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MiR-542-5p Inhibits Hyperglycemia and Hyperlipoidemia by Targeting FOXO1 in the Liver

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Purpose: This research was designed to investigate how miR-542-5p regulates the progression of hyperglycemia and hyperlipoidemia.

Materials and Methods: An in vivo model with diabetic db/db mice and an in vitro model with forskolin/dexamethasone (FSK/DEX)-induced primary hepatocytes and HepG2 cells were employed in the study. Bioinformatics analysis was conducted to identify the expression of candidate miRNAs in the liver tissues of diabetic and control mice. H&E staining revealed liver morphology in diabetic and control mice. Pyruvate tolerance tests, insulin tolerance tests, and intraperitoneal glucose tolerance test were utilized to assess insulin resistance. ELISA was conducted to evaluate blood glucose and insulin levels. Red oil O staining showed lipid deposition in liver tissues. Luciferase reporter assay was used to depict binding between miR-542-5p and forkhead box O1 (FOXO1).

Results: MiR-542-5p expression was under-expressed in the livers of db/db mice. Further in vitro experiments revealed that FSK/DEX, which mimics the effects of glucagon and glucocorticoids, induced cellular glucose production in HepG2 cells and in primary hepatocytes cells. Notably, these changes were reversed by miR-542-5p. We found that transcription factor FOXO1 is a target of miR-542-5p. Further in vivo study indicated that miR-542-5p overexpression decreases FOXO1 expression, thereby reversing increases in blood glucose, blood lipids, and glucose-related enzymes in diabetic db/db mice. In contrast, anti-miR-542-5p exerted an adverse influence on blood glucose and blood lipid metabolism, and its stimulatory effects were significantly inhibited by sh-FOXO1 in normal control mice.

Conclusion: Collectively, our results indicated that miR-542-5p inhibits hyperglycemia and hyperlipoidemia by targeting FOXO1.

Key Words: miR-542-5p, forkhead box O1, diabetes, hyperglycemia, hyperlipoidemia.

INTRODUCTION

The liver regulates the synthesis and metabolism of glucose and lipids to maintain carbohydrate and lipid homeostasis.[1] Studies have revealed that hepatic lipid metabolism is regulated by a delicate interplay among hormones, nuclear receptors, intracellular signaling pathways, and transcription factors.[2] MicroRNAs (miRNAs), a group of small non-coding RNAs, have been shown to regulate the proliferation, migration, invasion, and apoptosis of cells.[3] Accumulating evidence has revealed that miRNAs, including miR-122, miR-802, miR-26, and miR-676, can regulate insulin resistance, glucose tolerance, inflammatory process, and/or hepatic steatosis in the liver:[4-8] for example, hepatic metabolic proteins, such as PGC-1α, PPARα, and p110α, are regulated by miRNAs, leading to altered glucose and lipid metabolism.[4,9-11] However, the role of miRNAs in glucose metabolism remains largely unknown. Although bioinformatics analysis has revealed that miR-542-5p is under-expressed in diabetic mice, compared to control mice, it remains unknown whether miR-542-5p regulates glucose and lipid metabolism.

Forkhead box O1 (FOXO1) is known to regulate glucose homeostasis.[12] Therein, insulin activates the IR/PI3K/Akt pathway, thereby initiating the phosphorylation of FOXO1, which contributes to the nuclear exclusion and transcriptional suppression

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of FOXO1. To promote gluconeogenesis during fasting, FOXO1 induces the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) in the liver.[13] Mice with hepatic FOXO1 overexpression exhibit hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and hepatic steatosis.[4,14] Furthermore, FOXO1 targets lipogenesis-responsible genes, including sterol-response-binding-protein-1c (SREBP-1c) and fatty acid synthase (FAS).[14-16] SREBP-1c is translocated into the nucleus to bind with FAS promoter at serum response elements after proteolytic cleavage. Additionally, the expression of FAS and SREBP-1c has been found to be elevated with NAFLD overexpression.[17] Interestingly, miR-451 has been found to modulate the Akt-FOXO1-PEPCK/G6Pase pathway to regulate hepatic gluconeogenesis and glucose homeostasis.[18]

In this study, we show that miR-542-5p is under-expressed in the liver tissues of diabetic mice. We confirmed that FOXO1 is a downstream target of miR-542-5p in the development of hyperglycemia and insulin resistance through in vitro and in vivo experiments. The present findings may offer new pharmacological target for the prevention of hyperglycemia and insulin resistance.

MATERIALS AND METHODS

Bioinformatics analysis

GEO2R analysis was performed via the Omics website (http://www.omicsclass.com/article/209). Gene expression omnibus database analysis was performed as suggested in NCBI.

Cell culture

The primary culture of mice hepatocytes was performed as previously described.[19] Briefly, a two-step collagenase perfusion technique was used. At first, calcium-free medium containing a calcium chelator was perfused through the liver. Afterwards, collagenase was introduced into the liver lobes. Cells were cultured in DMEM medium containing 10% FBS, 7 ng/mL of glucagon, 0.5 U/mL of insulin, and 1% antibiotics. AML12, a mouse hepatocyte cell line, was grown in DMEM/F-12 medium (Gibco, Grand Island, NY, USA) supplemented with 10% FBS, 1×insulin-transferrin-seleniums (Gibco), 2 mmol/L of glutamine and 40 ng/mL of dexamethasone. HepG2 cells, human liver cells, were cultured in DMEM medium (Gibco) containing 10% fetal bovine serum (FBS) and 233.6 mg/mL of glutamine at 37°C, 5% CO_2.[20] Forskolin (FSK, 10 μM, Sigma-Aldrich, St. Louis, MO, USA) and dexamethasone (DEX, 100 nM, Sigma-Aldrich) were applied to mimic the action of glucagon and glucocorticoids as described previously.[14] Cell transfection was implemented using Lipofectamine 2000 reagent (Invitrogen, Waltham, MA, USA) in accordance with the manufacturer's protocol.

