

Original Research Article

IRF2BP2 attenuates gestational diabetes mellitus by activating AMPK signaling

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Abstract

Purpose: To investigate the role of interferon regulatory factor 2 binding protein 2 (IRF2BP2) in gestational diabetes mellitus (GDM).

Methods: Mice were injected intraperitoneally with streptozotocin to establish a model of GDM and then subjected to intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) to determine glucose and insulin tolerances. Lipid metabolism was evaluated by enzyme-linked immunosorbent assay (ELISA). The histomorphology of pancreatic islets was assessed by hematoxylin and eosin staining.

Results: IRF2BP2 was downregulated in pancreatic tissues of mice with GDM ($p < 0.001$). Mice in GDM group showed higher blood glucose levels than those in normal pregnancy group. However, overexpression of IRF2BP2 reduced glucose and insulin levels in mice with GDM. Overexpression of IRF2BP2 increased the level of high-density lipoprotein (HDL) and reduced triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) levels in mice with GDM ($p < 0.001$). The histopathological changes in the islets of mice with GDM were also ameliorated by overexpression of IRF2BP2. Overexpression of IRF2BP2 reduced IL-6, IL-1 β , and TNF- α levels and increased protein expression of p-AMPK in mice with GDM.

Conclusion: IRF2BP2 ameliorates the outcomes of GDM and suppressed inflammation in mice with GDM through activation of AMPK signaling. Thus, IRF2BP2 is a potential therapeutic strategy for the management of GDM.

Keywords: IRF2BP2, inflammation, gestational diabetes mellitus, AMPK, mice, insulin tolerance

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INTRODUCTION

Diabetes mellitus is characterized by impaired insulin secretion and abnormal metabolism of carbohydrates leading to insulin resistance and hyperglycemia [1]. Gestational diabetes mellitus (GDM) refers to dysregulated insulin secretion

and glucose uptake in women during pregnancy leading to operative deliveries, polyhydramnios, and preeclampsia in approximately 5 % of pregnant women [2]. GDM also affects offspring adversely causing dysregulated glucose metabolism, respiratory distress syndrome, macrosomia, birth trauma, and prematurity [2].

However, there is no effective treatment for GDM.

Insulin resistance was shown to play a role in the initiation and pathogenesis of GDM [3]. Placental inflammation, an important regulator of fetal development, was also shown to be involved in the development of insulin resistance and GDM [3]. Anti-inflammatory strategies were suggested for the clinical treatment of GDM [4].

Interferon regulatory factor 2 binding protein 2 (IRF2BP2) functions as a transcriptional corepressor and regulates a variety of cellular processes, including the immune response, inflammation, angiogenesis, the cell cycle, and cell death [5]. IRF2BP2 associated with lipid homeostasis and macrophage inflammation through binding to Krüppel-like factor 2 (KLF2) [6]. Loss of IRF2BP2 downregulated protein expression of the anti-inflammatory transcription factor KLF2 leading to the inflammatory phenotype in macrophages [6]. Moreover, IRF2BP2 associated with high-fat diet-induced hepatic inflammation, insulin resistance, and steatosis [7], and downregulation of IRF2BP2 contributed to hepatic dyslipidemia by promoting high-fat diet-induced inflammation [8]. However, the role of IRF2BP2 in GDM has rarely been studied. In this study, the effects of IRF2BP2 on the histomorphology of islets and placental inflammation in mice with GDM were investigated.

EXPERIMENTAL

Mouse GDM model

A total of 45 male SPF C57BL/6J mice and 90 female mice (8 weeks old, 22 – 25 g) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). The experiments were approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University (approval no. JD-LK-2020-041-02) and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [9]. The male and female mice (ratio of 1:2) were mated in cages and the day following mating was designated gestation day 0. Female mice were confirmed as pregnant by inspection of their uterine horns for embryos and then divided into GDM (n = 30) and control (n = 6) groups. After 12 h of fasting, mice in the control group were injected intraperitoneally with sodium citrate buffer and mice in the GDM group were injected intraperitoneally with 150 mg/kg streptozotocin (Sigma-Aldrich, St. Louis, MO, USA). Blood glucose level of the mice were measured using an ACCU-CHEK advantaged glucometer (Roche

Diagnostics, Risch-Rotkreuz, Switzerland) 3 days after the injection. Mice (n = 24) with glucose levels > 300 mg/dL or 16.7 mM were considered to have GDM.

