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Review

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

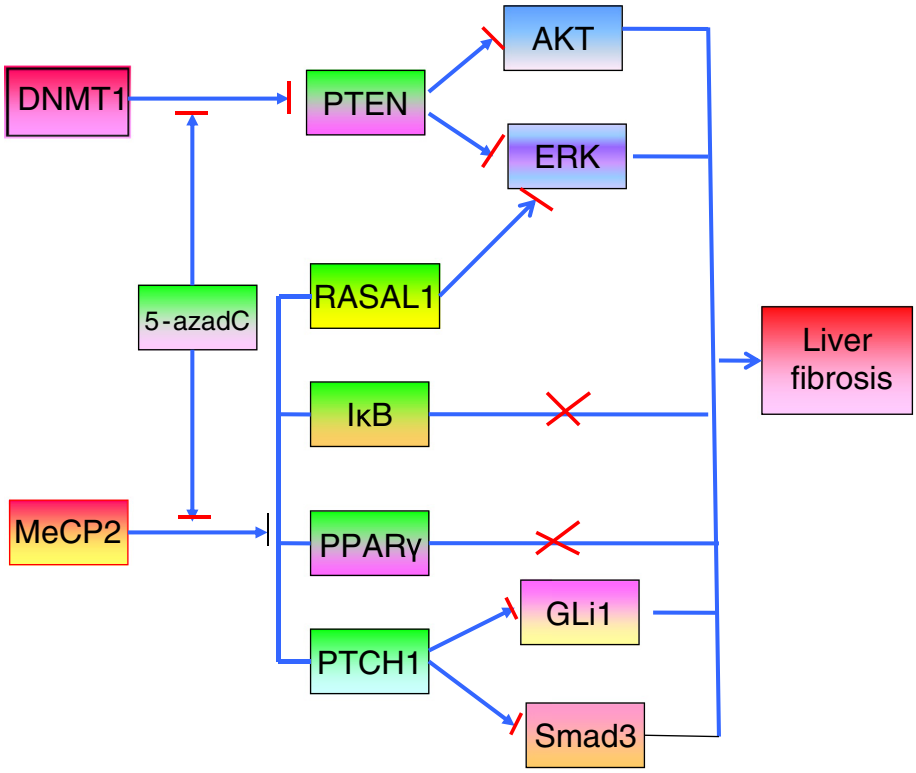

Fig. 1. Overview of the role of DNA methylation in pathobiology of liver fibrosis.

the methylated CpGs or indirectly by their activation upon treatment with demethylating agents such as 5-aza-2′-deoxycytidine (5-azadC). Transcriptional repression of some genes has been consistently shown to be due to promoters hypermethylation of genes in activated HSC and liver fibrosis. The role of these genes in pathobiology of liver fibrosis is reviewed in greater detail.

PTEN (Phosphatase and tensin homologue) was originally identified as a tumor suppressor gene frequently lost on chromosome 10q23 [35,36]. PTEN functions as a lipid phosphatase, dephosphorylating the 3′ position of phosphoinositide 3,4,5-triphosphate (PIP3) that becomes activated through the PI3K (phosphatidylinositol-3-kinase) and in turn triggers stimulation of AKT (also known as Protein kinase B) [37]. Moreover, PTEN inhibits the activation of ERK signaling pathways by its protein tyrosine phosphatase activity, thereby negatively regulating cell cycling, proliferation, focal adhesion, and cell migration [38]. Recently, we reported that the downregulation of PTEN gene expression by the promoter hypermethylation of PTEN was observed in activated HSC and the liver tissues from CCl_4-treated rats. The inhibition of DNMT1 by DNA methylation inhibitor 5-azadC or silencing DNMT1 gene decreased aberrant hypermethylation of the PTEN gene promoter and prevented the loss of PTEN expression, followed the repression of both ERK and AKT pathway in HSC-T6 cells [39]. These suggests that PTEN hypermethylation is a central event in liver fibrosis.

RASAL1 Ras GTPase activating-like protein 1 (RASAL1) gene is located at chromosome 12q23–24 and is a member of the RAS–GAP family, which catalyses Ras inactivation by binding to GTP–Ras and catalysing hydrolysis to GDP–Ras [40,41]. The downregulation of RASAL1 has been reported in many tumors, including bladder, liver, gastric, colorectal cancer and multiple cell lines [41–48]. Loss of RASAL1 activity has been correlated with hyperactive Ras in the colon and hepatocellular carcinoma lacking oncogenic Ras [43,45].Ohta et al. were able to confirm that ectopic expression of RASAL1 in transfected cells in culture could promote Ras inactivation, as well as suppression of signaling to the Ras downstream effector, ERK [45]. A recent study from our laboratory observed that treatment of PDGF-induced HSCs with 5-azadC prevents loss of RASAL1 expression that occurs during HSCs proliferation. In addition, silencing of MeCP2 increased RASAL1 in both mRNA and protein level in myofibroblasts [49]. These studies suggest that the expression of RASAL1 mediated by DNA methylation and MeCP2 may provide molecular mechanisms for HSC activation and liver fibrosis.

PTCH1: The gene patched1 (PTCH1) is an pivotal developmental regulator and tumor suppressor gene in vertebrates [50]. In mammals, Sonic hedgehog (Shh) have been identified and coded for secreted proteins which bind to multi-pass transmembrane receptors named PTCH1 [51,52]. The presence of Shh ligands and their binding to PTCH1 receptor or mutational inactivation of PTCH1 relieves the inhibition of Smoothened culminating in the activation of one or more of glioma-associated oncogene homolog 1 (Gli1) transcription factors that regulate the expression of downstream targets [53,54]. Methylation of PTCH1 is present in gastric cancers and pediatric medulloblastomas, however, treatment with 5-azadC increases the expression of PTCH1 [55,56]. Moreover, we demonstrated that the loss of PTCH1 expression was observed in CCL_4-treated liver in rats and TGF-β1-induced HSCs, however, 5-azadC treatment prevented loss of PTCH1 expression that occurred during HSCs activation. An aberrant methylation occurred in PTCH1 gene promoter contributes to the increase in Gli1 and Smad3 activity during HSC activation [57]. These results suggest that PTCH1 hypermethylation as a mechanism of HSC activation and liver fibrosis.

