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NAT10 promotes liver lipogenesis in mouse through N4-acetylcytidine modification of *Srebf1* and *Scap* mRNA

Zhouqi Wang¹, Xinxing Wan¹, Md Asaduzzaman Khan², Lin Peng³, Xiaoying Sun¹, Xuan Yi¹ and Ke Chen^{1*}

Abstract

Background Metabolic dysfunction associated steatotic liver disease (MASLD), closely linked to excessive lipogenesis, induces chronic liver disease. MASLD often cause other metabolic diseases, such as cardiovascular disease, diabetes and obesity. However, the mechanism of N-acetyltransferase 10 (NAT10)-mediated N4-acetylcytidine (ac4C) mRNA modification in lipogenesis of MASLD has not been fully elucidated. This study investigated the role of NAT10 in lipogenesis targeting mRNA ac4C modification.

Methods The expression of NAT10 in mouse liver was assessed after a 12-week high-fat diet. In addition, the expression of NAT10 also was detected after AML12 hepatocytes cells were treated with 150 $\mu\text{mol/L}$ palmitic acid (PA). The ac4C mRNA modification was performed by dot blotting. Oil red O staining and the mRNA expression of *Srebf1*, *Acaca* and *Fasn* were used to assess lipogenesis in AML12 cells with NAT10 overexpression or knockdown. acRIP-PCR and NAT10 RIP-PCR were used to verify the *Srebf1* and *Scap* mRNA ac4C modification by NAT10. Furthermore, the liver lipogenesis was evaluated by AAV-mediated target knockdown of NAT10 in mouse liver and treating a specific inhibitor, Remodelin.

Results This study revealed that NAT10 is significantly upregulated in liver lipogenesis after a 12-week high-fat diet. NAT10 and ac4C mRNA modification were also drastically increased in AML12 cells after treated with 150 $\mu\text{mol/L}$ PA. Silencing of NAT10 notably inhibited the lipogenesis in AML12 cells and AAV-mediated target knockdown of NAT10 in mouse liver. The acRIP-PCR and NAT10-RIP-PCR revealed that NAT10 ac4C modified *Srebf1* and *Scap* mRNA, the critical modulator of liver lipogenesis, to regulate liver lipogenesis. Besides, Remodelin strongly inhibited liver lipogenesis, including liver TG, serum ALT, AST, TG and TC level and glucose metabolism.

Conclusions NAT10 mediates ac4C modification of *Srebf1* and *Scap* mRNA, thereby affecting lipogenesis in the liver. This study provided a new target for the treatment of MASLD.

Keywords N-acetyltransferase 10, ac4C modification, *Srebf1*, *Scap*, Liver lipogenesis

*Correspondence:

Ke Chen
chenke520@yeah.net

¹Department of Endocrinology, The Third Xiangya Hospital of Central South University, No. 138 Tongzipo Road, Yuelu District, Changsha, Hunan 410013, People's Republic of China

²Department of Biochemistry and Microbiology, School of Health & Life Sciences, North South University, Plot-15, Block-B, Bashundhara, Dhaka 1229, Bangladesh

³Department of Nephrology, The First Hospital of Changsha, No. 311 Yingpan Road, Kaifu District, Changsha, Hunan 410005, People's Republic of China



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Background

The metabolic dysfunction associated steatotic liver disease (MASLD) has been extensively studied recently and named in 2023 [1]. The incidence rate of MASLD is about 39% worldwide, which induces liver cirrhosis, liver dysfunction and hepatocellular carcinoma [2, 3]. In addition, MASLD is strongly associated with most of the metabolic diseases, such as, obesity, diabetes, cardiovascular disease and other diseases [2, 4]. The pathological mechanism of MASLD include dysfunction of lipid uptake, transportation of fatty acids, hepatic lipogenesis and β -oxidation [5, 6], and among these, increased liver lipogenesis is one of critical pathological mode for MASLD that lead to excessive lipid deposition in the liver [7, 8]. There is a large number of studies to explore the molecular aspects contributing to MASLD [5, 9, 10], however, no studies have been reported the involvement of N4-acetylcytidine (ac4C) mRNA modification in MASLD.

The sterol regulatory element-binding proteins (SREBPs) are the core regulators of liver lipogenesis [11]. SREBPs include three subtypes: SREBP-1a, SREBP-1c, and SREBP-2 [12], among them, SREBP-1c is the predominant subtype in liver [13]. SREBPs binding to the SREBF chaperone (SCAP) and insulin induced genes (INSIGs) further form a SREBPs/SCAP/INSIGs complex, and SREBPs are transported to nucleus and activate the transcription process of acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 (SCD1) of lipogenesis [14].

Post-transcriptional modification of mRNA is extensively studied, and the most common expression patterns include N6-methyladenine (m6A), 5-ethylcytosine (m5C), N1-methyladenine (m1A) and N7-methylguanosine (m7G) and others [15–17]. In previous studies, ac4C-modified tRNAs and 18 S rRNAs were reported [18, 19], but in 2018, Arango et al. found that ac4C is also present in mRNA [20]. N-acetyltransferase-like protein 10 (NAT10) plays an activator or inhibitor as the “writer” of ac4C, and further affect mRNA stability and translation efficiency [21–23]. In addition, ac4C modification motifs are mainly enriched in the protein coding sequence (CDS) of mRNA, a small portion of motifs are enriched in 5'-untranslated region (5'-UTR) and 3'-UTR [24]. NAT10 acetylates the N4 nucleoside ac4C of mRNA cytosine in the target gene during the translation of mRNA, which affects the spatial conformation of its cytosine and further affects the binding to ornithine, thereby significantly increases or inhibits the structural stability of mRNA, and thus improves or decreases the translation efficiency [25, 26]. So far, it's evident that NAT10 regulates different diseases including myocardial remodeling, oocyte maturation, tumor progression via mRNA ac4C modification [27–31].

In a liver lipogenesis related study, Zhang et al. found that knockout of NAT10 lead to decreased hepatic steatosis in male offspring of high fat-diet induced mother mice [32]. In addition, knockdown of NAT10 drastically inhibit cellular lipid accumulation after human HepG2 cell were treated with palmitic acid (PA) [32]. In molecular level, by using NAT10-RNA immunoprecipitation (RIP)-PCR silencing of NAT10 inhibited *Cd36/FATP2* mRNA stability to prevent hepatic lipid uptake and steatosis [32]. Moreover, silencing of NAT10 blocked free fatty acid (FFA) metabolism in human MCF7 breast cancer cells via ac4C modification of ELOVL fatty acid elongase 6 (*ELOVL6*), Acyl-CoA synthetase long chain family member 1 (*ACSL1*), Acyl-CoA synthetase long chain family member 3 (*ACSL3*), Acyl-CoA synthetase long chain family member 4 (*ACSL4*), Acyl-CoA dehydrogenase short/branched chain (*ACADSB*) and Acetyl-CoA acetyltransferase 1 (*ACAT1*) mRNA [33]. The NAT10 inhibitor, Remodelin was also reported to participate in impeding mitochondrial lipid metabolism in cancer cells [34]. Although Zhang et al.'s research has shown that NAT10 can interact with *Cd36/FATP2* mRNA via NAT10-RIP-PCR, in HepG2 and MCF7 cells cancer cells [32, 33], whether NAT10 can directly regulate liver lipogenesis via mRNA ac4C modification was not verified.

In this study, NAT10 was found significantly upregulated in high-fat diet mice liver. NAT10 positively regulated lipogenesis in mice AML12 hepatocytes cells and mice liver lipogenesis via ac4C modified *Srebf1* and *Scap* mRNA. This study may provide a new treatment target for MASLD.

Materials and methods

Culture of AML12 and HepG2 cells and PA or Remodelin treatment

The AML12 and HepG2 cell were purchased from China National Collection of Authenticated Cell Cultures. AML12 cells were maintained in Gibco's DMEM/F-12 (Shanghai, China) media enriched with 10% fetal bovine serum (FBS, Sigma, F8687, Australia), ITS Liquid Media Supplement (Sigma, I3146, MO, USA) and 40 ng/ml Dexamethasone (Sigma, D4902, Shanghai, China). In addition, AML12 cells were treated with 150 μ mol/L PA (Sigma, P0500, Shanghai, China) or 20 μ mol/L Remodelin (Beyotime, SD1168, Shanghai, China) for 48 h. HepG2 cells were cultured in Gibco's DMEM (Shanghai, China) media containing 10% FBS.

Real-time quantitative reverse transcription PCR (RT-qPCR)

RNAs were isolated from AML12 cells or liver tissues using Triquick reagent (Solarbio, R1100, Beijing, China), and subsequently were reverse transcribed to cDNA (Solarbio, RP1100, Beijing, China). qPCR with 2 \times SYBR Green PCR Mastermix (Solarbio, SR1110, Beijing, China)

was used to detect relative mRNA expression, normalized to *Gapdh* expression. Primer sequences are provided in Supplementary Table 1.

Oil red O staining and FFA quantification

After AML12 cells were incubated with 150 $\mu\text{mol/L}$ PA for 48 h, the cells were first washed with PBS and fixed with 4% paraformaldehyde, followed by multiple washes with water. Next, the cells were then washed with water for 3 times, incubated with diluted Oil red O (Beyotime, C0157S, Shanghai, China) for 10 min, and were washed with water again. The photographs were taken with microscope (Leica DM IL LED Fluo, Wetzlar, Germany). The liver tissue slices were added to diluted Oil red O, and incubated for 20 min, decolorized with 70% alcohol, and then washed with water again. Next, the liver slices were further stained using hematoxylin. The cellular FFA content were detected with FFA content assay kit (Solarbio, BC0590, Beijing, China).

