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The Mechanisms of HSC Activation and Epigenetic Regulation of HSCs Phenotypes

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Abstract Epigenetics is a dynamically expanding field of science entailing numerous regulatory mechanisms controlling changes of gene expression in response to environmental factors. Over the recent years there has been a great interest in epigenetic marks as a potential diagnostic and prognostic tool or future target for treatment of various human diseases. There is an increasing body of published research to suggest that epigenetic events regulate progression of chronic liver disease. Experimental manipulation of epigenetic signatures such as DNA methylation, histone acetylation/methylation and the activities of proteins that either annotate or interpret these epigenetic marks can have profound effects on the activation and phenotype of HSC, key cells responsible for onset and progression of liver fibrosis. This review presents recent advances in epigenetic alterations, which could provide mechanistic insight into the pathogenesis of chronic liver disease and provide novel clinical applications.

Keywords Epigenetics · Hepatic stellate cells · Liver fibrosis · DNA methylation · MicroRNAs · Histone modyfications

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Introduction

Chronic liver disease (CLD) is one of the leading causes of mortality worldwide that is still on the rise; the term includes wide-ranging liver diseases, from steatosis, fibrosis and cirrhosis to hepatocellular cacrinoma [1]. The current major causes of CLD include viral infections (HBV, HCV), xenobiotics (alcohol, prescription drugs), metabolic disease (obesity-associated hepatic steatosis), inherited disorders (haemochromatosis, Wilson's disease) and autoimmune hepatitis [1]. Common to all of these injuries is a pathobiology that is triggered by hepatocellular damage which, if persistent, can establish a chronic inflammatory state. The majority of individuals do not progress beyond chronic hepatitis and compensate for lost tissue mass by the highly regenerative capacity of the liver. However, in a significant minority of people (10–20 %) the ongoing cell death and hepatitis stimulate the net deposition of extracellular matrix that can lead to fibrosis. If unchecked, the fibrotic process becomes progressive and self-sustaining resulting in the disturbance of normal tissue architecture and hepatic functions. End-stage liver disease is characterized by the maturation of fibrosis into cirrhosis where the profound loss of liver structure and function becomes life threatening and the risk of liver cancer dramatically increases [2]. The molecular mechanisms underlying CLD are still incompletely understood, with liver transplantation remaining the only effective treatment for the end stage of this disease.

When the liver is injured a wound healing response is mounted, which includes the generation of activated myofibroblasts that promote the formation of granulation tissue, a key intermediate step in the repair process [3]. It is now accepted that transdifferentiation of HSC is the major event responsible for production of hepatic myofibroblasts

[4]. In normal liver, HSC are quiescent perisinusoidal cells located within the space of Disse where they function to store retinoid and lipid droplets [5]. In response to tissue damage the quiescent HSC undergoes a dramatic reprogramming of its epigenome and transcriptome to enable its transdifferentiation to an ECM-producing myofibroblast [6]. The fate of the HSC-derived myofibroblast is then dictated by subsequent repair and injury. In the case of an acute transient injury, myofibroblasts are either cleared by apoptosis or alternatively a proportion may reverse their phenotype to a more quiescent state [4, 7]. However, if there is repeated injury to the liver, as in chronic disease, then HSC-derived myofibroblasts persist in the tissue and via both paracrine and autocrine pathways drive the formation of mature fibrotic matrix. In addition, new evidence suggests that the persistence of HSC-derived myofibroblasts may actively repress hepatocyte regeneration via their production of transforming growth factor β1 (TGFβ1) [8].

Cell phenotype and gene expression are governed by epigenetic mechanisms including DNA methylation, histone modifications and non-coding RNA [9, 10]. The term "epigenetics" is defined as heritable changes in gene expression without alteration in DNA sequence. These alterations change the structure of chromatin, which is a complex of DNA associated with proteins called histones [11]. The smallest unit of chromatin is the nucleosome, which consists of 147 bp of DNA wrapped around a core of eight histone molecules (two copies each of H2A, H2B, H3 and H4). The transcriptional state of chromatin is influenced by covalent modifications to either DNA or histones, which regulate gene expression [12]. Due to chromatin condensation DNA is tightly packed and poorly accessible to transcription factors or chromatin-associated proteins, which leads to transcriptional silencing [13]. Conversely, gene activation requires chromatin to be in unfolded state and as a result it is accessible to polymerases involved in gene transcription [14].