PPARγ: Transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) has four isoforms, γ1, γ2, γ3 and γ4, which differ only in their N-terminal sequence and have similar transcriptional activities [58]. PPARγ, a ligand-activated transcriptional factor that belongs to the nuclear hormone receptor superfamily, is predominantly present in liver and adipose tissue [59]. There are increasing evidences that the decrease of PPARγ expression was observed during HSC activation, however overexpression of PPARγ or PPARγ

ligands reversed the biochemical features of HSC activation and reduced collagen secretion both in vitro and in vivo [60–62]. Moreover, adipogenic transcriptional regulation of PPARγ by epigenetic modification occurred during HSC activation, because silencing of MeCP2 or 5-azadC treatment blocked HSC myofibroblastic transdifferentiation and prevented the diminished PPARγ expression in HSCs [23].The observations indicate that epigenetic modifications of PPARγ play in a pivotal role in liver fibrosis.

NFκB: Nuclear factor κB (NFκB) originally described as a nuclear transcription factor required for immunoglobulin kappa light chain transcription in B lymphocytes that bound to κB target DNA sites [63]. Since then it has been demonstrated that NF-κB is constitutively expressed in all cell types and regulates transcription of many cellular activation events critical for development, proper control of cell growth and proliferation, the immune response, control of apoptosis and survival, and stress responses to a variety of noxious stimuli [64,65]. Aberrant NFκB activity can lead to constitutive overproduction of proinflammatory cytokines, which is associated with a variety of chronic inflammatory disorders [66,67]. Moreover, constitutive activation of NFκB has been observed in patients with liver diseases such as hepatitis B, hepatitis C, or hepatocellular carcinoma [68]. Specific NFκB inhibitor proteins, known as IκBs, control NFκB signaling [69]. The inhibition of NFκB activity resulted in hepatic stellate cell apoptosis and decreased fibrogenesis. In contrast, NFκB activity is increased by the repression of IκB, which promotes stellate cell survival and therefore increased fibrosis [70]. This finding has been exploited by demonstrating that 5-azadC prevented loss of IκB expression that occurs during transdifferentiation to allow acquisition of proinflammatory characteristics. Moreover, ChIP analysis revealed IκB promoter is associated with transcriptionally repressed chromatin that converts to an active state with 5-azadC treatment. Interestingly, siRNA knockdown of MeCP2 elevated IκB a promoter activity, mRNA and protein expression in myofibroblasts. Further study demonstrated that MeCP2 interacts with IκB a promoter via a methyl-CpG-dependent mechanism and recruitment into a CBF1 corepression complex [23].

5. Erasure of aberrant DNA methylation in liver fibrosis

Since aberrant hypermethylation of specific gene contributes to HSC activation and fibrogenesis, erasing pathological methylation seems to be an attractive therapeutic strategy. Therefore, utilization of endogenous-de-methylating mechanisms may prove to be even more attractive in the future to possibly convert the fibrogenic program to physiologic repair and possibly facilitate regeneration of chronic liver disease. Recent studies suggest that there might be multiple pathways or mechanisms by which 5hmC and Tet family proteins regulate DNA methylation dynamics and gene transcription [71]. The Ten-Eleven-Translocation (TET) proteins TET1, TET2 and TET3, known as the mammalian homologous of the trypanosome proteins JBP1 and JBP2, generate hydroxyl-methyl-cytosine (5hmC) through addition of a hydroxyl group onto the methyl-group of 5mC [72,73]. In addition, TET proteins were reported to be able to further convert 5hmC to 5-formylcytosine and 5-carboxylcytosine, which can subsequently be recognized and excised by thymine DNA glycosylase (TDG) in vitro and in vivo [74–76]. Subsequent repair of the resulting abasic site by base excision repair (BER) can regenerate an unmethylated cytosine [77]. Recent studies suggest that TET proteins, important contributors in DNA demethylation process, have essential roles in neuro development and aging and are involved in human cancers [78,79]. A recent study have demonstrated that the decrease of PTCH1 gene expression due to increase CpG methylation after Tet1 depletion in Mouse Embryonic Stem Cells [80]. Moreover, the decrease of PTCH1 gene by DNA methylation was observed in activated HSC [57]. These results suggest that the decrease of PTCH1 by DNA methylation may be dependent on the loss of TET1 in liver fibrosis. In summary, CpG island promoter methylation can be erased in principle, an involvement of endogenous demethylating mechanisms in liver repair and liver fibrosis has not yet been explored but deserves further consideration.

6. Overview of miRNA

miRNA is a widely studied small, non-coding and single-strand RNA of 19–22 nucleotides [81]. Genes encoding miRNAs are transcribed as primary miRNA transcripts (pri-miRNAs) by the polymerase activity of Pol II [82,83]. Pri-miRNAs are processed by the "micro-processor complex"; a Class 2 RNase III endonuclease (Drosha) to produce stem-loop-structured miRNA precursors (pre-miRNAs) [84,85]. Pre-miRNAs are exported to the cytoplasm, where Dicer generates ~21 nucleotide double-stranded RNA intermediates [86]. Such double-stranded RNAs are processed further, and one strand, the mature miRNA, interacts with Argonaute (Ago) proteins to form miRNA–protein complexes (miRNPs) [87,88]. miRNA is found to result in translational repression of protein-coding genes via sequence-specific binding of its seed region to the untranslated region (UTR) of its target protein-coding genes [81,89]. miRNAs are involved in post-translational gene silencing by controlling mRNA translation into proteins, and hence participate in the regulation of various cellular functions, including development, apoptosis, proliferation and differentiation [90–93]. miRNAs can also be divided into tumor suppressive and oncogenic miRNA, depending on whether they target conventional tumour suppressors or oncogenes, respectively [94]. mRNA degradation occurs occasionally in animals when the miRNA guide template binds to the mRNA transcript and activate the RNase activity of its associated Ago protein. Translational repression is found more frequently in animals, and arises from the miRNA guide molecule in a RNA-induced silencing complex binding imperfectly with a sequence in the 3′-UTR of a target mRNA [95].

