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LncRNA MEG3 aggravates palmitate-induced insulin resistance by regulating miR-185-5p/Egr2 axis in hepatic cells

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Abstract. – OBJECTIVE: Long non-coding RNA (LncRNA) has been reported to play an important role in type 2 diabetes (T2D). We investigated the role of LncRNA maternally expressed gene 3 (MEG3) and its potential interaction with miR-185-5p in palmitate-induced hepatocyte insulin resistance.

PATIENTS AND METHODS: High-fat diet (HFD) mice and insulin resistant hepatocyte were employed. Relative mRNA expressions of MEG3, miR-185-5p, and early growth response proteins-2 (Egr2) were measured by qRT-PCR. Western blot was performed to evaluate Egr2 protein expression levels. Glycogen contents and plasma insulin levels were tested by the corresponding assay.

RESULTS: MEG3 and Egr2 were upregulated, but miR-185-5p was downregulated in palmitate-treated insulin resistance hepatocytes and HFD mice. MEG3 knockdown alleviated the influence of palmitate on insulin resistance *in vitro* and *in vivo*. miR-185-5p expression was upregulated upon MEG3 knockdown. Expression of Egr2 was positively correlated with MEG3 knockdown or overexpression, which could be negatively managed by abnormal expression of miR-185-5p.

CONCLUSIONS: Our data demonstrated that LncRNA MEG3 aggravated palmitate-induced insulin resistance by regulating miR-185-5p/Egr2 axis, providing new insights into T2D therapeutic strategies.

Key Words:
LncRNA MEG3, miR-185-5p, Egr2, Hepatic insulin resistance.

Introduction

The incidence rate of type 2 diabetes (T2D) has significantly increased worldwide since the last few decades, forming one of the main menaces to public health[1]. T2D is considered to be caused by interaction between genetic susceptibility and environmental factors, such as inappropriate diet and sedentary lifestyle[2]. Hepatic insulin resistance plays a vital role in the progress of T2D[3,4], dysfunction of which inhibits glycogenesis and motivates adipogenesis, and thus generating hyperglycemia and hypertriglyceridemia[5]. However, the molecular and cellular mechanisms of hepatic insulin resistance have not yet been fully elucidated.

Long non-coding RNA (LncRNA) is a non-decoding single-stranded RNA consisted of over 200 nucleotides with restricted protein coding potential[6,7]. LncRNAs participate in numerous biological processes, including cell proliferation, apoptosis, differentiation, etc[8]. Previous studies[9,10] have reported lncRNA is associated with management of hepatic glucose and lipid metabolism, and its aberrant expression might be related to the pathogenesis of T2D. A lncRNA named maternally expressed gene 3 (MEG3), is a maternally expressed imprinted gene that is associated with many liver diseases[11-13]. Whereas, MEG3 expression level and its biological functions in the process of hepatic insulin resistance remain unclear.

MicroRNAs are small non-coding single strand RNAs consisted of 20-22 nucleotides in length[14]. microRNAs mediate mRNA expression at transcriptional as well as post-transcriptional level, therefore promoting the management of target genes in physiological courses, such as cell development, differentiation, apoptosis, and proliferation[15,16]. Scholars have reported microRNAs were also related to various biological processes, including energy and fat metabolism, which are attracting more and more attention in diabetes and obesity research[17,18]. Moreover,

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miR-185-mediated inhibition of low-density lipoprotein (LDL) uptake has recently been reported to participate in the imbalance of cholesterol homeostasis, which might give rise to many metabolic disorders, especially diabetes[19]. Therefore, the exact role of miR-185 in insulin resistance regulation should be further established.

Early growth response proteins-2 (Egr2) elevated expression of suppressors of cytokine signaling-1 (SOCS-1), and damaged insulin signaling by inhibiting insulin receptor substrates (IRS)[20]. Egr2 has been reported to contribute to fat cell differentiation, Barbeau et al[21] speculated Egr2 contributed to insulin resistance. To date, few studies regarding relationship between Egr2 and insulin resistance have been performed. In this study, we investigated the effects of MEG3 on insulin resistance and its underlying mechanisms. Our results suggested MEG3 was upregulated in palmitate-induced hepatic insulin resistant, which exacerbated hepatic insulin resistance by regulating miR-185-5p/Egr2 axis in hepatic cells.

Materials and Methods

Animals

Male C57BL/6J mice were purchased from the Experimental Animal Center of Nantong University. Mice were fed at constant temperature and relative humidity (50-60% humidity, 22°C) at 12 h:12 h light-dark circulation. In addition, mice were given free drinking and provided a regular fat diet (NFD) or a high-fat diet (HFD) for 16 weeks. All animal procedures followed the Guidelines for the Care and Use of Laboratory Animals[22]. The study protocol was approved by the Institutional Review Boards of The First Affiliated Hospital of Xiamen University.

Body weight was measured weekly. Blood samples used to measure glucose and insulin assay were taken from the tail veins of mice after fasting overnight. Blood glucose was measured using an Accu-check (Roche, Basle, Switzerland) glucometer. Plasma insulin levels were assessed by the Ultrasensitive Mouse Insulin ELISA kit (Mercodia) based on the manufacturer's protocols[23].

Cell Culture and Insulin Resistant Cell Model

Human HepG2 cells and primary hepatocytes were purchased from the Institute of Biochemistry and Cell Biology of The Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium containing 10% fetal bovine serum (FBS), incubated at 37°C incubator supplemented with 5% CO_2. To establish an insulin-resistant hepatocyte cell model, hepatocytes were stimulated by palmitate (0.25 mM, Sigma-Aldrich, St. Louis, MO, USA) for 24 h, and treated with insulin (100 nM) for 20 min before harvest.

Transfection

Cells were transfected with the plasmids containing MEG3 or sh-RNA oligonucleotides using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA). sh-RNAs (including sh-MEG3, sh-miR-185-5p and scramble or miR-NC) were synthesized by GenePharma (Shanghai, China) with the aim to silence the MEG3 or miR-185-5p expression. Cells were seeded in a 6-well plant and transfected according to the manufacturer's protocol. 24 h post-transfection, cells were treated with or without palmitate (Sigma-Aldrich, St. Louis, MO, USA, 0.25 mM) for 24 h, then, treated with insulin (100 nM) for 20 min before harvest.