Immunoblotting

The AML12 cells or liver tissue protein samples were extracted with ProteoPrep[®] total protein isolation kit (Sigma, MO, USA), and the content was detected using the BCA quantitative analysis kit (Sigma, Shanghai, China). Subsequently, approximate 100 μg protein was separated by SDS-PAGE (70 V, 30 min then 120 V, 90 min) and transferred (300 mA, 60 min) and transferred to PVDF membranes (Millipore, MA, USA). The membrane was then blocked with 5% non-fat milk for 2 h, and incubated in diluted primary antibody including NAT10 (13365-1-AP), SREBP-1 (14088-1-AP), ACC1 (21923-1-AP), FASN (10624-2-AP) and β -actin (66009-1-Ig) [all provided by Proteintech, Wuhan, China], SCAP (Abcam, ab308060, MA, USA) and Cd36 (Abcam, ab255331, MA, USA). The next day, the membranes were incubated with HRP-labeled secondary antibodies for 1 h, and after washed for 3 times, chemiluminescence reaction was performed by using BeyoECL plus kit (Beyotime, P0018S, Beijing, China). Images were captured using the GelDoc XR Biorad system (Bio-Rad, CA, USA) for further analysis.

mRNA ac4C dot blot

The mRNA was isolated from about 10 μg of total RNA using Dynabeads[™] mRNA purification kit (Invitrogen, CA, USA), and mRNA was denatured at 95 $^{\circ}\text{C}$ for 5 min, and was placed at ice immediately for 1 min. Next, the mRNA was added to PVDF membranes (Millipore, MA, USA), the membrane was cross-linked for 3 times at ultraviolet 254 nm, and subsequently were blocked with 5% non-fat milk containing 0.1% PBST. The membrane was incubated with anti-ac4C antibody (Abcam, ab252215, MA, USA) overnight at 4 $^{\circ}\text{C}$. The membrane

was washed for 3 times and incubated using an anti-rabbit IgG-HRP (Sigma, MO, USA) for 1.5 h at room temperature, and then washed for 3 times. The membrane was incubated with ECL (Beyotime, P0018S, Shanghai, China) and 0.02% methylene blue (Solarbio, M8030, Beijing, China) was used to stain membrane. After that, membrane was washed with RNase-free water (Solarbio, R1600, Beijing, China) for 1.5 h, and the membrane was photographed with ChemiDoc imaging system (Bio-Rad, CA, USA).

Hepatocyte-targeted mouse adeno-associated virus (AAV) shRNA-NAT10 plasmid construction

The overexpressed NAT10 (OE-NAT10, NM_153126.4), OE-Srebf1, OE-Scap and overexpressed negative control plasmid (OE-NC), siRNA NAT10 (siRNA-NAT10) and the siRNA negative control plasmid (siRNA-NC) was constructed by Shanghai Sangong Biotechnology Co., Ltd, China. Hepatocyte-targeted AAV-shRNA-NAT10 and AAV-shRNA-NC plasmids were designed and constructed by Shanghai Gene Chemistry Co., Ltd, China. The NAT10 target sequence was 5'-GCTGGATTTGTTC CTGTCTAT-3', and element sequences was pAAV-ApoE/hAATp-EGFP-MIR155(MCS)-SV40 PolyA. The cells were seeded in six-well plate and when cells growth to 80–90% confluence and then were transfected by 50nM siRNA-NAT10 and siRNA-NC.

Cell transfection

When AML12 cells were grown in approximately 80% confluency, the cells were transfected with OE-NAT10, OE-Srebf1, OE-Scap, OE-NC, siRNA-NAT10 and siRNA-NC with Lipo 5000 (Solarbio, L3200, Beijing, China) according to operation manual. After 48 h, the cells were treated with or without 150 $\mu\text{mol/L}$ PA.

ac4C-specific RNA immunoprecipitation (RIP) PCR (acRIP-PCR) and NAT10-RIP-PCR

After AML12 cells transfected with siRNA-NAT10 or siRNA-NC for 48 h, the total RNA was isolated and mRNA was purified using a mRNA purification kit (Invitrogen, CA, USA). The acRIP (Millipore, 17–700, MA, USA) was performed. Briefly, the mRNA was incubated with anti-ac4C antibody (Abcam, ab252215, MA, USA) and anti-IgG antibody (Abcam, ab172730, MA, USA) using Protein G magnetic beads. The ac4C modified mRNA was collected and further were amplified using RT-qPCR. NAT10-RIP-PCR method was performed according to acRIP-PCR. The *Srebf1* and *Scap* primers were designed according to ac4C modification sites predicted by PACES software (<http://www.rnanut.net/paces/>). The primer sequences are listed in Supplementary Table 1.

RNA decay assay

After AML12 cells were transfected with siRNA-NAT10 and siRNA-NC for 48 h, the two group of cells were harvested after 5 µg/mL Actinomycin D (Selleck, S8964, TX, USA) treatment for 0, 2, 4, 6 h. The total RNA was extracted and reverse transcribed, and then the *Srebf1* and *Scap* expression were amplified using qPCR.

Dual-luciferase reporter assay

According to *Srebf1* and *Scap* mRNA ac4C motif site predicted by PACES software, the pGL3-basic-*Srebf1*-wild type (*Srebf1*-WT), pGL3-basic-*Scap*-wild type (*Scap*-WT), pGL3-basic-*Srebf1*-mutation (*Srebf1*-MUT, mutation sequence: CTGCTGGCAAGTCTC) and pGL3-basic-*Scap*-mutation (*Scap*-MUT, mutation sequence: CGACCGAATACCCGA) plasmid were constructed. Approximately 1 µg plasmid was transfected into HEK293T and evaluation of firefly luciferase and renilla luciferase was carried out using a dual-luciferase reporter assay kit (Solarbio, D0011, Beijing, China).

In vivo study

To explore NAT10 and mRNA ac4C expression in liver tissue following a high-fat diet in mice, 6-weeks-old male mice were fed by chow diet (D12450) ($n=5$) and high-fat diet (D12492), all feed were provided by Research Diets, NJ, USA ($n=5$) for 12 weeks, the liver tissue were collected at 12th week. In addition, for AAV mediated NAT10 hepatocyte-targeted silencing experiment, 6-weeks-old male C57BL/6J mice were injected with hepatocyte-targeted AAV-shRNA-NAT10 (4×10^{11} v.g, $n=5$) or AAV-shRNA-NC (4×10^{11} v.g, $n=5$) through tail vein injection, and then subsequently were fed with a high-fat diet for 12 weeks. Additionally, two group of mice were injected again using same dose at 4th week. Furthermore, for Remodelin gavage experiment, male 6-weeks-old C57BL/6J mice were gavage with DMSO ($n=5$) and Remodelin (100 mg/kg/day, $n=5$), respectively, and two groups also were fed with high-fat diet for 12 weeks. All animal procedures were conducted in accordance with ethical guidelines approved by the Animal Ethics Committee of The Third Xiangya Hospital of Central South University, Changsha, China, (Approval NO: 2023-S027).

Hematoxylin and eosin (H&E) staining

The liver tissues were fixed with 4% paraformaldehyde for overnight at 4 °C, and the liver tissue were paraffin imbedded and cut into 5µM sections. After deparaffinized and rehydrated, the specimens were stained with H&E staining kit (Solarbio, G1120, Beijing, China). Microscopic images were captured using a Leica DM IL LED Fluo microscope (Wetzlar, Germany).

Biochemical analysis

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were assayed by corresponding assay kits (BC1555 and BC1565, Solarbio, Beijing, China) along with the triglyceride (TG) and cholesterol (TC) assay kits (BC0625 and BC1985, Solarbio, Beijing, China). 100 mg of liver tissue were added 1mL of a mixture containing n-heptane and isopropyl alcohol at a volumetric ratio of 1:1, and were centrifuged at 10,000 rpm at 4°C. The supernatant was used to detect liver TG content.

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

For AAV mediated NAT10 hepatocytes targeted silencing experiment and Remodelin gavage experiment, after 12 weeks, mice were subjected to a fasting period of 12 h, the mice were then intraperitoneally injected with 1.5 g/kg of glucose and their blood glucose levels were monitored at various time points –0, 30, 60, 90, and 120 min post-injection. In addition, for ITT, after mice were fasted for 12 h, they were injected with 1 IU/kg of insulin (Novo Nordisk, Copenhagen, Denmark), the blood glucose levels were measured at the same intervals as the GTT experiment. Area under the curve (AUC) for GTT and ITT were calculated by Graphpad Prism 10 software.

Statistics analysis

The data analysis was conducted using GraphPad Prism 10 software. A Student's t-test was used to compare data from two groups, while one-way analysis of variance (ANOVA) was used to analyze and compare multiple groups. The significance level was set at $P < 0.05$ to determine statistically significant differences among the groups.

Results

NAT10 is elevated in liver tissue of high-fat diet mice

To investigate the NAT10 expression in liver, the NAT10 level were analyzed after mice were fed chow diet ($n=5$) or high-fat diet ($n=5$) for 12 weeks, the results revealed the *Nat10* mRNA and protein expression were drastically increased (Fig. 1A-B). In addition, ac4C dot blot revealed that the total mRNA ac4C modification of liver tissue was also strongly upregulated after mice were fed high-fat diet (Fig. 1C). Furthermore, after AML12 cells were treated with 150µmol/L PA, the *Nat10* mRNA, protein and total mRNA ac4C modification also were apparently increased (Fig. 1D-F). The results elucidated that high-fat diet elevated liver NAT10 expression and mRNA ac4C level. The results indicated that NAT10 may play an important role on liver lipogenesis.

