

# miRNA-770-5p expression is upregulated in patients with type 2 diabetes and miRNA-770-5p knockdown protects pancreatic $\beta$ -cell function via targeting BAG5 expression

MIN WANG, JILOU WEI, TING JI and KUI ZANG

Department of Critical Care Medicine, The First Affiliated Huai'an People's Hospital of Nanjing Medical University, Huai'an, Jiangsu 223300, P.R. China

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**Abstract.** MicroRNA (miR)-770-5p expression is increased in patients with type 2 diabetes mellitus (T2DM) compared with healthy controls; however, the roles and molecular mechanism underlying miR-770-5p in T2DM are not completely understood. In the present study, the reverse transcription-quantitative PCR (RT-qPCR) results indicated that miR-770-5p expression was significantly increased and Bcl-2 associated athanogene 5 (BAG5) expression was significantly decreased in the serum of patients with T2DM compared with healthy volunteers. TargetScan and a dual luciferase reporter gene system were used to predict and verify BAG5 as a target gene of miR-770-5p. Additionally, the RT-qPCR results demonstrated that miR-770-5p expression was significantly increased and BAG5 expression was significantly decreased in uric acid (UA)-treated Min6 cells compared with control cells. Min6 cells were transfected with miR-770-5p inhibitor and BAG5-small interfering (si)RNA to alter expression levels. The results indicated that miR-770-5p negatively regulated BAG5. The effect of miR-770-5p knockdown on UA-induced pancreatic  $\beta$ -cell damage and dysfunction was subsequently assessed. Min6 cells were transfected with miR-770-5p inhibitor or miR-770-5p inhibitor + BAG5-siRNA for 48 h, followed by treatment with or without 5 mg/dl UA for 24 h. Cell viability, apoptosis, apoptosis-related factor expression levels and insulin secretion were assessed. The results demonstrated that UA treatment significantly reduced cell viability, increased cell apoptosis and reduced insulin secretion in Min6 cells compared with the control group. miR-770-5p inhibitor significantly attenuated UA-induced injury and dysfunction

of Min6 cells, whereas BAG5 knockdown abolished the protective effects of miR-770-5p inhibitor on UA-damaged Min6 cells. In conclusion, miR-770-5p was highly expressed in the serum of patients with T2DM compared with healthy volunteers. In UA-treated pancreatic  $\beta$ -cells, compared with the inhibitor control group, miR-770-5p knockdown regulated the expression of apoptosis-related genes, increased cell viability, inhibited cell apoptosis and increased insulin secretion by targeting BAG5. Therefore, the present study suggested that miR-770-5p inhibitor may serve a protective role in T2DM.

## Introduction

Diabetes, whose global prevalence in adults has risen from 4.7% in 1980 to 8.5% in 2014, is currently one of the most prevalent non-communicable diseases threatening global human health (1). At present, the etiology and pathogenesis of type 2 diabetes mellitus (T2DM) are not completely understood. T2DM is characterized by insulin resistance and pancreatic  $\beta$ -cell hypofunction (2), and is often accompanied by a variety of complications, such as kidney disease, fundus disease and neuropathy, that cause damage to various organs and tissues in the body (3,4).

MicroRNAs (miRNAs/miRs) are single-stranded, endogenous non-coding RNAs that are 18-22 nucleotides in length, which regulate gene expression at the post-transcriptional level to exert their biological effects (5,6). miRNAs serve important roles in physiological alterations in the human body, and during the occurrence and development of various diseases, such as cancer, cardiovascular disease and diabetes (7).

Alterations in serum miRNA expression levels occur earlier compared with target genes, they are relatively stable in body fluids, and they display intercellular shuttle ability and tissue-specific specificity; therefore, miRNAs are well suited as biomarkers for the early detection and diagnosis of diseases (8). It has been reported that miRNAs serve important roles in the development of islet cell failure in patients with T2DM (9).

miR-770-5p is a form of mature miR-770 and the high evolutionary conservation of miR-770-5p suggests that it may be of particular biological significance (10). Several studies have demonstrated that miR-770 is clinically significant and

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*Correspondence to:* Mrs. Min Wang, Department of Critical Care Medicine, The First Affiliated Huai'an People's Hospital of Nanjing Medical University, 1 Huanghe West Road, Huai'an, Jiangsu 223300, P.R. China  
E-mail: wangmin011937@163.com

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serves a crucial role in the carcinogenesis and progression of different types of cancer, such as non-small cell lung, breast, ovarian and gastric cancer, as well as hepatocellular carcinoma (11-15). Moreover, previous studies have indicated the significant role of miR-770-5p in diabetic nephropathy and gestational diabetes mellitus (16-18), suggesting that miR-770-5p serves a key role in the regulation of cell function (11-18). It has also been reported that compared with healthy volunteers, miR-770-5p expression was significantly increased in patients with T2DM (9); however, the mechanism underlying miR-770-5p in patients with T2DM is not completely understood. As the function of islet cells serves critical roles in T2DM development (19,20), the aim of the present study was to investigate the expression of miR-770-5p in patients with T2DM, as well as its role in pancreatic  $\beta$ -cells to provide insight into potential therapeutic targets for T2DM.

