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Dysregulation of microRNA-770-5p influences pancreatic-β-cell function by targeting TP53 regulated inhibitor of apoptosis 1 in gestational diabetes mellitus

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the role of microRNA-770-5p (miR-770-5p) in gestational diabetes mellitus (GDM).

MATERIALS AND METHODS: In the present study, the expression levels of miR-770-5p in the peripheral blood from GDM women and healthy women were investigated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The relationship between TP53 regulated inhibitor of apoptosis 1 (TRIAP1) and miR-770-5p was determined using dual-luciferase reporter assay. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay and flow cytometry were used to detect pancreatic β-cell proliferation and apoptosis. Enzyme-linked immunosorbent assay (ELISA) was used to measure total insulin content and insulin secretion.

RESULTS: Our data indicated that miR-770-5p was up-regulated in GDM patients. TRIAP1 was a direct target of miR-770-5p and it was down-regulated in GDM patients. Besides, miR-770-5p negatively regulated the expression of TRIAP1 in INS-1 cells. Then, we explored the effects of miR-770-5p down-regulation on the insulin secretion of pancreatic β-cells, and the results showed that miR-770-5p inhibitor promoted the generation of insulin secretion or total insulin content in INS-1 cells, while these effects were significantly inhibited by TRIAP1-siRNA. Moreover, we found that miR-770-5p inhibitor enhanced INS-1 cell proliferation and suppressed cell apoptosis, whereas these effects were eliminated by TRIAP1-siRNA. Accordingly, miR-770-5p inhibitor decreased the expression of Bax, apoptotic peptidase activating factor 1 (APAF1) and increased Bcl-2 level in INS1 cells. These results were all reversed by TRIAP1-siRNA.

CONCLUSIONS: The data demonstrated that miR-770-5p was a vital regulator in pancreatic β-cell proliferation, apoptosis and insulin secretion by targeting TRIAP1, and dysregulation of miR-770-5p resulted in the development of GDM via APAF1 signaling pathway.

Key Words:
MicroRNA-770-5p, Pancreatic-β-cell, Gestational diabetes mellitus, TRIAP1.

Introduction

Gestational diabetes mellitus (GDM), defined as diabetes diagnosed during pregnancy, is one of the most normal complications that can develop during pregnancy [1-4]. The incidence of GDM worldwide is increasing, and 3-8% of all pregnancies are caused by GDM [5]. Despite great improvements have been made in the diagnosis and treatment of GDM, the pathogenesis of GDM is still not fully addressed. Therefore, it is important to investigate the mechanisms leading to the progression of GDM. Several studies have shown that GDM is related to pancreatic β-cell dysfunction which cannot secrete insulin normally[6]. Decrease of pancreatic β-cell function is characterized by enhanced apoptosis rates and defective insulin generation and secretion[7]. However, the specific mechanisms of pancreatic β cells dysfunction in the development of GDM are complicated and are not completely understood.

MicroRNAs (miRNAs), a class of small non-coding RNAs, about 20-22 nucleotides in length, negatively modulate gene expression by binding with the 3′-untranslated region (3′-UTR) of target mRNA for translational repression or degradation[8,9]. Increasing evidence has supported that miRNAs are participated in regulating

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metabolism and pathogenesis of various types of diabetes, including GDM [10-12]. Furthermore, a number of miRNAs have been demonstrated to be involved in insulin production and secretion, pancreatic β-cell proliferation and apoptosis and endocrine pancreas regeneration, such as miR-124a[13], miR-184[14], miR-375[15] and so on. MiRNAs dysregulation in circulation or placenta participate in the inflammatory response and lead to GDM. Therefore, more studies are needed to be conducted to explore the novel and prospective strategies for GDM treatment by targeting miRNAs network.

The purpose of this study was to investigate the modulating effect of miR-770-5p in GDM and its functions in pancreatic β cells, and further to explore the underlying mechanisms. Our results showed that miR-770-5p might serve as a potential candidate for GDM treatment and suggested that targeting APAF1 pathway might be a potential application for GDM.

Materials and Methods

Clinical Specimens Collection

Peripheral blood samples were collected from 30 patients with gestational diabetes mellitus and 30 healthy pregnancies from Huai'an First People's Hospital. The blood samples were immediately frozen and stored at –80°C until further analysis. Written informed consent was obtained from every patient and all patients approved the use of their blood samples in this study. The present study procedures were approved by Ethics Committee of Huai'an First People's Hospital.

Cell Culture

The INS-1 cell line, a well differentiated mouse insulinoma β-cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). INS-1 cells were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen, Carlsbad, CA, USA) supplemented with 11 mM glucose, 2 mM L-glutamine, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 55 μM β-mercaptoethanol, and 1% penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

Cell Transfection and Reagents

TRIAP1-siRNA, control-siRNA, miR-770-5p inhibitor and inhibitor control were purchased from GenePharma (Shanghai, China). Cell transfection was carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. 48 h after cell transfection, qRT-PCR and Western blot assay were performed to evaluate the transfection efficiency.

