



OPEN GADD45 β inhibits hepatic lipogenesis through the AMPK/SREBP1 pathway via reducing the ubiquitination-mediated degradation of SIRT1

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Metabolic dysfunction–associated steatotic liver disease (MASLD) is a globally increasing metabolic disorder associated with serious health complications. The molecular mechanisms linking stress-response proteins to hepatic lipogenesis in MASLD remain poorly understood. Here, we identified GADD45 β as a key suppressor of de novo lipogenesis through SIRT1 stabilization. In both methionine-choline-deficient (MCD) diet-fed mice and palmitic acid (PA)-treated hepatocytes, GADD45 β deficiency exacerbated lipid accumulation and upregulated lipogenic genes (SREBP1, FASN, ACC). Mechanistically, GADD45 β directly bound to SIRT1 and inhibited its ubiquitination, thereby prolonging SIRT1 protein stability. Enhanced SIRT1 stability increased AMPK phosphorylation, which suppressed SREBP1-mediated transcription of lipogenic targets. Crucially, hepatic overexpression of GADD45 β reversed PA-induced steatosis in vitro. Our study uncovered a GADD45 β /SIRT1-AMPK axis as a central regulator of hepatic lipogenesis, proposing GADD45 β as a therapeutic target for MASLD.

Keywords GADD45 β , MASLD, Lipogenesis, Ubiquitination, SIRT1

The increasing prevalence of metabolic dysfunction–associated steatotic liver disease (MASLD) is a significant global health concern. In accordance with recent consensus recommendations, the condition previously termed nonalcoholic fatty liver disease (NAFLD) is now defined as MASLD, and nonalcoholic steatohepatitis (NASH) is now termed metabolic dysfunction–associated steatohepatitis (MASH). Over the last decade, MASLD has become the predominant cause of chronic liver disease worldwide and now affects more than 25% of the global population¹. MASLD is often linked to obesity, insulin resistance, and metabolic syndrome². It is characterized by excessive lipid accumulation in hepatocytes, which can progress from simple steatosis to more severe forms, such as steatohepatitis and cirrhosis. The mechanisms underlying hepatic lipid metabolism are critical for understanding the pathophysiology of MASLD, yet they remain inadequately explored. De novo lipogenesis plays a crucial role in the development of MASLD³. This process converts excess glucose or fructose into fatty acids and triglycerides⁴ and is important for maintaining metabolic homeostasis; however, its increased activation may cause hepatic steatosis⁵. Therefore, inhibition of lipogenesis represents a potential therapeutic strategy for lipid metabolism-related diseases. Previous studies have focused primarily on signaling pathways involved in lipid regulation, such as sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK), whereas the specific contributions of growth arrest and DNA damage-inducible 45 β (GADD45 β) in this context remain poorly characterized^{6,7}.

GADD45 β , a member of the GADD45 family, participates in cellular stress responses, DNA repair, and apoptosis⁸. Recent studies have highlighted its potential involvement in glucose and lipid metabolism, suggesting that GADD45 β may be an important regulator in the pathogenesis of MASLD⁹. However, whether GADD45 β

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contributes to lipogenesis and thereby affects MASLD remains unclear. A significant gap persists regarding the mechanistic role of GADD45 β in hepatic lipid metabolism, particularly its interactions with established pathways such as SIRT1/AMPK/sterol regulatory element-binding protein 1 (SREBP1) signaling, which are crucial for maintaining metabolic homeostasis^{10,11}.

In this study, we provided evidence that SIRT1 was regulated by GADD45 β and demonstrated an important association between them in MASLD. GADD45 β interacted with SIRT1 and attenuated its ubiquitin–proteasome degradation, thereby enhancing its stability. GADD45 β deficiency decreased the activity of the SIRT1 target AMPK, resulting in lipogenesis exacerbation. Our findings highlight a critical role of GADD45 β in maintaining SIRT1 stability and limiting lipogenesis, providing new insights and potential therapeutic strategies for MASLD.

Materials and methods

Reagents and antibodies

Cycloheximide (CHX, Cat# M4879) was purchased from AbMole Bioscience, Inc. MG132 (Cat# S2619) was purchased from Selleck Chemicals. Palmitic acid (PA, Cat# S-A9165-5G) was purchased from Sigma-Aldrich. Compound C (CC, Cat# HY-13418) was purchased from Med ChemExpress. Antibodies against FASN (Cat# ARG55898, 1:1000), HSP90 (Cat# ARG55781, 1:1000), Flag Tag (Cat# ARG62342, 1:1000) and β -Actin (Cat# ARG65683, 1:1000) were purchased from Arigo. Antibodies against ubiquitin (Cat# 3936, 1:400), AMPK (Cat# 2532 S, 1:1000), and p-AMPK (Cat# 2531 S, 1:1000) were purchased from Cell Signaling Technology. Antibodies against SIRT1 (Cat# ab189494, 1:1000), SREBP1 (Cat# ab28481, 1:1000), and ACC1 (Cat# ab45174, 1:1000) were purchased from Abcam. Antibody against GADD45 β (Cat# sc377311, 1:1000) was purchased from Santa Cruz. HRP-conjugated secondary antibodies against mouse (Servicebio, GB23301) or rabbit (Servicebio, GB23303) were used.

Animal treatment

All animal experiments were approved by the Animal Research Ethics Committee of Shanghai Sixth People's Hospital and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study followed all institutional and national guidelines for animal research and complied with the ARRIVE guidelines to ensure ethical and reproducible practices¹².

Eight-week-old C57BL/6J mice weighing 23–28 g were purchased from Shanghai Sippe-Bk Lab Animal Co., Ltd. (Shanghai, China). Animals were maintained in a specific pathogen-free facility under a 12 h light/dark cycle. After one week of acclimatization, mice were randomly assigned to experimental groups. The methionine-choline-deficient (MCD) diet (A02082002B; Research Diets, USA) was administered for 4 weeks to induce MASH, and age-matched mice on a standard chow diet were used as controls.

To knock down hepatic GADD45 β , an adeno-associated virus (AAV8; OBiO Technology, Shanghai, China) carrying a short hairpin RNA targeting GADD45 β (pscAAV-U6-shGADD45 β -CMV-EGFP-tWPA) was constructed (AAV-shGADD45 β). The target sequence was 5'-GCGACAATGACATTGACATCG-3'. Control mice received an AAV-shNC construct (pscAAV-U6-shNC-CMV-EGFP-tWPA). Viruses (2×10^{11} PFU per mouse) were purified using cesium chloride gradient centrifugation, dialyzed in PBS containing 10% glycerol, and administered via tail vein injection 14 days before euthanasia. At the study endpoint, after 12 h of fasting, mice were euthanized by intraperitoneal injection of 3% sodium pentobarbital (60 mg/kg). Liver tissues were rapidly dissected, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent RNA and protein analysis.

All animal procedures were performed in adherence to the 3R principles (Replacement, Reduction, and Refinement). Sample size was determined based on prior pilot studies and ethical guidelines, with a minimum of eight mice per group considered sufficient to achieve statistical power while minimizing animal use. Although investigators were aware of group allocation during procedures and outcome assessment, all interventions and data collection followed standardized protocols to minimize bias. Humane endpoints were predefined, and any animals showing signs of severe distress or irreversible suffering were promptly euthanized to ensure animal welfare.

Biochemical assays

Blood samples collected from the mice were left at room temperature for 30 min and then centrifuged at 5000 rcf for 5 min at 4°C . The supernatant was centrifuged again at 10,000 rcf for 5 min at 4°C . Serum was carefully transferred into clean 1.5 mL EP tubes and stored at -20°C until use. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), and gamma-glutamyltransferase (γ -GT) were measured using a fully automated biochemical analyzer (Rayto Technologies, China).

Hematoxylin and eosin (H&E) and Oil Red O staining

For H&E staining, paraffin-embedded liver tissues were sectioned at 4 μm thickness. Slides were deparaffinized in xylene for 5 min (twice), rehydrated through a graded ethanol series (100%, 95%, 85%, 75%), and rinsed in distilled water. Sections were stained with hematoxylin for 7 min, rinsed, counterstained with eosin for 30 s, differentiated in acid ethanol for 30 s, washed in distilled water, dehydrated through graded alcohols, cleared in xylene, and mounted with neutral resin.