## Materials and methods

**Clinical samples.** Peripheral blood samples from 20 patients with T2DM (14 male patients and 8 female patients; age, 32-61 years) and 20 healthy subjects (14 male patients and 8 female patients; age, 29-64 years) were collected from the First Affiliated Huai'an People's Hospital of Nanjing Medical University (Huai'an, China) between May 2017 and September 2018. T2DM was diagnosed according to the World Health Organization criteria (21) and was defined as a fasting plasma glucose of  $\geq 7$  mmol/l (126 mg/dl), a 2-h glucose of  $\geq 11.1$  mmol/l (200 mg/dl) in the 75-g Oral Glucose Tolerance Test and/or HbA1c  $\geq 6.5\%$ . The inclusion criteria were as follows: body mass index  $< 40$  and no history of diagnosis of diabetes. The exclusion criteria were as follows: i) Type 1 diabetes; ii) other types of diabetes; iii) severe infections; iv) acute cerebrovascular disease; or iv) those who had recently undergone surgery. There were no significant differences in age and sex between patients with T2DM and healthy volunteers. The present study was approved by the Ethics Committee of The First Affiliated Huai'an People's Hospital of Nanjing Medical University (approval no. KY-P-2017-009-01). Written informed consent was obtained from each patient prior to sample collection. Blood samples were immediately frozen and stored at  $-80^{\circ}\text{C}$ . To detect the expression levels of miR-770-5p and BAG5 mRNA in patients with T2DM, serum was isolated from the peripheral blood samples by centrifugation at  $1,500 \times g$  for 10 min at  $4^{\circ}\text{C}$ .

**Cell culture and treatment.** Min6 cells (a mouse insulinoma cell line; American Type Culture Collection) were cultured in 5 mM glucose DMEM (Hyclone; Cytiva) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 50 mmol/l  $\beta$ -mercaptoethanol (Sigma-Aldrich; Merck KGaA), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Sigma-Aldrich; Merck KGaA) at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ .

Uric acid (UA) was prepared as previously reported (22). Briefly, UA sodium salt (10-100 g/ml; Sigma-Aldrich; Merck KGaA) was added to prewarmed EBM-2 medium (Lonza Group, Ltd.;  $37^{\circ}\text{C}$ ). The mixture was agitated at  $37^{\circ}\text{C}$  for 30 min and passed through a sterile  $0.22\text{-}\mu\text{m}$  filter.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was isolated from cells or serum samples using

TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA (1  $\mu\text{g}$ ) was reverse transcribed into cDNA using the PrimeScript RT reagent kit (Takara Bio, Inc.) according to the manufacturer's protocol. Subsequently, qPCR was performed using SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> (Takara Bio, Inc.). The primer sequences used for qPCR were as follows: U6 forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse, 5'-CGCTTCACGAATTTGCGTGTTCAT-3'; GAPDH forward, 5'-CTTTGGTATCGTGGAAGGACTC-3' and reverse, 5'-GTAGAGGCAGGGATGATGTTCT-3'; miR-770-5p forward, 5'-CCAGTACACGTGTTCAG-3' and reverse, 5'-GAACATGTCTGCGTATCTC-3'; BAG5 forward, 5'-TGTCCCCGGGTTTAGGGGTGTTTC-3' and reverse, 5'-TTCACAAGCACTGTCCCCGCC-3'; Bcl-2 forward, 5'-TTGGATCAGGGAGTTGGAAG-3' and reverse, 5'-TGTCCCTACCAACCAGAAGG-3'; and Bax forward, 5'-CGTCCACCAAGAAGCTGAGCG-3 and reverse 5'-CGTCCACCAAGAAGCTGAGCG-3'. The following thermocycling conditions were used for qPCR: Initial denaturation at  $95^{\circ}\text{C}$  for 10 min; followed by 35 cycles of  $95^{\circ}\text{C}$  for 15 sec and  $55^{\circ}\text{C}$  for 40 sec. miRNA and mRNA expression levels were quantified using the  $2^{-\Delta\Delta\text{Ct}}$  method (23) and normalized to the internal reference genes U6 and GAPDH, respectively.

**Dual luciferase reporter assay.** TargetScan (version 7.2; targetscan.org/vert\_72) was used to predict the potential targets of miR-770-5p, and the binding sites between miR-770-5p and BAG5 were identified. To investigate the association between miR-770-5p and BAG5, a dual luciferase reporter assay was performed. The binding site for miR-770-5p in the 3'-untranslated region (UTR) of BAG5 was constructed using synthetic oligonucleotides (Beijing Augct DNA-Syn Biotechnology Co., Ltd.) and cloned into the pmirGLO Dual-Luciferase expression vector (Promega Corporation) to generate wild-type (WT)-BAG5 (5'-UAGUUGGAGGAUGA AUACUGGAG-3'). The mutated 3'-UTR sequence of BAG5 was cloned into the pmirGLO Dual-Luciferase expression vector to generate mutated (MUT)-BAG5 (5'-UAGUUGGAG GAUGAAACUACCAG-3'). Min6 cells ( $5 \times 10^4$ ) cultured in 24-well plates were co-transfected with 100 nM miR-770-5p mimic (5'-UCCAGUACCACGUGUCAGGGCCA-3') or 100 nM mimic control (5'-UCGCUUGGUGCAGGUCGG GAA-3') and 1 ng WT-BAG5 or 1 ng MUT-BAG5 reporter plasmids using the Lipofectamine<sup>®</sup> 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. At 48 h post-transfection, cells were harvested, and a dual luciferase assay system (Promega Corporation) was used to detect luciferase activities according to the manufacturer's protocol. Luciferase activity was normalized to that of *Renilla* luciferase activity.

**Western blotting.** Western blotting was used to measure protein expression levels. Briefly, cells were lysed using RIPA lysis buffer (Shanghai Yeasen Biotechnology Co., Ltd.) and centrifuged at  $12,000 \times g$  at  $4^{\circ}\text{C}$  for 5 min. Protein concentrations were determined using a bicinchoninic acid assay. Total protein (40  $\mu\text{g}$  per lane) was separated via 10% SDS-PAGE and transferred to PVDF membranes (Invitrogen; Thermo Fisher Scientific, Inc.). Following blocking in 5% skimmed dry milk at room temperature for 1 h, the membranes were incubated

overnight at 4°C with primary antibodies targeted against the following: Caspase-3 (cat. no. 14220; 1:1,000; Cell Signaling Technology, Inc.), cleaved caspase-3 (cat. no. 9664; 1:1,000; Cell Signaling Technology, Inc.), Bcl-2 (cat. no. 3498; 1:1,000; Cell Signaling Technology, Inc.), Bax (cat. no. 2772; 1:1,000; Cell Signaling Technology, Inc.), BAG5 (cat. no. ab97660; 1:1,000; Abcam) and  $\beta$ -actin (cat. no. 4970; 1:1,000; Cell Signaling Technology, Inc.). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibodies (cat. no. ab205718; 1:2,000; Abcam) for 1 h at room temperature. Protein bands were visualized using an ECL chemiluminescence kit (Thermo Fisher Scientific, Inc.). Protein expression was semi-quantified using Gel-Pro Analyzer densitometry software (version 6.3; Media Cybernetics, Inc.) with  $\beta$ -actin as the loading control.