Animal model

Sixteen-week-old male diabetic db/db and control db/m mice were purchased from Shanghai SLACS Co., Ltd (Shanghai, China). All animal experiments conformed with the Guide for the Care and Use of Laboratory Animals, were approved by the Ethics Review Board of Xixi Hospital of Hangzhou, and conformed to the principles and regulations as described previously.[21] The mice were housed at a constant room temperature (23±2°C) and relative humidity (50±10%) at a fixed 12-h light/dark. The mice were given free access to food and water and were allowed to accommodate to the new environment for 2 weeks before experimentation.

miR-542-5p mimics, inhibitor, and the construction and transfection of adenovirus overexpressing miR-542-5p/FOXO1 3'UTR

The relative sequences are as follows: miR-542-5p mimic: 5'-UCGGGGAUCAUCAUGUCACGAGA-3', miR-542-5p inhibitor: 5'-UCUCGUGACAACACAUCCCCGA-3'. sh-FOXO1: 5'-GGAGGUAUGAGUCAGUAUAUU-3'. The miR-542-5p mimic and miR-542-5p inhibitor were synthesized by Sangon Biotech (Shanghai, China). The adenovirus overexpressing miR-542-5p and FOXO1 3' untranslated region (3'UTR) expression system were constructed into pmirGLO (Promega, Madison, WI, USA) by Hanbio Co., Ltd (Shanghai, China). The transfection was performed as described previously.[22]

Luciferase assay

Luciferase reporter assay was conducted according to a previous study.[23] Briefly, prior to transfection, the 3'UTR of FOXO1 and the mutated 3'UTR were amplified. 100 nM miR-542-5p mimic or NC mimics were added to each well in 12-well plates. Cell lysates were prepared for measurement of luciferase activity after treatment for 48 h.

RNA isolation and quantitative reverse transcription-quantitative polymerase chain reaction

Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's protocol, and Premix Ex Taq DNA polymerase for qPCR (RR039B, Takara, Tokyo, Japan) was applied. The RT primer for miR-542-5p was 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAUCUCGU-3'. qPCR primers were as follows: miR-542-5p, F: 5'-TCGGGGATCATCATGTC-3', R: 5'-GTGCAGGGTCCGAGGT-3'; FOXO1, F: 5'-CCACATTCAACAGGCAGCAG-3', R: 5'-CCATCCACATCGAGGCTCC-3'. GAPDH and U6 served as internal references. All sequences were synthesized by Sangon Biotech (Shanghai).

H&E staining

After sacrificing the mice, 4% buffered paraformaldehyde was used to fix the liver tissues for 24 h. Afterwards, paraffin was used to embed tissues, and 5-μm thick continuous sections were obtained.

Triglyceride, glucose, and insulin analysis

After treatment, liver tissues were collected and weighed. Hepatic and plasma liver triglyceride (TG) levels were measured using commercial kits from Cayman Chemical (Ann Arbor, MI, USA). Intraperitoneal injection (1.5 mg/g of body weight) of sodium pyruvate (Sigma-Aldrich) after a 16-h fast was applied for pyruvate tolerance tests (PTT). Regular human insulin (1.0 U/kg of body weight, Sigma-Aldrich) was injected into mice after a 6-h fast for insulin tolerance tests (ITT). Intraperitoneal glucose tolerance test (IPGTT) was conducted by introducing glucose (2 mg/g of body weight) by intraperitoneal injection. A portable blood glucose meter (Sigma-Aldrich) was applied to determine blood glucose levels. Commercial ELISA kits from R&D systems (Minneapolis, MN, USA) were purchased and used to determined plasma insulin levels.

Western blotting

After cell samples were boiled for 5 min, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to separate total protein equivalents. After being transferred to polyvinylidene fluoride membranes, cells were incubated with the following primary antibodies: FOXO1 antibody (ab52857, Abcam, Cambridge, MA, USA), PEPCK (PA5-18426, Thermo Fisher, Waltham, MA, USA), G6Pase (ab83690, Abcam), SREBP-1c (ab28481, Abcam), acetyl-CoA acetyltransferase 2 (Acat2, ab131215, Abcam), lecithin-cholesterol acyltransferase (LCAT, ab51060, Abcam), and FAS (ab22759, Abcam), followed by a HRP-conjugated secondary antibody. Primary anti-GAPDH antibody (sc-32233, Santa Cruz Biotechnology, Dallas, TX, USA) served as the internal control. Signals were detected using enhanced chemiluminescence (Bio-Rad, Hercules, CA, USA) and were evaluated with ImageJ software (1.51K).

Statistical analysis

Data are presented as a mean±standard deviation. Differences were analyzed by unpaired two-tailed Student's t-test and one-way/two-way analysis of variance with Tukey's post hoc test. Differences were considered significant when $p<0.05$.