For Oil Red O staining, frozen liver Sect. (7 μm) were fixed in 4% paraformaldehyde for 10 min, washed with distilled water, and incubated with freshly prepared Oil Red O working solution (Sigma-Aldrich; 0.5 g Oil Red O dissolved in 100 mL isopropanol, diluted 3:2 with distilled water, filtered before use) for 8–10 min. Sections were differentiated in 75% ethanol, rinsed with PBS, counterstained with hematoxylin for 90 s, and mounted using glycerol gelatin. Slides were examined under an Olympus light microscope.

Cell culture and treatment

HepG2 cells were cultured in DMEM/high glucose (Cat# SH30243.01; HyClone) supplemented with 10% fetal bovine serum (Cat#04-001-1ACS; Biological Industries) and 1% penicillin-streptomycin (Cat#C0222; Beyotime, China) in a 5% CO₂ incubator at 37 °C. Cells were treated with fatty acid-free bovine serum albumin or 400 μM PA for 24 h. Intracellular lipid droplets were stained with Oil Red O and visualized under a light microscope (Olympus, Tokyo, Japan).

Cell transfection

pSLenti-GADD45β-3xFlag (GADD45β), control pSLenti-MCS-3xFlag (vector), pLenti-U6-siGADD45β-CMV (siGADD45β:GCATACTCCTTCCACGTTA), and control pSLenti-U6-shNC2-CMV (siNC: TTCTCCGAACG TGTCACGT) were constructed by OBiO Technology (Shanghai, China). Transfections were performed using Lipofectamine[®] LTX & PLUS[™] Reagent (Cat# A12621; Invitrogen, NY, USA) according to the manufacturer's instructions. Briefly, when HepG2 cells reached 70–80% confluence, solutions A (2.5 μg plasmid DNA, 2.5 μL PLUS reagent, and 125 μL Opti-MEM) and solution B (5 μL LTX reagent and 125 μL Opti-MEM) were prepared separately, combined, and incubated for 5 min before being added dropwise to the cell culture medium. Medium was replaced after 6–8 h. Cells were collected 48 h later for RNA and protein extraction.

In addition, two lentivirus (LV) constructs, LentiCRISPRv2-SIRT1-sgRNA (LV-shSIRT1) and a blank vector (LV-shNC), were purchased from Fengbio Biotechnology Co., Ltd. (Hunan, China). HepG2 cells at 80% confluence were transduced with these constructs at a multiplicity of infection of 50. After 24 h, the medium was replaced with fresh medium containing FBS, and puromycin (30 μg/mL; Cat# HY-K1057; MedChemExpress) was applied to select successfully transfected cells. The culture was continued for 2 days, after which cells were harvested and lysed for Western blotting.

Western blot analysis

Total protein extracts from liver tissues and HepG2 cells were prepared using RIPA buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl₂, 2 mM EDTA, 1 mM NaF, 1% NP40, and 0.1% SDS. Equal amounts of protein were denatured in loading buffer, separated by 10–12% SDS-PAGE, and transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 5% nonfat milk for 1 h at room temperature and then incubated with primary antibodies overnight at 4 °C. After five washes with PBS containing Tween-20, membranes were incubated with HRP-conjugated secondary antibodies for 1 h. Protein bands were visualized using an enhanced chemiluminescence system.

Coimmunoprecipitation (Co-IP)

Cells were washed with PBS and lysed on ice for 30 min with IP lysis solution (Beyotime) supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific). After centrifugation, equal amounts of lysates were incubated overnight at 4 °C with the corresponding antibodies or control IgG, followed by incubation with 20 μl of A/G agarose beads (Cat# 88802; Thermo Fisher Scientific) for 4 h. Immunocomplexes were pelleted, washed three times with lysis buffer, and eluted with SDS loading buffer (Cat# BL502A; Biosharp Life Sciences, Hefei, Anhui, China). Samples were then analyzed by immunoblotting with the indicated antibodies.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells or liver tissues using TRIzol reagent and reverse transcribed into cDNA with PrimeScript[™] RT Master Mix (Cat# R433-01; Vazyme) according to the manufacturer's instructions. qRT-PCR was performed with SYBR Premix Ex Taq[™] (Cat#Q312-02; Vazyme), and results were analyzed using LightCycler 480 software (Roche Diagnostics GmbH, Mannheim, Germany). Relative gene expression was quantified using the 2^{-ΔΔC_t} method, with GAPDH as the internal control. Primer sequences are listed in Table S1.

Statistics

Statistical analyses were performed using GraphPad InStat Software (San Diego, CA, USA). Data are presented as the means ± SEM. For animal and cellular experiments, comparisons between two groups were made using a two-tailed unpaired Student's t-test. Western blot and morphological images are representative of at least three experiments. The normality of quantitative data was assessed using the Kolmogorov-Smirnov test. For multiple group comparisons, one-way ANOVA was applied. Statistical significance was expressed as **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Results

Hepatic GADD45β expression is decreased in MASLD

By accessing the GEO database, two MASLD datasets, GSE48452 and GSE33814, were analyzed, comprising 37 healthy controls, 39 MASLD patients, and 31 MASH patients. Notably, GADD45β was consistently downregulated at the mRNA level in both MASLD and MASH groups (Fig. 1A,B). To further validate this finding, GADD45β mRNA and protein levels were examined in mouse liver samples. Consistent with the human data, GADD45β expression was substantially reduced in the livers of MCD diet-fed mice compared with controls (Fig. 1C,D).

Hepatic knockdown of GADD45β exacerbates steatohepatitis in MCD mice

To investigate the role of hepatic GADD45β in steatohepatitis, C57BL/6J mice fed an MCD diet were injected with adenoviral shRNA targeting GADD45β via the tail vein, which effectively reduced hepatic GADD45β expression (Fig. 2A,B). Although body weight remained unchanged, liver weight, liver index, and hepatic TG content were significantly increased in the knockdown group compared with controls (Fig. 2C–E). Consistently, H&E and Oil Red O staining revealed markedly aggravated hepatic steatosis in GADD45β-deficient mice