In addition to the direct binding of miRNAs to their target genes, miRNAs can regulate gene expression by binding to RNA-binding proteins (RBPs) that usually act as transcriptional activators or repressors [96]. MiRNAs is identified to bind protein domains that usually recognize and bind RNA secondary structures, acting as decoys and therefore preventing the positioning of the RBPs and abrogating their function [97,98].

7. The crosstalk between DNA methylation and microRNAs in liver fibrosis

7.1. miRNAs target DNMTs, MeCP2 in liver fibrosis

There are accumulating data that some miRNAs target, directly or indirectly, effectors of the epigenetic machinery such as DNMTs and MeCP2 [99–102] (Fig. 2). DNMTs targeted directly by some miRNAs is confirmed firstly in cancer research. For example, one published study showed that in lung cancer, the miR-29 family directly targets DNMT3A and 3B, thereby leading to down-regulation of these genes, reduction of global DNA methylation, and reexpression of the DNA hypermethylated and silenced tumor suppressor genes FHIT and WWOX [100]. In addition, several groups have shown that that some miRNAs target, indirectly, DNMTs. Enforced expression of miR-29b in acute myeloid leukemia cells resulted in marked reduction of the expression of DNMT1, DNMT3A, and DNMT3B at both RNA and protein levels. Although down-regulation of DNMT3A and DNMT3B was the result of a direct interaction of miR-29b with the 3′ untranslated regions of these genes, no predicted miR-29b interaction sites were found in the DNMT1 3′ untranslated regions. Further experiments revealed that miR-29b down-regulates DNMT1 indirectly by targeting Sp1, a transactivator of the DNMT1 gene [103]. These results are also in agreement with the work of Qin H et al. who reported miR-29b negatively regulates DNMT1 expression by targeting sp1 in T cells. The overexpression of miR-29b contributes to the reduction of DNMT1 levels and thereby DNA hypomethylation

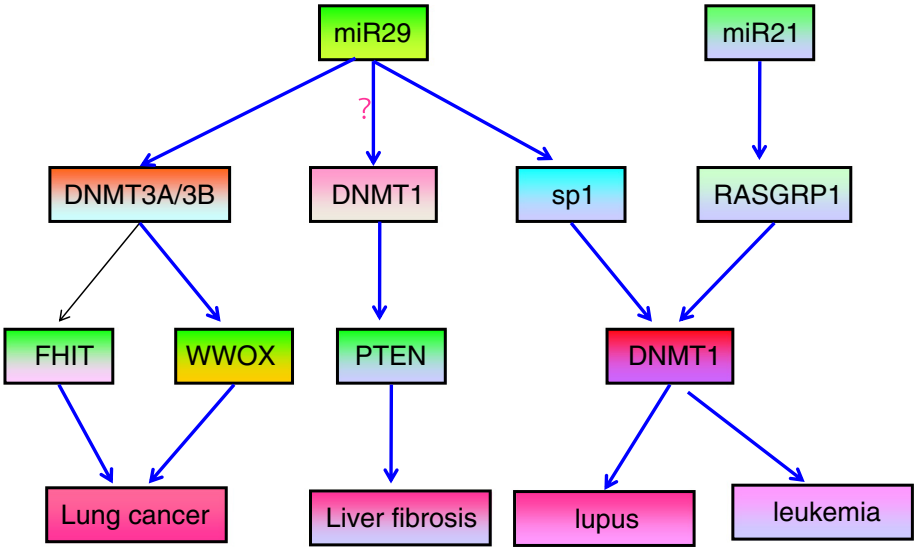

Fig. 2. MiRNAs repressed gene expression by targeting, directly or indirectly, DNMTs and MeCP2 in various diseases.

in SLE [104]. It is interesting to point out that miR-21 overexpressed in CD4+ T cells from both patients with lupus and lupus-prone MRL/lpr mice, which promote cell hypomethylation by repressing DNMT1 expression. Further experiments revealed that miR-21 indirectly downregulated DNMT1 expression by targeting an important autoimmune gene, RASGRP1, which mediated the Ras-MAPK pathway upstream of DNMT1 [105].

In the pathogenesis of liver fibrosis, an increasing evidence suggest that miRNAs are involved in the control of DNA methylation by targeting the DNA methylation machinery. MiR-29 was significantly down-regulated in livers of CCl_4-treated mice as well as in mice that underwent bile duct ligation and transition of HSC into myofibroblastic, however transfection of a miR-29b precursor markedly attenuated the expression of Col1a1 and Col1a2 mRNAs and additionally blunted the increased expression of α-SMA, DDR2, FN1, ITGB1, and PDGFR-β, which are key genes involved in the activation of HSCs [106–108].

Recently, we have demonstrated that DNMT1 was upregulated in the fibrotic livers from CCl_4-treated livers and in cultured HSCs after activation. Knockdown of DNMT1 by RNAi inhibited HSC activation and proliferation, and reversed the methylation of PTEN gene promoter and subsequently restored PTEN gene expression [39]. These studies suggest that the loss of miR-29 may contribute to the activation of DNMT1 during liver fibrosis. Nevertheless, additional mechanisms are necessary to attain such an effect remains to be fully elucidated.

7.2. Aberrant methylation of miRNAs in liver fibrosis

Downregulation of miRNA expression in cancers may be controlled by diverse mechanisms, ranging from epigenetic modification, gene mutation or copy number loss to defective miRNA biogenesis or post-transcriptional processing [109].

