Published in final edited form as:

Free Radic Biol Med. 2017 December ; 113: 71–83. doi:10.1016/j.freeradbiomed.2017.09.016.

Inhibition of hepatocyte nuclear factor 1b induces hepatic steatosis through DPP4/NOX1-mediated regulation of superoxide

Zi Long^{a,1}, Meng Cao^{a,1}, Shuhao Su^a, Guangyuan Wu^a, Fansen Meng^a, Hao Wu^a, Jiangzheng Liu^a, Weihua Yu^a, Kamran Atabai^{b,c}, and Xin Wang^{a,*}

^aDepartment of Toxicology, Shaanxi Key Lab of Free Radical Biology and Medicine, the Ministry of Education Key Lab of Hazard Assessment and Control in Special Operational Environment, School of Public Health, Fourth Military Medical University, Xi'an 710032, China

^bCardiovascular Research Institute, University of California, San Francisco, United States

^cDepartment of Medicine, University of California, San Francisco, United States

Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder that is closely associated with insulin resistance and type 2 diabetes. Previous studies have suggested that hepatocyte nuclear factor 1b (HNF1b) ameliorates insulin resistance. However, the role of HNF1b in the regulation of lipid metabolism and hepatic steatosis remains poorly understood. We found that HNF1b expression was decreased in steatotic livers. We injected mice with lentivirus (LV) expressing HNF1b shRNA to generate mice with hepatic knockdown of HNF1b. We also injected high fat (HF) diet-induced obese and db/db diabetic mice with LV expressing HNF1b to overexpress HNF1b. Knockdown of HNF1b increased hepatic lipid contents and induced insulin resistance in mice and in hepatocytes. Knockdown of HNF1b worsened HF diet-induced increases in hepatic lipid contents, liver injury and insulin resistance in mice and PA-induced lipid accumulation and impaired insulin signaling in hepatocytes. Moreover, overexpression of HNF1b alleviated HF diet-induced increases in hepatic lipid content and insulin resistance in mice. Knockdown of HNF1b increased expression of genes associated with lipogenesis and endoplasmic reticulum (ER) stress. DPP4 and NOX1 expression was increased by knockdown of HNF1b and HNF1b directly bound with the promoters of DPP4 and NOX1. Overexpression of DPP4 or NOX1 was associated with an increase in lipid droplets in hepatocytes and decreased expression of DPP4 or NOX1 suppressed the effects of knockdown of HNF1b knockdown on triglyceride (TG) formation and insulin signaling. Knockdown of HNF1b increased superoxide level and decreased glutathione content, which was inhibited by downregulation of DPP4 and NOX1. N-acetylcysteine (NAC) suppressed HNF1b knockdown-induced ER stress, TG formation and insulin resistance. Palmitic acid (PA) decreased HNF1b expression which was inhibited by NAC. Taken together, these studies demonstrate that HNF1b plays an essential role in controlling

*Correspondence to: Department of Toxicology, School of Public Health, Fourth Military Medical University, Changle West Road 169, Xi'an 710032, China. xinwang@fmmu.edu.cn (X. Wang).

¹Coauthors.

Disclosure statement

The authors declared no conflict of interest.

hepatic TG homeostasis and insulin sensitivity by regulating DPP4/NOX1 mediated generation of superoxide.

Keywords

Hepatocyte nuclear factor 1b; Nonalcoholic fatty liver disease; Lipogenesis; Endoplasmic reticulum stress; Dipeptidyl peptidase 4; Nicotinamide adenine dinucleotide phosphate oxidase 1; Superoxide

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder worldwide [1]. It is estimated that NAFLD accounts for up to 20% of the total population in the United States and 15% in China [2]. 10–15% of NAFLD patients have nonalcoholic steatohepatitis (NASH), which can progress to liver cirrhosis and hepatocellular carcinoma [3]. NAFLD is characterized by excessive fat accumulation in hepatocytes, mainly in the form of triglycerides (TGs) [4]. Uncontrolled lipogenesis contributes to development of NAFLD under several pathophysiological conditions, including diabetes, obesity, and insulin resistance [4–6]. Disorders of hepatic lipid metabolism are closely associated with NAFLD. However, the mechanisms underlying the pathogenesis of NAFLD are incompletely understood and effective preventive and therapeutic strategies are lacking.

Hepatocyte nuclear factor 1b (HNF1b), also named as vHNF1, HNF1 β , TCF2 and LF-B3, is a member of the homeodomain-containing superfamily of liver-enriched transcription factors, which are highly conserved across species from yeast to human [7]. HNF1b recognizes the sequence 5'-GTTAATNATTAAC-3' and mediates sequence-specific DNA binding through its POU-specific (Pit-1, OCT1/2, UNC-86; POU5) and atypical POU homeodomain (POUH) [8]. Truncated or loss-of-function HNF1b alleles cause maturity-onset diabetes of the young (MODY) 5, which is characterized by an early age of onset, usually at a mean age of 17–25.8 years (30–66%), genital malformations (12.0–62.5%), and an autosomal dominant mode of inheritance [9–13]. Some genome-wide association studies have shown that variants of HNF1b are associated with type 2 diabetes [14–17], while the opposite has been observed in different populations [18]. In addition, a large population-based cohort study demonstrates that genetic risk variants of HNF1b are significantly associated with lipoprotein traits, such as lipoprotein subclasses and particle composition [19]. In our previous study, we found that downregulation of HNF1b was involved in polychlorinated biphenyls (PCB)-153-induced oxidative stress and lipid accumulation in livers [20]. Overexpression of HNF1b increased GPx1 expression, decreased superoxide level, decreased sterol regulatory element-binding protein-1 (SREBP-1), fatty acid synthase (FAS) and acetyl CoA carboxylase expression, and inhibited PCB-153-resulted oxidative stress, NF- κ B-mediated inflammation, and final glucose/lipid metabolic disorder [20]. However, the role of HNF1b in the regulation of lipid metabolism and hepatic steatosis remains poorly understood.

In order to elucidate the role of HNF1b in the pathogenesis of NAFLD and associated metabolic dysfunction, we injected mice with lentivirus (LV) expressing HNF1b shRNA to

generate mice with liver knockdown of HNF1b. We also injected high fat (HF) diet-induced obese and db/db diabetic mice with LV expressing HNF1b to overexpress HNF1b. We observed that knockdown of HNF1b increased increase of hepatic lipid contents and induced insulin resistance in mice and in hepatocytes. In addition, knockdown of HNF1b worsened HF diet-induced increases in hepatic lipid content, liver injury and insulin resistance in mice and PA-induced lipid accumulation and disturbance of insulin signaling in hepatocytes. Moreover, overexpression of HNF1b alleviated HF diet-induced increases in hepatic lipid content and insulin resistance in mice. Our findings support the concept that HNF1b activators may have potential therapeutic benefit for the treatment of NAFLD.

2. Materials and methods

2.1. Animals and treatment

C57BL/6J mice were purchased from the Animal Center of Fourth Military Medical University. db/db mice were obtained from Model Animal Research Center of Nanjing University. For HF diet feeding, C57 mice were fed a chow diet or an HF diet (45% kcal from fat; Research Diets, New Brunswick, NJ) for 8 or 12 weeks. LVs expressing HNF1b or HNF1b shRNA were constructed commercially (Genechem, Shanghai, China). Each mouse was injected with 5×10^6 PFU LV through caudal vein in 500 μ l phosphate buffered saline (PBS) twice in four weeks. 8-week old C57 mice were injected with LVs expressing HNF1b shRNA. C57 mice feeding with HF diet for 12 weeks and db/db diabetic mice were injected with LVs expressing HNF1b. C57 mice feeding with HF diet for 8 weeks were injected with LVs expressing HNF1b shRNA. For the detection of insulin signaling, mice were intraperitoneally injected with 10 U/kg insulin 30 min before euthanization. Unless otherwise stated, male mice were used and all mice were fasted for overnight before euthanization. All animal experiments were approved by the institutional animal care and use committee at Fourth Military Medical University.

