MicroRNA-152 regulates insulin secretion and pancreatic β cell proliferation by targeting PI3Kα

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Abstract. An increasing number of microRNAs (miRNAs/miRs) are reported to have important roles in diabetes. Glucose-stimulated insulin secretion and pancreatic β cell proliferation are essential in the control of metabolic disorder, however, the underlying molecular mechanisms remain unclear. The present study investigated the function of miR-152 in diabetes. The results of reverse transcription-quantitative polymerase chain reaction demonstrated that miR-152 levels in the blood were markedly reduced in patients with diabetes compared with nondiabetic controls. In addition, a high blood glucose concentration was significantly associated with reduced miR-152 expression. Furthermore, overexpression of miR-152 using miR-152 mimics promoted the proliferation of INS-1 and MIN6 cells, as determined by an MTT assay, in addition to insulin secretion, while knockdown of miR-152 using an inhibitor led to the opposite effects. Phosphatidylinositol 3-kinase (PI3K) signaling has been reported to inhibit insulin secretion, however, the regulation of PI3K in the pancreatic β cell is poorly understood. The present study identified that PI3K catalytic subunit α (PI3Kα) was a direct target gene of miR-152 using a luciferase reporter assay, and miR-152 inhibited the expression of PI3Kα at the protein level, which was determined by western blotting. Therefore, the regulation of insulin secretion and pancreatic β cell proliferation may occur via the miR-152/PI3Kα axis. The overexpression of PI3Kα in INS-1 and MIN6 cells partially reduced the effects of miR-152 overexpression on insulin secretion. Consistently, PI3Kα levels were reduced in murine pancreatic islets following treatment with 20 mM glucose, and increased in blood samples from patients with diabetes compared with healthy individuals. In conclusion, the results of the present study demonstrate that miR-152 may have an important role in pancreatic β cell function, and established an association between miR-152 and the PI3Kα axis. Therefore, targeting PI3Kα may be a potential therapeutic option for diabetes.

Introduction

Globally, type 2 diabetes (T2D) is a common chronic disease, which is characterized by high blood glucose levels and insulin resistance (1). Insulin is involved in the pathogenesis of T2D and metabolic syndrome. An inappropriate amount of insulin as a result of b-cell dysfunction is the hallmark of T2D. Decreased glycogen level is a hallmark of insulin resistance in hepatocytes (2). Members of the phosphatidylinositol 3-kinase (PI3K) family function in the regulation of islet mass and function (3). Although three different PI3K isoforms are expressed in the endocrine pancreas (4), the class 1 PI3K isoforms are the most investigated groups, with opposing roles in the regulation of insulin secretion (5). For example, the PI3K catalytic subunit α (PI3Kα/PIK3CA), also termed p110α, acts as a negative regulator of insulin secretion, while PI3Kβ/PIK3CB, also termed p110β, is a positive regulator of insulin secretion through distinct mechanisms that are separated from the catalytic activity (6,7). However, the regulation of PI3Kα in T2D is poorly understood.

MicroRNAs (miRNA/miRs) are a group of endogenous non-coding small RNAs that have a length of between 21 and 23 nucleotides and act as key regulators of post-transcriptional gene expression (8). miRNAs function by base pairing to the 3' untranslated region of the target mRNA and act as specific gene silencers (9). Various studies have indicated that miRNAs regulate the multiple aspects in various metabolic diseases, including diabetes mellitus, endothelial function, obesity and metabolic syndrome (10,11). Among miRNAs, miR-152 was reported to regulate hepatic insulin resistance and glucose metabolism, and high glucose levels reduced the expression of miR-152 and subsequently impaired the synthesis of glycogen in hepatocytes (12). However, the mechanism underlying the miR-152 regulation cascade remains unclear.

The investigation of the molecular mechanisms involved in T2D strongly indicates that excess genes impair cell functions, leading to metabolically relevant cellular dysfunction, inflammation and oxidative stress.