DNA hypermethylation of CpG sites within CpG islands is known as an epigenetic aberration resulting in silencing of tumor-suppressive miRNA (TS-miRNA) in cancer cells, in the same manner as that of many classical tumor-suppressor gene (TSG) [110,111]. Furthermore, downregulation of miRNA expression could be reverted by treatment with demethylating agents such as 5-azadC, suggesting that expression of these miRNAs was regulated by DNA hypermethylation [112]. Although the genomic distances between the 5′-end of intergenic miRNA genes or host genes harboring intronic miRNA and their proximal CpG islands vary, these distances might provide more important information for the understanding of epigenetic dysregulation of tumor-suppressor miRNA genes by promoter DNA methylation [113]. Therefore, a better understanding of epigenetic inactivation of specific tumor-suppressor miRNA genes is essential for the treatment in liver fibrosis.

The expression of miR-146a was downregulated in HSC in response to TGF-β1 stimulation and CCL_4-treated rat liver [114]. In vitro, miR-146a overexpression in NKTL cell lines, SNK6 and YT, inhibited NFκB activity, suppressed cell proliferation, induced apoptosis, and enhanced chemosensitivity. TNF receptor-associated factor 6, a target of miR-146a and a known NFκB activator, was downregulated by miR-146a in SNK6 and YT cells [115]. In addition, The HSC transfected with miR-146a mimics exhibited attendated TGF-β1-induced α-SMA expression and the proliferation of HSC and increased HSC apoptosis [114]. Promoter methylation of miR-146a gene was observed in SNK6 and YT cells, as well as in NKTL tissues with low miR-146a expression and miR-146a expression was induced by the conversion of methylation status with a demethylating agent in SNK6 and YT cells [115]. In addition, the promoter-associated CpG island of the miR-194 cluster was hypermethylated, and hence, upon hypomethylation treatment, the miR-194 cluster could be re-expressed in human myeloma cell lines [116]. Expression of miRNA-194 was reduced in HSC isolated from fibrotic rats compared with sham-operated animals. Moreover, miRNA-194 overexpressed in LX-2 cells, and their ability to inhibit cell proliferation, the expression of α-SMA, a marker for activation, and collagen type I, a marker for ECM secretion, was determined [24]. These studies suggest that the downregulation of some miRNAs may be attributed to promoter methylation of miRNAs in liver fibrosis.

In hematological cancers, Chim et al. studied the methylation status of the miR-34a in a broad spectrum of primary samples, consisting of AML, chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), MM, non-Hodgkin's lymphoma (NHL) and Philadelphia chromosome-negative (Ph−) myeloproliferative diseases (MDS). Both of these studies showed that the promoter-associated CpG island of the miR-34a was unmethylated in normal controls but aberrantly methylated in 50% of the hematological cancer cell lines, including human myeloma cell lines (HMCLs). Treatment with 5-aza-2′-deoxycytidine led to demethylation of the miR-34a promoter and consequent re-expression of the pri-miR-34a transcript in cells homozygously methylated for the miR-34a [117,118]. In addition, treatment of normal human hepatocytes (N-Heps) and cholangiocytes human intrahepatic biliary epithelial cells (HiBECs) with ethanol and lipopolysaccharide induced a significant increase of miR-34a expression. Overexpression of miR-34a decreased ethanol-induced apoptosis in both N-Heps and HiBECs. The 5′-promoter region of miR-34a was noted to be embedded within a CpG island, the expression level of miR-34a was

significantly increased after demethylation treatment in N-Heps and HiBECs. Furthermore, modulation of miR-34a also altered expression of matrix metalloproteases 1 and 2, the mediators involved in hepatic remodeling during alcoholic liver fibrosis [119].

7.3. The interaction DNA methylation with microRNAs

More and more evidences showed that a functional crosstalk between DNA methylation and miRNAs was involved in cancer development. For example, Xiang Y et al. demonstrated that miR-152 was significantly downregulated in the cisplatin-resistant ovarian cell lines, whereas the overexpression of miR-152 increased cisplatin sensitivity of cisplatin-resistant ovarian cell lines by inhibiting proliferation and promoting apoptosis, then we further confirmed that these miRNAs functioned through suppressing DNMT1 directly [120]. In addition, the expression of miR-152 was frequently down-regulated in HBV-related HCC tissues in comparison with adjacent noncancerous hepatic tissues and was inversely correlated to DNMT1 expression in HBV-related HCCs. The forced expression of miR-152 in liver cell lines resulted in a marked reduction of DNMT1 expression by directly targeting the 3′ untranslated regions of DNMT1. This in turn led to a decrease in global DNA methylation, whereas inhibition of miR-152 caused global DNA hypermethylation and increased the methylation levels of two tumor suppressor genes, glutathione S-transferase pi 1 (GSTP1) and E-cadherin 1 (CDH1) [121]. These results suggest that the interaction of miRNAs with DNMTs regulate gene expression. Interestingly, the expression of miR-152 was specifically downregulated in NiS-transformed cells via promoter DNA hypermethylation, whereas ectopic expression of miR-152 caused a marked reduction of DNMT1 expression, which led to a significant decrease of cell growth in NiS-transformed cells. Further experiments revealed that miR-152 directly downregulated DNMT1 expression by targeting the 3′ untranslated regions of its transcript. Treatment of DNMT inhibitor, 5-azadC, or depletion of DNMT1 led to increased miR-152 expression by reversion of promoter hypermethylation, DNMT1 and MeCP2 binding to miR-152 promoter in NiS-transformed cells. Moreover, inhibition of miR-152 expression could increase DNMT1 expression and result in an increase in DNA methylation, DNMT1 and MeCP2 binding to miR-152 promoter, which significantly increased cell growth in 16HBE cells [122]. These results suggest that a functional crosstalk between miRNAs and DNA methylation is involved in controlling gene expression.