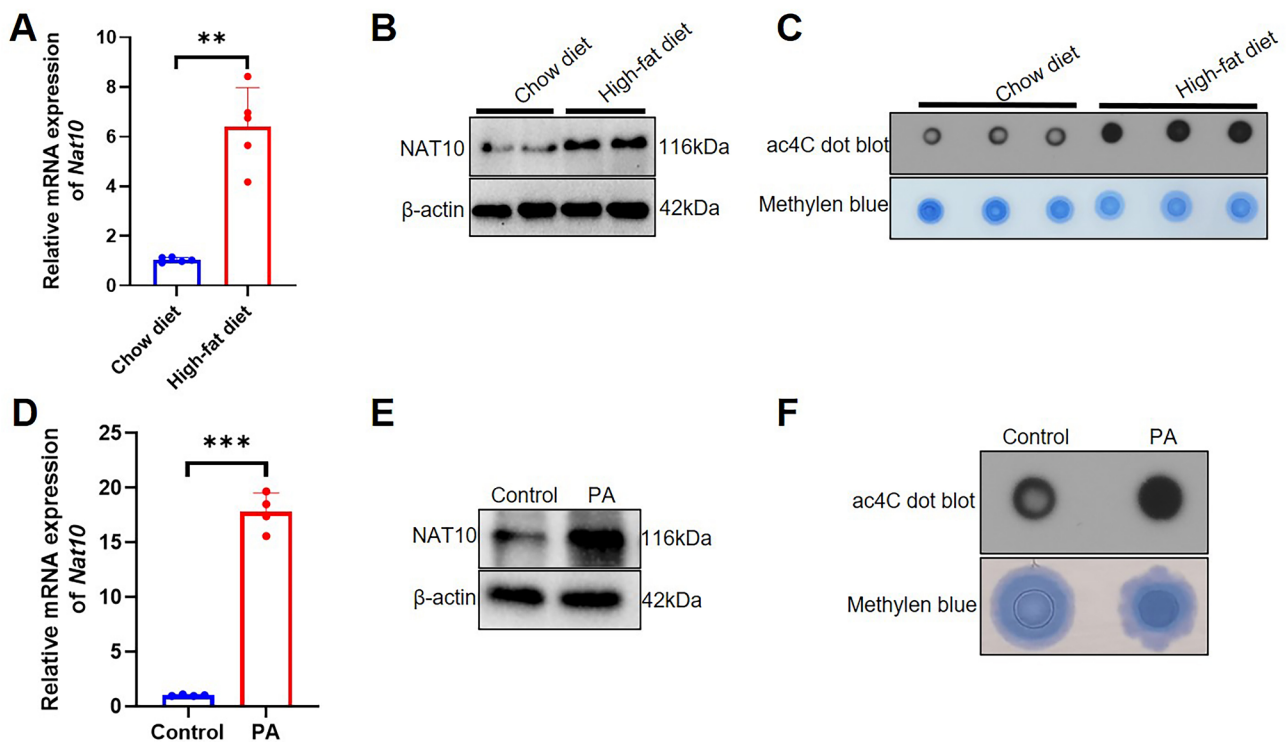


Fig. 1 NAT10 and mRNA ac4C level were upregulated in liver of high-fat mice. **(A)** *Nat10* mRNA, **(B)** protein and **(C)** mRNA ac4C modification was enhanced in liver tissue after mice were fed with high-fat diet for 12 weeks. **(D)** *Nat10* mRNA, **(E)** protein and **(F)** mRNA ac4C modification was increased after AML12 cells was induced by 150 μ mol/L PA. Data were represented as mean \pm SEM. ** $P < 0.01$, *** $P < 0.001$

NAT10 radically induces AML12 cell lipogenesis

To further explore the role of NAT10 on lipogenesis in vitro, NAT10 was overexpressed or silenced in AML12 cells for 48 h, and were treated with 150 μ mol/L PA, and Oil red O staining results showed that NAT10 significantly promoted lipid accumulation in AML12 cells, and conversely, silencing of NAT10 strongly inhibited lipogenesis in AML12 cells (Fig. 2A). The cellular content of FFA was greatly enhanced or reduced in NAT10 overexpressed or silenced in AML12 cells respectively after 150 μ mol/L PA treatment (Fig. 2B). Additionally, some critical molecules of hepatocyte lipogenesis, such as, *Srebf1*, acetyl-CoA carboxylase alpha (*Acaca*), and *Fasn* mRNA and protein, including Cd36, were also positively regulated by NAT10, especially *Srebf1* mRNA and precursor and mature SREBP-1 were increased or decreased even without PA intervention (Fig. 2C-F). Furthermore, knockdown of NAT10 suppressed mRNA ac4C modification, which was revealed by using ac4C dot blot (Fig. 2G). In addition, Oil red O staining displayed that a specific inhibitor of NAT10, Remodelin, strongly repressed AML12 cell lipogenesis (Fig. 2H), decreased cellular FFA contents (Fig. 2I), and inhibited *Srebf1*, *Acaca*, *Fasn* mRNA and protein, including Cd36 expression (Fig. 2J-K). The results demonstrated that NAT10 notably promoted lipogenesis in AML12 cells.

NAT10-mediated ac4C *Srebf1* mRNA modification promotes AML12 lipogenesis

To further explore the molecular mechanism of NAT10 regulated lipogenesis, some important genes who participate in hepatocyte lipogenesis, were evaluated by PACES website prediction. *Srebf1* mRNA was predicted as a target gene ac4C modified by NAT10 (score: 0.3281) (Fig. 3A). The prediction site was found located at 1939 to1953 of mRNA sequence, belonging to CDS. The motif sequence “CTGCTCCAGCGTCTC” is a characteristic ac4C motif “CXXCXXCXX” structure (Fig. 3B). To verify whether *Srebf1* mRNA was ac4C modified by NAT10, after silencing NAT10, acRIP-PCR was used to detect the *Srebf1* mRNA ac4C modification level. The results proved that knockdown of NAT10 downregulated *Srebf1* mRNA ac4C modification level (Fig. 3C). Moreover, NAT10-RIP-PCR also confirmed that NAT10 was able to bind to *Srebf1* mRNA and silencing of NAT10 decreased *Srebf1* mRNA (Fig. 3D). Besides, the RNA decay assay exhibited that silencing of NAT10 significantly decreased *Srebf1* mRNA half-life time (Fig. 3E). Next, to explore whether NAT10 can directly bind to *Srebf1* mRNA ac4C modification site, dual-luciferase reporter assay elucidated that mutated *Srebf1* mRNA decrease the combination with NAT10, which strongly supported the fact that NAT10 can bind to the motif of *Srebf1* mRNA (Fig. 3F). Furthermore, overexpression or inhibition of NAT10 increased

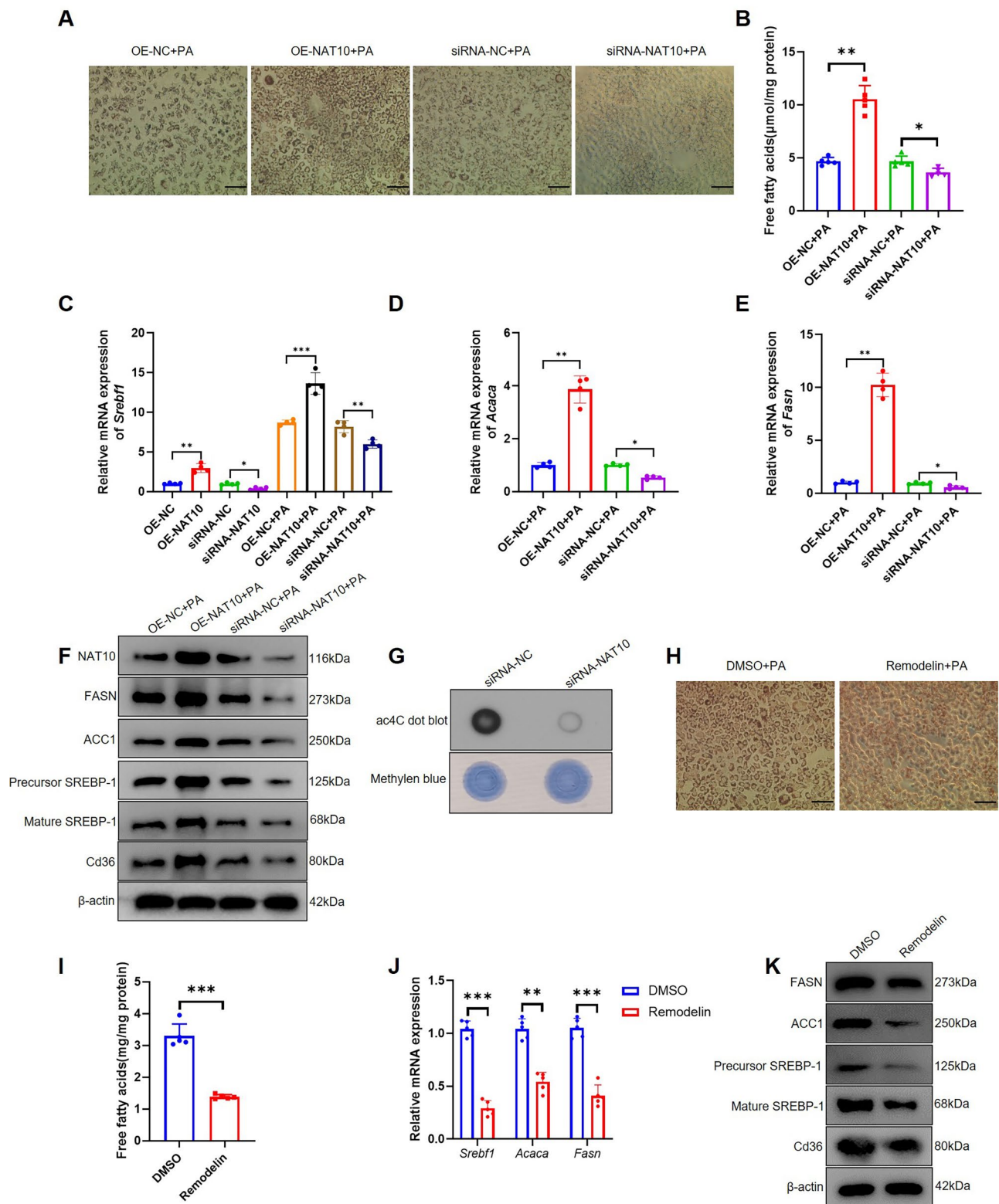


Fig. 2 NAT10 improves AML12 cells lipogenesis and mRNA ac4C modification. **(A)** Overexpressing or silencing of NAT10 promoted or inhibited AML12 cell lipid droplets form (Oil red O staining, scale bar = 50 μm), **(B)** cellular FFA content, **(C)** *Srebf1*, **(D)** *Acaca*, **(E)** *Fasn* mRNA and **(F)** protein including Cd36 expression after AML12 were intervened with 150 μmol/L PA. **(G)** Knockdown of NAT10 decreased mRNA ac4C modification in AML12 cells. **(H)** 20 μmol/L Remodelin inhibited AML12 cellular lipid droplet forms (Oil red O staining, scale bar = 50 μm), **(I)** cellular FFA content, **(J)** *Srebf1*, *Acaca* and *Fasn* mRNA and **(K)** protein including Cd36 expression. Data were represented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