Mice with GDM were then divided into four groups: GDM (n = 6), GDM + vector (mice were tail vein injected with pcDNA empty vector; n = 6), GDM + OE-IRF2BP2 (mice were tail vein injected with pcDNA-IRF2BP2; n = 6), GDM + OE-IRF2BP2 + compound C (mice were tail vein injected with pcDNA-IRF2BP2 and injected intraperitoneally with 5 mg/kg compound C once a day for 1 week; n = 6). The pcDNA vectors were packaged with lentivirus from GenePharma (Shanghai, China) and 2×10^7 IU of virus were injected into the mice.

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

For the IPGTT, mice on gestation day 10 were injected intraperitoneally with 2 g/kg glucose after 6 h of fasting and blood glucose levels were measured with a glucometer at 30, 60, 90, and 120 min after glucose injection. For the IPITT, mice were injected intraperitoneally with 0.75 U/kg insulin after 6 h of fasting and blood glucose levels were measured with a glucometer at 30, 60, 90, and 120 min after insulin injection.

Measurement of serum glucose and insulin

On gestation day 20, blood samples were collected from mice through their eyeballs. Serum glucose levels were measured with a glucometer and serum insulin levels were measured using an ELISA kit (Beijing Solarbio Science and Technology Co. Ltd., Beijing, China).

Evaluation of blood lipid indices and inflammatory factors

Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were assessed using mouse ELISA kits (Shanghai Guang Rui Biological Technology, Shanghai, China). IL-6, IL-1 β , and TNF- α levels were also evaluated using ELISA kits.

Hematoxylin and eosin staining

On gestation day 20, the mice were anesthetized and their pancreases were removed. Pancreatic tissues were fixed with 10 % formaldehyde overnight and embedded in paraffin. The tissues were sliced into 5 μ m thick sections. The

sections were then dewaxed with xylene and hydrated using an alcohol gradient. Hematoxylin and eosin (Sigma-Aldrich) were used to stain the sections and morphological changes in the islets were observed using a light microscope (Olympus, Tokyo, Japan).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Pancreatic tissues were lysed and a TRIzol kit (Life Technologies, Carlsbad, CA, USA) was used to isolate RNAs. RNAs were synthesized into cDNAs. IL-6, IL-1 β , and TNF- α mRNA levels were assessed using the PreTaq II kit (Takara, Dalian, Liaoning, China) and the primers shown in Table 1.

Western blot

Pancreatic tissues were lysed in RIPA buffer (Sigma-Aldrich). Proteins were isolated from the tissues and protein concentrations were determined by BCA Protein Assay Kit (Sigma-Aldrich). Proteins were separated by 10 % SDS-PAGE. Samples were transferred onto nitrocellulose membranes, and the membranes were blocked in 5 % bovine serum albumin. Membranes were probed with specific antibodies overnight at 4 °C: anti-IRF2BP2 and anti-GAPDH (1:2000) and anti-p-AMPK and anti-AMPK (1:3000). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:4000). Immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich). All the antibodies were acquired from Abcam.

Statistical analysis

All experiments were performed in triplicate. Data are expressed as mean \pm SEM and were analyzed by Student's t test or one-way analysis of variance (ANOVA) using SPSS software. *p* values < 0.05 were considered statistically significant.

RESULTS

IRF2BP2 reduced glucose and insulin levels in mice with GDM

IRF2BP2 protein expression was downregulated

in the pancreases of mice with GDM compared to the control mice (Figure 1 A). Injection with OE-IRF2BP2 upregulated IRF2BP2 expression (Figure 1 A). Mice in the GDM group showed higher blood glucose levels than mice in the normal pregnancy group based on the IPGTT (Figure 1 B) and the IPGTT (Figure 1 C). However, overexpression of IRF2BP2 reduced blood glucose levels in mice with GDM (Figure 1 B and C). Moreover, injection with OE-IRF2BP2 attenuated streptozotocin-induced increases in fasting blood glucose and insulin (Figure 1 D and E).