The role of epigenetic mechanisms in hepatic myofibroblast transdifferentiation has been previously demonstrated in studies showing that the methyl CpG binding protein 2 (MeCP2) facilitates myofibroblast transdifferentiation by silencing peroxisome proliferator-activated receptor gamma (PPARgamma) gene, a master regulator of adipogenic phenotype of the quiescent HSC [15]. Moreover, the MeCP2-regulated histone methyltransferase ASH1 is a key transcriptional activator that upregulates the expression of profibrogenic genes by stimulating Histone 3 Lysine 4 methylation (H3K4me) and therefore guides a transcriptionally permissive state of chromatin [16].

Cirrhosis, the final outcome of liver fibrosis is highly variable between patients, with only a minority progressing to this end stage, irrespective of disease aetiology [17–19]. Factors that contribute to variation in progression of CLD include age-of-onset of disease, sex, a multitude of lifestyle-associated factors (e.g. smoking, weight, alcohol consumption, co-morbidities etc.), genetic influences and environmental factors that modulate gene expression via epigenetic mechanisms [9, 10, 20, 21]. Furthermore, it is possible that population variability may be influenced by environmentally-induced factors transmitted between generations via heritable epigenetic marks [22••]. However, the exact mechanism for this type of transgenerational epigenetic transmission is still to be discovered. In this review, we consider recent discoveries concerning the epigenetic mechanisms that regulate the HSC phenotype and the fibrogenic process which may vary considerably between individuals to influence disease progression.

Overview of DNA Methylation

DNA methylation at position five of the cytosine ring occurs at most CpG dinucleotides in the mammalian genome. DNA methylation of the promoter region is generally associated with repressed chromatin state and inhibition of gene expression, whereas methylation within gene body may be responsible for gene activation [23]. Methylation can inhibit gene expression by two mechanisms, including transcriptional repression through the interference with the binding of transcription factors or via the recruitment of methyl-binding proteins (MeCP2, MBD1-4), which interact with chromatin silencing complexes [23]. Two classes of DNA methyltransferases (DNMTs) are responsible for introducing cytosine methylation. The de novo methyltransferases DNMT3A and DNMT3B stimulate and catalyse the addition of methyl groups at previously unmethylated CpG sites, whereas DNMT1 is responsible for the maintenance of methylation patterns onto the daughter strand during DNA replication [24]. Until recently DNA methylation was considered as a stable mark; however, the discovery of TET enzymes that convert methylCpG to hydroxymethylCpG has revealed the highly dynamic nature of this epigenetic mark [25].

DNA methylation is essential for embryonic viability and is involved in key cellular processes, including transcriptional repression, X-chromosome inactivation and imprinting. Alterations in DNA methylation have been associated with a variety of human diseases including imprinting disorders and cancers [26]. Both global hypomethylation and hypermethylation have been linked with cancer. Global hypomethylation is responsible for genomic instability, whereas hypermethylation of CpGs islands leads to direct silencing of tumour suppressor genes [26].

DNA Methylation in Liver Fibrosis

DNA methylation and its associated regulatory proteins are now considered to be mechanistically important in fibrosis, in particular there is strong evidence of a pivotal orchestrating role for MeCP2 in myofibroblast activation and fibrogenesis. MeCP2 is a methyl-DNA-binding protein which due to its association with Rett syndrome has been mostly extensively examined in neurons [27]. The most compelling evidence for a role for MeCP2 in fibrosis are the reports that MeCP2-deficient mice are protected from liver [15] and lung fibrosis [28]. Moreover, MeCP2 is functionally implicated in myofibroblast differentiation in the heart [29] and eye [30]. Nevertheless, there is only a basic knowledge available as to how MeCP2 controls the myofibroblast phenotype. MeCP2 is upregulated in the earliest phases of HSC transdifferentiation via post-transcriptionally mechanisms, it then binds to methylated-CpG sites in the HSC genome and can subsequently recruit protein complexes which remodel chromatin [15]. A key target of MeCP2 in HSC is the PPARgamma, gene which must be transcriptionally silenced for the HSC to lose its quiescent phenotype and undergo transdifferentiation [31]. MeCP2 can also act as an activator of gene transcription in both neurons and HSC; however, the mechanisms responsible for this effect are still unclear. In neurons, MeCP2 binds to 5-hydroxymethylcytosine (5hmC), a modification of DNA methylation that may be associated with transcriptionally active genes, however, it is not understood how this combination of MeCP2 and 5hmC influences chromatin structure [32••]. In HSC it is proposed that, MeCP2 positively regulates expression of the histone methyltransferase, ASH1, which is responsible for attachment of methyl group to histone H3 lysine 4, a signature of actively transcribed genes. During HSC transdifferentiation, ASH1 binds to the regulatory regions of alpha smooth muscle actin (αSMA), collagen 1A1, tissue inhibitor of metalloproteinase-1 (TIMP1) and TGFβ1 genes. Depletion of this methyltransferase causes decreased expression of these profibrogenic genes indicating it is likely to be a downstream mediator of MeCP2-dependent activation of transcription [16].