2.2. Cell culture, treatment and transient transfection

AML-12 cells were purchased from the American Type Culture Collection (ATCC, USA). Cells were cultured in DMEM/F-12 medium containing 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μ g/ml) at 37 °C in a humidified 5% CO₂ atmosphere. Cells were transfected with LV expressing HNF1b shRNA and purified with puromycin to establish cell lines with stable knockdown of HNF1b. Transient transfection of pCMV-DPP4, pCMV-NOX1, shDPP4 or shNOX1 (GeneChem, Shanghai, China) was performed using Turbofect Transfection Reagents (Thermo Scientific, USA) according to the manufacturer's protocol. Cells were treated with 20 μ M palmitic acid (PA) or oleic acid (OA) for 4 h to stimulate lipid accumulation. Cells were treated with 100 μ M PA for 24 h to stimulate cellular insulin resistance.

2.3. Histology and lipid staining in tissues and cells

Liver tissues were fixed in 10% formalin and embedded in paraffin. Paraffin-embedded sections were stained with hematoxylin and eosin using a standard protocol. For lipid staining in tissues, frozen sections were prepared and stained with Oil Red O (Sigma, St.

Louis, MO). For lipid staining in cells, cells were fixed in 10% paraformaldehyde and stained with Oil Red O or BODIPY 493/503 (Life Technologies, USA).

2.4. Biochemical determination

The levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and hepatic TG were measured using colorimetric assay kits (Nanjing Jiancheng, China).

2.5. Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT)

At the end of the experiment, IPGTT and IPITT were conducted to assess the metabolic activity in response to glucose or insulin load. Mice were fasted for 12 h, and basal level of blood glucose was measured using tail blood drops. Then, mice were intraperitoneally injected with D-glucose (Sigma, 1 g/kg), or insulin (Novolin Novolin R, 0.75 U/kg body weight, 0.75 U/kg body weight). Blood glucose level at 30, 60, and 120 min time points were measured using blood samples dropped from tails.

2.6. Glucose uptake

Glucose uptake was measured by a fluorescent glucose 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) method using a glucose uptake assay kit (Cayman Chemical, USA) according the manufacturer's protocols. Cells were incubated with 2-NBDG in the presence or absence of 100 nM insulin at 37 °C for 30 min 2-NBDG content was determined using a microplate fluorimeter (Infinite M200; Tecan, Hillsborough, NC).

2.7. Real-time PCR and western blot

Real-time PCR and western blot were performed as we previously did [21]. For PCR, primers used were shown in Supporting Table S1. Antibody dilution: β -actin: Santa Cruz, 1:500; HNF1b: Prosci Technology, 1: 1000; GRP78: Bioworld Technology, 1:500; CHOP: Bioworld Technology, 1:500; eIF2 α : Bioworld Technology, 1:500; P-eIF2 α : Santa Cruz, 1:500; PERK: Bioworld Technology, 1:500; P-PERK: Abcam, 1:1000; Akt: Cell Signaling Technology, 1:1000; P-Akt (S473): Cell Signaling Technology, 1:1000; PPAR α : Santa Cruz, 1:500; PPAR γ : Santa Cruz, 1:500; Srebp-1: Santa Cruz, 1:500; FAS: Cell Signaling Technology, 1:1000; FABP4: Cell Signaling Technology, 1:1000.

2.8. ChIP-PCR assay

HNF1b binding in the promoters of DPP4 and NOX1 was assessed by a ChIP assay using the Chromatin Immunoprecipitation Assay kit (Thermo Scientific, USA) according to the manufacturer's protocols. Subsequent PCR was conducted as detailed above. IgG binding was used as negative control.

2.9. Statistical analysis

Results were expressed as mean $\pm$ SEM. The statistical significance of differences between two groups was assessed by Student's *t*-tests. Differences between more than two groups were analyzed by one-way analysis of variance (ANOVA) followed by the Newman Keuls

multiple-comparison post hoc test using Graph-Pad Prism software. Data were considered to be statistically significant if $p < 0.05$.

3. Results

3.1. HNF1b expression is reduced in steatotic livers

To investigate whether HNF1b is associated with lipid accumulation in livers, we examined hepatic HNF1b expression in genetically obese db/db mice and HF diet-fed obese mice. As expected, db/db mice and HF diet-fed obese mice had severe liver steatosis (Supporting Fig. S1A and B). In both db/db mice (Fig. 1A, B) and HF diet-fed mice (Fig. 1C, D), hepatic HNF1b messenger RNA (mRNA) level was reduced by greater than 50% (Fig. 1A, C) and HNF1b protein level was reduced by approximately 80% (Fig. 1B, D). PA and OA induced a significant increase in lipid accumulation in AML-12 hepatocytes (Supporting Fig. S1C and D). HNF1b mRNA level was markedly reduced by PA and OA treatment (Fig. 1E and F). We further analyzed the expression of HNF1b in fasting-induced fatty liver in mice. Here, decreased expression of HNF1b was also observed in the livers of mice after 24 h and 48 h fasting (Fig. 1G). Together, the data indicate that HNF1b expression is reduced in steatotic livers, suggesting a role of HNF1b in the regulation of hepatic lipid metabolism.

3.2. Overexpression of HNF1b alleviates fatty liver and improves glucose tolerance

To determine whether HNF1b plays a role in hepatic lipid metabolism, we generated a lentivirus (LV) expressing HNF1b. db/db mice and HF diet-fed mice were injected with LV-HNF1b via tail vein. Overexpression of HNF1b was confirmed (Supporting Fig. S2A and B). Fasting blood glucose levels (Supporting Fig. S2E), body weights (Supporting Fig. S2C, F), and liver weights (Supporting Fig. S2D, G) were not significantly altered except for the fasting blood glucose levels (Supporting Fig. S2B) in db/db mice. HE and Oil Red O staining showed that hepatic lipid content was decreased in LV-HNF1b-injected db/db (Fig. 2A), HF diet-fed (Fig. 2B) and fasted (Fig. 2C) mice. Hepatic TG levels in db/db (Fig. 2D), HF diet-fed (Fig. 2E) and fasted (Fig. 2F) mice were decreased by LV-HNF1b injection. Overexpression of HNF1b remarkably improved both glucose tolerance (Fig. 2G) and insulin sensitivity (Fig. 2H), with the area under the curve (AUC) being significantly reduced (Fig. 2G, H, right panel). Consistent with improved glucose homeostasis and insulin sensitivity, HNF1b overexpression increased insulin-stimulated hepatic phosphorylation of Akt in db/db mice (Fig. 2I) and HF diet-fed mice (Fig. 2J). The data suggests that overexpression of HNF1b alleviates fatty liver and improves glucose tolerance in both genetic and diet-induced mice model of metabolic disorder.