RNA Extraction and qRT-PCR

RNAs were extracted from carcinoma tissues using TRIzol Reagent Kit (Invitrogen, Carlsbad, CA, USA). Reverse Transcription Kit (TaKaRa, Dalian, China) was utilized to reversely transcribe RNA into cDNA. qRT-PCR assay was performed using SYBR Perimix Ex Taq (TaKaRa, Dalian, China) directed by manufacturer's instructions. PCR cycling programs were set up as follows: 95°C for 10 min, 40 cycles of 95°C for 50 s, annealing and elongation at 72°C, 10 min. GAPDH were considered as internal control. Primers of LncRNA MEG3, miR-185-5p, and Egr2 mRNA were listed in Table I. ABI 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA) was applied for data analysis. Each experiment was performed for three times.

Western Blot

Total proteins acquired from cells using reactant RIPA (Beyotime, Haimen, China) containing 1% protease inhibitor cocktail (Roche, Basel, Switzerland) and PMSF (Roche, Basle, Switzerland). Protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto 0.22 mm nitrocellulose filters (Sigma-Aldrich, St. Louis, MO, USA). Membrane was blocked using 5% milk in Tris-Buffered Saline (TBS) at

Table I. The primers used in this study.

Genes	Primers	Sequences (5'-3')
MEG3	Forward	5′-CTGCCCATCTACACCTCACG-3′
	Reverse	5′-CTCTCCGCCGTCTGCGCTAGGGGCT-3′
miR-185-5p	Forward	5'-GAAGGATCCGCATGAGAGGGTGTTGGAATGC -3'
	Reverse	5'- GGAGAATTCGTGCAGGGGCAGCAGACC -3'
Egr2 mRNA	Forward	5'-TCCCAGTAACTCTCAGTGGTT-3'
	Reverse	5'-TGCCATCTCCGGCCA-3'

25°C for 1 h, and incubated with specific primary antibodies (Egr2, AKT, p-AKT, GSK-3β, p-GSK-3β) for 16 h at 4°C, then co-incubated with horseradish peroxidase (HRP)-coupled secondary antibodies (1:5000, GE Healthcare, Livonia, MI, USA) for 2 h at 37°C. Protein bands of interest were developed and recorded by Bio-Rad imaging system.

Glucose Uptake Assay

Glucose uptake amount in insulin-induced hepatocyte was determined using Glucose Colorimetric/Fluorometric Assay Kit (BioVision, San Francisco, CA, USA). Briefly, HepG2 cells were treated with insulin (100 nM) for 20 min. Then, 30 ml test specimens were added into 96-well plate, and the volume was adjusted to 50 ml/well by Glucose Assay Buffer. 50 ml reaction system consisted of glucose assay buffer 46 ml, glucose probe 2 ml and glucose enzyme mix 2 ml. These 50 ml reaction system buffers were added to glucose standard well and test specimens well and were incubated for 30 min at 37°C in dark. Finally, the absorbance (OD 570 nm), as well as the fluorescence (Ex/Em=535/590 nm), were measured by microplate reader.

Luciferase Reporter Assay

Luciferase was applied to detect the expression of target gene. First, pRL-TK Renilla luciferase plasmids, firefly luciferase reporter plasmids, and miRNA were co-transfected in cell model. Luciferase activity was detected using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Data were standardized for transfection efficiency by division of firefly luciferase activity with that of Renilla luciferase.

Statistical Analysis

SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA) was performed for statistical analysis. Results showed as the means ± S.D. (n = 3). When comparing two groups, Student's *t*-test was implemented to calculate differences; while comparing multiple groups, one-way ANOVA analysis was performed. $p<0.05$ was considered as statistically significant in this study (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

Results

MEG3 and Egr2 Expression Were Upregulated While miR-185-5p were Downregulated in HFD Mice

To investigate whether MEG3 is involved in hepatic insulin resistance, we established a mouse model of HFD-induced insulin resistance. C57BL/6J mice aged 4 weeks were raised on HFD or NFD diet for 16 weeks, then fasting glucose and insulin levels were tested. As shown in Figure 1A, HFD mice exhibited significantly heavier body weight than that of NFD mice after 11 weeks of feeding (*$p<0.05$). In addition, HFD mice impaired fasting blood glucose and plasma insulin levels (Figure 1B). Expressions of MEG3, miR-185-5p, and Egr2 in C57BL/6J mice liver tissue were measured by qRT-PCR and Western blot. mRNA levels of MEG3 (Figure 1C) and Egr2 (Figure 1D) were notably elevated in HFD mice compared to NFD mice (***$p<0.001$), while miR-185-5p expression (***$p<0.001$) were significantly downregulated in HFD mice (Figure 1E). Similarly, Egr2 protein expression (***$p<0.001$) also exhibited higher level in HFD mice than that in NFD mice (Figure 1F). Furthermore, relevance of MEG3, miR-185-5p and Egr2 expression levels in HFD mice were assessed. There were negative relations (R=-0.6699, $p=0.0341$) between MEG3 and miR-185-5p expression (Figure 1G) as well as between miR-185-5p and Egr2 mRNA (R=-073, $p=0.0165$) (Figure 1H). However, MEG3 and Egr2 mRNA expression were positively correlated (R=0.8697, $p=0.0011$) (Figure 1I).