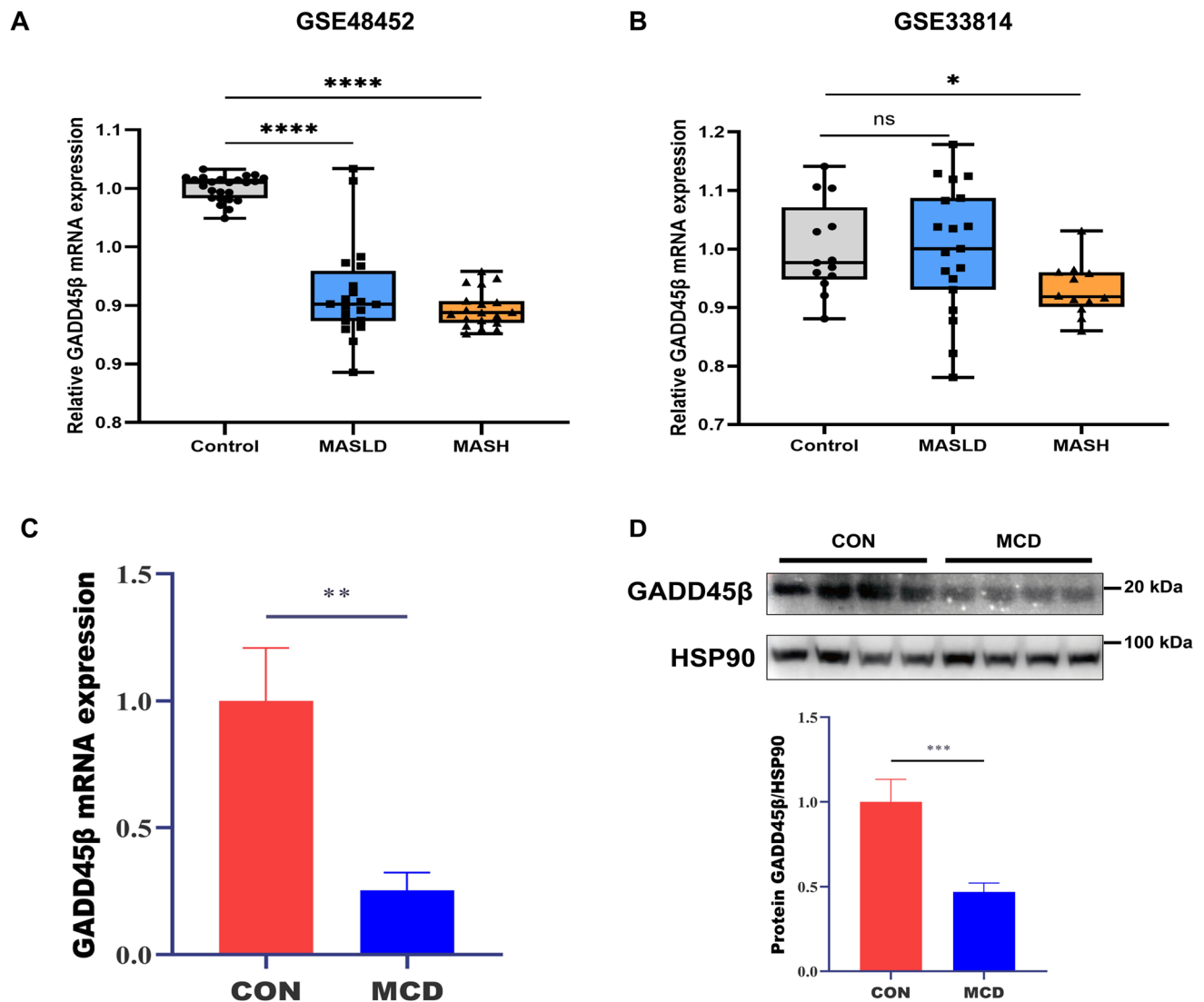


Fig. 1. Hepatic GADD45β expression was decreased in MASLD. (A, B) GADD45β mRNA expression levels in two different MASLD GEO datasets. (C) GADD45β mRNA expression levels in the livers of control diet-fed (CON) and methionine- and choline-deficient (MCD) diet-fed mice ($n = 6$). (D) GADD45β protein expression levels in the livers of control diet-fed mice (CON) and methionine- and choline-deficient diet-fed mice (MCD) ($n = 6$). $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

(Fig. 2F,G). Interestingly, while serum TG, TC, ALT, and AST levels showed no significant differences between groups (Fig. 2H–K), γ GT levels were significantly elevated in the GADD45β-knockdown group (Fig. 2L), suggesting worsened liver injury. Collectively, these findings demonstrate that hepatic GADD45β knockdown aggravates steatohepatitis in MCD-fed mice.

Hepatic knockdown of GADD45β enhances hepatic lipogenic gene expression in MCD diet-fed mice

To investigate how GADD45β knockdown aggravates hepatic steatosis, we compared the expression of lipogenesis-related genes in the livers of GADD45β-knockdown and control mice fed an MCD diet. MCD feeding alone significantly upregulated hepatic SREBP1 mRNA expression, and liver-specific knockdown of GADD45β further increased its expression. Liver-specific knockdown of GADD45β also significantly elevated the mRNA levels of additional lipogenic genes, FASN and ACC (Fig. 3A). However, the hepatic mRNA levels of genes related to fatty acid oxidation, fatty acid transport, and lipolysis were unaffected (Fig. 3A). Western blot analysis confirmed increased hepatic protein levels of SREBP1, FASN, and ACC1 in GADD45β-deficient mice, consistent with the mRNA changes (Fig. 3B). These results indicate that liver-specific knockdown of GADD45β enhances hepatic lipogenesis in MCD diet-fed mice.

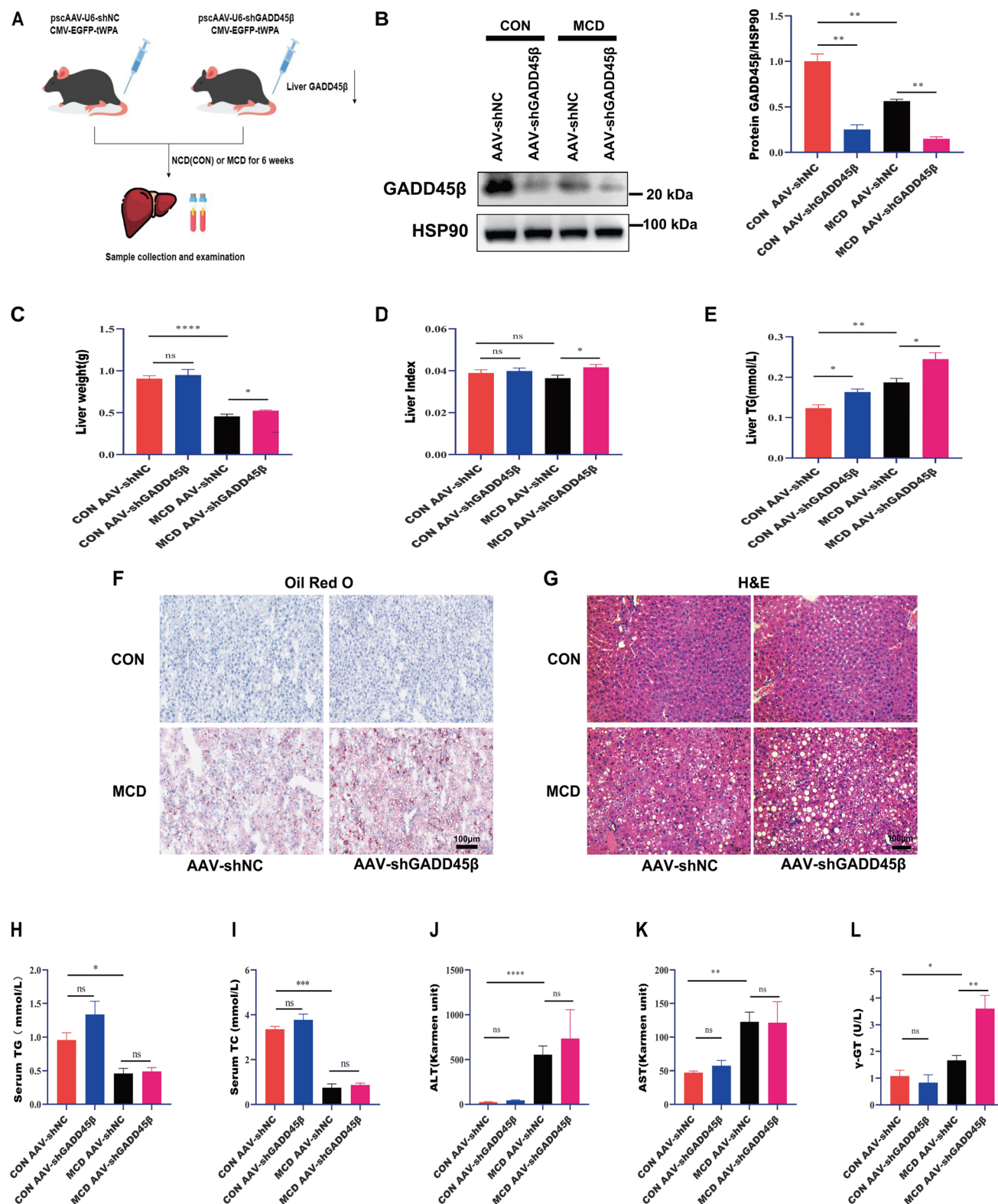


Fig. 2. Hepatic knockdown of GADD45β aggravated steatohepatitis in MCD diet-fed mice. **(A)** Schematic Diagram of Animal Experimental Procedures **(B)** GADD45β expression in liver tissues of GADD45β hepatic knockdown mice compared with the control group (n = 6). **(C–E)** Liver weight, liver weight/body weight ratio (liver index), and liver TG levels of each group (n = 6). **(F, G)** Representative images of H&E and Oil Red O staining of liver sections from each group (n = 5); scale bar, 100 μm. **(H–L)** Serum TG, TC, ALT, AST and γGT levels in each group (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001.