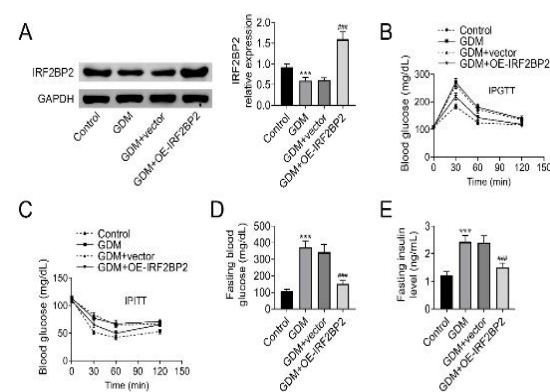


Figure 1: IRF2BP2 reduced glucose and insulin levels in mice with GDM. (A) IRF2BP2 protein expression was downregulated in the pancreases of mice with GDM, and injection with OE-IRF2BP2 upregulated IRF2BP2 expression. (B) Mice in the GDM group showed higher blood glucose levels than mice in the normal pregnancy group based on the IPGTT, and overexpression of IRF2BP2 reduced the blood glucose levels. (C) Mice in the GDM group showed higher blood glucose levels than mice in the normal pregnancy group based on the IPITT, and overexpression of IRF2BP2 reduced the blood glucose levels. (D) Overexpression of IRF2BP2 reduced the fasting blood glucose levels in mice with GDM. (E) Overexpression of IRF2BP2 reduced the fasting insulin levels in mice with GDM. ***, ### *p* < 0.001. GDM: gestational diabetes mellitus

IRF2BP2 ameliorated histopathological changes in the islets of mice with GDM

Lipid metabolism was dysregulated in mice with GDM as demonstrated by increases in TG (Figure 2 A), TC (Figure 2 B), and LDL (Figure 2 D) and a decrease in HDL (Figure 2 C).

Table 1: qRT-PCR primers

Gene	Forward	Reverse
IL-6	5'-ACAACACGCGCTTCCCTAC-3'	5'-CATTTCCACGATTTCCCAGA-3'
IL-1 β	5'-GCCCTACCAACCCACATTCTCA-3'	5'-GTCGGAGATTCGTAGCTGGAT-3'
TNF- α	5'-CCCAGGGACCTCTCTAATC-3'	5'-ATGGGCTACAGGCTTGCTACT-3'
GAPDH	5'-CGCTAACATCAAATGGGGTG-3'	5'-TTGCTGACAATCTTGAGGGAG-3'

However, overexpression of IRF2BP2 reduced levels of TG (Figure 2 A), TC (Figure 2 B), and LDL (Figure 2 D) and increased HDL (Figure 2 C). Hematoxylin and eosin staining showed that islets in the control group were regular morphology and that the cells were orderly with round nuclei, clear chromatin, and uniform size (Figure 2 E). However, in the streptozotocin-induced GDM mice, the islets were irregular morphology and the cells were disorderly with nuclear shrinkage, vacuolar degeneration, and infiltration of inflammatory cells (Figure 2 E). Overexpression of IRF2BP2 ameliorated histopathological changes in the islets of mice with GDM (Figure 2 E).

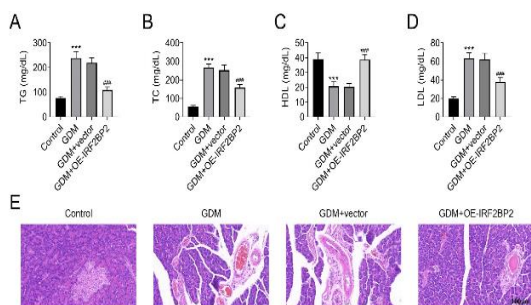


Figure 2: IRF2BP2 ameliorated histopathological changes in the islets of mice with GDM. (A) Overexpression of IRF2BP2 reduced TG levels in mice with GDM. (B) Overexpression of IRF2BP2 reduced TC levels in mice with GDM. (C) Overexpression of IRF2BP2 increased HDL levels in mice with GDM. (D) Overexpression of IRF2BP2 reduced LDL levels in mice with GDM. (E) Overexpression of IRF2BP2 ameliorated the histopathological changes in the islets of mice with GDM; $***p < 0.001$, $###p < 0.001$

IRF2BP2 reduced inflammation in mice with GDM

IL-6, IL-1 β , and TNF- α mRNA expression levels were upregulated in the islets of mice with GDM (Figure 3 A). Overexpression of IRF2BP2 reduced IL-6, IL-1 β , and TNF- α expression levels (Figure 3A). Moreover, IRF2BP2 decreased serum IL-6, IL-1 β , and TNF- α levels in mice with GDM (Figure 3 B).

