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ORIGINAL RESEARCH

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Knockdown of HNF1A improves type 2 diabetes combined with non-alcoholic fatty liver and glucose and lipid metabolism disorders by modulating the PI3K/AKT/mTOR signaling pathway

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is one abnormal buildup of fat within the liver, independent of excessive alcohol intake. In type 2 diabetes, the presence of NAFLD can exacerbate chronic kidney diseases and mortality in patients. Hepatocyte nuclear factor 1 homeobox A (HNF1A) predominantly expressed in the liver, owns one crucial role in liver development, function and tumorigenesis. However, the precise regulatory role of HNF1A on the progression of type 2 diabetes combined with NAFLD keep dimness. This investigation uncovered that HNF1A levels, both in protein and mRNA expressions, were elevated in high-fat diet plus hyperglycemia (HFG) mice. Furthermore, liver steatosis was strengthened in the HFG group, which was mitigated following the HNF1A inhibition. Knockdown of HNF1A ameliorated glucose and lipid metabolism disorders in HFG mice. Lastly, the study observed an stimulation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway in HFG mice, but this change was neutralized uponHNF1A silencing. In conclusion, knockdown of HNF1A improved type 2 diabetes combined with NAFLD, as well as disorders in glucose and lipid metabolism, and retarded the PI3K/AKT/mTOR signaling pathway. These finding demonstrated that HNF1A may be one serviceable target for ameliorating type 2 diabetes combined with NAFLD.

Keywords

Type 2 diabetes; HNF1A; Non-alcoholic fatty liver; PI3K/AKT/mTOR pathway

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is featured by liver steatosis that develops with no excessive alcohol consumption or other liver disease [1]. Globally, it is estimated that approximately 25% population in global is afflicted with NAFLD [2]. NAFLD can result into fatty liver injury, which can generate non-alcoholic steatohepatitis (NASH) and ultimately to hepatocellular carcinoma [3]. NAFLD is closely linked to the onset of metabolic disorders, containing insulin resistance, obesity, type 2 diabetes and inflammation [4]. The morbidity of NAFLD in type 2 diabetes patients is present in >70% [5]. The coexistence of type 2 diabetes and NAFLD exacerbates metabolic dysfunction, liver failure, and the happen of cardiovascular diseases [6]. Therefore, there is an urgent requirement for effective treatments or molecular targets to mitigate the complications of type 2 diabetes in conjunction with NAFLD.

Hepatocyte nuclear factor 1 homeobox A (HNF1A) has been discovered to exist in the liver, and participate into the development, function, and tumor growth of liver [7]. Numerous studies have investigated the regulatory functions of HNF1A in both the pancreas and liver. For instance, HNF1A has been shown to inhibit pancreatic cancer by promoting the differentiation of acinar cells through recruiting lysine demethylase 6A (KDM6A) [8]. HNF1A also exhibits specific target in modulating pancreatic beta cells and hepatocytes [9]. Furthermore, HNF1A mutations are associated with a subset of hepatocellular adenomas that exhibit tumor steatosis [10]. Moreover, HNF1A can mitigate steatohepatitis by triggering the degradation of TANK-binding kinase 1 (TBK1) and dampening the innate immune response [11]. In addition, it inhibits the transcription of peroxisome proliferator-activated receptor γ (PPARγ), thereby restraining liver cancer associated with steatosis [12]. Besides, homozygous HNF1A-deficient mice own growth retardation and hepatomegaly [13]. Importantly, HNF1A mutations have been implicated in abnormal blood glucose levels in diabetes mellitus [14]. But, the precise regulatory impacts of HNF1A on the progression of type 2 diabetes complicated by NAFLD remain unclear and warrant further investigations.

In conclusion, this work was subjected to explore the regulatory functions of HNF1A in type 2 diabetes combined

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with NAFLD and associated pathway. The findings may supply valuable opinions into the potential of HNF1A as a therapeutic target for improving the management of type 2 diabetes combined with NAFLD.

2. Materials and methods

2.1 Animal model

The C57BL/6J mice (male, n = 24, 6 weeks) were offered by Vital River (Beijing, China).