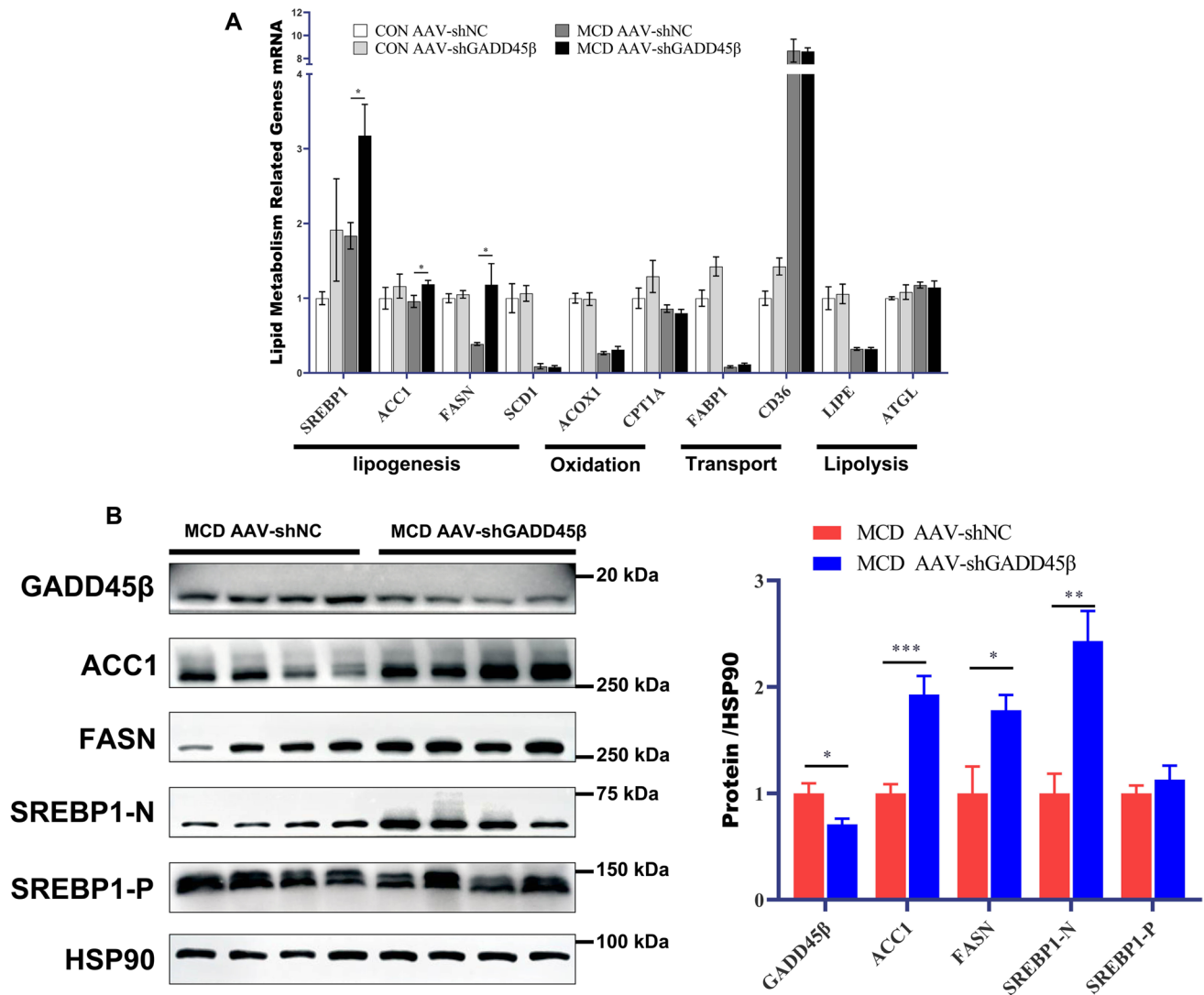


Fig. 3. Hepatic knockdown of GADD45 β increased hepatic lipogenic gene expression in MCD diet-fed mice. **(A)** Quantitative PCR analysis of the mRNA expression levels of lipid metabolism-related genes in the liver tissue of different groups ($n = 6$). **(B)** Western blot of ACC1, FASN, nuclear SREBP1 (SREBP1-N) and cytoplasmic SREBP1 (SREBP1-P) in the liver tissues of different groups ($n = 6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

GADD45 β inhibits PA-induced steatosis and lipogenesis in hepatocytes

To confirm the function of GADD45 β in hepatocytes, HepG2 cells were transfected with a GADD45 β plasmid. Western blot analysis verified a significant increase in GADD45 β protein levels after transfection (Fig. 4F). Cells were then treated with 400 μ M PA for 24 h to induce steatosis. Oil Red O staining revealed that GADD45 β overexpression significantly reduced intracellular lipid accumulation. Consistently, PA stimulation significantly elevated intracellular TG content, which was blocked by GADD45 β overexpression (Fig. 4A–C). These results indicate that GADD45 β upregulation protects hepatocytes from PA-induced steatosis.

The effect of GADD45 β on lipogenesis was further examined. qRT-PCR and Western blot analyses demonstrated that GADD45 β overexpression significantly suppressed PA-induced expression of lipogenic regulators, including SREBP1, ACC1, and FASN (Fig. 4F–H). Conversely, knockdown of GADD45 β enhanced the expression of these genes after PA treatment (Fig. 4A, I–K), which was accompanied by increased lipid accumulation and aggravated steatosis in hepatocytes (Fig. 4A, D, E). These findings collectively suggest that GADD45 β exerts a protective effect against hepatic steatosis by inhibiting lipogenesis.

GADD45 β mediates lipogenesis via the SIRT1/AMPK/SREBP1 pathway in hepatocytes

To determine the molecular mechanism by which GADD45 β regulates hepatic lipogenesis, we first examined its effects on the SIRT1/AMPK pathway and downstream SREBP1. In vivo, hepatic knockdown of GADD45 β in MCD-fed mice markedly reduced SIRT1 expression and the p-AMPK/AMPK ratio as shown by both western blotting and immunohistochemistry (Fig. 5A–C). Consistently, in PA-treated HepG2 cells, GADD45 β

