

Original Article

Effects of miR-22-3p targeted regulation of Socs3 on the hepatic insulin resistance in mice with gestational diabetes mellitus

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Abstract: Objective: Gestational diabetes mellitus (GDM) is one of the common complications of pregnant women, with serious threatening to pregnant women and newborns. The pathogenesis of GDM remains unclear now. This study aims to explore the effects of miR-22-3p targeted regulation of suppressors of cytokine signaling 3 (Socs3) on the hepatic insulin resistance (HIR) in mice with GDM. Methods: Healthy SPF C57BL/6J mice were selected to establish GDM model and divided into 7 groups: Normal group, Model group, NC-(negative control) mimic group, miR-22-3p mimic group, NC-pcDNA3.0 group, pcDNA3.0-Socs3 group, and miR-22-3p mimic + pcDNA3.0-Socs3 group. The islet morphology, and the expressions of miR-22-3p, Socs3 mRNA and Socs3 protein in the islet tissues were detected by HE staining, qRT-PCR and Western blot. Fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured. Oral glucose tolerance test (OGTT) was performed to detect FBG and fasting insulin (FINS) contents, and insulin resistance (HOMA-IR) was calculated. Results: Compared with the Normal group, the model group had decreased levels of miR-22-3p and HDL-C, while increased levels of Socs3 mRNA and protein expressions, OGTT, FBG, FINS, and HOMA-IR, TG and TC (all $P < 0.05$). Compared with the Model group, the above indicators (OGTT, FBG, FINS, HOMA-IR, TG, TC and HDL-C) were improved in the miR-22-3p mimic group, but worsened in the pcDNA3.0-Socs3 group (all $P < 0.05$). Conclusion: miR-22-3p can down-regulate the expression of Socs3, thereby inhibiting HIR in GDM mice.

Keywords: miR-22-3p, suppressors of cytokine signaling 3, gestational diabetes mellitus, hepatic insulin resistance, effect

Introduction

Gestational diabetes mellitus (GDM) is one of the common complications of pregnant women, and it refers to the abnormal glucose tolerance that occurs or is first discovered during pregnancy [1]. GDM is a polygenic disease characterized by insufficient insulin secretion and insulin resistance (IR), and it usually occurs during the middle and late pregnancy [2]. The main clinical manifestations of this disease include overweight of pregnant women, and recurrent episodes of vulvovaginal and vaginal candidiasis infection [3]. This disease seriously endangers the health of newborns and pregnant women, and can cause spontaneous abortion,

fetal macrosomia, gestational hypertension (GH) and excessive quantities of amniotic fluid, as well as neonatal asphyxia and congenital infection [4]. The prevalence of GDM is rising, increased by more than 30% in the last two decades [5]. Therefore, it is of great significance to explore the pathogenesis of GDM for the early intervention and active prevention and treatment.

The pathogenesis of GDM is still not clear now, which may be resulted from the combined effects of genetic and environmental factors [5]. Studies have pointed out that the pathogenesis of GDM is related to the decrease of insulin secretion and IR [6]. IR is the main pathological

feature of GDM and plays a key role in GDM's occurrence and development, leading to the decline of the body's ability to process glucose [7]. Insufficient insulin in pregnant women can't resist the IR, causing the increase of blood glucose and eventually leading to GDM [8]. Therefore, this study explored the treatment for GDM from IR, the potential pathogenesis of GDM.

Suppressors of cytokine signaling 3 (Socs3) has been widely studied in recent years, especially in the aspects of glucose metabolism and IR, and Socs3 may be a therapeutic target for these diseases. Socs3, a novel type of cytokine signal transduction inhibitory molecules in the families of Socs, is located at 17q25.3 and has a length of 3,303 bp [9]. Researchers have found that, in general, the intracellular expression level of Socs3 is rather low, but with the stimulation of cytokines and hormones, the expression level of Socs3 usually rises rapidly within 15-20 min [10]. The expression level of Socs3 mRNA was significantly increased in the visceral adipose tissues of GDM patients [11].

microRNA (miRNA) is a class of endogenous non-coding small RNA molecule and it plays an important role in the pathogenetic process of GDM [12]. Studies have found that the abnormal expression of miRNAs is closely related to the occurrence and development of pregnancy-related diseases such as placental dysplasia [13, 14]. We used TargetScan online prediction website to screen the upstream miRNA of Socs and we found a possible binding site between Socs and miR-22-3p. The effects of miR-22-3p on the hepatic insulin resistance (HIR) of mice with GDM were studied in this experiment. There are few studies on miR-22-3p, mainly found in severe acute pancreatitis, non-small cell lung cancer, and liver cancer [15-17]; it is the first time to investigate the effects of miR-22-3p on GDM in this study.

In this paper, the effects of miR-22-3p targeted regulate Socs3 on the HIR of mice with GDM were investigated.

Materials and methods

Dual-luciferase reporter gene assay

TargetScan online prediction website (http://www.targetscan.org/vert_71/) was used to

analyze the target genes of miR-22-3p, and dual-luciferase reporter gene assay was conducted to verify whether Socs3 is the direct target gene of miR-22-3p. The 3'UTR fragments of Socs3 were cloned and amplified, and the PCR products were cloned to the multiple cloning sites in the downstream of pmirGLO (E1330; Promega Corporation, USA) Luciferase gene and named Wt-Socs3. The specific binding sites in miR-22-3p and Socs3 predicted by bioinformatics website (TargetScan (http://www.targetscan.org/vert_72/)) were mutated and named Mut-Socs3. pRL-TK vector (Ranilla luciferase; E2241; Promega, USA) was used as the internal reference. miR-22-3p mimic and NC-mimic were co-transfected with the luciferase reporter vectors into 293T cells, and the luciferase activity in each group was detected.