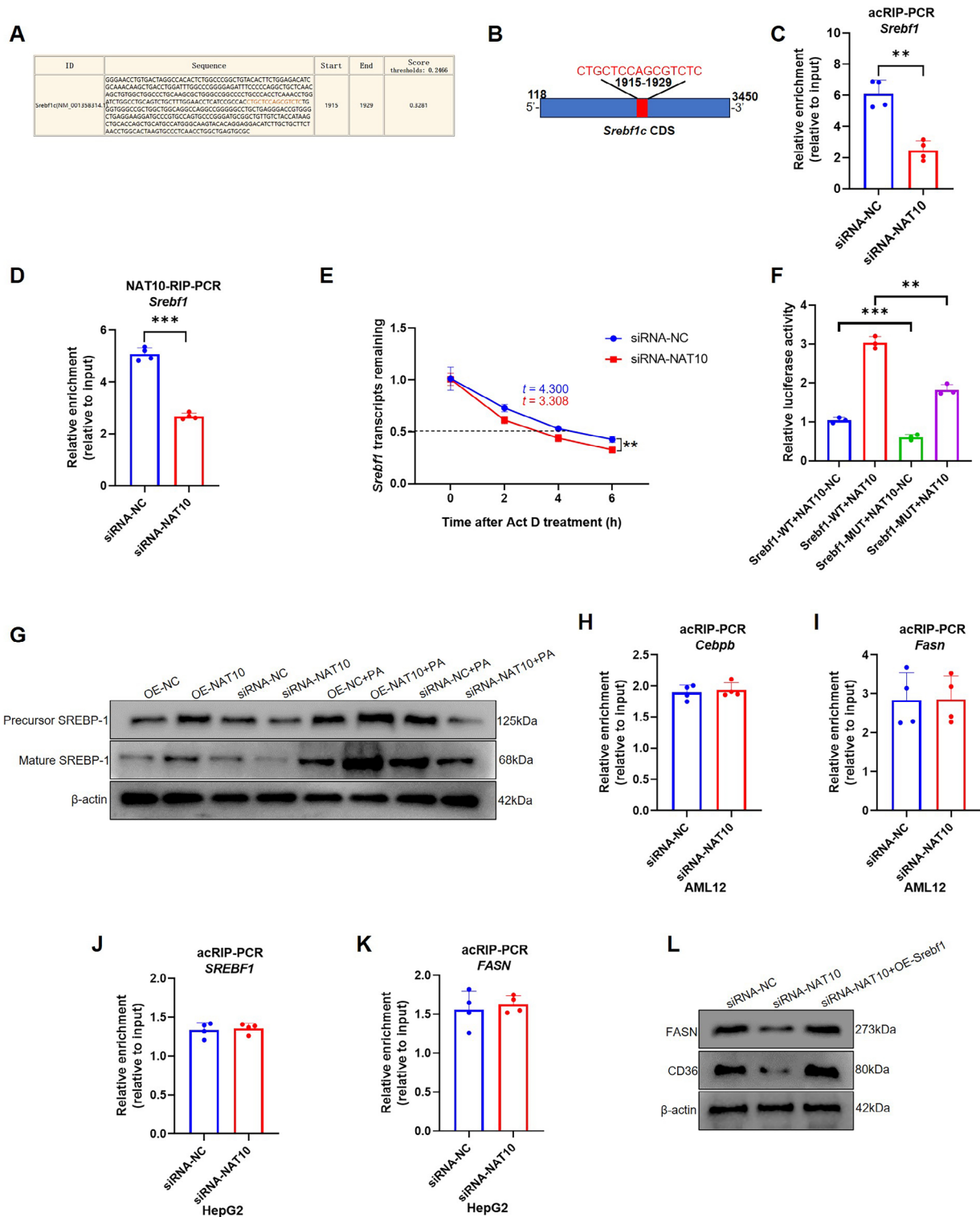


Fig. 3 NAT10 ac4C modified *Srebf1* mRNA in AML12 cells. **(A)** The NAT10 ac4C modification motif and **(B)** schematic diagram of *Srebf1* mRNA was predicted using PACES software. **(C)** NAT10-mediated *Srebf1* mRNA ac4C modification was verified using acRIP-PCR. **(D)** NAT10 bound with *Srebf1* mRNA and inhibition of NAT10 decreased *Srebf1* mRNA were detected by NAT10-RIP-PCR. **(E)** The *Srebf1* mRNA stability was detected with RNA decay assay. **(F)** NAT10 directly bound to *Srebf1* mRNA ac4C modification motif through dual-luciferase reporter assay. **(G)** SREBP-1 expression was verified with or without 150 μ mol/L PA treatment. **(H)** acRIP-PCR verified that mice *Cebpb*, **(I)** mice *Fasn* and **(J)** human *SREBF1* and **(K)** *FASN* (HepG2 cells) did not be ac4C modified by NAT10. **(L)** The rescue experiment showed that *FASN* and *Cd36* protein expression after AML12 was transfected with siRNA-NAT10 and OE-Srebf1. Data were represented as mean \pm SEM. ** $P < 0.01$, *** $P < 0.001$

or decreased with or without PA-induced the precursor and mature SREBP-1 protein expression (Fig. 3G). The results illustrated that NAT10 induced lipogenesis in AML12 cells via ac4C modification of *Srebf1* mRNA. In addition, mice CCAAT/enhancer binding protein beta (*Cebpb*) (score: 0.4322), *Fasn* (score: 0.5839) and human *SREBF1* (score: 0.2318) and *FASN* (score: 0.6972) were predicted as possibly ac4C modified, using PACES software. However, despite high prediction score, acRIP-PCR verified that NAT10 did not affect their mRNA ac4C modification (acRIP-PCR of human *SREBF1* and *FASN* were performed in HepG2 cells) (Fig. 3H-K). In addition, the rescue experiment showed overexpression of

Srebf1 was able to partially increase *FASN* and *Cd36* protein expression after AML12 was knockdown of NAT10 (Fig. 3L).

NAT10 ac4C modified *Scap* mRNA

In addition, another critical hepatocyte lipogenesis factor, mouse *Scap*, was also predicted as a target gene, ac4C modified by NAT10 (score: 0.2613) (Fig. 4A). The motif sequence, CGACGACGCCCCCGA, is located from 2807 to 2821 of *Scap* mRNA, also positioned at CDS (Fig. 4B). acRIP-PCR revealed that silencing of NAT10 inhibited *Scap* mRNA ac4C modification (Fig. 4C). NAT10 also was verified that it can bind to *Scap* mRNA and silencing

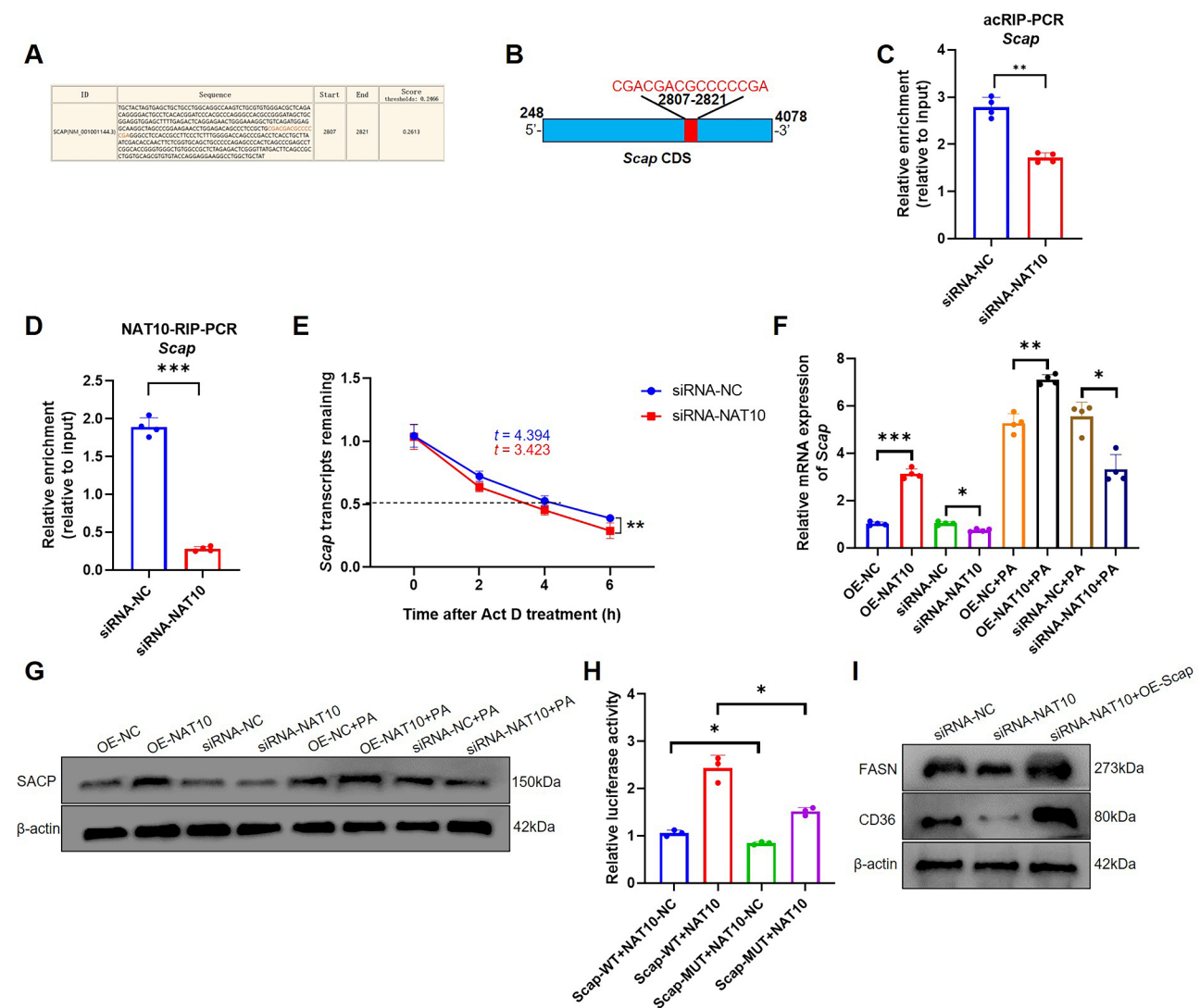


Fig. 4 NAT10 regulated *Scap* mRNA via ac4C modification. **(A)** The *Scap* mRNA ac4C modification site was predicted using PACES software. **(B)** Location of *Scap* mRNA ac4C motif. **(C)** acRIP-PCR was used to test that NAT10 ac4C modified *Scap* mRNA. **(D)** NAT10-RIP-PCR verified NAT10 protein bound with *Scap* mRNA and knockdown of NAT10 decreased *Scap* mRNA. **(E)** RNA decay assay detected *Scap* mRNA stability after silencing of NAT10. **(F)** *Scap* mRNA and **(G)** SCAP protein expression was verified with or without 150 μ mol/L PA treatment. **(H)** Dual-luciferase reporter assay validated NAT10 regulated *Scap* mRNA via its ac4C modification motif site. **(I)** The rescue experiment illustrated that overexpression of *Scap* was able to partial increase *FASN* and *Cd36* protein expression after AML12 was knockdown by NAT10. Data were represented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

of NAT10 decrease *Scap* mRNA via NAT10-RIP-PCR (Fig. 4D). In addition, inhibition of NAT10 attenuated *Scap* mRNA stability and mRNA and protein level expression (Fig. 4E-G). Furthermore, NAT10 was validated to directly bind to *Scap* mRNA ac4C modification through dual-luciferase reporter assay (Fig. 4H). The rescue experiment also showed that upregulated *Scap* was able to partially improve FASN and Cd36 protein expression after NAT10 knockdown in AML12 cells (Fig. 4I). The results indicated that NAT10 regulated *Scap* mRNA ac4C modification in AML12 cells.

