Received: 7 December 2016 | Accepted: 23 August 2017

DOI: 10.1111/liv.13581

WILEY Liver INTERNATIONAL

Hepatitis C virus core protein induces hepatic steatosis via Sirt1-dependent pathway

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Funding information
This work was supported by the strategic priority research program (XDB13030000 to WJ), Beijing Municipal Administration of Hospitals (DFL20151701, ZYLX201402 to JC), Beijing Natural Science Foundation (7161006 to JC), National Natural Science Foundation of China (81700508 to XY).

Handling Editor: Francesco Negro

Abstract

Background & Aims: Hepatic steatosis is a common feature of patients with chronic hepatitis C. Previous reports have shown that the overexpression of hepatitis C virus core-encoding sequences (hepatitis C virus genotypes 3a and 1b) significantly induces intracellular triglyceride accumulation. However, the underlying mechanism has not yet been revealed.

Methods: To investigate whether Sirt1 is involved in hepatitis C virus-mediated hepatic steatosis, the overexpression of hepatitis C virus core 1b protein and Sirt1 and the knockdown of Sirt1 in HepG2 cells were performed. To confirm the results of the cellular experiment liver-specific Sirt1 KO mice with lentivirus-mediated hepatitis C virus core 1b overexpression were studied.

Results: Our results show that hepatitis C virus core 1b protein overexpression led to the accumulation of triglycerides in HepG2 cells. Notably the expression of PPARγ2 was dramatically increased at both the mRNA and protein levels by hepatitis C virus core 1b overexpression. The protein expression of Sirt1 is an upstream regulator of PPARγ2 and was also significantly increased after core 1b overexpression. In addition, the overexpression or knockdown of Sirt1 expression alone was sufficient to modulate p300-mediated PPARγ2 deacetylation. In vivo studies showed that hepatitis C virus core protein 1b-induced hepatic steatosis was attenuated in liver-specific Sirt1 KO mice by downregulation of PPARγ2 expression.

Conclusions: Sirt1 mediates hepatitis C virus core protein 1b-induced hepatic steatosis by regulation of PPARγ2 expression.

KEYWORDS
deacetylation, hepatitis C virus, Sirt1, PPARγ2

1 | INTRODUCTION

Triglycerides (TGs), as the most reliable energy source for the body, participate in key metabolic functions such as thermal insulation and fatty acid deposition. Increased serum TG levels are involved in the development of hepatic steatosis.[1,2]

Chronic hepatitis C virus (HCV) infection is a common health problem and a leading cause of cirrhosis and hepatic carcinogenesis.

Abbreviations: CHX, cycloheximide; cKO, conditional knockout; CPT1, Carnitine O-palmitoyltransferase 1; DMEM, Dulbecco's modified eagle medium; GTT, glucose tolerance test; H&E, hematoxylin and eosin; HFD, high-fat diet; HRP, horseradish peroxidase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARγ2, peroxisome proliferator-activated receptor gamma 2; qPCR, quantitative real-time PCR; Sirt1, NAD-dependent deacetylase sirtuin-1; WT, wild type; HCV G1b, hepatitis C virus genotype 1b.

Chuanhai Zhang, Jingjing Wang and Hanlin Zhang are contributed equally to this work.

HCV infection alters lipid metabolism and leads to dyslipidemia, hepatic steatosis and advanced fibrosis.[3] Persistent viral infections tend to induce liver dysfunction and may further result in cirrhosis and hepatocellular carcinoma.[4,5] Hepatic steatosis is one of the primary aetiologies of induced liver diseases. Compared to other chronic liver diseases, the occurrence of hepatic steatosis is prevalent in patients with chronic hepatitis C.[6] The core protein of HCV localizes to the surface of lipid droplets and contributes to hepatic steatosis by interfering with intracellular lipid metabolism.[7] The overexpression of HCV core genes in HEPG2 cells leads to the accumulation of lipid droplets.[8] The core protein also induces hepatic steatosis in HCV transgenic mice.[3] This suggests that the core protein plays a critical role in the development of hepatic steatosis. However, the precise mechanism of hepatic steatosis caused by HCV remains unclear.

The mammalian genome consists of seven sirtuins, collectively referred to as Sirt1 to Sirt7,[9] where Sirt1 is a nuclear NAD+-dependent protein deacetylase[10] that plays an important role in regulating transcription in various key metabolites. Many studies have shown that Sirt1 is deacetylate for many non-histones, including PPARγ,[11] SREBP1,[12] p53,[13-15] NFκB,[16] FOXO transcription factors,[17,18] PGC-1α,[19] LXR,[20] CLOCK[21] and TORC2 through inhibit or activate the transcriptional activity of its target genes, thereby involving different metabolic and stress pathways. Numerous reports have demonstrated that Sirt1 increases PGC-1α activity by deacetylation, thereby enhancing liver gluconeogenesis, fatty acid oxidation and mitochondrial functions.[22-24] In addition, it has been shown that Sirt1 affects lipid metabolism by regulating the PPARα/PGC-1α signalling pathway and the LKB1/AMPK signalling pathway.[25,26] PPARγ (peroxisome proliferative activated receptor γ) is modulated by Sirtuins to inhibit adipogenesis,[27] and Sirt1 affects the differentiation of muscle and neuronal stem cells.[28] PGC-1α (proliferator-activated receptor gammacoactivator-1α) is a known target of Sirt1-dependent deacetylation[19,29] and this coactivator also plays a fundamental role in regulating fatty acid oxidation and gluconeogenesis pathways in the liver. Thus, there might be a significant role for Sirt1 in the regulation of PPARγ2 and PGC-1α activity during fat mobilization and fatty acid oxidation.[22,27] However, the role of Sirt1 in hepatic steatosis occurring with chronic hepatitis C remains unclear. Given the several common features between hepatic lipid mobilization and hepatic steatosis in chronic hepatitis C, we sought to investigate whether Sirt1 possibly plays an important role in the development of hepatic steatosis with chronic hepatitis C.

