dot1L knockout rescued Ang II-induced and myocardial ischemia-induced cardiac fibrosis by decreasing H3K79me3 enrichment in forkhead box O3 (*FOXO3*) and thus inhibiting FOXO3 expression. Pharmacological targeting for Dot1L alleviated ECM deposition and inhibited cardiac fibroblast activation by epigenetically suppressing FoxO3a in cardiac fibrosis[49]. In renal tubular epithelial cells and myofibroblasts, treatment with EPZ5676 or Dot1L siRNA inhibited H3K79me3 enrichment in Snail, Twist and Notch1 promoter and thus inhibited their expression. Targeting Dot1L attenuated renal fibrosis *via* inhibiting renal fibroblasts and epithelial-to-mesenchymal transition (EMT)[50]. Additionally, *Salvia miltiorrhiza* and *Carthamus tinctorius* extract (SCE) treatment downregulated H3K4me3 and H3K36me3 at *Smad3* promoter in cardiac fibroblasts and thus inhibited *Smad3* transcription, which prevented myocardial fibrosis and adverse remodeling after myocardial infarction[51]. MM-102 or OICR-9429, both of which are MLL1/WDR5 protein−protein interaction inhibitors, could suppress the transcription of $p16^{INK4a}$ *via* decreasing H3K4me3, and attenuate kidney ischemia−reperfusion injury-related renal fibrosis and inflammation[52]. Meanwhile, sinefungin, a SET7/9 inhibitor were reported to reverse H3K4me1 modification and then suppressed TGF-$\beta$ expression, which inhibited collagen deposition and ameliorated peritoneal fibrosis[53]. Therefore, the potential therapeutic effects of H3K79me2/3, H3K36me3, H3K4me1, and H3K4me3 on renal fibrosis, pulmonary fibrosis, cardiac fibrosis, and peritoneal fibrosis suggest a potential therapeutic avenue for "Activator" in treating liver fibrosis.

### 3.2. *Histone acetylation in liver fibrosis*

In the past decades, histone acetylation has been found to be involved in various diseases, including liver fibrosis (Fig. 4). The imbalance between acetylation and deacetylation of histone proteins, addition or removal of an acetyl group on histone lysine residue, is dominated by histone acetyltransferase (HAT) and histone deacetylases (HDACs)[54]. Additionally, bromodomain and extra-terminal (BET) proteins act as 'readers' and mediate histone acetylation by interacting with acetylated nucleosomes and transmitting acetylated lysine signals[55]. Histone acetylation has seemed as an active marker of gene transcription. By adding acetyl groups to lysine residues, the positive charge of histone was decreased and the binding between histone and negatively charged DNA was disturbed, leaving the underlying DNA exposed[56]. Chromatin is loosed into active transcription and the activities of transcription factors are upregulated after histone acetylation, which establishes a transcriptional connection between transcription factors and chromatin[57]. Several histone lysine residues could be acetylated, including H3K27, H3K9, H3K4 and H3K36 and so on. Hereafter, we mainly focus on well-investigated H3K27 and

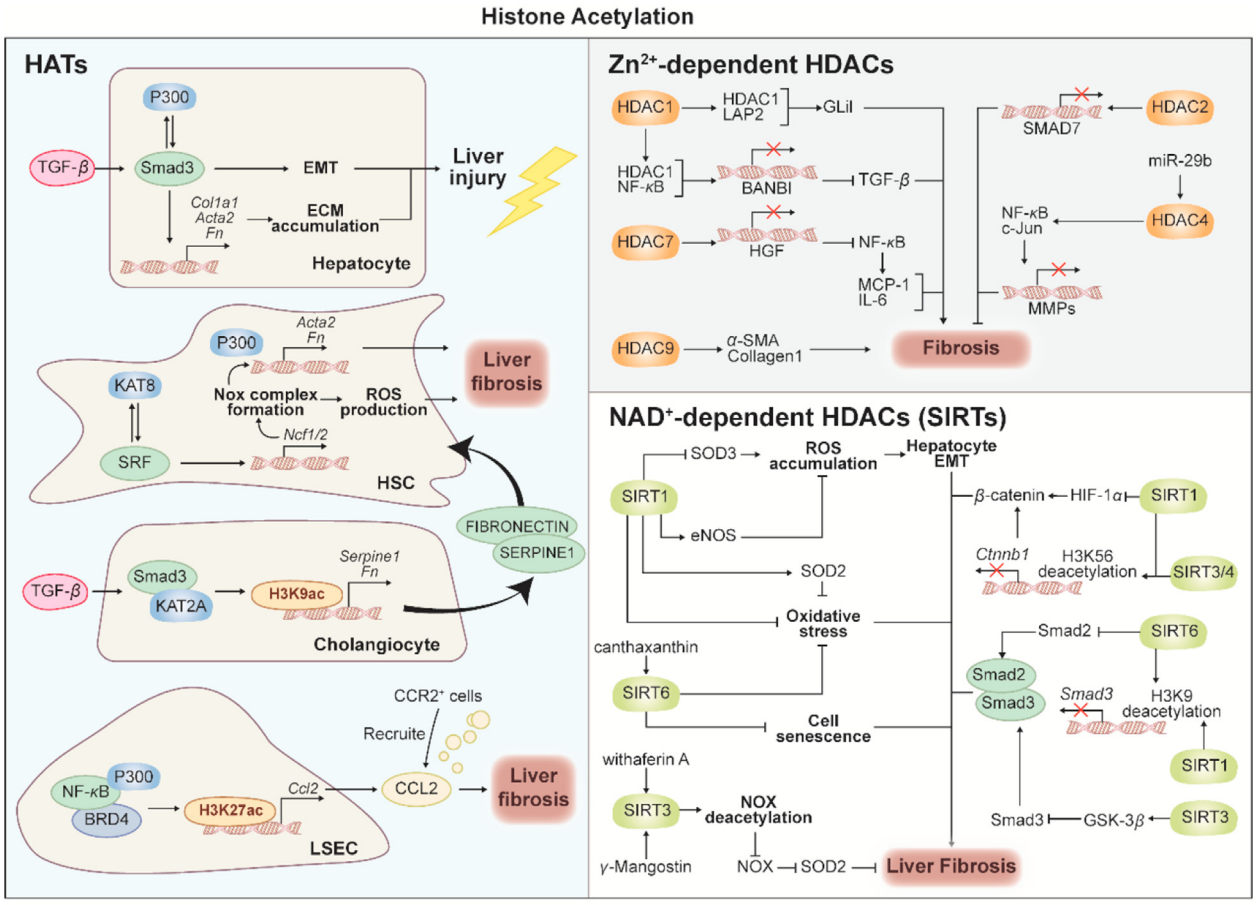


**Figure 4** Histone acetylation in liver fibrosis. This diagram represents the roles of histone acetylation in liver fibrosis. Acetylation of histone is mediated by a dynamic balance between HATs and HDACs. HATs, including P300, KAT2A and KAT8, regulate several fibrosis-related genes including *Acta2*, *Ncf1* and *Serpine1* by catalyzing H3K9ac and H3K27ac. HDACs are divided into 2 categories $Zn^{2+}$-dependent HDACs and $NAD^{+}$-dependent SIRTs. Several HDACs, including HDAC1, HDAC2, HDAC4, HDAC7, HDAC9 and SIRTs, including SIRT1, SIRT3, SIRT4 and SIRT6, are involved in fibrosis process *via* targeting TGF-$\beta$-Smads pathway, EMT, oxidative stress, cell senescence and HIF/$\beta$-catenin pathway.

H3K9 acetylation and are committed to clarifying these potential modifications in liver fibrosis progression.