3.3. HNF1b knockdown exacerbated HF diet-induced fatty liver and glucose intolerance

To determine whether downregulation of HNF1b promotes HF diet-induced hepatic lipid accumulation, we generated a lentivirus (LV) expressing shHNF1b. HF diet-fed mice were injected with LV-HNF1b via tail vein. Knockdown of HNF1b was confirmed (Supporting Fig. S3A) and fasting blood glucose level (Supporting Fig. S3B) and liver weight (Supporting Fig. S3D) were not significantly altered. Interestingly, knockdown of HNF1b decreased body weight in HF diet-fed mice (Supporting Fig. S3C). HE and Oil Red O staining showed that hepatic lipid contents were increased in LV-shHNF1b-injected HF diet-

fed (Fig. 3A) mice. Hepatic TG level in HF diet-fed (Fig. 3B) mice was increased by knockdown of HNF1b. Bodipy and Oil Red O staining showed that knockdown of HNF1b increased lipid accumulation in AML-12 hepatocytes treated by OA or PA (Fig. 3C). Knockdown of HNF1b worsened both glucose intolerance (Fig. 3D) and insulin resistance (Fig. 3E), with AUC being significantly increased (Fig. 3D, E, right panel). The data suggest that knockdown of HNF1b worsens fatty liver and glucose intolerance in obese mice.

3.4. Knockdown of HNF1b results in fatty liver and impairment of glucose tolerance

To evaluate whether knockdown of HNF1b induces hepatic lipid accumulation, we injected C57BL/6J mice with LV-shHNF1b. Reduced expression of HNF1b was confirmed (Supporting Fig. S4A) and fasting blood glucose level, body weight and liver weight were not significantly altered (Supporting Fig. S4B, C, D). Knockdown of HNF1b resulted in a significant increase in hepatic lipid content in mice, as evidenced by HE and Oil Red O staining (Fig. 4A) mice. Hepatic TG levels were increased by knockdown of HNF1b (Fig. 4B). Reduced expression of HNF1b induced a significant increase in Bodipy staining in AML-12 hepatocytes, indicating increased lipid accumulation (Fig. 4C). Knockdown of HNF1b results in significant impairment of glucose tolerance (Fig. 4D) and insulin sensitivity (Fig. 4E), with AUC being significantly increased (Fig. 4D, E, right panel). Insulin-stimulated glucose uptake in AML-12 hepatocytes was markedly reduced by the knockdown of HNF1b (Fig. 4F). Consistently, reduced expression of HNF1b resulted in decreased insulin-stimulated phosphorylation of Akt in livers of mice (Fig. 4G) and AML-12 hepatocytes (Fig. 4H). Taken together, these data suggest that reduced hepatic expression of HNF1b results in fatty liver, impairment of glucose tolerance and insulin resistance in vivo and in vitro.

3.5. Knockdown of HNF1b worsened HF diet-induced liver injury, resulted in ER stress and increased lipogenesis-related gene expression

Knockdown of HNF1b had no significant effect on serum level of alanine aminotransferase (ALT)/aspartate aminotransferase (AST) in mice fed a normal chow diet (Fig. 5A, B). However, knockdown of HNF1b resulted in a marked increase in ALT and AST concentrations in plasma of mice fed with HF diet (Fig. 5A, B). Knockdown of HNF1b induced a significant increase in the expression of 78 kDa glucose-regulated protein (GRP78), C/EBP homologous protein (CHOP), activating transcription factor 6 (ATF6), and the phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) (Fig. 5C), indicating the induction of endoplasmic reticulum (ER) stress. In addition, HNF1b knockdown increased the basal and HF diet-induced expression of fatty acid metabolism-related genes, including peroxisome proliferator-activated receptor α/γ , Srebp-1, FAS, and fatty acid binding protein 4 (FABP4) (Fig. 5D, E, F, G, H). The protein expression of these genes were also increased by downregulation of HNF1b (Fig. 5I). These data suggest that reduced expression of HNF1b results worsened HF diet-induced liver injury, induced ER stress and increased fatty acid and TG synthesis-related gene expression.

3.6. Upregulation of DPP4 was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder

To further explore the underlying molecular mechanisms of HNF1b downregulation-mediated fatty and metabolic disorder, we performed Affymetrix-based global gene expression analysis on AML-12 cells with HNF1b knockdown. Transcriptome analysis identified 141 more than 1.5-fold differentially expressed genes (DEGs) between LV-Ctrl-AML-12 and LV-shHNF1b-AML-12 cells (Table 1). Among those DEGs, we focused on dipeptidyl peptidase 4 (DPP4), an important target in the treatment of type 2 diabetes (Table 1). We validated the altered expression of DPP4 and showed that HNF1b knockdown markedly increased the expression of DPP4 (Fig. 6A, B). In livers of both genetic and HF diet-induced obese mice, the expression of DPP4 was significantly increased (Fig. 6C, D, E, F). Upregulation of DPP4 was associated with a significant increase in lipid formation in AML-12 hepatocytes (Fig. 6G and Supporting Fig. S5). Silence of DPP4 using shRNAs (Supporting Fig. S6) was associated with HNF1b knockdown-resulted increase of lipid accumulation (Fig. 6H), decrease of insulin-stimulated glucose uptake (Fig. 6I) and Akt phosphorylation (Fig. 6J) in AML-12 hepatocytes. Furthermore, we revealed that the level of DPP4 promoter expression in HNF1b antibody immunoprecipitated mixture was significantly higher than that of IgG, indicating that HNF1b exhibited a direct binding in the promoters of DPP4 (Fig. 6K). In the presence of PA, the binding of HNF1b in the promoters of DPP4 was significantly reduced (Fig. 6K), which may contribute to increased expression of DPP4. The results demonstrated that upregulation of DPP4 was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder.

3.7. Upregulation of NOX1/superoxide was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder

In the transcriptome analysis, we also found that nicotinamide adenine dinucleotide phosphate oxidase 1 (NOX1) was increased by HNF1b knockdown (Table 1). We validated the upregulated expression of NOX1 and showed that HNF1b knockdown increased NOX1 expression (Fig. 7A, B). In livers of both db/db mice and HF diet-resulted obese mice, the expression of NOX1 was significantly increased (Fig. 7C, D, E, F). Upregulation of NOX1 (Supporting Fig. S7) resulted in a significant increase in lipid formation in AML-12 hepatocytes (Fig. 7G). Silence of NOX1 (Supporting Fig. S8) prohibited the increase of lipid accumulation (Fig. 7H), decrease of insulin-stimulated glucose uptake (Fig. 7I) and Akt phosphorylation (Fig. 7J) induced by HNF1b knockdown in AML-12 hepatocytes. Furthermore, we revealed that the level of NOX1 promoter expression in HNF1b antibody immunoprecipitated mixture was significantly higher than that of IgG, indicating that HNF1b exhibited a direct binding in the promoters of NOX1 (Fig. 7K). In the presence of PA, the binding of HNF1b in the promoters of NOX1 was significantly reduced (Fig. 7K), which may contribute to increased expression of NOX1. The results demonstrated that upregulation of NOX1 was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder.