RESULTS

MiR-542-5p is under-expressed in diabetic mice

Based on the GSE17035 dataset, the expression profiles of miRNAs in the livers of diabetic and control mice were identified. Screened by the condition that logFC values were less than -2, miR-542-5p, miR-879, and miR-222 were selected. As shown in Fig. 1A, bioinformatics analysis in GSE17035 showed that miR-542-5p was under-expressed in the liver tissue of diabetic mice. H&E staining in Fig. 1B revealed increased cell sizes in the livers of db/db mice, compared to db/m mice, confirming that db/db mice could be used in further experiments. We then detected expression profiles of miR-542-5p, miR-879, and miR-222 in mice livers. Only miR-542-5p was significantly under-expressed

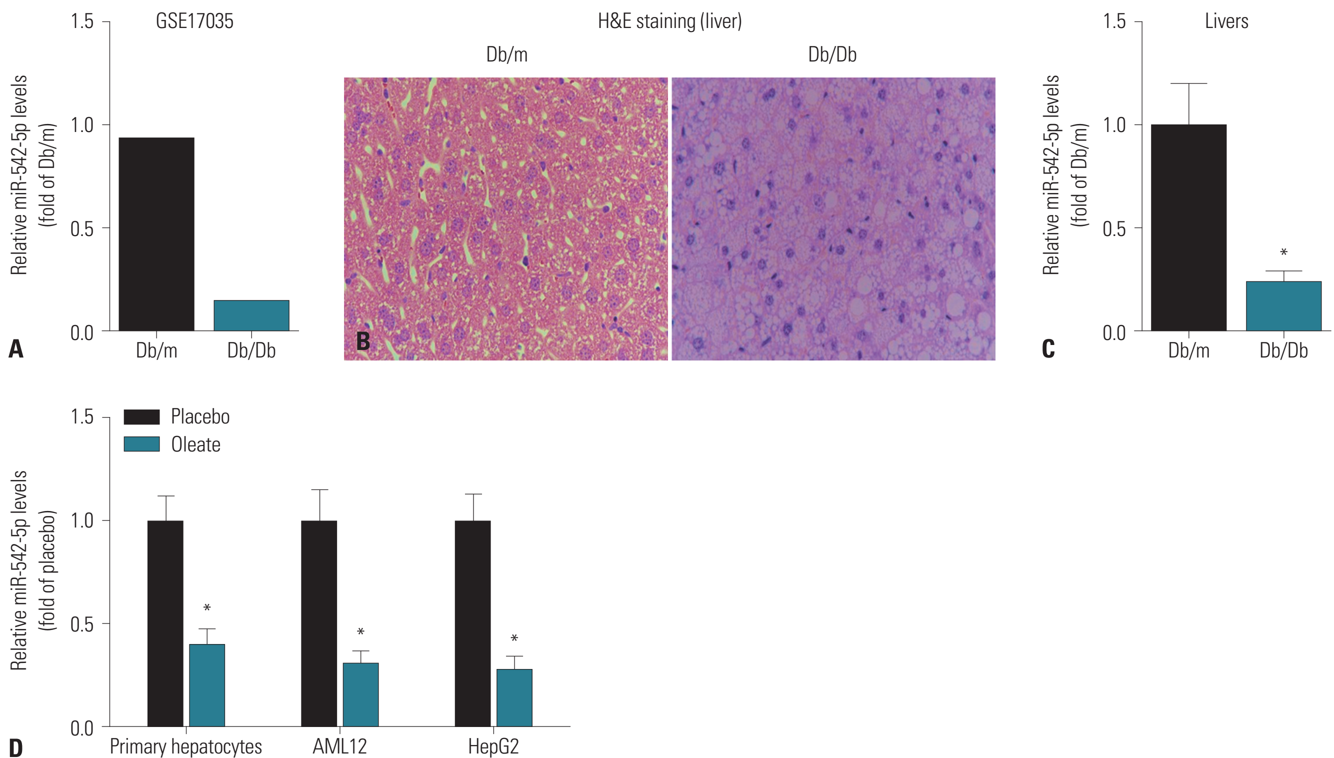

Fig. 1. MiR-542-5p is under-expressed in the liver of db/db mice. (A) MiR-542-5p expression in mice livers in GEO2R microarray analysis. (B) H&E staining highlights morphological changes in the liver of diabetic and control mice. (C) MiR-542-5p expression in the liver of db/db and db/m mice. *$p<0.05$, compared with that in db/m group. (D) The influence of oleate on miR-542-5p expression in primary hepatocytes. n=5–6. *$p<0.05$ vs. db/m group.

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in db/db mice relative to control mice (Fig. 1C, Supplementary Fig. 1A and B, only online). Thus, miR-542-5p was established as the research subject and was explored in the following experimental assays. Furthermore, oleate, the major physiological toxic free fatty acid for human β cells, reduced miR-542-5p content in primary hepatocytes, AML12, and HepG2 cells (Fig. 1D).

Involvement of miR-542-5p in glucose metabolism in hepatocytes and in HepG2 cells

We then explored whether miR-542-5p regulated glucose and lipid metabolism. Fig. 2A shows that transfection of miR-542-5p mimics and miR-542-5p inhibitor worked efficiently, significantly increasing and decreasing miR-542-5p expression, respectively, in primary hepatocytes and in HepG2 cells. Fig. 2B and C shows that FSK/DEX enhanced cellular glucose. This effect was significantly decreased by miR-542-5p, while anti-miR-542-5p further enhanced cellular glucose in primary hepatocytes and in HepG2 cells. Moreover, FSK/DEX significantly increased PEPCK levels, and this effect was significantly decreased by miR-542-5p. Meanwhile, anti-miR-542-5p significantly enhanced the effect of FSK/DEX on PEPCK protein levels in hepatocytes (Fig. 2D and E) and HepG2 cells (Fig. 2F and G). Similarly, FSK/DEX significantly increased G6Pase protein levels, which significantly decreased by miR-542-5p overexpression. The down-