### 3.2.1. *Histone acetylation mediated by HATs*

HATs, which catalyze histone acetylation, can be divided into three categories: p300/CBP, Gcn5-related *N*-acetyltransferases (GNATs) superfamily and MYST proteins[58]. P300/CBP proteins could stimulate histones acetylation at the enhancer and promoter regions of target genes and act as a transcription coactivator by cooperating with several transcription factors, including NF-$\kappa$B, AP1 and STAT. GNAT superfamily includes PCAF, Gcn5, Elp3, Hpa2 and Hat1, and MYST superfamily is composed of Esa1, Sas2, Sas3, Tip60, MOF, MOZ, MORF and HBO1. These HATs are illustrated to be involved in multiple diseases, while, to be more specific, p300 (also called KAT3B), CBP (also known as KAT3A), Gcn5 (also called KAT2A), MOF (also called KAT8) and PCAF (also called KAT2B) were closely associated with the progression and development of fibrosis[59].

P300, a transcriptional coactivator, initiated transcriptional regulation to promote the function of hepatic cells in liver fibrosis. As a direct target of TGF-$\beta$, p300 mediated transcriptional activation of fibrotic genes, including *Acta2*, *Fn*, and *Col1a1*, by interacting with Smad3 in fibroblasts[60]. Further study demonstrated that p300 interacted with Smad3 and thus enhanced TGF-$\beta$-induced EMT in hepatocytes[61]. The binding partners of p300 include transcription factor NF-$\kappa$B and epigenetic reader protein bromodomain containing 4 (Brd4). The complex of p300-NF-$\kappa$B-Brd4 is involved in pathological inflammatory in liver fibrosis. Indeed, Brd4 directly bonded with p300 and recruited NF-$\kappa$B to the acetylated histone lysine, contributing to the formation and function of this acetyltransferase complex[62]. During fibrosis, p300 and its binding partners, NF-$\kappa$B and Brd4, are required for the transcription of inflammatory factor C−C motif Chemokine 2 (*Ccl2*) in $CCl_4$-stimulated liver sinusoidal endothelial cells (LSECs). Released from LSECs, *Ccl2* subsequently recruited inflammatory cells, including $CCR2^{+}$ monocyte/macrophage, into the sinusoids. These inflammatory cells activated HSCs by releasing factors such as TNF-$\alpha$ and TGF-$\beta$, which further promoted portal hypertension and liver fibrosis. Mechanically, chromatin immunoprecipitation showed that p300 upregulated *Ccl2* expression by catalyzing H3K27ac at the *Ccl2* promoter. Furthermore, Gao and his colleagues established LSEC-specific p300 deletion mice and further confirmed that p300 absence inhibited portal hypertension and hepatic fibrosis *via* reducing the hepatic accumulation of CCR2-positive monocyte/macrophage[63]. Previous studies also demonstrated the reliance of serum response factor (SRF), a transcription

factor in myofibroblast transdifferentiation, on p300 for promoter binding. More importantly, SRF also interacted with KAT8 (known as MOF) and thereby recruited KAT8 to promoters of several genes, including *NCF1* and *NCF2*. As members of NADPH oxidase (NOX), NCF1 and NCF2 promoted ROS production and HSC activation by organizing the NOX2 complex. Following HSC-specific SRF knockout, the reduction of H3K27ac and H4K16ac signatures was observed on the *NCF1* and *NCF2* promoters, leading to NCF1 and NCF2 transcriptional repression. Consistently, KAT8 knockdown by siRNA significantly downregulated pro-fibrogenic genes, including *Acta2* and *Col1a1*, by decreasing *Ncf1* and *Ncf2* expression in primary mouse HSCs and LX-2[64].

H3K9ac was mediated by GNAT family members lysine acetyltransferase 2A (KAT2A) and KAT2B (also known as GCN5 and PCAF, respectively). As a master regulator of fibrosis, TGF-$\beta$ directly activated HSCs and stimulated cholangiocyte transformation into reactive and secretory states, and therefore facilitating cholangiocyte-HSCs crosstalk. In TGF-$\beta$-stimulated cholangiocytes, the transcription of HSCs activators *Fn* and *Serpine1* were promoted due to the enrichment of H3K9ac in promoters of these genes. Chromatin immunoprecipitation assay illustrated that KAT2A, which is predominantly expressed in cholangiopathies, was recruited to and acetylated H3K9 near the promoters of *Fn* and *Serpine1* with the help of Smad3 and thereby promoted TGF-$\beta$-stimulated genes transcription in cholangiocytes. Subsequently, Fibronectin and SERPINE1 were released by cholangiocytes and swallowed by HSCs, leading to HSCs activation and biliary fibrosis in *Mdr2*$^{-/-}$ mice[65].

### 3.2.2. *Histone deacetylation mediated by HDACs*

Opposite to HATs, histone deacetylation, catalyzed by HDACs, leads to gene repression *via* inducing chromatin compaction and subsequently preventing the binding of transcription factors[66]. To date, HDACs are divided into two categories and four families: Class I HDACs, including HDAC1, HDAC2, HDAC3, and HDAC8; Class II HDACs, including HDAC4, HDAC5, HDAC7, HDAC9, HDAC6, HDAC10; Class III HDACs, including SIRT1−7; and Class IV HDACs, including HDAC11[67]. Among these HDACs, Class I HDACs are predominantly expressed and located in nucleus, while Class II HDACs are located in cytoplasm and transferred to nucleus in response to cellular signals. Class III HDACs are different from other HDACs due to their requirement of cofactor nicotinamide adenine dinucleotide $(NAD)^+$ in catalyzing histone deacetylation. Finally, HDAC11 is the sole member of Class IV localizing in the nucleus and uniquely shares sequence homology with the catalytic domains of both Class I and Class II. Numerous studies proposed the complex changes of HDACs in liver fibrosis. For example, HDAC1 and HDAC2 were significantly increased in early stages of HSC activation, and the upregulation of HDAC8 was found at later time points of HSC activation[68]. HDAC9 and HDAC10 were downregulated in HSC transdifferentiation, while HDAC4 and HDAC7 remained constantly expressed[68]. Additionally, recent studies illustrated that HDAC1, 2, 4, 5, 6, 8, 9 were increased during liver fibrosis, yet HDAC2, 6, 8 were decreased accompanied with increased HDAC11 during fibrosis resolution. Inconsistent understandings of the pathogenic roles of HDACs-mediated deacetylation hindered the discovery of novel therapeutic approaches targeting HDACs in liver fibrosis.