Decreased expression of miR-132 by promoter hypermethylation was confirmed pancreatic cancerous tissues and in PANC1 and SW1990 cells. Moreover, cancerous tissues showed significantly lower Sp1-binding affinity to the miR-132 promoter, relative to non-tumor samples. Proliferation and colony formation of pancreatic cancer cells were suppressed in cells transfected with miR-132 mimics and enhanced in cells transfected with miR-132 inhibitor by negatively regulating the Akt-signaling pathway [123]. Moreover, a recent study showed that a rapid increase in MeCP2 protein associated with downregulation of miR-132 was induced in preconditioning ischemia [124]. Block of miR132-mediated repression increased MeCP2 and brain-derived neurotrophic factor (BDNF) levels in cultured rat neurons and the loss of MeCP2 reduced BDNF and miR132 levels in vivo [101]. This feedback loop may provide new and profound insights in the interaction of MeCP2 with miRNAs.

Intrauterine growth restriction (IUGR) would in rats alter the levels of MeCP2 mRNA and protein as well as miR132 and that this will be associated with changes in PPARγ mRNA levels, MeCP2 occupancy at the PPARγ promoters, and PPARγ H3K9Me3 [101]. Transcriptional silencing of PPARγ gene is required for conversion of HSC into myofibroblasts, whereas forced over-expression of PPARγ in hepatic myofibroblasts leads to reversion of transdifferentiation, with down-regulation of type I collagen, loss of proliferation and reacquisition of their adipogenic characteristics [60,62]. 5-azadC prevented loss of PPARγ expression that occurs during transdifferentiation to allow acquisition of profibrogenic characteristics. Interestingly, MeCP2 is recruited to the 5′ end of PPARγ, where it promotes methylation by H3K9 and recruits the transcription repressor HP1α during liver fibrosis. Loss of miR132 expression in myofibroblasts during liver injury was confirmed in HSC isolated from BDL- and CCl_4-injured rat livers, however transfection of miR132 diminished the expression of MeCP2 protein. This treatment was also accompanied by increased PPARγ expression [22,23]. These results suggest that a functional crosstalk between miRNAs and MeCP2 via a double-negative feedback loop is involved in the regulation of gene expression in liver fibrosis.

In summary, a functional crosstalk between miRNAs and DNA methylation via a double-negative feedback loop is involved in controlling gene expression (Fig. 3).

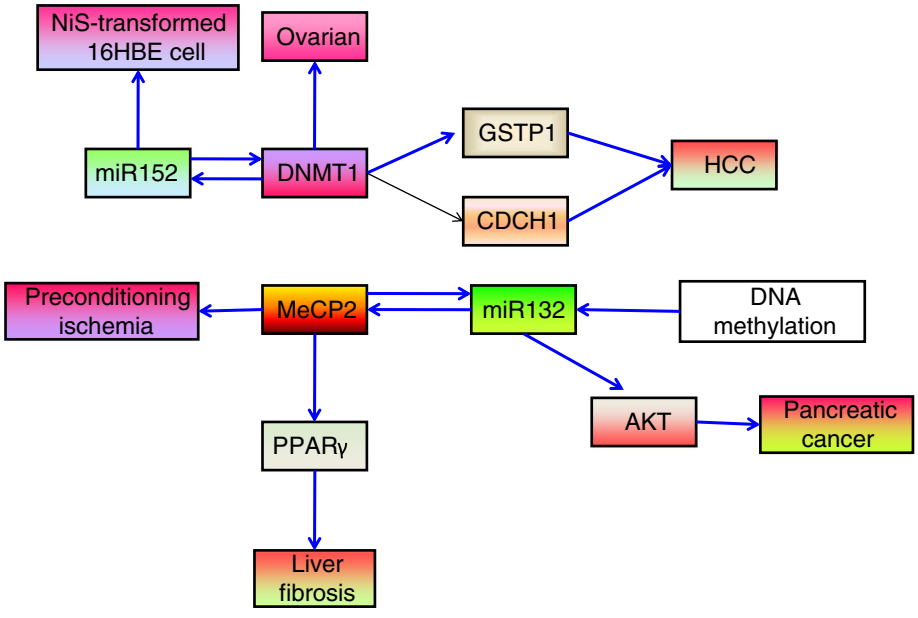

Fig. 3. Overview of a functional crosstalk between miRNAs and DNA methylation is involved in the regulation of gene expression.

8. Conclusion and prospective

In summary, current data on DNA methylation of specific gene in liver fibrosis focus on the loss of tumor-suppressor genes due to promoter DNA hypermethylation. Epigenetic inactivation of these TGS is involved in the pathogenesis and progression of liver fibrosis. In addition, epigenetic silencing of miRNAs by promoter hypermethylation is involved in the regulation of key pathways involved in liver fibrosis. Therefore, the use of epigenetic drugs was able to induce re-expression of these TGS and miRNAs could provide a therapeutic advantage for these patients. Indeed, DNA methylation can be reversed by demethylating agents 5-azacytidine or decitabine, and therapeutic benefits have been demonstrated in patients with myelodysplastic syndrome [125]. Moreover, these data also suggest the potential use of tumor-suppressor miRNA mimics as a liver fibrosis therapy in tumors lacking certain critical tumor-suppressor miRNAs.

Moreover, in this review, we, in particular, emphasize the role of the interaction of miRNA with DNA methylation in the mechanism of disease occurs, since epigenetic dysregulation of tumor-suppressor miRNA genes are found to be due to promoter DNA methylation and miRNAs can target, directly or indirectly, MeCP2 and DNMTs in specific condition. We foresee that the study of the crosstalk between miRNAs and DNA methylation and the complex pattern of the epigenetic networks may result in novel hypotheses about pathogenesis liver fibrosis and may offer a new promise for the treatment of liver fibrosis.