3.8. DPP4/NOX1-mediated generation of superoxide was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder

We have previously found that downregulation of HNF1b is associated with oxidative stress. In the current study, we also examined the possible role of redox changes in HNF1b-exhibited regulation of hepatic lipid metabolism. Knockdown of HNF1b increased superoxide level in AML-12 hepatocytes (Fig. 8A). Silence of both DPP4 and NOX1 inhibited the high level of DHE staining by HNF1b knockdown in AML-12 hepatocytes, indicating that DPP4 and NOX1 were involved in HNF1b knockdown-associated superoxide generation (Fig. 8B and C). Moreover, HNF1b knockdown resulted in a significant decrease of glutathione, an important antioxidant protein (Fig. 8D and E). Silence of both DPP4 and NOX1 inhibited the reduction of GSH level induced by HNF1b knockdown in AML-12 hepatocytes (Fig. 8D and E). The results indicated that upregulation of DPP4 and NOX1 was responsible for HNF1b knockdown-induced oxidative stress in AML-12 hepatocytes. Furthermore, we examined the role of superoxide generation in HNF1b knockdown-induced lipid accumulation and insulin resistance. We revealed that treatment with N-acetylcysteine (NAC), a well-known antioxidant, could significantly suppress HNF1b knockdown-induced ER stress, lipid formation and insulin resistance (Fig. 8F, G and H). The results indicated that DPP4/NOX1-mediated generation of superoxide was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder. In the next step, we also determined the effect of fatty acids on superoxide level and HNF1b expression. The results showed that PA exposure resulted in a significant increase of DHE staining in AML-12 hepatocytes, which was inhibited by the antioxidant NAC (Fig. 8I). The expression of HNF1b was markedly decreased by PA, which effect was inhibited by NAC (Fig. 8J). The results indicated that PA downregulated HNF1b through production of superoxide and this may cause a vicious circle of downregulation of HNF1b and production of superoxide, leading enhancement of ER stress, lipid accumulation and insulin resistance (Fig. 8K).

4. Discussion

Although numerous papers have reported the pivotal role of HNF1b in MODY5 and the association between variants of HNF1b and type 2 diabetes, the molecular mechanisms remain unclear. Kornfeld et al. discovered that shRNA-mediated reduction of HNF1b in liver resulted in glucose intolerance, impaired insulin signaling and promoted hepatic gluconeogenesis [22]. In turn, hepatic overexpression of HNF1b improved insulin sensitivity in db/db mice [22], suggesting an unexpected role for HNF1b in the control of obesity-associated hepatic insulin sensitivity. However, whether abnormal expression of HNF1b is associated with hepatic steatosis has not been established.

In this study, we examined the pathophysiological functions and molecular mechanisms of HNF1b in the development of fatty liver and metabolic disorder. We observed that HNF1b expression was decreased in fatty liver under pathological conditions in genetic and diet-induced obese mice and in fasted mice, indicating that reduced HNF1b expression was associated with the development of hepatic steatosis. We also found that knockdown of HNF1b increased hepatic lipid content and induced insulin resistance in mice and in a AML-12 hepatocyte cell line. In addition, knockdown of HNF1b exacerbated HF diet-

induced hepatic lipid accumulation, liver injury and insulin resistance in mice and PA-induced lipid accumulation and disturbance of insulin signaling in AML-12 hepatocytes. Moreover, overexpression of HNF1b alleviated HF diet-induced hepatic lipid contents and insulin resistance in mice. These results demonstrated that HNF1b protects against hepatic steatosis and insulin resistance.

PPAR γ is the central regulator of fatty acid and lipogenesis through regulating a series of target genes, including Srebp-1, FAS and FABP4 [23,24]. In contrast, PPAR α is responsible for fatty acid oxidation and lipolysis. The findings showed that knockdown of HNF1b increased the expression of all these factors. The results indicated that knockdown of HNF1b may induce an increase in both lipogenesis and lipolysis compared to basal condition, and the process of lipogenesis may be predominant. The effect of knockdown of HNF1b on lipolysis needs to be further thoroughly investigated, which may be a consequence of excessive lipid accumulation and could also contribute to insulin resistance. Lipogenesis and ER stress are reciprocally regulated [25] and ER stress is critical for protein homeostasis. However, abnormal ER stress may promote fat accumulation, insulin resistance and inflammation [25]. ER stress is reported to be involved in lipogenesis [26] through proteolytic cleavage of Srebp-1 [27]. ER stress is also involved in the development of insulin resistance. Lipogenesis resulting in lipid droplet accumulation in the ER is known to cause ER stress [28,29]. Exogenous FABP4 induces ER stress [30]. Activation of PPAR α/γ could either promote or inhibit ER stress [31–35]. We found that downregulation of HNF1b increased lipogenesis-related regulators and activated ER stress. The evidence suggests that enhancement of lipogenesis and ER stress underlie the mechanism by which reduced hepatic expression of HNF1b leads to hepatic steatosis and insulin resistance.

To explore the molecular mechanism of HNF1b-exhibited regulation of lipid metabolism and insulin sensitivity, we performed transcriptome analysis and found that DPP4 was significantly increased after the knockdown of HNF1b. DPP4 degrades glucagon-like peptide-1, which stimulates insulin secretion from pancreatic β -cells. DPP4 inhibitors have been introduced for the treatment of type 2 diabetes [36]. Hepatic DPP4 mRNA expression is significantly greater in NAFLD patients than in control subjects and is negatively correlated with homeostatic model assessment (HOMA-IR) and positively correlated with serum cholesterol levels [36]. Inhibition of DPP4 attenuates hepatic steatosis in ob/ob mice [37] and ER stress in db/db mice [38]. We further reported that overexpression of DPP4 increased lipid droplets in AML-12 hepatocytes and downregulation of DPP4 suppressed knockdown of HNF1b-induced TG formation and disturbance of insulin signaling. The promoter region of the DPP4 gene contains functional HNF1 binding sites [38]. Interestingly, HNF1a is a positive transcriptional regulator of DPP4 [38]. Conversely, our results suggest that HNF1b suppresses expression of DPP4. Therefore, it appears that HNF1a and HNF1b differentially regulate the expression of specific gene targets with the ratio of HNF1a and HNF1b likely determining target gene expression levels. Whether there is direct interaction between HNF1a and HNF1b in the regulation of DPP4 expression and the subsequent lipogenesis needs further investigation.

Oxidative stress is a fundamental biological process that promotes the development of fatty liver, obesity and insulin resistance [39,40]. We also found that knockdown of HNF1b

resulted in increase of su-peroxide level which could be attributed to increased expression of DPP4 and NOX1. The treatment of NAC significantly inhibited HNF1b knockdown-induced ER stress, steatosis, and insulin resistance, suggesting that the production of superoxide played a central role in the adverse effect of HNF1b knockdown. NADPH oxidases are major source of intracellular superoxide [41]. NOX1-mediated production of superoxide is involved in various pathological processes. However, the role of NOX1/superoxide axis in hepatic lipid metabolism and insulin resistance has not been given adequate attention. We reported that hepatic NOX1 expression was increased in genetic and diet-induced obese mice. Overexpression of NOX1 increased lipid droplets in AML-12 hepatocytes and downregulation of NOX1 suppressed knockdown of HNF1b-induced TG formation and impaired of insulin signaling. One possibility is that HNF1b functions as a transcriptional repressor of NOX1, resulting in increase of superoxide generation and subsequent hepatic lipogenesis and insulin resistance. In addition, we found that silence of DPP4 inhibited superoxide generation induced by HNF1b knockdown in AML-12 hepatocytes, indicating that DPP4 also involved in HNF1b knockdown-associated superoxide generation. Previous studies have reported that DPP4 inhibitors played an antioxidant role mainly through regulating expression of antioxidant enzymes and mitochondrial ROS generation [42,43]. These results indicated that generation of superoxide induced by DPP4 also contributed to the ER stress, hepatic lipid accumulation and insulin resistance. In addition to our findings, there are several literatures reporting the relationship between DPP4 and steatosis. Gemigliptin, an inhibitor of DPP4, improved hepatic steatosis and insulin resistance in HF diet-fed mice, by AMP-activated protein kinase-dependent and c-Jun N-terminal kinase-dependent mechanisms [44]. In addition, DPP-4 inhibition could ameliorate hepatic steatosis and insulin resistance by suppressing hepatic TG and diacylglycerol accumulation through enhanced mitochondrial carbohydrate utilization and hepatic TG secretion/export with a concomitant reduction of uric acid production [45]. In future studies, the mechanism underlying DPP4-induced superoxide generation and whether there exist other mechanisms responsible for DPP4-exhibited hepatic steatosis and insulin resistance in response to HNF1b downregulation are to be clarified. We also revealed that PA induced superoxide generation, which was responsible for the downregulation of HNF1b expression. Treatment of NAC significantly restored the expression of HNF1b in PA-treated hepatocytes. The results indicated that fatty acids promote superoxide generation, decrease HNF1b expression, which could upregulate DPP4 and NOX1, leading to enhancement of superoxide generation and lipid metabolic disorder and forming a vicious circle.