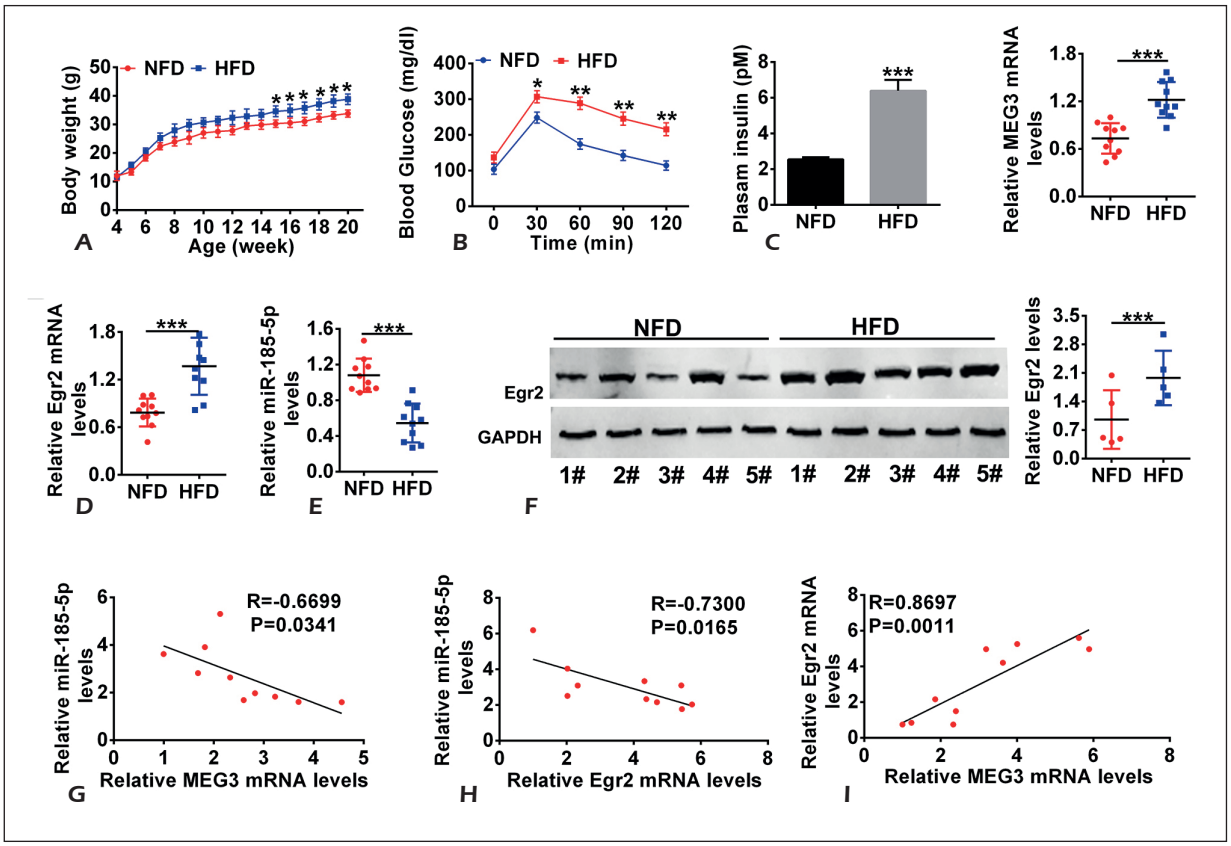

Figure 1. Expression of MEG3, Egr2, and miR-185-5p in HFD mice. C57BL/6J mice were raised in NFD or HFD diet for 16 weeks. **A**, Body weight changes every week. **B**, Blood glucose and plasma insulin level after fasting for 12 hr. **C-E**, Relative MEG3, miR-185-5p and Egr2 mRNA levels in HFD and NFD mice were detected via qRT-PCR. **F**, Egr2 protein levels in HFD and NFD mice were assessed via Western blot. **G**, Correlation of MEG3 and miR-185-5p expression. **H**, Correlation of miR-185-5p and Egr2 mRNA expression. **I**, Correlation of MEG3 and Egr2 mRNA expression (Mean ± SD; *p<0.05, **p<0.01 vs. NFD mice).

Expression of MEG3 and Egr2 Were Regulated, but miR-185-5p Was Downregulated in Insulin Resistance Hepatocytes

Glucose uptake abilities in primary hepatocytes and HepG2 cells were analyzed, indicating insulin-induced glucose uptake were significantly inhibited (**p<0.01) by palmitate (Figure 2A). Furthermore, mRNA expression levels of MEG3, miR-185-5p, and Egr2 in insulin-resistant hepatocytes were measured. As indicated in Figure 2B, MEG3 mRNA level was notably up-regulated (***p<0.001) in palmitate-treated HepG2 cells and primary hepatocytes. In contrast, miR-185-5p level was significantly decreased (Figure 2C). Next, the expression of Egr2 and the commonly used marker of insulin signaling were analyzed in palmitate-treated HepG2 cells and primary hepatocytes. As hoped, Egr2 protein level significantly added (***p<0.001) in palmitate-treated HepG2 cells and primary hepatocytes (Figure 2D). In addition, insulin-induced phosphorylation of AKT (**p<0.01) and GSK-3β (*p<0.05) were weakened by palmitate treatment in HepG2 cells and primary hepatocytes.

MEG3 Knockdown Alleviates Palmitate-Induced Hepatocyte Insulin Resistance

To examine the effect of MEG3 on hepatocyte insulin resistance, therefore MEG3 expression and subsequent phosphorylation statuses of AKT and GSK-3β were analyzed. As shown in Figure 3A, MEG3 expression was significantly inhibited upon MEG3 knockdown (sh-MEG31# and sh-MEG32#) in HepG2 cells and primary hepatocytes compared to cells overexpressing MEG3. Glucose uptake assay suggested MEG3 knockdown weakened the ability of insulin-induced glucose uptake (Figure 3B). Western blot showed MEG3 knockdown could reverse the downregulation of AKT (p-AKT) and palmitate-induced GSK-3β (p-GSK-3β) activation (Figure 3C). These data suggested MEG3 played an