IRF2BP2 ameliorated reproductive changes in mice with GDM

Mice in the GDM group had smaller litters (Figure 4 A) with higher birth weights (Figure 4 B) than the control mice. However, overexpression of IRF2BP2 increased the litter sizes (Figure 4 A) and decreased the birth weights (Figure 4 B) in mice with GDM.

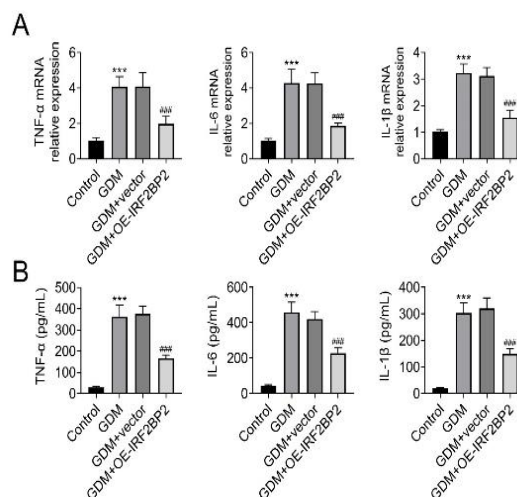


Figure 3: IRF2BP2 reduced inflammation in mice with GDM. (A) Overexpression of IRF2BP2 reduced IL-6, IL-1 β , and TNF- α mRNA expression levels in mice with GDM. (B) Overexpression of IRF2BP2 reduced serum IL-6, IL-1 β , and TNF- α levels in mice with GDM; $***p < 0.001$, $###p < 0.001$

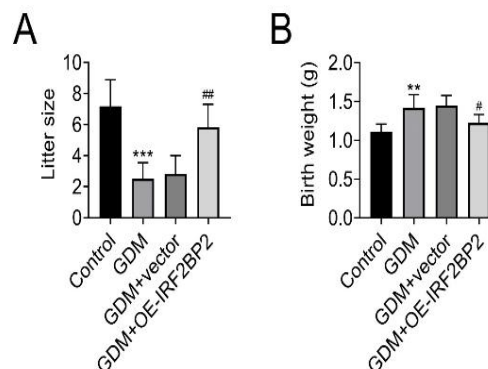


Figure 4: IRF2BP2 ameliorated reproductive changes in mice with GDM. (A) Overexpression of IRF2BP2 increased the litter sizes in mice with GDM. (B) Overexpression of IRF2BP2 reduced the birth weights in mice with GDM. $**P < 0.01$, $***p < 0.001$ vs. control, $\#p < 0.01$, $##p < 0.001$. $##p$ vs. GDM + vector

IRF2BP2 activated AMPK in mice with GDM

Phosphorylated AMP-activated protein kinase (p-AMPK) protein expression was lower in the islets of mice with GDM than in the control mice (Figure 5 A), and overexpression of IRF2BP2 increased p-AMPK expression (Figure 5 A). However, treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reductions in fasting blood glucose (Figure 5 B), insulin (Figure 5 C), TG (Figure 5 B), TC (Figure 5 E), IL-6 (Figure 5 F), and IL-1 β (Figure 5 G).

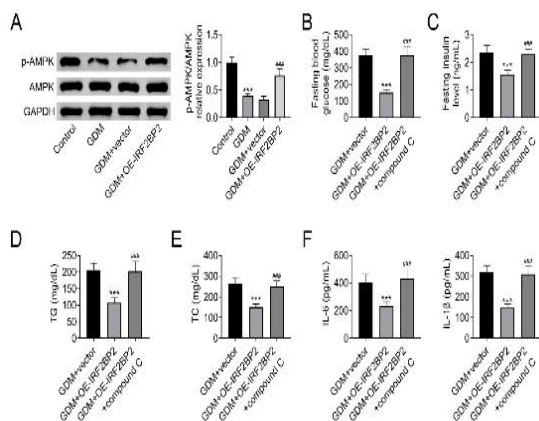


Figure 5: IRF2BP2 activated AMPK in mice with GDM. (A) Overexpression of IRF2BP2 increased p-AMPK protein expression in mice with GDM. (B) Treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reduction in fasting blood glucose in mice with GDM. (C) Treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reduction in fasting insulin in mice with GDM. (D) Treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reduction in TG in mice with GDM. (E) Treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reduction in TC in mice with GDM. (F) Treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reduction in IL-6 in mice with GDM. (G) Treatment with compound C, an inhibitor of AMPK, attenuated the IRF2BP2-induced reduction in IL-1β in mice with GDM. *** vs. control, $p < 0.001$. ### vs. GDM + vector, $p < 0.001$

DISCUSSION

IRF2BP2 has been associated with hepatic dyslipidemia, insulin resistance, and inflammation in high-fat diet-induced mice [7,8]. This study found that overexpression of IRF2BP2 exerted an anti-inflammatory effect, reduced glucose and insulin levels, and restored histopathological changes, reproductive function, and lipid metabolism to normal in mice with GDM.