MTT Assay

INS-1 cells were transfected with inhibitor control, miR-770-5p inhibitor, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA. After transfection for 48 h, INS-1 cells were treated with 10 μl MTT (5 mg/ml) solution and continuously incubated for further 4 h. After that, the solution was removed and 100 μl dimethylsulfoxide (DMSO) was added to each well to solubilize the formazan product. Finally, the optical density (OD) at the wavelength of 490 nm was measured by FLUOstar® Omega Microplate Reader (BMG LABTECH, Ortenberg, Germany) after 15 min of vibration mixing following the manufacturer's instructions. The experiment was repeated three times.

Flow Cytometry Analysis

INS-1 cells were transfected with inhibitor control, miR-770-5p inhibitor, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA for 48 h, then INS-1 cell apoptosis was evaluated by using An annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) was used to quantify cell apoptosis and data were analyzed by applying CellQuest software.

Dual-Luciferase Reporter Assay

TargetScan bioinformatics software (http://www.targetscan.org/vert_71) was performed to predict the potential target genes of miR-770-5p, and we observed the binding sites between TRIAP1 and miR-770-5p. Then, the TRIAP1-3'UTR, which contains the miR-770-5p binding sites or mutated target sites were synthesized by genomic PCR and cloned into pGL-3-Luc (Promega, Madison, WI, USA) to construct the reporter vector TRIAP1-wild-type (TRIAP1-WT) or TRIAP1-mutated-type (TRIAP1-MUT). The INS-1 cells were co-transfected with TRIAP1-WT or TRIAP1-MUT combined with miR-770-

5p mimic or mimic control using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's manual. 48 h after cell transfection, Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was applied to detect the luciferase activity.

ELISA

INS-1 cells were transfected with inhibitor control, miR-770-5p inhibitor, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA for 48 h, then the INS-1 cells were treated with basal glucose (3.3 mM) or stimulatory glucose (16.7 mM) for 1 h. Subsequently, total insulin secretion in cells was detected using ELISA kits (BioLegend, Inc., San Diego, CA, USA) following the product instructions. After cells were sonicated in acid ethanol (2% H_2SO_4), they were followed by 3 freeze/thaw cycles, and then centrifuged for 10 min at 10,000 × g, the level of insulin in supernatant was detected by ELISA.

QRT-PCR

Total RNA from INS-1 cells or blood samples was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Then, 200 ng of total RNA was used to produce cDNA with the PrimeScript™ RT-PCR Kit (TaKaRa, Otsu, Shiga, Japan). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 small nuclear RNA (U6) were used as internal references for mRNA and miRNA, respectively. Primers were purchased from Sangon Biotech (Shanghai, China). qPCR was performed using a SYBR Green PCR Master Mix Kit (TaKaRa, Otsu, Shiga, Japan) under the ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Primer sequences were listed as following: miR-770-5p forward, 5'-CCAGTACCACGTGTCAG-3'; Reverse, 5'-GAACATGTCTGCGTATCTC-3'; TRIAP1 forward, 5'-GCACCGACCTCTTCAAGC-3'; Reverse, 5'-CCATGAACTCCAGTCCTTCA3'; U6 forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3'; Reverse, 5'-CGCTTCACGAATTTGCGTGTCAT-3'; GAPDH forward, 5'-CTTTGGTATCGTGGAAGGACTC-3'; Reverse, 5'-GTAGAGGCAGGGATGATGTTCT-3'; APAF1 forward, 5'-AACCAGGATGGGTCACCATA-3'; Reverse, 5'-ACTGAAACCCAATGCACTCC-3'; Bax forward, 5'-CGTCCACCAAGAAGCTGAGCG-3'; Reverse, 5'-CGTCCACCAAAGCTGAGCG-3'; Bcl-2 forward, 5'-TTGGATCAGGGAGTTGGAAG-3'; Reverse, 5'-TGTCCCTACCAACCAGAAGG-3'. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative gene expression.

Western Blot Analysis

Proteins from INS-1 cells or blood samples were isolated using Radio Immunoprecipitation Assay (RIPA) buffer (Sigma-Aldrich, St. Louis, MO, USA). We used Bicinchoninic Acid Protein Assay Kit (Pierce, Rockford, IL, USA) to determine the protein samples concentration. Then, the protein samples were mixed with 5× loading buffer, boiled at 100°C for 5 min, centrifuged at 13,500 rpm at 4°C for 2 min, separated by 10% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride (PVDF) membrane. Subsequently, the membranes were incubated at 4° C overnight with primary antibodies (1: 1000 dilution) against APAF1, Bcl-2, Bax, and GAPDH (all Cell Signaling Technology Inc., Danvers, MA, USA), respectively. After washing, the membranes were incubated with a secondary antibody for 2 h at room temperature. Finally, proteins were assessed by the enhanced chemiluminescence (ECL) Western blotting detection kits (Merck Millipore, Billerica, MA, USA) according to the manufacturer's protocol.