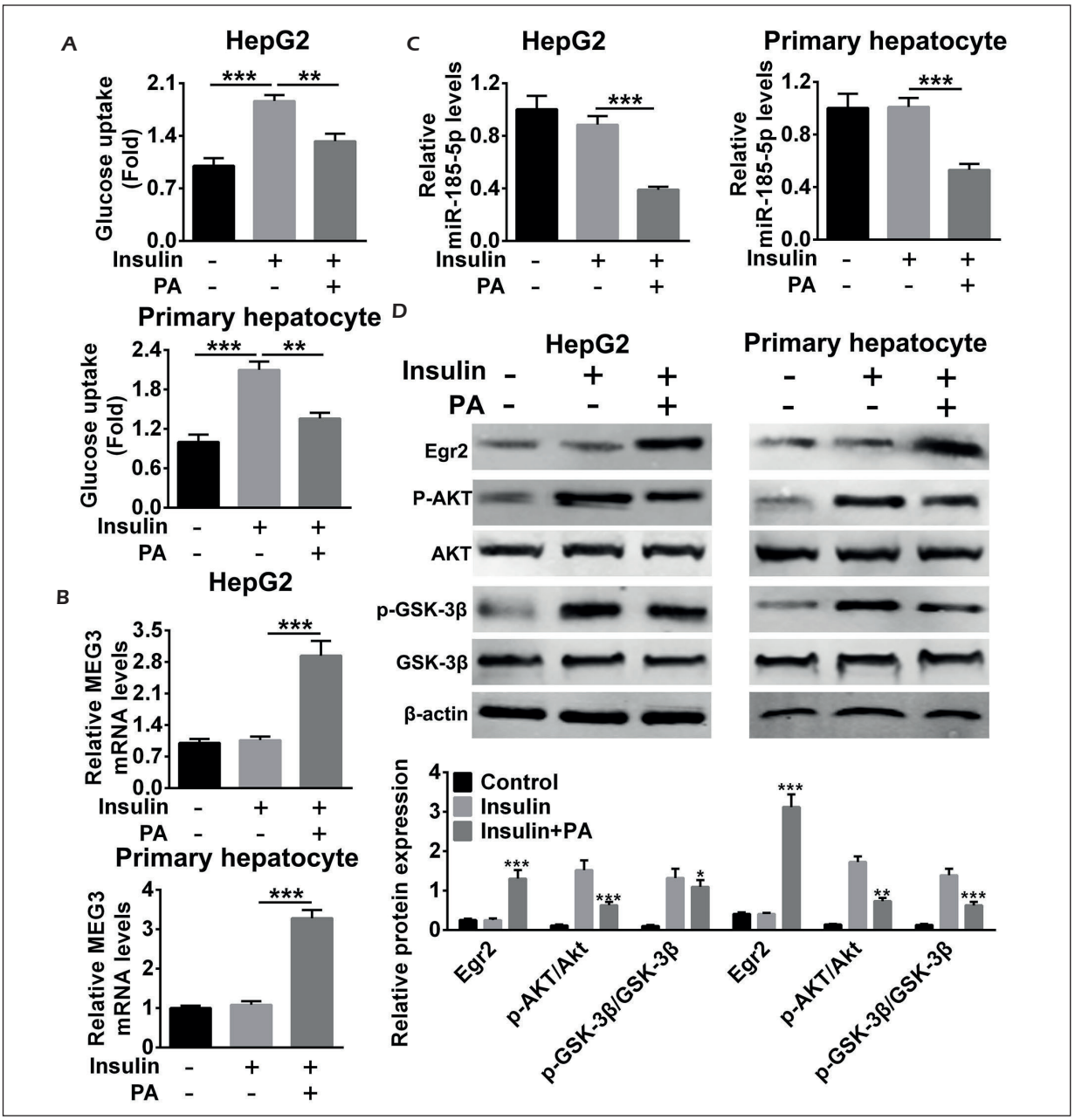

Figure 2. Expression of MEG-3, Egr2, and miR-185-5p in palmitate-treated insulin resistance hepatocytes. ***A***, Glucose uptake ability was measured with glucose fluorometric assay kit. ***B***-***C***, Relative expressions of MEG3 and miR-185-5p were detected via qRT-PCR. ***D***, Protein levels of Egr2 and insulin-stimulated phosphorylation of AKT and GSK-3βin palmitate-treated HepG2 cells and primary hepatocytes were measured with Western blotting (Mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

important part in deteriorating palmitate-induced insulin resistance.

MEG3 Promoted Insulin Resistance by Decreasing miR-185-5p Expression in Hepatocytes

We took advantage of the miRDB site database to explore the targets of miR-185-5p. MEG3-wt or MEG3-mut mRNA bears the miR-185-5p binding sites (Figure 4A). miR-185-5p expression was significantly higher in miR-185-5p group than that in miR-NC group (Figure 4B). The reporter gene system was adopted to certify the database predictions. Results showed that miR-185-5p significantly inhibited the MEG3-wt 3'UTR luciferase activity but did not influence the MEG3-mut 3'UTR luciferase activity (Figure 4C). RNA pull down experiments show miR-185-

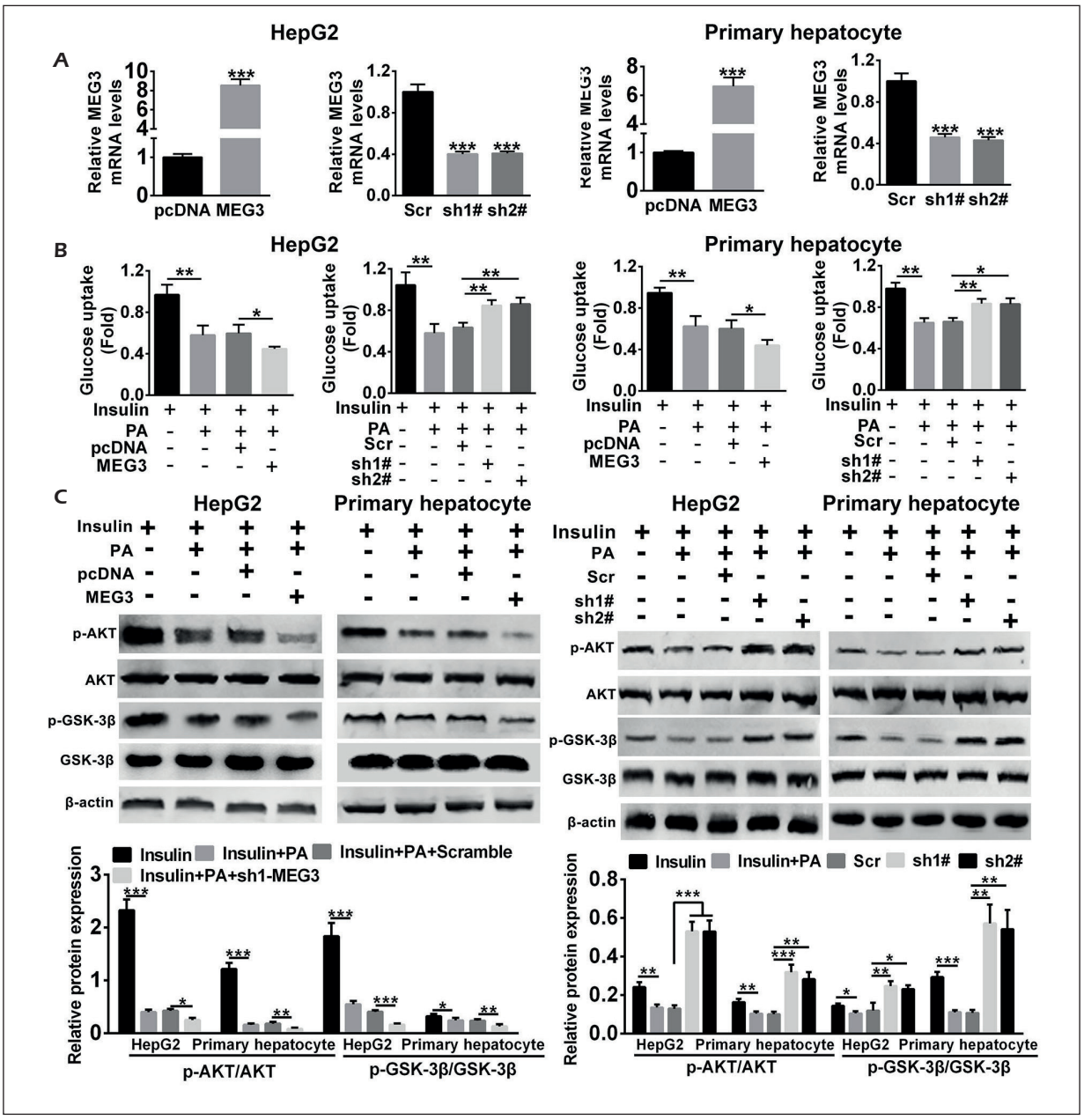

Figure 3. MEG3 Knockdown alleviates palmitate-induced hepatocyte insulin resistance. **A**, MEG3 expression increased after transfected by pcDNA-MEG3 and decreased expression of MEG3 after transfected by sh-MEG3 (sh-MEG31# and sh-MEG32#). **B-C**, The glucose uptake ability and protein levels of phosphorylation of AKT and GSK-3β in palmitate-treated HepG2 cells and primary hepatocytes after transfected by pcDNA-MEG3 and sh-MEG3 (sh-MEG31# and sh-MEG32#) (Mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