*3.2.2.1. Zinc-dependent HDACs.* The $Zn^{2+}$-dependent HDACs were contained in Class I, Class II, and Class IV and involved in liver fibrosis progression and resolution. Many researchers have illustrated the regulation of histone deacetylation and further clarified the fundamental mechanism of $Zn^{2+}$-dependent HDAC in the progression of liver fibrosis by integrating previous studies[68]. The hallmark of fibrotic lesions is constitutive TGF-$\beta$ signaling and HDACs are involved in liver fibrosis by interfering with TGF-$\beta$-related genes. Accompanied with H3K9ac hyperexpression, miR-29a overexpression inhibited HDAC4 expression and its nuclear translocation and thereby improved TGF-$\beta$-stimulated HSC activation in LX-2[69]. During HSC activation, HDAC4 was significantly enriched in the promoter regions of MMPs, enzymes responsible for collagen degradation, and inhibited their expression by repressing the recruitment of transcription factors, including c-JUN and NF-$\kappa$B, resulting in abnormal collagen accumulation[70]. HDAC9 was also involved in the transcriptional suppression of TGF-$\beta$ targets genes, including $\alpha$-SMA and collagen, which was illustrated by introducing HDAC9 knockdown by siRNA in LX-2[71]. Smad7 was a negative regulator of the TGF-$\beta$/Smads signaling pathway, and HDAC2 contributed to TGF-$\beta$-stimulated HSC activation *via* participating in Smad7 transcriptional repression[72]. Glioma-associated oncogene homolog 1 (Glil) are the profibrotic downstream of TGF-$\beta$. HDAC1 combined with lamina-associated polypeptide 2a (LAP2) and enriched in the promoter of Glil, leading to Glil hyperexpression and subsequent HSC activation[73]. Persistent inflammation almost always precedes liver fibrosis and TLR4 activates NF-$\kappa$B and subsequently stimulates proinflammatory cytokines though adaptor protein MyD88. As mentioned above, BAMBI is a negative regulator in the TGF-$\beta$ signaling pathway. HDAC1 interacted with NF-$\kappa$B to form a transcriptional repression complex in the BAMBI promoter and thereby upregulated TGF-$\beta$ signaling by decreasing BAMBI expression[74]. Additionally, hepatocyte growth factor (HGF) was regarded as an antifibrogenic mediator and significantly suppressed the TGF-$\beta$ signaling pathway by inhibiting latent TGF-$\beta$1 activation. HDAC7 was enriched in promoter region of HGF and epigenetically repressed HGF expression and thus promoting NF-$\kappa$B-stimulated expression of proinflammatory genes MCP-1 and IL-6[75].

*3.2.2.2. NAD-dependent HDACs.* Class III HDACs, also known as sirtuins (SIRTs), are localized to several subcellular compartments, including the nucleus (SIRT1, 2, 3, 6 and 7), cytoplasm (SIRT1 and 2), and mitochondria (SIRT3, 4 and 5), and SIRTs-mediated deacetylation can target both histones and non-histone proteins with the synergy of NAD. Owing to their dependence on NAD as the major substrate, SIRTs are linked with energy-sensing-related degenerative disorders. Indeed, SIRTs were reported to be involved in multiple organ fibrosis, especially liver fibrosis, through multiple mechanisms, including EMT, oxidative stress, cell senescence and HIF-$\beta$-catenin.

Increasing evidence demonstrated that SIRT1, SIRT3, and SIRT6 attenuated liver fibrosis by influencing the TGF-$\beta$ pathway[76,77]. SIRT1 overexpression significantly blocked liver fibrosis by stimulating apoptosis and inhibiting the proliferation of activated HSCs, and SIRT1 knockdown enhanced $CCl_4$-induced liver fibrosis[76,78]. Under TGF-$\beta$ stimulation, Smad2/3 are phosphorylated and further bind to SMAD4 to form a complex, and the Smad2/3/4 complex triggers downstream genes transcription *via* relocating to the nucleus. SIRT1 decreased Smad3 expression by stimulating H3K9 deacetylation at Smad3 promoter and thereby inhibited the Smad2/3/4 complex formation and subsequent HSC activation[79]. Recent studies further demonstrated that SIRT1 is

involved in the suppression of TGF-$\beta$-induced myofibroblast transdifferentiation by deacetylating EZH2 and thus reducing the stability of EZH2. In $CCl_4$-and TGF-$\beta$-induced liver fibrosis, the variation in SIRT1 levels exhibited an opposite trend to the expression levels of EZH2. Mechanically, EZH2 acetylation at lysine 348 increased its stability and enhanced the PRC2 compound formation. SIRT1 disrupted EZH2 stability and played the hepatoprotective role through deacetylation. The SIRT1−EZH2 pathway may represent an attractive therapeutic target, suggesting the cooperative relationship between histone methylation and acetylation in liver fibrosis[80]. SIRT3 overexpression increased expression of glycogen synthase kinase 3$\beta$ (GSK3$\beta$), a negative regulator of TGF-$\beta$1, and blocked TGF-$\beta$1 signaling, attenuating liver fibrosis in $CCl_4$-induced mice. In detail, GSK3$\beta$ was critically acetylated at residue K5, which negatively regulated GSK3$\beta$ activity in TGF-$\beta$-stimulated HSC. SIRT3 is directly bound to and deacetylated GSK3$\beta$, thus reversing its activity and decreasing its downstream Smad3 expression[81]. SIRT6 was markedly decreased in patients as fibrosis progressed to cirrhosis, culture-activated and TGF-$\beta$-induced primary HSCs. SIRT6 stimulated H3K9 deacetylation at the *Smad3* promoter and thereby inhibited *Smad3* transcription. Meanwhile, SMAD3 acetylation was concurrently depended on and promoted by TGF-$\beta$-induced phosphorylation. SIRT6 also downregulated SMAD3 expression and thus alleviated liver fibrosis by directly deacetylating SMAD3 at key lysine residues K333 and K378[82]. Interestingly, recent studies have reported that SIRT6 may be an endogenous negative feedback regulator of TGF-$\beta$/Smad3. Lysine K333 and K378 of Smad3 were not necessary for SMAD3 to influence SIRT6, illustrating the coexistence of bidirectional regulation between SMAD3 and SIRRT6. SIRT6 limited TGF-$\beta$-stimulated fibrotic changes, while Smad3 formed a complex with SPTBN1 and bound to the promoter region of SIRT6, promoting SIRT6 transcription[83]. In addition to Smad3, recent studies illustrated that Smad2 acetylation plays a role in TGF-$\beta$-induced HSC activation. Lysine 54 in MH1 domain of Smad2 was the major position for phosphorylation, and acetylation of lysine 54 was required for phosphorylation and nuclear localization of Smad2. SIRT6 significantly inhibited Smad2 lysine 54 acetylation and subsequently alleviated TGF-$\beta$/Smads signaling pathway[84]. SIRT6 was also reported to interfere with Hippo pathway and acted as an anti-fibrosis factor. In cholestatic fibrosis, excess toxic bile acids may activate TGF-$\beta$/Smads and Yes-associated protein (YAP) signaling. YAP and transcriptional coactivator with PDZ-binding motif (TAZ) were implicated in Hippo pathway activation and liver fibrosis. SIRT6 downregulated the expression of YAP and TAZ by stimulating deacetylation, resulting in hepatic fibrosis-genes repression and liver fibrosis inhibition[85]. Furthermore, under pathological conditions, estrogen-related receptor $\gamma$ (ERR$\gamma$) was increased and aggravated liver fibrosis by continually stimulating bile acid production. SIRT6 activation ameliorated BDL-induced cholestatic fibrosis by deacetylating orphan nuclear receptor ERR$\gamma$ and inhibiting its transcriptional activity[86].

Under oxidative stress, continuous ROS accumulation contributes to the activation of SIRTs, while SIRTs, including SIRT1, SIRT3 and SIRT6, are demonstrated to alleviate liver fibrosis *via* negatively regulating oxidative stress. In $CCl_4$-stimulated rat fibrosis, oxidative stress induced the loss of fenestration in hepatic sinusoidal endothelial cells and promoted fibrogenesis. The overexpression of SIRT1 significantly reversed these processes and thus exerting therapeutic potential[87]. Furthermore, excessive oxidative stress is strongly associated with aging and exacerbates cellular dysfunction, tissue failure and fibrosis. SIRT1 was found downregulated in alcoholic-induced liver injury and fibrosis associated with aging and enhancing SIRT1 activity has yielded prominently prospects for new therapies[88]. HSCs isolated from middle-aged mice expressed lower levels of SIRT1 protein and were more susceptible to spontaneous activation *in vitro*[89]. EMT refers to a process wherein epithelial cells transform into mesenchymal cells, promoting liver fibrosis, which is also closely related to oxidative stress. Recently, EMT have been suggested to be the downstream event of SIRT1 regulation. Enforced expression of SIRT1 inhibited superoxide dismutase 3 (SOD3) deficiency-induced hepatocyte EMT and HSC activation, and depletion of SIRT1 counteracted the inhibitory effect of SOD3 *in vitro*[90]. Paradoxically, the activation of SIRT1/p53 signaling is required for TGF-$\beta$ stimulated hepatocyte EMT, which contributed to liver fibrosis in rat[91]. In addition to liver fibrosis, SIRT1 could enhance SOD2-dependent anti-oxidative mechanism and inhibited high glucose-induced cardiac fibrosis by reducing ROS accumulation[92]. SIRT1 also increased eNOS level and therefore alleviated UUO-induced renal fibrosis[93], providing the possibility for the therapeutic roles of SIRT1 in the liver.