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References

[1] K. Iwaisako, D.A. Brenner, T. Kisseleva, Journal of Gastroenterology and Hepatology 27 (Suppl. 2) (2012) 65–68.
[2] X. Kong, N. Horiguchi, M. Mori, B. Gao, Frontiers in Physiology 3 (2012) 69.
[3] F.J. Eng, S.L. Friedman, American Journal of Physiology. Gastrointestinal and Liver Physiology 279 (2000) G7–G11.
[4] S.L. Friedman, Journal of Biological Chemistry 275 (2000) 2247–2250.
[5] J.A. Fallowfield, American Journal of Physiology. Gastrointestinal and Liver Physiology 300 (2011) G709–G715.
[6] J.J. Maher, Seminars in Liver Disease 21 (2001) 417–426.
[7] J. Mann, D.A. Mann, Advanced Drug Delivery Reviews 61 (2009) 497–512.
[8] S.J. Forbes, M. Parola, Best Practice & Research. Clinical Gastroenterology 25 (2011) 207–217.
[9] Y.P. Han, L. Zhou, J. Wang, S. Xiong, W.L. Garner, S.W. French, et al., Journal of Biological Chemistry 279 (2004) 4820–4828.
[10] R. Blomhoff, T. Berg, Methods in Enzymology 190 (1990) 58–71.
[11] S.L. Friedman, Gastroenterology 134 (2008) 1655–1669.
[12] M.R. Alison, P. Vig, F. Russo, B.W. Bigger, E. Amofah, M. Themis, et al., Cell Proliferation 37 (2004) 1–21.
[13] S.L. Friedman, Physiological Reviews 88 (2008) 125–172.
[14] T. Lamireau, A. Desmouliere, P. Bioulac-Sage, J. Rosenbaum, Archives of Pediatrics 9 (2002) 392–405.
[15] N. Nieto, Hepatology 44 (2006) 1487–1501.
[16] N. Nieto, S.L. Friedman, A.I. Cederbaum, Journal of Biological Chemistry 277 (2002) 9853–9864.
[17] E. Mormone, J. George, N. Nieto, Chemico-Biological Interactions 193 (2011) 225–231.
[18] J.S. Duffield, S.J. Forbes, C.M. Constandinou, S. Clay, M. Partolina, S. Vuthoori, et al., The Journal of Clinical Investigation 115 (2005) 56–65.
[19] F.J. Eng, S.L. Friedman, Seminars in Liver Disease 21 (2001) 385–395.
[20] D. Fritz, B. Stefanovic, Journal of Molecular Biology 371 (2007) 585–595.
[21] C.J. Guo, Q. Pan, D.G. Li, H. Sun, B.W. Liu, Journal of Hepatology 50 (2009) 766–778.
[22] J. Mann, D.C. Chu, A. Maxwell, F. Oakley, N.L. Zhu, H. Tsukamoto, et al., Gastroenterology 138 (2010) 705–714, (714 e1-4).
[23] J. Mann, F. Oakley, F. Akiboye, A. Elsharkawy, A.W. Thorne, D.A. Mann, Cell Death and Differentiation 14 (2007) 275–285.
[24] S.K. Venugopal, J. Jiang, T.H. Kim, Y. Li, S.S. Wang, N.J. Torok, et al., American Journal of Physiology. Gastrointestinal and Liver Physiology 298 (2010) G101–G106.
[25] Y. Li, J. Zhu, G. Tian, N. Li, Q. Li, M. Ye, et al., PLoS Biology 8 (2010) e1000533.
[26] R. Lister, M. Pelizzola, R.H. Dowen, R.D. Hawkins, G. Hon, J. Tonti-Filippini, et al., Nature 462 (2009) 315–322.
[27] T. Liloglou, N.G. Bediaga, B.R. Brown, J.K. Field, M.P. Davies, Cancer Letters (2012), http://dx.doi.org/10.1016/j.canlet.2012.04.018 (in press).
[28] Z.X. Chen, A.D. Riggs, Journal of Biological Chemistry 286 (2011) 18347–18353.
[29] J.K. Kim, M. Samaranayake, S. Pradhan, Cellular and Molecular Life Sciences 66 (2009) 596–612.
[30] L. Lopez-Serra, M. Esteller, British Journal of Cancer 98 (2008) 1881–1885.
[31] R. Kanwal, S. Gupta, Clinical Genetics 81 (2012) 303–311.
[32] J.D. Lewis, R.R. Meehan, W.J. Henzel, I. Maurer-Fogy, P. Jeppesen, F. Klein, et al., Cell 69 (1992) 905–914.
[33] X. Nan, H.H. Ng, C.A. Johnson, C.D. Laherty, B.M. Turner, R.N. Eisenman, et al., Nature 393 (1998) 386–389.
[34] J.M. LaSalle, D.H. Yasui, Epigenomics 1 (2009) 119–130.
[35] J. Li, C. Yen, D. Liaw, K. Podsypanina, S. Bose, S.I. Wang, et al., Science 275 (1997) 1943–1947.
[36] P.A. Steck, M.A. Pershouse, S.A. Jasser, W.K. Yung, H. Lin, A.H. Ligon, et al., Nature Genetics 15 (1997) 356–362.
[37] A. Carracedo, A. Alimonti, P.P. Pandolfi, Cancer Research 71 (2011) 629–633.
[38] A. Besson, S.M. Robbins, V.W. Yong, European Journal of Biochemistry 263 (1999) 605–611.
[39] E.B. Bian, C. Huang, T.T. Ma, H. Tao, H. Zhang, C. Cheng, et al., Toxicology and Applied Pharmacology 264 (2012) 13–22.