Altered patterns of DNA methylation during HSC activation have been found at specific loci by studying the methylated CpGs or by treatment with DNA demethylating agent, 5-aza-2′-deoxycytidine (5-azadC) [33]. 5-azadC is incorporated into DNA during repilication and inhibits DNA methyltransferase activity, therefore, genes are demethylated and reactivated. 5azadC reverses epigenetic repression of the PPARgamma gene in hepatic myofibroblast, indicating an inhibitory effect on HSC activation [33]. 5-azadC also blocks HSC proliferation by preventing loss of expression of proliferation-associated genes including Ras GTPase activating-like protein 1 (RASAL1) [34], Patched 1 (PTCH1) [35] and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) [36], which are hypermethylated and consequently decreased in expression during HSC activation. Knockdown of MeCP2 increased the expression of RASAL1 and PTCH1 in myofibroblasts, suggesting that DNA methylation and MeCP2 may provide molecular mechanism for silencing these genes and a possible pathway by which myofibroblasts are activated [34, 35]. Decreased expressions of RASAL1 also contributes to renal fibrosis progression [37], suggesting RASAL1 as a potential diagnostic marker to predict fibrosis.

Medical plant extracts may have many potential antifibrotic effects; however, their mechanisms of action are still to be determined. The Chinese herbal prescription Yang-Gan-Wan, which contains rosmarinic acid and baicalin as active ingredients, prevents and reverses HSC activation by stimulating re-expression of PPARgamma [38]. Both rosmarinic acid and baicalin inhibit MeCP2 binding to the PPARgamma promoter and its suppression; prevent expression of EZH2, a histone methyltransferase that controls the repressive H3K27me3 mark which is also involved in PPARgamma gene silencing [38]. Curcumin, a polyphenol isolated from yellow pigment of the spice turmeric, is able to suppress liver fibrosis through upregulation of PTEN [36]. PTEN is a tumour suppressor and a negative regulator of liver fibrosis, hypermethylation of the PTEN promoter is responsible for the loss of PTEN expression during HSC activation. Curcumin treatment also increases expression of miR-29b, which is involved in the hypomethylation of PTEN through downregulation of DNMT3b, this leads to suppression of HSC activation [36].

DNA methylation is an established regulator of gene transcription, and an epigenetic mark which has an important role in liver fibrosis. Given that DNA methylation is a highly dynamic modification it is likely that functionally instructive alterations in the HSC methylome occur during transdifferentiation, however, at present this concept has not been experimentally investigated. Genome-wide studies of changes in DNA methylation during HSC activation are now warranted as they would bring new insights into the molecular events underpinning fibrogenesis and may provide biomarkers for disease progression as well as potential drug targets.

Non-Coding RNAs (ncRNAs)

Numerous microRNAs (miRNAs) are involved in a variety of biological processes relevant to fibrosis, such as proliferation, apoptosis, TGFβ signalling and extracellular matrix deposition [39, 40]. miRNAs are small (22