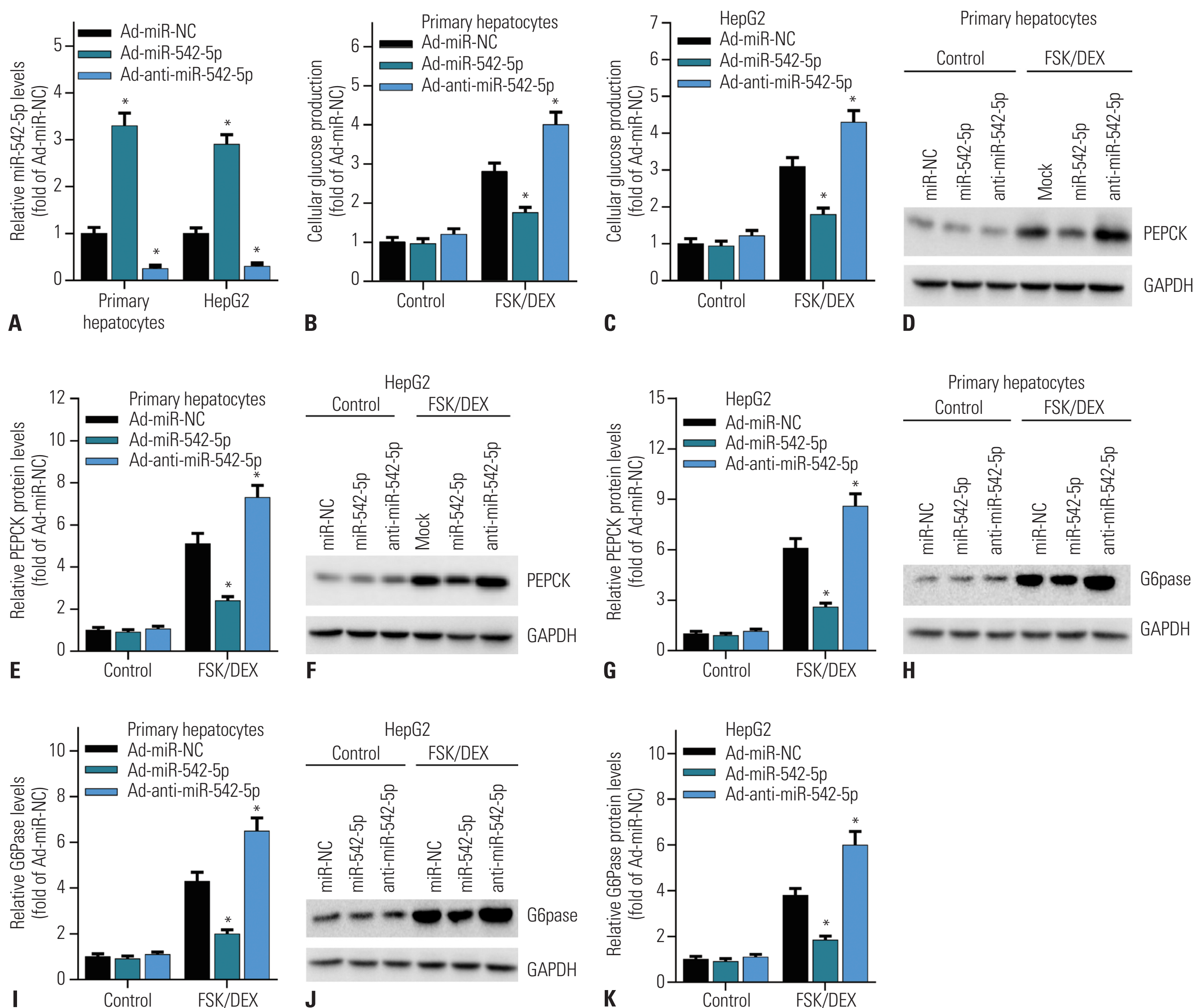

Fig. 2. MiR-542-5p regulates glucose production in hepatocytes. (A) The overexpression and knockdown efficiency of miR-542-5p in primary hepatocytes and HepG2 cells. The influences of overexpressed and inhibited miR-542-5p on glucose content in primary hepatocytes (B) and in HepG2 cells (C) were detected by ELISA. Western blot (D) and quantitative data (E) demonstrate the influence of miR-542-5p and anti-miR-542-5p on FSK/DEX-induced PEPCK expression in primary hepatocytes. Western blot analysis (F and G) shows the influences of overexpressed and inhibited miR-542-5p on FSK/DEX-induced PEPCK expression in HepG2 cells. Western blot analysis (H and I) reflects the influences of overexpressed and inhibited miR-542-5p on FSK/DEX-induced G6Pase expression in primary hepatocytes. Western blot analysis (J and K) demonstrates the influences of overexpressed and inhibited miR-542-5p on FSK/DEX-induced G6Pase expression in HepG2 cells. n=5–6. *$p<0.05$ vs. Ad-Mock group in the same treatment. FSK, forskolin; DEX, dexamethasone, PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose 6-phosphatase.

regulation of miR-542-5p significantly enhanced G6Pase protein levels in primary hepatocytes (Fig. 2H and I) and HepG2 cells (Fig. 2J and K). Also, we detected the expression of lipid metabolism-related proteins, including Acat2[24] and LCAT.[25] Interestingly, miR-542-5p decreased the expression of Acat2 and elevated the expression of LCAT in primary hepatocytes and HepG2 cells, while anti-miR-542-5p exerted the opposite effects on the expression of Acat2 and LCAT (Supplementary Fig. 1C, only online). Altogether, these findings suggested that miR-542-5p alleviates glucose metabolism and lipid metabolism in FSK/DEX-induced hepatocytes and in HepG2 cells.