**Cell transfection and treatment.** Min6 cells were transfected with 100 nM miR-770-5p inhibitor (5'-UGGCCUGACACG UGGUACUGGA-3'; Shanghai GenePharma Co., Ltd.), 100 nM inhibitor control (5'-CAGUACUUUUGUGUAGUACAA-3'; Shanghai GeneChem Co., Ltd.), 2  $\mu$ M control-small interfering (si)RNA (cat. no. sc-37007; Santa Cruz Biotechnology, Inc.), 2  $\mu$ M BAG5-siRNA (cat. no. sc-72605; Santa Cruz Biotechnology, Inc.) or 100 nM miR-770-5p inhibitor + 2  $\mu$ M BAG5-siRNA using Lipofectamine® 3000 reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. At 48 h post-transfection, transfection efficiency was determined via RT-qPCR.

After transfection, cells were treated with 5 mg/dl UA at 37°C for 24 h and then subjected to subsequent experiments. Cells without any treatment were considered as the control.

**MTT assay.** Min6 cell viability was measured using an MTT assay (Beyotime Institute of Biotechnology). Min6 cells were seeded ( $5 \times 10^3$  cells/well) into 96-well plates and incubated overnight in DMEM supplemented with 10% FBS. Following treatment and transfection, 20  $\mu$ l MTT solution (5 mg/ml in distilled water) was added to each well and incubated for 4 h at 37°C. The medium was removed and 150  $\mu$ l DMSO was added to dissolve purple formazan. The optical density was measured at a wavelength of 490 nm using a POLARstar OPTIMA multifunctional micro-plate reader (BMG Labtech GmbH).

**Apoptosis analysis.** Min6 cell apoptosis was measured by performing flow cytometry. Min6 cells ( $10^6$ ) were digested using 0.2% trypsin, followed by washing with pre-cooled PBS. Cells were stained using an Annexin V-FITC/PI kit (cat. no. 70-API101-100; Multisciences (Lianke) Biotech Co., Ltd.) according to the manufacturer's protocol. In brief, Min6 cells were stained with 5  $\mu$ l Annexin V-FITC and 10  $\mu$ l PI at room temperature for 5 min in the dark. Apoptotic cells (early and late apoptosis) were analyzed using a FACSCalibur flow cytometer (BD Biosciences) and CellQuest software version 5.1 (BD Biosciences).

**Glucose-stimulated insulin secretion assay.** Min6 cells were incubated in glucose-free Krebs Ringer bicarbonate (KRB) buffer (115 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM NaHCO<sub>3</sub>, 16 mM HEPES and 2.56 mM

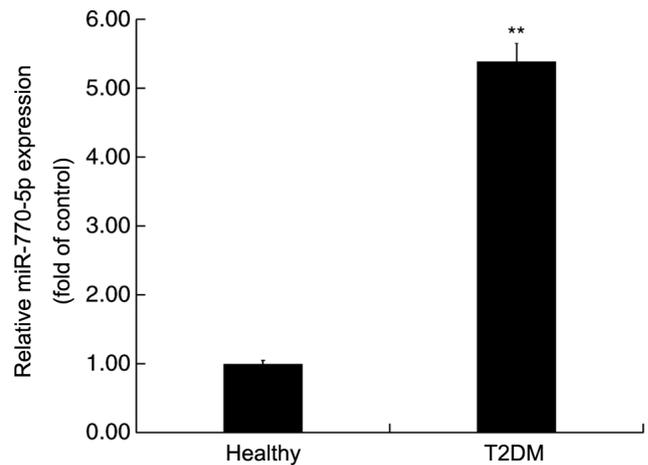


Figure 1. miR-770-5p expression levels in the peripheral blood of patients with T2DM. Reverse transcription-quantitative PCR was performed to detect the expression levels of miR-770-5p in the peripheral blood of patients with T2DM and healthy volunteers. \*\*P<0.01 vs. healthy. miR, microRNA; T2DM, type 2 diabetes mellitus.

CaCl<sub>2</sub>; Sigma-Aldrich; Merck KGaA) supplemented with 0.2% BSA (Sigma-Aldrich; Merck KGaA) at 37°C for 1 h. Subsequently, cells were treated with KRB buffer with basal glucose (3.3 mM) or high concentration glucose (16.7 mM; Sigma-Aldrich; Merck KGaA) at 37°C for 1 h. The supernatants were collected by centrifugation at 4°C for 10 min at 500 x g, and subsequently insulin concentrations were determined using an Insulin ELISA kit (cat. no. PI602; Beyotime Institute of Biotechnology) according to the manufacturer's protocol.

**Statistical analysis.** Statistical analyses were performed using SPSS software (version 20.0; IBM Corp.). Comparisons between two groups were analyzed using an unpaired Student's t-test. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean  $\pm$  SD of three independent experiments. P<0.05 was considered to indicate a statistically significant difference.

## Results

**miR-770-5p is highly expressed in the serum of patients with T2DM.** RT-qPCR was performed to detect the expression levels of miR-770-5p in the serum of patients with T2DM and healthy volunteers. miR-770-5p expression levels in the serum of patients with T2DM were significantly higher compared with healthy volunteers (Fig. 1).