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Materials and methods

Cell culture. INS-1 and MIN6 cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM; 5 mmol/l glucose; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.), 5.5 mM 2-mercaptoethanol, 100 U/ml penicillin and 0.1 mg/ml streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C in a humidified chamber atmosphere with 5% CO_2.

Reagents and transfection of INS-1 and MIN6 cells. The miR-152 mimic, miR-152 inhibitor, and miRNA mimic and inhibitor controls, were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The miR-152 mimic, GCAGTC TCAGTGCATGACAGA, miR-152 inhibitor, CCAUCUUUA CCAGACAGUGUUA, inhibitor controls CAGUACUUUUGU GUAGUAC. HiPerFect transfection reagent (Qiagen GmbH, Hilden, Germany) was used for the transfection of miR-152 mimic and inhibitors, and controls. MIN6/INS-1 cells were cultured by plating 6x105 cells into 100-mm Petri dishes. A total of 250 pmol miR-152 mimic or inhibitor, or respective controls were transfected into cells. At 48 h following transfection, the expression of miR-152 and target genes were detected reverse transfection-quantitative polymerase chain reaction (RT-qPCR). Insulin (1 nmol/l) was purchased from United States Biological (Salem, MA, USA).

Blood sample collection. The present study was approved by the Ethics Committee of the Second Clinical Medical College, Yangtze University (Jingzhou, China) and written informed consent was obtained from all patients and healthy volunteers. Blood samples were collected from patients with T2D (n=50), of whom 24 were female and 26 were male, and patients were aged between 45 and 60. Normal subjects (n=20), of which 10 were female and 10 were male, were aged between 44 and 60. The samples were collected between January 2014 and November 2015 in Jingzhou Central Hospital. Patients with serious kidney diseases, malignancy and acute heart failure were excluded.

Experimental animals. A total of 40 Male C57BL/6J mice (age, 12 weeks; weight, 20-25 g) were provided by the Chinese Academy of Sciences affiliated Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China), and were originally purchased from Jackson Laboratory (Bar Harbor, ME, USA). All animal procedures were performed in accordance with the National Institutes of Health Animal Care and Use Guidelines (13) and approved by the Animal Ethics Committee at the Shanghai Institute of Geriatrics (Shanghai, China). Mice were housed in a clean animal laboratory at the Second Clinical Medical College, Yangtze University Experimental Animal Center. Mice were housed under specific pathogen free conditions with controlled room temperature (22.0±1.0°C), 0.05% CO_2, a 12 h light/dark cycle (light, 8 am-8 pm), humidity of 40-60% and free access to water and food. Mice islets were isolated from the pancreata according to a previously described method by Lau *et al* (14). Adenovirus with miR-152 overexpression construct or the miR-152 inhibitor, and the relative controls were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China) and were transfected in freshly 2 ug isolated mouse pancreatic islets at a dose of $1x10^9$ plaque forming units in 0.2 ml PBS for 72 h and collected for the following experiments.

Determination of glucose in blood samples. The glucose levels were determined by a routine laboratory method. The blood glucose levels in whole blood were examined at the Department of Clinical Laboratory of Jingzhou Central Hospital, The Second Clinical Medical College, Yangtze University using a Glucose assay kit (BioVision, Inc., Milpitas, CA, USA), according to the manufacturer's protocol.

Western blot analysis. Total protein was extracted from INS-1 and MIN6 cells using radioimmunoprecipitation assay lysis buffer (Sigma-Aldrich; Merck KGaA). After centrifugation at 12,000 x g for 20 min at 4°C, the supernatant was collected. The BCA protein assay was used to determine the protein concentration. Following mixing with 4X SDS loading buffer, proteins (30 μg) in each group were heated at 100°C for 5 min and separated on 10% SDS-polyacrylamide gels and transferred to a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA). Following blocking with 8% non-fat dry milk at room temperature for 30 min, the membranes were probed with primary antibodies overnight at 4°C, followed by incubation with horseradish peroxidase (HRP)-conjugated second antibodies at room temperature for 1 h and detection with Signal Boost™ Immunoreaction Enhancer Kit (407207, EMD Millipore). The signals were recorded using X-ray film. The following antibodies were employed: PI3Kα (cat. no. sc-7174; Santa Cruz Biotechnology, Inc., Dallas, TX, USA, 1:1,000), β-actin (cat. no. sc-130300; Santa Cruz Biotechnology, Inc, 1:2,000), goat anti-mouse IgG-HRP, (cat. no. sc-2005; Santa Cruz Biotechnology, Inc. 1:3,000) and goat anti-rabbit IgG-HRP (cat. no. sc-2004; Santa Cruz Biotechnology, Inc. 1:3,000).