NAT10 promoted liver lipogenesis via *Srebf1* and *Scap* mRNA ac4C modification in vivo

Liver-specific knockout of NAT10 has been shown to reduce liver steatosis in male offspring mice after maternal high-fat diet [30]. However, there is no direct evidence that NAT10 participate in liver lipogenesis via mRNA ac4C modification. To explore whether NAT10 regulate liver lipogenesis in vivo, hepatocyte-targeted AAV-shRNA-NAT10 ($n=5$) and AAV-shRNA-NC ($n=5$) were injected through tail vein of mice at 0 and 4th week, while simultaneously fed a high-fat diet for 12 weeks (Fig. 5A). The results showed that although body weight was not significantly different in two groups, liver size was smaller and lighter in weight in AAV-shRNA-NAT10 than AAV-shRNA-NC group (Fig. 5B-D). In addition, the pathological staining like H&E and Oil red O staining showed that silencing of NAT10 inhibited liver lipid droplet formation (Fig. 5E-F). Moreover, TG content of liver, serum ALT, AST, TC and TG concentration were decreased in AAV-shRNA-NAT10 (Fig. 5G-K). Besides, GTT results showed that blood glucose level in AAV-shRNA-NC was similar to AAV-shRNA-NAT10 group except for at 30 min, however, there was no difference in AUC of GTT, ITT and AUC of ITT. These results indicated normal glucose tolerance and insulin sensitivity (Fig. 5L-O). In molecular level, acRIP-PCR results revealed that silencing of NAT10 decreased *Srebf1* and *Scap* mRNA ac4C modification (Fig. 5P, Q). In addition, knockdown of NAT10 reduced *Srebf1*, *Scap*, *Acaca* and *Fasn* mRNA and protein expression in liver (Fig. 5R, S). The results suggested that NAT10 inhibited liver lipogenesis through the downregulation of *Srebf1* and *Scap* mRNA ac4C modification.

Remodelin significantly inhibits high-fat diet induced liver lipogenesis through *Srebf1* and *Scap* mRNA ac4C modification

To test Remodelin, the inhibitor of NAT10, whether can inhibit liver lipogenesis after mice were fed with high-fat diet. The mice were gavage with Remodelin (100 mg/kg/day, $n=5$) or DMSO ($n=5$) and high-fat dieted for 12 weeks (Fig. 6A). The results elucidated that Remodelin

significantly inhibited mice weight, liver size and weight (Fig. 6B-D). H&E and Oil red O staining showed that Remodelin almost completely inhibited liver lipid droplets form (Fig. 6E-F). In addition, liver TG, serum ALT, AST, TC and TG were also apparently decreased in Remodelin treated group compared to DMSO treated group (Fig. 6G-K). In glucose metabolism, Remodelin also improved glucose tolerance compared to DMSO group (Fig. 6L, M). Moreover, in ITT experiment, Remodelin decreased blood glucose level via exogenous insulin in 0, 30, 60 min, although there was no difference in AUC of ITT, illustrating increased insulin sensitivity (Fig. 6N, O). Furthermore, acRIP-PCR showed that Remodelin also inhibited *Srebf1* and *Scap* mRNA ac4C modification in liver (Fig. 6P, Q). In addition, Remodelin inhibited *Srebf1*, *Scap*, *Acaca* and *Fasn* mRNA and protein expression in liver (Fig. 6R, S). The results showed that Remodelin decreased the liver lipogenesis via ac4C modification of *Srebf1* and *Scap* mRNA. The molecular mechanism of NAT10 regulation of liver lipogenesis is depicted in Fig. 7.

Discussion

In a recent study, Zhang et al. revealed that knockout of NAT10 in maternal mice inhibited male offspring liver hepatic steatosis, and in human HepG2, downregulation of NAT10 prevented lipid accumulation via NAT10 bound to *Cd36/FATP2* mRNA via NAT10-RIP-PCR [32]. However, there is no direct evidence to show that NAT10 regulates liver adipogenesis by ac4C modified downstream genes. In MCF7 cells, a human breast cancer cells, knockdown of NAT10 inhibited FFA metabolism targeting ac4C modified *ELOVL6*, *ACSL1*, *ACSL3*, *ACSL4*, *ACADSB* and *ACAT1* mRNA [33]. In all, NAT10 has been verified to positively regulate lipogenesis in HepG2 and MCF7 cells. However, HepG2 and MCF7 cells both belong to cancer cells, and whether NAT10 is involved in regulating lipogenesis in normal liver cells has not been studied yet.

To find the direct evidence of NAT10 regulation of liver lipogenesis via mRNA ac4C modification, the expression of NAT10 was evaluated in liver after mice were fed high-fat diet, the results showed that NAT10 also was induced, and mRNA ac4C modification level was elevated in mice liver. In addition, NAT10 and mRNA ac4C modification also was upregulated after AML12 cells were treated with 150 $\mu\text{mol/L}$ PA for 48 h. Thus, the results implied that NAT10 may act as a critical factor that regulate liver lipogenesis through mRNA ac4C modification. Although NAT10 has been reported to regulate FFA metabolism in cancer cells [33], whether NAT10 participate in regulating others genes of lipogenesis, has not been studied well. Some critical genes of lipogenesis were analyzed via PACES software, and found mice *Srebf1*, *Scap*, *Cebpb*,

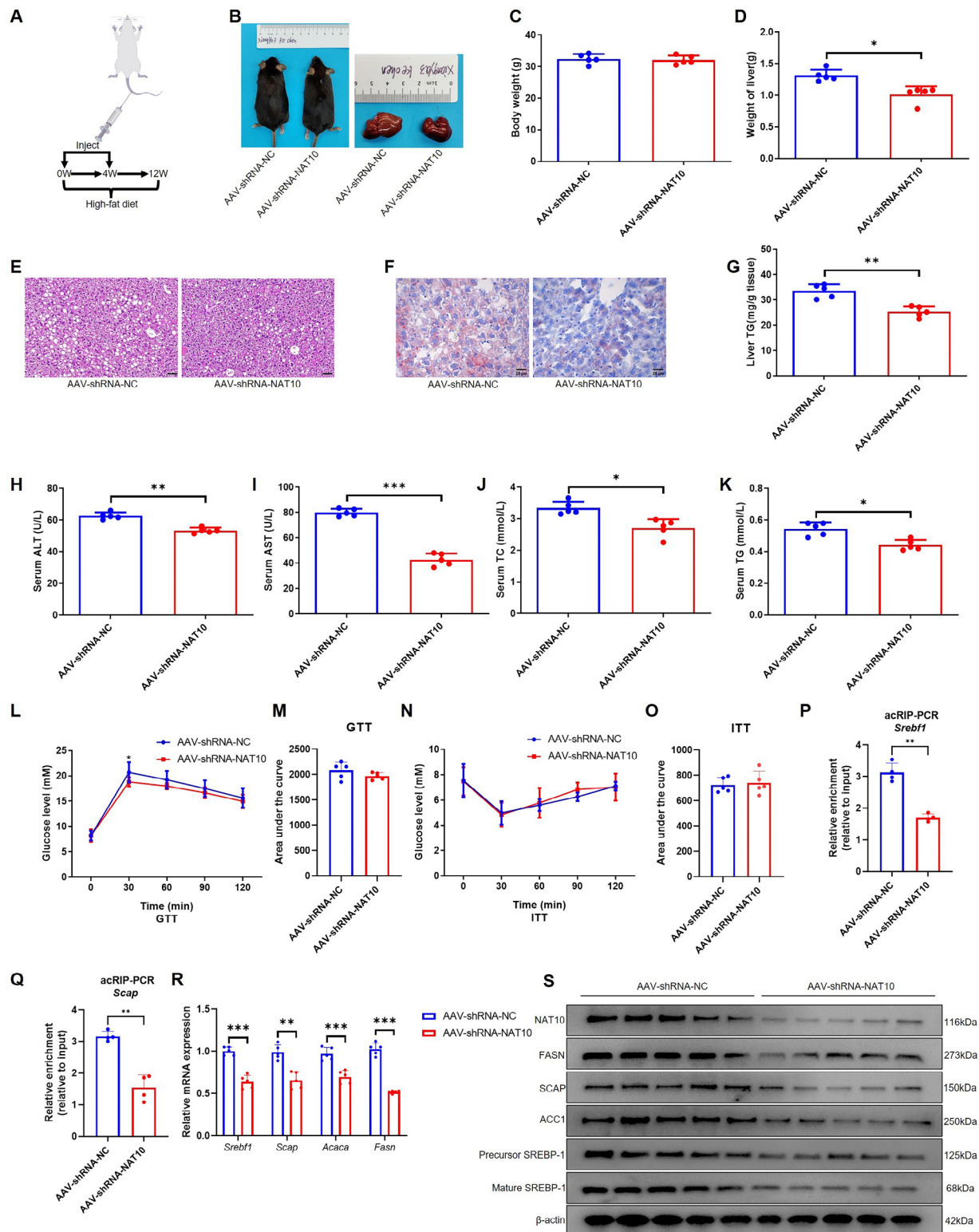


Fig. 5 Silencing of NAT10 ac4C modified *Srebf1* and *Scap* mRNA to inhibit mice liver lipogenesis. **(A)** The protocol of AAV-shRNA-NAT10 injection in vivo (the cartoon image was provided by www.figdraw.com). **(B)** The mice and liver size after mice were injected with AAV-shRNA-NAT10 and AAV-shRNA-NC and fed with high-fat diet for 12 weeks. **(C)** The body weight and **(D)** liver weight in two groups. **(E)** H&E (scale bar = 50 μm) and **(F)** Oil red O staining (scale bar = 25 μm) in liver. **(G)** The liver TG, **(H)** serum ALT, **(I)** AST, **(J)** TC, **(K)** TG, **(L)** GTT, **(M)** GTT-AUC, **(N)** ITT and **(O)** ITT-AUC in two groups. **(P)** Knockdown of NAT10 inhibited *Srebf1* and **(Q)** *Scap* mRNA ac4C modification in liver tissue using acRIP-PCR. **(R)** Knockdown of NAT10 blocked *Srebf1*, *Scap*, *Acaca* and *Fasn* mRNA and **(S)** protein expression in liver. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