**BAG5 is a target gene of miR-770-5p.** TargetScan was used to predict the possible targets of miR-770-5p. miR-770-5p had numerous potential target genes, including BAG5 (Fig. 2A). As a member of the BAG protein family, which has been reported to enhance cell proliferation and survival (24), BAG5 is involved in several diseases via regulation of cell proliferation and gene expression (25-28). Islet  $\beta$ -cell destruction and apoptosis serve an important role in the development of DM (29). However, the role of BAG5 in T2DM, and the relationship between miR-770-5p and BAG5 are not completely understood. Thus,

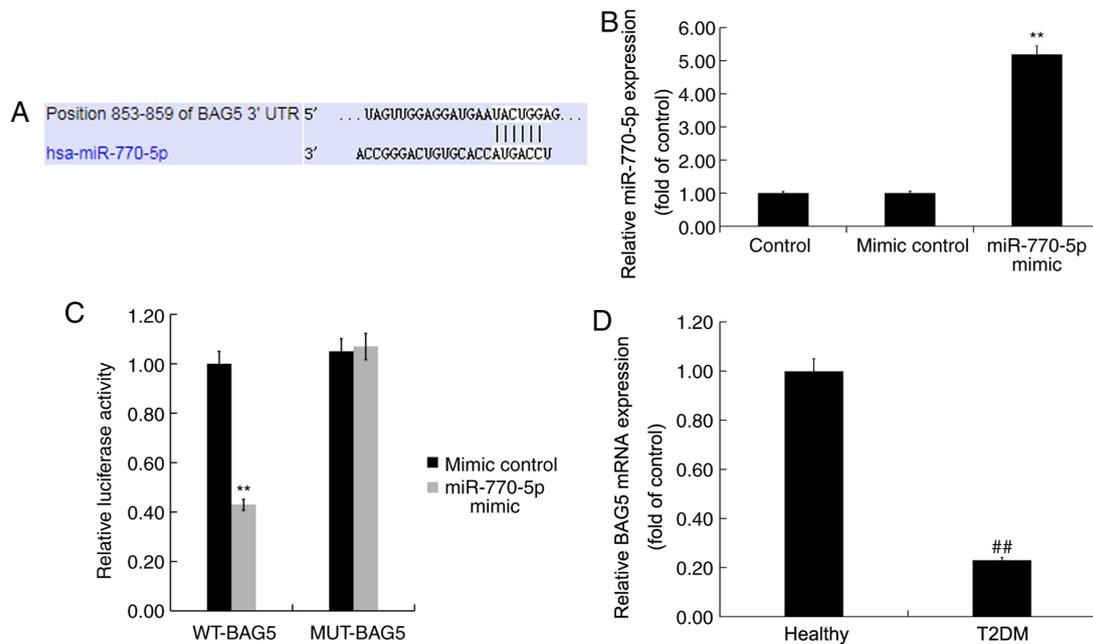


Figure 2. BAG5 is a target gene of miR-770-5p. (A) TargetScan was used to predict the binding sites between BAG5 and miR-770-5p. (B) Transfection efficiency of miR-770-5p mimic. (C) Dual luciferase reporter assays were performed to verify the interaction between BAG5 and miR-770-5p. (D) Reverse transcription-quantitative PCR was performed to detect BAG5 expression levels in the peripheral blood of patients with T2DM and healthy volunteers. \*\*P<0.01 vs. mimic control; ##P<0.01 vs. healthy. BAG5, Bcl-2 associated athanogene 5; miR, microRNA; T2DM, type 2 diabetes mellitus; WT, wild-type; MUT, mutant; UTR, untranslated region.

BAG5 was selected for further examination in the present study. The dual luciferase reporter assay is a well-established experimental method for identifying whether a miRNA directly binds to its target gene (30,31). Therefore, to determine whether miR-770-5p directly targeted BAG5, a dual luciferase reporter assay was used. Compared with the mimic control group, miR-770-5p mimic significantly increased miR-770-5p expression levels in Min6 cells (Fig. 2B). Subsequently, Min6 cells were co-transfected with WT-BAG5 or MUT-BAG5 and miR-770-5p mimic or mimic control. The results indicated that miR-770-5p mimic- + WT-BAG5-transfected cells displayed significantly reduced levels of luciferase activity compared with mimic control- + WT-BAG5-transfected cells (Fig. 2C). By contrast, the luciferase activities of miR-770-5p mimic- + MUT-BAG5-transfected cells were not significantly altered compared with mimic control- + MUT-BAG5-transfected cells, suggesting that miR-770-5p directly targeted the 3'-UTR of BAG5.

RT-qPCR was performed to detect the mRNA expression levels of BAG5 in the serum of patients with T2DM and healthy volunteers. BAG5 mRNA expression levels in the serum of patients with T2DM were significantly decreased compared with healthy volunteers (Fig. 2D).

*UA treatment upregulates miR-770-5p expression and downregulates BAG5 expression in Min6 cells.* Min6 cells were treated with UA (5 mg/dl) for 24 h. Compared with the control group, miR-770-5p expression was significantly increased in the UA treatment group (Fig. 3A). By contrast, the mRNA and protein expression levels of BAG5 were decreased in UA-treated Min6 cells compared with control cells (Fig. 3B and C).

*miR-770-5p negatively regulates BAG5 expression in Min6 cells.* Min6 cells were transfected with control-siRNA, BAG5-siRNA, miR-770-5p inhibitor, inhibitor control or BAG5-siRNA + miR-770-5p inhibitor for 48 h. The RT-qPCR results demonstrated that miR-770-5p inhibitor significantly decreased miR-770-5p expression levels in Min6 cells compared with inhibitor control (Fig. 4A). BAG5-siRNA markedly reduced BAG5 mRNA and protein expression levels in Min6 cells compared with control-siRNA (Fig. 4B and C). Moreover, compared with the inhibitor control group, miR-770-5p inhibitor increased the mRNA (Fig. 4D) and protein (Fig. 4E) expression levels of BAG5 in Min6 cells, which was reversed by co-transfection with BAG5-siRNA.