RNA isolation and RT-qPCR. Enriched miRNA was isolated using an miRNA isolation kit using whole blood samples from T2D patients and healthy controls (Takara Bio, Inc., Otsu, Japan). Total RNA was extracted from the pancreatic tissues or blood samples from T2D patients or cell lines using TRIzol method (Invitrogen; Thermo Fisher Scientific, Inc.). The concentration and purity of RNA was confirmed. First-strand cDNA synthesis was performed using a reverse transcription kit (iScript cDNA Synthesis kit; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following temperature protocol was used for reverse transcription: 25°C for 10 min, 42°C for 30 min and 85°C for 3 min. qPCR was performed using the TB Green™ Premix Dimer Eraser™, according to the manufacturer's protocol (RR091A, Takara Bio, Inc.) based on an ABI 7500 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Cycling parameters were as follows: 95°C for 5 min and then 40 cycles of 95°C for 15 sec and annealing/extension at 60°C for 1 min. The U6 small nucleolar RNA or GAPDH were used as the reference genes. The relative gene expression was normalized to U6 or GAPDH using the $2^{-\Delta\Delta Cq}$ method (15). PI3Ka 5'-ATATGATGCAGCCATTGACC-3' (forward)

5'-CTCCTGAAACCTCTCAAATTCTC-3' (reverse) GAPDH, GAGAAGTATGACAACAGCCTC-3' (forward) and 5'-ATG GACTGTGGTCATGAGTC-3' (reverse). miR-152, 5'-ACT CTCGAGGCTTCTAAGCTGGGAACTTTGTC-3' (forward) and 5'-ACTGAATTCCGCTTGTCTTGGACATATGGC ACT-3' (reverse); U6, 5'-CTCGCTTCGGCAGCACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse). Each reaction was performed in triplicate.

MTT assay. An MTT kit (Roche Diagnostics, Indianapolis, IN, USA) was used to determine the proportion of viable MIN6 and INS-1 cells, $5x10^3$ cells were seeded into 12-well plates, according to manufacturer's protocol. MTT (500 μg/ml) was added to DMEM medium for 3 h at 37°C. At the end of the incubation, MTT reagent was removed and the cells were dissolved in dimethyl sulfoxide. The absorbance value was measured at 570 nm on a microplate reader (Perkin Elmer EnSpire; Perkin Elmer, Inc., Waltham, MA, USA). Experiments were performed at least three times.

Insulin secretion measurements. Insulin secretion measurements were performed at 37°C in Krebs-Ringer bicarbonate buffer (115 mmol/l NaCl, 5 mmol/l KCl, 24 mmol/l NaHCO$_3$, 2.5 mmol/l CaCl$_2$, 1 mmol/l MgCl$_2$, 10 mmol/l HEPES and 0.1% bovine serum albumin (Pierce; Thermo Fisher Scientific, Inc. pH 7.4). A total of 10 mouse islets were preincubated for 2 h in KRB per group, followed by incubation for 1 h in these basal buffers and further 30 min or 24 h incubation with 3 or 20 mM glucose. Acid/ethanol extraction was used to extract insulin from islets. Insulin secretion was also measured in 10^6 INS-1 and MIN6 cells following incubation with 3 or 20 mM glucose for 2 h incubation at 37°C. Samples were stored at -20°C until insulin levels were detected using an ELISA kit (cat. no. 80-INSMSU-E01, Mouse Ultrasensitive Insulin ELISA, ALPCO, Salem, NH, USA). Each experiment was repeated at least three times.