In the HFG group, a total of 18 mice were kept with a high-fat diet (HFD, 58Y1, 60 kcal% fat, TestDiet, MO, USA) for eight weeks to trigger obesity, followed by intraperitoneal injections of streptozotocin (STZ, 40 mg/kg, 2 injections, S0130, Sigma; St. Louis, MO, USA) to trigger hyperglycemia (>300 mg/dL) [15]. The HFD/STZ mouse model was utilized to mimic late-stage type 2 diabetes. Post one week, 6 mice were randomly selected in the HFD + hyperglycemia (HFG) group. The siRNAs-HNF1A (si-HNF1A, A10003, Gene Parma, Shanghai, China) or negative control (si-NC, A06001, Gene Parma, Shanghai, China) was transfected through Lipofectamine 2000 (11668019, Invitrogen, Carlsbad, CA, USA). The HFG mice plus si-NC were the HFG + si-NC group (n = 6), and HFG mice plus si-HNF1A were the HFG + si-HNF1A group (n = 6). For the sham group, six mice were maintained on a normal, unrestricted diet without any treatment. After eight weeks, all mice were euthanized. Blood and liver samples were collected for subsequent experiments, and livers were weighted.

2.2 Western blot

Proteins were extracted from liver tissues using radio immunoprecipitation assay (RIPA) buffer. Next, 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed for the separation of proteins, then migrating proteins to polyvinylidene difluoride (PVDF) membranes (Beyotime, Shanghai, China). Following membrane sealing, the primary antibodies were placed for 12 hours incubation, followed by incubation with the secondary antibodies (1/2000; ab7090) for 2 hours. Eventually, protein expression was visualized through one chemiluminescence detection kit (34577, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The primary antibodies:

HNF1A (1/1000; ab272693; Abcam, Shanghai, China), p-mTOR (1/1000; ab109268), mTOR (1/1000; ab32028), p-AKT (1/500; ab38449), AKT (1/500; ab8805), p-PI3K (1/1000; ab235266), PI3K (1/1000; ab86714) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1/1000; ab8245).

2.3 RT-qPCR (reverse transcription-quantitative polymerase chain reaction)

The RNAs by the TRIzol reagent (15596018, Invitrogen, Carlsbad, CA, USA) was extracted from liver tissues. The PrimeScript™ RT Master Mix kit (RR036A, Takara, Dalian, China) was employed for generating cDNA from RNAs. The SYBR Premix Ex Taq™ (RR820A, Takara, Dalian, China) was adopted for qPCR. Lastly, the HNF1A mRNA expression was determined under the $2^{-\Delta\Delta Ct}$ method.

The primer sequences:

HNF1A: forward, 5′-GCCCCTTCATGGCAACCA-3′, and reverse, 5′-CTCTCCCAGGCCAACGT-3′;

GAPDH: forward, 5′-GCACCGTCAAGCTGAGAAC-3′, and reverse, 5′-TGGTGAAGACGCCAGTGGA-3′.

2.4 Detection of insulin, glucose and HOMA-IR

The insulin level was measured using an insulin Enzyme-Linked Immunosorbent Assay (ELISA) kit (ab277390, Abcam, Shanghai, China). Blood glucose was determined under the FreeStyle Lite blood glucose meter (Abbot Diabetes Care, Inc., South Loop Road Alameda, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated: HOMA-IR = (Insulin (μU/mL) × Glucose (mmol/L))/22.5.

2.5 H&E staining

Liver tissues were fixed using 10% formalin (F8775, Sigma-Aldrich, St. Louis, MO, USA) before being processed. Next, the 4-μm sections of liver tissues were mixed with hematoxylin & eosin (H&E) staining solution. H&E images were then determined under a microscope (BX41, Olympus Corporation, Tokyo, Japan).

2.6 Detection of blood biochemistry

Blood samples were subjected to centrifugation, and the resulting plasma was used to assess the concentrations of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), triacylglycerol (TG), total cholesterol (TCHO) and high-density lipoprotein (HDL) under the fully automatic dry biochemical analyzer (Fuji DRI-CHEM 4000, Fujifilm, Tokyo, Japan).

