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# New advances of DNA methylation in liver fibrosis, with special emphasis on the crosstalk between microRNAs and DNA methylation machinery☆

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ABSTRACT

Epigenetics refers to the study of heritable changes in the pattern of gene expression that is controlled by a mechanism specifically not due to changes the primary DNA sequence. Well-known epigenetic mechanisms include DNA methylation, post-translational histone modifications and RNA-based mechanisms including those controlled by small non-coding RNAs (miRNAs). Recent studies have shown that epigenetic modifications orchestrate the hepatic stellate cell (HSC) activation and liver fibrosis. In this review we focus on the aberrant methylation of CpG island promoters of select genes is the prominent epigenetic mechanism to effectively silence gene transcription facilitating HSC activation and liver fibrosis. Furthermore, we also discuss epigenetic dysregulation of tumor-suppressor miRNA genes by promoter DNA methylation and the interaction of DNA methylation with miRNAs involved in the regulation of HSC activation and liver fibrosis. Recent advances in epigenetics alterations in the pathogenesis of liver fibrosis and their possible use as new therapeutic targets and biomarkers.



**Contents**



*Abbreviations:* miRNAs, small non-coding RNAs; HSC, hepatic stellate cell; ECM, extracellular matrix; α-SMA, α-smooth muscle actin; TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemotactic protein-1; DNMTs, DNA methyltransferases; MBD, methyl-CpG-binding domain; 5-azadC, 5-aza-2′-deoxycytidine; PTEN, Phosphatase and tensin homologue; PI3K, Phosphatidylinositol-3-kinase; RASAL1, Ras GTPase activating-like protein 1; PTCH1, patched1; Gli1, glioma-associated oncogene homolog 1; PPARγ, Transcription factor peroxisome proliferator-activated receptor- γ; NFκB, Nuclear factor κB; TET, Ten-Eleven- Translocation; TDG, thymine DNA glycosylase; BER, base excision repair; pri-miRNAs, primary miRNA transcripts; miRNPs, miRNA–protein complexes; UTR, untranslated region; RBPs, RNA-binding proteins; TS-miRNA, tumor-suppressive miRNA; TSG, tumor-suppressor gene; GSTP1, glutathione S-transferase pi 1; CDH1, E-cadherin 1; BDNF, brain-derived neurotrophic factor; IUGR, Intrauterine growth restriction.



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## 1. Introduction

Liver fibrosis results from persistent liver jury, including viral hepatitis, alcohol abuse, metabolic diseases, autoimmune diseases, and cholestatic liver diseases [1]. During fibrosis progression, inflammation and liver injury trigger complex cellular events that result in collagen deposition and the disruption of the normal liver architecture [2]. Over the last two decades, sinusoidal resident hepatic stellate cells (HSCs) have been commonly recognized as the major source of extracellular matrix (ECM). In the normal liver, HSCs are quiescent, vitamin A-storing adipogenic cells, However, following a fibrogenic stimulus, HSCs undergo a complex activation process associated with morphological changes from a quiescent vitamin A-storing cell to that of an activated myofibroblast-like cell [3,4]. HSC activation is also associated with a dramatic increase in the synthesis and deposition of ECM components, marked upregulation of α-smooth muscle actin (α-SMA), collagen, tissue inhibitors of metalloproteinases (TIMP1) and desmin, production of profibrogenic cytokines/growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), as well as pro-inflammatory molecules including interleukin (IL)-6, intercellular adhesion molecule-1 (ICAM-1) and monocyte chemotactic protein-1 (MCP-1) [5–7].

Because HSC activation and liver fibrosis are orchestrated by the same signals, for example by growth factors such as TGF-β, the molecular mechanisms which exert global control of HSC activation and liver fibrosis incompletely understood. Recent works from our group and from others implicated that epigenetic modifications play an important role in determining HSC activation and liver fibrosis (Fig. 1). Here we review insights into the role of epigenetics in HSC activation and liver fibrosis.

