DNA methylation of SAB gene and its correlation to the progression of NASH

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Abstract

Increased SAB (SH3BP5) gene expression and protein level correlates with the progression and severity of non-alcoholic steatohepatitis (NASH) in diet induced mouse model and human non-alcoholic fatty liver disease (NAFLD). However, the mechanism of regulation of SAB gene expression has not yet been examined. Two CpG islands are predicted at the promoter region immediately upstream of the transcription start site and at intron1 of SAB gene. In addition, specific methylation sites were identified in the data base of whole genome bisulfite sequencing (WGBS) of liver from C57BL/6N embryos and young adults (ENCODE). Thus, DNA methylation might be involved in the regulation of SAB gene expression. Methylated cytosine in DNA regulates gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factors. DNA methylation was examined by methylation specific primers (MSP) after bisulfite conversion followed by unbiased amplification of genomic DNA of chow or high fat high carbohydrate diet fed mice. This experiment laid the work of future analysis such as bisulfite sequencing and quantitative analysis by qPCR using methylation specific probe.

Background

DNA (CpG) methylation is one of the fundamental mechanisms of repression of gene expression. Hypo-methylation of four CpG sites of *SH3BP5* (*SAB*) in peripheral blood occurs in early-stage lung adenocarcinoma in humans. However, regulation of *SAB* gene expression by CpG methylation in human and mouse liver has not yet been examined in the context of NAFLD. CpG methylation at the transcriptional start site (TSS) blocks transcription initiation. In fact, DNA methylation adds an additional level of stability to epigenetic states. *De novo* DNA methylation is achieved by DNA methyltransferase DNMT1, DNMT3A, DNMT3B, and requires a nucleosome which is a substrate, and S-adenosylmethionine (SAM) which is a methyl donor. Active TSSs are depleted of nucleosome and therefore lack the substrate for *de novo* methylation.

Enzyme-mediated DNA methylation and demethylation

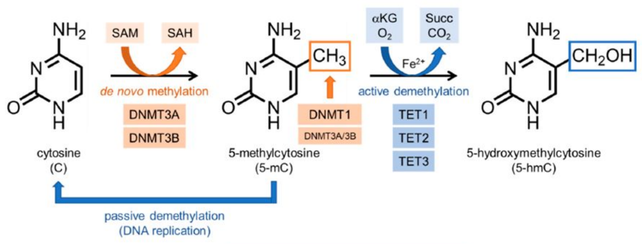

Figure 1. DNA methylation and demethylation reaction diagram. DNA methylation occurs at the carbon 5 of cytosine in CpG dinucleotides. DNA methyltransferases (DNMTs) catalyze the methylation reaction by transferring a methyl group to cytosine (C) using S-adenosyl methionine (SAM) as methyl donor and producing S-adenosyl homocysteine (SAH). As a product, 5-methylcytosine (5-mC) is generated. DNMTs also maintains the status of DNA methylation. DNA demethylation is a multi-step oxidation process mediated by ten-eleven translocation (TET) methylcytosine dioxygenases (active demethylation). TET enzymes use Fe^{2+} and α-ketoglutarate (KG) as co-substrates and generates succinate (Succ) and CO2. In the first step of demethylation process, the 5-mC is converted to 5-hydroxymethylcytosine (5-hmC) (shown), and further TET-dependent oxidation leads to the conversion of 5-hmC into 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) (not shown). After several oxidation reactions mediated by TET enzymes, the methyl group is removed by base excision repair mechanism. 5-mC or 5-hmC can also be converted to unmethylated cytosine during DNA replication, through a passive DNA demethylation.

Mapping CpG methylation sites on mouse Sab gene promoter

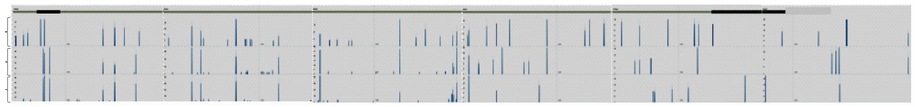

Figure 2. Mapping CpG methylation sites on mouse *Sab* gene promoter, first exon, first intron and second exon obtained from methylation database (ENCODE). Whole genome bisulfite sequencing of liver at various embryonic age and adult (8 weeks) of mouse *Sab* gene indicates methylation of several CpG sites (shown at blue bar) in *Sab* DNA. Methylated CpG were identified in two CpG islands located at the promoter and gene body. Consistency of several CpG methylation sites from embryonic to adult age suggests the role of DNA methylation in repression of *Sab* gene expression. CpG methylation site (□) at *Sab* transcriptional start region (−177 and −182 nt of *Sab* promoter**)** was examined for methylation specific PCR (MS-PCR)

Decreased DNA-methylation at transcriptional start region of Sab in High-Fat High-Calorie (HFHC) diet fed mouse liver

SAB (SH3BP5) is a key pivotal gene involved in progression and severity of NASH. Knockdown or knockout prevents or reverses diet induced NASH. Therefore, to explore the transcriptional regulation of SAB gene, we examined the transcriptional activation regulators such as transcription activators/repressors and DNA methylation. DNA (CpG) methylation is one of the fundamental mechanisms of repression of gene expression. However, regulation of SAB gene expression by CpG methylation in human and mouse liver has not yet been examined in the context of NAFLD.