Luciferase reporter assay. TargetScan Human version 7.0 (www.targetscan.org) predicted that PI3Kα was a potential target of miR-152. The wild-type (WT) or mutant (MUT; without miR-152 binding site) human PI3Kα 3'UTR sequences were synthesized using QuikChange Multi Site-Directed Mutagenesis kit (Agilent Technologies, Inc., Santa Clara, CA, USA) and separately cloned into the pGL-3 luciferase reporter plasmid (Promega Corporation, Madison, WI, USA). The recombinant plasmids were termed pGL3-PI3Kα-WT and pGL3-PI3Kα-MUT. These plasmids were co-transfected with 50 nm miR-152 mimic or inhibitor or their negative control using Lipofectamine® 2000. Cell lysates were prepared and luciferase assays were performed 48 h following transfection. Luciferase activity was normalized to Renilla luciferase activity.

Statistical analysis. All statistical analyses were performed using GraphPad prism 6.0 (GraphPad Software, Inc. La Jolla, CA, USA). Data are presented as the mean ± standard deviation and P<0.05 was considered to indicate a statistically significant difference. One-way analysis of variance, followed by Fisher's least significant difference test, was performed to determine the statistical significance of differences between
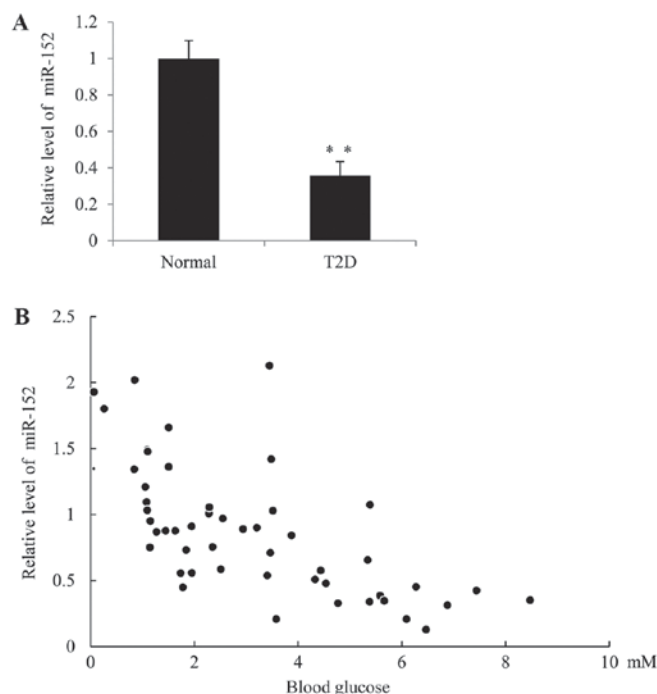

Figure 1. The expression of miR-152 was significantly downregulated in patients with T2D. (A) Reverse transcription-quantitative polymerase chain reaction was performed to measure the miR-152 levels in whole blood samples from patients with T2D (n=50) and healthy volunteers (n=20). (B) A significant inverse correlation was observed between the expression of miR-152 and blood glucose concentration in patients with T2D, as determined by Pearson's correlation analysis. R=0.5424, P=0.0078 **P<0.01 vs. normal group. miR, microRNA; T2D, type 2 diabetes; normal group, healthy volunteers.

two groups. Pearson's correlation analysis was performed to determine the correlation between miR-152 and glucose levels in the blood of patients with T2D and healthy controls.