Key points

- HCV core protein 1b overexpression led to increases in PPARγ2 expression
- HCV core protein 1b increases Sirt1-dependent PPARγ2 deacetylation
- HCV core protein 1b-induced Sirt1 protein is an upstream regulator of PPARγ2.
- HCV core protein 1b-induced hepatic steatosis was attenuated in liver-specific Sirt1 KO mice by downregulation of PPARγ2 expression.

2 | MATERIALS AND METHODS

2.1 | Animal experiments

The Sirt1 allele with a floxed exon4 (Cheng et al, 2003)[30] was backcrossed five times onto the C57BL/6 background and then bred with albumin-cre mice, which express Cre recombinase driven by the albumin promoter (Jackson Laboratory, Bar Harbor, ME, USA). Liver-specific Sirt1 knockout mice (cKO) were generated in the F1 generation and then expanded by repeated breeding. cKO mice and their age-matched littermate controls ($Cre^{-/-}$, Sirt1 flox/flox) older than 6 weeks of age were injected with HCV core 1b or empty control lentivirus via the tail vein and were fed ad libitum with either a standard laboratory chow diet or a high-fat diet (D12492i) for 15 weeks. Mice were sacrificed under mild ether anaesthesia. All animal studies were approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

2.2 | Cell culture

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) (Gibco, Life Technologies, Carlsbad, CA, USA), 100 units/mL penicillin and 100 mg/mL streptomycin (Gibco, Life Technologies) at 37°C in a humid atmosphere containing 5% CO_2.

2.3 | Lentivirus package and transfection

HEK293FT cells (Sci-Tech, Shanghai, China) were used to generate the lentivirus package. The cells were maintained in basic DMEM high glucose, supplemented with 10% FBS, 1× antibiotic/antimycotic solution and 10 μmol/L nonessential amino acid. The coding region of the HCV core 1b sequence was amplified from a plasmatic cDNA sample of HCV core 1b derived from a patient and was cloned into the pCDH-CMV-MCS-EF1-copGFP Lentivector (System Biosciences, San Francisco, CA, USA).[18] The constructed recombinant shuttle plasmid pCDH-CMV-MCS-EF1-copGFP and lentivirus helper plasmid were cotransfected into HEK293FT cells to produce virus. Forty-eight hours after transient transfection, the fresh lentivirus-containing supernatant was harvested for future use. Titre of lentiviral particles used for cell transduction and mice infection is 10^8 TU (transducing units) per millilitre and the dose for cell transduction was given 10 μL per 10^6 cells or mice was given 100 μL per mouse.

2.4 | Intracellular or liver total triglyceride assessments

HepG2 cells were collected after 48 hours of lentivirus transfection. For total liver triglyceride assessments, approximately 0.5 g

of liver tissue was collected after the mice were sacrificed. The triglyceride (TG) contents were assayed with the Adipogenesis Assay Kit (Sigma-Aldrich, St. Louis, MO, USA), according to the manufacturers' instructions and was normalized to total protein concentrations.

2.5 | Glucose tolerance test

Mice were fasted for 16 hours (17:00-09:00) with free access to drinking water and were then injected with D-glucose (2 g/kg for chow diet mice and 1.5 g/kg for high-fat diet mice) intraperitoneally. Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 minutes after the ip glucose injection by using an Accu-Chek glucose monitor (Roche Diagnostics Corp., Pleasanton, CA, USA).

2.6 | Total fat distribution

All mice were anaesthetized with an intra-abdominal injection of Avertin, and whole body fat mass was measured using non-radiotracer computerized tomography (MesoQMR23-060H-I; Nuimag Corp., Shanghai, China).

2.7 | Histological analysis

To detect neutral lipids in cells, HepG2 cells were fixed overnight in 4% paraformaldehyde after 48 hours of transfection with HCV core 1b or control vector. Then, cells were stained with 0.2% (w/v) Oil-Red O (Sigma-Aldrich) for 10 minutes at room temperature. For hepatic histopathological analyses, Oil Red O staining was performed on frozen liver sections to detect the presence of fat. Liver tissues were fixed with 4% paraformaldehyde (PFA) overnight and sliced into 20 μm sections. Then, the sections were washed in PBS solution for 1 minute followed by 60% isopropyl alcohol for 2 minutes, and then stained with oil red O for 10 minutes at 37°C. Then, slices were submerged in 60% isopropyl alcohol for 2 minutes, washed with PBS solution for 2 minutes, and then stained with hematoxylin for 5 minutes. After a final 2 minutes wash in PBS solution a cover slip was mounted.