Statistical Analysis

All the above experiments were performed three times. Data were presented as the mean ± standard deviation (SD) and analyzed by using SPSS software version 18.0 (IBM Corp., Armonk, NY, USA). Differences were estimated with Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test among groups. $p<0.05$ indicated statistically significant difference.

Results

MiR-770-5p Expression was Up-Regulated in Blood Samples From Patients with Gestational Diabetes Mellitus

In the present study, 30 gestational diabetes mellitus patients and 30 healthy pregnancies peripheral blood samples were collected to determine miR-770-5p expression levels by qRT-PCR. As shown in Figure 1, the miR-770-5p expression was significantly higher in the peripheral blood samples of GDM patients compared with the healthy pregnancies (Figure 1).

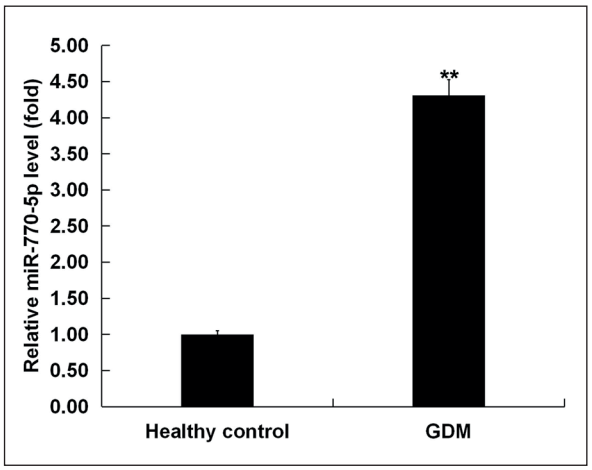

Figure 1. MiR-770-5p was up-regulated in gestational diabetes mellitus patients. The expression levels of miR-770-5p was validated by qRT-PCR in peripheral blood samples from 30 gestational diabetes mellitus patients (GDM) and healthy pregnancies (Healthy control). All experiments were carried out three times. Data were expressed as the mean ± SD.**p< 0.01 *vs.* Healthy control.

TRIAP1 was a Direct Target of MiR-770-5p and MiR-770-5p Suppressed TRIAP1 Expression by Targeting its 3'UTR

To investigate the role of miR-770-5p in pancreatic β cells, we performed TargetScan to analyze the potential targets of miR-770-5p. We found that TRIAP1 was a possible target of miR-770-5p (Figure 2A). To better validate whether TRIAP1 was a direct target of miR-770-5p, luciferase reporter assay was performed. We firstly observed that miR-770-5p mimic significantly enhanced miR-770-5p expression in INS-1 cells (Figure 2B). Then, the results indicated that transfection of the miR-770-5p mimic markedly decreased the luciferase activities of cells co-transfected with TRIAP1 with wild-type 3'-UTR, whereas, there was no significant difference in the luciferase activities of cells co-transfected with TRIAP1 containing the mutant 3'-UTR and miR-770-5p mimic (Figure 2C).

We then conducted qRT-PCR analysis to detect the TRIAP1 mRNA levels in the peripheral blood samples of GDM patients. As shown in Figure 2D, the mRNA levels of TRIAP1 significantly decreased in the peripheral blood samples of GDM patients compared with that in the healthy pregnancies. In summary, these results showed that TRIAP1 was a direct target of miR-770-5p in INS-1 cells. Meanwhile, TRIAP1 was down-regulated in GDM patients.

TRIAP1-siRNA Reversed the Incremental Effects of MiR-770-5p Inhibitor in Pancreatic β-Cells

To further explore the relevance of miR-770-5p/TRIAP1 in pancreatic β-cells dysfunction, control-siRNA, TRIAP1-siRNA, miR-770-5p inhibitor or inhibitor control was transfected into INS-1 cells for 48 h. We conducted qRT-PCR and western blot assays to assess the transfection efficiency. As shown in Figure 3A, compared with the control group, miR-770-5p inhibitor significantly down-regulated the miR-770-5p level in INS-1 cells. Meanwhile, the mRNA and