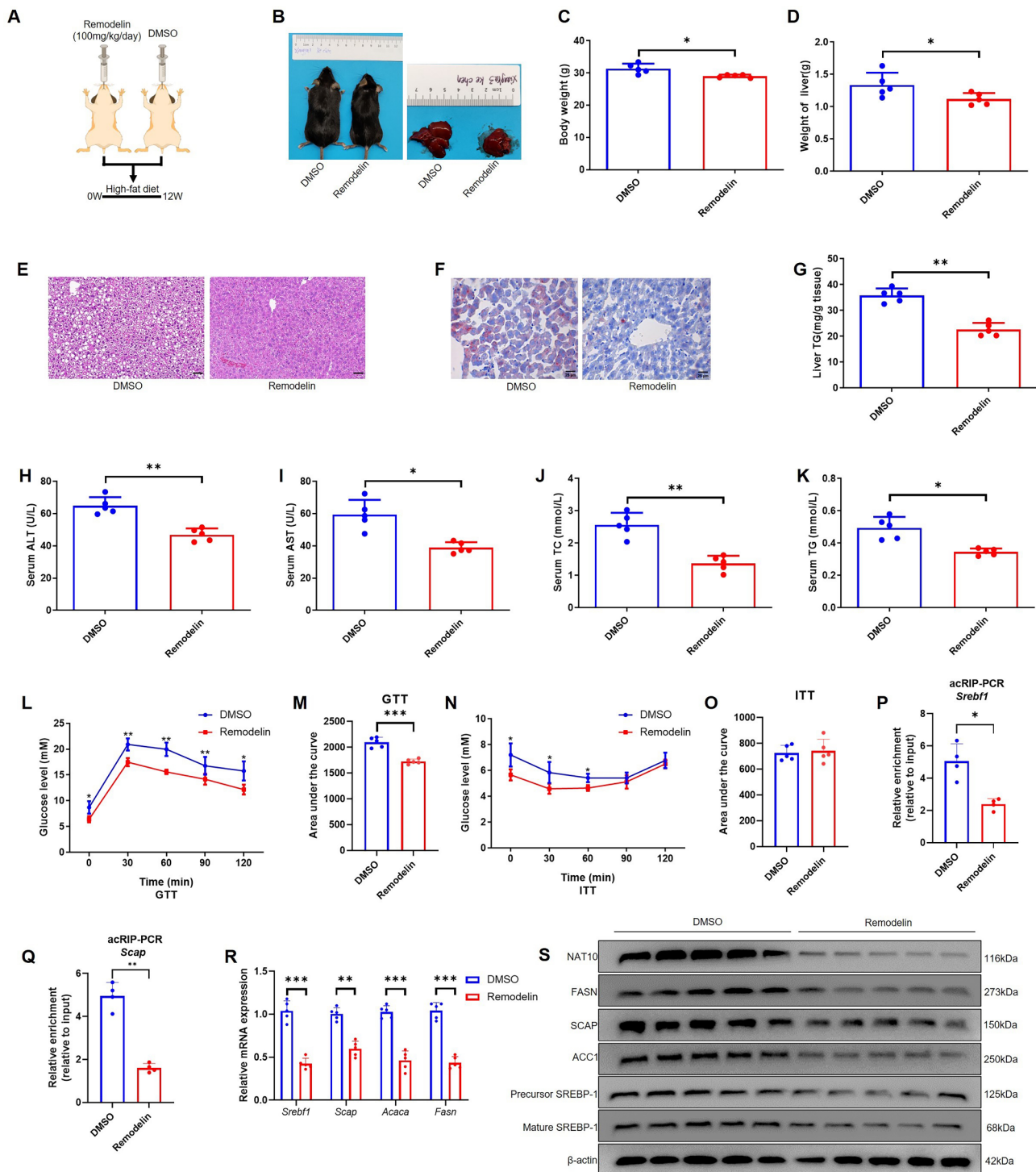


Fig. 6 Remodelin prevented liver lipogenesis through targeting inhibit *Srebf1* and *Scap* mRNA ac4C modification. **(A)** The schematic diagram of Remodelin gavage experiment in vivo (the cartoon image was provided by www.figdraw.com). **(B)** The mice and liver size in Remodelin and DMSO gavage group. **(C)** The body weight and **(D)** liver weight in two groups. **(E)** H&E (scale bar = 50 μ m) and **(F)** Oil red O staining (scale bar = 25 μ m) in liver. **(G)** The liver TG, **(H)** serum ALT, **(I)** AST, **(J)** TC, **(K)** TG, **(L)** GTT, **(M)** GTT-AUC, **(N)** ITT and **(O)** ITT-AUC in two groups. **(P)** acRIP-PCR verified *Srebf1* mRNA and **(Q)** *Scap* mRNA ac4C modification in liver tissue. **(R)** Remodelin impeded *Srebf1*, *Scap*, *Acaca* and *Fasn* mRNA and **(S)** protein expression in liver. Data were represented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

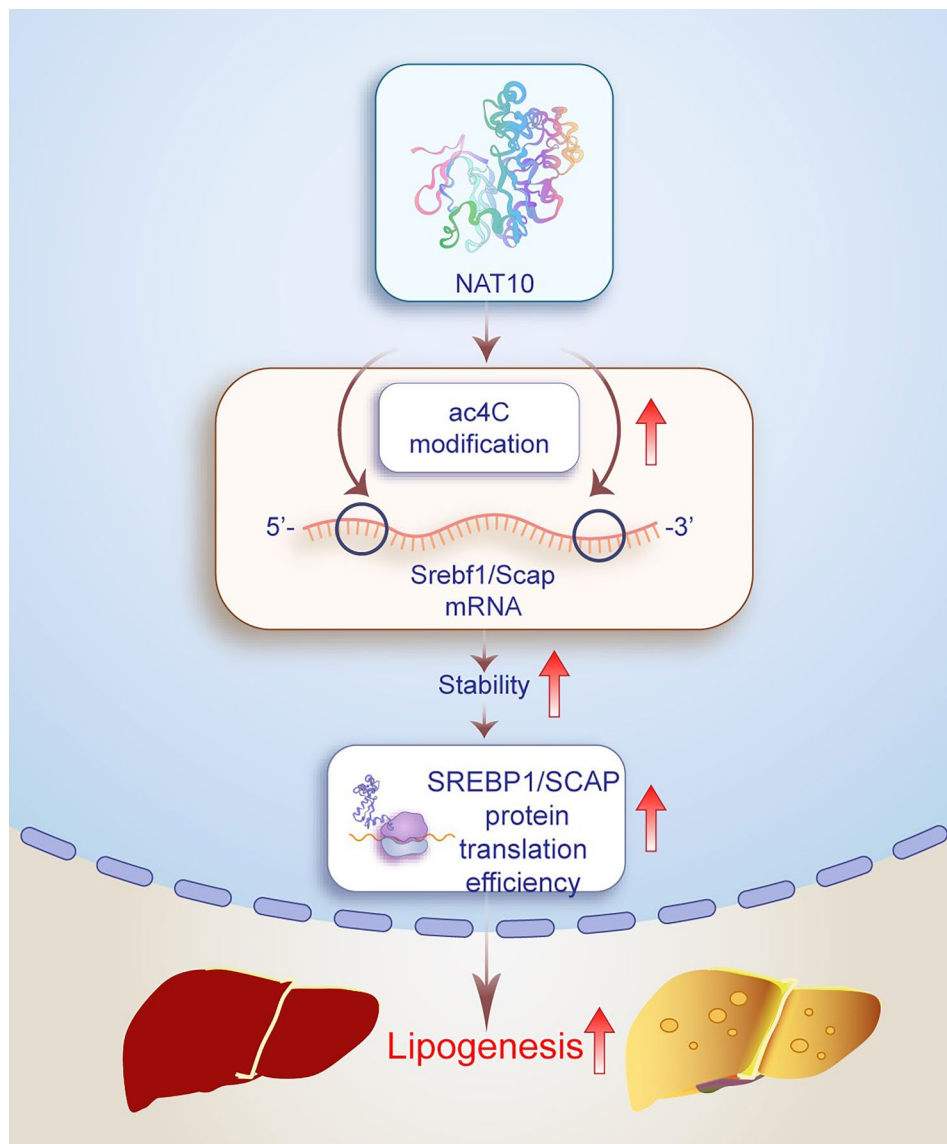


Fig. 7 The molecular mechanism scheme of NAT10-dependent ac4C modification in liver lipogenesis. NAT10 ac4C modifies *Srebf1* and *Scap* mRNA and promotes *Srebf1* and *Scap* mRNA stability, further increasing SREBP-1 and SCAP protein translation efficiency, hence, elevates downstream lipogenesis genes expression in liver

Fasn and human *SREBF1* and *FASN* are possible genes which were ac4C modified by NAT10. The results verified that *Srebf1* and *Scap* mRNA were two target genes which were ac4C modified by NAT10. However, although there was high prediction score of mice *Cebpb*, *Fasn* and human *SREBF1* and *FASN*, the acRIP-PCR verified that these genes are not ac4C modified by NAT10. In addition, as a downstream target gene of *Srebf1*, in this study, *Fasn* mRNA was downregulated after silencing of NAT10. This inhibitory effect was directly related to the reduction of *Srebf1*, rather than being modified by ac4C mRNA modification of NAT10.