Experimental animals

Healthy SPF C57BL/6J mice (Experimental Animal Center of Guangzhou University of Chinese Medicine, China), weighing 20-25 g, were included in this study. All animals were bred under specific pathogen-free conditions (IVC system, UK) with a normal diet. All mice were raised in a controlled environment (25 ± 1°C, humidity of 40-70%), under alternating 12 h of light and darkness, and were given free diet and water. This study was approved by the Animal Ethics Committee of the Fourth Hospital of Shijiazhuang.

Establishment of GDM model

Female mice aged 8 weeks (n = 90) and male mice of the same age (n = 45) were mated in a cage with a ratio of 2:1. The occurrence of vaginal tamponade in the next morning was considered to be day 0.5 of pregnancy. After confirmation of pregnancy, the pregnant mice were randomly divided into two groups: control group (Normal group, n = 10) and GDM group (n = 80). After 12 h of fasting, the GDM group received an intraperitoneal injection of newly prepared streptozotocin (150 mg/kg; Sigma, St. Louis, MO, USA) within 30 min to induce GDM model [18]; the Normal group received an injection of excipient (50 mM sodium citrate, pH 4.5). Three days after injection, blood glucose levels were measured from caudal venous blood using Accu-Chek Performa blood glucose meter (Roche Diagnostics, Germany). The success of GDM model was defined as stable of glucose

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level ≥ 16.7 mM or 300 mg/dL for three days [18].

A total of 60 mice selected from the successful GDM model were divided into 6 groups, 10 in each group: Model group (model mice), NC- (negative control) mimic group (model mice with tail vein injection of negative control vector), miR-22-3p mimic group (model mice with tail vein injection of miR-22-3p mimic), NC-pcDNA3.0 group (model mice with tail vein injection of pcDNA3.0 empty vector), pcDNA3.0-Socs3 group (model mice with tail vein injection of pcDNA3.0-Socs3), and miR-22-3p mimic + pcDNA3.0-Socs3 group (model mice with tail vein injection of miR-22-3p mimic and pcDNA3.0-Socs3). miR-22-3p mimic (5'-TCATTTCTCGCTCGATTCT-3'), pcDNA3.0-Socs3 (5'-TGCGGAATCCATCGTATCC-3'), NC-pcDNA3.0 and NC-mimic (5'-GACTCAGATTCTG GAAC-3') were packaged by lentivirus with a virus injection of 2×10^7 IU (all purchased from Shanghai GenePharma Co., Ltd., China). All pregnant mice were given free water and normal diet.

HE staining

After the mice were anesthetized with 3% pentobarbital sodium (P3761, Sigma, USA), blood samples were obtained via eyeball blood collection for other experiments. After blood collection, the mice died due to excessive blood loss under anesthesia. Then the pancreas was quickly removed, and the pancreatic tissues were fixed with 10% formaldehyde solution. The pancreatic tissues were taken to prepare paraffin sections with a thickness of 5 μ m. Then the paraffin sections were spread out at 45°C, picked up, baked for 1 h at 60°C, dewaxed with xylene and stained with hematoxylin (Beijing Solarbio Science & Technology Co., Ltd., China). Details are as follows. The sections were dewaxed with xylene, hydrated in gradient alcohol, and stained with hematoxylin for 2 min. Then, the sections were washed for 10 s, differentiated with 1% hydrochloric acid alcohol for 30 s, washed for 2 s, and stained with eosin (Beijing Solarbio Science & Technology Co., Ltd., China) for 2 min. After washed again, the sections were dehydrated with gradient alcohol, cleared with xylene, and mounted with permount mounting medium for the observation of morphological changes of islet cells under ordinary optics microscope (XP-330, Shanghai Binyu Optical Instrument Co., Ltd., China).

Oral glucose tolerance test (OGTT)

The mice were fasted for 12 h overnight before OGTT. Glucose (2 g/kg body weight) was given by oral gavage. The tail vein blood samples were obtained to measure glucose levels at 0, 30, 60, and 120 min after glucose gavage, and then the oral glucose tolerance curve was plotted. The area under the curve was calculated and the differences between the groups were compared. Glucose area under the curve = $1/4 \times$ fasting blood glucose (FBG) value + $1/2 \times$ 0.5 h blood glucose value + $4/3 \times$ 1 h blood glucose value + 2 h blood glucose value [19].

Detection of FBG

Blood samples of all female mice were collected on gestational day 18. After 12 h of fasting, the mice were anesthetized with 3% pentobarbital sodium (P3761; Sigma, USA), and the blood was sampled from eyeball extirpating. The blood samples were placed at room temperature for 2 h, centrifuged at $10,000 \times g$ for 20 min, and the serum was stored in a 1 mL EP tube at -80°C. FBG was determined by glucose oxidase assay (Biosino Bio-technology and Science Inc., China). Glucose is oxidized into H_2O_2 and gluconic acid with the action of glucose oxidase. H_2O_2 reacts with 4-Aminoantipyrine and phenol to produce quinone-imine under the catalysis of peroxidase. The optical density (OD) was measured at a wavelength of 505 nm by Biochemical Analyzer (7171A; Hitachi, Japan), and the OD was directly proportional to the glucose content.

Detection of fasting insulin (FINS)

Blood samples of all female mice were collected on gestational day 18. The concentration of FINS was determined by double antibody sandwich Elisa (BC1710; Beijing Solarbio Science & Technology Co., Ltd., China). All procedures were conducted according to the instructions of the kit. Standard substance, sample and HRP-labeled insulin antibody were successively added into the microporous plate to form the complex, and then the TMB substrate was added for color development. The OD value was detected at 450 nm wavelength by the enzyme marker. The concentration of FINS in the sample was calculated from the standard curve.