Taken together, our current studies demonstrate that HNF1b plays an essential role in controlling hepatic TG homeostasis by regulating DPP4/NOX1/superoxide. In addition to regulating hepatic lipid metabolism, rescue of reduced hepatic HNF1b expression in db/db or HF diet-fed mice improved glucose tolerance and insulin sensitivity. Accordingly, we propose that HNF1b is required to maintain lipid balance in the liver, and its function in lipid metabolism has important implications for the prevention and treatment of NAFLD associated with diabetes and obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 21677176 and No. 31400724), The Innovative Talents Promotion Plan in Shaanxi Province (No. 2017KJXX-42), The Outstanding Youth Project of Chinese PLA (No. 16QNP116), and by the NIH/NIDDK (RO1DK104857 to KA).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.freeradbiomed.2017.09.016>.

References

1. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease, QJM: Mon. J Assoc Physicians. 2010; 103:71–83.
2. Fan JG, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. J Hepatol. 2009; 50:204–210. [PubMed: 19014878]
3. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006; 43:S99–s112. [PubMed: 16447287]
4. Musso G, Gambino R, Cassader M. Emerging molecular targets for the treatment of nonalcoholic fatty liver disease. Annu Rev Med. 2010; 61:375–392. [PubMed: 20059344]
5. Moon YA, Liang G, Xie X, Frank-Kamenetsky M, Fitzgerald K, Koteliensky V, Brown MS, Goldstein JL, Horton JD. The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in animals. Cell Metab. 2012; 15:240–246. [PubMed: 22326225]
6. Tang JJ, Li JG, Qi W, Qiu WW, Li PS, Li BL, Song BL. Inhibition of SREBP by a small molecule, betulin, improves hyperlipidemia and insulin resistance and reduces atherosclerotic plaques. Cell Metab. 2011; 13:44–56. [PubMed: 21195348]
7. Tronche F, Yaniv M. HNF1, a homeoprotein member of the hepatic transcription regulatory network, Bioessays: News Rev. Mol Cell Dev Biol. 1992; 14:579–587.
8. Rey-Campos J, Chouard T, Yaniv M, Cereghini S. vHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1. EMBO J. 1991; 10:1445–1457. [PubMed: 1673926]
9. Bell GI, Xiang KS, Newman MV, Wu SH, Wright LG, Fajans SS, Spielman RS, Cox NJ. Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q. Proc Natl Acad Sci USA. 1991; 88:1484–1488. [PubMed: 1899928]
10. Edghill EL, Oram RA, Owens M, Stals KL, Harries LW, Hattersley AT, Ellard S, Bingham C. Hepatocyte nuclear factor-1beta gene deletions—a common cause of renal disease. Nephrol Dial Transplant. 2008; 23:627–635. [PubMed: 17971380]
11. Edghill EL, Bingham C, Ellard S, Hattersley AT. Mutations in hepatocyte nuclear factor-1beta and their related phenotypes. J Med Genet. 2006; 43:84–90. [PubMed: 15930087]
12. Bingham C, Hattersley AT. Renal cysts and diabetes syndrome resulting from mutations in hepatocyte nuclear factor-1beta. Nephrol Dial Transplant. 2004; 19:2703–2708. [PubMed: 15496559]
13. Bellanne-Chantelot C, Clauin S, Chauveau D, Collin P, Daumont M, Douillard C, Dubois-Laforgue D, Dusselier L, Gautier JF, Jadoul M, Laloi-Michelin M, Jacquesson L, Larger E, Louis J, Nicolino M, Subra JF, Wilhem JM, Young J, Velho G, Timsit J. Large genomic rearrangements in the hepatocyte nuclear factor-1beta (TCF2) gene are the most frequent cause of maturity-onset diabetes of the young type 5. Diabetes. 2005; 54:3126–3132. [PubMed: 16249435]
14. Han X, Luo Y, Ren Q, Zhang X, Wang F, Sun X, Zhou X, Ji L. Implication of genetic variants near SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, FTO, TCF2, KCNQ1, and WFS1 in type 2 diabetes in a Chinese population. BMC Med Genet. 2010; 11:81. [PubMed: 20509872]

15. Lauenborg J, Grarup N, Damm P, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T. Common type 2 diabetes risk gene variants associate with gestational diabetes. *J Clin Endocrinol Metab.* 2009; 94:145–150. [PubMed: 18984664]
16. Wang C, Hu C, Zhang R, Bao Y, Ma X, Lu J, Qin W, Shao X, Lu J, Xu J, Lu H, Xiang K, Jia W. Common variants of hepatocyte nuclear factor 1beta are associated with type 2 diabetes in a Chinese population. *Diabetes.* 2009; 58:1023–1027. [PubMed: 19168595]
17. Bonnycastle LL, Willer CJ, Conneely KN, Jackson AU, Burrill CP, Watanabe RM, Chines PS, Narisu N, Scott LJ, Enloe ST, Swift AJ, Duren WL, Stringham HM, Erdos MR, Riebow NL, Buchanan TA, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Boehnke M, Collins FS. Common variants in maturity-onset diabetes of the young genes contribute to risk of type 2 diabetes in Finns. *Diabetes.* 2006; 55:2534–2540. [PubMed: 16936201]
18. Wen J, Ronn T, Olsson A, Yang Z, Lu B, Du Y, Groop L, Ling C, Hu R. Investigation of type 2 diabetes risk alleles support CDKN2A/B, CDKAL1, and TCF7L2 as susceptibility genes in a Han Chinese cohort. *PLoS One.* 2010; 5:e9153. [PubMed: 20161779]
19. Stancakova A, Paananen J, Soininen P, Kangas AJ, Bonnycastle LL, Morken MA, Collins FS, Jackson AU, Boehnke ML, Kuusisto J, Ala-Korpela M, Laakso M. Effects of 34 risk loci for type 2 diabetes or hyperglycemia on lipoprotein subclasses and their composition in 6,580 nondiabetic Finnish men. *Diabetes.* 2011; 60:1608–1616. [PubMed: 21421807]
20. Wu H, Yu W, Meng F, Mi J, Peng J, Liu J, Zhang X, Hai C, Wang X. Polychlorinated biphenyls-153 induces metabolic dysfunction through activation of ROS/NF- κ B signaling via downregulation of HNF1b. *Redox Biol.* 2017; 12:300–310. [PubMed: 28285191]
21. Wang X, Liu J, Long Z, Sun Q, Liu Y, Wang L, Zhang X, Hai C. Effect of diosgenin on metabolic dysfunction: role of ERbeta in the regulation of PPARgamma. *Toxicol Appl Pharmacol.* 2015; 289:286–296. [PubMed: 26408789]
22. Kornfeld JW, Baitzel C, Konner AC, Nicholls HT, Vogt MC, Herrmanns K, Scheja L, Haumaitre C, Wolf AM, Knippschild U, Seibler J, Cereghini S, Heeren J, Stoffel M, Bruning JC. Obesity-induced overexpression of miR-802 impairs glucose metabolism through silencing of Hnf1b. *Nature.* 2013; 494:111–115. [PubMed: 23389544]
23. Pettinelli P, Videla LA. Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. *J Clin Endocrinol Metab.* 2011; 96:1424–1430. [PubMed: 21325464]
24. Yamazaki T, Shiraishi S, Kishimoto K, Miura S, Ezaki O. An increase in liver PPARgamma2 is an initial event to induce fatty liver in response to a diet high in butter: PPARgamma2 knockdown improves fatty liver induced by high-saturated fat. *J Nutr Biochem.* 2011; 22:543–553. [PubMed: 20801631]
25. Bozaykut P, Sahin A, Karademir B, Ozer NK. Endoplasmic reticulum stress related molecular mechanisms in nonalcoholic steatohepatitis. *Mech Ageing Dev.* 2016; 157:17–29. [PubMed: 27393639]
26. Bobrovnikova-Marjon E, Hatzivassiliou G, Grigoriadou C, Romero M, Cavener DR, Thompson CB, Diehl JA. PERK-dependent regulation of lipogenesis during mouse mammary gland development and adipocyte differentiation. *Proc Natl Acad Sci USA.* 2008; 105:16314–16319. [PubMed: 18852460]
27. Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, Ferre P, Foufelle F. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. *J Clin Investig.* 2009; 119:1201–1215. [PubMed: 19363290]
28. Gonzalez P, Mader I, Tchoghandjian A, Enzenmuller S, Cristofanon S, Basit F, Debatin KM, Fulda S. Impairment of lysosomal integrity by B10, a glycosylated derivative of betulinic acid, leads to lysosomal cell death and converts autophagy into a detrimental process. *Cell Death Differ.* 2012; 19:1337–1346. [PubMed: 22343715]
29. Ferre P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metab.* 2010; 12(Suppl 2):83–92. [PubMed: 21029304]
30. Bosquet A, Guaita-Esteruelas S, Saavedra P, Rodriguez-Calvo R, Heras M, Girona J, Masana L. Exogenous FABP4 induces endoplasmic reticulum stress in HepG2 liver cells. *Atherosclerosis.* 2016; 249:191–199. [PubMed: 27155388]