SREBP-1 is a critical and central modulator that participate in liver lipogenesis, when there is low sterol level

in hepatocytes, SCAP-mediated SREBP-1 is transported from ER to Golgi, and release an amino-terminal domain to nucleus and further activate the downstream genes involved in lipogenesis [9, 35, 36]. It's evident that promoting SREBP-1 or SCAP can activate liver lipogenesis, while silencing SREBP-1 or SCAP can inhibit liver lipogenesis [35, 37, 38]. In this study, both of mice *Srebf1* and *Scap* mRNA were validated as two target genes, which were ac4C modified by NAT10, and the ac4C motifs are all located at CDS. Previous studies also reported that NAT10 positively regulate target gene's mRNA, of which motifs are located in CDS stability and mRNA expression level [20, 39–41]. In this study, NAT10 ac4C modified mice *Srebf1* and *Scap* mRNA and also positive

regulation of their expression. Additionally, NAT10 bind to *Cd36/FATP2* mRNA to affect lipid uptake in HepG2 cells and ac4C modified *ELOVL6*, *ACSL1*, *ACSL3*, *ACSL4*, *ACADSB* and *ACAT1* mRNA to affect mitochondrial FFA metabolism in cancer cells [32–34]. However, although human *ELOVL6* mRNA was ac4C modified, mouse *Elovl6* mRNA was not been predicted in presence of ac4C modification motifs. In this study, NAT10 can ac4C modify mice *Srebf1* and *Scap* mRNA, therefore, the results revealed that NAT10 can regulate liver lipogenesis through ac4C modification of multiple genes.

Remodelin, the only developed NAT10 inhibitor, has been widely applied to study NAT10's molecular function, Remodelin was found to improve Hutchinson-Gilford Progeria Syndrome cell phenotype [42], and also Remodelin was found able to prevent tumor procession [27, 43–45]. Moreover, Remodelin also was found not toxic for cell and mice experiments [40, 46–48]. Therefore, Remodelin may be used for a potential small molecular compound to treat MASLD. But it is noteworthy that in this study, some mice's hair partially turned white after Remodelin gavage treatment for 12 weeks. From here, the potential systemic effects and long-term safety of Remodelin is needed to be explored further. In a recent study, Liu et al. found Panobinostat had a more efficient affinity with NAT10 and inhibitory effect than Remodelin, as an anti-tumor drug, Panobinostat may not be suitable for treatment of MASLD [49]. So, developing new NAT10 inhibitor in vivo and treatment of MASLD is needed by further research.

In addition, although targeted knockdown of NAT10 in liver showed to decrease liver size and lipogenesis including liver TG, serum TG, TC, ALT and AST, there was no difference in body weight and glucose metabolism. However, Remodelin strongly prevented liver lipogenesis, hepatic damage and decreased glucose tolerance. Remodelin treatment was more efficient than targeted knockdown of NAT10 in liver, possible reason is that the inhibitory effect of Remodelin on NAT10 is stronger than targeted liver knockout of NAT10. In addition, the efficient and widespread systemic inhibition of NAT10 by Remodelin, especially in adipose tissue, may further improve metabolism.

Study strengths and limitations

This study revealed that NAT10 can regulate mice liver lipogenesis via ac4C modified *Srebf1* and *Scap* mRNA. Also, the knockdown of NAT10, as well as the administration of Remodelin, an effective NAT10 inhibitor, successfully inhibited liver lipogenesis in vivo. However, in this study, acRIP-seq was not used to comprehensively examine the ac4C-modified mRNAs involved in lipogenesis in AML12 and HepG2 cells. It was observed that some mice exhibited partial whitening of their hair

following Remodelin treatment, suggesting that the safety profile of NAT10 inhibitors such as Remodelin requires further assessment.

Conclusions

In summary, in this study provides direct evidence for NAT10 regulated mice liver lipogenesis in vitro and in vivo, and knockdown of NAT10 decreased ac4C modified *Srebf1* and *Scap* mRNA, and further decreasing their mRNA stability. Additionally, Remodelin or development of new NAT10 inhibitor may be a promising method to treat for MASLD. In all, the present study will provide a new intervention target for the treatment of MASLD.

Abbreviations

AAV	Adeno-associated virus
ac4C	N4-acetylcytidine
Acaca	Acetyl-CoA carboxylase alpha
ACADSB	Acyl-CoA dehydrogenase short/branched chain
ACAT1	Acetyl-CoA acetyltransferase 1
ACC1	Acetyl-CoA carboxylase 1
ACSL1	Acyl-CoA synthetase long chain family member 1
ACSL3	Acyl-CoA synthetase long chain family member 3
ACSL4	Acyl-CoA synthetase long chain family member 4
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
CDS	Coding sequence
Cebpb	CCAAT/enhancer binding protein beta
DMSO	Dimethyl sulfoxide
ELOVL6	ELOVL fatty acid elongase 6
Fasn	Fatty acid synthase
FBS	Fetal bovine serum
FFA	Free fatty acids
GTT	Glucose tolerance test
H&E	Hematoxylin and eosin
INSIGs	Insulin induced genes
ITT	Insulin tolerance test
m1A	N1-methyladenine
m5C	5-ethylcytosine
m6A	N6-methyladenine
m7G	N7-methylguanosine
MASLD	Metabolic dysfunction associated steatotic liver disease
NAT10	N-acetyltransferase 10
PA	Palmitic acid
RIP	RNA immunoprecipitation
SCAP	SREBF chaperone
SCD1	Stearoyl-CoA desaturase-1
SREBPs	Sterol regulatory element-binding proteins
Srebf1	Sterol regulatory element binding transcription factor 1
TC	Total cholesterol
TG	Triglyceride
UTR	Untranslated region

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12944-024-02360-1>.

Supplementary Material 1

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Not applicable.

Author contributions

All authors were involved in the development of the primary manuscript and revision. ZQW-performed the experiments, data analysis, manuscript drafting. XXW- performed the experiments, data analysis. AK-wrote the manuscript, LP-performed the experiments, XYS-performed the experiments, XY-performed the experiments, KC-performed the experiments, data analysis, manuscript drafting, supervision, planning, data interpretation. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The animal experiments conducted at The Third Xiangya Hospital of Central South University, Changsha, China adhered to strict ethical guidelines set forth by the Ethical Committee for Animal Experiments. Approval for the experiments was granted under Approval NO: 2023-S027.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Rinella ME, Lazarus JV, Ratzliff V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology*. 2023;78:1966–86. <https://doi.org/10.1097/hep.0000000000000520>.
- Sogabe M, Okahis T, Kagawa M, Sei M, Ueda H, Yokoyama R, et al. Association of variabilities in body mass index and waist circumference with newly achieved remission of metabolic dysfunction-associated fatty liver disease. *Diabetes Metab Syndr*. 2024;18:103036. <https://doi.org/10.1016/j.dsx.2024.103036>.
- Eslam M, Sarin SK, Wong VW, Fan JG, Kawaguchi T, Ahn SH, et al. The Asian Pacific Association for the study of the liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. *Hepatol Int*. 2020;14:889–919. <https://doi.org/10.1007/s12072-020-10094-2>.
- Kaya E, Yilmaz Y. Metabolic-associated fatty liver Disease (MASLD): a multi-systemic disease beyond the liver. *J Clin Transl Hepatol*. 2022;10:329–38. <https://doi.org/10.14218/jcth.2021.00178>.
- Kuchay MS, Choudhary NS, Mishra SK. Pathophysiological mechanisms underlying MASLD. *Diabetes Metab Syndr*. 2020;14:1875–87. <https://doi.org/10.1016/j.dsx.2020.09.026>.
- Gutiérrez-Cuevas J, Santos A, Armendariz-Borunda J. Pathophysiological molecular mechanisms of obesity: a link between MASLD and NASH with Cardiovascular diseases. *Int J Mol Sci*. 2021;22:11629. <https://doi.org/10.3390/ijms222111629>.
- Shao Y, Yao Z, Zhou J, Yu M, Chen S, Yuan Y, et al. A novel small compound TOIDC suppresses lipogenesis via SREBP1-dependent signaling to curb MASLD. *Nutr Metab (Lond)*. 2022;19:80. <https://doi.org/10.1186/s12986-022-00713-0>.
- Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. *Mol Metab*. 2021;50:101238. <https://doi.org/10.1016/j.molmet.2021.101238>.
- Badmus OO, Hillhouse SA, Anderson CD, Hinds TD, Stec DE. Molecular mechanisms of metabolic associated fatty liver disease (MASLD): functional analysis of lipid metabolism pathways. *Clin Sci (Lond)*. 2022;136:1347–66. <https://doi.org/10.1042/cs20220572>.
- Filipovic B, Marjanovic-Haljilji M, Mijac D, Lukic S, Kapor S, Kapor S, et al. Molecular aspects of MASLD-New insights on Pathogenesis and Treatment. *Curr Issues Mol Biol*. 2023;45:9132–48. <https://doi.org/10.3390/cimb45110573>.
- Li N, Li X, Ding Y, Liu X, Diggler K, Kisseleva T, et al. SREBP regulation of lipid metabolism in Liver Disease, and therapeutic strategies. *Biomedicines*. 2023;11:3280. <https://doi.org/10.3390/biomedicines11123280>.
- Araki M, Nakagawa Y, Saito H, Yamada Y, Han SI, Mizunoe Y, et al. Hepatocyte- or macrophage-specific SREBP-1a deficiency in mice exacerbates methionine- and choline-deficient diet-induced nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol*. 2022;323:G627–39. <https://doi.org/10.1152/ajpgi.00090.2022>.
- Zhai M, Zhang C, Cui J, Liu J, Li Y, Xie K, et al. Electromagnetic fields ameliorate hepatic lipid accumulation and oxidative stress: potential role of CaMKK β /AMPK/SREBP-1c and Nrf2 pathways. *Biomed Eng Online*. 2023;22:51. <https://doi.org/10.1186/s12938-023-01114-x>.
- Moslehi A, Hamidi-Zad Z. Role of SREBPs in Liver diseases: a mini-review. *J Clin Transl Hepatol*. 2018;6:332–8. <https://doi.org/10.14218/jcth.2017.00061>.
- Wang MK, Gao CC, Yang YG. Emerging roles of RNA methylation in Development. *Acc Chem Res*. 2023;56:3417–27. <https://doi.org/10.1021/acs.accounts.3c00448>.
- Li J, Zhang H, Wang H. N(1)-methyladenosine modification in cancer biology: current status and future perspectives. *Comput Struct Biotechnol J*. 2022;20:6578–85. <https://doi.org/10.1016/j.csbj.2022.11.045>.
- Liu Y, Zhao Y, Wu R, Chen Y, Chen W, Liu Y, et al. mRNA m5C controls adipogenesis by promoting CDKN1A mRNA export and translation. *RNA Biol*. 2021;18:711–21. <https://doi.org/10.1080/15476286.2021.1980694>.
- Thalalla Gamage S, Howpay Manage SA, Chu TT, Meier JL. Cytidine Acetylation across the Tree of Life. *Acc Chem Res*. 2024;57:338–48. <https://doi.org/10.1021/acs.accounts.3c00673>.
- Ma CR, Liu N, Li H, Xu H, Zhou XL. Activity reconstitution of Kre33 and Tan1 reveals a molecular ruler mechanism in eukaryotic tRNA acetylation. *Nucleic Acids Res*. 2024;52:5226–40. <https://doi.org/10.1093/nar/gkae262>.
- Arango D, Sturgill D, Alhusaini N, Dillman AA, Sweet TJ, Hanson G, et al. Acetylation of Cytidine in mRNA promotes translation efficiency. *Cell*. 2018;175:1872–86. <https://doi.org/10.1016/j.cell.2018.10.030>.
- Xie L, Zhong X, Cao W, Liu J, Zu X, Chen L. Mechanisms of NAT10 as ac4C writer in diseases. *Mol Ther Nucleic Acids*. 2023;32:359–68. <https://doi.org/10.1016/j.omtn.2023.03.023>.
- Zhang Y, Lei Y, Dong Y, Chen S, Sun S, Zhou F, et al. Emerging roles of RNA ac4C modification and NAT10 in mammalian development and human diseases. *Pharmacol Ther*. 2024;253:108576. <https://doi.org/10.1016/j.pharmt.2023.108576>.
- Arango D, Sturgill D, Yang R, Kanai T, Bauer P, Roy J, et al. Direct epitranscriptomic regulation of mammalian translation initiation through N4-acetylcytidine. *Mol Cell*. 2022;82:2797–814. <https://doi.org/10.1016/j.molcel.2022.05.016>.
- Luo J, Cao J, Chen C, Xie H. Emerging role of RNA acetylation modification ac4C in diseases: current advances and future challenges. *Biochem Pharmacol*. 2023;213:115628. <https://doi.org/10.1016/j.bcp.2023.115628>.
- Sas-Chen A, Thomas JM, Matzov D, Taoka M, Nance KD, Nir R, et al. Dynamic RNA acetylation revealed by quantitative cross-evolutionary mapping. *Nature*. 2020;583:638–43. <https://doi.org/10.1038/s41586-020-2418-2>.
- Karthiya R, Wasil SM, Khandelia P. Emerging role of N4-acetylcytidine modification of RNA in gene regulation and cellular functions. *Mol Biol Rep*. 2020;47:9189–99. <https://doi.org/10.1007/s11033-020-05963-w>.
- Shi J, Yang C, Zhang J, Zhao K, Li P, Kong C, et al. NAT10 is involved in Cardiac Remodeling through ac4C-Mediated transcriptomic regulation. *Circ Res*. 2023;133:989–1002. <https://doi.org/10.1161/circresaha.122.322244>.
- Jiang X, Cheng Y, Zhu Y, Xu C, Li Q, Xing X, et al. Maternal NAT10 orchestrates oocyte meiotic cell-cycle progression and maturation in mice. *Nat Commun*. 2023;14:3729. <https://doi.org/10.1038/s41467-023-39256-0>.
- Jin C, Wang T, Zhang D, Yang P, Zhang C, Peng W, et al. Acetyltransferase NAT10 regulates the Wnt/ β -catenin signaling pathway to promote colorectal cancer progression via Ac(4)C acetylation of KIF23 mRNA. *J Exp Clin Cancer Res*. 2022;41:345. <https://doi.org/10.1186/s13046-022-02551-7>.
- Miao D, Shi J, Lv Q, Tan D, Zhao C, Xiong Z, et al. NAT10-mediated ac(4)C-modified ANKZF1 promotes tumor progression and lymphangiogenesis in clear-cell renal cell carcinoma by attenuating YWHAE-driven cytoplasmic retention of YAP1. *Cancer Commun (Lond)*. 2024;44:361–83. <https://doi.org/10.1002/cac2.12523>.