2.7 Statistical analysis

The GraphPad Prism Software 9 (GraphPad Software, San Diego, CA, USA) was employed for statistical analysis. Data were displayed as mean ± standard deviation (SD). The student's *t*-test or one-way analysis of variance (ANOVA) was utilized for differences analysis. The $p < 0.05$ was set as statistically significant.

3. Results

3.1 HNF1A owned the uplifted expression in HFG mice

The HNF1A protein expression was notarized to be higher in HFG mice ($p < 0.01$, $F = 2.544$) (Fig. 1A). Moreover, the HNF1A mRNA expression was also elevated in HFG group ($p < 0.01$, $F = 110.1$) (Fig. 1B). In short, HNF1A existed the elevated expression in HFG mice.

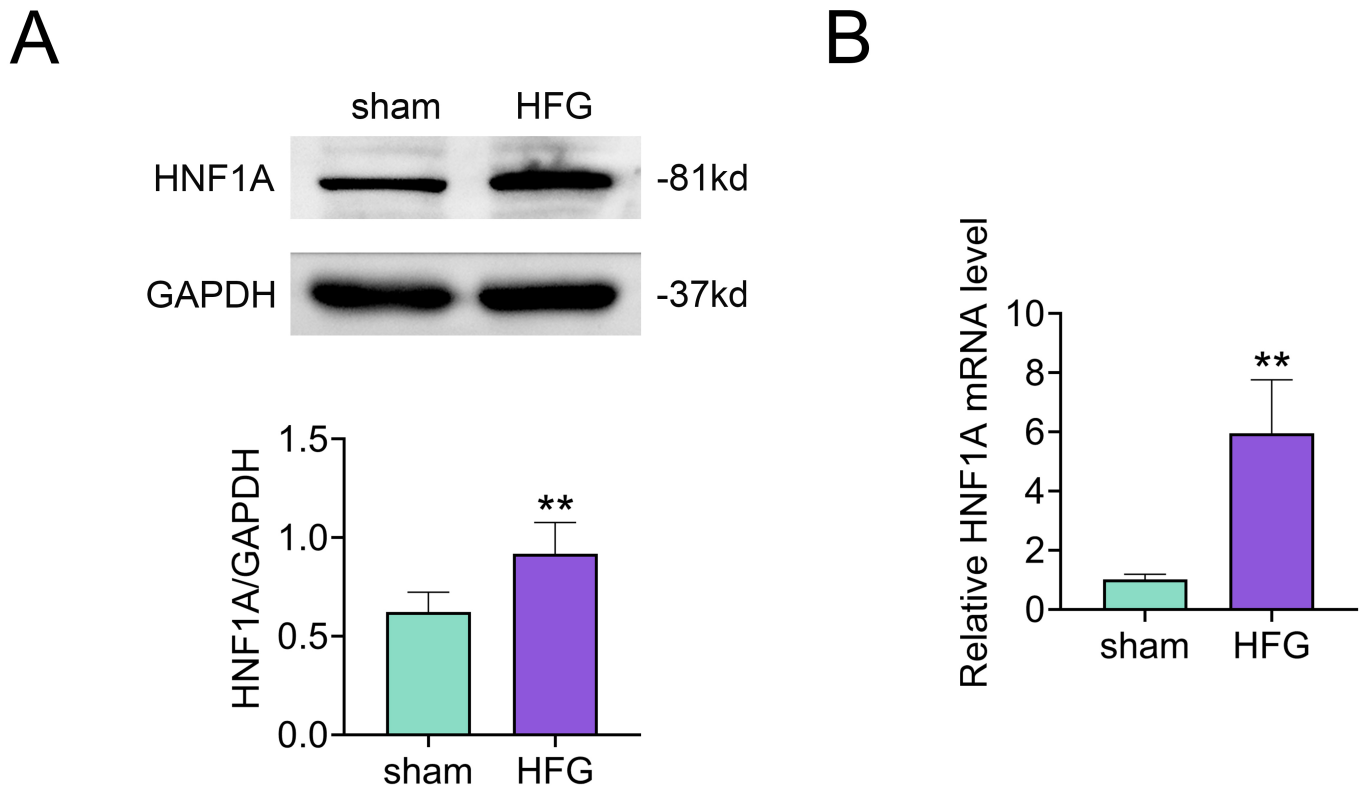

FIGURE 1. HNF1A exhibited the elevated expression in HFG mice. (A) The protein expression of HNF1A was verified in the sham and HFG groups by western blot. (B) The mRNA expression of HNF1A was examined in the sham and HFG groups by RT-qPCR. $^{**}p < 0.01$. HNF1A: Hepatocyte nuclear factor 1 homeobox A; HFG: high-fat diet plus hyperglycemia; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