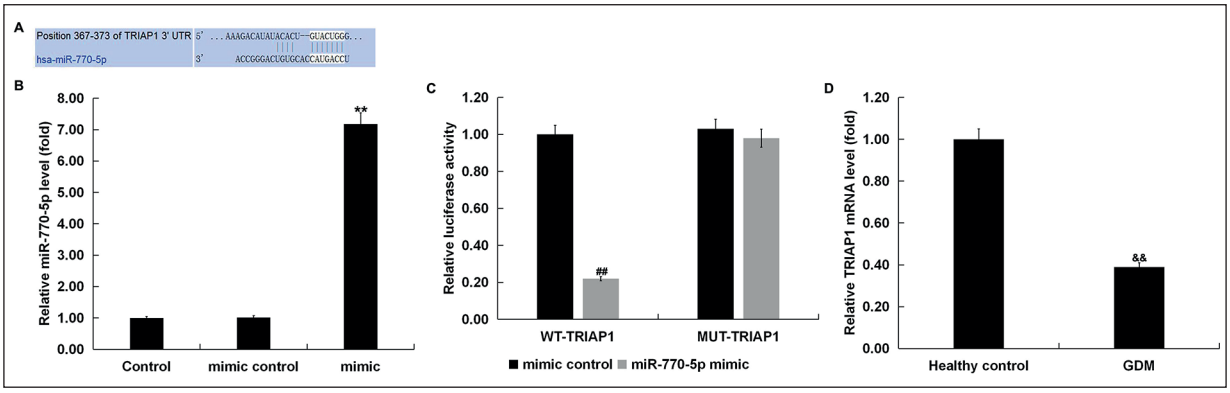

Figure 2. MiR-770-5p targeted the TRIAP1 transcript 3'UTR. **(A)** Binding sites of miR-770-5p in the 3′-UTR of TRIAP1 predicted by bioinformatics tools. **(B)** INS-1 cells were transfected with miR-770-5p mimic or mimic control, then the level of miR-770-5p in INS-1 cells was detected using qRT-PCR. **(C)** Relative luciferase activity of a reporter containing a wild-type TRIAP1 3'UTR or a mutant TRIAP1 3'UTR was measured by Dual Luciferase Assay. **(D)** The mRNA level of TRIAP1 in peripheral blood samples of gestational diabetes mellitus patients (GDM) and healthy pregnancies (Healthy control) was determined by qRT-PCR assay. The results were depicted as the mean ± SD of three independent experiments. **p<0.01 *vs.* Control; $^{\#\#}p$<0.01 *vs.* mimic control; $^{\&\&}p$<0.01 *vs.* Healthy control.