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Table 1. Primer sequence

	Sequence
miR-22-3p	F: 5'-GGAGGTGCGCTGGTCTC-3' R: 5'-GGGACGGAAGGGCAGA-3'
Socs3	F: 5'-TGCAACCAACGCATCGGATC-3' R: 5'-GTTTCGATGCAGGAGCGTAGG-3'
U6	F: 5'-CGCTTCTGCCACACAGG-3' R: 5'-ACACTCGAGATTACGTG-3'
GAPDH	F: 5'-AGGTCCGGTGAACGGATTG-3' R: 5'-GGGGTCGTTGATGGCAACA-3'

Note: Socs3, suppressors of cytokine signaling 3.

Detection of insulin resistance

Homeostasis model assessment (HOMA) was used to calculate insulin resistance (HOMA-IR). $HOMA-IR = FBG * FINS / 22.5$ [20]. The higher HOMA-IR value represents lower sensitivity of peripheral tissues to insulin and stronger IR.

Detection of blood lipid indexes

Blood samples of all female mice were collected on gestational day 18. Mouse triglyceride (TG) ELISA kit, mouse total cholesterol (TC) ELISA kit, and mouse high-density lipoprotein cholesterol (HDL-C) ELISA kit (all purchased from Shanghai Guang Rui Biological Technology Co., Ltd., China) were used to measure the serum concentrations of TG, TC and HDL-C, respectively. The procedures were performed in accordance with the instructions of the kit. Standard substance, sample and HRP-labeled antibodies (TG, TC and HDL-C antibodies) were successively added into the microporous plate to form the complexes, and then the TMB substrate was added for color development. The OD value was detected at 450 nm wavelength by the enzyme marker. The concentrations of TG, TC and HDL-C in the sample were calculated from the standard curves, respectively.

qRT-PCR

The total RNA of pancreatic tissues was extracted from each group using a Trizol kit (Invitrogen Company, USA), and its purity and concentration were determined. RNA was reversely transcribed into cDNA using RT reverse transcription kit (11939823001; Merck, USA). The reaction mixture (10 μ L) consisted of 0.5 μ L each of the forward and reverse primers, 1 μ L cDNA, 3 μ L ddH₂O, and 5 μ L (2 \times) SYBR[®] Premix Ex Taq[™] II. The reaction conditions were: pre-denatur-

ation at 95°C for 4 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and after 35 cycles elongation at 72°C for 5 min. U6 and GAPDH were selected as reference genes for quantitating miR-22-3p and Socs3, respectively. The relative expression of each gene was calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences of detected genes are listed in **Table 1**, and the primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd., China. $\Delta\Delta Ct = \Delta Ct_{\text{other group}} - \Delta Ct_{\text{control group}}$, $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{reference gene}}$

Western blot

The islet tissue of each group was collected, and the total protein was extracted with the kit (BB-3121; Shanghai BestBio Co., Ltd., China). The protein concentration was determined using the BCA kit (20201ES76; Yesen Biotechnology (Shanghai) Co., Ltd., China). The protein samples (20 μ g/well) were fractionated by 8% SDS-PAGE for 1 h and the protein bands were transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk and then incubated with primary antibodies overnight at 4°C. The primary antibodies were Socs3 (ab14939; 1:2,000, Abcam, UK) and GAPDH (ab181602; 1:5,000, Abcam, USA). After thrice washes with PBST, the membrane was incubated with horseradish peroxidase-labeled goat anti-mouse secondary antibody IgG (ab6789; 1:1,000, Abcam, Cambridge, MA, UK) for 1 h at room temperature. Following three PBST washes, the membrane was reacted with ECL (ECL808-25; Biomiga, USA) and exposed in a dark room. GAPDH were regarded as the internal reference. The relative expression of each protein was calculated as ratio of protein band gray-scale value to internal reference band gray-scale value.

Statistical analysis

Data were processed using SPSS20.0 (IBM SPSS Statistics, Chicago, IL, USA). Measurement data were expressed as the mean \pm standard deviation ($\bar{x} \pm sd$). Unpaired data with normal distribution and homogeneity in two groups were compared by unpaired t-test. Comparison of data among multiple groups was done using one-way analysis of variance (ANOVA) and pairwise comparison was performed using Tukey's post-hoc test. Difference was statistically significant when $P < 0.05$.

