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REVIEW

DNA methylation in liver diseases

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Abstract

Recently, growing evidences show that the combination of epigenetic and genetic abnormalities contribute together to the development of liver diseases. DNA methylation is a very important epigenetic mechanism in human beings. It refers to addition of the methyl groups to DNA and mainly occurs at cytosine adjacent to guanine. DNA methylation is prevalent across human genome and is essential for the normal human development, while its dysfunction is associated with many human diseases. A deep understanding of DNA methylation may provide us deep insight into the origination of liver diseases. Also, it may provide us new tools for diseases diagnosis and prognosis prediction. This review summarized recent progress of DNA methylation study and provided an overview of DNA methylation and liver diseases. Meanwhile, the association between DNA methylation and liver diseases including hepatocellular carcinoma, liver fibrosis, nonalcoholic steatohepatitis and liver failure were extensively discussed. Finally, we discussed the potential of DNA methylation therapeutics for liver diseases and the value of DNA methylation as biomarkers for liver diseases diagnosis and prognosis prediction. This review aimed to provide the emerging DNA methylation information about liver diseases. It might provide essential information for managing and care of these patients.

Key words: DNA methylation; Liver diseases; Hepatocellular carcinoma; Liver fibrosis; Nonalcoholic steatohepatitis; Liver failure

Core tip: This review summarized recent progress of DNA methylation study and provided an overview of DNA methylation and liver diseases. The association between DNA methylation and liver diseases including hepatocellular carcinoma, liver fibrosis, nonalcoholic steatohepatitis or liver failure were extensively discussed. We also discussed the potential of DNA methylation as biomarkers and therapeutic targets for liver diseases. This review aimed to provide the emerging DNA methylation information about liver diseases. It might provide essential information for managing and care of these patients.

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INTRODUCTION

Because of the high prevalence, liver diseases have been studied systematically during the past few decades. Many studies focus on genetic defects[1] and genome-wide association studies do provide us great information about the pathogenesis of liver diseases[2]. However, many questions which cannot be totally illustrated by genetic mechanism still exist, which lead researchers to initiate