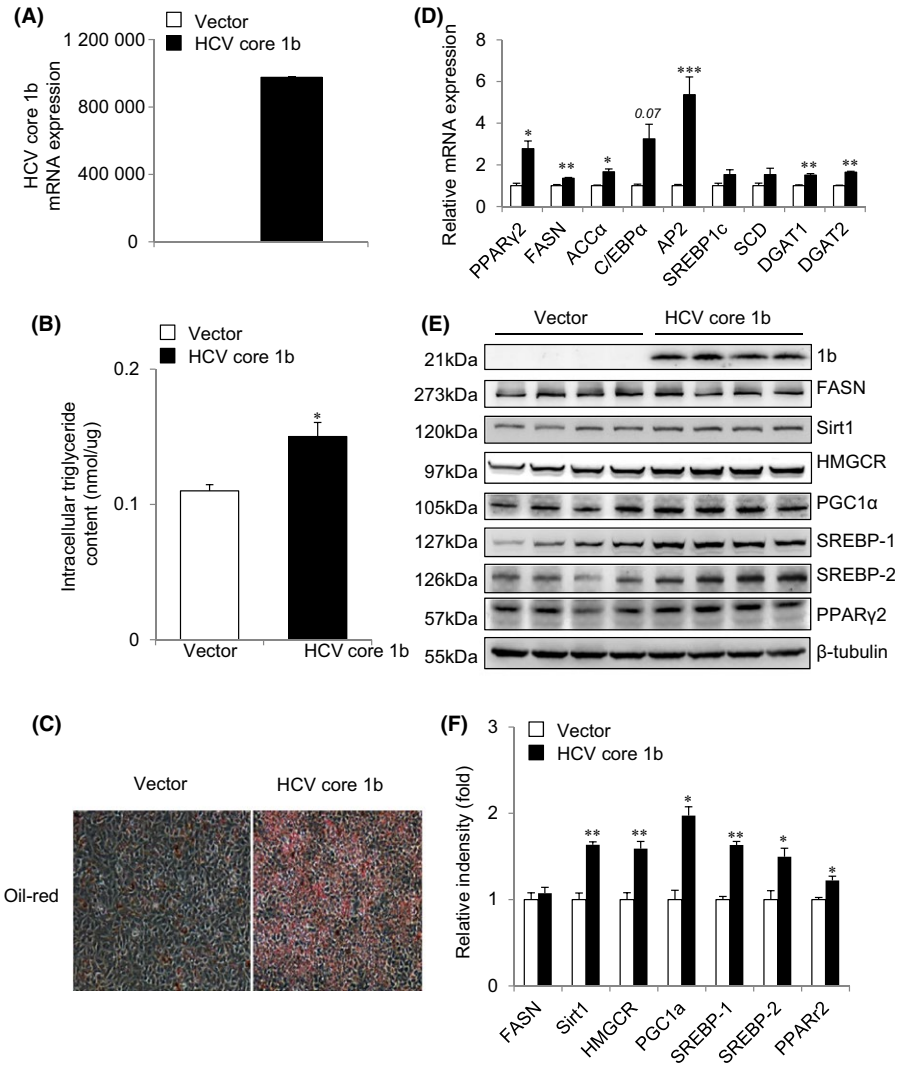


FIGURE 1 Hepatitis C virus (HCV) core protein 1b overexpression led to increases in PPARγ2 expression. Overexpression of HCV core protein 1b (A) induced significant triglyceride accumulation (B,C) and expression of genes (D) and proteins (E,F) that are involved in fatty acid oxidation and lipogenesis in the HepG2 cells. (Data are shown as the mean ± SEM, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$)

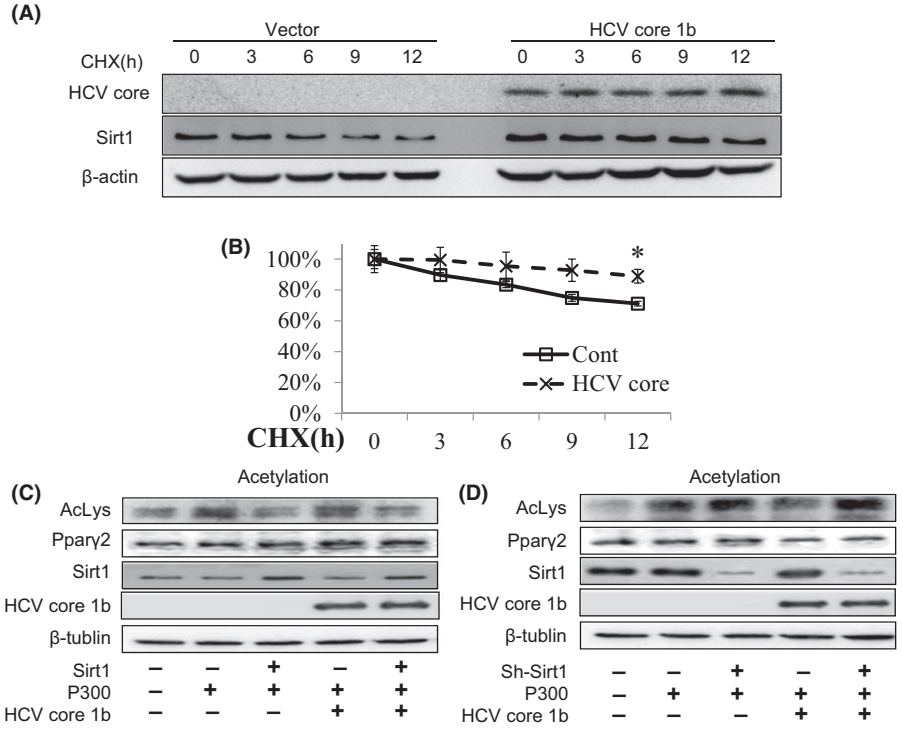


FIGURE 2 Hepatitis C virus (HCV) core protein 1b increases Sirt1-dependent PPARγ2 deacetylation. Sirt1 protein stability was significantly lengthened with HCV core 1b overexpression in the presence of cycloheximide (CHX) (A,B). Overexpression or knockdown of Sirt1 expression modulates p300-mediated PPARγ2 deacetylation (C,D). (Data are shown as the mean ± SEM, n = 3, **P* < .05)

2.8 | Blood analysis

The blood samples were collected by cardiac exsanguination under Avertin anaesthesia, and the plasma samples were frozen and stored at −80°C until further analysis.

2.9 | Gene expression analysis

Total RNA was isolated using the RNeasy Mini Kit. cDNA was synthesized using random hexamers (Invitrogen, Carlsbad, CA, USA) for subsequent real-time quantitative PCR analysis (with a Prism VIIA7; Applied Biosystems Inc, Foster City, CA, USA). PCR products were detected using Sybr Green and results were normalized to cyclophilin expression. Primers were designed using Primer Quest (Integrated DNA Technologies Inc, Coralville, IA, USA). Primer sequences are available in Table S2.