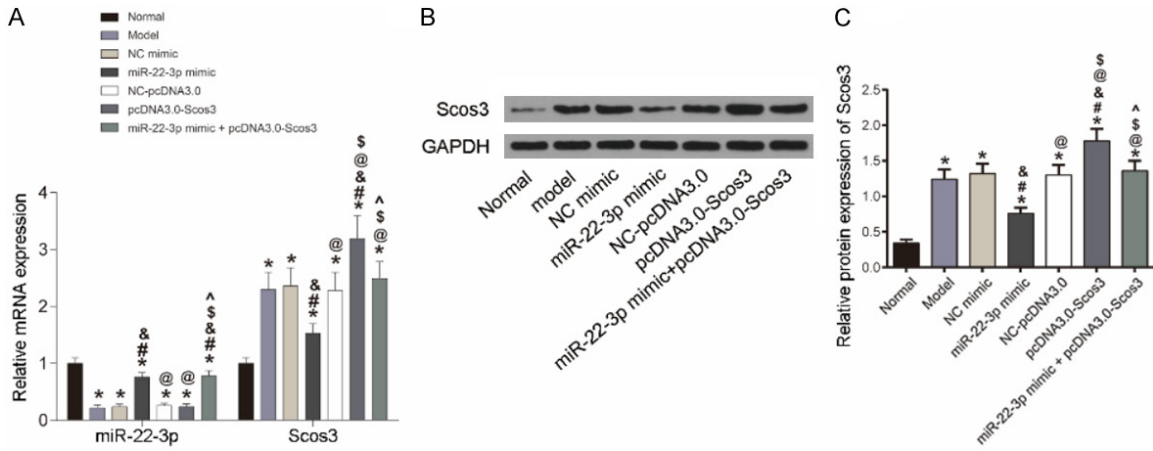


Figure 1. The expressions of miR-22-3p, Socs3 mRNA and Socs3 protein detected by qRT-PCR and Western blot. A. The histograms of qRT-PCR results; B. Western blot bands; C. The histograms of Western blot results. Compared with the Normal group, *P < 0.05; compared with the Model group, #P < 0.05; compared with the NC-mimic group, &P < 0.05; compared with the miR-22-3p mimic group, @P < 0.05; compared with the NC-pcDNA3.0 group, \$P < 0.05; compared with the pcDNA3.0-Socs3 group, ^P < 0.05. Socs3: suppressors of cytokine signaling 3.

Results

The changes of miR-22-3p and Socs3 expression levels in model mice

To verify the over-expressions of miR-22-3p and socs3 after injection of miR-22-3p mimic or pcDNA3.0-Socs3, the expressions of miR-22-3p, Socs3 mRNA, and Socs3 protein were detected by qRT-PCR and Western blot (Figure 1). Compared with the Normal group, the miR-22-3p expressions were significantly reduced, and the mRNA and protein expressions of Socs3 were increased significantly in the other groups (all P < 0.05). Compared with the Model group, the miR-22-3p expressions of miR-22-3p mimic and miR-22-3p mimic + pcDNA3.0-Socs3 groups were increased significantly, the mRNA and protein expressions of Socs3 in the miR-22-3p mimic group were significantly decreased, while the mRNA and protein expressions of Socs3 in the pcDNA3.0-Socs3 group were increased significantly (all P < 0.05). There was no difference between the Model and NC-mimic group in terms of the above indicators (all P > 0.05). These results indicated that model mice had low level of miR-22-3p expression, but high level of Socs3 expressions, and miR-22-3p upregulation treatment could inhibit Socs3 expression.

miR-22-3p upregulation and Socs3 inhibition alleviated the tissue damages of model mice

To investigate the effects of miR-22-3p and Socs3 expression upregulations on the histo-

morphology of islets, islet morphology of each group was observed by HE staining (Figure 2). The Normal group had regular islet morphology, orderly cell arrangement, uniform size, clear chromatin and mostly round nuclei. As for the Model, NC-mimic, NC-pcDNA3.0, and miR-22-3p mimic + pcDNA3.0-Socs3 group, the morphology of the islet tissue was irregular with different degrees of atrophy, the cells were disordered arrangement with vacuolar degeneration, and the number of cells was reduced, with nuclear pyknosis and lysis, mainly infiltrated by inflammatory cells such as lymphocytes and monocytes. miR-22-3p mimic group had relative regular islet morphology, reduced number of cells, and less vacuolar degeneration; while compared with the Model group, pcDNA3.0-Socs3 had more irregular islet morphology, larger degree of atrophy and damage, and severer infiltration of inflammatory cells. Therefore, miR-22-3p upregulation could alleviate the tissue damages of model mice, while Socs3 up-regulation would deteriorate the damages.

miR-22-3p targets and down-regulates the expression of Socs3

To further explore the relationship between miR-22-3p and Socs3, the specific binding sites of miR-22-3p and Socs3 were predicted by TargetScan online prediction website (Figure 3A) and the dual-luciferase reporter gene assay was performed to verify the results of bioinformatic prediction (Figure 3B). Compared with the NC mimic group, the luciferase activity was

miR-22-3p targeted regulation of Socs3 on HIR

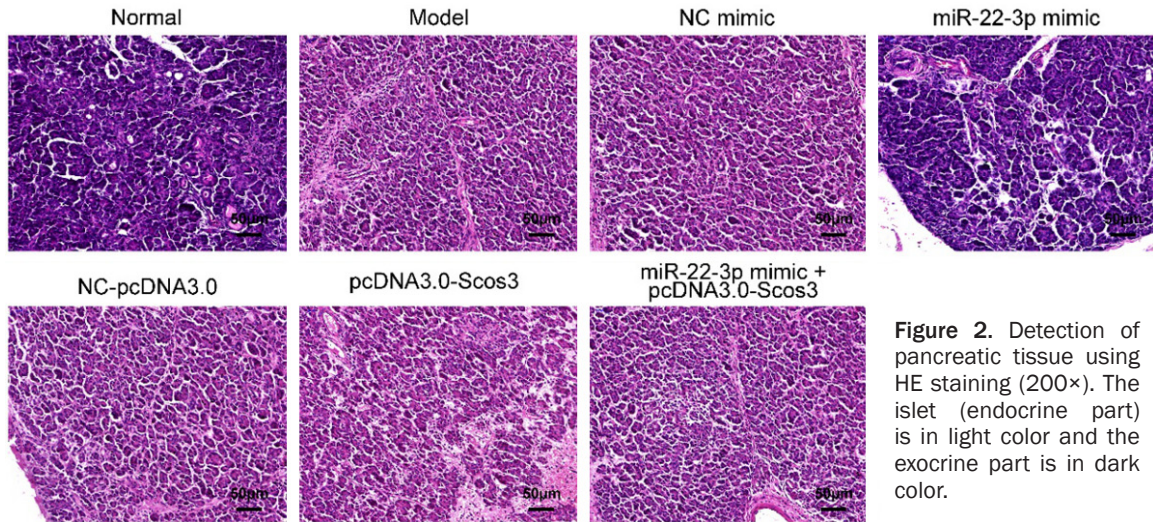


Figure 2. Detection of pancreatic tissue using HE staining (200×). The islet (endocrine part) is in light color and the exocrine part is in dark color.