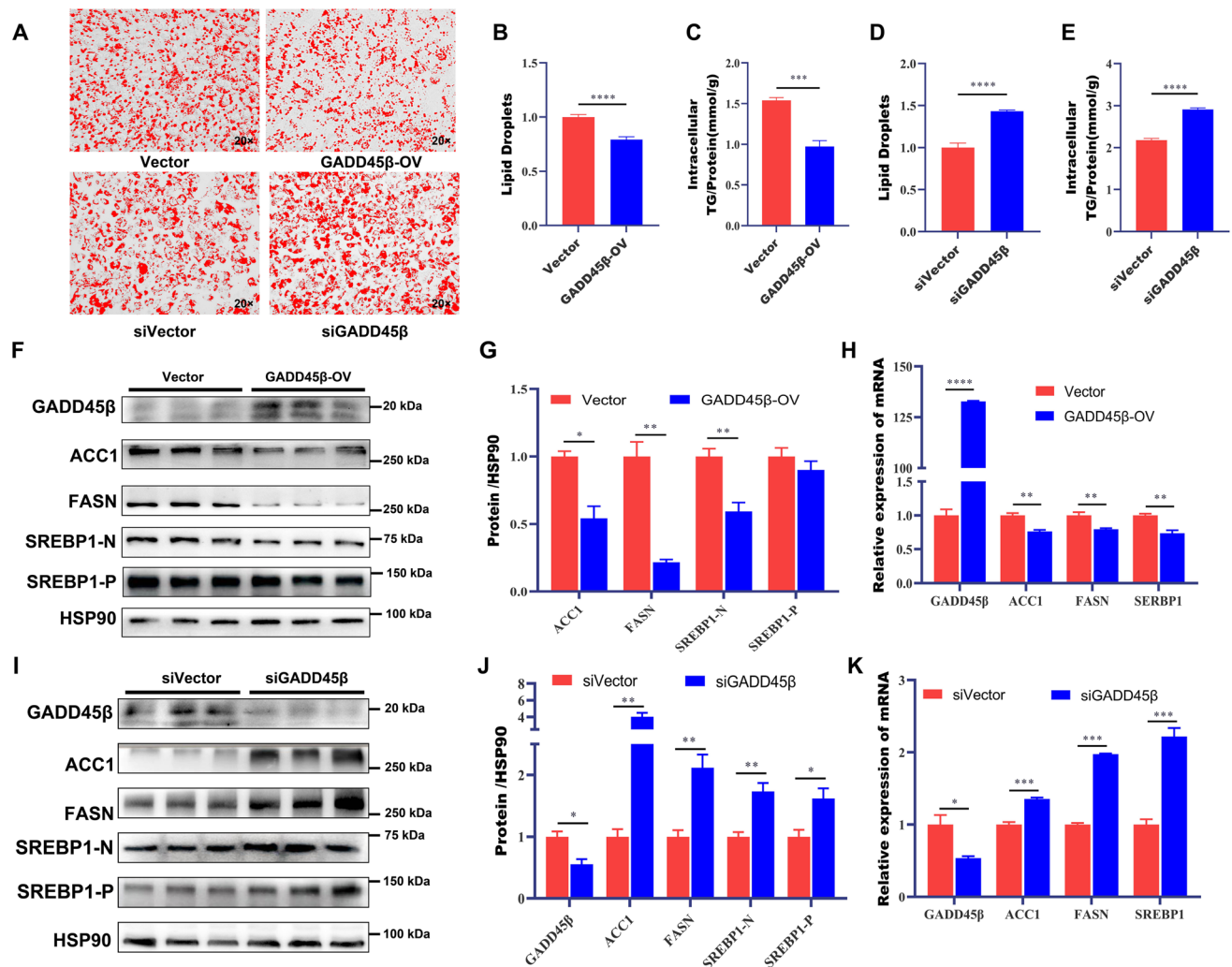


Fig. 4. GADD45β inhibited palmitic acid-induced steatosis and lipogenesis in hepatocytes. **(A)** Oil Red O staining and area quantification of GADD45β-overexpressing or GADD45β-knockdown HepG2 cells after treatment with PA. **(B–E)** Lipid drops, intracellular TG levels, and relative quantification of GADD45β-overexpressing or GADD45β-knockdown HepG2 cells after treatment with PA. **(F–H)** Protein and mRNA expression levels of FASN, ACC1, and SREBP1 in PA-induced GADD45β-overexpressing HepG2 cells ($n = 3$). **(I–K)** Protein and mRNA expression levels of FASN, ACC1, and SREBP1 in PA-induced GADD45β-knockdown HepG2 cells ($n = 3$).

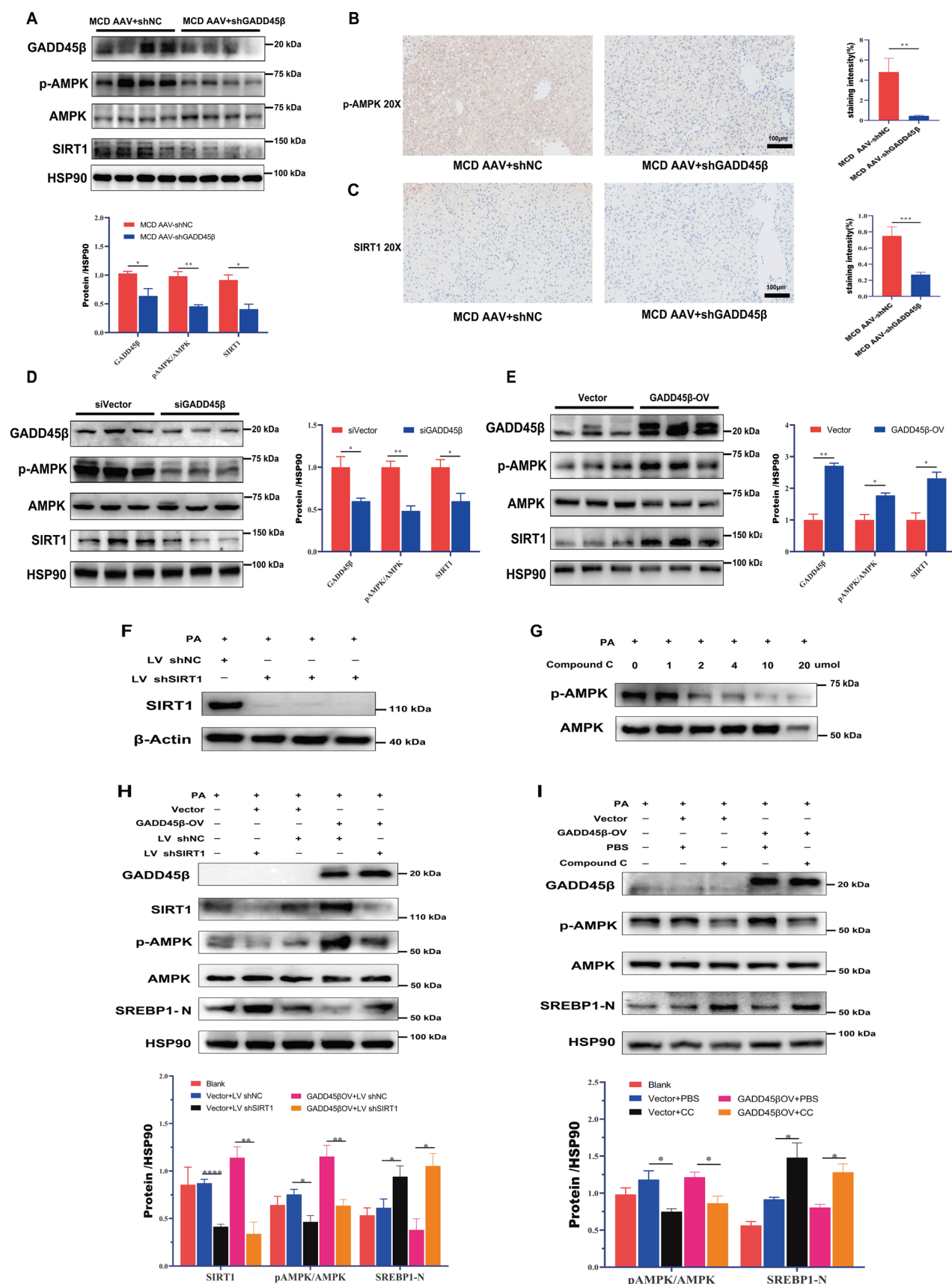
silencing decreased SIRT1 and p-AMPK/AMPK expression and increased SREBP1 levels, whereas GADD45β overexpression enhanced SIRT1/AMPK signaling and suppressed SREBP1 expression (Fig. 5D,E). These data indicate that GADD45β positively regulates the SIRT1/AMPK pathway and thereby inhibits SREBP1-mediated lipogenesis.

To further confirm whether SIRT1 functions as an upstream regulator of AMPK, rescue experiments were performed. Lentiviral knockdown of SIRT1 in PA-stimulated HepG2 cells abrogated the increase in p-AMPK/AMPK and the suppression of SREBP1 conferred by GADD45β overexpression (Fig. 5F,G). These results identify SIRT1 as a key upstream mediator of the p-AMPK/AMPK axis.

Finally, to evaluate whether AMPK is essential for the protective role of GADD45β, HepG2 cells were treated with the AMPK inhibitor CC. Inhibition of AMPK abolished the effects of GADD45β overexpression, as indicated by decreased p-AMPK and restored SREBP1 expression (Fig. 5H,I). Together, these findings demonstrate that GADD45β suppresses lipogenesis in hepatocytes through the SIRT1/AMPK/SREBP1 signaling pathway.

GADD45β directly interacts with SIRT1

Although GADD45β overexpression increased SIRT1 protein levels, it did not affect SIRT1 mRNA expression as determined by qRT-PCR (Fig. 6A). We therefore hypothesized that GADD45β may physically associate with SIRT1. Co-IP assays in PA-induced HepG2 cells confirmed this interaction, as GADD45β was pulled down with SIRT1 and reciprocally, SIRT1 was detected in anti-GADD45β immunoprecipitates (Fig. 6B). These findings indicate that GADD45β directly interacts with SIRT1.



GADD45β upregulates SIRT1 levels by inhibiting ubiquitin–proteasome degradation

To exclude the influence of protein synthesis on SIRT1 levels, and because SIRT1 can be regulated through ubiquitination, we further examined whether GADD45β affects SIRT1 stability. HepG2 cells transfected with GADD45β plasmids were treated with CHX, a protein synthesis inhibitor, and SIRT1 protein levels were

Fig. 5. GADD45 β mediated lipogenesis via the SIRT1/AMPK/SREBP1 pathway in hepatocytes. (A) Western blot analysis of p-AMPK, AMPK, and SIRT1 expression in liver tissues from GADD45 β -knockdown and control mice (n = 6). (B, C) Representative IHC staining and relative quantification of p-AMPK and SIRT1 in liver sections from the indicated individuals. (n = 6). (D) Western blot of p-AMPK, AMPK, and SIRT1 expression in GADD45 β -knockdown HepG2 cells treated with PA (n = 6). (E) Western blot of p-AMPK, AMPK, and SIRT1 expression in GADD45 β -overexpressing HepG2 cells treated with PA (n = 6). (F) Western blot analysis of SIRT1 expression in PA-induced HepG2 cells infected with LV-shSIRT1 or LV-shControl. (G) SIRT1, p-AMPK/AMPK, and SREBP-1 expression in PA-induced SIRT1 knockdown and GADD45 β -overexpressing HepG2 cells (n = 6). (H) Western blot of p-AMPK/AMPK expression in PA-induced HepG2 cells treated with multiple doses of Compound C. (I) p-AMPK/AMPK and SREBP-1 expression in PA-induced GADD45 β -overexpressing and Compound C-treated HepG2 cells (n = 6).