In this study, injection with streptozotocin, a diabetogenic agent, increased blood glucose and insulin levels and disturbed lipid metabolism through downregulation of HDL and upregulation of TG, TC, and LDL in mice. Moreover, in streptozotocin-induced mice, the pancreatic islets were morphologically irregular. IRF2BP2 was downregulated in liver tissues of mice treated with a high-fat diet and loss of IRF2BP2 accelerated high-fat diet-induced increases in liver lipid, fasting glucose, and insulin levels [7]. Furthermore, an IRF2BP2 deletion induced glucose intolerance and insulin resistance in mice [7].

The present study demonstrated that IRF2BP2 was downregulated in the pancreases of mice with GDM and that overexpression of IRF2BP2 reduced fasting glucose and insulin levels, ameliorated glucose intolerance and insulin resistance, and attenuated dysregulated lipid metabolism in mice with GDM. The histopathological changes in mice with GDM were also alleviated by IRF2BP2. High birth weights and reduced litter sizes of offspring are regarded as characteristics of GDM [10]. Overexpression of IRF2BP2 attenuated the streptozotocin-induced decrease in litter size and increase in birth weight in GDM mice suggesting that IRF2BP2 protects against GDM.

Placental tissue has been shown to secrete cytokines IL-6, IL-8, IL-1β, and TNF-α, which contribute to maternal insulin resistance in GDM [3]. IL-1β promoted secretion of IL-8 and IL-6 and induced apoptosis of islet β cells, and IL-8 and IL-6 stimulated infiltration of inflammatory cells to aggravate the inflammatory injury [3]. Suppression of IL-6, IL-1β, and TNF-α ameliorated GDM [11]. Deletion of IRF2BP2 exacerbated high-fat diet-induced increases in IL-1β and TNF-α in mice [8]. This study showed that overexpression of IRF2BP2 reduced expression of IL-6, IL-1β, and TNF-α in mice with GDM indicating the anti-inflammatory effect of IRF2BP2 against GDM. Oxidative stress has also been implicated in the pathogenesis of GDM [12], and antioxidant strategies alleviated GDM [13]. Because knockout of IRF2BP2 enhanced oxidative stress in high-fat diet-induced mice [8], IRF2BP2 may also have an anti-oxidant effect on GDM.

AMPK is an important enzyme in fat biosynthesis and oxidation that promotes uptake of muscle glucose and inhibits production of hepatic glucose [14]. Dysregulation of AMPK signaling associated with metabolic disorders, such as insulin resistance and type 2 diabetes [14]. Downregulation of AMPK contributed to hyperglycemia in GDM through regulation of glucose transporter 3 [15], and activation of AMPK signaling ameliorated insulin resistance and inflammation in GDM [16,17]. IRF2BP2 activated AMPK signaling and exerted anti-apoptotic and anti-inflammatory effects against sepsis-induced cardiomyopathy [18]. Overexpression of IRF2BP2 reduced protein expression of p-AMPK in mice with GDM. Inhibition of AMPK reversed the IRF2BP2-induced reductions in fasting blood glucose, insulin, TG, TC, IL-6, and IL-1β levels in mice with GDM.

CONCLUSION

Overexpression of IRF2BP2 alleviates symptoms of GDM, restores glucose and insulin levels, and suppresses inflammation through activation of AMPK signaling. Therefore, IRF2BP2 may be a potential therapeutic target for GDM. However, the cellular effect of IRF2BP2 on human islets should be investigated in future research.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shuyun Zhang and Yunjie Wang designed and carried out the study. Ling Zhou supervised the data collection and analyzed and interpreted the data. Ziyang Yu and Aihua Gao prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript. Shuyun Zhang and Yunjie Wang contributed equally to the work and should be considered co-first authors.

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