A

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score
Position 1201-1207 of <i>Socs3</i> 3' UTR	5' ... UAUGGGGCUGGGUGGGCAGCUG ...		
<i>mmu-miR-22-3p</i>	3' UGUCAAGAAGUGACCGUCGAA	7mer-m8	-0.17

B

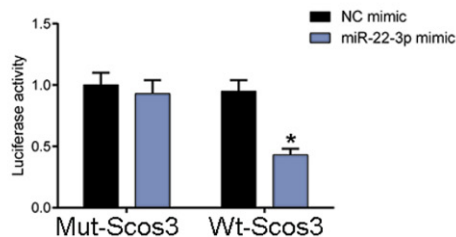


Figure 3. miR-22-3p targets and down-regulates the expression of *Socs3*. A. The sequence of 3'-UTR for the pairing of miR-22-3p and *Socs3*; B. Luciferase activity detected by dual-luciferase reporter gene assay. Compared with the NC mimic group, * $P < 0.05$. *Socs3*: suppressors of cytokine signaling 3.

significantly decreased in the miR-22-3p mimic group with co-transfection of Wt-Socs3 ($P < 0.05$), while the luciferase activity remained unchanged in the miR-22-3p mimic group with co-transfection of Mut-Socs3 plasmid ($P > 0.05$). The results delineated that miR-22-3p can targetedly down-regulate the expression of *Socs3*.

miR-22-3p upregulation could decrease the level of blood glucose and severe insulin resistance in model mice by inhibiting Socs3

To investigate the effects of miR-22-3p and *Socs3* expression upregulations on the level of blood glucose and severe insulin resistance, on gestational day 18, the changes of FBG and FINS were detected, and HOMA-IR was calculated (**Figure 4**). Compared with the Normal group, the FBG, FINS and HOMA-IR in the other groups were significantly increased (all $P < 0.05$). Compared with the Model group, the

three indicators were significantly reduced and increased in the miR-22-3p mimic and pcDNA3.0-Socs3 groups respectively (all $P < 0.05$). There was no intergroup difference in the three indications among the Model, NC-mimic, NC-pcDNA3.0, and miR-22-3p mimic + pcDNA3.0-Socs3 groups (all $P > 0.05$). Compared with the miR-22-3p mimic group, the three indicators were significantly increased in the miR-22-3p mimic + pcDNA3.0-Socs3 group (all $P < 0.05$). These results indicated that miR-22-3p upregulation could alleviate the high level of blood glucose and severe insulin resistance in model mice, while *Socs3* up-regulation would deteriorate these symptoms.

miR-22-3p upregulation could improve the blood glucose regulating ability of model mice OGTT inhibiting Socs3

To investigate the effects of miR-22-3p and *Socs3* expression upregulations on the blood

miR-22-3p targeted regulation of Socs3 on HIR

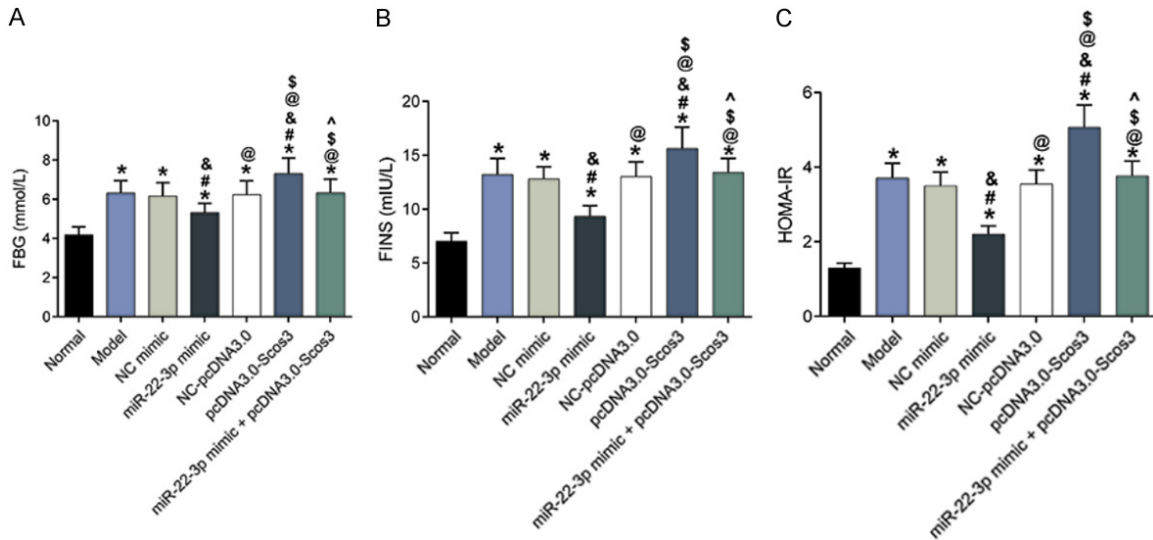


Figure 4. The changes of FBG, FINS and HOMA-IR. A. The changes of FBG; B. The changes of FINS; C. The changes of HOMA-IR. Compared with the Normal group, *P < 0.05; compared with the Model group, #P < 0.05; compared with the NC-mimic group, &P < 0.05; compared with the miR-22-3p mimic group, @P < 0.05; compared with the NC-pcDNA3.0 group, \$P < 0.05; compared with the pcDNA3.0-Socs3 group, ^P < 0.05. FBG: fasting blood glucose; FINS: fasting insulin; HOMA-IR: homeostasis model assessment insulin resistance; Socs3: suppressors of cytokine signaling 3.