Involvement of miR-542-5p in glucose and lipid metabolism in vivo

Next, we aimed to examine whether miR-542-5p regulates glucose and lipid metabolism in vivo. As shown in Fig. 3A, miR-542-5p was under-expressed in liver and in inducible white adipose tissue, but exhibited no significant change in expression in muscle tissue from db/db mice. Fig. 3B and C shows that adenovirus overexpressing miR-542-5p significantly decreased blood glucose and serum insulin level in diabetic mice. PTT, ITT, and IPGTT analyses revealed that miR-542-5p effectively cleared glucose from the blood, compared to the Ad-miR-NC group, sug-

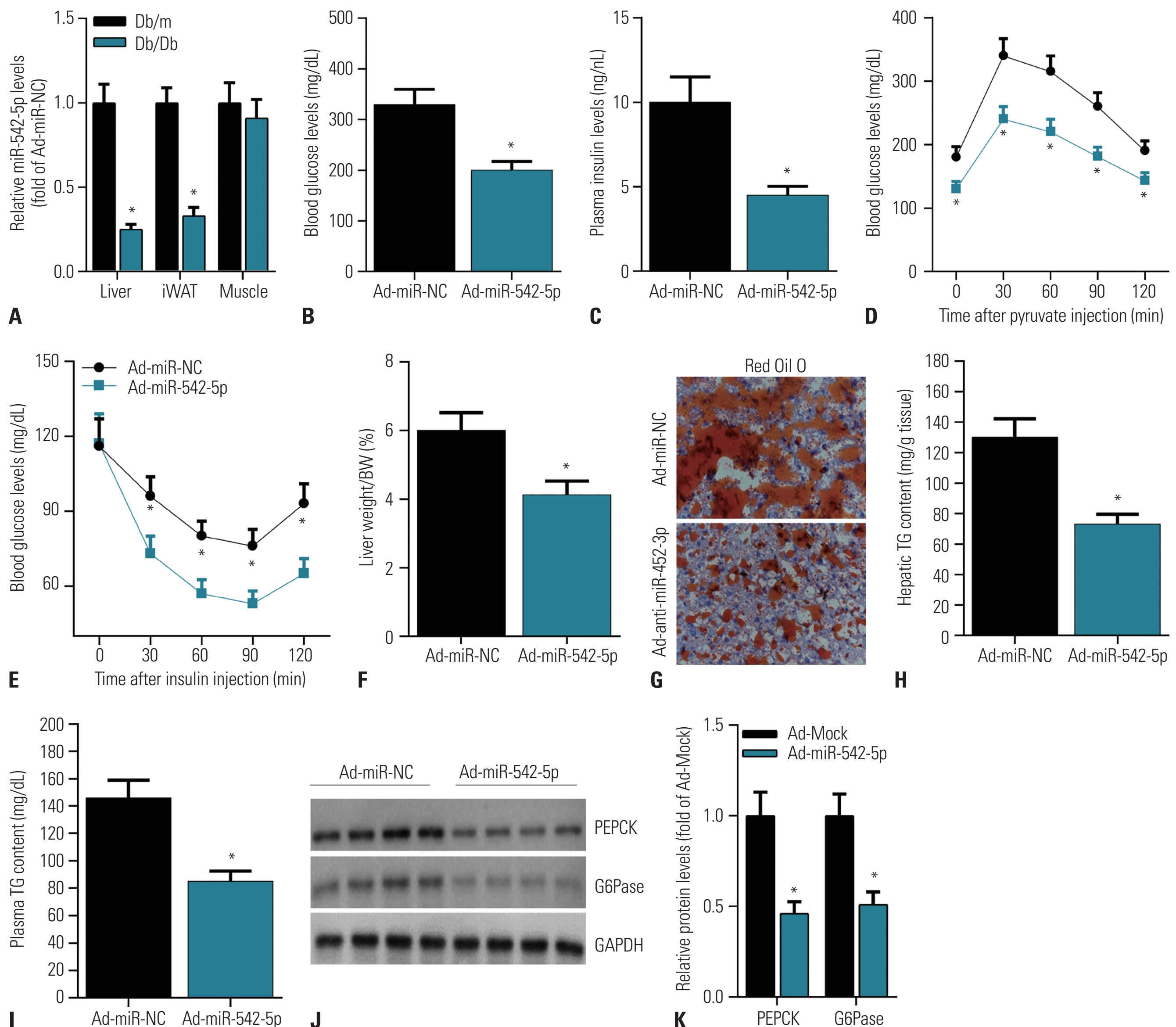

Fig. 3. Overexpression of miR-542-5p regulates hyperglycemia and insulin resistance and lipid droplet accumulation in vivo. (A) Quantitative data demonstrate miR-542-5p expression in liver, inducible white adipose, and muscle tissues in qPCR analysis. Influence of miR-542-5p on blood glucose (B) and insulin (C) levels in mice was detected by ELISA. Influence of miR-542-5p on blood glucose level in PTT (D) and in ITT (E). (F) Influence of miR-542-5p on liver weight. (G) Influence of miR-542-5p on adipose deposition in red oil O staining. (H and I) ELISA was used to detect hepatic and plasma TG content. Western blot (J) and quantitative data (K) demonstrate the expression of PEPCK and G6Pase by miR-542-5p overexpression in liver tissues. n=5–6. *p<0.05 vs. Ad-Mock group. iWAT, inducible white adipose tissue; BW, body weight; TG, triglyceride; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose 6-phosphatase; PTT pyruvate tolerance tests; ITT, insulin tolerance tests.