2.10 | Western blot analysis

Cell and tissue lysates were prepared using RIPA buffer (150 mmol/L sodium chloride, 1.0% TritonX-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mmol/L Tris and a protease and phosphatase inhibitor cocktail; Roche Diagnostics Corp.). Protein concentrations were measured with a BCA assay kit (Pierce Diagnostics Corp, Pleasanton, CA, USA). Proteins were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, MA, USA). Membranes were blocked in 5% skim milk in TBST (0.02 mol/L Tris base, 0.14 mol/L NaCl, 0.1% Tween 20, pH 7.4) followed by incubated with primary antibodies overnight at 4°C and then with secondary antibodies conjugated with HRP. The following primary antibodies were used: Anti-Hepatitis C Virus Core 1b (Mouse monoclonal; Abcam, Cambridge, UK), anti-FASN (Rabbit monoclonal; Abcam), anti-Sirt1 (Mouse monoclonal; CST, Danvers, MA, USA), anti-HMGCR (Rabbit monoclonal; Abcam), anti-SREBP1 (Rabbit monoclonal; Abcam), anti-SREBP2 (Rabbit monoclonal; Abcam), PPARγ2 (Rabbit monoclonal; CST), Anti-PGC1α (Rabbit polyclonal; Abcam), anti-ACC (Rabbit monoclonal; CST) anti-β-actin (mouse monoclonal; Sigma-Aldrich) and anti-β-Tubulin (Rabbit monoclonal; CST). Signals were detected with super signal west pico chemiluminescent substrate (Pierce).

2.11 | Co-immunoprecipitation studies

HepG2 cells were transfected with HCV core 1b vector. Cell lysates were collected in RIPA buffer 48 hours after transfection. HCV core 1b protein was immunoprecipitated using antihepatitis C virus core 1b antibody (Mouse monoclonal; Abcam) and then detected with using antihepatitis C virus core 1b antibody (Mouse monoclonal; Abcam) and anti-Sirt1 antibody (Mouse monoclonal, CST).

2.12 | Immunoprecipitation and PPARγ2 acetylation

HepG2 cells were transfected with pCMV-HA-Sirt1 (0.5 μg), sh-Sirt1 (PLKO.1-puro) (0.5 μg) or pECE-SV40-Flag-p300 (0.5 μg) with or without pCDH-CMV-HCV G1b vector. The sequence of the HCV core 1b protein is as follows: MSTNPKPQRKTKRNTNRRPQDVKFPGGG QIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIPKARRPE GRAWAQPGYPWPLYGNEGLGW. Twenty-four hours after transfection, cells were transfected, and then cell lysates were collected with IP buffer (50 mmol/L HEPES [pH 7.9], 150 mmol/L NaCl, 10% glycerol, 1 mmol/L EGTA, 1% Triton X-100, 0.5% NP-40, 1 μmol/L trichostatin A and protease inhibitor cocktail [Roche, Basel, Switzerland]), followed by incubation with PPARγ2 antibody overnight at 4°C. Immunoprecipitates