31. Chan SM, Sun RQ, Zeng XY, Choong ZH, Wang H, Watt MJ, Ye JM. Activation of PPAR α ameliorates hepatic insulin resistance and steatosis in high fructose-fed mice despite increased endoplasmic reticulum stress. *Diabetes*. 2013; 62:2095–2105. [PubMed: 23349482]
32. Ikeda J, Ichiki T, Takahara Y, Kojima H, Sankoda C, Kitamoto S, Tokunou T, Sunagawa K. PPAR γ agonists attenuate palmitate-induced ER stress through up-regulation of SCD-1 in macrophages. *PLoS One*. 2015; 10:e0128546. [PubMed: 26061913]
33. Kim BM, Maeng K, Lee KH, Hong SH. Combined treatment with the Cox-2 inhibitor niflumic acid and PPAR γ ligand ciglitazone induces ER stress/caspase-8-mediated apoptosis in human lung cancer cells. *Cancer Lett*. 2011; 300:134–144. [PubMed: 21067863]
34. Maganti AV, Tersey SA, Syed F, Nelson JB, Colvin SC, Maier B, Mirmira RG. Peroxisome proliferator-activated receptor- γ activation augments the beta-cell unfolded protein response and rescues early glycemic deterioration and beta cell death in non-obese diabetic mice. *J Biol Chem*. 2016; 291:22524–22533. [PubMed: 27613867]
35. Tang WX, Wang LK, Wang YQ, Zong ZJ, Gao ZX, Liu XS, Shen YJ, Shen YX, Li YH. Peroxisome proliferator-activated receptor- α activation protects against endoplasmic reticulum stress-induced HepG2 cell apoptosis. *Mol Cell Biochem*. 2014; 385:179–190. [PubMed: 24068329]
36. Miyazaki M, Kato M, Tanaka K, Tanaka M, Kohjima M, Nakamura K, Enjoji M, Nakamura M, Kotoh K, Takayanagi R. Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. *Mol Med Rep*. 2012; 5:729–733. [PubMed: 22179204]
37. Ohyama T, Sato K, Yamazaki Y, Hashizume H, Horiguchi N, Kakizaki S, Mori M, Kusano M, Yamada M. MK-0626, a selective DPP-4 inhibitor, attenuates hepatic steatosis in ob/ob mice. *World J Gastroenterol*. 2014; 20:16227–16235. [PubMed: 25473177]
38. Wu YJ, Guo X, Li CJ, Li DQ, Zhang J, Yang Y, Kong Y, Guo H, Liu DM, Chen LM. Dipeptidyl peptidase-4 inhibitor, vildagliptin, inhibits pancreatic beta cell apoptosis in association with its effects suppressing endoplasmic reticulum stress in db/db mice, *Metab. Clin Exp*. 2015; 64:226–235.
39. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature*. 2006; 440:944–948. [PubMed: 16612386]
40. Tsendensodnom O, Sadler KC. ROS: redux and paradox in fatty liver disease. *Hepatology*. 2013; 58:1210–1212. [PubMed: 23703804]
41. Wang X, Hai C. Redox modulation of adipocyte differentiation: hypothesis of “Redox Chain” and novel insights into intervention of adipogenesis and obesity. *Free Radic Biol Med*. 2015; 89:99–125. [PubMed: 26187871]
42. Wu C, Hu S, Wang N, Tian J. Dipeptidyl peptidase4 inhibitor sitagliptin prevents high glucose-induced apoptosis via activation of AMP-activated protein kinase in endothelial cells. *Mol Med Rep*. 2017; 15:4346–4351. [PubMed: 28440488]
43. Ku HC, Chen WP, Su MJ. DPP4 deficiency exerts protective effect against H₂O₂ induced oxidative stress in isolated cardiomyocytes. *PLoS One*. 2013; 8:e54518. [PubMed: 23359639]
44. Hwang HJ, Jung TW, Kim BH, Hong HC, Seo JA, Kim SG, Kim NH, Choi KM, Choi DS, Baik SH, Yoo HJ. A dipeptidyl peptidase-IV inhibitor improves hepatic steatosis and insulin resistance by AMPK-dependent and JNK-dependent inhibition of LECT2 expression. *Biochem Pharmacol*. 2015; 98:157–166. [PubMed: 26297911]
45. Arora AR, Habibi J, Ford DA, Nistala R, Lastra G, Manrique C, Dunham MM, Ford KD, Thyfault JP, Parks EJ, Sowers JR, Rector RS. Dipeptidyl peptidase-4 inhibition ameliorates Western diet-induced hepatic steatosis and insulin resistance through hepatic lipid remodeling and modulation of hepatic mitochondrial function. *Diabetes*. 2015; 64:1988–2001. [PubMed: 25605806]

Fig. 1.

Reduced expression of HNF1b was correlated with lipid accumulation in livers and AML-12 hepatocytes. (A) The mRNA levels of HNF1b in livers of WT and db/db mice. $n = 6$. (B) The protein levels of HNF1b in livers of WT and db/db mice. $n = 6$. (C) The mRNA levels of HNF1b in livers of control and HF fed mice. $n = 6$. (D) The protein levels of HNF1b in livers of control and HF fed mice. $n = 6$. (E) The mRNA levels of HNF1b in PA-treated AML-12 hepatocytes. $n = 6$. (F) The mRNA levels of HNF1b in OA-treated AML-12 hepatocytes. $n = 6$. (G) The protein levels of HNF1b in livers of mice after 8, 12, 24 and 48-h fasting. $n = 3$. * $P < 0.05$.