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gesting that miR-542-5p improved insulin sensitivity (Fig. 3D and E, Supplementary Fig. 1D, only online). MiR-542-5p significantly decreased liver weight in diabetes db/db mice (Fig. 3F). Red oil O, which was used to stain lipids, showed that miR-542-5p decreased lipid deposition in the liver (Fig. 3G). Moreover, miR-542-5p decreased hepatic and plasma TG contents (Fig. 3H and I) and decreased PEPCK and G6Pase protein levels in db/db mice livers (Fig. 3J and K). These results suggested that miR-542-5p improves glucose and lipid metabolism in vivo.

miR-542-5p targets FOXO1

Next, the potential targets of miR-542-5p were explored. Fig. 4A shows that FOXO1 3'UTR harbors a miR-542-5p binding site. As shown in Fig. 4B, miR-542-5p overexpression reduced the luciferase activity of FOXO1-WT, while miR-542-5p downregulation significantly elevated that in primary hepatocytes and HepG2 cells. However, these effects were abrogated when 3'UTR of FOXO1 was mutated. Consistently, miR-542-5p reduced FOXO1 protein levels, while anti-miR-542-5p increased that in primary

Binding sites of miR-542-5p on FOXO1 3'UTR

miR-542-5p 3'-AGAGCACUGU**ACUACUAG**GGGCU-5'

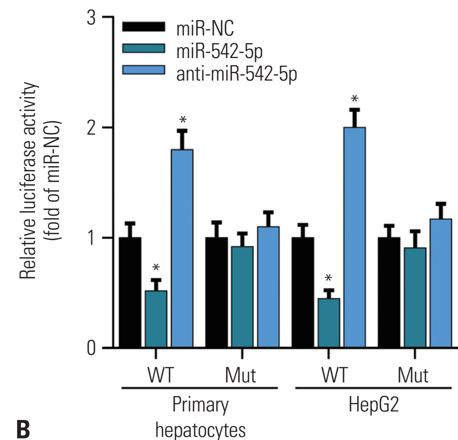
Human FOXO1 3'UTR WT 5'-CUUCCCACUG**UGAUGAUC**AUUUUU-3'
Human FOXO1 3'UTR Mut 5'-CUUCCCACUG**UCUUCUUG**AUUUUU-3'

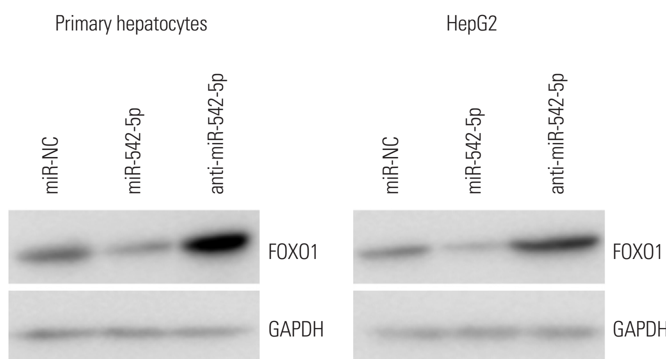
miR-542-5p 3'-AGAGCAC**UGUACUA**CUAGGGGCU-5'

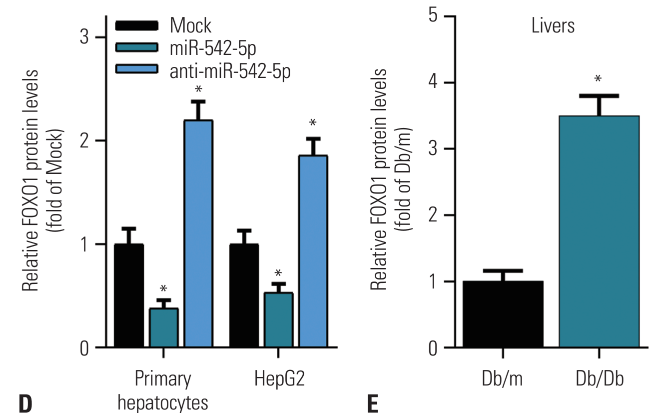
Human FOXO1 3'UTR WT 5'-UUAUUGU**ACAUGAU**UGCUUUGUGA-3'
Human FOXO1 3'UTR Mut 5'-UUAUUGU**AGUUCUU**UGCUUUGUGA-3'

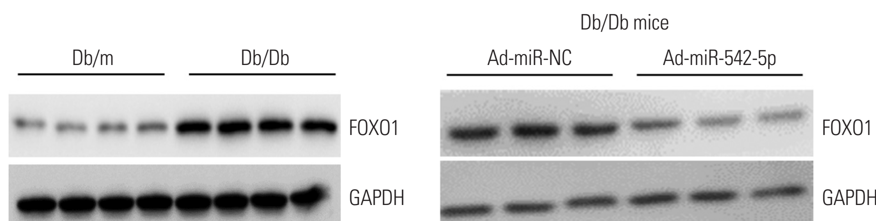
Mouse FOXO1 3'UTR WT 5'-UUAUUGU**ACAUGAU**UGCUCUGUGA-3'