*miR-770-5p inhibitor alleviates UA-induced injury and dysfunction in Min6 cells.* To explore the effect of miR-770-5p knockdown on UA-induced Min6 cell injury and dysfunction, Min6 cells were transfected with inhibitor control, miR-770-5p inhibitor, or miR-770-5p inhibitor + BAG5-siRNA for 48 h, and subsequently treated with UA (5 mg/dl) for 24 h. Cell viability, apoptosis and insulin secretion were assessed. Compared with UA treatment alone, miR-770-5p inhibitor significantly increased cell viability (Fig. 5A) and decreased cell apoptosis (Fig. 5B and C) in UA-treated Min6 cells. Similarly, miR-770-5p inhibitor significantly increased insulin secretion from UA- and glucose-treated Min6 cells compared with UA treatment alone (Fig. 5D). Compared with the control group, UA treatment markedly increased Bax and cleaved-caspase-3 protein expression levels, reduced Bcl-2 protein expression levels and upregulated the ratio of cleaved-caspase-3/caspase-3 in Min6 cells (Fig. 6A and B). miR-770-5p inhibitor markedly decreased Bax and cleaved-caspase-3 protein expression

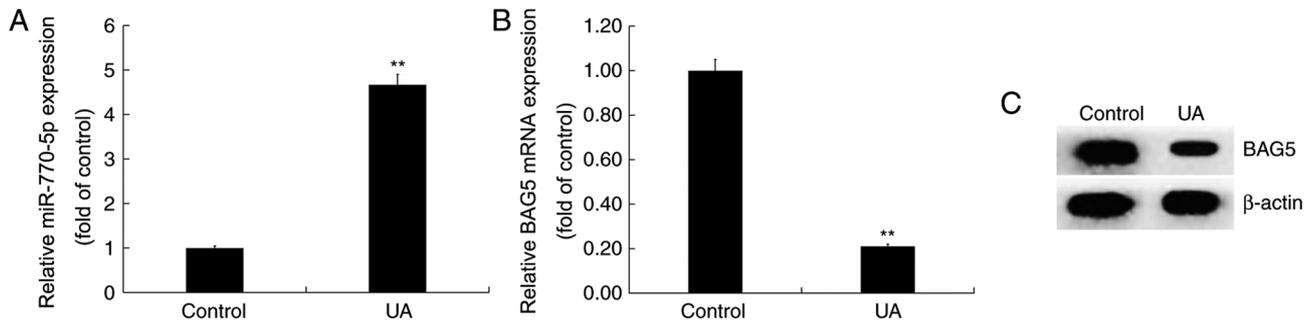


Figure 3. Effect of UA treatment on miR-770-5p and BAG5 expression levels in Min6 cells. Min6 cells were treated with UA (5 mg/dl) for 24 h. (A) miR-770-5p expression levels were detected via RT-qPCR. BAG5 (B) mRNA and (C) protein expression levels were determined via RT-qPCR and western blotting, respectively. \*\* $P < 0.01$  vs. control group. UA, uric acid; miR, microRNA; BAG5, Bcl-2 associated athanogene 5; RT-qPCR, reverse transcription-quantitative PCR.