Table 1 List of microRNAs involved in liver fibrosis

microRNA in liver fibrosis		
Upregulated miRNA	Target in liver fibrosis	References
miR-181b	p27	Wang et al. [46]
miR-9	CXCR5; CXCL6	Noetel et al. [47]
miR-128	CXCL2; CCL5; CXCL11; CCL4	
miR-125b	CXCL2; CXCL1	
miR-21	SMAD 7, correlation with MMP-2; PTEN	Zhang et al. [49], Wei et al. [48]
miR-33a	PPAR-α; correlation with Col1A1, α-SMA	Li et al. [50]
miR-221/222	p27; PTEN; correlation with Col1a1	Ogawa et al. [51]
miR-214-5p	Correlation with MMP-2; α-SMA, TGF-β1	Iizuka et al. [52]
miR-571	CREBBP	Roderburg et al. [53]
miR-652	Not known	
Downregulated miRNA	Target in liver fibrosis	References
miR-122	P4HA1	Li et al. [54]
miR-133a	Correlation with collagens (Col1a1, Col5a3)	Roderburg et al. [53]
miR-146a	SMAD4	He et al. [56]
miR-29	PDGF-C; IGF-I; Col1a1; Col4a1, correlation with phospho -FADD; cleaved caspase-3, 8; Bax, Bcl-2, PARP, NFκB	Kwiecinski et al. [57], Tiao et al. [73]
miR-19b	TGFβRII	Lakner et al. [58]
miR-126	VEGF-A	Guo et al. [74]

nucleaotides long) non-coding RNA molecules that inhibit gene expression by targeting the 3′-untranslated region (3′-UTR) of numerous mRNAs to affect their stability and translation. A single miRNA may bind to a number of mRNAs transcripts and it is estimated that miRNAs regulate the expression of more than one-third of all human genes [41]. Dysregulation of miRNAs is often associated with human disease [42, 43] and recent studies suggest that miRNAs are functional in HSC transdifferentiation and liver fibrosis [44]. Microarray assays have revealed 12 upregulated miRNAs (miR-874, miR-29C*, miR-501, miR-349, miR-325-5p, miR-328, miR-138, miR-143, miR-207, miR-872, miR-140 and miR-193) and nine that are downregulated (miR-341, miR-20b-3p, miR-15b, miR-16, miR-375, miR-122, miR-146a, miR-92b and miR-126), during activation of rat HSCs [45]. However, potentially there are many more miRNA involved in control of fibrosis including miR-181b [46], miR-9, miR-125b, miR-128 [47], miR-21 [48, 49], miR-33a [50], miR-221/222 [51], miR-214-5p, miR-199a [52], miR-571 and miR-652 [53] which have been show to be expressed in HSC-derived myofibroblasts. By contrast miR-122 [54], miR-133a [55], miR-146a [56], miR-29 [57] and miR-19b [58] are thought to be associated with quiescent phenotype of HSC (Table 1).

TGFβ1 is a key driver of HSC transdifferentiation and is a regulator of microRNA expression [59]. TGFβ stimulates miR-181b, a regulator of cell proliferation via its control of p27 [46]. HSC expression of miR-33a correlates with TGFβ1-induced expression of α1 (I) collagen (Col1A1) and α-SMA, while PPARalpha is another putative target of miR-33a [50]. Treatment of HSC with TGFβ1 significantly downregulates miR-133a and miR-146a, which leads to decreased levels of type I collagen [55, 56]. It has been predicted that SMAD4 is a target of miR-146a [56]. The microRNA miR-19b is a negative regulator of TGFβ signalling by targeting TGFβ receptor II [58]. These discoveries indicate complex regulation of TGFβ1 signalling by a network of upstream and downstream miRNAs that may provide new strategies for targeting TGFβ1-driven fibrosis. However, more likely is that miRNAs will be exploited for biomarker development and in this context both miR-181b and miR-133a are elevated in the serum of cirrhosis patients compared to healthy controls [46, 55]. This finding could be helpful to design new diagnostic markers, however, first it is important to study how expression level of these miRNAs is related to the stage of liver fibrosis.