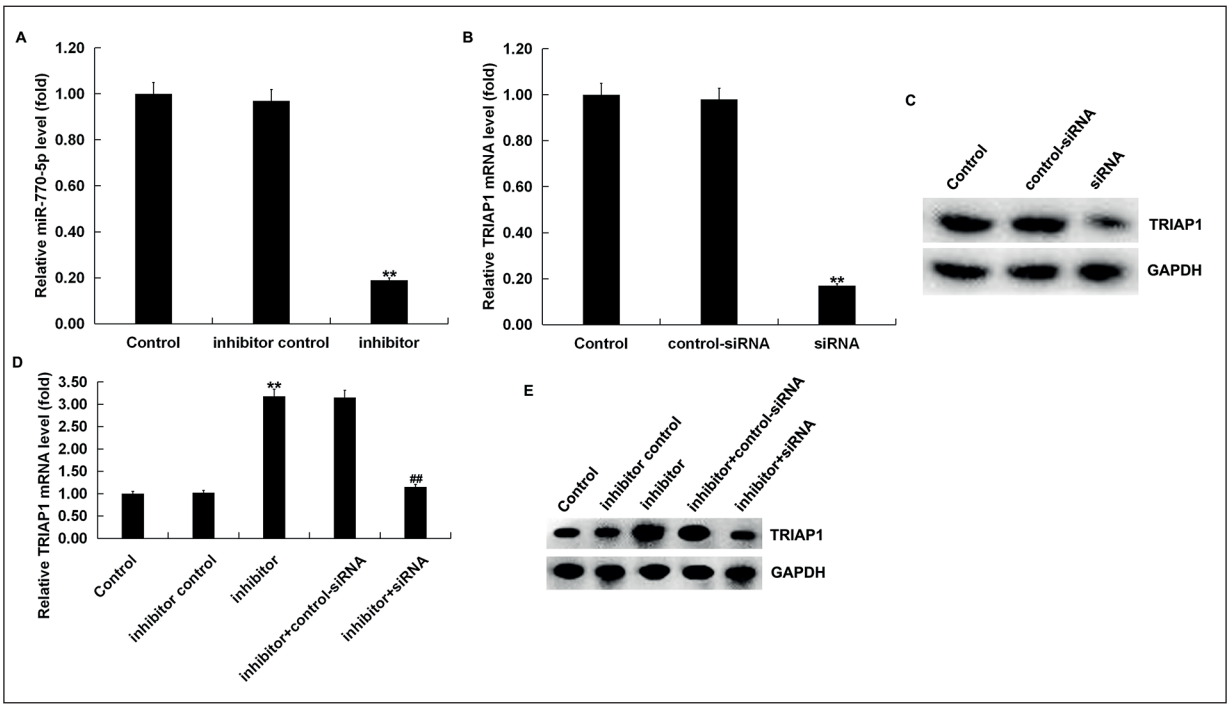

Figure 3. Effect of miR-770-5p inhibitor on TRIAP1 expression in INS-1 cells. INS-1 cells were co-transfected with the control-siRNA, TRIAP1-siRNA, inhibitor control or miR-770-5p inhibitor for 48 h. qRT-PCR and Western blot assays were conducted to evaluate the transfection efficiency. **(A)** The expression of miR-770-5p was tested in miR-770-5p inhibitor group or control group. **(B-C)** The TRIAP1 mRNA levels and protein expression were detected by qRT-PCR and Western blot assays in INS-1 cells. **(D-E)** qRT-PCR and Western blotting assays were carried out to measure the TRIAP1 expression in INS-1 cells transfected with miR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA. All experiments were performed three times. Data were displayed as the mean ± SD; $^{**}p<0.01$ *vs.* Control; $^{\#\#}p<0.01$ *vs.* inhibitor.

protein levels of TRIAP1 in INS-1 cells were remarkably decreased by TRIAP1-siRNA (Figure 3B and C). Besides, results from qRT-PCR and western blot assay indicated that both the mRNA and protein levels of TRIAP1 were up-regulated when the cells were transfected with miR-770-5p inhibitor compared to the control group and TRIAP1-siRNA eliminated these increases (Figure 3D and E). Taken together, we found that TRIAP1 could interfere with miR-770-5p expression in INS-1 cells.

MiR-770-5p Inhibitor Enhanced Insulin Secretion and Total Insulin Content

To investigate whether miR-770-5p affected the function of INS-1 cells in regulating metabolism. INS-1 cells were transfected with miR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA, or miR-770-5p inhibitor+TRIAP1-siRNA for 48 h and then treated with stimulatory glucose or basal glucose for 1 h. We measured the levels of insulin in miR-770-5p down-expressed INS-1 cells. As shown in Figure 4A, the total insulin content in INS-1 cells was increased in miR-770-5p inhibitor group compared to the control group, while the effects were reversed by TRIPA1-siRNA. As is well-known, high glucose will induce insulin secretion, we found that miR-770-5p inhibitor further enhanced insulin secretion in response to stimulatory glucose or basal glucose in INS-1 cells. Meanwhile, TRIPA1-siRNA abolished the effects of miR-770-5p inhibitor on insulin secretion (Figure 4B). These results demonstrated that miR-770-5p inhibitor enhanced insulin secretion in INS-1 cells after glucose stimulation.

TRIAP1-Sirna Reversed the Effect of Mir-770-5p Inhibitor on Cell Proliferation and Apoptosis in Pancreatic β-Cells

Then, we further investigated the effects of miR-770-5p on the proliferation and apoptosis of INS-1 cells. INS-1 cells were transfected with miR-770-5p inhibitor, inhibitor control, miR-770-