Results

The expression of miR-152 is significantly downregulated in patients with T2D. In order to investigate the function of miR-152 in diabetes, RT-qPCR was performed to measure miR-152 levels in the plasma of patients with T2D and healthy volunteers. As demonstrated in Fig. 1A, the expression of miR-152 was significantly decreased in patients with T2D compared with the control group. Furthermore, a significant inverse correlation between the miR-152 expression and blood glucose concentration was observed in patients with T2D, R=0.5424, P=0.0078 (Fig. 1B).

miR-152 enhances the insulin secretion and proliferation of pancreatic β cell lines. In order to investigate the exact function of miR-152 in pancreatic β cells, two pancreatic β cell lines, INS-1 and MIN6, were used. miR-152 mimic or miR-152 inhibitor, or respective controls, were transfected into INS-1 and MIN6 cells. At 48 h after transfection, RT-qPCR was performed to determine the miR-152 expression levels. As demonstrated in Fig. 2A and B, the expression of miR-152 was significantly higher in the miR-152 mimic-transfected cells, while the expression of miR-152 was significantly lower in miR-152 inhibitor-transfected cells, compared with respective
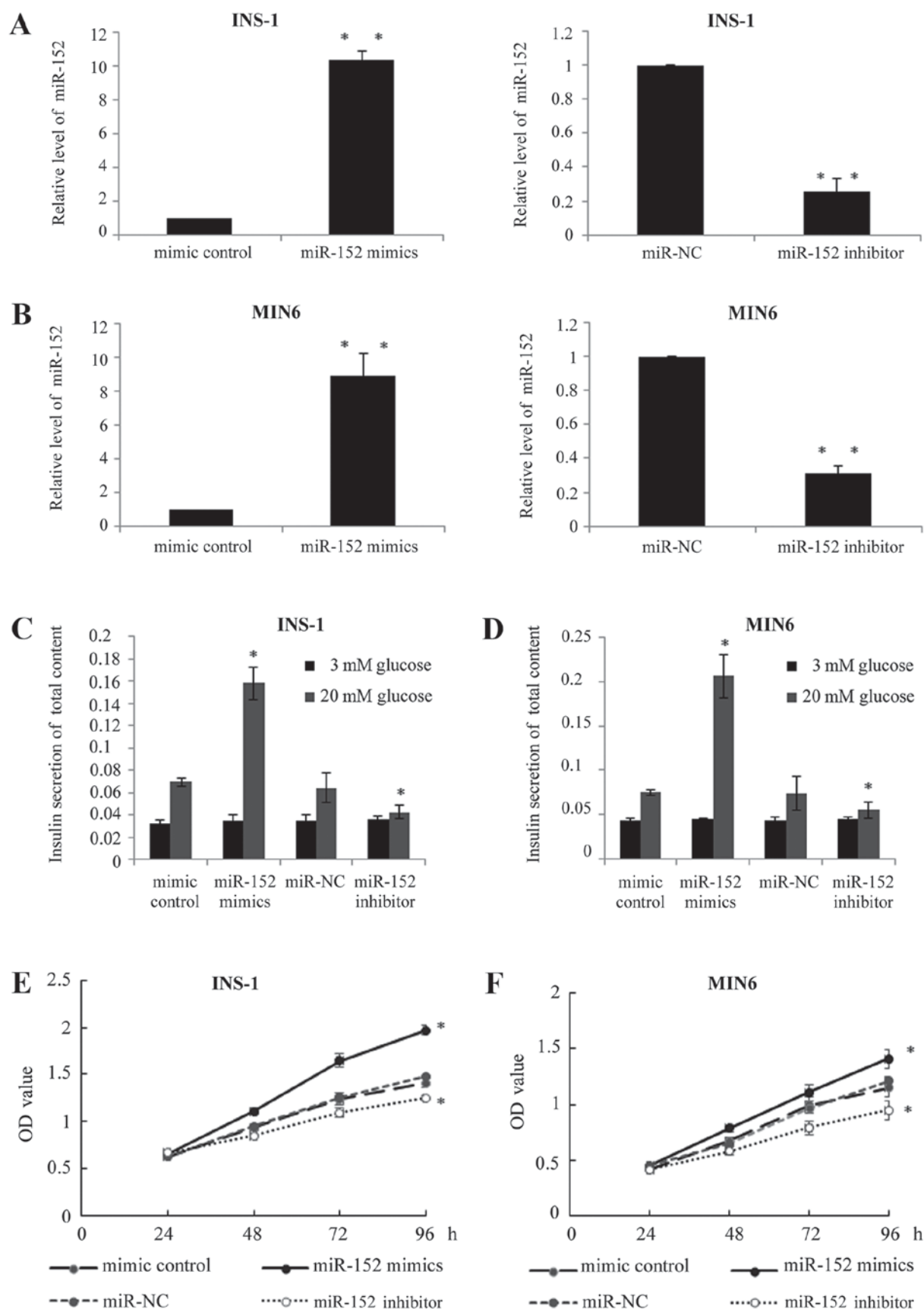
Figure 2. miR-152 enhanced the insulin secretion and proliferation of pancreatic β cell lines. Reverse transcription-quantitative polymerase chain reaction was performed to measure the miR-152 levels in (A) INS-1 and (B) MIN6 cells transfected with miR-152 mimic, miRNA mimic control, miR-152 inhibitor or miR-NC inhibitor control, n=5. (C) INS-1 and (D) MIN6 cells were transfected with miR-152 mimic, miRNA mimic control, miR-152 inhibitor or miR-NC inhibitor control for 48 h, followed by incubation with 3 or 20 mM glucose for 1 h. Glucose-stimulated insulin secretion was subsequently determined in each group, n=3. MTT assay was performed to investigate cell proliferation in (E) INS-1 and (F) MIN6 cells transfected with miR-152 mimic, miRNA mimic control, miR-152 inhibitor or miR-NC inhibitor control, n=5. *P<0.05 and **P<0.01 vs. mimic control or miR-NC. miR, microRNA; NC, negative control; miRNA, microRNA; OD, optical density.