It is now emerging that there are now many different types of non-coding RNAs including the long non-coding RNAs (lncRNAs) that are predicted to contribute to disease pathologies [60]. LncRNAs are transcripts of over 200 bp in length that are involved in diverse biological processes such as epigenetic modifications of DNA by recruiting

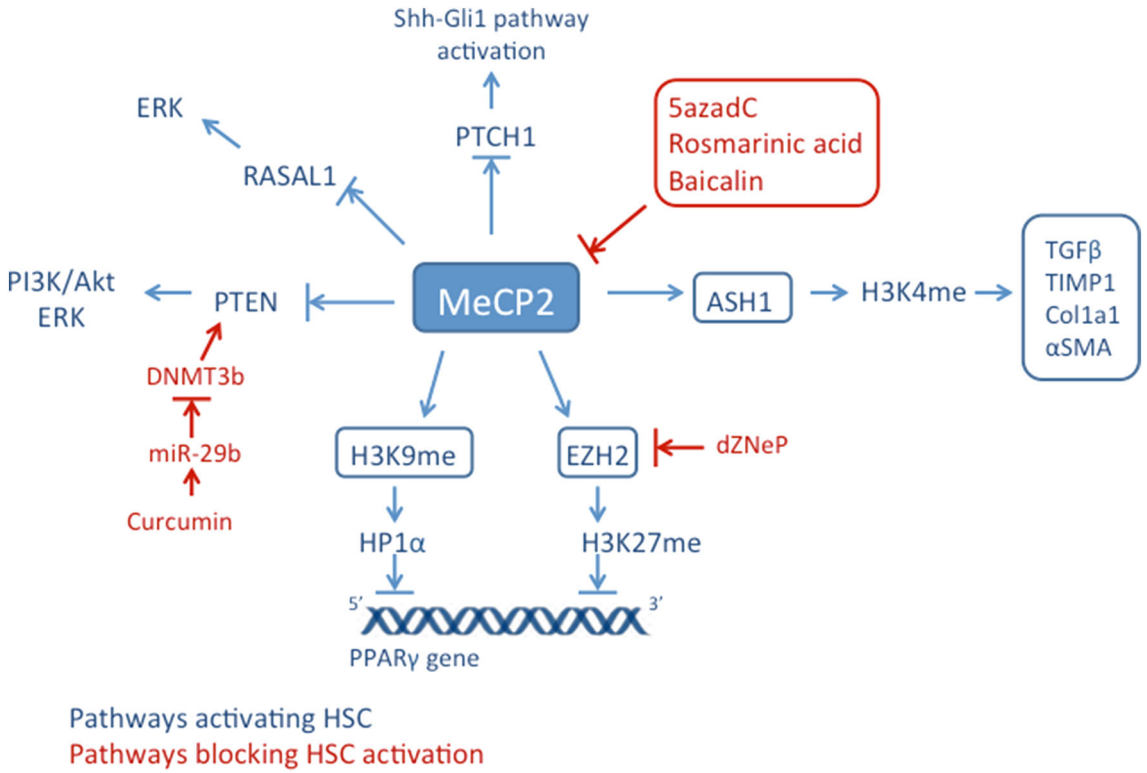

Fig. 1 Overview of MeCP2 role in liver fibrosis. MeCP2 upregulates expression of EZH2 resulting in silencing of the PPARgamma gene; MeCP2 also positively regulates expression of histone methyltransferase, ASH1, which is involved in activation of profibrogenic genes (TGFβ1, TIMP-1, Col1a1, αSMA) by trimethylating H3K4. In addition, MeCP2 promotes liver fibrosis by repressing expression of RASAL1, PTEN and PTCH1, which causes activation of ERK and Shh-Gli1 pathways and consequently leads to HSC activation

chromatin remodelling complexes to specific loci, regulation of chromatin accessibility in a process that involves histone modification enzymes and RNA polymerase or X-chromosome inactivation [60, 61]. The association between disruption of lncRNAs patterns and human disease has been already reported and in the future may prove to be of importance in liver fibrosis.

Histone Modifications

Amino-terminal tails of histones contain residues that are subjected to a diverse number of posttranslational modifications, including acetylation, methylation, phosphorylation and ubiquitination among others [9, 10]. Epigenetic control of gene expression at the level of chromatin modification is highly complex and in part entails attachment of the aforementioned chemical groups to histones; therefore, specific enzymes controlling histone modifications may play a crucial role in transdifferentiation of HSC. Histone acetylation is almost always linked with gene activation, whereas histone methylation is more modulatory with the ability to influence both the silencing and activation of genes [62]. There are many known epigenetic enzymes which carry out the attachment (histone methyltransferases) or removal (histone demethylases) of methyl groups to different lysine residues within histones [62]. The effect of histone modifications on chromatin structure depends on the specific lysine residue modified. Enrichment of histone trimethylation at H3K4, H3K36 and H3K79 is associated with active gene transcription [63]. By contrast, increase in histone trimethylation at H3K9, H4K20 and H3K27 are linked with gene silencing [64, 65].