SIRT3 blocked ROS-induced HSC transdifferentiation and ECM deposition by inhibiting oxidative stress. In detail, SIRT3 enhanced superoxide dismutase 2 (SOD2) activity through deacetylation and decreased NOX activity, which prevents the expression of fibrosis-related genes, including *Acta2* and *Fn*[94]. Therefore, Withaferin A[95] and $\gamma$-mangostin[96] acted as SIRT3 agonists and thereby prevented $CCl_4$-induced liver fibrosis *via* inhibiting oxidative stress. Meanwhile, SIRT6 overexpression also protected $CCl_4$-induced liver fibrosis *in vivo* and SIRT6 knockout mice spontaneously developed liver injury, as characterized by remarkable increase of oxidative stress and inflammation[97]. Canthaxanthin has been illustrated to increase SIRT6 expression to alleviate $CCl_4$-induced liver fibrosis[98].

These SIRTs have also been illustrated to influence $\beta$-catenin-associated pathway, major pathways involved in fibrosis responses. Empagliflozin alleviated thioacetamide-induced hepatotoxicity and liver fibrosis *via* increasing SIRT1 and thereby downregulating HIF-1$\alpha$/$\beta$-catenin axis[99]. SIRT1 deficiency promoted fibrosis and inflammation factors in glomerular mesangial cells *via* promoting HIF-1$\alpha$ and HIF-2$\alpha$ expression[100,101]. In addition to liver fibrosis, $\beta$-catenin target genes (*Fsp1* and *Axin2*) are upregulated by TGF-$\beta$, and SIRT6 prevented $\beta$-catenin transcription and thus inhibited fibrosis-related genes *via* binding with *Ctnnb1* promoter and thereby stimulating H3K56 deacetylation in tubular epithelial cells[102]. In Ang−II-induced cardiac fibrosis and TGF-$\beta$-stimulated renal fibrosis, SIRT1 and SIRT3 also alleviated fibrosis responses by catalyzing H3K56 deacetylation and epigenetically downregulating $\beta$-catenin expression[103,104]. Thus, further investigations on how SIRTs aggravate liver fibrosis by affecting $\beta$-catenin-associated pathways are urgently required.

However, unlike other members of SIRTs, SIRT2 is considered as a promoter in multi-organ fibrosis progression and inhibition of SIRT2 is demonstrated as an effective anti-fibrosis strategy. SIRT2 was abnormally increased in human fibrotic liver tissues and SIRT2 knockout downregulated fibrosis-related genes, including $\alpha$-SMA and collagen[105]. SIRT2 activated the extracellular regulated protein kinases (ERK) pathway by stimulating ERK protein deacetylation. Thus, the degradation of c-MYC, the downstream target of ERK, was decreased, leading to c-MYC hyperexpression and fibrogenesis[105]. In idiopathic pulmonary fibrosis, SIRT2 inhibition, mediated by inhibitor AGK2 or siRNA, suppressed expression of *Acta2* and *Fn via* downregulating TGF-$\beta$/Smad3[106].

While the underlying mechanism of SIRT2 deficiency in anti-fibrosis is still unclear, and the cause of its inconsistent effects with other SIRTs needs to be revealed.

## 4. RNA modification in liver fibrosis

As both targets and enactors of expression change, RNA, including mRNA, microRNA (miRNA), and long ncRNA (lncRNA), modulate gene expression through epigenetic mechanisms in liver fibrosis (Fig. 5). miRNA forms a RNA-induced silencing complex through interaction with argonaute, leading to the destabilization of the 3′ UTR in the target mRNA and subsequent inhibition of translation[107]. LncRNA regulates gene expression *via* recruiting histone-modifying enzymes to the chromatin, including HMT, HDMT, HAT and HDAC. Most recently, the field of mRNA epigenetic modifications has gained significant attention, with a particular focus on $N^6$-methyladenosine (m6A) RNA modification. This modification is known to modulate gene expression by impacting mRNA nuclear export, translation, as well as the splicing and stability of ncRNAs[108].

### 4.1. *mRNA methylation in liver fibrosis*

$m^6A$ modification is a dynamic process regulated by methyltransferases, demethylases and $m^6A$ binding proteins, serving as a post-transcriptional mechanism in regulating mRNA stability[109]. The WT1-associated protein (WTAP), methyltransferase like 3 (METTL3), METTL4 and METTL14 all function as methyltransferases, while demethylases mainly include alkB homolog 5 (ALKBH5) and obesity-associated protein (FTO). Following methylation, modified mRNAs will be recognized and bound by $m^6A$ binding proteins, such as heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs), YTH-domain-containing protein 1/2 (YTHDC1/2) and YTH-domain-family 1/2/3 (YTHDF1/2/3), and the cytosol transportation and translation of these mRNAs will be greatly regulated.

RNA $m^6A$ methylation has recently been reported to play a regulatory role in the activation of HSCs and then promote the progression of liver fibrosis. Yang and his colleagues[110] established a spontaneous NASH, liver fibrosis, and HCC in type 2 diabetes mellitus (DM2) mouse model and confirmed that METTL3/14 overexpression promoted the production of triglyceride and cholesterol and ECM accumulation *via* increasing the protein levels of ATP citrate lyase (ACLY) and stearoyl-CoA desaturase1 (SCD1). $m^6A$ sequencing analysis confirmed that METTL3/14 overexpression bonded and stabilized mRNA of ACLY and SCD1 through $m^6A$ modification. Detailly, the coding sequence (CDS) of ACLY and 3′ UTR of SCD1 showed the relatively higher $m^6A$ enrichment, which was confirmed