[40] S. Kupzig, D. Deaconescu, D. Bouyoucef, S.A. Walker, Q. Liu, C.L. Polte, et al., Journal of Biological Chemistry 281 (2006) 9891–9900.
[41] M. Seto, M. Ohta, T. Ikenoue, T. Sugimoto, Y. Asaoka, M. Tada, et al., International Journal of Cancer 128 (2011) 1293–1302.
[42] A. Bernards, J. Settleman, Gastroenterology 136 (2009) 46–48.
[43] D.F. Calvisi, S. Ladu, E.A. Conner, D. Seo, J.T. Hsieh, V.M. Factor, et al., Journal of Hepatology 54 (2011) 311–319.
[44] H. Chen, X.W. Yang, H. Zhang, Q. Yang, Z. Wang, Y. Liu, et al., Oncology Letters 3 (2012) 535–540.
[45] M. Ohta, M. Seto, H. Ijichi, K. Miyabayashi, Y. Kudo, D. Mohri, et al., Gastroenterology 136 (2009) 206–216.
[46] F. Qiao, X. Su, X. Qiu, D. Qian, X. Peng, H. Chen, et al., Oncology Reports 28 (2012) 1475–1481.
[47] J. Zhu, Z. Jiang, F. Gao, X. Hu, L. Zhou, J. Chen, et al., PLoS One 6 (2011) e28223.
[48] B.B. Ma, F. Sung, Q. Tao, F.F. Poon, V.W. Lui, W. Yeo, et al., Investigational New Drugs 28 (2010) 107–114.
[49] H. Tao, C. Huang, J.J. Yang, T.T. Ma, E.B. Bian, L. Zhang, et al., Toxicology 290 (2011) 327–333.
[50] M. Hammerschmidt, A. Brook, A.P. McMahon, Trends in Genetics 13 (1997) 14–21.
[51] F.A. Grieco, M. Moretti, G. Sebastiani, L. Galleri, I. Spagnuolo, G. Scafetta, et al., Diabetes/Metabolism Research and Reviews 27 (2011) 755–760.
[52] T. Shimokawa, F. Rahnama, P.G. Zaphiropoulos, FEBS Letters 578 (2004) 157–162.
[53] J.T. Huse, E.C. Holland, Nature Reviews. Cancer 10 (2010) 319–331.
[54] M.F. Roussel, M.E. Hatten, Current Topics in Developmental Biology 94 (2011) 235–282.
[55] S.J. Diede, J. Guenthoer, L.N. Geng, S.E. Mahoney, M. Marotta, J.M. Olson, et al., Proceedings of the National Academy of Sciences of the United States of America 107 (2010) 234–239.
[56] P. Du, H.R. Ye, J. Gao, W. Chen, Z.C. Wang, H.H. Jiang, et al., World Journal of Gastroenterology 15 (2009) 3799–3806.
[57] J.J. Yang, H. Tao, C. Huang, K.H. Shi, T.T. Ma, E.B. Bian, et al., Cellular Signalling 25 (2013) 1202–1211.
[58] R.M. Evans, G.D. Barish, Y.X. Wang, Nature Medicine 10 (2004) 355–361.
[59] S. Sugii, R.M. Evans, FEBS Letters 585 (2011) 2121–2128.
[60] S. Hazra, S. Xiong, J. Wang, R.A. Rippe, V. Krishna, K. Chatterjee, et al., Journal of Biological Chemistry 279 (2004) 11392–11401.
[61] F. Marra, E. Efsen, R.G. Romanelli, A. Caligiuri, S. Pastacaldi, G. Batignani, et al., Gastroenterology 119 (2000) 466–478.
[62] T. Miyahara, L. Schrum, R. Rippe, S. Xiong, H.F. Yee Jr., K. Motomura, et al., Journal of Biological Chemistry 275 (2000) 35715–35722.
[63] R. Sen, D. Baltimore, Cell 47 (1986) 921–928.
[64] P.A. Baeuerle, Cell 95 (1998) 729–731.
[65] P.A. Baeuerle, T. Henkel, Annual Review of Immunology 12 (1994) 141–179.
[66] M. Karin, A. Lin, Nature Immunology 3 (2002) 221–227.
[67] P.P. Tak, G.S. Firestein, The Journal of Clinical Investigation 107 (2001) 7–11.
[68] B. Sun, M. Karin, Oncogene 27 (2008) 6228–6244.
[69] H.J. Dyson, E.A. Komives, IUBMB Life 64 (2012) 499–505.
[70] S.L. Friedman, Journal of Gastroenterology and Hepatology 21 (Suppl. 3) (2006) S79–S83.
[71] L. Tan, Y.G. Shi, Development 139 (2012) 1895–1902.
[72] S. Ito, A.C. D'Alessio, O.V. Taranova, K. Hong, L.C. Sowers, Y. Zhang, Nature 466 (2010) 1129–1133.
[73] M. Tahiliani, K.P. Koh, Y. Shen, W.A. Pastor, H. Bandukwala, Y. Brudno, et al., Science 324 (2009) 930–935.
[74] Y.F. He, B.Z. Li, Z. Li, P. Liu, Y. Wang, Q. Tang, et al., Science 333 (2011) 1303–1307.
[75] A. Inoue, Y. Zhang, Science 334 (2011) 194.
[76] S. Ito, L. Shen, Q. Dai, S.C. Wu, L.B. Collins, J.A. Swenberg, et al., Science 333 (2011) 1300–1303.
[77] L. Shen, Y. Zhang, Current Opinion in Cell Biology (2013), http://dx.doi.org/10.1016/j.ceb.2013.02.017 (in press).
[78] S.R. Kinney, S. Pradhan, Advances in Experimental Medicine and Biology 754 (2013) 57–79.
[79] K.E. Szulwach, X. Li, Y. Li, C.X. Song, H. Wu, Q. Dai, et al., Nature Neuroscience 14 (2011) 1607–1616.