## 2. The pathogenesis of liver fibrosis

Liver fibrosis, irrespective of aetiology, is a dynamic and highly integrated molecular, tissue and cellular process that leads to progressive accumulation of ECM components in an attempt to limit hepatic damage in chronic liver diseases [8]. The terminal outcome of liver fibrosis is liver cirrhosis, a condition characterized by distortion of the normal architecture, septae and nodule formation, altered blood flow, portal hypertension, hepatocellular carcinoma and ultimately liver failure [9]. The hepatic stellate cell (HSC) is the main fibrogenic cell type orchestrating the deposition of ECM in the injured liver and it also has been identified as a primary effector in liver inflammation [4].

HSCs are resident perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells [10]. These cells are strategically positioned to intimately interact with hepatocytes, endothelial cells, and nerve endings through their numerous processes extending across the space of Disse [11]. Under pathological conditions, including injury, inflammation, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, quiescent HSC have been reported to undergo a particular process of activation which involves significant changes in morphology and phenotypical responses observed in either human or rat HSC when cultured on plastic substrate [12–14].

Several factors have been identified to promote HSC activation. Damage to hepatocytes and Kupffer cell activation are still considered the main effectors driving HSC activation [15,16]. Mediators released from damaged hepatocytes, such as lipid peroxidation products, intermediate metabolites of drugs or hepatotoxins, acetaldehyde and 1-hydroxyethyl radical from alcohol metabolism as well as reactive oxygen species (hydrogen peroxide, superoxide radical and others are strong inducers of HSC activation [17].Once activated by bacterial products, Kupffer cells secrete a large number of pro-inflammatory and fibrogenic mediators. Activation of HSC by macrophage-derived TGF-β or insulin-like growth factor is an early feature of fibrogenesis which promotes a switch in HSC gene expression to initiate matrix remodeling [18].

Advances of understanding gene regulation in HSCs has paralleled the dramatic expansion of knowledge about both traditional mechanisms of gene regulation, including transcription factor activity, localization and modification, as well as epigenetic regulation of gene expression by DNA methylation, histone modification and microRAN interactions [19–24]. Elucidating the precise molecular mechanisms underlying HSC activation and liver fibrosis is translating into fruitful new therapeutic approaches.

## 3. Overview of DNA methylation

The methylation of the C5 position of the cytosine base with S-adenosyl methionine as the methyl donor is found in approximately 70–80% of CpG dinucleotides in somatic mammalian cells and to some extent in non-CpG sequences in embryonic stem cells [25,26]. DNA methylation is currently the most widely studied form of epigenetic programming. The methylation of cytosine residues within CpG sequences is catalysed by DNA methyltransferases (DNMTs) [27]. In mammals, five members of the DNMT family have been identified: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. Among these proteins, only DNMT1, DNMT3A, and DNMT3B exhibit methyltransferase activity.DNMT3a and DNMT3b target unmethylated CpGs and therefore are termed de novo methyltransferases, while DNMT1 maintains DNA methylation during replication by copying the methylation pattern of the parent DNA strand onto the newly synthesized strand [28,29].

DNA methylation of the promoter regions is generally related to transcriptional repression through different mechanisms, including the inhibition of transcription factor binding and the recruitment of methyl-CpG-binding domain (MBD) proteins and their associated complexes [30]. So far, six methyl-CpG-binding proteins, including MeCP2, MBD1, MBD2, MBD3, MBD4 and Kaiso, have been reported in mammals [31]. MeCP2 is a member of a small family of methylated DNA-binding domain proteins that was first described through its affinity for DNA sequences containing methylated 5′-CpG-3′dinucleotides [32]. The ability of MeCP2 to bind methylated DNA has been interpreted in the context of transcriptional repression and silencing of specific target genes.In addition, MeCP2 binds the corepressor mSin3A, which is thought to recruit histone deacetylases, providing a mechanism for the transcriptional repression of genes with methylated CpG sites [33]. Interestingly, MeCP2 was shown to associate with the transcriptional activator CREB1 at the promoter of somatostatin, a gene upregulated in Mecp2 duplication mice, thereby suggesting a potential activation mechanism [34].

## 4. Methylated genes in liver fibrosis

Abnormal patterns of DNA methylation in liver fibrosis have been recognized over the last few years and so far a number of aberrantly hypermethylated genes have been discovered. These genes have been found to be hypermethylated either by direct examination of



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