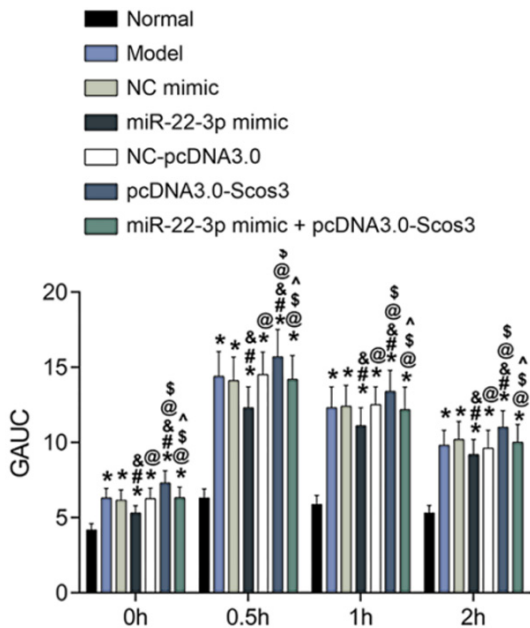


Figure 5. The changes of blood glucose level. Compared with the Normal group, *P < 0.05; compared with the Model group, #P < 0.05; compared with the NC-mimic group, &P < 0.05; compared with the miR-22-3p mimic group, @P < 0.05; compared with the NC-pcDNA3.0 group, \$P < 0.05; compared with the pcDNA3.0-Socs3 group, ^P < 0.05. GAUC: glucose area under the curve; Socs3: suppressors of cytokine signaling 3.

glucose regulating ability of model mice, the OGTT were detected (Figure 5). Compared with the Normal group, the blood glucose level of the Model group was increased significantly and reached the highest level at 0.5 h after oral glucose load, and then decreased at 1 h and 2 h after oral glucose load (all P < 0.05). Compared with the Model group, the blood glucose level of the miR-22-3p mimic group was decreased significantly at 0.5 h, 1 h, and 2 h after oral glucose load, while that of the pcDNA3.0-Socs3 group was increased significantly at the same time points (all P < 0.05). No intergroup difference was seen in the NC-mimic, NC-pcDNA3.0, and miR-22-3p mimic + pcDNA3.0-Socs3 groups in terms of the above indicator (all P > 0.05). These results indicated that miR-22-3p upregulation could improve the blood glucose regulating ability of model mice, while Socs3 up-regulation would deteriorate it.

miR-22-3p upregulation could improve the blood lipid metabolism of model mice by inhibiting Socs3

To investigate the effects of miR-22-3p and Socs3 expression upregulations on the blood lipid metabolism, on gestational day 18, the blood lipid indexes including TG, TC, HDL-C

miR-22-3p targeted regulation of Socs3 on HIR

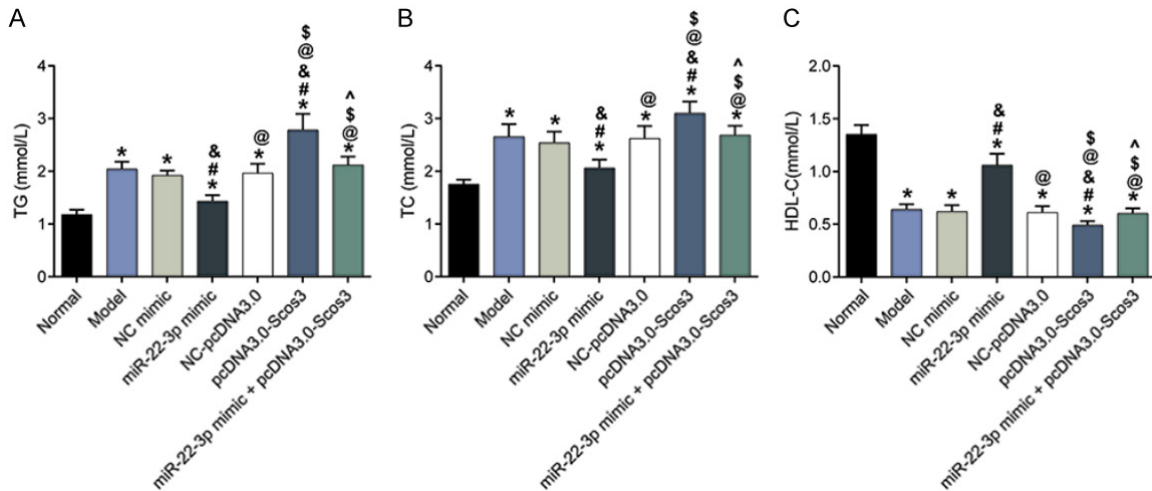


Figure 6. The content of blood lipid indexes. A. TG content; B. TC content; C. HDL-C content. Compared with the Normal group, * $P < 0.05$; compared with the Model group, # $P < 0.05$; compared with the NC-mimic group, \$ $P < 0.05$; compared with the miR-22-3p mimic group, @ $P < 0.05$; compared with the NC-pcDNA3.0 group, ^ $P < 0.05$. Socs3: suppressors of cytokine signaling 3; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol.

were measured and the results were shown in **Figure 6**. Compared with Normal group, there were significantly increased TG and TC, and significantly reduced HDL-C in the other groups (all $P < 0.05$). Compared with the Model group, TG and TC were significantly decreased, and HDL-C was significantly increased in the miR-22-3p mimic group (all $P < 0.05$); while the three indicators showed opposite trend in the pcDNA3.0-Socs3 group (all $P < 0.05$). There was no difference among the Model, NC-mimic, NC-pcDNA3.0, and miR-22-3p mimic + pcDNA3.0-Socs3 groups in terms of the above indicators (all $P > 0.05$). Compared with the miR-22-3p mimic group, there were increased TG and TC, and significantly decreased HDL-C in the miR-22-3p mimic + pcDNA3.0-Socs3 group (all $P < 0.05$). These results indicated that miR-22-3p upregulation could improve the blood lipid metabolism of model mice, while Socs3 up-regulation would deteriorate it.