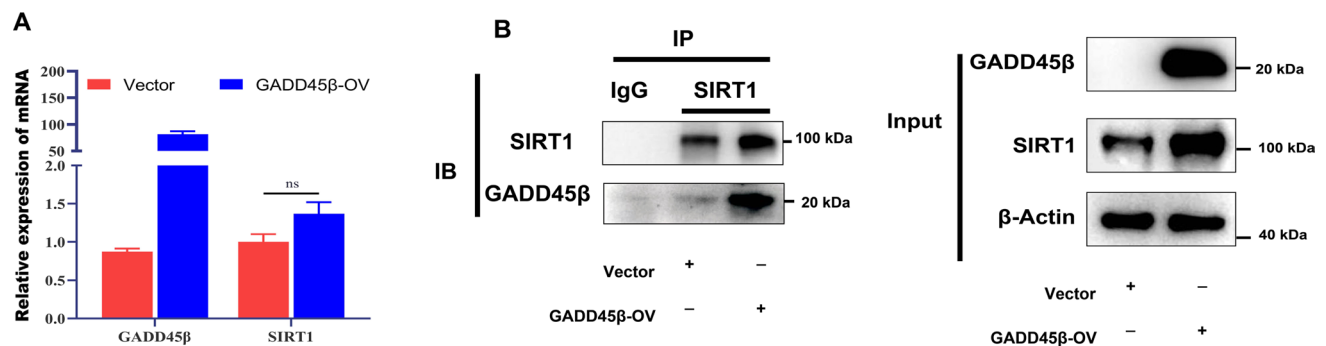


Fig. 6. GADD45 β directly interacted with SIRT1. (A) GADD45 β and SIRT1 mRNA expression in HepG2 cells after the overexpression of GADD45 β . (B) Coimmunoprecipitation (co-IP) of GADD45 β with SIRT1 proteins in HepG2 cells. The co-IP and western blot data shown are representative of at least 3 independent experiments with consistent results.

measured. As expected, GADD45 β significantly prolonged the half-life of SIRT1, which was decreased by CHX (Fig. 7A). Conversely, GADD45 β knockdown decreased SIRT1 stability in PA-induced HepG2 cells (Fig. 7B), suggesting that GADD45 β maintains SIRT1 levels by inhibiting its degradation rather than promoting protein synthesis.

The K48-linked ubiquitin-proteasome system (UPS) is a major intracellular protein degradation mechanism. As shown in Fig. 7C, in the condition of exposing to PA, treatment HepG2 cells with the proteasome inhibitor MG132, GADD45 β overexpression significantly increased SIRT1 protein expression levels compare with the control group, implicating that the UPS pathway may involve in this process. Furthermore the ubiquitin immunoprecipitation further revealed that PA exposure mediates SIRT1 ubiquitination, whereas GADD45 β overexpression reverses these effects. All of these results indicate that GADD45 β increases SIRT1 levels by inhibiting its ubiquitin-proteasome-mediated degradation.

Based on these results, we propose a mechanistic model: reduced GADD45 β expression in hepatocytes of MASH patients and MCD-fed mice diminishes its interaction with SIRT1, leading to enhanced ubiquitin-proteasome-mediated degradation of SIRT1. The resulting loss of SIRT1 impairs AMPK phosphorylation, thereby augmenting SREBP1-mediated de novo fatty acid synthesis. This cascade increases lipogenesis and exacerbates hepatic steatosis (Fig. 8).

Discussion

MASLD has emerged as a significant global health concern due to its complex pathogenesis, which involves insulin resistance, oxidative stress, and inflammatory pathways. With the increasing prevalence of obesity and metabolic syndrome, MASLD is recognized not only as a hepatic disorder but also as a major risk factor for cardiovascular diseases, type 2 diabetes, and other metabolic disorders. This underscores the urgent need to understand its mechanisms and develop effective therapeutic strategies^{13,14}. In this study, we identified GADD45 β as a novel factor contributing to the development and progression of MASLD. GADD45 β is an acidic stress-responsive protein involved in DNA repair, cell cycle regulation, apoptosis, inflammation, and stress responses through protein-protein interactions¹⁵. Several studies have also implicated GADD45 β in lipid metabolism. Dong et al. have demonstrated that GADD45 β was downregulated in multiple clinical datasets and in a murine MASLD model, where its expression mitigated lipid accumulation and insulin resistance in high-fat diet (HFD)-induced disease⁶. Fuhrmeister et al. have reported that under fasting conditions, GADD45 β binds FABP1, promoting its cytoplasmic retention, thereby inhibiting fatty acid uptake and enhancing lipid metabolism¹⁶. Kim et al. have revealed that GADD45 β suppresses hepatic gluconeogenesis by stabilizing and activating forkhead box protein O1 (FoxO1), while GADD45 β knockout increased expression of lipogenesis-related genes. Furthermore, GADD45 β has been identified as an inducible coactivator of the constitutive androstane receptor (CAR), promoting rapid liver growth. Cai et al. have also reported that CAR-mediated metabolic regulation depends