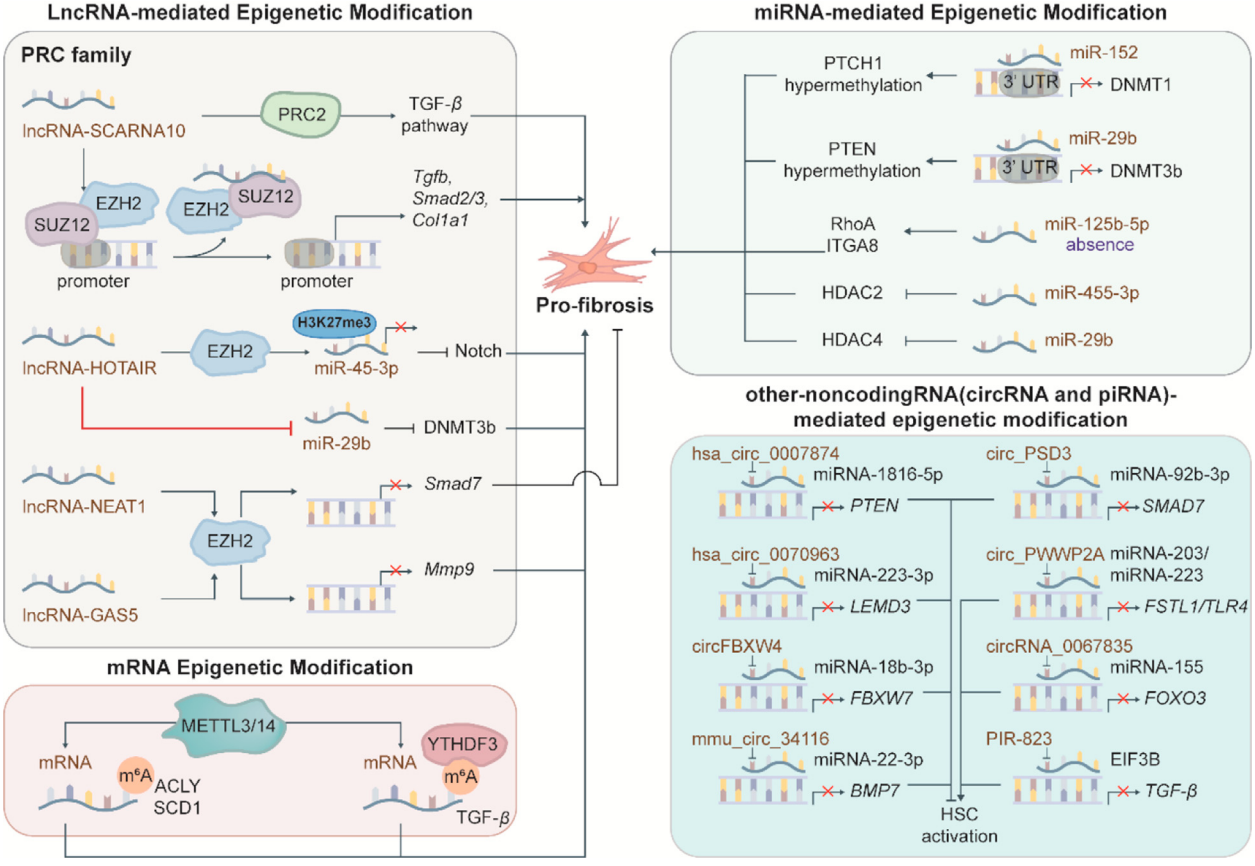


**Figure 5** Scheme diagram of RNA modification in fibrosis. Mounting evidence shows that RNA modification plays a vital role in the fibrosis process. Several miRNAs, including miR-152, miR-29b and miR-125b-5p, promote fibrosis by interacting with DNA methyltransferases and histone deacetylases. LncRNAs, including lncRNA-SCARNA10, lncRNA-HOTAIR, lncRNA-NEAT1, and lncRNA-GAS5, mainly regulate the PRC2 family. Several circRNAs, including hsa_circ_0007874, hsa_circ_0070963, circFBXW4, mmu_circ_34116, circ_PSD3 and circ_PWWP2A inhibited HSC activation *via* combining with miRNA and thus reversed miRNA-mediated transcription suppression.

by luciferase reporter assay[110]. The upregulation of methyltransferases METTL3 and METTL14 and global RNA hypermethylation were also observed in HFD-stimulated NASH and LPS-activated KCs. In active-KCs, NF-$\kappa$B p65 directly promoted the expression of METTL3 and METTL14 by binding to their promoter. Upregulated METTL3 and METTL14 then increased m$^6$A modification in the *TGF-$\beta$* mRNA 5′UTR with the help of reader protein YTHDF3 and subsequently promoted cap-independent translation of TGF-$\beta$. This finding was further verified in METTL3/14 conditional knockout KCs[111].

Recently, it is found that ferroptosis, a novel form of programmed cell death, functions as a two-edged sword in liver fibrosis. It has been reported that excessive hepatic iron deposition aggravates acetaminophen-induced liver fibrosis. While other studies indicated that ferroptosis could be considered as a new strategy to eliminate activated HSCs and regulate autophagy. Under the stimulus, different types of selective autophagy increased, including nuclear receptor coactivator 4 (NCOA4)-dependent ferritinophagy, RAB7A-dependent lipophagy, and p62-dependent clockophagy and chaperone-mediated autophagy, promoting ferroptosis *via* stimulating iron accumulation and lipid peroxidation[112]. Therefore, molecules triggered HSC ferroptosis might be able to alleviate liver fibrosis by regulating autophagy-related targets. Min and his colleagues identified that m$^6$A reader YTHDF1 increased the mRNA stability of BECN1, an autophagy-related gene, by recognizing its coding region and thus triggering autophagy activation and ferroptosis in HSC. Recognized ferroptosis inducer has been demonstrated to promote ferroptosis-related elimination of activated-HSC by increasing YTHDF1 expression[113].

### 4.2. *ncRNA-mediated epigenetic modification in liver fibrosis*

Recently, emerging evidence hint at a significant role for ncRNA in liver fibrosis and increasing emphasis is being placed on the role of ncRNA as epigenetic regulators[114]. A total of 32,461 differentially expressed ncRNAs have been identified by comparing fibrotic and normal liver samples in NASH patients, suggesting a strong link between ncRNA expression and fibrosis and a heightened risk of cirrhosis[115]. Consistently, more than 3600 differentially expressed ncRNAs were described during human HSC quiescence and activation[115] and 381 ncRNAs were recognized in HSC under the conditions of TGF-$\beta$ stimulation[1]. Indeed, gene expression is regulated not only by proteins but also by ncRNAs. Among them, miRNA and lncRNA are the most well-studied ncRNA. During fibrogenesis, the relationship between miRNA and lncRNA HSC activation has been widely disclosed (Table 2).

#### 4.2.1. *miRNA-directed epigenetic modification*

miRNAs regulate target expression by affecting transcription and mRNA stability, thus modifying DNA methylation and histone modification in liver fibrosis. A recent study reported that some miRNAs stimulated DNA hypomethylation state and therefore decreased fibrogenic activities by downregulating methyltransferases, including DNMT1 and DNMT3b[115]. Increased miR-152-stimulated by salvianolic acid B directly decreased DNMT1 expression *via* binding to the 3′UTR of DNMT1. Therefore, PTCH1, a negative regulatory factor of the fibrosis-related Hedgehog pathway, underwent DNA hypomethylation and hyperexpression due to the absence of DNMT1. DNMT1 knockout and miR-152 inhibitor were consistently demonstrated to inhibit EMT in liver fibrosis[132]. Additionally, miR-29b was decreased in liver fibrosis associated with chronic HBV infection. While curcumin upregulated miR-29b and thus led to the suppression of activated HSCs. In detail, miR-29b directly downregulated DNMT3b expression, contributing to hypomethylation of phosphatase and tensin homolog deleted on chromosome (PTEN) DNA, as illustrated by luciferase activity assays[133]. Interestingly, epigenetic silencing of miRNA stimulated by DNA methylation also plays an important role in the miRNA-directed regulation of liver fibrosis. It has been observed that the CpG island in miR-125b-5p promoter was hypermethylated, and thus its expression was suppressed in NAFLD livers and hepatocytes. Cai