A









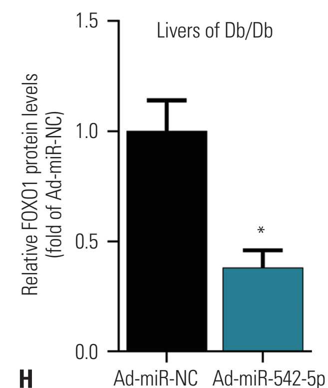


Fig. 4. miR-542-5p targets 3'UTR of FOXO1 in vitro. (A) Binding sequences between miR-542-5p and 3'UTR of FOXO1. (B) Influence of overexpressed and inhibited miR-542-5p on luciferase activity of native and mutant FOXO1 3'UTR in primary hepatocytes and HepG2 cells. Western blot analysis (C and D) demonstrates the influence of overexpressed and inhibited miR-542-5p on FOXO1 protein expression. Western blot analysis (E and F) reveals increased FOXO1 protein levels in the livers of db/db and db/m mice. Western blot images (G) and quantitative data (H) demonstrate the influence of miR-542-5p on FOXO1 protein level in the liver of db/db mice. n=5–6. *$p<0.05$ vs. Ad-Mock group. FOXO1, forkhead box O1.

hepatocytes and HepG2 cells (Fig. 4C and D). In vivo studies showed that the livers of diabetic mice contained high protein levels of FOXO1 (Fig. 4E and F). Meanwhile, miR-542-5p significantly inhibited FOXO1 protein levels in the livers of diabetic db/db mice (Fig. 4G and H). Additionally, we conducted luciferase reporter assay again to explore whether miR-542-5p binds with SREBP-1c and FAS, and the results demonstrated that miR-542-5p did not bind with SREBP-1c or FAS at the 3'UTR (Supplementary Fig. 1E, only online). Thus, we concluded that miR-542-5p regulates SREBP-1c and FAS expression via FOXO1. Accordingly, we deemed that miR-542-5p targets FOXO1 and inhibits FOXO1 expression.