[80] Y. Xu, F. Wu, L. Tan, L. Kong, L. Xiong, J. Deng, et al., Molecular Cell 42 (2011) 451–464.
[81] V. Ambros, Nature 431 (2004) 350–355.
[82] G.M. Borchert, W. Lanier, B.L. Davidson, Nature Structural and Molecular Biology 13 (2006) 1097–1101.
[83] Y. Lee, M. Kim, J. Han, K.H. Yeom, S. Lee, S.H. Baek, et al., EMBO Journal 23 (2004) 4051–4060.
[84] R.I. Gregory, K.P. Yan, G. Amuthan, T. Chendrimada, B. Doratotaj, N. Cooch, et al., Nature 432 (2004) 235–240.
[85] Y. Lee, J. Han, K.H. Yeom, H. Jin, V.N. Kim, Cold Spring Harbor Symposia on Quantitative Biology 71 (2006) 51–57.
[86] E. Lund, S. Guttinger, A. Calado, J.E. Dahlberg, U. Kutay, Science 303 (2004) 95–98.
[87] D.P. Bartel, Cell 136 (2009) 215–233.
[88] R.W. Carthew, E.J. Sontheimer, Cell 136 (2009) 642–655.
[89] D.P. Bartel, Cell 116 (2004) 281–297.
[90] N. Felli, F. Pedini, P. Romania, M. Biffoni, O. Morsilli, G. Castelli, et al., Haematologica 94 (2009) 479–486.
[91] R. Garzon, C.M. Croce, Current Opinion in Hematology 15 (2008) 352–358.
[92] M.T. Le, H. Xie, B. Zhou, P.H. Chia, P. Rizk, M. Um, et al., Molecular and Cellular Biology 29 (2009) 5290–5305.
[93] R. Schickel, B. Boyerinas, S.M. Park, M.E. Peter, Oncogene 27 (2008) 5959–5974.
[94] R.L. Yim, Y.L. Kwong, K.Y. Wong, C.S. Chim, Frontiers in Genetics 3 (2012) 233.
[95] S. Srivastava, G.J. Tsongalis, P. Kaur, Clinical Biochemistry (2013), http://dx.doi.org/10.1016/j.clinbiochem.2013.03.007, (in press).
[96] A. Soriano, L. Jubierre, A. Almazan-Moga, C. Molist, J. Roma, J.S. de Toledo, et al., Pharmacological Research (2013), http://dx.doi.org/10.1016/j.phrs.2013.03.006 (in press).
[97] M. Beitzinger, G. Meister, Cell 140 (2010) 612–614.
[98] A.M. Eiring, J.G. Harb, P. Neviani, C. Garton, J.J. Oaks, R. Spizzo, et al., Cell 140 (2010) 652–665.
[99] F. Balaguer, A. Link, J.J. Lozano, M. Cuatrecasas, T. Nagasaka, C.R. Boland, et al., Cancer Research 70 (2010) 6609–6618.
[100] M. Fabbri, R. Garzon, A. Cimmino, Z. Liu, N. Zanesi, E. Callegari, et al., Proceedings of the National Academy of Sciences of the United States of America 104 (2007) 15805–15810.
[101] M.E. Klein, D.T. Lioy, L. Ma, S. Impey, G. Mandel, R.H. Goodman, Nature Neuroscience 10 (2007) 1513–1514.
[102] S. Varambally, Q. Cao, R.S. Mani, S. Shankar, X. Wang, B. Ateeq, et al., Science 322 (2008) 1695–1699.
[103] R. Garzon, S. Liu, M. Fabbri, Z. Liu, C.E. Heaphy, E. Callegari, et al., Blood 113 (2009) 6411–6418.
[104] H. Qin, X. Zhu, J. Liang, J. Wu, Y. Yang, S. Wang, et al., Journal of Dermatological Science 69 (2013) 61–67.
[105] W. Pan, S. Zhu, M. Yuan, H. Cui, L. Wang, X. Luo, et al., Journal of Immunology 184 (2010) 6773–6781.
[106] M. Kwiecinski, N. Elfimova, A. Noetel, U. Tox, H.M. Steffen, U. Hacker, et al., Laboratory Investigation 92 (2012) 978–987.
[107] C. Roderburg, G.W. Urban, K. Bettermann, M. Vucur, H. Zimmermann, S. Schmidt, et al., Hepatology 53 (2011) 209–218.
[108] Y. Sekiya, T. Ogawa, K. Yoshizato, K. Ikeda, N. Kawada, Biochemical and Biophysical Research Communications 412 (2011) 74–79.
[109] S. Deng, G.A. Calin, C.M. Croce, G. Coukos, L. Zhang, Cell Cycle 7 (2008) 2643–2646.
[110] J.C. Chuang, P.A. Jones, Pediatric Research 61 (2007) 24R–29R.
[111] J.G. Herman, S.B. Baylin, The New England Journal of Medicine 349 (2003) 2042–2054.
[112] A. Lujambio, M. Esteller, Cell Cycle 6 (2007) 1455–1459.
[113] K. Kozaki, J. Inazawa, Cancer Science 103 (2012) 837–845.
[114] Y. He, C. Huang, X. Sun, X.R. Long, X.W. Lv, J. Li, Cellular Signalling 24 (2012) 1923–1930.
[115] J.H. Paik, J.Y. Jang, Y.K. Jeon, W.Y. Kim, T.M. Kim, D.S. Heo, et al., Clinical Cancer Research 17 (2011) 4761–4771.
[116] F. Pichiorri, S.S. Suh, A. Rocci, L. De Luca, C. Taccioli, R. Santhanam, et al., Cancer Cell 18 (2010) 367–381.
[117] C.S. Chim, K.Y. Wong, Y. Qi, F. Loong, W.L. Lam, L.G. Wong, et al., Carcinogenesis 31 (2010) 745–750.
[118] K.Y. Wong, L. Yu, C.S. Chim, Epigenomics 3 (2011) 83–92.
[119] F. Meng, S.S. Glaser, H. Francis, F. Yang, Y. Han, A. Stokes, et al., American Journal of Pathology 181 (2012) 804–817.
[120] Y. Xiang, N. Ma, D. Wang, Y. Zhang, J. Zhou, G. Wu, et al., Oncogene (2013), http://dx.doi.org/10.1038/onc.2012.575 (in press).
[121] J. Huang, Y. Wang, Y. Guo, S. Sun, Hepatology 52 (2010) 60–70.
[122] W. Ji, L. Yang, J. Yuan, M. Zhang, D. Qi, X. Duan, et al., Carcinogenesis 34 (2013) 446–453.
[123] S. Zhang, J. Hao, F. Xie, X. Hu, C. Liu, J. Tong, et al., Carcinogenesis 32 (2011) 1183–1189.
[124] T.A. Lusardi, C.D. Farr, C.L. Faulkner, G. Pignataro, T. Yang, J. Lan, et al., Journal of Cerebral Blood Flow and Metabolism 30 (2010) 744–756.
[125] G. Garcia-Manero, P. Fenaux, Journal of Clinical Oncology 29 (2011) 516–523.