5p enrichment elevated under MEG3 overexpression (Figure 4D). To discover the relationship between MEG3 and miR-185-5p, qRT-PCR was performed to measure the miR-185-5p expression in HepG2 cells and primary hepatocytes after transfected by pcDNA and sh-MEG3(sh-MEG31# and sh-MEG32#). We discovered miR-185-5p expression significantly inhibited under MEG3 overexpression. Similarly, miR-185-5p expression could increase under MEG3 knockdown (Figure 4E), suggesting the combination of MEG3 and miR-185-5p reduced miR-185-5p level. Next, we further explored the effects of MEG3 on miR-185-5p by transfecting palmitate-treated HepG2 cells

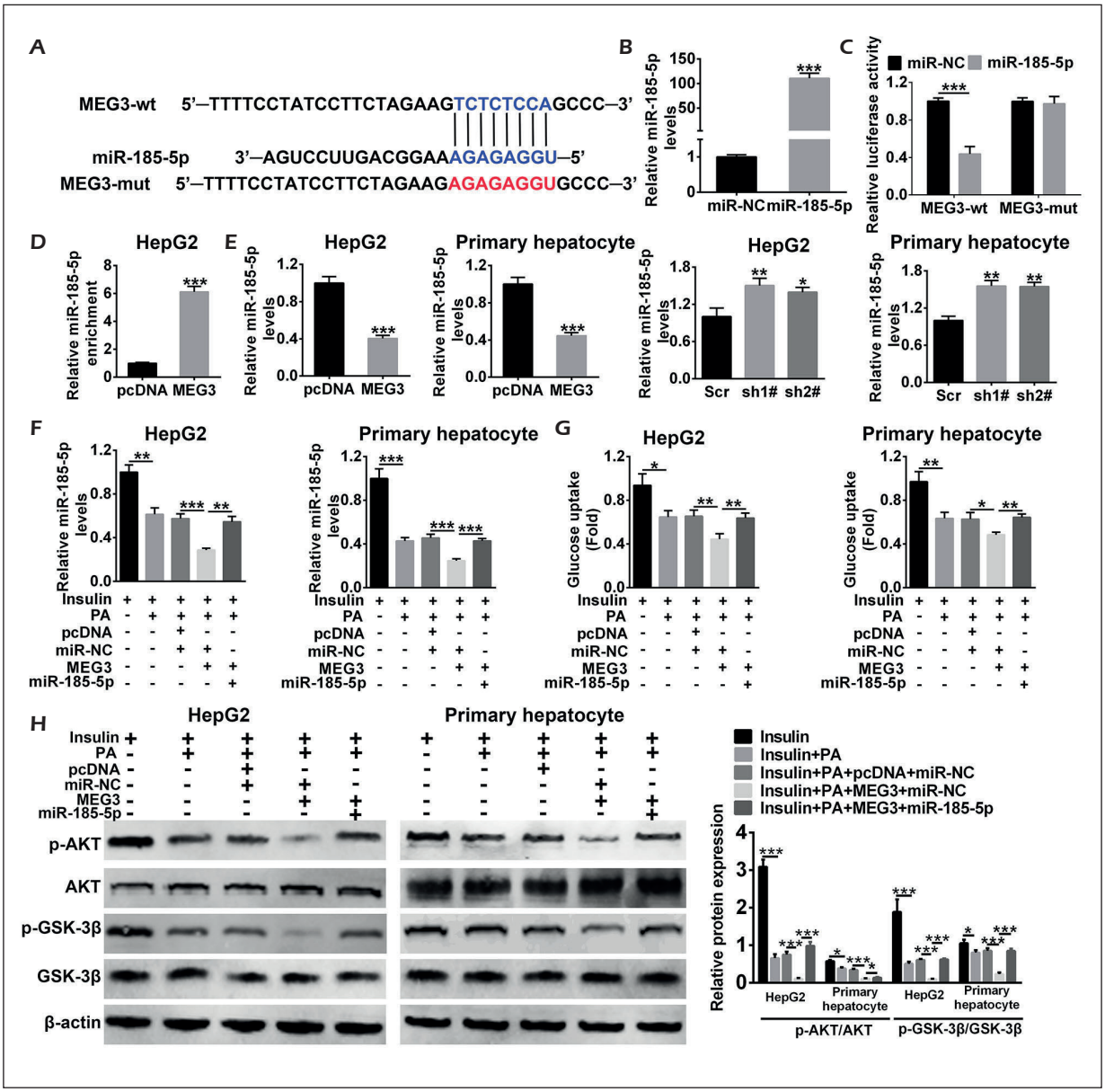

Figure 4. MEG3 promoted insulin resistance by decreasing miR-185-5p expression in hepatocytes. **A**, Conserved miR-185-5p binding sites in the MEG3-wt or MEG3-mut mRNA. **B**, miR-185-5p expression after transfected with miR-185-5p mimic in HEK-293T. **C**, Luciferase activity in lysates of HEK-293T cells after transfected with construct encoding MEG3-wt or MEG3-mut. **D**, RNA pull down experiment to detect miR-185-5p enrichment. **E**, miR-185-5p expression were analyzed in HepG2 cells and primary hepatocytes after transfected by pcDNA-MEG3 or sh-MEG3(sh-MEG31# and sh-MEG32#). **F-H**, miR-185-5p expression, glucose uptake ability and protein levels of phosphorylation of AKT and GSK-3β in palmitate -treated HepG2 cells and primary hepatocytes after transfected by pcDNA-MEG3 and pcDNA-miR-185-5p (Mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

and primary hepatocytes with vector expressing MEG3 and miR-185-5p. miR-185-5p overexpression significantly reversed the effect of MEG3 on decreasing miR-185-5p (Figure 4F). Furthermore, MEG3 overexpression reduced glucose uptake and miR-185-5p overexpression weakened MEG3 level inhibited glucose uptake (Figure 4G), indicating MEG3 promoted insulin resistance by reducing miR-185-5p levels. After overexpression of MEG3, the phosphorylation status of AKT and GSK-3β were reduced, and the ability of MEG3 to decrease the phosphorylation levels of AKT and GSK-3β was attenuated upon miR-185-5p overexpression (Figure 4H).