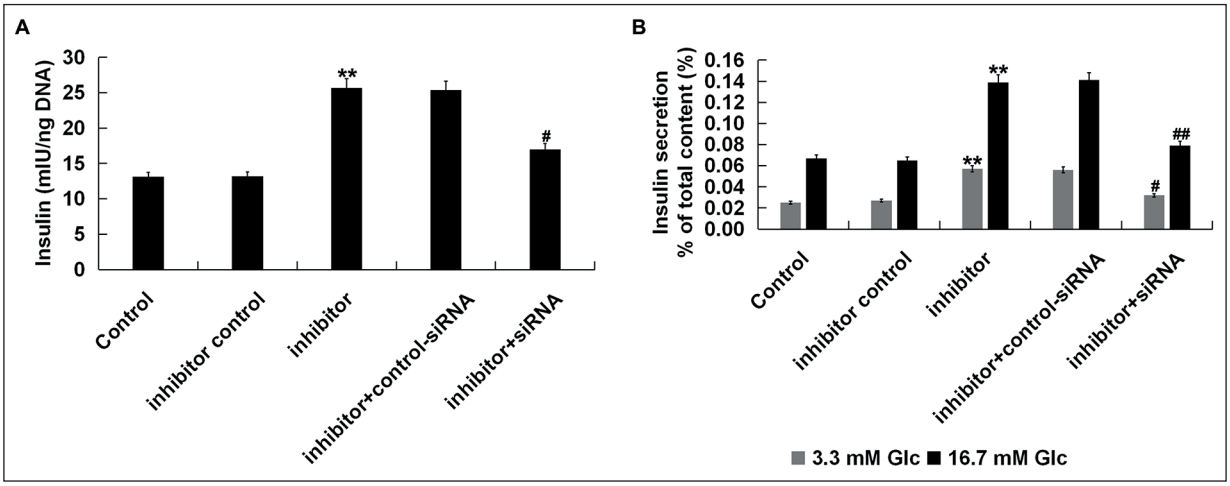

Figure 4. MiR-770-5p inhibitor enhanced the functions of pancreatic β cells by targeting TRIAP1. MiR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA was transfected into INS-1 cells pre-treated with 3.3 or 16.7 mM glucose. **(A)** Total insulin content was determined by ELISA assay in INS-1 cells. **(B)** Insulin secretion was detected by ELISA assays in INS-1 cells. The experiments were repeated for three times. All data were presented as the mean ± SD. **p<0.01 *vs.* Control; $^{\#}$, $^{\#\#}p$<0.05, 0.01 *vs.* inhibitor.

5p inhibitor+control-siRNA, or miR-770-5p inhibitor+TRIAP1-siRNA for 48 h. Findings suggested that miR-770-5p inhibitor significantly increased INS-1 cell viability and this effect was abolished by TRIAP1-siRNA (Figure 5A). Meanwhile, miR-770-5p inhibitor significantly reduced INS-1 cell apoptosis and TRIAP1 silencing eliminated this effect in INS-1 cells (Figure 5B and C). These data suggested that miR-770-5p inhibitor enhanced insulin secretion through promoting the growth and suppressing apoptosis of INS-1 cells.

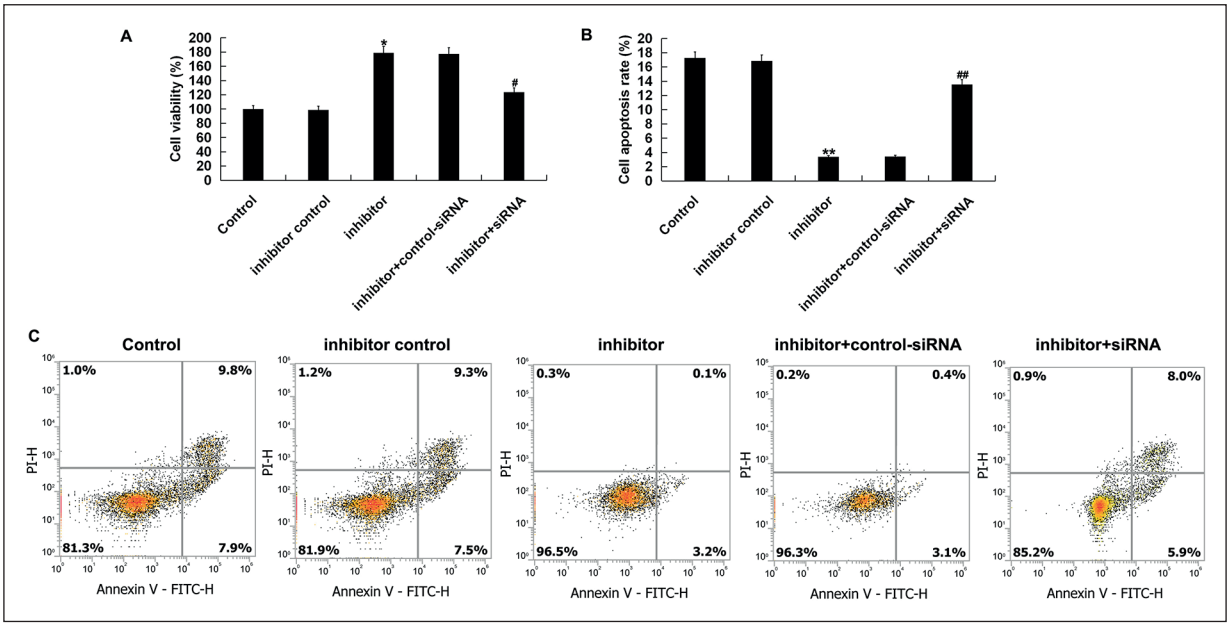

Figure 5. Effects of miR-770-5p inhibitor on INS-1 cell proliferation and apoptosis. MiR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA was transfected into INS-1 cells for 48 h. **(A)** MTT assay was used to determine cell viability. Cell apoptosis was evaluated by flow cytometric analysis, and cell apoptosis rate was calculated **(B)** and representative pictures of apoptosis from flow cytometry were shown **(C)**. Data were represented as the mean ± SD of three independent experiments. *, **p<0.05, 0.01 *vs.* Control; $^{\#}$, $^{\#\#}p$<0.05, 0.01 *vs.* inhibitor.