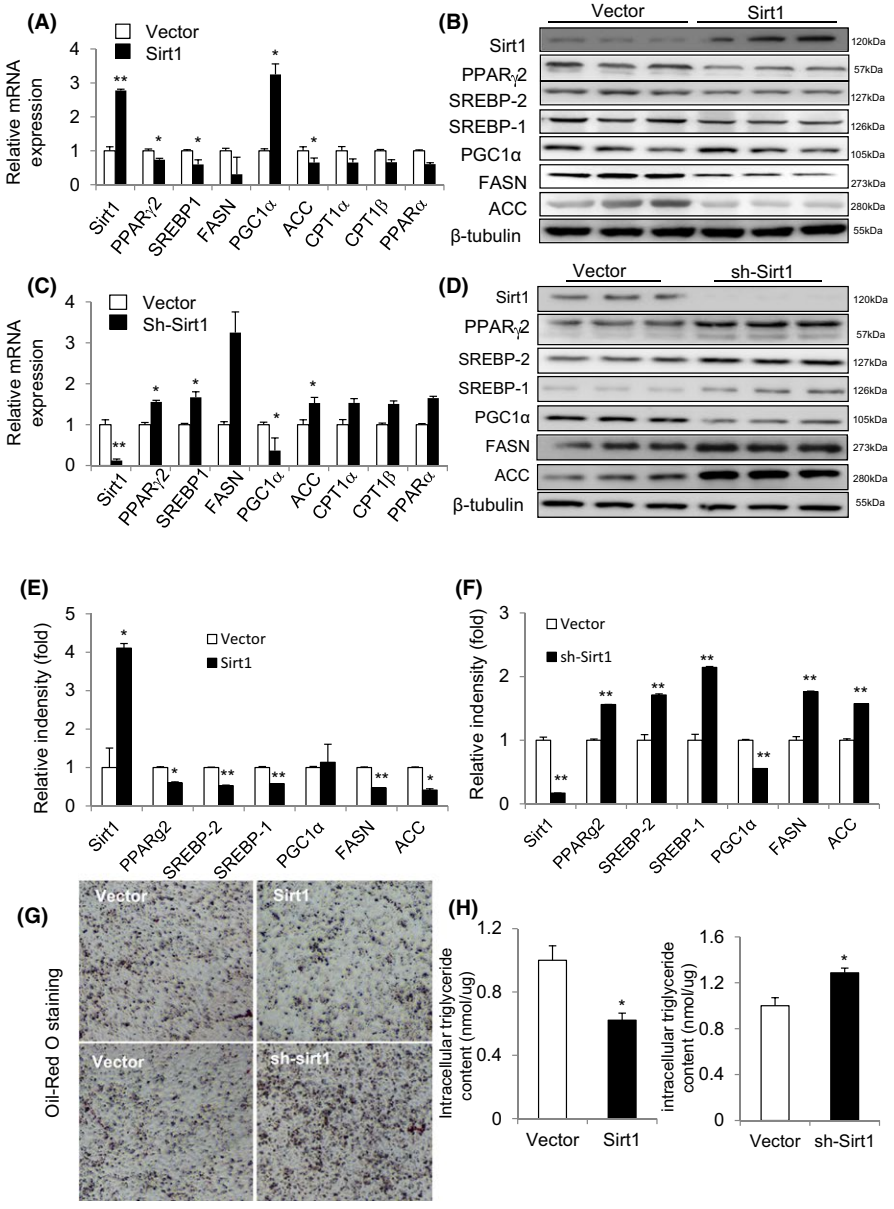


FIGURE 3 Sirt1 regulates PPARγ2 expression. Overexpression of Sirt1 decreases but knockdown of Sirt1 increases both mRNA and protein expression of PPARγ2 (A-D). Gene and protein involved in lipid metabolism, such as SREBP1, SREBP2, PGC1α, FASN and ACC, also increased or decreased by overexpress or knockdown Sirt1 (A-D). Quantification of the blots of three independent experiments by relative intensity (E,F). Quantify the steatosis by oil-red-O standing and detect the dosage of triglycerides in conditions of either overexpression or silencing of Sirt1 (G and H) (Data are shown as the mean ± SEM, n = 3, $^{*}P < .05$, $^{**}P < .01$)

were analysed by western blot using antibodies against HCV core 1b, Sirt1, PPARγ2 and acetyl-lysine (Millipore).

2.13 | Statistical analyses

Comparisons between groups were made by one-way ANOVA; follow-up comparisons were made using Tukey's post hoc test or Student's *t* tests. A difference between groups of $P < .05$ was considered significant.

3 | RESULTS

3.1 | Hepatitis C virus core protein 1b overexpression led to increases in PPARγ2 expression

It is well known that hepatic steatosis is a common feature of patients with chronic hepatitis C infection. Previous reports have shown that the overexpression of HCV core-encoding sequences (genotypes 3a and 1b) significantly induce intracellular triglyceride accumulation.[31] However, the precise molecular mechanisms have not yet been revealed. To investigate possible mechanisms, we first successfully overexpressed core protein 1b in HEPG2 cells (Figure 1A). We also confirmed significant TG accumulation after core 1b overexpression using a TG content assay (Figure 1B) and Oil-red-O staining (Figure 1C). In line with these observations, fatty acid metabolism-related mRNA and protein expression were significantly increased after core 1b overexpression (Figure 1D-F). Notably, among these genes, the key adipogenic transcription factor PPARγ2 was significantly upregulated at both the mRNA and protein levels (Figure 1D-F). As a regulator of PPARγ2, Sirt1 protein expression was significantly increased after core 1b overexpression, which was consistent with our previous findings.[32] These results indicate that core 1b overexpression led to increases in TG accumulation via the upregulation of fatty acid metabolism-related protein expression.

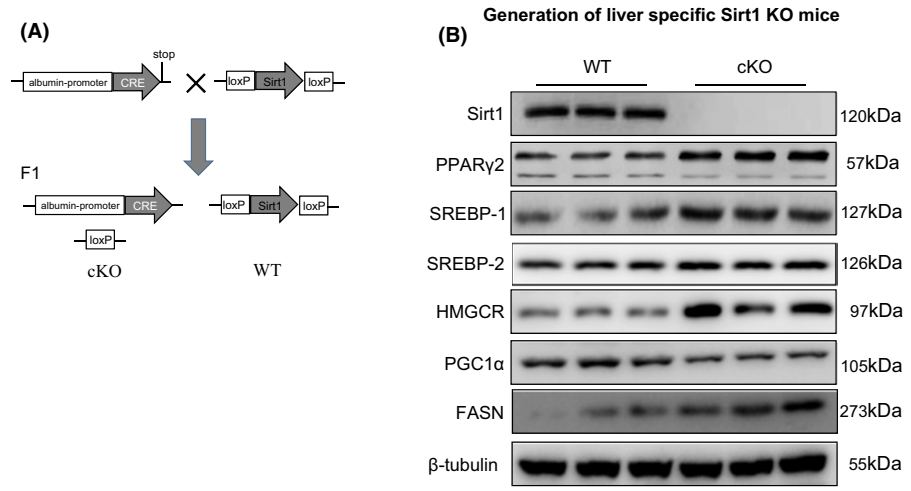


FIGURE 4 Generation of liver-specific Sirt1 KO mice. Liver-specific Sirt1 KO mice conditional knockout (cKO) was generated by crossing Sirt1-flox mice to liver-specific albumin-CRE mice (A). Western blot analysis confirmed the specific ablation of Sirt1 protein expression in the liver (B)

3.2 | Hepatitis C virus core protein 1b increases Sirt1-dependent PPARγ2 deacetylation