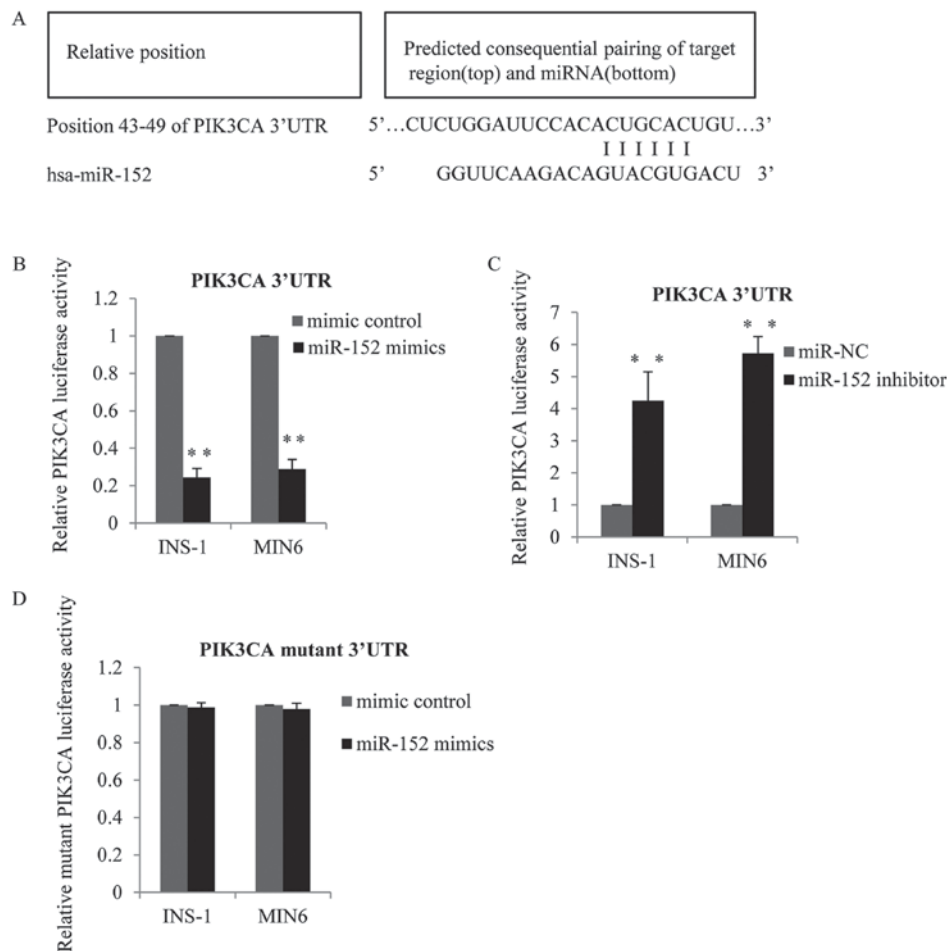Figure 3. Identification of PI3Kα as a target gene of miR-152. (A) PI3Kα was predicted to be a potential target gene of miR-152. Matches in seed regions are marked. The wild-type PI3Kα 3'-UTR with the putative miR-152 binding sites was subcloned into the pMiR-report vector, and a luciferase reporter assay was performed in MIN6 and INS-1 cells transfected with (B) miR-152 mimic or miRNA mimic control, or (C) miR-152 inhibitor or miR-NC inhibitor control, n=3. (D) Mutant PI3Kα 3'-UTR was subcloned into the pMiR-report vector, and a luciferase reporter assay was performed in MIN6 cells and INS-1 cells transfected with miR-152 mimic or miRNA mimic control, n=3. **P<0.01 vs. mimic control or miR-NC. PI3Kα, phosphatidylinositol 3-kinase catalytic subunit α; miR, microRNA; UTR, untranslated region; miRNA, microRNA; NC, negative control.