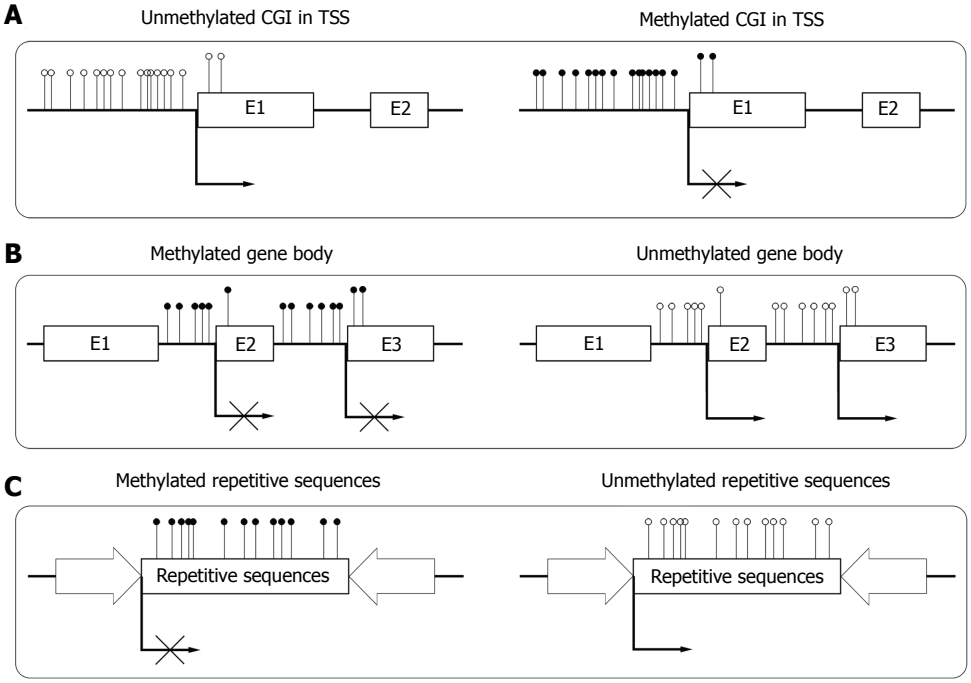

Figure 1 DNA methylation pattern in different parts of the genomes. The normal conditions are presented in the left column and aberrant conditions are shown on the right. The black dots represent methylated CpG sites and the white circles represent unmethylated CpG sites. A: In normal cells, CpG islands (CGI) in transcriptional start site (TSS) usually remain unmethylated, allowing transcription. Aberrant methylation often links to long-term stabilization of transcriptional silencing and loss of gene function both physically and pathologically; B: In normal cells, gene bodies are CpG-poor and extensively methylated, increasing elongation efficacy. Aberrant demethylation of gene bodies may facilitates spurious initiations of transcription; C: In normal cells, repetitive sequences of genome are highly methylated, preventing chromosomal instability or gene disruption. Aberrant demethylation of repetitive sequences may reactivate endoparasitic sequences.

the study of epigenetic variation. Recent studies showed that the combination of genetic and epigenetic variants contributed together to the susceptibility and progression of liver diseases[3-5]. Epigenetics refers to the heritable changes of gene expression without changes in gene sequence[6]. DNA methylation is a very important epigenetic mechanism in human and distribute widely across human genome. It is of crucial important for normal development, genomic imprinting as well as inactivation of X-chromosome[7-9]. Meanwhile, aberrant DNA methylation usually associates with many human diseases[10]. The goal of this article is to review the studies associated with DNA methylation and liver diseases. Finally, we look into the future prospect that DNA methylation may bring to the detection and treatment of liver diseases.

DNA METHYLATION AND ITS MECHANISM

DNA methylation which refers to addition of the methyl groups to DNA is firstly introduced in 1970s[11,12]. In invertebrates and fungi, DNA methylation only presents in small proportion of genome and varies among different clades[13,14]. In vertebrate genome, it presents in almost everywhere across the genome. Mainly, DNA methylation occurs at cytosine adjacent to guanine (CpG dinucleotides)[15]. In human genome, The CpG dinucleotides are very rare (approximately 1%). They are nonuniformly distributed and tend to cluster together to form CpG island (CGI). CGI refers to a 200-bp region in DNA which is characterized by high G+C content (more than 50%) and high observed CpG/expected CpG ratio (at least 0.6)[16]. Previous studies showed that CGIs existed in more than half of the genes in vertebrate genomes. Until now, the exact role of gene methylation in gene regulation remains largely unclear[17].

DNA methylation in transcriptional start sites

Until now, most of the studies on DNA methylation focus on CGIs in the transcriptional start sites (TSSs) of genes. In human genome, about 60% of gene TSSs contain CGIs and usually remain unmethylated in normal cells. Methylation of these CGIs often result in long-term stabilization of transcriptional silencing and loss of gene function both physically and pathologically[18] (Figure 1A). CpG island shore is defined as lower CpG density region which is close (approximately 2 kb) to the CGI. Recent studies show that most tissue specific methylation occurs at CpG island shores[19,20]. Aberrant DNA methylation at CpG island shores correlate even more strongly with gene expression than CGI[21].