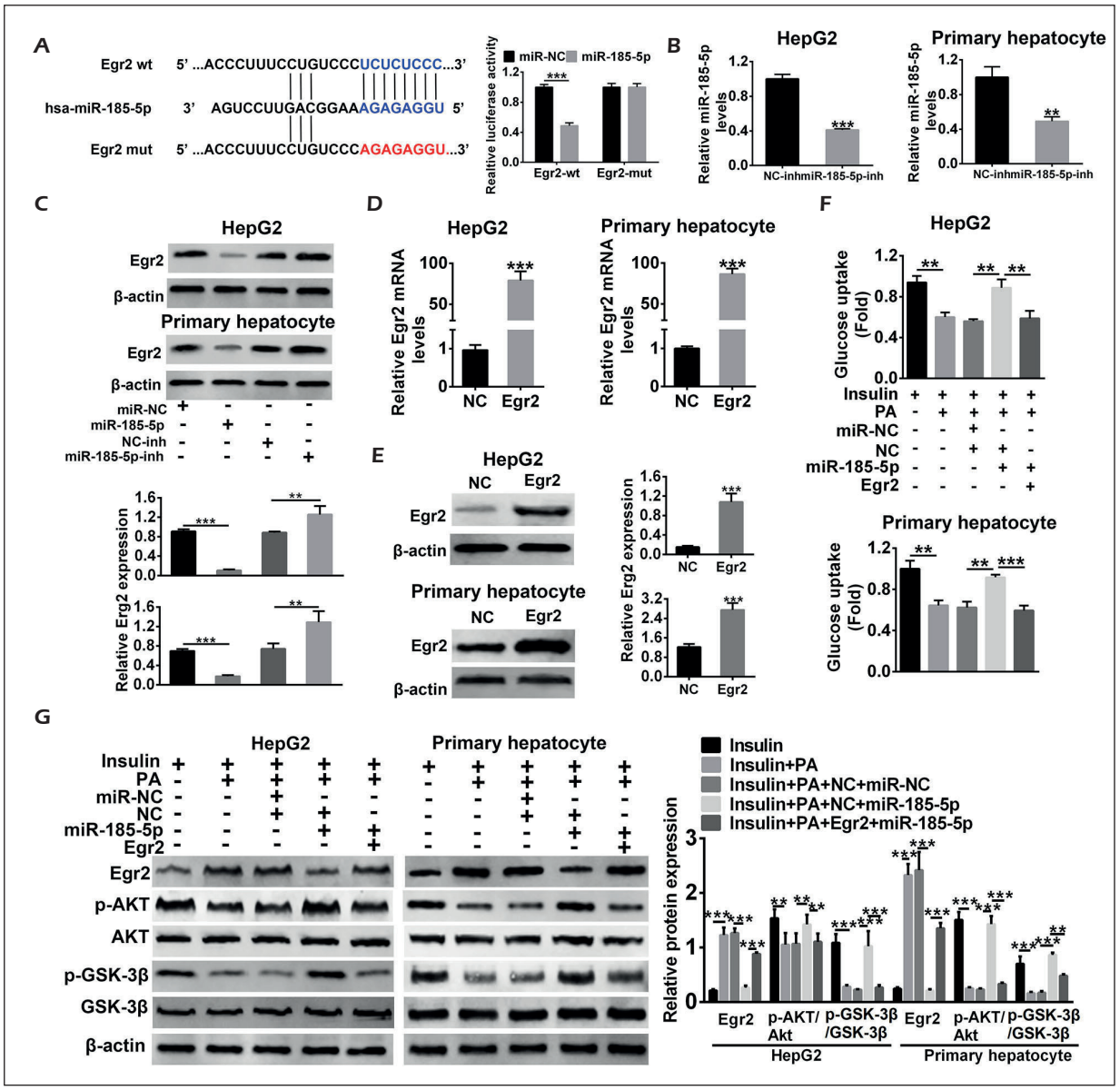

Figure 5. miR-185-5p alleviated insulin resistance by targeting Egr2. **A**, Luciferase activity in lysates of HEK-293T cells after transfected with construct encoding Egr2-wt or Egr2-mut. **B**, qRT-PCR detection of miR-185-5p expression after miR-185-5p inhibitor transfection. **C**, Protein levels of Egr2 in HepG2 and primary hepatocyte cells after transfected with miR-185-5p mimic or miR-185-5p inhibitor. **D**-**G**, Egr2 expression, glucose uptake ability and protein levels of phosphorylation of AKT and GSK-3β in palmitate -treated HepG2 cells and primary hepatocytes after transfected by pcDNA-MEG3 and pcDNA-miR-185-5p (Mean ± SD; **p<0.01, ***p<0.001).

miR-185-5p Alleviated Insulin Resistance by Targeting Egr2

Targetscan database of predicted microRNA Targets was utilized to measure the targets of miR-185-5p. Egr2-wt or Egr2-mut mRNA bears miR-185-5p binding sites (Figure 5A). miR-185-5p significantly inhibited the Egr2-wt 3'UTR luciferase activity but did not affect the Egr2-mut 3'UTR luciferase activity.

To further explore the relationship between miR-185-5p and Egr2, Western blot analysis was performed to measure the Egr2 expression in HepG2 cells and primary hepatocytes after transfected with miR-185-5p mimic or miR-185-5p inhibitor. qRT-PCR showed miR-185-5p expression was significantly downregulated under miR-185-5p inhibitor treatment (Figure 5B). Western blot analysis showed miR-185-5p mimics significantly