3.2 Suppression of HNF1A relieved fatty liver in HFG mice

The inhibition capacity of si-HNF1A was displayed in Fig. 2A ($p < 0.01$, $F = 10.21$). HFG mice exhibited an increase in liver weight ($p < 0.01$, $F = 2.282$), but this change was attenuated after HNF1A suppression ($p < 0.01$, $F = 6.980$) (Fig. 2B). Additionally, H&E staining revealed significant microvesicular and macrovesicular steatosis in the HFG group, which was alleviated upon HNF1A inhibition (Fig. 2C). Moreover, the insulin level was descended, the glucose level was ascended as well as the HOMA-IR was enhanced in HFG mice, but these influences were reversed after HNF1A knockdown (Fig. 2D). Taken together, the suppression of HNF1A alleviated fatty liver symptoms in HFG mice.

3.3 Knockdown of HNF1A ameliorated glucose and lipid metabolism disorders in HFG mice

The elevated levels of GOT and GPT in the bloodstream signal liver injury, while the increased levels of TG, TCHO, and HDL suggest the presence of dyslipidemia. It was demonstrated that GOT, GPT, TG, TCHO and HDL levels were all augmented in HFG mice, but these effects were offset after HNF1A restriction ($p < 0.01$) (Fig. 3). In a word, the reduction of HNF1A expression improved the disorders of glucose and lipid metabolism in HFG mice.

3.4 Silencing of HNF1A retarded the PI3K/AKT/mTOR pathway

The markedly augmented protein expression levels of p-mTOR/mTOR, p-AKT/AKT and p-PI3K/PI3K were existed in HFG mice, but these impacts were neutralized after silencing HNF1A ($p < 0.01$) (Fig. 4). Overall, the inhibition of HNF1A resulted in the suppression of the PI3K/AKT/mTOR pathway.

4. Discussion

HNF1A has been elaborated to exhibit regulatory impacts in diversiform diseases [10–14]. Nevertheless, its specific regulatory role in the context of type 2 diabetes complicated by NAFLD is not fully investigated. In this work, it was revealed that HNF1A exhibited the elevated protein and mRNA expressions in HFG mice.

Liver steatosis is a critical feature of type 2 diabetes combined with NAFLD [16]. Extensive research has been conducted on modulating liver steatosis to improve this condition. For instance, semaglutide has been shown to against hepatic steatosis by targeting the miR-5120/ABHD6 pathway in mice with type 2 diabetes combined with NAFLD [17]. Traditional Mongolian medicine has also been reported to reduce liver steatosis in this condition [18]. In addition, circ0004535/miR-1827/CASP8 axis participates into the improvement of liver steatosis in type 2 diabetes combined with NAFLD [19]. Luseogliflozin has also demonstrated efficacy in