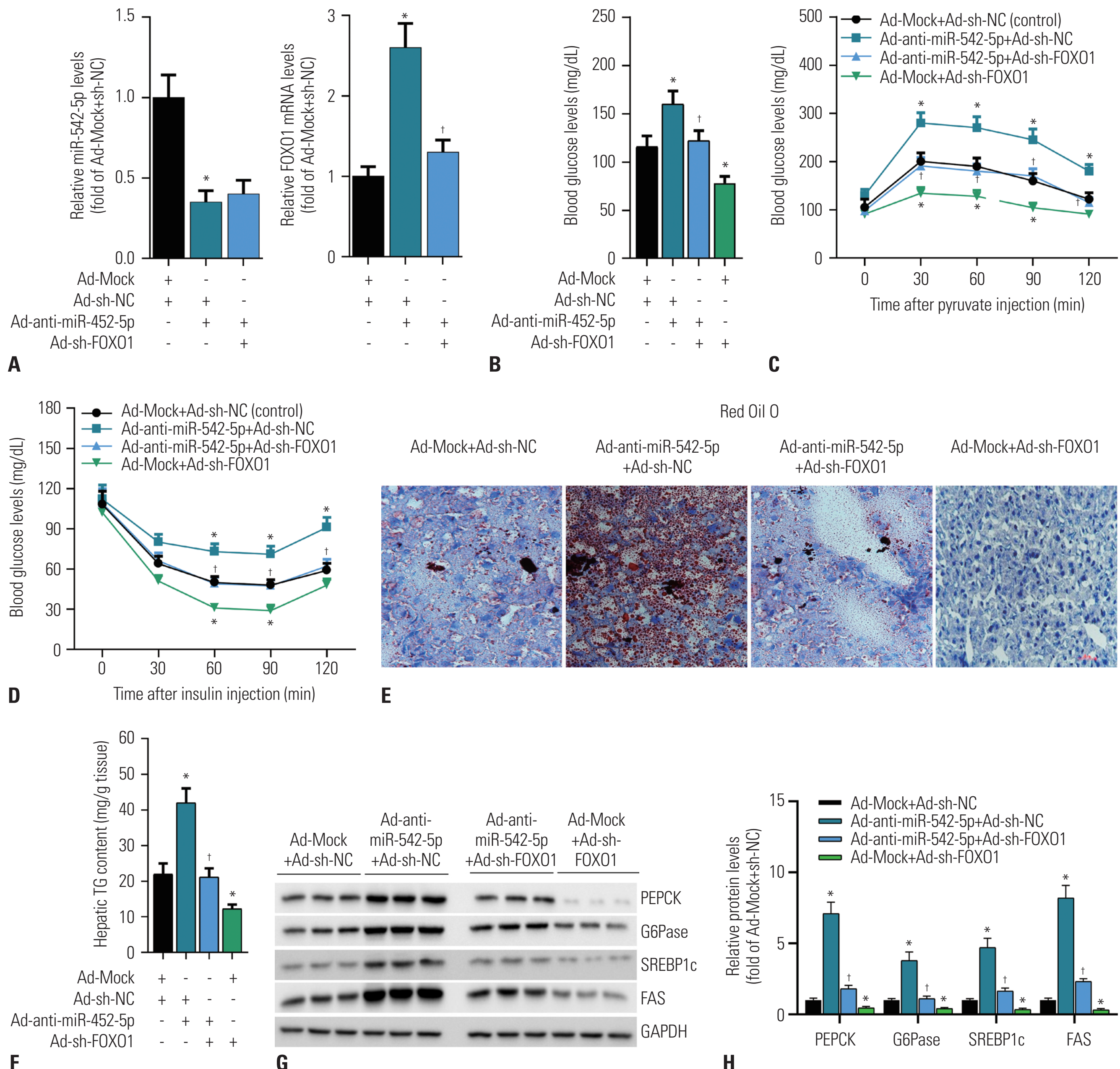


Fig. 5. Anti-miR-542-5p regulates hyperglycemia and insulin resistance and lipid droplet accumulation through targeting FOXO1 in vivo. (A) MiR-542-5p levels (left panel) and FOXO1 mRNA levels (right panel) in HepG2 cells by indicated transfections were measured by RT-qPCR. (B) ELISA was used to detect blood glucose. Quantitative data indicate the inhibitory effects of sh-FOXO1 on blood glucose levels in pyruvate tolerance tests (C) and insulin tolerance tests (D) in mice. (E) Red oil O staining images show that sh-FOXO1 inhibits anti-miR-542-5p-induced liver lipid accumulation. (F) ELISA reveals TG content under the influence of sh-FOXO1. Western blot analysis (G and H) demonstrates the influence of sh-FOXO1 on expression of PEPCK, G6Pase, SREBP-1c, and FAS. n=5-6. $^*p<0.05$ vs. sh-vector+Mock group, $^\dagger p<0.05$ vs. Ad-anti-miR-192-5p+Ad-sh-NC group and Ad-Mock+Ad-sh-FOXO1 group in A-H. FOXO1, forkhead box O1; TG, triglyceride; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose 6-phosphatase; SREBP-1c, sterol-response-binding-protein-1c; FAS, fatty acid synthase.

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MiR-542-5p regulates FOXO1 to inhibit hepatic steatosis and insulin resistance

Whether miR-542-5p regulates glucose and lipid metabolism by targeting FOXO1 was further explored. Fig. 5A demonstrates that silencing of miR-542-5p reduced miR-542-5p levels and that shRNA targeting FOXO1 did not further decrease miR-542-5p levels. Anti-miR-542-5p enhanced FOXO1 mRNA levels, which were repressed by sh-FOXO1. Fig. 5B shows that inhibition of miR-542-5p reversed the suppressive effects of sh-FOXO1 on blood glucose contents. Furthermore, anti-miR-542-5p increased blood glucose in both PTT and ITT experiments, which was previously inhibited by sh-FOXO1 (Fig. 5C and D). Additionally, anti-miR-542-5p elevated adipose deposition (Fig. 5E) and hepatic TG content (Fig. 5F) in the liver, and these effects were reversed by sh-FOXO1. Consistently, anti-miR-542-5p elevated the protein levels of PEPCK, G6Pase, SREBP-1c, and FAS, and such effects were reversed by sh-FOXO1 (Fig. 5G and H). All the findings suggest that miR-542-5p regulates glucose and lipid metabolism by targeting FOXO1 in vivo.

DISCUSSION

Research has shown that miR-542-5p is closely associated with prion-induced dysregulation of cholesterol homeostasis.[26] MiR-542-5p is indispensable for hepatic differentiation.[27] Ectopic overexpression of miR-542-5p stimulates conversion of human umbilical cord lining-derived mesenchymal stem cells into mature induced hepatocytes.[28] We found that miR-542-5p is underexpressed in diabetic mice livers. As FSK/DEX has been shown to elevate cellular glucose production and expression of PEPCK and G6Pase in primary hepatocytes,[29] we verified that FSK/DEX elevated cellular glucose production and expression of PEPCK and G6Pase in primary hepatocytes and in HepG2 cells. Notably, all these stimulatory effects of FSK/DEX were remarkably counteracted by miR-542-5p. Additionally, miR-542-5p targeted FOXO1 3'UTR and decreased FOXO1 expression in primary hepatocytes and HepG2 cells. Finally, in vivo studies demonstrated that miR-542-5p overexpression reduces blood glucose, plasma insulin, liver weight, lipid accumulation, and hepatic and plasma TG contents in diabetic mice livers. In contrast, anti-miR-542-5p exerted the opposite effects.

The present study demonstrated that miR-542-5p regulates glucose metabolism and lipid metabolism by decreasing blood glucose, plasma insulin, liver weight, lipid accumulation, and hepatic and plasma TG content in diabetic mice livers. Further, mechanistic study revealed that miR-542-5p targets FOXO1 3'UTR and inhibits FOXO1 expression. Similar mechanisms underlying interactions for other miRNAs in glucose metabolism or lipid metabolism are presented in Supplementary Table 1 (only online).

Interestingly, accumulating data has indicated that FOXO1 exerts significant regulatory effects in the regulation of glucose metabolism, as well as the initiation and development of gluconeogenesis, in the liver.[13-16] One study showed that increases in FOXO1 expression in chondrocytes lead to diabetes-impaired fracture by increasing the expression of RANKL.[30] Meanwhile, a SIRT1-FOXO1-ATG14 pathway has been found to regulate autophagy to prevent lipid metabolism dysfunction in HepG2 injury,[31] and AKT/FOXO1 and cAMP/AMPK/SREBP1 pathways have been shown to modulate lipid metabolism in largemouth bass.[32]

In summary, our findings demonstrated that miR-542-5p targets and degrades FOXO1 mRNA to regulate glucose metabolism or lipid metabolism in diabetic rats. The miR-542-5p-FOXO1 axis might offer new insights into the prevention of hyperglycemia and insulin resistance.

AUTHOR CONTRIBUTIONS

Conceptualization: Fang Tian and Hui-Min Ying. Data curation: Fang Tian and Hui-Min Ying. Formal analysis: Fang Tian and Hui-Min Ying. Funding acquisition: Hui-Min Ying. Investigation: Fang Tian. Methodology: Yuan-Yuan Wang. Project administration: Yuan-Yuan Wang and Bo-Ning Cheng. Resources: Fang Tian and Hui-Min Ying. Software: Juan Chen. Supervision: Hui-Min Ying. Validation: all authors. Visualization: Fang Tian. Writing—original draft: Fang Tian and Hui-Min Ying. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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