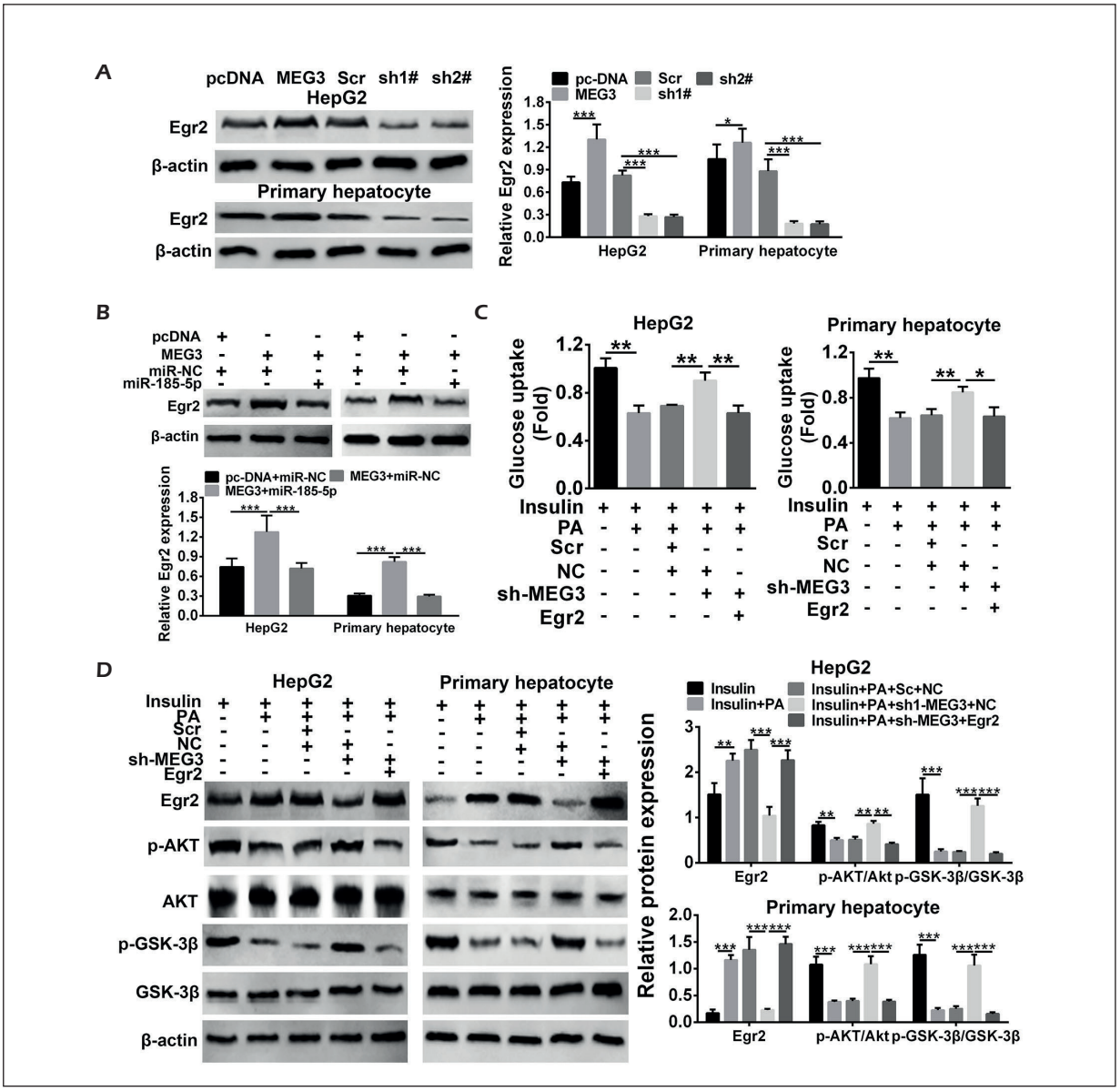

Figure 6. MEG3 aggravates insulin resistance in hepatocytes through upregulating Egr2. **A**, Egr2 expression in HepG2 cells and primary hepatocytes after transfected by pcDNA and sh-MEG3(sh-MEG31# and sh-MEG32#). **B**, Egr2 expression in HepG2 cells and primary hepatocytes after overexpression of MEG3 and miR-185-5p. **C**-**D**, Glucose uptake ability and protein levels of phosphorylation of AKT and GSK-3β in palmitate -treated HepG2 cells and primary hepatocytes upon MEG3 knockdown and Egr2 overexpression (Mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

downregulated Egr2 expression, and miR-185-5p inhibitor could restore Egr2 expression (Figure 5C), suggesting miR-185-5p inhibited Egr2 expression by targeting Egr2.

To further determine the correlativity between miR-185-5p and Egr2 in insulin-resistant hepatocytes. Human HepG2 cells and primary hepatocytes were incubated with palmitate to produce insulin resistance. qRT-PCR (Figure 5D) and Western blot (Figure 5E) analysis showed Egr2 expression significantly elevated compared to that in negative controls. In addition, miR-185-5p expression contributed to glucose uptake, while Egr2 overexpression reduced the ability of miR-185-5p of glucose uptake (Figure 5F), illustrating miR-185-5p attenuated insulin resistance by inhibiting Egr2 in hepatocytes. We also discovered that phosphorylation levels of AKT and GSK-3β were enhanced upon miR-185-5p expression, which could be restored by Egr2 overexpression (Figure 5G).

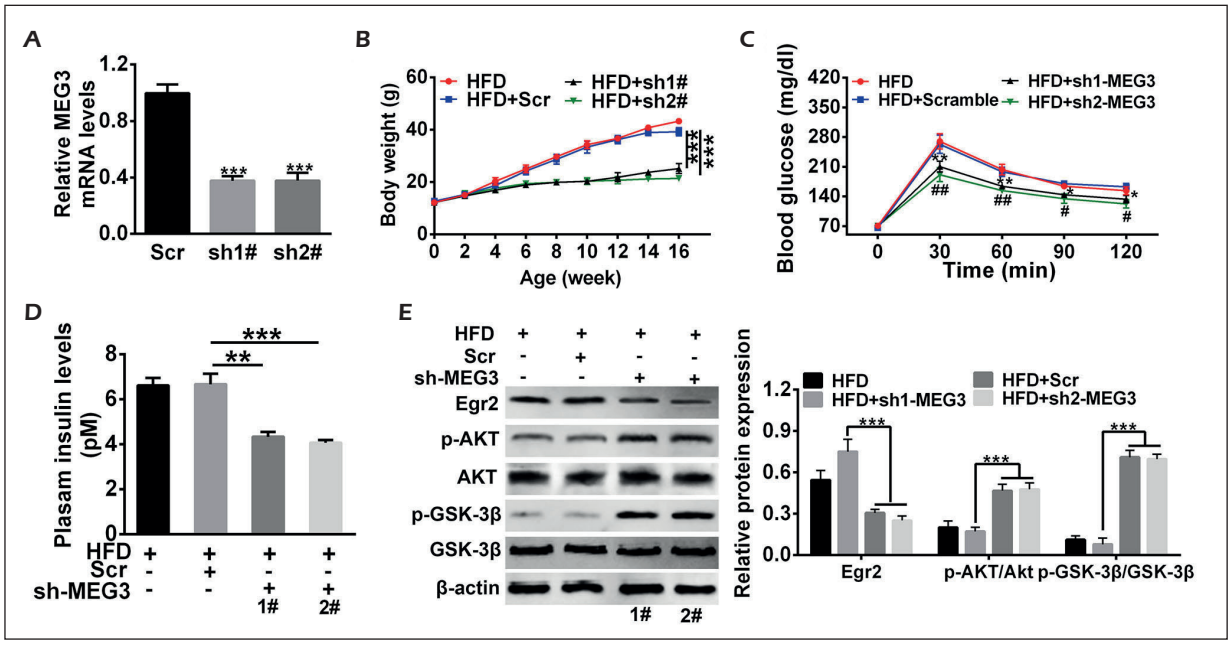

Figure 7. MEG3 knockdown significantly relieves insulin resistance in vivo. **A**, qRT-PCR detection of MEG3 expression after Knockdown of MEG3. **B**-**D**, Body weight changes, blood glucose and plasma insulin level of C57BL/6J mice. **E**, The expression of Egr2, AKT, p-AKT, GSK-3β and p-GSK-3β in liver tissues (Mean ± SD; **p<0.01, ***p<0.001).