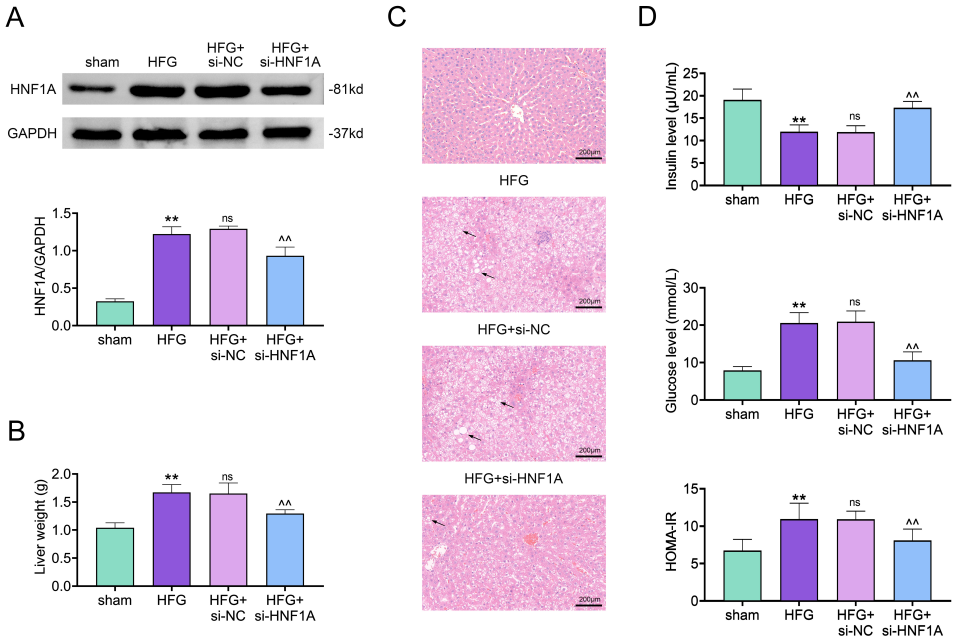

FIGURE 2. Suppression of HNF1A relieved fatty liver in HFG mice. Groups were separated into the sham, HFG, HFG + si-NC and HFG + si-HNF1A group. (A) The knockdown efficiency of HNF1A was ascertained by western blot. (B) The liver weight was tested. (C) The pathological changes of liver were evaluated by H&E staining. (D) The insulin, glucose and HOMA-IR was confirmed. $^{**}p < 0.01$ *vs.* the sham group; $^{\wedge\wedge}p < 0.01$ *vs.* the HFG + si-NC group; no significance (ns): the HFG group *vs.* the HFG + si-NC group. HNF1A: Hepatocyte nuclear factor 1 homeobox A; HFG: high-fat diet plus hyperglycemia; si-NC: si-negative control; HOMA-IR: homeostasis model assessment of insulin resistance; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

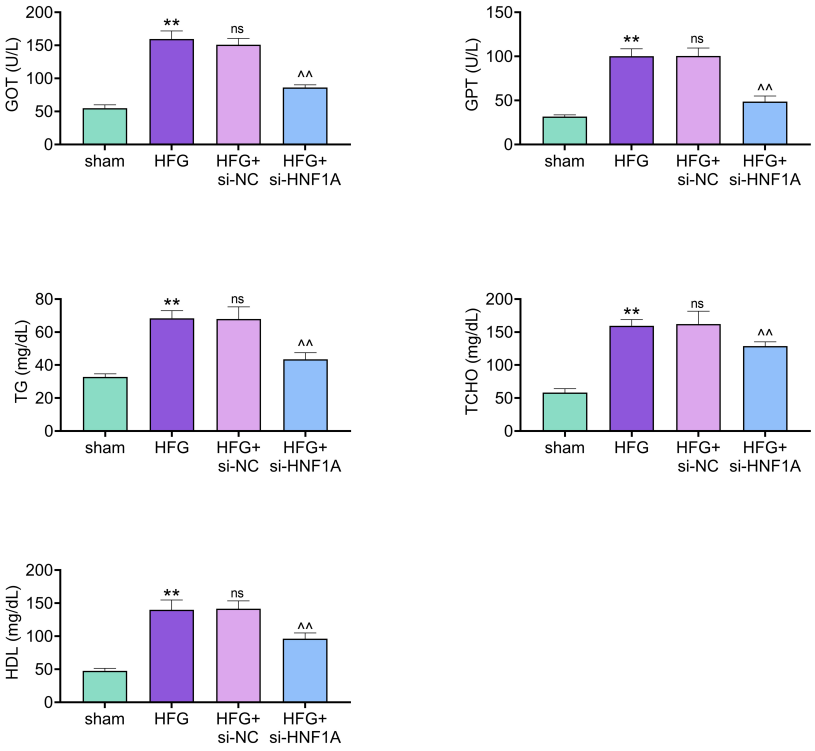

FIGURE 3. Knockdown of HNF1A ameliorated glucose and lipid metabolism disorders in HFG mice. Groups were separated into the sham, HFG, HFG + si-NC and HFG + si-HNF1A group. The GOT, GPT, TG, TCHO and HDL levels in blood were measured. $^{**}p < 0.01$ *vs.* the sham group; $^{\wedge\wedge}p < 0.01$ *vs.* the HFG + si-NC group; no significance (ns): the HFG group *vs.* the HFG + si-NC group. GOT: glutamate oxaloacetate transaminase; GPT: glutamate pyruvate transaminase; TG: triacylglycerol; TCHO: total cholesterol; HDL: high-density lipoprotein.