There are about 40% of human genes which do not contain bona fide CGI at their TSSs[16]. Compared with genes that contained CGIs, the role of methylation in genes without CGIs at the TSSs has not been well demonstrated. More studies still need to be performed on genes without CGIs. Studies revealed that maspin gene had a promoter that did not reach the criteria for CGI

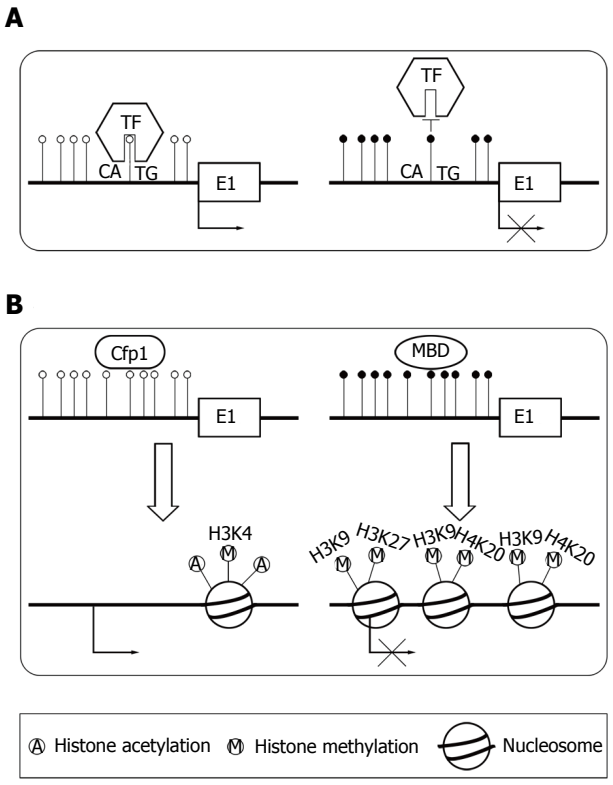

Figure 2 Transcriptional suppression mechanisms of DNA methylation in TSSs. The normal conditions are presented in the left column and aberrant conditions are shown on the right. The black dots represent methylated CpG sites and the white circles represent unmethylated CpG sites. A: In normal cells, transcription factors (TF) bind to unmethylated binding site, allowing transcription. Aberrant methylated binding site prevent TF binding to its normal sites; B: In normal cells, unmethylated CpG island can recruit CpG binding proteins (Cfp1) and trigger histone modifications characterized by high levels of acetylation and trimethylated H3K4, H3K36 and H3K79. Finally, it forms a structure suitable for transcription. Aberrant methylated recruit methyl-CpG-binding domain (MBD) proteins and trigger histone modifications characterized by high levels of H3K9, H3K27 and H4K20 methylation and low levels of acetylation. It represses the transcriptional permissiveness of chromatins and results in gene silencing.

and hypermethylation of this promoter was strongly correlated with its tissue specific expression[22]. However, *MAGE* gene was found to be unregulated by methylation in the promoters which do not satisfy CGIs.

There are two primary means by which DNA methylation in TSSs repress transcription. The transcription factors[23] control gene expression level. DNA methylation can directly preclude the transcription factors binding to its normal sites[24,25] (Figure 2A). For example, transcription factor YY1 which is essential for the imprinting of *Peg3* gene can bind to PEG3-DMR sequence in the first intron[24]. *In vivo*, the methylation of PEG3-DMR sequence precludes the binding of YY1, which result in the repression of maternal allele. In paternal allele, YY1 can effectively bind to the unmethylated PEG3-DMR sequence. Alternatively, DNA methylation can recruit specific proteins and induce a repressive chromatin structure[9] (Figure 2B). In normal condition, unmethylated CGIs can recruit CpG binding proteins, which form a structure suitable for transcription[26]. When CGIs are methylated, they can recruit methyl-CpG-binding domain (MBD) proteins[14,27]. Then, MBD proteins could recruit the histone modifying as well as chromatin remodeling complex to the methylated positions, which result in transcriptional silencing by repressing the transcriptional permissiveness of chromatins.