Discussion

GDM is becoming a common disease all around the world. The screen of GDM in pregnant patients is often performed in the middle and late pregnancy, thus it is urgent to find efficient and feasible clinical treatment methods. Currently, the oral hypoglycemic agents are the main treatment for GDM, but its effectiveness and safety need to be further confirmed. The treatment of GDM has its own uniqueness lies

on the condition of pregnant women and the health of the fetus.

The correlation between pregnancy-related diseases and miRNA expression has become a clinical hotspot. Studies have found that miR-26b was associated with the occurrence and development of GH and high expression level of miR-26b was presented in serum of GH patients; therefore, miR-26b may be an important molecular mechanism in the formation of GH [21]. Researches on GDM found that the expressions of miR-41, miR-200a, and miR-375 were closely related to the blood glucose related indicators, and their abnormal expressions indicated the susceptibility of GDM, providing a certain reference for the early diagnosis of GDM [22, 23]. This study found that miR-22-3p regulated GDM through its targeted down-regulation of Socs3. The specific binding sites of miR-22-3p and Socs3 were predicted by TargetScan online prediction website. The luciferase activity of the miR-22-3p mimic group with co-transfection of Wt-Socs3 was significantly decreased, while the luciferase activity remained unchanged in the miR-22-3p mimic group with co-transfection of Mut-Socs3 plasmid, indicating that miR-22-3p can targetedly down-regulate the Socs3.

In this study, compared with the Model group, OGTT, FBG, FINS, HOMA-IR, TG and TC were significantly decreased, and HDL-C was signifi-

cantly increased in the miR-22-3p mimic group; the above indicators showed opposite trend in the pcDNA3.0-Socs3 group. The higher HOMA-IR value represents lower sensitivity of peripheral tissues to insulin and stronger IR. Though the model mice had islet damages which may lead to insulin secretion insufficiency, the severe IR significantly slowed down the insulin mentalism, thus the model mice still had high FINS level. It is predicted that miR-22-3p targetedly down-regulate the Socs3 to improve GDM through affecting HIR of mice. IR is the core pathogenesis of GDM, and overexpression of Socs-3 may interfere with the effect of insulin and lead to IR [24]. Previous studies have confirmed that the reduced expression level of Socs-3 can regulate blood glucose [25]. The study of the expression and significance of Socs3 in placental tissue of GDM patients has found that the overexpression of Socs3 inhibits insulin signal transduction and promotes IR [26]. The mechanism of Socs3-mediated IR involves: 1) inhibition of insulin receptor substrate -1 (IRS-1) phosphorylation; 2) competitive inhibition of STAT5b; 3) mediated IRS-1 ubiquitination and increased degradation of IRS-1 protein; 4) negative feedback regulation of JAK2/SATA3 signal transduction pathway; 5) affecting leptin signal transduction [27].

However, this study lacks sufficient evidence owing to few studies on the relationship between miR-22-3p and diabetes. Moreover, we are not sure if our findings applied to only GDM or can it be found also in T2DM with pregnancy as the mice model we established are Type I diabetes mellitus, it remains to be further confirmed.

The pathogenesis of GDM is closely related to Socs3; Socs3 is overexpressed in GDM patients and miR-22-3p can down-regulate the expression of Socs3, thereby inhibiting HIR of GDM mice.

Disclosure of conflict of interest

None.

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References

[1] Yao H, Xu D, Zhu Z and Wang G. Gestational diabetes mellitus increases the detection rate

and the number of oral bacteria in pregnant women. *Medicine (Baltimore)* 2019; 98: e14903.

- [2] Zhang T, Ji C and Shi R. miR-142-3p promotes pancreatic β cell survival through targeting FOXO1 in gestational diabetes mellitus. *Int J Clin Exp Pathol* 2019; 12: 1529-1538.
- [3] Zhu WW, Yang HX, Wang C, Su RN, Feng H and Kapur A. High prevalence of gestational diabetes mellitus in Beijing: effect of maternal birth weight and other risk factors. *Chin Med J (Engl)* 2017; 130: 1019-1025.
- [4] Zhao L, Zhang Y, Gu ZR and Li YG. Effects of effective blood glucose control on pregnancy outcomes and neonatal complications in pregnant women with gestational diabetes. *Int J Clin Exp Med* 2019; 12: 4387-4391.
- [5] Zhu Y and Zhang C. Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective. *Curr Diab Rep* 2016; 16: 7.
- [6] Li L, Lee SJ, Kook SY, Ahn TG, Lee JY and Hwang JY. Serum from pregnant women with gestational diabetes mellitus increases the expression of FABP4 mRNA in primary subcutaneous human pre-adipocytes. *Obstet Gynecol Sci* 2017; 60: 274-282.
- [7] Sun YL, Zhou FM and Wang HR. Mechanism of pomegranate ellagic polyphenols reducing insulin resistance on gestational diabetes mellitus rats. *Am J Transl Res* 2019; 11: 5487-5500.
- [8] Jeon EJ and Lee JH. Response: adipokines and insulin resistance according to characteristics of pregnant women with gestational diabetes mellitus. *Diabetes Metab J* 2018; 42: 90-91.
- [9] Ozkul C, Guclu-Gunduz A, Irkeç C, Fidan I, Aydin Y, Ozkan T and Yazici G. Effect of combined exercise training on serum brain-derived neurotrophic factor, suppressors of cytokine signaling 1 and 3 in patients with multiple sclerosis. *J Neuroimmunol* 2018; 316: 121-129.
- [10] do Carmo JM, da Silva AA, Freeman JN, Wang Z, Moak SP, Hankins MW, Drummond HA and Hall JE. Neuronal suppressor of cytokine signaling 3: role in modulating chronic metabolic and cardiovascular effects of leptin. *Hypertension* 2018; 71: 1248-1257.
- [11] Rancourt RC, Ott R, Ziska T, Schellong K, Melchior K, Henrich W and Plagemann A. Visceral adipose tissue inflammatory factors (TNF-Alpha, SOCS3) in gestational diabetes (GDM): epigenetics as a clue in GDM pathophysiology. *Int J Mol Sci* 2020; 21: 479.
- [12] Kappeler BIG, Regitano LCA, Poleti MD, Cesar ASM, Moreira GCM, Gasparin G and Coutinho LL. MiRNAs differentially expressed in skeletal muscle of animals with divergent estimated breeding values for beef tenderness. *BMC Mol Biol* 2019; 20: 1.