MEG3 Aggravates Insulin Resistance in Hepatocytes Through Upregulate Egr2 Level

To explore the relationship between MEG3 and Egr2, Western blot analysis was performed to assess Egr2 protein level in HepG2 cells and primary hepatocytes after transfected with pcDNA and sh-MEG3 (sh-MEG31# and sh-MEG32#). Upon MEG3 overexpression, Egr2 level was increased, and this effect could be re-inhibited by MEG3 knockdown (Figure 6A) indicating MEG3 promoted Egr2 expression. Moreover, Egr2 expression in HepG2 cells and primary hepatocytes were detected after overexpression of MEG3 and miR-185-5p. Ability of MEG3 to promote Egr2 expression could be impaired by miR-185-5p overexpression (Figure 6B), indicating MEG3 promoted Egr2 expression by inhibiting miR-185-5p level. We next examined the glucose uptake ability as well as phosphorylation statuses of AKT and GSK-3β in palmitate-treated HepG2 cells and primary hepatocytes under MEG3 knockdown and Egr2 overexpression. MEG3 knockdown increased glucose uptake, which could be reversed Egr2 overexpression (Figure 6C), indicating MEG3 aggravated insulin resistance by promoting Egr2 expression. Similarly, MEG3 knockdown enhanced the phosphorylation levels of AKT and GSK-3β, decreased Egr2 expression (Figure D).

Knockdown of MEG3 Significantly Alleviates Insulin Resistance In Vivo

To investigate whether MEG3 regulate liver insulin resistance *in vivo*, C57BL/6J mice were raised on HFD and sh-MEG3 (sh-MEG31# and sh-MEG32#) interference fragment were injected into the tail vein. qRT-PCR showed MEG3 expression was significantly decreased upon MEG3 knockdown (Figure 7A). In sh-MEG3 group, body weight (Figure 7B), blood glucose content (Figure 7C) and serum insulin level (Figure 7D) were found to be downregulated. These *in vivo* results showed MEG3 knockdown alleviated insulin resistance in HFD fed model mice. In addition, Egr2 expression was decreased and the phosphorylation levels of AKT and GSK-3β were increased under MEG3 knockdown (Figure 7E).

Discussion

Excess nutrient intake is a significant factor for T2D pathogenesis[24]. As a starting factor for T2D, insulin resistance has been observed in adipose, muscle, hepar, and other tissues. During T2D, liver was the first organ that exhibited resistance, then muscle and finally fat[25]. T2D patients usually present damaged glucose production regulation and increased gluconeogenesis because

of raised free fatty acids (FFA) level in plasma[26]. Palmitate is one of the most abundant saturated fatty acids in plasma[27]. In this study, palmitate was adopted to dispose HepG2 cells to imitate the T2D pathogenesis. Our results demonstrated that MEG-3 and Egr2 were up-regulated, while miR-185-5p was downregulated, in palmitate-treated insulin resistance hepatocytes and HFD mice.

LncRNAs are important regulators of T2D and insulin resistance[10,28]. MEG3 is a maternally expressed imprinting gene. However, effects of lncRNA expression on insulin resistance have not been fully studied[29]. We discovered MEG-3 was increased in palmitate-treated insulin resistance hepatocytes and HFD mice. Elevated MEG-3 aggravated the inhibition of glucose uptake. MEG3 knockdown alleviated the influence of palmitate on the phosphorylation of AKT and GSK-3β and thus reversing palmitate-induced insulin resistance in hepatocytes. Then, MEG3 knockdown significantly inhibited insulin resistance in C57BL/6J mice. Collectively, our results suggested MEG3 played an important part in deteriorating palmitate-induced insulin resistance.

Previous researches reported LncRNA MEG3 served as linker for pools of active some miRNAs, thereby altering miRNA target gene expressions. LncRNA MEG3 functions as an endogenous RNA of miR-181a, which suppresses the proliferation, migration, and invasion of gastric cancer cells[30]. Zhang et al[31] discovered MEG3 expression was decreased in cervical cancer, as well as affected cell proliferation and apoptosis by modulating miR-21. Qin et al[32] illustrated that MEG3 repress the phosphatase and tensin homologues production to suppress the glioma cells proliferation, migration, and invasion by targeting miR-19a. In this work, we demonstrated miR-185-5p expression was up-regulated upon MEG3 knockdown. Consistently, miR-185-5p expression could be inhibited MEG3 over-expression. Combined our study with previous reports, we speculated MEG3 functioned as a key modulator for miR-185-5p in insulin resistance.

Accumulating evidence reported Egr2 expression increased under insulin resistance[33]. Our study was consistent with previous investigations, demonstrating Egr2 expression were upregulated in HFD mice and insulin resistance hepatocytes. As a member of the EGR family, Egr2 enhanced insulin resistance in palmitate-treated HepG2 cells via JAK2/STAT3/SOCS-1 pathway[33]. To investigate the changes of MEG3 and miR-185-5p with Egr2 in palmitate-treated insulin resistance, we next explored relationships between miR-185-5p, Egr2 and MEG3 in insulin-resistant hepatocytes, showing Egr2 expression was positively influenced by MEG3, while negatively managed by abnormal miR-185-5p expression. Egr2 was a downstream target gene of miR-185-5p and its expression could be suppressed by miR-185-5p. To sum up, we suggested Egr2 was an ultimate responder for MEG3/miR-185-5p and mediated palmitate-induced insulin resistance.

Conclusion

We observed that elevated MEG3 expression aggravated palmitate-induced insulin resistance by regulating miR-185-5p/Egr2 axis in hepatic cells, thus providing new insights into novel therapeutic candidate targeting MEG3 for future T2D treatment from bench to clinic.

Acknowledgments

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Conflict of Interests

The authors declare that there are no conflicts of interest.

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