Sirt1 deacetylases PPARγ2 at Lys268 and Lys293, thereby controlling the balance from energy storage to energy expenditure.[11] We first asked whether HCV core 1b has any effect on Sirt1 protein stability. With empty vector treatment, Sirt1 protein degraded after 12 hours in the presence of cycloheximide (CHX), a eukaryote protein synthesis inhibitor. However, HCV core 1b overexpression significantly delayed Sirt1 protein degradation, even in the presence of CHX (Figure 2A,B). Next, we investigated whether HCV core 1b participates in Sirt1-dependent PPARγ2 deacetylation. Consistent with a previous report,[11] the overexpression or knockdown of Sirt1 expression alone was sufficient to modulate p300-mediated PPARγ2 deacetylation (Figure 2C,D). Interestingly, HCV core 1b protein overexpression further enhanced Sirt1-mediated PPARγ2 deacetylation (Figure 2C,D). These results imply that HCV core 1b protein directly participates in Sirt1-mediated PPARγ2 deacetylation. Next, we asked whether Sirt1 had any effect on PPARγ2 expression. Sirt1 was overexpressed and/or knocked-down in HEPG2 cells. The results showed that overexpression Sirt1 decreases but knockdown Sirt1 increases both mRNA and protein expression of PPARγ2 (Figure 3A-D). These results indicate that Sirt1 regulates PPARγ2 expression. Both gene and protein involved in lipid metabolism, such as SREBP1, SREBP2, PGC1α, FASN and ACC, also increased or decreased by overexpression or knockdown of Sirt1 (Figure 3A-D). In addition, we evaluated the steatosis by oil-red-O standing and measured the level of triglycerides in conditions of either overexpression or silencing of Sirt1 (Figure 3G,H). These results clearly show that Sirt1 is involved in the regulation of TG metabolism in HepG2 cells.

3.3 | Generation of liver-specific Sirt1 KO mice

The results of the cellular experiment suggested that HCV core 1b increases hepatic TG accumulation via increasing Sirt1-mediated PPARγ2 expression. To further investigate this important point in vivo, we generated liver-specific Sirt1 KO mice (cKO) by crossing Sirt1-flox mice with liver-specific albumin-CRE mice (Figure 4A), and KO efficiency was confirmed by the complete loss of Sirt1 protein expression (Figure 4B). Notably, we found that PPARγ2 expression was significantly upregulated in the livers of cKO mice compared to WT mice (Figure 4B), which was consistent with the results of the above cellular experiment (Figure 3A-D).

3.4 | Hepatitis C virus core protein 1b-induced hepatic steatosis is attenuated in liver-specific Sirt1 KO mice

Accumulating evidence indicates that injecting lentivirus through the mouse tail vein can effectively deliver lentivirus to the liver. Thus, this method is frequently used for the liver-specific delivery of genes. As anticipated, HCV core 1b lentivirus injection led to the liver-specific overexpression of core 1b protein (Figure 5A). We perform the immunostaining of HCV core protein on liver sections and check the expression of HCV core1b protein in different tissues after HCV core1b lentiviral transduction (Fig. S1). The result of immunostaining showed that more than 80% liver cells were infected with HCV lentivirus (Fig. S1A). Western blot shows that HCV core1b is overexpressed highly in the liver and other tissues such as BAT and kidney are slightly expressed, but are negligible compared to the expression in the liver (Fig. S1B).

In addition, PPARγ2 expression was induced by HCV core 1b lentivirus administration, whereas it was diminished in cKO mice fed either a normal chow diet or a high-fat diet (HFD) (Figures 5A and S2A). These results further imply that Sirt1-mediated PPARγ2 expression plays an important role in HCV genotype 1b-induced hepatic steatosis. Importantly, body weight (Figures 5B and S2B), total body fat content (Figures 5C and S2C), liver weight (Figures 5D and S2D) and liver triglyceride (TG) content (Figures 5E,F and S2E,F) were significantly increased in cKO mice compared to WT mice that were injected with empty vector lentivirus regardless of diet condition (normal chow diet and high-fat diet). These results suggest that cKO mice are prone to