**Table 2** ncRNAs involved in the regulation of liver fibrosis.

| ncRNA | Target | Pathway or regulatory mechanism | Output | Ref. |
|---|---|---|---|---|
| miRNA | | | | |
| miR-542-3p | BMP-7 | Decreases BMP-7 expression | Promotes liver fibrosis | 116 |
| miR-125b | RhoA | Activates RhoA pathway | Activates HSC | 117 |
| miR-199 | TGF-$\beta$ | Activates TGF-$\beta$ pathway | Promotes liver fibrosis | 118 |
| miR-200 | TGF-$\beta$ | Activates TGF-$\beta$ pathway | Promotes liver fibrosis | 118 |
| miR-21 | SMAD7 | Increases SMAD7 expression | Activates HSC | 119 |
| miR-129-5p | Collagen I | Reduces collagen I expression | Promotes liver fibrosis | 120 |
| miR-454 | Collagen I<br>$\alpha$-SMA | Reduces collagen I and $\alpha$-SMA expression | Promotes cirrhosis progression | 121 |
| miR-378-3p | Gli3 | Reduces Gli3 expression | Inhibits HSC activation | 122 |
| miR-222 | ICAM-1 | Decreases ICAM-1 expression | Promotes HSC proliferation and differentiation | 123 |
| miR-185 | SREBF1 | Increases SREBF1 expression | Promotes liver fibrosis | 124 |
| LncRNA | | | | |
| H19 | Let-7<br>ZEB1 | Increases expression of let-7 and ZEB1 | Promotes cholestatic liver fibrosis | 125,126 |
| MEG3 | SMO | Decreases SMO expression | Inhibits EMT in liver fibrosis | 127 |
| GAS5 | miR-222 | Competes with miR-222 and | Inhibits HSC activation and proliferation | 128 |
| Gm5091 | miR-27b/23b/24 | Increases TGF-$\beta$ by sponging to miR-27b/23b/24 | Alleviates alcoholic hepatic fibrosis | 129 |
| Lnc-LFAR1 | Smadd2/3<br>phosphorylation | Promotes Smadd2/3 phosphorylation | Promotes liver fibrosis | 130 |
| NEAT1 | miR-122<br>miR-29b | Increases miR-122 and miR-29b | Promotes liver fibrosis progression | 131 |

et al.[134] established the NAFLD mouse model and demonstrated that miR-125b-5p absence promoted liver fibrosis in NAFLD *via* activating RhoA and ITGA8. It is worth noting that some miRNAs act as the upstream factor of DNMTs and serve as the regulator of histone modification enzymes. miR-455-3p was reported to reduce the expression of profibrotic genes by binding to the 3′UTR of HDAC2 and upregulating the HDAC2 expression in activated HSCs[72]. Similarly, the interaction between miR-29b and HDAC4 in diabetic nephropathy was illustrated by luciferase assay. MiR-29b targeted 3′ untranslated region of HDAC4 and subsequently attenuated HDAC4 expression and renal fibrosis[135]. Overall, these studies indicate the involvement of miRNAs in epigenetic regulation in liver fibrosis, while the underlying mechanisms and specific targets have only been explored in a few studies. Further investigations on how varied miRNAs regulate liver fibrosis by affecting multifarious epigenetic modifications are still urgently required.

#### 4.2.2. *LncRNA-directed epigenetic modification*

Accompanied by liver fibrogenesis, lncRNAs are involved in the promotion or suppression of liver fibrosis *via* mutual interaction with miRNAs and proteins. LncRNAs have been illustrated to interact with miRNAs as 'sponges' or competing endogenous RNA (ceRNA), thus altering the expression and function of miRNAs. Meanwhile, the majority of lncRNAs can interact with RNA-binding proteins (RBPs) to form ribonucleoprotein (RNP) complex, medicating mRNA stability, translation and post-translation. Notably, lncRNAs are involved in epigenetic regulation through interactions with epigenetic enzymes and its-associated miRNAs. It has been illustrated that lncRNA-ACTA2-AS1 contributed to ductular reaction and paracrine HSC activation by interacting with H3K27 acetyltransferase p300 and transcription factor Elk1. In detail, lncRNA-ACTA2-AS1 combined with Elk1 and subsequently guided p300 to the promoters of DR and fibrosis-related genes, including Platelet-derived growth factor $\beta$ (*Pdgfb*), *Acta2*, *Fn* and *Serpine1*, which then catalyzed H3K27ac at binding sites for Elk1 (CCGGAA) and thus promoted the transcription of these genes in cholangiocytes[136]. As a typical lncRNA, homeobox transcript antisense RNA (HOTAIR) expression was upregulated in HSCs. Meanwhile, HOTAIR strikingly contributed to HSC activation and upregulated the expression of $\alpha$-SMA and collagen I by promoting DNA methylation of PTEN and suppressing PTEN expression *in vitro* and *in vivo*. Detailly, HOTAIR, a ceRNA of miR-29b, downregulated miR-29b expression and attenuated its suppression on DNMT3b in HSCs. Restored DNMT3b subsequently catalyzed DNA methylation of PTEN and decreased its transcription, which was illustrated by HOTAIR knockdown mice[137]. Furthermore, HOTAIR was demonstrated to stimulate myofibroblast activation by interacting with EZH2 in systemic sclerosis characterized by vascular fibrosis. Wasson and his colleagues illustrated that upregulated HOTAIR inhibited miR-34a, functions as a Notch1 suppressor, and thus activated classical pro-fibrosis targets Notch signaling in fibroblasts. Mechanically, HOTAIR guided EZH2 to the promoter of miR-34a to catalyze H3K27me3 and thereby suppressed miR-34a transcription and increased downstream Notch1 expression, which was reversed by EZH2 inhibitor GSK126[138]. In addition to EZH2, other members of the PRC2 family, such as SUZ12 polycomb repressive complex 2 subunit (SUZ12), has been demonstrated to be involved in liver fibrosis through the interaction with various lncRNAs. LncRNA-SCARNA10 was increased in the serum and liver of patients with advanced hepatic fibrosis and functioned as a positive regulator of the TGF-$\beta$ signaling pathway. Mechanically, LncRNA-SCARNA10 combined with SUZ12 and EZH2 and subsequently guided them away from the promoters of genes, including *Tgfb*, *Smad2*, *Smad3* and *Col1a1*. Accompany with the unbinding of methylases, H3K27me3 was reversed and thus, the genes transcription was restarted, which promoted liver fibrosis progression[139]. In addition, epigenetic regulation mediated by lncRNAs through direct interaction with PRC2 family members were observed in fibrogenesis of other different organs, inspiring the investigation of these mechanisms in liver fibrosis. Similarly, lncRNA-NEAT1 promoted cardiac fibrosis by recruiting EZH2 to the promoter region of *Smad7* and subsequently inhibiting *Smad7* expression[140]. In diabetic nephropathy-related renal fibrosis, lncRNA-GAS5 recruited EZH2 to the *Mmp9* promoter region and inhibited the *Mmp9* expression, alleviating renal fibrosis progression[141]. Collectively, these results have verified that lncRNAs have great potential in guiding PRC2-mediated gene-specific epigenetic modification and targeting the complex of specific lncRNA and PRC2 may provide a promising therapeutic target against liver fibrosis, which is more selective than directly targeting the catalytic activities of PRC2.