Fig. 2.

Overexpression of HNF1b alleviates lipid accumulation in livers and improves glucose tolerance in db/db and HF diet-fed mice. HE and Oil Red O staining of livers in db/db mice (A), HF diet-fed mice (B) and fasted mice (C) with LV-Ctrl or LV-HNF1b injection. $n = 8$. TG content in livers in db/db mice (D), HF diet-fed mice (E) and fasted mice (F) with LV-Ctrl or LV-HNF1b injection. $n = 8$. IPGTT (G) and IPITT (H) in HF diet-fed mice with LV-Ctrl or LV-HNF1b injection. $n = 6$. Insulin-stimulated phosphorylation of Akt (Ser-473) in

livers in db/db mice (I) and HF diet-fed mice (J) with LV-Ctrl or LV-HNF1b injection. n = 3.
*P < 0.05.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 3.

Knockdown of HNF1b promotes lipid accumulation in livers and glucose intolerance in HF diet-fed mice. HE and Oil Red O staining (A) and TG content (B) in livers in HF diet-fed mice with LV-Ctrl or LV-shHNF1b injection. $n = 8$. (C) Bodipy staining of AML-12 hepatocytes treated by OA or PA with LV-Ctrl or LV-shHNF1b transfection. $n = 6$. IPGTT (D) and IPITT (E) in HF diet-fed mice with LV-Ctrl or LV-HNF1b injection. $n = 6$. * $P < 0.05$.

Fig. 4. Knockdown of HNF1b results in lipid accumulation in livers and glucose intolerance in mice. HE and Oil Red O staining of livers (A) and TG content in livers (B) in C57BL/6 J mice with LV-Ctrl or LV-shHNF1b injection. $n = 8$. (C) Bodipy staining of AML-12 hepatocytes with LV-Ctrl or LV-shHNF1b transfection. $n = 6$. IPGTT (D) and IPITT (E) in C57BL/6 J mice with LV-Ctrl or LV-shHNF1b injection. $n = 6$. (F, G, and H) Insulin-stimulated glucose uptake (F), phosphorylation of Akt (Ser-473) in livers in C57BL/6 J mice with LV-Ctrl or LV-shHNF1b injection (G) or in AML-12 hepatocytes with LV-Ctrl or LV-shHNF1b transfection (H). $n = 3$. * $P < 0.05$.

Fig. 5. Knockdown of HNF1b results in liver injury and ER stress and activates lipogenesis. ALT (A) and AST (B) content in serum of C57BL/6J mice with LV-Ctrl or LV-shHNF1b injection fed with HF diet or not. $n = 8$. (C) Protein expression of ER stress markers in livers of C57BL/6J mice with LV-Ctrl or LV-shHNF1b injection. $n = 3$. mRNA (D, E, F, G and H) and protein (I) expression of lipogenesis-related regulators. $n = 6$. * $P < 0.05$.

Fig. 6.

Upregulation of DPP4 was involved in HNF1b silencing-induced hepatic lipid accumulation and metabolic disorder. The mRNA (A) and protein (B) levels of DPP4 in AML-12 hepatocytes with LV-Ctrl or LV-shHNF1b transfection. $n = 3$. The mRNA (C) and protein (D) levels of DPP4 in livers of WT and db/db mice. $n = 3$. The mRNA (E) and protein (F) levels of DPP4 in livers of chow and HF diet-fed mice. $n = 3$. (G) Bodipy staining of AML-12 hepatocytes treated with pCMV-DPP4 or empty plasmids transfection. $n = 6$. (H) Bodipy staining of LV-Ctrl and LV-shHNF1b AML-12 hepatocytes treated with shDPP4 or control shRNAs transfection. $n = 6$. Insulin-stimulated glucose uptake (I) and Akt phosphorylation (J) in LV-Ctrl and LV-shHNF1b AML-12 hepatocytes treated with shDPP4 or control shRNAs transfection. (K) HNF1b binding in the promoters of DPP4 in was detected using CHIP assay. $n = 3$. * $P < 0.05$.

Fig. 7.

Upregulation of NOX1 was involved in HNF1b silencing-induced hepatic lipid accumulation and metabolic disorder. (A and B) The mRNA and protein levels of NOX1 in AML-12 hepatocytes with LV-Ctrl or LV-shHNF1b transfection. $n = 3$. (C and D) The mRNA and protein levels of NOX1 in livers of WT and db/db mice. $n = 3$. (E and F) The mRNA and protein levels of NOX1 in livers of chow and HF diet-fed mice. $n = 3$. (G) Bodipy staining of AML-12 hepatocytes treated with pCMV-NOX1 or empty plasmids transfection. $n = 6$. (H) Bodipy staining of LV-Ctrl and LV-shHNF1b AML-12 hepatocytes treated with shNOX1 or control shRNAs transfection. $n = 6$. Insulin-stimulated glucose uptake (I) and Akt phosphorylation (J) in LV-Ctrl and LV-shHNF1b AML-12 hepatocytes treated with shNOX1 or control shRNAs transfection. (K) HNF1b binding in the promoters of DPP4 in was detected using CHIP assay. $n = 3$. * $P < 0.05$.

Fig. 8. DPP4/NOX1-mediated generation of superoxide was involved in HNF1b silence-induced hepatic lipid accumulation and metabolic disorder. (A) DHE staining of AML-12 hepatocytes with LV-Ctrl or LV-shHNF1b transfection. $n = 6$. (B) DHE staining of HNF1b KD AML-12 hepatocytes transfected with shDPP4. $n = 6$. (C) DHE staining of HNF1b KD AML-12 hepatocytes transfected with shNOX1. $n = 6$. (D) GSH content in HNF1b KD AML-12 hepatocytes transfected with shDPP4. $n = 6$. (E) GSH content in HNF1b KD AML-12 hepatocytes transfected with shNOX1. $n = 6$. (F) Protein expression of ER stress markers in HNF1b KD AML-12 hepatocytes treated with NAC. $n = 3$. (G) Bodipy staining of HNF1b KD AML-12 hepatocytes treated with NAC. $n = 6$. (H) Insulin-stimulated glucose uptake in HNF1b KD AML-12 hepatocytes treated with NAC. $n = 6$. (I) DHE staining of AML-12 hepatocytes treated with PA and NAC. $n = 6$. (J) Protein expression of HNF1b in AML-12 hepatocytes treated with PA and NAC. $n = 3$. (K) Schematic figure of the

mechanism underlying HNF1b-exhibited regulation of steatosis and insulin resistance. *P < 0.05.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Differential mRNA expression LV-shHNF1b VS LV-Ctrl (> 1.5 fold).