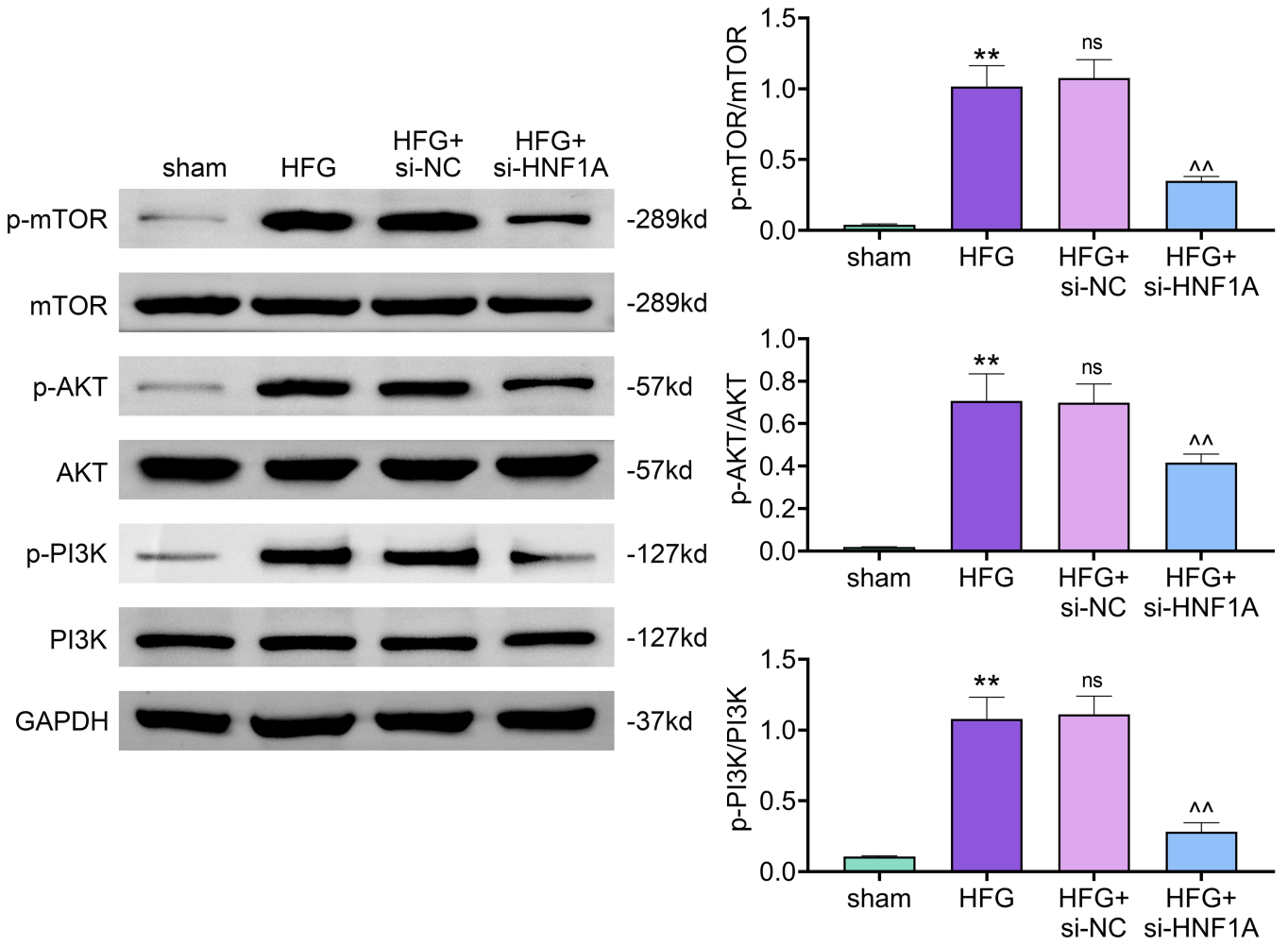

FIGURE 4. Silencing of HNF1A retarded the PI3K/AKT/mTOR pathway. Groups were separated into the sham, HFG, HFG + si-NC and HFG + si-HNF1A group. The protein expressions of p-mTOR, mTOR, p-AKT, AKT, p-PI3K and PI3K were ascertained by western blot. $^{**}p < 0.01$ *vs.* the sham group; $^{\wedge\wedge}p < 0.01$ *vs.* the HFG + si-NC group; no significance (ns): the HFG group *vs.* the HFG + si-NC group. HFG: high-fat diet plus hyperglycemia; si-NC: si-negative control; HNF1A: Hepatocyte nuclear factor 1 homeobox A; p-mTOR: phosphorylation-mammalian target of rapamycin; p-AKT: phosphorylation-protein kinase B; p-PI3K: phosphorylation-phosphatidylinositol 3-kinase.

mitigating liver fat accumulation in these mice [20]. Similarly, in this study, it was also demonstrated that liver steatosis was strengthened in the HFG group, but this effect was counteracted after HNF1A inhibition. Knockdown of HNF1A in HFG mice resulted in an improvement of disorders in glucose and lipid metabolism.

The PI3K/AKT/mTOR pathway is the pivotal pathway to join into the progression of multifarious diseases, including type 2 diabetes and NAFLD. For instance, a synthetic analogue of pioglitazone has been shown to modulate this pathway to alleviate diabetes mellitus induced by streptozotocin [21]. Resolvin D1 has also been found to affect the PI3K/AKT/mTOR pathway to retard nicotinamide-streptozotocin-triggered type 2 diabetes mellitus [22]. Additionally, S1PR2 has been implicated in regulating this pathway to promote the progression of NAFLD-related hepatocellular carcinoma [23]. Besides, overexpression of HIF-2α targets the PI3K/AKT/mTOR pathway, exacerbating the progression of NAFLD-related hepatocellular carcinoma [24]. Scoparone has also been identified as a compound that targets this pathway to improve hepatic inflammation and autophagy in non-alcoholic steatohepatitis [25]. Consistent with these studies, our research uncovered that the PI3K/AKT/mTOR pathway was evoked in HFG mice, a phenomenon that was attenuated following the silencing of HNF1A.