#### 4.2.3. *Other ncRNA-directed epigenetic modification*

Indeed, in addition to miRNA and lncRNA, circular RNA (circRNA) and piwi-interacting RNA (piRNA) may also play vital important roles in epigenetic modifications by affecting miRNA and lncRNA or directly affecting gene transcription. circRNAs are characterized by a covalently closed continuous loop and act through miRNA sponges or ceRNA mechanisms[142]. circRNA, named hsa_circ_0007874, increased PTEN transcription and thus inhibited HSC activation. Detailly, luciferase reporter assay and pull-down assays illustrated that hsa_circ_0007874 bound with miR-181b-5p in cytoplasm of HSC and further suppressed the inhibition of miR-181b-5p on *PTEN*[143]. As a miR-223-3p sponge, hsa_cir_0070963 bound with miR-223-3p and thus suppressed the inhibition effects of miR-223-3p on LEM domain containing 3 (*LEMD3*), which inhibited HSC activation in hepatic fibrosis[144]. Circ F-Box And WD Repeat Domain Containing 4 (CircFBXW4) was downregulated in liver fibrogenesis and was illustrated to bind to miR-18b-3p in primary mouse HSCs. Enforcing expression of circFBXW4 inhibited HSC activation by reducing the suppression of miR-18b-3p on *FBXW7*[145]. Similarly, mmu_circ_34116 inhibited HSC activation by binding with miR-22-3p and targeting miR-22-3p/BMP7 axis[146]. CircPSD3 bound with miR-92b-3p and thus suppressed its inhibition on Smad7, which alleviating hepatic fibrosis[147]. Meanwhile, circ-PWWP2A was significantly upregulated in TGF-$\beta$ and LPS-stimulated HSC and was suggested to promoted HSC activation and proliferation by sponging miR-203 and miR-223 and increasing follistatin-like 1 (*FSTL1*) and *TLR4*[148]. Recently, a circRNA microarray identified that circRNA-0067835 promoted HSC activation by binding with miR-155 and reversing *FOXO3* expression in thymosin beta 4 (T$\beta$4)-stimulated HSC[149]. Regarding piRNAs, Xue and his colleagues isolated primary HSCs from carbon tetrachloride and bile duct ligation-stimulated mice and demonstrated that piR-823 increased TGF-$\beta$ expression and activated HSCs *via* binding with eukaryotic initiation factor 3B (*EIF3B*)[150]. Collectively, ncRNA network is more than just the interaction of cirRNAs and piRNAs with miRNA. Investigating the further connections among different ncRNAs could be a promising research direction in the future.

## 5. Promising therapeutic directions and challenges ahead

Based on all the evidence summarized above, it is not difficult to observe that many studies have clarified the epigenetic mechanisms involved in the pathogenesis of liver fibrosis. However, therapeutic strategies targeting epigenetic regulation to treat liver fibrosis have yet to be developed. Among epigenetic targets, specific inhibitors of EZH2, including DzNep, GSK126, GSK926, and GSK343 have been reported (Table 3). The first EZH2 inhibitor DzNep, an *S*-adenosyl-L-homocysteine (SAH) hydrolase inhibitor, inhibits EZH2 *via* repressing SAM-dependent histone methyltransferase activity[151]. Subsequently, highly selective SAM competitive inhibitors including GSK926 and GSK343 have been generated, which own a 2-pyridone core structure[152,153]. Among these inhibitors, GSK126 is demonstrated to be highly selective when compared with other inhibitors and its safety was evaluated by a phase 1 clinical trial.

Indeed, histone methyltransferases play essential roles in the pathophysiology of cancer, thus, different types of specific inhibitors were initially developed for cancers. For example, the inhibitor of H3K36 methyltransferases SETD2, EZM0414, the inhibitors of H3K79 methyltransferases, including EPZ5676, SGC0946 and EPZ004777, and the inhibitors of KAT2A, YF-2, SR-18292 and CPTH2, have been demonstrated to be applied in antitumor treatments[157]. These inhibitors are gradually being evaluated in clinical trials and are promising to be used in further liver fibrosis therapeutics. Additionally, genetic approaches targeting methyltransferases were employed to alleviate liver fibrosis in basic studies, but no such candidate therapies have been demonstrated. For instance, G9a is responsible for the three methylation states of H3K9 and multiple studies confirmed that siRNA-G9a was the commonly used antifibrotic regimen[158]. While inhibitors of G9a have not yet been clearly characterized, suggesting that developing specific inhibitors of G9a may capture the windvane of further antifibrotic studies.

Notably, several pharmacological activators or inhibitors have been used to regulate histone acetylation, and HDAC enzyme inhibitors, including HNHA, Vorinostat and VPA, are a powerful group of chemotherapeutic in clinical (Table 4).

It is not difficult to see the HDAC inhibitors are mainly broad-spectrum, targeting one class of HDACs or several HDACs, which hampers the development of specific anti-fibrosis drugs. Meanwhile, the roles of SIRTs, class III HDACs, are relatively consistent in diseases, and the SIRT activators and inhibitors have been identified (Table 5). At the same time, in anti-fibrosis studies, the plausible therapeutic or pathogenic roles of SIRTs are primarily verified by gene manipulation (overexpression or knockout). Thus, evaluating the antifibrotic activities of SIRTs regulators and developing specific SIRTs inhibitors instead of pan-HDACs inhibitors may become a feasible strategy.

In recent decades, numerous epigenetic studies have demonstrated the potential of $m^6A$ demethylase, including ALKBH5 and FTO, as therapeutic targets and several inhibitors have been reported to regulate $m^6A$ in multiple diseases. Surprisingly, researchers have summarized the inhibitors of ALKBH5 and FTO and organized several detailed and comprehensive reviews[173–176]. According to these reviews, available inhibitors of FTO and ALKBH5 are mainly 20G analogs or substrate-competitive inhibitors, and have been demonstrated to inhibit the proliferation of cancer cells, such as R-2HG, FB23-2, CS1, CS2 and NSC48890 in leukemia cells, MA2 in GSCs and MO-I-500 in breast cancer cells[173]. While only few inhibitors have been tested in fibrosis therapeutics. For example, ALKBH5 inhibitor IOX1 promoted $m^6A$ modifications of chemokine CCl28 mRNA and thus enhanced its stability. Therefore, IOX1 upregulated $T_{reg}$ recruitment and inhibited inflammatory cells *via* increasing CCl28 expression, which alleviated Ischemia–reperfusion injury-stimulated renal fibrosis mouse model[177]. It is noteworthy that radiation-induced ALKBH5 could inhibit toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) $m^6A$ methylation and thus activate NF-$\kappa$B–Smad2 pathway to activate HSCs[178]. It may attract interest whether ALKBH5 inhibitors could disturb TIRAP–NF-$\kappa$B–Smad2 pathway to suppress HSC activation and liver fibrosis for further studies. Collectively, further work is required to determine the anti-fibrosis effects of these antitumor $m^6A$ demethylase inhibitors in HSC activation and liver fibrosis mouse model.

Although molecular inhibitors of epigenetic-related enzymes are being evaluated and are expected to be used in liver diseases, it still faces considerable challenges. Currently, the molecular activators and inhibitors, including EZH2 inhibitors, HDAC inhibitors, SIRT activators and inhibitors are often used in cancer therapy, which has not been extended to liver fibrosis-related research. As aforementioned, the selectivity and specificity of these agonists and inhibitors need to be significantly improved. In addition, a more detailed understanding and analysis of epigenetic regulation mechanisms are required to ensure that the clinical use of these agonists and inhibitors reaches expected outcomes.

There may be therapeutic approaches by targeting the integrative effects between different epigenetic mechanisms, for

**Table 3** Small molecules with EZH2-inhibiting activity.

| EZH2 inhibitor | Disease | Fibrosis-related output | Status | Ref./Identifier |
|---|---|---|---|---|
| DzNep | Tumor | Inhibits HSC cell cycle arrest | Drug discovery | 34 |
| GSK126 | Lymphomas, multiple myeloma | Inhibits hepatocyte autophagy and HSC activation | I clinical trial | 154 |
| GSK503 | Tumors | Inhibits TGF-$\beta$ pathway | Drug discovery | 155 |
| EPZ-6438 | Lymphomas, advanced solid tumors | Induces HSC G2/M arrest and apoptosis | II clinical trial | 156 |
| MC4343 | Tumors | _ | I clinical trial | NCTO3854474 |
| MC4355 | Tumors | _ | II clinical trial | NCTO3213665 |
| CPI-1205 | B-cell lymphoma | _ | II clinical trial | NCT02395601 |
| CPI-360 | Tumors | _ | I clinical trial | NCTO2860286 |
| CPI-169 | Tumors | _ | Pre-clinical | WO2013120104A2 |
| EPZ005687 | Lymphoma, Solid tumors | _ | Pre-clinical | US20130317026A1 |