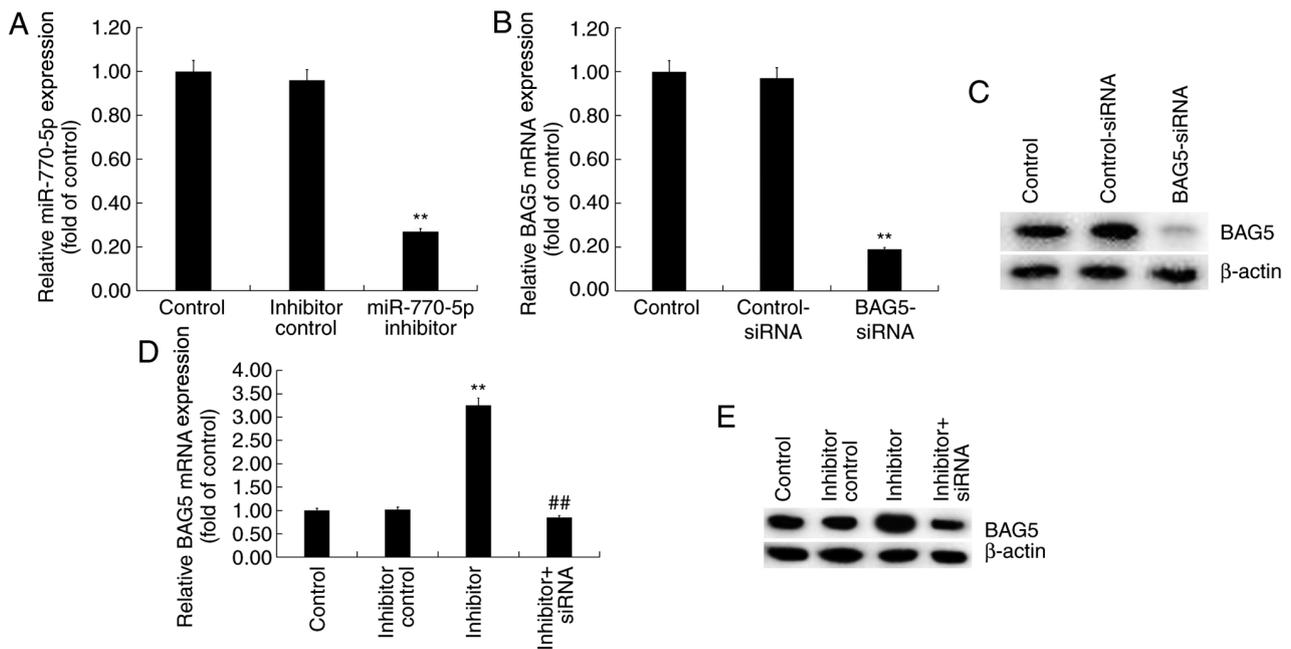


Figure 4. Regulatory effect of miR-770-5p on BAG5 expression in Min6 cells. Min6 cells were transfected with control-siRNA, BAG5-siRNA, inhibitor control, miR-770-5p inhibitor or BAG5-siRNA + miR-770-5p inhibitor for 48 h. (A) Transfection efficiency of miR-770-5p inhibitor. The transfection efficiency of BAG5-siRNA was assessed via (B) RT-qPCR and (C) western blotting. BAG5 (D) mRNA and (E) protein expression levels were determined via RT-qPCR and western blotting, respectively. \*\* $P < 0.01$  vs. control; ## $P < 0.01$  vs. inhibitor. miR, microRNA; BAG5, Bcl-2 associated athanogene 5; siRNA, small interfering RNA; RT-qPCR, reverse transcription-quantitative PCR.

levels, increased Bcl-2 protein expression levels, and downregulated the ratio of cleaved-caspase-3/caspase-3 in UA-treated Min6 cells compared with the UA + inhibitor control treatment group. Moreover, compared with the control group, UA treatment significantly increased Bax mRNA expression levels and decreased Bcl-2 mRNA expression levels in Min6 cells (Fig. 6C and D). However, miR-770-5p inhibitor significantly decreased Bax expression levels and increased Bcl-2 mRNA expression levels in UA-treated Min6 cells compared with UA treatment alone. Furthermore, miR-770-5p inhibitor-mediated effects on UA-treated Min6 cells were significantly reversed by co-transfection with BAG5-siRNA.

## Discussion

According to relevant epidemiological statistics, the number of new cases of diabetes mellitus (DM) worldwide is increasing

on a yearly basis (32). Although multiple pathogenic mechanisms underlying DM complications have been identified, the clinical results achieved are limited and the exact pathogenesis is not completely understood. With continuous advances in molecular biotechnology, the discovery of miRNAs has provided a novel insight into the investigation of DM (33). An increasing number of studies have demonstrated that miRNAs serve important roles in the pathological processes underlying DM (9,33,34). In the present study, the expression of miR-770-5p and its target gene BAG5 in patients with T2DM was assessed. miR-770-5p expression was significantly increased in the serum of patients with T2DM compared with healthy volunteers. By contrast, BAG5 expression was significantly decreased in the serum of patients with T2DM compared with healthy volunteers.

The relationship between T2DM and serum UA levels has been investigated (35). A number of studies have reported