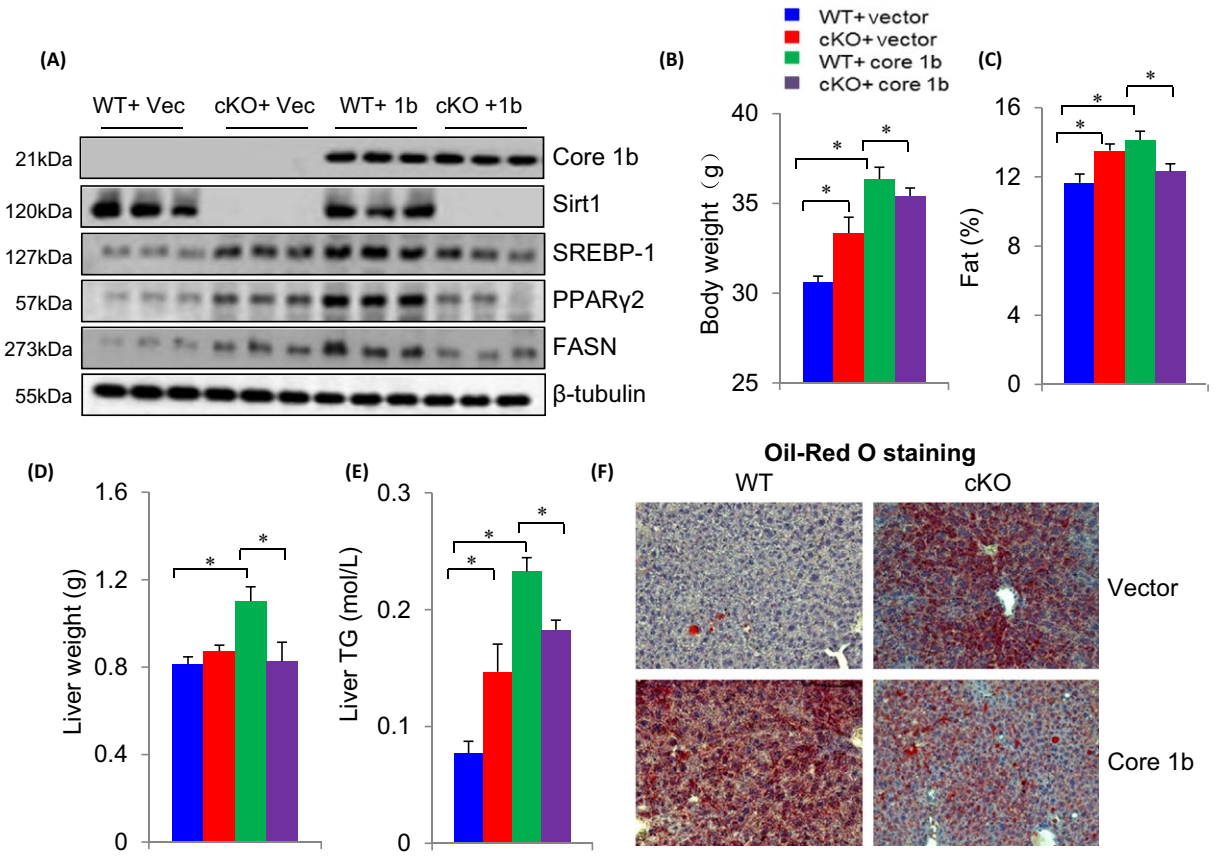


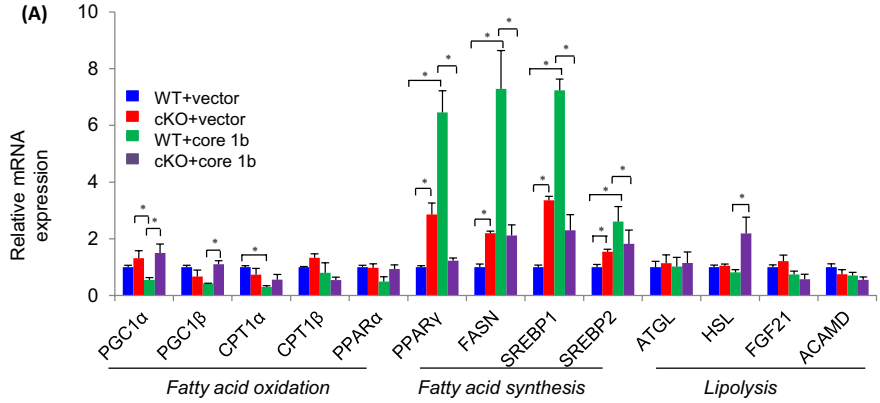
FIGURE 5 Hepatitis C virus (HCV) core protein 1b-induced hepatic steatosis is attenuated in liver-specific Sirt1 KO mice. HCV core 1b lentivirus injection led to liver-specific overexpression of HCV 1b protein (A). PPARγ2 and fatty acid metabolism-related protein expression were diminished in conditional knockout (cKO) mice with HCV core 1b lentivirus (Cko + Core 1b) compared to wild-type (WT) mice with HCV core 1b lentivirus injection (WT + Core 1b) (A). Body weight (B), total fat mass (C), liver weight (D) and liver TG content (E,F) were significantly reduced in cKO mice with HCV core 1b lentivirus (Cko + Core 1b) compared to WT mice with the lentivirus injection (WT + Core 1b). (Data are shown as the mean ± SEM, n = 3, $^*P < .05$)

develop to obesity related to hepatic steatosis. This was further supported by results showing the upregulation of PPARγ2 expression in cKO livers (Figures 5A, 6A,G and S2A,G). Liver adiposity and hepatic steatosis were further aggravated by HCV core 1b lentivirus injection in WT mice, whereas they were largely ameliorated in cKO mice (Figures 5B-F and S2B-F). Alterations in adiposity often affect glucose homeostasis. We next investigated glucose homeostasis using a intraperitoneal glucose tolerance test (GTT). Our data demonstrated that HCV core 1b lentivirus injection led to marginal glucose intolerance in WT mice but not in cKO mice (Figures 6B,C and S2H,I). Consistently, plasma lipid profiles further support that Sirt1 KO ameliorated HCV core 1b-induced dyslipidemia (Tables 1 and S1). Taken together, these results highlight that HCV core protein 1b-induced hepatic steatosis was attenuated in liver-specific Sirt1 KO mice via the downregulation of PPARγ2 expression.

4 | DISCUSSION

Chronic HCV infection is a risk factor for the development of hepatic steatosis and hepatocellular carcinoma.[33] However, the exact molecular pathogenesis of chronic HCV infection-mediated hepatic steatosis is still unclear. In this study, we demonstrated that HCV core protein-induced Sirt1 expression governs PPARγ2 expression by modulating PPARγ deacetylation. Furthermore, HCV core protein 1b-induced hepatic steatosis is attenuated in liver-specific Sirt1 KO mice via the downregulation of PPARγ2 expression. To our knowledge, this is the first study to show that the Sirt1-PPARγ2 pathway is a potential target for the treatment of HCV-mediated hepatic steatosis.

Several studies have revealed the correlation of Sirt1 and hepatic metabolic disorders induced by hepatitis C virus core protein.[32,34-36] Consistent with these studies, we found that overexpression of HCV 1b upregulates protein levels of Sirt1, a regulator of PPARγ2 in HepG2 cells (Figure 1D-F). In line with these observations, we found that HCV core 1b overexpression led to a significant augmentation of both mRNA and protein levels of PPARγ2 (Figure 1D-F). In parallel, core 1b overexpression led to increased TG accumulation. These findings highlight that the HCV-Sirt1-PPARγ2 axis plays an important role in hepatic TG accumulation. It has been proposed that Sirt1 controls energy balance by deacetylation of PPARγ2.[11] Accordingly, we found that HCV core 1b significantly increases Sirt1 expression by enhancing Sirt1 protein stability (Figure 2A,B). In addition, Sirt1-mediated