Mir-770-5p Inhibitor Affected APAF1 Pathway in Pancreatic β-Cells by Targeting TRIAP1

To further explore the molecular mechanism of miR-770-5p inhibitor reduced cell apoptosis in pancreatic β-cells, the expression of Bax, APAF1, and Bcl-2 in APAF1 pathway were determined by western blot assay or qRT-PCR. INS-1 cells were transfected with miR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA, or miR-770-5p inhibitor+TRIAP1-siRNA for 48 h. As shown in Figure 6A, APAF1, and Bax protein levels in pancreatic β-cells were markedly suppressed by miR-770-5p inhibitor, while the protein level of Bcl-2 was increased and these effects were reversed by TRIAP1 silencing. Similar results were observed from qRT-PCR analysis (Figure 6B-D).

Discussion

Gestational diabetes mellitus (GDM), regarded as any degree of carbohydrate intolerance in pregnancy[16], is one of the leading causes of death in diabetic patients[17]. While, at present, the treatment of GDM is still not satisfactory. Furthermore, several studies have evaluated that miRNAs are involved in multiple types of diseases and a lot of miRNAs have been demonstrated to be involved in the regulation of pancreatic β-cell development and function during pregnancy[18-20]. For example, Li et al[21] reported that miR-19a-3p enhanced the insulin secretion of pancreatic β-cells and promoted pancreatic β-cell proliferation, while the apoptosis of pancreatic β-cells was inhibited through the inhibition of suppressor of cytokine signaling 3 (SOCS3). Although many