DNA methylation in gene bodies

Although CGIs also exist within gene bodies[28], most gene bodies are CpG-poor and extensively methylated. Studies showed that high level of gene body methylation was positively correlated with transcription, which meant it might associate with gene activation[29,30]. Zilberman *et al*[31] found that the methylation of gene body could increase elongation efficiency and prevent spurious initiations of transcription (Figure 1B). Shukla *et al*[32] illustrated that methylation between exons and introns was involved in regulating splicing[33]. Other studies reported that the methylation in gene body could be an important mechanism for managing promoter usage[34]. The high methylation level in gene body was essential for the elongation of a transcript.

DNA methylation in repetitive sequence

Repetitive elements comprise up to 45% of human genome[35], which mainly consist of interspersed repeats and tandem repeats. In normal somatic cells, repetitive sequences of genome are highly methylated. The deeply methylated condition is essential for the stability of chromosome and normal gene expression[36] (Figure 1C). Demethylation of repetitive sequences in genome may result in different kinds of diseases[37,38].

The inheritance of DNA methylation

DNA methylation is an important way to store hereditary information. Although it does not change gene sequence, it can propagate the methylation mark during cell divisions[39]. The DNA methylation inheritance process is catalyzed by DNA methyltransferase (DNMT) enzyme family. Manly, there are five members in DNMT enzyme family, DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L. DNMT1, DNMT3a, DNMT3b serve as methyltransferase. Each of the three DNMTs is essential for normal human development[7,40]. Studies revealed that loss of methylation resulted from the inactivation of DNMTs could result in apoptosis of somatic cell[41] and cancer cells[42]. However, it showed that DNMTs were not essential for the survival of embryonic stem cells[43].

Bestor *et al*[44] firstly cloned DNMT1 in 1988 from mouse cells. Later studies revealed that DNMT1 expressed mostly at S phase of cell cycle[45] and mainly acted as maintenance DNMT. Interacting with the DNA polymerase processing factor proliferating cell nuclear antigen and ubiquitin-like plant homeodomain and RiNG finger domain containing protein 1 (UHRF1), DNMT1 methylated the hemimethylated sites during DNA semi-reserved replication[46,47]. Soon after replication, DNMT3a and DNMT3b bound to methylated DNA and corrected methylation sites missed by DNMT1 and completed the

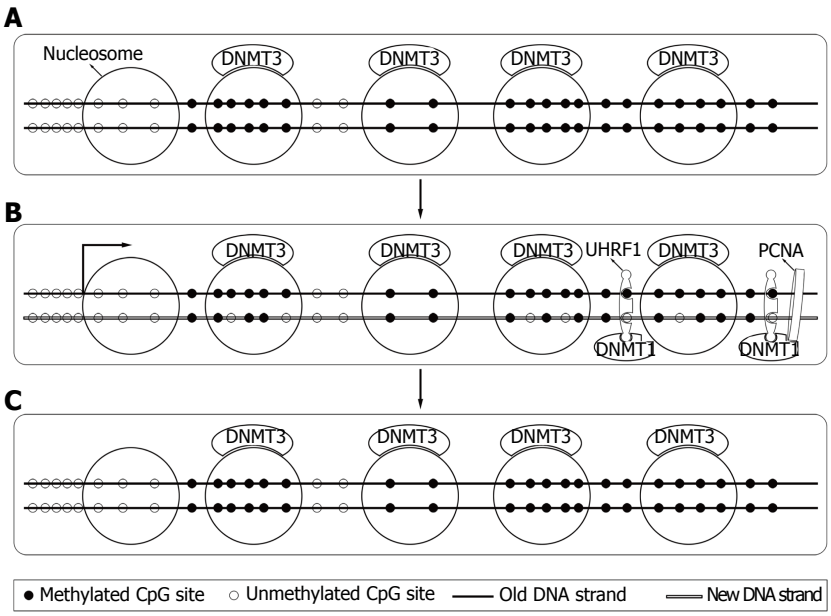

Figure 3 The maintenance of DNA methylation pattern. A: In somatic cells, DNA methyltransferase (DNMT) 3 (DNMT 3a and DNMT 3b) are bound to nucleosomes containing methylated DNA; B: During DNA semi-reserved replication, DNMT1 interact with the DNA polymerase processing factor proliferating cell nuclear antigen (PCNA) and ubiquitin-like plant homeodomain and RiNG finger domain containing protein 1 (UHRF1) and methylate the hemimethylated sites; C: Soon after DNA semi-reserved replication, DNMT3 correct methylation sites missed by DNMT1 and complete the process.

process[48,49] (Figure 3). DNMT1 was essential for both normal somatic cells and cancer cells and a knockout of DNMT1 could cause their death[41,42].