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- [13] Poirier C, Desgagné V, Guérin R and Bouchard L. MicroRNAs in pregnancy and gestational diabetes mellitus: emerging role in maternal metabolic regulation. *Curr Diab Rep* 2017; 17: 35.
- [14] He Y, Bai J, Liu P, Dong J, Tang Y, Zhou J, Han P, Xing J, Chen Y and Yu X. miR-494 protects pancreatic β -cell function by targeting PTEN in gestational diabetes mellitus. *EXCLI J* 2017; 16: 1297-1307.
- [15] Ulivi P, Petracci E, Marisi G, Baglivo S, Chiari R, Billi M, Canale M, Pasini L, Racanicchi S, Vagheggini A, Delmonte A, Mariotti M, Ludovini V, Bonafè M, Crinò L and Grignani F. Prognostic role of circulating mirnas in early-stage non-small cell lung cancer. *J Clin Med* 2019; 8: 131.
- [16] Chen J, Wu FX, Luo HL, Liu JJ, Luo T, Bai T, Li LQ and Fan XH. Berberine upregulates miR-22-3p to suppress hepatocellular carcinoma cell proliferation by targeting Sp1. *Am J Transl Res* 2016; 8: 4932-4941.
- [17] Qin T, Fu Q, Pan YF, Liu CJ, Wang YZ, Hu MX, Tang Q and Zhang HW. Expressions of miR-22 and miR-135a in acute pancreatitis. *J Huazhong Univ Sci Technol Med Sci* 2014; 34: 225-233.
- [18] Salim HM, Fukuda D, Yagi S, Soeki T, Shimabukuro M and Sata M. Glycemic control with ipragliflozin, a novel selective SGLT2 inhibitor, ameliorated endothelial dysfunction in streptozotocin-induced diabetic mouse. *Front Cardiovasc Med* 2016; 3: 43.
- [19] Fu SH, Chen YT, Chiang CH and Hsu BR. Enhancing engraftment of neonatal porcine xenograft with CTLA4Ig and nordihydroguaiaretic acid. *Transplant Proc* 2016; 38: 3283-3285.
- [20] Wang Y, Feng Q, Niu X, Xu K, Wang Y, Wang J, Li Q, Mao Y and Gao S. The preventive effect of zuogui wan on offspring rats' impaired glucose tolerance whose mothers had gestational diabetes mellitus. *Evid Based Complement Alternat Med* 2016; 2016: 9417362.
- [21] Zhou S, Sun L, Cao C, Wu P, Li M, Sun G, Fei G, Ding X and Wang R. Hypoxia-induced microRNA-26b inhibition contributes to hypoxic pulmonary hypertension via CTGF. *J Cell Biochem* 2018; 119: 1942-1952.
- [22] Mi Y, Guo N, He T, Ji J, Li Z and Huang P. miR-410 enhanced hESC-derived pancreatic endoderm transplant to alleviate gestational diabetes mellitus. *J Mol Endocrinol* 2015; 55: 219-229.
- [23] Peng HY, Li HP and Li MQ. High glucose induces dysfunction of human umbilical vein endothelial cells by upregulating miR-137 in gestational diabetes mellitus. *Microvasc Res* 2018; 118: 90-100.
- [24] Blumenstein M, Bowen-Shauver JM, Keelan JA and Mitchell MD. Identification of suppressors of cytokine signaling (SOCS) proteins in human gestational tissues: differential regulation is associated with the onset of labor. *J Clin Endocrinol Metab* 2002; 87: 1094-1097.
- [25] Park EJ, Park SY, Joe EH and Jou I. 15d-PGJ2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia. *J Biol Chem* 2003; 278: 14747-14752.
- [26] Kuzmicki M, Telejko B, Wawrusiewicz-Kurylonek N, Citko A, Lipinska D, Pliszka J, Wilk J, Kalejta K, Lemancewicz A, Grabiec M, Pyszczepko-Wawreszuk AM, Skibicka J, Kretowski A, Gorska M and Szamatowicz J. The expression of suppressor of cytokine signaling 1 and 3 in fat and placental tissue from women with gestational diabetes. *Gynecol Endocrinol* 2012; 28: 841-844.
- [27] Rehman K, Akash MSH, Liaqat A, Kamal S, Qadir MI and Rasul A. Role of interleukin-6 in development of insulin resistance and type 2 diabetes mellitus. *Crit Rev Eukaryot Gene Expr* 2017; 27: 229-236.