5. Conclusions

Our findings reveal that knockdown of HNF1A enhances the condition of type 2 diabetes complicated by NAFLD, and ameliorates disorders in glucose and lipid metabolism, while also inhibiting the PI3K/AKT/mTOR signaling pathway. Nevertheless, this project is not without its limitations, including the absence of additional phenotypic assessments, cell culture models, other animal models, and clinical studies. Future research will delve into the broader impacts of HNF1A and the underlying molecular regulatory mechanisms in type 2 diabetes complicated by NAFLD. The deeply explorations will offer novel understandings in clinical treatment for type 2 diabetes complicated with NAFLD.

ABBREVIATIONS

NAFLD, Non-alcoholic fatty liver disease; HNF1A, Hepatocyte nuclear factor 1 homeobox A; HFG, high-fat diet plus hyperglycemia; NASH, non-alcoholic steatohepatitis; HFD, high-fat diet; STZ, streptozotocin; RT-qPCR, Reverse Transcription-quantitative Polymerase Chain Reaction; H&E, hematoxylin & eosin; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; TG, triacylglycerol; TCHO, total cholesterol; HDL, high-density lipoprotein; SD, standard deviation; ANOVA, one-way analysis of variance.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

GXL—designed the study and carried them out. GXL, XXJ, HLG, QYS, LZ, ZZL and ZGQ—supervised the data collection, analyzed the data, interpreted the data. GXL, GDZ and XHJ—prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of Suzhou Ninth People's Hospital (Suzhou Ninth Hospital Affiliated to Soochow University) (Approval no. KY2024-003-01). The patients provided informed consent and agreed to publication of the details of this research.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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