31. Liu Y, Wang X, Liu Y, Yang J, Mao W, Feng C, et al. N4-acetylcytidine-dependent GLMP mRNA stabilization by NAT10 promotes head and neck squamous cell carcinoma metastasis and remodels tumor microenvironment through MAPK/ERK signaling pathway. *Cell Death Dis.* 2023;14:712. <https://doi.org/10.1038/s41419-023-06245-6>.
32. Zhang QR, Zhang JB, Shen F, Xue R, Yang RX, Ren TY, et al. Loss of NAT10 alleviates maternal high-fat diet-induced hepatic steatosis in male offspring of mice. *Obes (Silver Spring).* 2024;32:1349–61. <https://doi.org/10.1002/oby.24041>.
33. Dalhat MH, Mohammed MRS, Alkhatabi HA, Rehan M, Ahmad A, Choudhry H, et al. NAT10: an RNA cytidine transferase regulates fatty acid metabolism in cancer cells. *Clin Transl Med.* 2022;12:e1045. <https://doi.org/10.1002/ctm.21045>.
34. Dalhat MH, Mohammed MRS, Ahmad A, Khan MI, Choudhry H. Remodelin, a N-acetyltransferase 10 (NAT10) inhibitor, alters mitochondrial lipid metabolism in cancer cells. *J Cell Biochem.* 2021;122:1936–45. <https://doi.org/10.1002/jcb.30155>.
35. Chandrasekaran P, Weiskirchen R. The role of SCAP/SREBP as central regulators of lipid metabolism in hepatic steatosis. *Int J Mol Sci.* 2024;25:1109. <https://doi.org/10.3390/ijms25021109>.
36. Ferré P, Phan F, Fougelle F. SREBP-1c and lipogenesis in the liver: an update. *Biochem J.* 2021;478:3723–39. <https://doi.org/10.1042/bcj20210071>.
37. Ferré P, Fougelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metab.* 2010;12(Suppl 2):83–92. <https://doi.org/10.1111/j.1463-1326.2010.01275.x>.
38. Moon YA, Liang G, Xie X, Frank-Kamenetsky M, Fitzgerald K, Kotliansky V, et al. The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in animals. *Cell Metab.* 2012;15:240–6. <https://doi.org/10.1016/j.cmet.2011.12.017>.
39. Zhang H, Chen Z, Zhou J, Gu J, Wu H, Jiang Y, et al. NAT10 regulates neutrophil pyroptosis in sepsis via acetylating ULK1 RNA and activating STING pathway. *Commun Biol.* 2022;5:916. <https://doi.org/10.1038/s42003-022-03868-x>.
40. Zhang M, Yang K, Wang QH, Xie L, Liu Q, Wei R, et al. The cytidine N-Acetyltransferase NAT10 participates in peripheral nerve Injury-Induced Neuropathic Pain by stabilizing SYT9 expression in primary sensory neurons. *J Neurosci.* 2023;43:3009–27. <https://doi.org/10.1523/jneurosci.2321-22.2023>.
41. Zheng X, Wang Q, Zhou Y, Zhang D, Geng Y, Hu W, et al. N-acetyltransferase 10 promotes colon cancer progression by inhibiting ferroptosis through N4-acetylation and stabilization of ferroptosis suppressor protein 1 (FSP1) mRNA. *Cancer Commun (Lond).* 2022;42:1347–66. <https://doi.org/10.1002/cac2.12363>.
42. Balmus G, Larriue D, Barros AC, Collins C, Abrudan M, Demir M, et al. Targeting of NAT10 enhances healthspan in a mouse model of human accelerated aging syndrome. *Nat Commun.* 2018;9:1700. <https://doi.org/10.1038/s41467-018-03770-3>.
43. Liu Y, Huang H, Zhang CB, Fan HN. N-acetyltransferase 10 promotes the progression of oral squamous cell carcinoma through N4-acetylcytidine RNA acetylation of MMP1 mRNA. *Cancer Sci.* 2023;114:4202–15. <https://doi.org/10.1111/cas.15946>.
44. Deng M, Zhang L, Zheng W, Chen J, Du N, Li M, et al. Helicobacter pylori-induced NAT10 stabilizes MDM2 mRNA via RNA acetylation to facilitate gastric cancer progression. *J Exp Clin Cancer Res.* 2023;42:9. <https://doi.org/10.1186/s13046-022-02586-w>.
45. Zong G, Wang X, Guo X, Zhao Q, Wang C, Shen S, et al. NAT10-mediated AXL mRNA N4-acetylcytidine modification promotes pancreatic carcinoma progression. *Exp Cell Res.* 2023;428:113620. <https://doi.org/10.1016/j.yexcr.2023.113620>.
46. Larriue D, Britton S, Demir M, Rodriguez R, Jackson SP. Chemical inhibition of NAT10 corrects defects of laminopathic cells. *Science.* 2014;344:527–32. <https://doi.org/10.1126/science.1252651>.
47. Akimoto K, Miyata A, Kangawa K, Matsuo H, Koga Y, Matsuoka Y, et al. Plasma and right auricle concentrations of atrial natriuretic polypeptide in children with cardiac diseases. *Eur J Pediatr.* 1988;147:485–9. <https://doi.org/10.1007/bf00441972>.
48. Tsai K, Jaguva Vasudevan AA, Martinez Campos C, Emery A, Swanstrom R, Cullen BR. Acetylation of cytidine residues boosts HIV-1 gene expression by increasing viral RNA Stability. *Cell Host Microbe.* 2020;28:306–12. <https://doi.org/10.1016/j.chom.2020.05.011>.
49. Liu H, Xu L, Yue S, Su H, Chen X, Liu Q, et al. Targeting N4-acetylcytidine suppresses hepatocellular carcinoma progression by repressing eEF2-mediated HMGB2 mRNA translation. *Cancer Commun (Lond).* 2024;44:1018–41. <https://doi.org/10.1002/cac2.12595>.

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