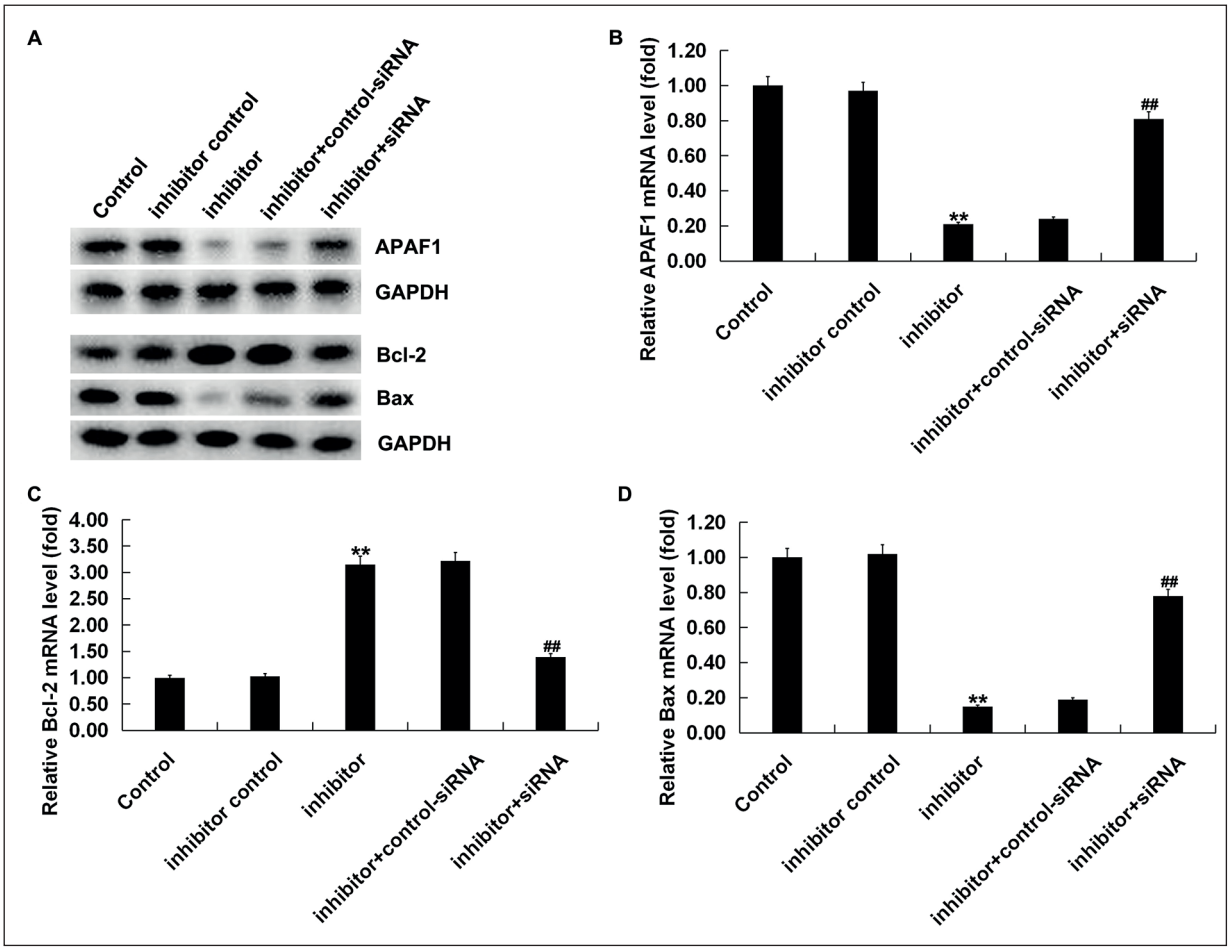

Figure 6. Effects of miR-770-5p inhibitor on APAF1 pathway in pancreatic β-cells. MiR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA or miR-770-5p inhibitor+TRIAP1-siRNA was transfected into INS-1 cells for 48 h. Then, Western blot assay **(A)** and qRT-PCR assays **(B-D)** were performed to detect the protein expression and mRNA levels of APAF1, Bcl-2, and Bax in pancreatic β-cells, respectively. All experiments were performed in triplicate. Results showed the mean ± SD of three independent experiments. $^{**}p<0.01$ *vs.* Control; $^{\#\#}p<0.01$ *vs.* inhibitor.

miRNAs have been identified as vital functional elements in the development of GDM, the molecular mechanisms by which miRNAs regulated this disease are not completely understood and further investigations are required to explain the roles of these miRNAs in the regulation of signaling pathways related to GDM pathology. Meanwhile, the role of miR-770-5p in GDM remains unknown. Therefore, in the present study, we aimed to investigate the role of miR-770-5p in pancreatic β-cells in GDM.

First, we detected the level of miR-770-5p in the peripheral blood samples of GDM pregnancies, and the results showed that miR-770-5p was up-regulated in GDM patients compared with healthy pregnancies, indicating the potential role of miR-770-5p in the development of GDM. Then, in order to investigate the precise role of miR-770-5p in GDM, we conducted TargetScan and dual-luciferase reporter assay to predict and confirm the target genes of miR-770-5p. Our data confirmed that TRIAP1 was a direct target of miR-770-5p and it was down-regulated in the peripheral blood samples of GDM pregnancies. We speculated that down-regulated miR-770-5p expression might play an important role in pancreatic β-cells in GDM. To verify our hypothesis, we transfected miR-770-5p inhibitor, inhibitor control, miR-770-5p inhibitor+control-siRNA, or miR-770-5p inhibitor+TRIAP1-siRNA into INS-1 cells. The results demonstrated that miR-770-5p inhibitor increased the TRIAP1 expression, while this increase was eliminated by TRIAP1-siRNA. It was reported that the inadequate β-cells adaptation to peripheral insulin resistance is likely to be the leading cause of GDM and the abundance of GDM is negatively related to the blood glucose concentration. Thus, we next detected the insulin secretion and total insulin content in INS-1 cells after stimulatory glucose or basal glucose treatment. Our results provided the evidence that miR-770-5p inhibitor promoted the insulin secretion and total insulin content levels in pancreatic β-cells. This analysis was in accordance with the findings of Zhang et al[22].

TRIAP1 is a small, conserved protein containing 76 amino acids and plays a vital role in reducing cell death[23]. Moreover, TRIAP1 could regulate apoptotic pathways via preventing the formation of the APAF1, cytochrome c, and Caspase 9 apoptosome complex[24]. Other studies also indicated that TRIAP1 was involved in diabetes development[25]. Therefore, next, we explored the effects of miR-770-5p inhibitor or TRIAP1-siRNA on the proliferation and apoptosis of pancreatic β-cells and investigated whether APAF1 pathway was involved in. Findings from our study suggested that inhibition of miR-770-5p promoted INS-1 cell proliferation and suppressed the apoptosis of INS-1 cells by targeting TRIAP1, indicating the inhibitory effects of miR-770-5p inhibitor on pancreatic β-cells apoptosis. In addition, we found that miR-770-5p inhibitor inhibited the expression of Bax and APAF1 in pancreatic β-cells, while the Bcl-2 levels were significantly increased. It was worth mentioning that TRIAP1 silencing abolished all these effects miR-770-5p inhibitor of on pancreatic β-cells. These results suggested that TRIAP1 played a critical role in the function of pancreatic β cells. Taken together, our data illuminated that miR-770-5p was up-regulated in GDM patients and it might be a vital regulator in pancreatic β-cell proliferation, apoptosis and insulin secretion by targeting TRIAP1.

In summary, our research demonstrated an unrecognized role of miR-770-5p in GDM by targeting TRIAP1 and APAF1 pathway, and miR-770-5p might be a potential therapeutic target for the treatment of GDM. This report helped us to better understand the role of miRNAs in GDM and provided more theoretically foundation for targeted therapies. In future studies, other targets of miR-770-5p in GDM should be explored to fully illustrate the roles of miR-770-5p in INS-1 cells.

Conclusions

MiR-770-5p down-regulation plays a protective role in GDM *in vitro* by regulating TRIAP1/APAF1 pathway. Therefore, miR-770-5p may be a novel and potential therapeutic target for GDM treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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