After the cloning of DNMT1, studies found that embryonic stem cells could still methylate retroviral DNA de novo even without DNMT1[50]. DNMT3a and DNMT3b were found in later studies[40]. They were regarded as de novo DNMT and functioned to set up normal methylation pattern during embryonic development. They were abundant in embryonic stem cell and less expressed in differentiated cells[51]. Other DNMTs like DNMT3L possessed no methylation catalytical activities. But Bourc'his *et al*[52] found that DNMT3L was crucial for establishment of maternal genomic imprinting.

DNA METHYLATION AND HEPATOCELLULAR CARCINOMA

In hepatocellular carcinoma (HCC), DNA methylation is characterized by a genome wide hypomethylation and a site specific hypermethylation[53]. Until now, many studies for presenting the DNA methylation patterns in HCC have been published.

Hypomethylation

Compared with normal liver tissue, DNA methylation in HCC is characterized by global hypomethylation. The hypomethylation of intergenic areas, repetitive DNA sequences[54], introns[55] and promoter CGI of specific oncogene[56] are responsible for the global hypomethylation. Global hypomethylation mainly result in chromosomal instability, loss of genomic imprinting[57,58] and reactivation of transposable elements, which may contribute to the development of cancer.

Previous studies revealed that the demethylation of chromosome 1 heterochromatin DNA was associated with the q-arm copy gain[59] in HCC. Also, a number of hypomethylated tumor-promoting genes, including HPA[60], MAT2A[61], VIM[62] and SNCG[63] have been identified in primary human HCC.

Hypermethylation

In tumor suppressor gene, the hypermethylation of CGIs in TSSs result in the loss of gene function, which is crucial for the origin of cancer[64]. The inactivation of tumor suppressor genes caused by hypermethylation of CGI in TSS exist in almost every type of human cancers[65]. Hypermethylation may affect the process of cell cycle regulation, DNA repair, angiogenesis, programmed cell death and tumor cell invasion. The genes silenced by hypermethylation in human cancers are often those who are essential for the maintenance of stem cell characteristics and/or the maturation of adult cells during cell renewal[65,66]. Silencing of these genes may result in the initiation of tumors through distribution of abnormal stem cells and/or abnormal of normal cell differentiation.

Until now, many tumor suppressor genes have been identified to be hypermethylated in HCC. Table 1 presents a group of frequently methylated genes in HCC.

DNA METHYLATION AND LIVER FIBROSIS

In liver fibrosis, aberrant DNA methylation has been studied for a few years. Until now, a number of aberrantly methylated genes have already been recognized. Through direct or indirect examination methods (treated with demethylating agents such as 5-aza-2'-deoxycytidine), these genes were identified to be aberrantly methylated. In activated hepatic stellate cell (HSC), transcriptional repression of some genes was indentified to be due to promoter hypermethylation of them.