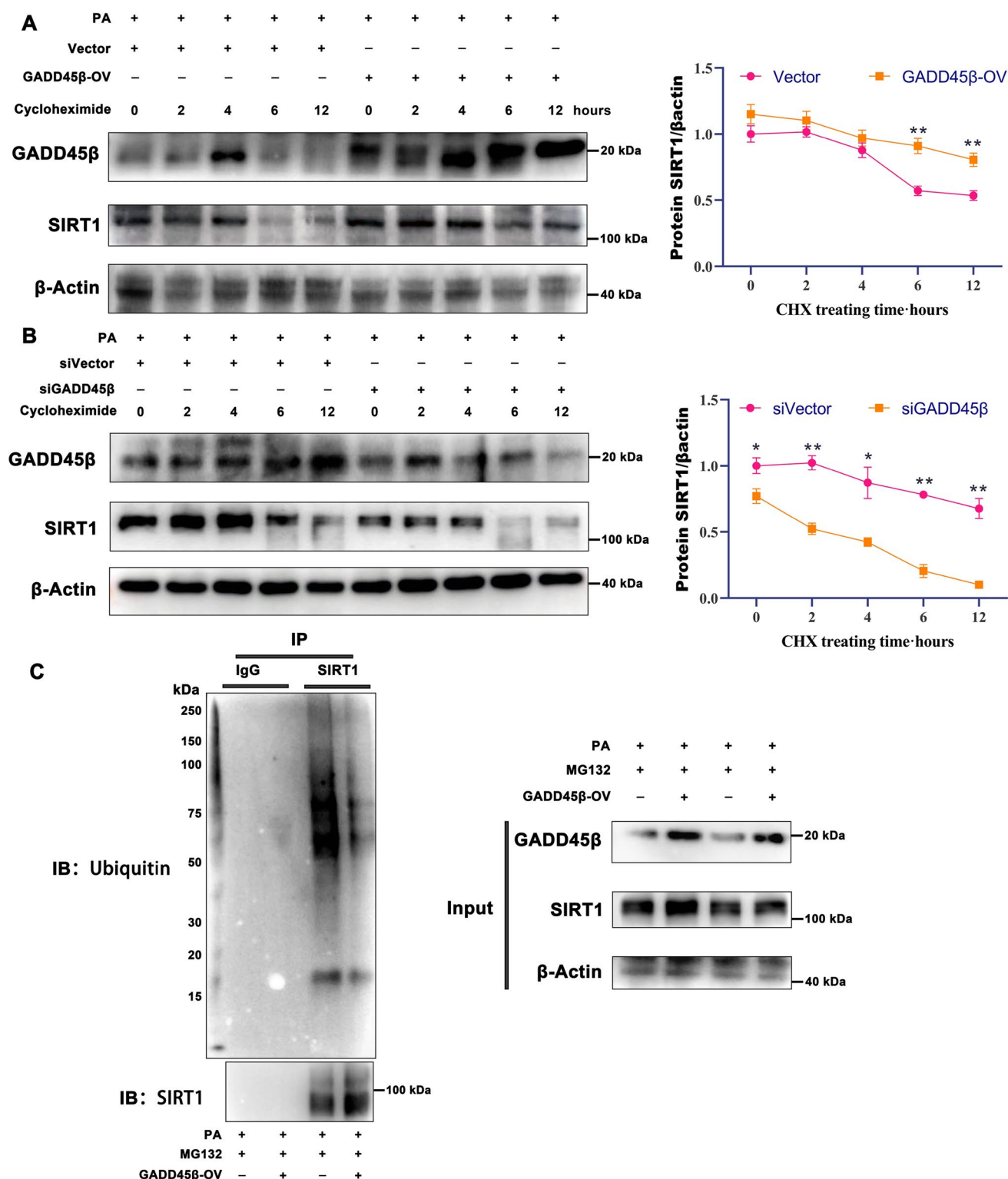


Fig. 7. GADD45 β facilitates SIRT1 stability by inhibiting its ubiquitin-proteasome degradation. **(A)** Western blots of PA-induced GADD45 β -overexpressing HeG2 cells treated with cycloheximide (CHX; 20 μ g/ml) for the indicated time periods and semiquantification of SIRT1 levels. **(B)** Western blots of PA-induced GADD45 β -knockdown HeG2 cells treated with cycloheximide (CHX; 20 μ g/ml) for the indicated time periods and semiquantification of SIRT1 levels. **(C)** Coimmunoprecipitation (co-IP) of SIRT1 with ubiquitin proteins in PA-induced GADD45 β -overexpressing HepG2 cells after treatment with 10 μ M MG132 for 8 h ($n = 3$). The Co-IP and western blot data shown are representative of 3 independent experiments with consistent results.

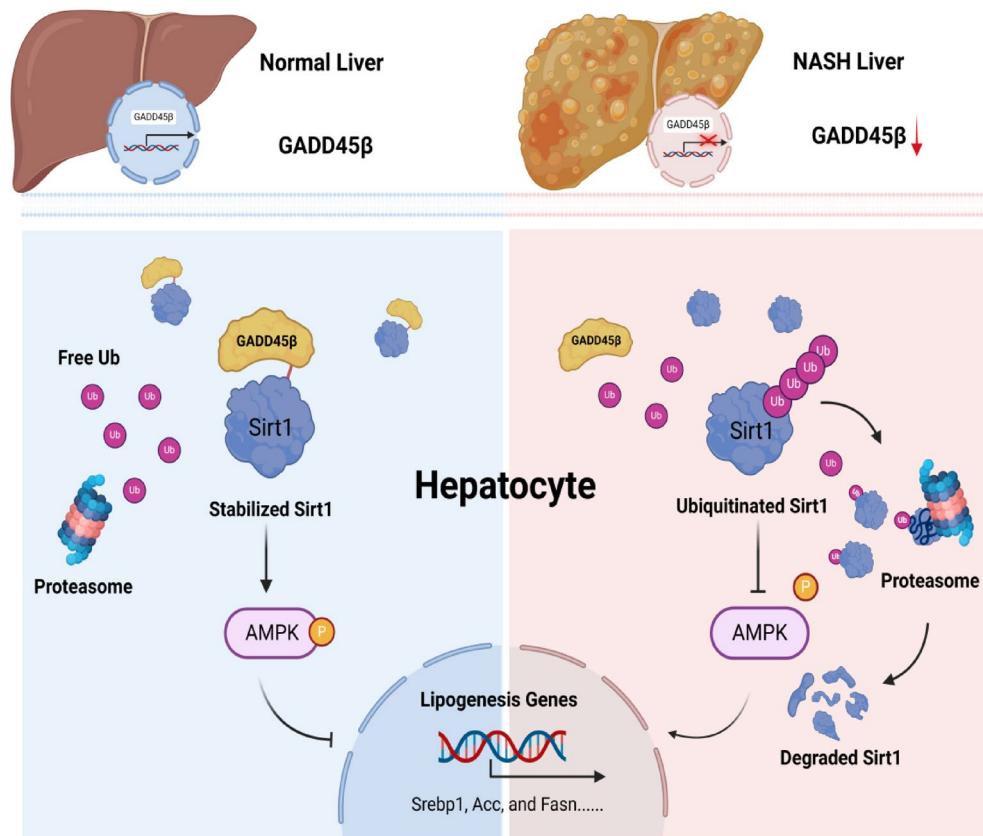


Fig. 8. Working model of GADD45 β in the regulation of hepatic steatosis. GADD45 β expression is decreased in hepatocytes fed an MCD diet. A decrease in GADD45 β does not interact with Sirtuin 1 (SIRT1) and is subsequently degraded by ubiquitination. This results in increased lipogenesis and thereby synergistically aggravates fatty acid accumulation and hepatic steatosis.

on GADD45 β , as GADD45 β knockout blunted the weight reduction and insulin sensitivity induced by the CAR agonist TCPOBOP in HFD-fed mice, mechanistically linked to lipogenesis inhibition in hepatocytes¹⁷. However, further mechanistic studies remain limited. In the present study, we observed that GADD45 β mRNA and protein levels progressively decreased in human and mouse MASLD livers as the disease advanced from MASLD to MASH. Using a liver-specific GADD45 β knockdown model under an MCD diet, we found that steatosis was aggravated compared with control diet-fed mice. This was accompanied by increased hepatic expression of lipogenic genes, including SREBP1, FASN, and ACC, consistent with previous findings. In vitro, GADD45 β overexpression in HepG2 cells significantly suppressed fatty acid-induced expression of these genes, whereas GADD45 β knockdown enhanced their expression and lipid accumulation. These data indicate that GADD45 β is involved in hepatic lipid metabolism and fat deposition in MASLD.

To clarify how GADD45 β influences hepatic lipid metabolism, we investigated its relationship with AMPK, a heterotrimeric kinase that regulates cellular energy balance through lipid metabolism. AMPK plays a key role in promoting hepatic fatty acid oxidation, inhibiting cholesterol and TG synthesis, and repressing lipogenesis¹⁸. Among its downstream targets, SREBP-1c is a key transcription factor for lipogenesis in the liver¹⁹. AMPK reduces lipogenic gene expression by phosphorylating SREBP-1c at Ser372, thereby preventing its proteolytic activation²⁰. Furthermore, AMPK may suppress SREBP-1c expression through mTOR and LXR α ²¹. Once activated, SREBP1 translocates to the nucleus and stimulates the transcription of lipogenic genes such as ACC, FASN, and stearoyl-CoA desaturase (SCD1)^{22–24}. Although AMPK is known to strongly influence lipogenic gene expression, it has not been established whether GADD45 β regulates lipid metabolism through this pathway. Our study revealed that protein levels associated with the AMPK/SREBP1 axis were significantly altered in vitro and in vivo under MASLD conditions, and these changes were further modulated by GADD45 β . Moreover, treatment with CC, an ATP-competitive inhibitor of AMPK²⁵, abolished GADD45 β -induced activation of AMPK and its inhibitory effect on lipogenesis. These results indicate that GADD45 β may ameliorate lipid metabolism dysfunction via the AMPK/SREBP1 pathway in MASLD.