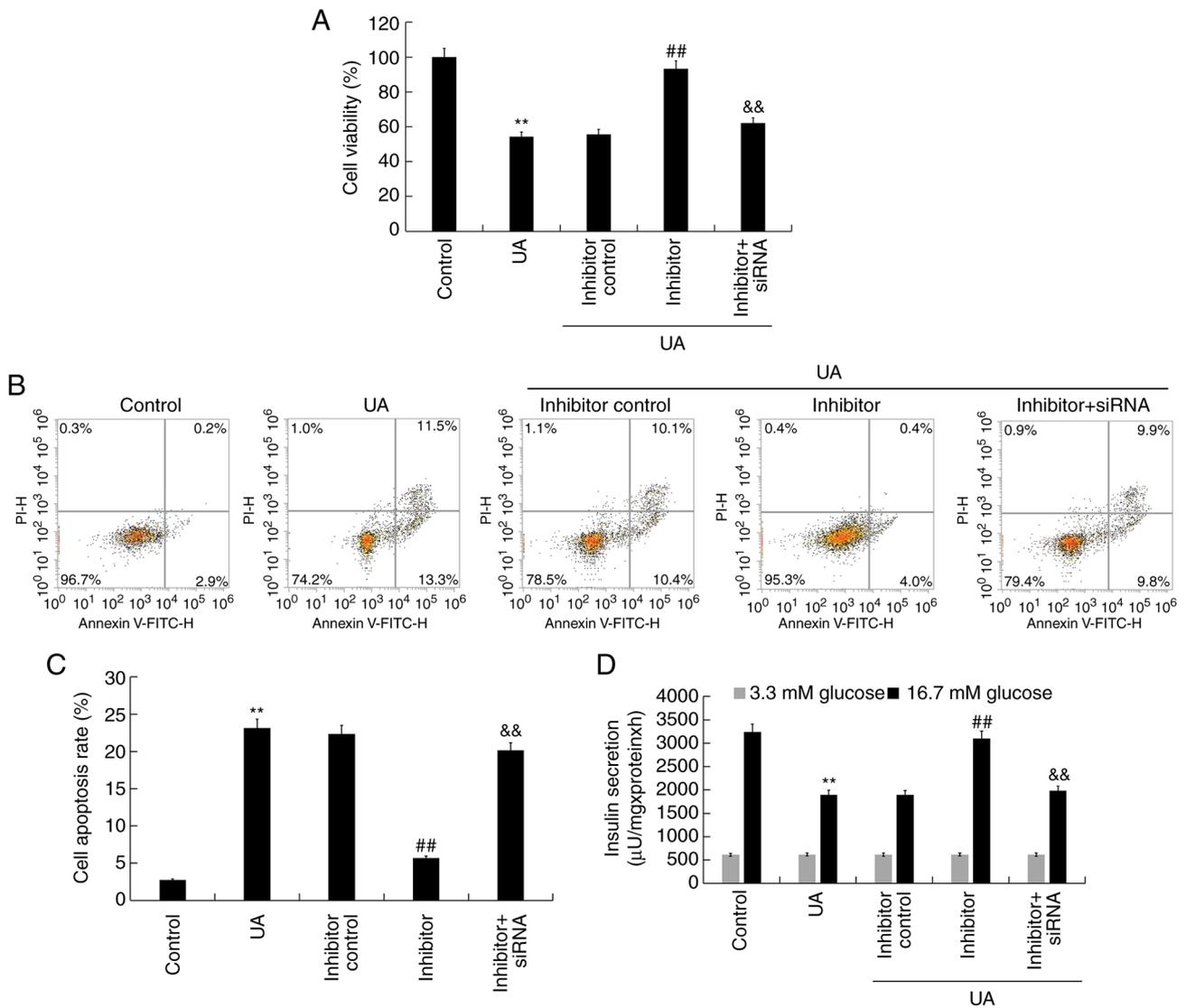


Figure 5. Effect of UA and miR-770-5p inhibitor on Min6 cells. Following transfection, Min6 cells were treated with UA (5 mg/dl) for 24 h. (A) Cell viability was detected by performing an MTT assay. Cell apoptosis was (B) determined by flow cytometry and (C) quantified. (D) A glucose-stimulated insulin secretion assay was performed to detect the insulin secretion. \*\* $P < 0.01$  vs. control; ## $P < 0.01$  vs. UA; && $P < 0.01$  vs. inhibitor. UA, uric acid; miR, microRNA; siRNA, small interfering RNA.

that the risk of T2DM was greatly increased under high UA conditions (36,37). Enhanced UA levels not only resulted in insulin resistance but also negatively affected pancreatic  $\beta$ -cell survival and insulin release (38). The Min6 cell line has been used to investigate pancreatic  $\beta$ -cell function *in vitro* (38,39). In the present study, UA-treated Min6 cells were used to explore the effect of miR-770-5p on UA-induced pancreatic  $\beta$ -cell damage and dysfunction.

Islet  $\beta$ -cell destruction, apoptosis and dedifferentiation can result in the loss of islet  $\beta$ -cells, which decreases insulin secretion resulting in blood sugar level disorders, ultimately leading to diabetes and the associated complications (29). miRNAs destruct multiple target genes, such as C-C chemokine receptor type 7 and CD247, in islet  $\beta$ -cells (40). Apoptosis refers to the autonomous and orderly death of cells under the regulation of genes in order to maintain the homeostasis of the internal environment (41). Islet  $\beta$ -cell apoptosis is a primary cause of DM and islet  $\beta$ -cell function damage is also closely associated with DM. Islet  $\beta$ -cell function damage mechanisms may be

associated with oxidative stress, glucose toxicity, lipotoxicity and hypoxia. Inflammation caused by these mechanisms may initially maintain and repair the function of pancreatic islet  $\beta$ -cell, but persistent inflammatory reactions can cause damage and permanent destruction to islet  $\beta$ -cells (42,43). Several miRNAs have been reported to be involved in islet  $\beta$ -cell apoptosis, including miR-23a-3p, miR-23b-3p and miR-149-5p. These miRNAs are associated with type 1 diabetes mellitus via downregulating Bcl-2 expression, which promotes islet  $\beta$ -cell apoptosis (44). miR-770-5p, which has been studied in several types of cancer, such as non-small cell lung, breast, ovarian and gastric cancer, as well as hepatocellular carcinoma (11-15), was reported to serve key roles in diabetic nephropathy and gestational diabetes mellitus (16-18). A recent study indicated that compared with healthy volunteers, miR-770-5p expression was upregulated in patients with gestational diabetes mellitus, and it can regulate INS-1 insulinoma cell proliferation, apoptosis and insulin secretion by targeting tumor protein p53 regulated inhibitor of apoptosis 1 (18). However, the roles and