As previously mentioned in this review, one of the ways in which MeCP2 promotes liver fibrosis is by silencing of PPARγ [15]. MeCP2 binds directly to the PPARγ promoter (Fig. 1) where it facilitates methylation of H3K9, this modification in turn provides binding sites for heterochromatin protein 1α (HP1α) and promotes transcriptional silencing. MeCP2 also stimulates expression of EZH2, which is recruited to the downstream coding region of PPARgamma gene where it increases the H3K27me3 mark and mediates polycomb-regulated transcriptional silencing. These two MeCP2-regulated epigenetic mechanisms, therefore, combine to ensure shut-down of PPARgamma expression by inhibiting both the initiation of transcription at the promoter and elongation of transcription along the gene body. In line with this, 3-deazaneplanocin (dZNep), a

potent inhibitor of EZH2, irreversibly blocks HSC activation [33]. However, treatment of skin fibroblasts with dZNep induces the expression of collagen and leads to skin fibrosis [66]. These apparently paradoxical effects of dZNep on fibrogenic mechanisms may be indicative of its potential for effects on several histone methyltransferases [67] or may reflect cell-specific functions for EZH2 and downstream H3K27 methylation.

Bile duct ligation (BDL), a experimental model of cholestatic liver disease, induces the hepatic expression of TGFβ1 and is linked with increased levels of active chromatin marks (H3K4me1, H3K4me2 and H3K4me3) and decreased levels of repressive marks (H3K9me2 and H3K9me3) at the TGFβ1 promoter [68]. BDL injury is also associated with increased expression of SET7/9, a H3K4 methyltransferase that is recruited to TGFβ1 promoter. SET7/9 gene knockdown significantly decreases BDL-induced TGFβ1 gene expression, serum enzymes and liver collagen content indicating a key role for this enzyme in fibrogenesis [68]. Retinoblastoma binding protein 2 (RBP2) is also an H3K4 demethylase and has been found in cirrhotic rat livers where its expression is stimulated by TGFβ. Depletion of RBP2 leads to reduced HSC proliferation and decreased levels of αSMA and vimentin, suggesting that RBP2 may be a potential target in treatment of liver fibrosis [69].

Histone acetylation is closely associated with transcriptional activity and is controlled by two groups of enzymes histone acetyltransferases (HAT) and histone deacetylases (HDAC) [10]. HDACs are significantly upregulated in chronic liver disease and the histone deacytylase inhibitor Trichostatin A suppresses HSC activation and proliferation which is associated with reduced expression of profibrogenic genes such as αSMA and collagen 1A1 [70, 71]. Additionally, MC1568, a compound with specificity towards class II HDACs, inhibits HSC proliferation and collagen secretion through the induction of microRNA-29, the latter having an antifibrotic function [57, 72]. HDAC inhibitors have become a centre of research interest, however, their functions are not completely understood. In order to use them as therapeutics, it is necessary to further study their activities and substrates, and also to generate HDAC inhibitors with greater specificity and lower toxicity.

Conclusions

Epigenetics is a dynamically expanding field of science, not only in terms of basic biology of chromatin but also in clinical environment where scientists are trying to discover changes underlying the pathobiology of their diseases of interest. There is increasing amount of evidence to show that epigenetic events control HSC activation and progression of liver fibrosis. Most recent research on epigenetics presents studies on three systems including DNA methylation, microRNA and histone modifications. However, it is important to emphasize the role of interactions between these systems and take into consideration additional epigenetic influences such as transcription factors, histone remodelling complexes and other non-coding RNAs (long non-coding RNAs) as well as environmental interactions, which mould the phenotype of an individual. Therefore, in order to use epigenetics in clinical applications such as biomarkers, prognostic indicators or therapeutics, it is critical to study the crosstalk between these epigenetic elements. Enhanced knowledge of the complex epigenetic networks on cell phenotype and disease may introduce novel hypotheses relating to pathogenesis of liver fibrosis, could provide mechanistic insights into diagnosis, novel drug targets and provide a future treatment of many liver diseases.

Compliance with Ethics Guidelines

Conflict of Interest Agata Page, Derek A. Mann, and Jelena Mann declare they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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