Even though GADD45 β affects the AMPK signaling pathway, AMPK activity is regulated primarily by phosphorylation, and GADD45 β does not possess phosphatase activity. In addition, as a scaffold protein, GADD45 β does not directly regulate transcription, prompting us to identify downstream factors potentially affected by GADD45 β . We found that SIRT1, a known GADD45 β -binding partner, mediated its effects on the AMPK/SREBP1 pathway. This is a key finding of our study in understanding how GADD45 β regulates lipogenesis in MASLD. SIRT1 is a class III histone deacetylase that requires nicotinamide adenine (NAD⁺) to

deacetylate histone and non-histone proteins involved in diverse metabolic processes and stress responses^{26,27}. It is widely expressed in multiple tissues and has been implicated in metabolic and age-related diseases²⁸. SIRT1 regulates adipocyte differentiation, hepatic lipid metabolism, systemic inflammation, nutrient sensing, circadian rhythms, and particularly lipogenesis²⁹. Often described as a “master metabolic regulator”, it maintains lipid homeostasis by modulating several metabolic proteins^{30,31}. Previous studies have demonstrated that SIRT1 inhibits lipogenic enzymes such as SREBP-1c, thereby alleviating hepatic lipid dysregulation^{32–34}, and can also suppress ACC and FASN in MASLD³⁵. Importantly, AMPK, the natural regulator of SREBP1, is activated indirectly by SIRT1 through deacetylation of its upstream kinase³⁶. Thus, SIRT1 amplifies AMPK activity to reinforce lipid homeostasis^{37,38}. These findings align with clinical evidence showing that SIRT1 activators can alleviate fatty liver in MASLD patients, highlighting its pivotal role in lipid-related disease through the AMPK pathway^{39,40}. In this study, disruption of SIRT1 expression revealed that the impact of GADD45 β on hepatic lipid synthesis was mediated through the SIRT1/AMPK signaling pathway. We further confirmed the interaction between GADD45 β and SIRT1 in both animal and cell models of steatosis. Consistent with previous reports, the MCD diet significantly downregulated hepatic SIRT1 expression. In GADD45 β liver-specific knockdown mice, SIRT1 expression was reduced, and lipogenic genes were significantly increased. Conversely, GADD45 β overexpression in HepG2 cells reversed the PA-induced suppression of SIRT1 and upregulation of lipogenic genes. Together, these results suggest that GADD45 β regulates hepatic steatosis by stabilizing SIRT1 expression and subsequently modulating downstream lipogenic genes.

Another novel finding of our study is that GADD45 β was involved in the ubiquitination and stabilization of SIRT1. We found that GADD45 β not only interacted with SIRT1 but also modulated its protein stability. Previous studies have shown that SIRT1 is regulated through ubiquitin-proteasome degradation and that control of its ubiquitination is crucial for maintaining stability. Ubiquitination is a key signal for protein degradation, with ubiquitin ligases catalyzing the covalent attachment of ubiquitin to substrates and extending polyubiquitin chains. In MASLD, SIRT1 acts as a substrate of E3 ligases, undergoing K48-linked ubiquitination and degradation, which exacerbates hepatic steatosis⁴¹. Proteomic studies have identified multiple E3 ligases that target SIRT1, including MDM2 (under oxidative stress-induced cell senescence)⁴², SMURF2 (to inhibit proliferation and tumor formation)⁴³, CUL4 (to promote autophagy)⁴⁴, and COP1 (to exacerbate lipid toxicity)⁴⁵. Our results provide the first evidence that GADD45 β regulates SIRT1 ubiquitination. GADD45 β attenuated the PA-induced SIRT1 ubiquitination, thereby preserving SIRT1 protein expression and preventing proteasomal degradation. This novel mechanism highlights GADD45 β as a stabilizer of SIRT1, extending its role beyond transcriptional and metabolic regulation. Although our study revealed that GADD45 β bound to SIRT1 and influenced its ubiquitination, GADD45 β itself is not a ubiquitin ligase. Previous studies suggest that the association of adaptor proteins can provide additional layers of regulation to ubiquitin ligase activity⁴⁶. Ubiquitin ligases often function as part of complexes that include substrate receptors or adaptor proteins, which guide substrate recognition and catalyze ubiquitination and subsequent degradation⁴⁷. Therefore, we speculate that GADD45 β may act as an adaptor protein, facilitating ubiquitin ligase-mediated regulation of SIRT1. Among known E3 ligases, MDM2, SMURF2, COP1, and CUL4B have been implicated in SIRT1 turnover under different stress and metabolic conditions, including oxidative stress, lipotoxicity, and tumorigenesis^{43,48–50}. These findings suggest that similar mechanisms may operate in hepatocytes, and that GADD45 β could modulate SIRT1 stability by interacting with one or more of these ligase complexes. Proteomic screening and targeted co-IP assays are necessary to determine which ligase mediates this effect in MASLD. Additionally, the specific ubiquitination site on SIRT1 that is affected by GADD45 β warrants further exploration.

Notably, the GADD45 family consists of three isoforms, GADD45 α , GADD45 β , and GADD45 γ , that are structurally homologous and share partially overlapping functions in stress responses⁵¹. GADD45 α activates AMPK to enhance fatty acid oxidation and energy expenditure while suppressing lipogenesis, protecting against acetaminophen- and diet-induced liver injury⁵². Its downregulation under ER stress or fibrosis promotes steatosis and inflammation⁵³. GADD45 β also suppresses hepatic lipogenesis by regulating the SIRT1/AMPK/SREBP1 axis, as shown in our study. Similarly, GADD45 γ is expressed in the liver and induced by stress, but its role in hepatic lipid metabolism remains undefined. Thus, GADD45 α and GADD45 β may exert related but mechanistically distinct effects in hepatic lipid metabolism.

Evidence also suggests that GADD45 β regulates multiple metabolic and pathological processes in the liver. It promotes gluconeogenesis through TET1-PGC-1 α demethylation, contributing to hyperglycemia in obesity and diabetes⁵⁴. Stabilization by HSP72 improves insulin resistance and reduces triglyceride accumulation in MASLD⁵⁵. GADD45 β also inhibits liver fibrosis by attenuating hepatocyte injury, inflammatory responses, and fibrogenic signaling pathways⁵⁶. Metformin protects against acetaminophen-induced hepatotoxicity via GADD45 β -dependent JNK inhibition⁵⁷. Since metformin improves insulin sensitivity and steatosis in MASLD⁵⁸, these findings support a protective role of GADD45 β in limiting inflammation, fibrosis, and progression to MASH.

This study has several limitations. First, palmitate-treated HepG2 cells model lipotoxic stress rather than classical de novo lipogenesis, and the relevance of our findings to glucose-mediated de novo lipogenesis requires further clarification. Second, the MCD model does not robustly activate de novo lipogenesis, and GADD45 β may influence lipid accumulation via alternative pathways, such as fatty acid uptake or oxidation, which were not functionally explored. Third, genetic rescue experiments targeting SREBP1 or ACC are necessary to establish the causal role of this pathway in mediating the GADD45 β knockdown phenotype, but could not be performed due to current resource constraints. Fourth, while GADD45 β expression was analyzed at the mRNA level using human GEO datasets, direct validation in clinical liver tissues at the protein level was not feasible, limiting translational strength. Lastly, as HepG2 cells are hepatocarcinoma-derived, their regulatory fidelity may not fully reflect that of normal hepatocytes. Future studies using human liver tissues, primary hepatocytes, or organoid models are warranted to validate the GADD45 β /SIRT1 axis under more physiologically relevant conditions.

In conclusion, this study underscores the important role of GADD45 β in modulating hepatic lipogenesis through its interaction with SIRT1. By stabilizing SIRT1 and preventing its ubiquitin–proteasome degradation, GADD45 β enhances SIRT1 activity and activates the SIRT1/AMPK signaling pathway, thereby suppressing lipogenesis. These findings provide novel insights into the molecular mechanisms of hepatic lipid metabolism and identify GADD45 β as a potential therapeutic target for the management of MAFLD. Given the increasing global burden of metabolic liver disorders, further exploration of GADD45 β function may support the development of targeted interventions to mitigate disease progression.

Data availability

RNA sequencing data were obtained from the GEO database (GSE33814, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33814> and GSE48452, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48452>). All other data generated or analyzed during this study are included in the published manuscript and the Supplementary information files.

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Author contributions

Yuanyuan Xiao, Renjie Wang, and Li Wei conceptualized the study. Yuanyuan Xiao, Renjie Wang, Jun Yin, and Li Wei designed the methodology. Yuanyuan Xiao, Renjie Wang, and Chaoyu Zhu performed validation. Yuanyuan Xiao, Renjie Wang, Chaoyu Zhu, Qianqian Wang, Xinyi Wang, Wenjing Song, Shouxia Li, and Fusong Jiang conducted the investigation and data collection. Yuanyuan Xiao and Renjie Wang drafted the manuscript. Jun Yin and Li Wei revised the manuscript. Yuanyuan Xiao and Renjie Wang prepared the figures and tables. Jun Yin and Li Wei supervised the project. Li Wei provided resources and administered the project. Yuanyuan Xiao, Chaoyu Zhu, Qianqian Wang, Xinyi Wang, and Li Wei acquired funding.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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