Gene_ID	log2Fold Change	GeneName
Upregulation		
ENSRNOG00000049138	4.4531	-/-
ENSRNOG00000042478	3.7787	Adam22
ENSRNOG00000026607	3.2274	Tnfsf18
ENSRNOG00000026415	2.8054	Col14a1
ENSRNOG00000030111	2.8024	Cyp11b2
ENSRNOG00000007041	2.601	Abcg2
ENSRNOG00000030763	2.5474	Dpp4
ENSRNOG00000014686	2.5119	Kcnd3
ENSRNOG00000023725	2.3159	-/-
ENSRNOG00000014835	2.2131	Il1rl1
ENSRNOG00000042971	2.1859	LOC363337
ENSRNOG00000046231	2.1292	Cacna1s
ENSRNOG00000003745	2.0778	Atf3
ENSRNOG00000001963	2.0617	Mx2
ENSRNOG00000008622	2.011	Creb5
ENSRNOG00000010183	1.9334	Fam198b
ENSRNOG00000008673	1.9019	Arpc3
ENSRNOG00000046985	1.8951	-/-
ENSRNOG00000020272	1.8733	5330417C22Rik
ENSRNOG00000016823	1.8551	-/-
ENSRNOG00000049177	1.8524	AABR07024972.1
ENSRNOG00000047124	1.8066	Atp2a1
ENSRNOG00000025994	1.7499	Krt80
ENSRNOG00000004296	1.7449	Nt5c1b
ENSRNOG00000037097	1.6978	Wfdc18
ENSRNOG00000004703	1.6381	Muc15
ENSRNOG00000015829	1.5999	Trpm1
ENSRNOG00000016390	1.5951	Eef1e1
ENSRNOG00000015036	1.5919	Ctgf
ENSRNOG00000009963	1.5914	Ctps1
ENSRNOG00000019022	1.5884	Fam89a
ENSRNOG00000004731	1.5546	Ano3
ENSRNOG00000015514	1.5409	Bcat1
ENSRNOG00000045895	1.5149	Dcp2
ENSRNOG00000048706	1.5029	Nox1
Downregulation		
ENSRNOG00000016000	-6.6314	Atp5f1
ENSRNOG00000014375	-3.834	Adgrb2

Gene_ID	log2Fold Change	GeneName
ENSRNOG00000049484	-3.7842	Atp9a
ENSRNOG00000008323	-3.6783	Pitpnm3
ENSRNOG00000049942	-3.6645	RGD1564899
ENSRNOG00000024536	-3.3828	Ccbe1
ENSRNOG00000020519	-3.2338	Olfm2
ENSRNOG00000031475	-3.1344	Col16a1
ENSRNOG00000004110	-3.1342	Trib2
ENSRNOG00000002364	-3.1027	Rnf112
ENSRNOG00000020459	-3.0466	-/-
ENSRNOG00000017208	-2.9617	Cspg4
ENSRNOG00000014156	-2.9073	Fut7
ENSRNOG00000003897	-2.8156	Col1a1
ENSRNOG00000022552	-2.7772	-/-
ENSRNOG00000020270	-2.7279	-/-
ENSRNOG00000027380	-2.7007	Upk1b
ENSRNOG00000016828	-2.684	Cmtm5
ENSRNOG00000004865	-2.673	Pkd1l1
ENSRNOG00000019985	-2.6607	Asic4
ENSRNOG00000014293	-2.5562	Nkd1
ENSRNOG00000013694	-2.3527	Ntng2
ENSRNOG00000013166	-2.3517	Wnt4
ENSRNOG00000007113	-2.3499	-/-
ENSRNOG00000013380	-2.3028	Rhov
ENSRNOG00000011750	-2.2965	Fam180a
ENSRNOG00000039388	-2.2796	Prmt4
ENSRNOG00000005046	-2.2186	Tspan13
ENSRNOG00000033835	-2.21	Dnm1
ENSRNOG00000022704	-2.2088	Esyt3
ENSRNOG00000004346	-2.2003	Notch3
ENSRNOG00000007377	-2.1931	Slit3
ENSRNOG00000019435	-2.1082	Psd
ENSRNOG00000025059	-2.1077	Nxph4
ENSRNOG00000017319	-2.0698	Mertk
ENSRNOG00000015175	-2.0597	-/-
ENSRNOG00000005872	-2.0353	Tcf7
ENSRNOG00000017528	-2.026	Gpr157
ENSRNOG00000014034	-1.993	Olfml2a
ENSRNOG00000020850	-1.9863	-/-
ENSRNOG00000008697	-1.9558	Nov
ENSRNOG00000005392	-1.9419	Ngfr
ENSRNOG00000025404	-1.9182	Dppa4
ENSRNOG00000019181	-1.9101	Synpo

Gene_ID	log2Fold Change	GeneName
ENSRNOG00000028624	-1.8982	Kif26b
ENSRNOG00000043249	-1.8864	LOC100360908
ENSRNOG00000013140	-1.8848	Pdzd2
ENSRNOG00000003114	-1.8744	B4galt4
ENSRNOG00000007657	-1.8687	Col27a1
ENSRNOG00000002316	-1.8333	-/-
ENSRNOG00000003386	-1.8181	Rbfox3
ENSRNOG00000020901	-1.7885	S1pr5
ENSRNOG00000030478	-1.7544	-/-
ENSRNOG00000019120	-1.7503	Hmgcs2
ENSRNOG00000000433	-1.7277	Prrt1
ENSRNOG00000046949	-1.7264	Kcnb1
ENSRNOG00000033330	-1.7168	Skida1
ENSRNOG00000019689	-1.7111	Vwf
ENSRNOG00000001156	-1.7095	Msi1
ENSRNOG00000023851	-1.7087	Igsf3
ENSRNOG00000019217	-1.7055	Adrb2
ENSRNOG00000000906	-1.7019	Medag
ENSRNOG00000003031	-1.7004	Atp2b4
ENSRNOG00000005861	-1.6857	Hsd11b1
ENSRNOG00000021536	-1.6712	Plxdc1
ENSRNOG00000024055	-1.6605	-/-
ENSRNOG00000021102	-1.6564	Scn1b
ENSRNOG00000005747	-1.6545	Il27ra
ENSRNOG00000015718	-1.6389	RGD1307461
ENSRNOG00000022624	-1.6371	-/-
ENSRNOG00000002208	-1.6369	Shroom3
ENSRNOG00000007886	-1.6366	Orm1
ENSRNOG00000003562	-1.6348	Susd4
ENSRNOG00000013333	-1.6325	-/-
ENSRNOG00000008534	-1.628	Dusp15
ENSRNOG00000009298	-1.6129	Fbxo44
ENSRNOG00000016838	-1.6058	Pla2g5
ENSRNOG00000019810	-1.6058	Des
ENSRNOG00000029366	-1.5998	Prrt2
ENSRNOG00000025587	-1.5972	Plagl1
ENSRNOG00000028526	-1.5963	Mansc1
ENSRNOG00000015408	-1.5945	Vil1
ENSRNOG00000020030	-1.589	Crif1
ENSRNOG00000017431	-1.5844	RGD1304884
ENSRNOG00000002331	-1.57	Aldh3a1
ENSRNOG00000002981	-1.5658	-/-

Gene_ID	log2Fold Change	GeneName
ENSRNOG00000011659	-1.5636	Alpk3
ENSRNOG00000004589	-1.5618	Galnt16
ENSRNOG00000028627	-1.5612	Hmcn1
ENSRNOG00000006741	-1.5566	Podn11
ENSRNOG00000007290	-1.5551	Atp1a2
ENSRNOG00000002215	-1.5535	Mylk
ENSRNOG00000020247	-1.5487	Cnih2
ENSRNOG00000024931	-1.547	Ccdc88b
ENSRNOG00000049437	-1.5372	Gpc1
ENSRNOG00000003935	-1.536	-/-
ENSRNOG00000002946	-1.5321	Socs3
ENSRNOG00000016957	-1.5275	Igfbp2
ENSRNOG00000020482	-1.5275	Nfatc4
ENSRNOG00000016687	-1.5214	Ssc5d
ENSRNOG00000019850	-1.5141	Speg
ENSRNOG00000018789	-1.5121	-/-
ENSRNOG00000007199	-1.5102	-/-
ENSRNOG00000013141	-1.5043	Eno2