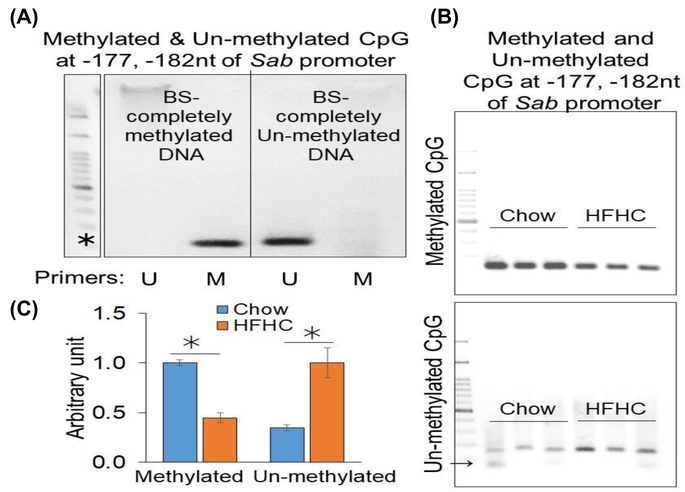

Figure 3. Decreased DNA-methylation at transcriptional start region of *Sab* in HFHC fed mouse liver. (A) Mouse liver genomic-DNA (gDNA) was extracted and prepared for BS-conversion of completely un-methylated and methylated DNA. Briefly, *BS-completely un-methylated DNA* was prepared by whole gDNA amplification (WGA) by REPLI-g Mini DNA polymerase (Qiagen) followed by Dpn1 digestion of methylated gDNA, BS conversion, and WGA of BS-converted DNA. *BS-Completely CpG methylated DNA* was prepared by in-vitro methylation of gDNA by CpG methyltransferase (M.SssI) with SAM followed by BS conversion, and WGA of BS-converted DNA. The final REPLI-g WGA amplified the DNA to the saturated level and equals the concentration. Methylation specific primer (M) and un-methylation specific primer (U) for the CpG methylation site at *Sab* TSS region (□) shown in Figure 2 was designed by MethPrimer. The result shows that primer (M) specifically identified methylated CpG at −177 and −182 nt on *Sab* promoter. **(B,C) Decreased methylated and increased unmethylated CpG at −177 and −182 nt of *Sab* promoter in HFHC 16 weeks fed mouse liver.** gDNA extracted from chow or HFHC 16 weeks fed mice liver were performed BS conversion followed by WGA of DNA. Methylation status of the CpG −177 and −182 nt from TAC (ATG) codon of *Sab* in the TSS region (□) was examined by MS-PCR. Unmethylated DNA PCR was performed separately. (→) = primer dimers. □,P<0.05, *t* test, N = 3

Tunicamycin induced ER stress decreases methylated CpG at −177 and −182 nt of Sab promoter

Low non-toxic dose of tunicamycin induces SAB gene transcription activation in AML-12 mouse hepatocytes and primary mouse hepatocytes. Therefore, we examined the DNA methylation status when SAB gene is activated by ER stress inducer tunicamycin.

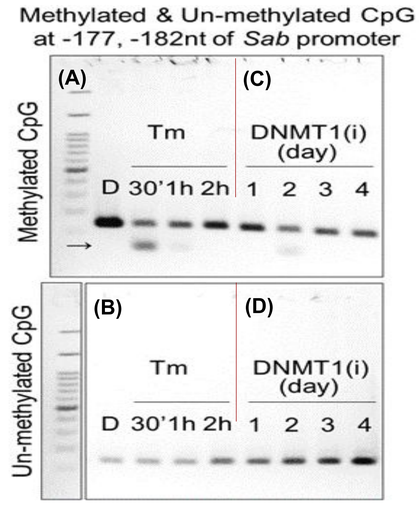

Figure 4 . Tunicamycin induced ER stress decreases methylated CpG at −177 and −182 nt of *Sab* promoter. (A,B) Time course of methylated and un-methylated CpG at (-177, -182 nt) by tunicamycin exposure in AML-12 cells. Cells were treated with DMSO (D) or Tm 10μg/ml for 0.5, 1, 2hr and examined the methylation status at *Sab* promoter by MS-PCR. Tm dramatically decrease CpG methylation at 0.5hr and start to recover at 2hr after Tm exposure. Un-methylated CpG increase was at 2hr. **(C,D) Inhibition of DNMT1, DNMT1(i), decreased CpG methylation.** Cells were treated with DNMT1 inhibitor (5-Azacytidine) 5μM for 1-4 days and examined the methylation status at *Sab* promoter by MS-PCR. DNMT1(i) decreased methylated CpG at 24hr after exposure compared to DMSO and decline more in longer incubation. Un-methylated CpG gradually increased.

Summary

- SAB (SH3BP5) gene expression is regulated by DNA methylation.
- Decreased SAB gene promoter DNA methylation in liver occurs in HFHC diet fed mice suggesting that DNA methylation mechanism is downregulated in HFHC diet fed liver.
- ER stress occur in HFHC diet fed liver and ER stress may be a contributing factor of downregulation of DNA methylation.

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