**Table 4** Small molecules with HDAC-inhibiting activity.

| HDAC inhibitor | Target | Disease | Fibrosis-related output | Status | Ref. |
|---|---|---|---|---|---|
| HNHA | Pan | Tumor | Improves hepatic function survival | FDA approval | 159 |
| Vorinostat | Pan | Cutaneous T-cell lymphoma, glioma | Inhibits TGF-$\beta$/SMAD signaling | FDA approve | 160 |
| VPA | Class I HDACs | Epilepsies, Partial | Inhibits TGF-$\beta$ and TNF-$\alpha$ signaling | FDA approve | 161 |
| Entinostat | Class I HDACs | Breast cancer, lymphoma | Inhibits hepatocyte death and type 2 inflammation | III clinical trial | 162 |
| BRD4884 | Class I HDACs | Memory disorders | Inhibits hepatocyte death and type 2 inflammation | Pre-clinical | 162 |
| NW21 | Class I HDACs | Hematologic neoplasms, solid tumors | Inhibits hepatocyte death and type 2 inflammation | Drug discovery | 162 |
| Largazole | Pan | Tumor | Inhibits VEGF signaling | Drug discovery | 163 |
| Parthenolide | HDAC4 | Tumor | Downregulates TGF-$\beta$ and upregulates CYP7A1 | Drug discovery | 164 |
| Valproate | Pan | Epilepsies | Inhibits HSC activation | Drug discovery | 165 |
| MC1568 | HDAC4/5/6 | Tumor | Inhibits HSC activation | Drug discovery | 166 |

instance, the interaction between DNA methylation and histone modification. Studies have demonstrated that combined effects of CpG binding protein Mecp2 and histone methyltransferase EZH2 are involved in PPAR$\gamma$ epigenetic repression and subsequent HSC activation. Mecp2 classically functions as a PPAR$\gamma$ transcriptional repressor by directly binding to the CpG island of PPAR$\gamma$ and thus mediating DNA methylation. Mann et al.[179] established *Mecp2*$^{-/y}$ and EZH2 deletion mice and identified stimulation of EZH2 and subsequent H3K27me3 in the downstream exon of PPAR$\gamma$ gene as a second mechanism through which Mecp2 achieves transcriptional silencing. Yang et al.[180] further reported polyphenolic rosmarinic acid reversed PPAR$\gamma$ expression and thus alleviated cholestatic fibrosis by reducing expression and recruitment of Mecp2 in PPAR$\gamma$ promoter and decreasing EZH2-mediated H3K27me3 at 3′exon, which suggested the possibility of Mecp2-EZH2 inhibition as effective anti-fibrosis strategies. In addition to the Mecp2-EZH2 targeting inhibitor, the simultaneous targeting of H3K9 methyltransferase G9a and DNA methyltransferase DNMT1 has been reported to alleviate liver fibrosis. During HSC activation, the serine−glycine metabolic pathway was indeed important for collagen synthesis, and TGF-$\beta$ promoted HSC activation by decreasing gluconeogenic enzymes genes fructose-bisphosphatase 1 (*FBP1*) and phosphoenolpyruvate carboxykinase (*PEPCK*) and metabolic regulator peroxisome proliferator-activated receptor $\gamma$ coactivator 1$\alpha$ (*PGC-1*$\alpha$). Mechanically, G9a and DNMT1 exited in a stable complex and colocalized at the promoter of several genes, including FBP1, PEPCK and PGC-1$\alpha$. By forming a complex, G9a delivered transcriptional suppressor H3K9me2 signature and meanwhile, DNMT1 stimulated DNA methylation on these genes, which synergistically suppressed gene transcription. Thus, focused on the corporation between G9a and DNMT1, Marina and his

**Table 5** Small molecules with SIRT-activating or SIRT-inhibiting activities.

| Compound name | Target | Disease | Fibrosis-related output | Status | Ref./identifier |
|---|---|---|---|---|---|
| SIRT activators | | | | | |
| Resveratrol | SIRT1, SIRT3, SIRT5 | Osteoarthritis | Increases IL-10 to reprogramme macrophages | III clinical trial | 167 |
| Sildenafil | SIRT1, SIRT3 | Huntington | Increases GSH and SOD and decreases TNF-$\alpha$ | II clinical trial | 168 |
| JP-022 | SIRT1, SIRT2, SIRT3 | Alzheimer's diseases | _ | Pre-clinical | WO2016028910A1 |
| SRT1460 | SIRT1 | Diabetes mellitus, type 2 | _ | Pre-clinical | CN103145738A |
| R-Cu | SIRT1 | Alzheimer diseases | _ | II clinical trial | CTRI/2019/07/020289 |
| SIRT inhibitors | | | | | |
| MDL-800 | SIRT6 | Hepatocellular carcinoma | Inhibits phosphorylation and nuclear localization of SMAD2 | Pre-clinical | 84 |
| EH-301 | SIRT5 | Acute kidney injury | Inhibits skin fibroblasts | II clinical trial | 169 |
| SP-624 | SIRT6 | Depressive disorder | _ | II clinical trial | NCT04510298, NCT04479852 |
| AGK2 | SIRT2 | Tumor | _ | Pre-clinical | 170 |
| Cambinol | Pan | Leukoencephalopathy, progressive multifocal | _ | Drug discovery | 171 |
| Ex-527 | SIRT1 | Tumor | _ | Drug discovery | 172 |

colleagues observed that CM272, a novel/classic G9a/DNMT1 inhibitor, reversed the expression of FBP1, PEPCK and PGC-1$\alpha$ and thus inhibited the fibrotic phenotypes[181]. While several "Activator" histone methylation and active histone acetylation signatures have also been reported to interact with DNA methylation[182,183]. Overall, it remains unclear which factor—DNA methylation or histone modification—contributes more significantly to the outcome resulting from the interaction between the two. On the one hand, DNA methylation is the modification of gene sequence, which is more direct than histone modification in gene transcription regulation. On the other hand, histone modification is catalyzed by multiple enzymes and targets multiple sites of histones, providing more possibilities for regulating gene expression. The current understanding of these interaction effects is insufficient to support the development of therapeutic strategies. Further research is needed to deepen our knowledge in this area.

## 6. Conclusions

In summary, increasing evidence has comprehensively revealed the vital role of epigenetics in the progression of fibrosis, highlighting the complexity of underlying mechanisms and emphasizing the therapeutic potential of epigenetic modulation in the treatment of liver fibrosis. However, the findings are not fully consistent. Further characterization of the specificity of epigenetic modifications would offer novel perspectives for developing effective and highly selective epigenetic therapies and may represent a promising approach with bright prospects for the treatment of chronic liver diseases.

## Acknowledgements

This study was supported by the National Key Research and Development Program on Modernization of Traditional Chinese Medicine (Grant No. 2022YFC3502104, China). This study also received supports from VA Merit Award 5 I01 BX005730 and National Institutes of Health Grant R01 R01DK-057543 to Huiping Zhou (USA), National High-Level Talents Special Support Program to Xiaojiaoyang Li (China), and Fundamental Research Funds for the Central Universities (Grant No. 2023-JYB-JBZD-046 to Xiaojiaoyang Li, China). Huiping Zhou is a VA Research Career Scientist (IK6BX004477, USA).

## Author contributions

Xiaojiaoyang Li, Huiping Zhou, and Runping Liu conceived the original idea and supervised the study. Runping Liu and Yajing Li prepared the manuscript and figures. Qi Zheng and Mingning Ding revised figures. All data were generated in-house and no paper mill was used. All authors have approved the final manuscript.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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