Until now, genome-wide studies of DNA methylation associated with HSC activation were limited. Aberrant

Table 1 A selection of methylated genes in hepatocellular carcinoma

Gene	Location	Function	Methylation frequency	Ref.
GSTP1	11q13.2	Detoxification	41%-58%	[85-87]
SOCS1	16p13.13	Cytokine inhibitor	60%	[88]
RASSF1A	3p21.3	Apoptosis	54%-95%	[89,90]
E-Cadherin	16q22.1	Cell adhesion	33%-67%	[91,92]
APC	5q22.2	Signal transduction	46%	[93]
p16	9q21.3	CDK inhibitor	17%-83%	[94,95]
SFRP1	8p11.21	Signal transduction	59.50%	[96]
WIF-1	12q14.3	Signal transduction	61.90%	[97]
MGMT	10q26	DNA repair	22%-39%	[98,99]
TFPI2	7q21.3	Protease inhibitor	46.50%	[100]

methylation associated with HSC activation had been reported at specific loci such as the phosphatase and tensin homologue (*PTEN*) and patched1 (*PTCH1*) genes. These genes were aberrantly methylated in the myofibroblast and associated with the decreased of gene expression[67,68]. Our previous study revealed that aberrant promoter methylation of PPAR gamma gene was significantly associated with liver fibrosis in patients with chronic hepatitis B[69]. Other genes like Ras GTPase activating-like protein 1 (*RASAL1*) gene were also found to be aberrantly hypermethylated in liver fibrosis[70].

DNA METHYLATION AND NONALCOHOLIC STEATOHEPATITIS

So far, the relationship between DNA methylation and metabolic diseases was firmly established. Ahrens *et al*[71] used array-based DNA methylation and mRNA expression profiling to analyze the liver tissues from patients with non-alcoholic fatty liver disease (NAFLD) (n = 45) and health controls (n = 18). Aberrant methylation and decreased mRNA expression were seen for nine genes, which included genes for key enzymes in intermediate metabolism (ACLY, PC and PLCG1) and insulin or insulin-like signaling (IGFBP2, IGF1 and PRKCE)[71]. Studies showed that supplementation of diets lack of methyl donors could induce DNA hypomethylation and the development of steatosis in mice. However, supplementation of diets with methyl donors could prevent the development of NAFLD, suggesting that differences in the DNA methylation status might be a potential factor for individual susceptibilities to hepatic steatosis[72,73]. The supplementation of the maternal diet with methyl donors could induce aberrant methylation in adulthood and protect offspring from suffering obesity[74].

DNA METHYLATION AND LIVER FAILURE

Recent studies found that the aberrant methylation of several genes might participate in the development of liver failure. The aberrant promoter methylation of some anti-inflammatory genes might result in the down-regulate gene expression and inhibit their protective role in liver injury. Our previous study found that glutathione-S-transferase P1 (GSTP1) promoter hypermethylation occurred in patients with acute on chronic hepatitis B liver failure (ACHBLF) which might facilitate oxidative stress associated liver damage[75]. A study performed by Fan *et al*[76] showed that hypomethylation of IFN-γ gene promoter in peripheral blood mononuclear cells might be associated with the onset of ACHBLF. Qi *et al*[77] found that the aberrant hypermethylation of glutathione-S-transferase P1 (*GSTM3*) gene occurred in ACHBLF, which was correlated with their disease severity.

FURTHER PROSPECTS AND SUMMARY

The development of liver diseases is a multifactorial process characterized by the combination and integration of a multitude of alterations including genetic and epigenetic changes. In the past decades, there were exponential increases in the interest and progress of DNA methylation. Studies already revealed the potential role that DNA methylation played in the normal human development and initiation of diseases. DNA methylation-based biomarkers were proposed for disease risk assessment[78], early detection[79,80], prognostic prediction[81] and treatment outcome prediction of liver diseases[82]. Meanwhile, there was hope for developing therapeutic agents to manipulate aberrant DNA methylation patterns and to treat malignancies[6]. In 1970s, Constantinides *et al*[83] reported 5-azacytidine had remarkable effects on differentiated states of cells. In 2005, Brueckner *et al*[84] reported the drug RG101 could also reactivate tumor suppressor gene by inhibiting human DNA methyltransferase. Therefore, combined genetic and epigenetic information may help clinicians to prevent liver diseases developing in at-risk individuals and from passing on unhealthy DNA methylation characteristics to offsprings.

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