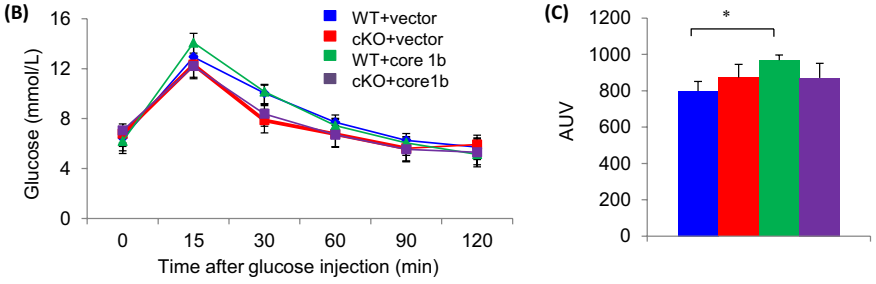


FIGURE 6 Hepatitis C virus (HCV) core protein 1b-induced glucose intolerance is improved in liver-specific Sirt1 KO mice. Fatty acid synthesis-related gene expression showed a similar pattern (A). HCV core 1b lentivirus injection led to glucose intolerance in wild-type mice but not in conditional knockout mice (B,C). (Data are shown as the mean ± SEM, n = 3, **P* < .05)

TABLE 1 Plasma profiles

	CHO (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	FFA (mmol/L)
WT + Vector	1.96 ± 0.14	1 ± 0.091	1.51 ± 0.1037	0.21 ± 0.03	0.39 ± 0.10
KO + Vector	3.5 ± 0.21*	1.1 ± 0.18	3.32 ± 0.2311*	0.26 ± 0.04	0.61 ± 0.17
WT + 1b	2.86 ± 0.19*	1.1 ± 0.13	2.38 ± 0.2257*	0.37 ± 0.03	0.57 ± 0.20
KO + 1b	3.43 ± 0.49*	1.3 ± 0.21	2.71 ± 0.2618*	0.427 ± 0.17	0.41 ± 0.19*,**

Plasma profiles were measured at the end of the experiment.

*Indicates a significant difference compared with the WT + Vector group and **indicates a significant difference compared with the WT + 1b group. (Data are shown as the mean ± SEM, n = 7-8/group, * or ***P* < .05.)

PPARγ2 deacetylation was further enhanced by core 1b overexpression (Figure 2D,E). This is the first time to link HCV 1b, PPARγ2 and Sirt1 to explain the possible mechanism of occurrence in hepatitis C-induced hepatic steatosis and clearly show their interaction. However, the molecular mechanism of hepatitis C-induced hepatic steatosis is complex and need more detailed research.

To understand the role of Sirt1 in liver cells, we carried out plasmid intervention on the expression of Sirt1 in HepG2 cells. We found that the overexpression of Sirt1 decreases but the knockdown of Sirt1 increases both mRNA and protein expression of PPARγ2 (Figure 3A-D). These results indicate that Sirt1 partly governs PPARγ2 expression. In addition, the expression of a series of genes and proteins involved in lipid metabolism, such as SREBP1, SREBP2, PGC1α, FASN and ACC, is correspondingly upregulated or downregulated according to overexpression or knockdown of Sirt1. The steatosis and TG were evaluated in HepG2 cells in conditions of either overexpression or silencing of SIRT1 (Figure 3G,H). Our results further illustrate the relationship between Sirt1 and lipid metabolism in liver cells, which laid a foundation for our further study of how Sirt1 participates in the regulation of HCV 1b-induced hepatic steatosis. Importantly, we show here that HCV core protein 1b-induced hepatic steatosis is attenuated in liver-specific Sirt1 KO mice by downregulation of PPARγ2 expression. However, the method of lentivirus via tail vein injection for the overexpression of HCV core 1b has some shortcomings, which cannot rule out the overexpression of 1b in other tissues and the impact of lentivirus on other organizations. But we checked the expression of HCV core1b protein in different tissues after HCV core1b lentiviral transduction by western blot. The results show that HCV core1b is overexpressed highly in the liver and other tissues are slightly or none expressed which is negligible compared to liver. So, we are pleased with this situation, but more in-depth and accurate method needed in the future research.

In addition to hepatic steatosis, several studies have reported the correlation of hepatitis C virus and diabetes mellitus.[37-39] As we all know, obesity and lipid metabolism disorders often affect

glucose homeostasis. We investigated glucose homeostasis via GTT (Figures 6B,C and S2H,I). Our data show that HCV core 1b led to marginal glucose intolerance in WT mice but not in cKO mice. There is still no direct evidence that there is a definite relationship between HCV and diabetes mellitus, but the crossover of hepatitis C and diabetes is worthy of attention and further study. In addition, the absence of Sirt1 in the liver did not or very slightly affect the body glucose homeostasis (Figures 6B,C and S2H,I). It has been established that Sirt1 is involved in numerous biological functions, at both the genetic and cellular levels. Small molecules that modulate Sirt1 have been used in multiple preclinical studies, notably for the treatment of cardiovascular diseases and tumors.[40,41] Because Sirt1 is involved in a very complex biological network, scientists are bringing their attention to develop Sirt1 activators and/or inhibitors to achieve the desired therapeutic purposes. In this regards, development of a liver-specific Sirt1 inhibitor might be a new therapeutic option for hepatitis C-mediated hepatic steatosis.

In summary, we have shown that hepatic Sirt1 plays an important role in the regulation of lipid homeostasis in response to HCV core 1b lentivirus injection. Our findings provide a direct link between SIRT1 and hepatic steatosis with HCV G1b infection. Our study shows that Sirt1 is involved in the hepatitis C-induced hepatic diseases. Therefore, modulation of Sirt1 activity might be another option to improve hepatitis C-induced hepatic diseases.

CONFLICT OF INTEREST

The authors disclose no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Zhang C, Wang J, Zhang H, et al. Hepatitis C virus core protein induces hepatic steatosis via Sirt1-dependent pathway. *Liver Int*. 2018;38:803–812. https://doi.org/10.1111/liv.13581