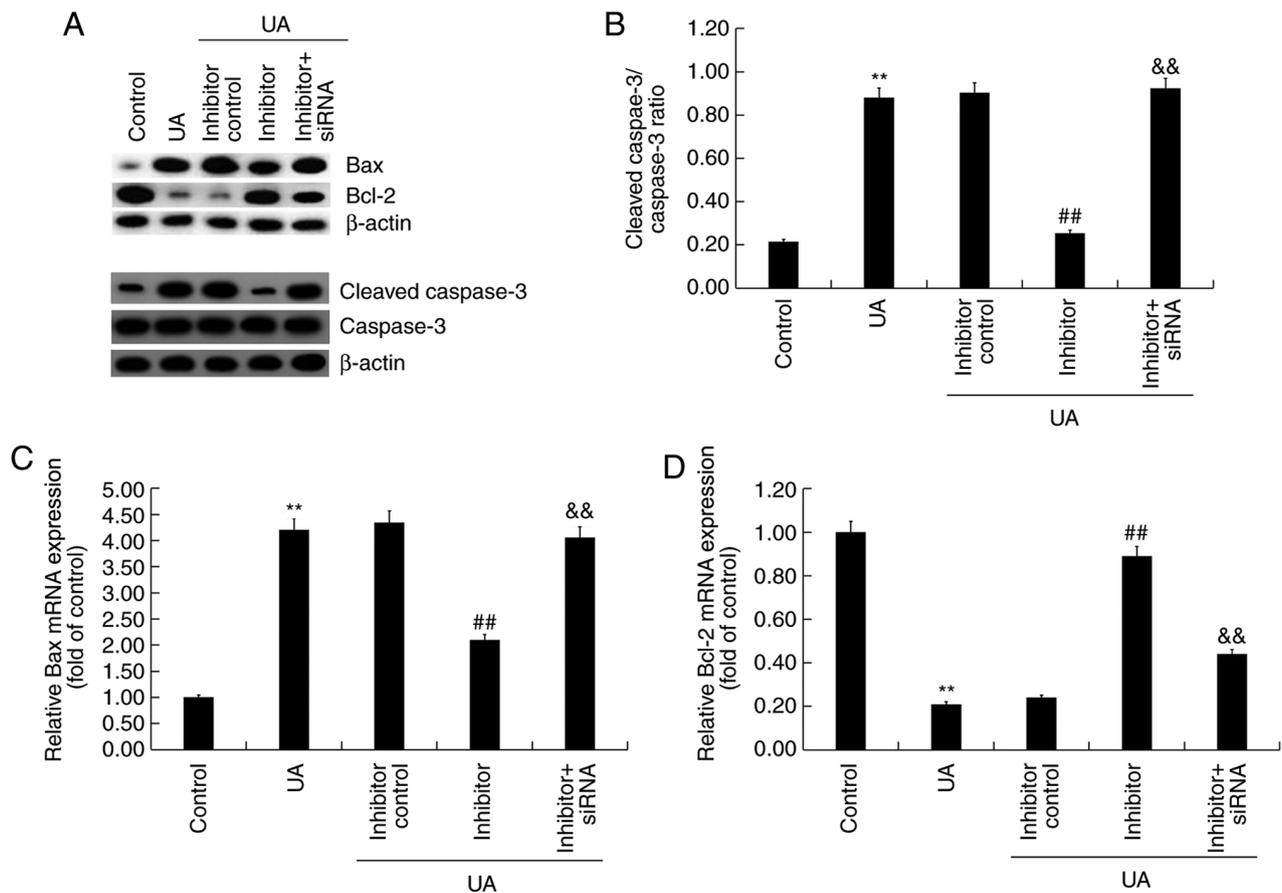


Figure 6. Effect of UA and miR-770-5p inhibitor on apoptosis-related gene expression levels in Min6 cells. Following transfection, Min6 cells were treated with UA (5 mg/dl) for 24 h. (A) Western blotting was performed to measure the protein expression levels of Bax, Bcl-2, cleaved-caspase-3 and caspase-3. (B) The ratio of cleaved-caspase-3/caspase-3. Reverse transcription-quantitative PCR were performed to measure (C) Bax and (D) Bcl-2 mRNA expression levels. \*\*P<0.01 vs. control; ##P<0.01 vs. UA; &&P<0.01 vs. inhibitor. UA, uric acid; miR, microRNA; siRNA, small interfering RNA.

mechanism underlying miR-770-5p in pancreatic  $\beta$ -cells are not completely understood.

In the present study, BAG5 was identified as a direct target of miR-770-5p. BAG5 expression was significantly downregulated in patients with T2DM and UA-treated Min6 cells compared with healthy volunteers and control cells, respectively. BAG5 is a member of the BAG protein family, which enhance cell proliferation and survival (24). Previous studies have demonstrated that BAG5 is involved in several diseases, including cancer and Alzheimer's disease, via regulation of apoptosis and gene expression (25-28). Therefore, it was hypothesized that miR-770-5p may serve an important role in T2DM via regulating islet  $\beta$ -cell apoptosis by targeting BAG5. The effects of miRNA-770-5p on islet  $\beta$ -cell damage and dysfunction were determined by assessing cell viability using an MTT assay, detecting apoptosis via flow cytometry, and measuring the expression levels of apoptosis-associated genes and proteins via RT-qPCR and western blotting, respectively. The results demonstrated that UA treatment reduced cell viability, promoted apoptosis, increased the cleaved-caspase-3/caspase-3 ratio and Bax expression levels, and decreased Bcl-2 expression levels in Min6 cells compared with the control group. However, UA treatment-mediated effects were significantly attenuated by transfection with miR-770-5p inhibitor. Moreover, miR-770-5p-mediated effects on UA-treated Min6 cells were significantly reversed by co-transfection with BAG5-siRNA. The aforementioned results were consistent with

a previous study that reported that miRNAs are involved in islet  $\beta$ -cell destruction and apoptosis (44).

Insulin is a protein hormone secreted by islet  $\beta$ -cells that are stimulated by endogenous or exogenous substances (45). Insulin serves an important role in the balance of blood sugar levels in the human body, whereby insufficiency results in hyperglycemia, DM and DM complications (46). Previous studies have reported that miRNAs can regulate the synthesis and secretion of insulin (47,48) and regulate the blood sugar balance in the human body (49). miRNAs not only promote insulin synthesis and secretion, but also serve a negative regulatory role in insulin synthesis and secretion (50-52). In the present study, the effects of miR-770-5p on insulin secretion were determined. The results demonstrated that compared with the control group, UA treatment significantly reduced insulin secretion in Min6 cells, which was significantly reversed by transfection with miR-770-5p inhibitor. However, miR-770-5p inhibitor-mediated effects on insulin secretion in UA-treated Min6 cells were significantly reversed by co-transfection with BAG5-siRNA. A key limitation of the present study was that a UA + miR-770-5p inhibitor + control-siRNA group was not included.

In conclusion, the results of the present study demonstrated that miR-770-5p expression was significantly upregulated in patients with T2DM compared with healthy volunteers. Compared with the inhibitor control group, miR-770-5p knock-down increased cell viability, reduced cell apoptosis, regulated

the expression of apoptosis-related genes and increased insulin secretion in UA-treated pancreatic  $\beta$ -cells by targeting BAG5, thus suppressing the development and/or progression of T2DM. The results of the present study provided novel insight into potential prevention and treatment strategies for T2DM. However, the present study was only a preliminary study of the role of miR-770-5p in T2DM. Therefore, *in vivo* studies are required to confirm the role of miR-770-5p in T2DM. Additionally, the relationship between miR-770-5p and BAG5 expression levels and the clinicopathological characteristics of patients with T2DM should be investigated in future studies.

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### Availability of data and materials

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

### Authors' contributions

MW contributed to designing the study, collecting, analyzing and interpreting the data, and preparing the manuscript. JW, TJ and KZ contributed to collecting, analyzing and interpreting the data. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The First Affiliated Huai'an People's Hospital of Nanjing Medical University (approval no. KY-P-2017-009-01). Written informed consent was obtained from each patient prior to sample collection.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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