control groups. Furthermore, in response to glucose stimulation (20 mM), transient transfection of miR-152 mimics significantly increased the insulin secretion, while the transfection of miR-152 inhibitor reduced insulin secretion stimulated by high glucose levels, compared with the respective transfection control groups (Fig. 2C and D). In addition, the effects of miR-152 on cell viability were also investigated using an MTT assay to confirm whether overexpression or knockdown of miR-152 affected cell viability. The results demonstrated that miR-152 overexpression markedly enhanced cell proliferation, while knockdown of miR-152 reduced cell proliferation, compared with their respective control groups (Fig. 2E and F).

Identification of PI3K-alpha as a target gene of miR-152. The above observations indicated that miR-152 may be required for glucose-stimulated insulin secretion and pancreatic β cell proliferation. Therefore, the present study subsequently investigated the specific genes suppressed by miR-152. TargetScan software was used to predict the putative target genes of miR-152. As demonstrated in Fig. 3A, PI3Kα was identified as a potential target gene of miR-152. To verify the targeting of PI3Kα by miR-152, wild-type PI3Kα 3'-untranslated region (UTR) containing the predicted miR-152 binding sites, or mutated versions of the PI3Kα 3'-UTR without the predicted miR-152 binding sites, were cloned into firefly luciferase reporter plasmids. Results of the luciferase reporter activity assay demonstrated that co-transfection of INS-1 and MIN6 cells with the wild-type PI3Kα 3'-UTR and miR-152 mimic reduced the luciferase activity, compared with control mimic-transfected cells (Fig. 3B). By contrast, co-transfection with the wild-type 3'-UTR and miR-152 inhibitors enhanced PI3Kα 3'-UTR luciferase activity, compared with the transfection control group (Fig. 3C). However, luciferase activity was not altered in cells that were co-transfected with the mutant PI3Kα 3'-UTR vector and miR-152 mimics, compared with the mimic control transfection group (Fig. 3D).

PI3Kα is involved in the miR-152-mediated pancreatic β cell proliferation and insulin secretion. It is established that the major function of miRNA is the negative regulation of target genes at the post-transcriptional level; therefore, the present study investigated the effects of miR-152 on the endogenous protein expression of PI3Kα. Results of western blot analysis demonstrated that, in miR-152 overexpressing INS-1 and MIN6
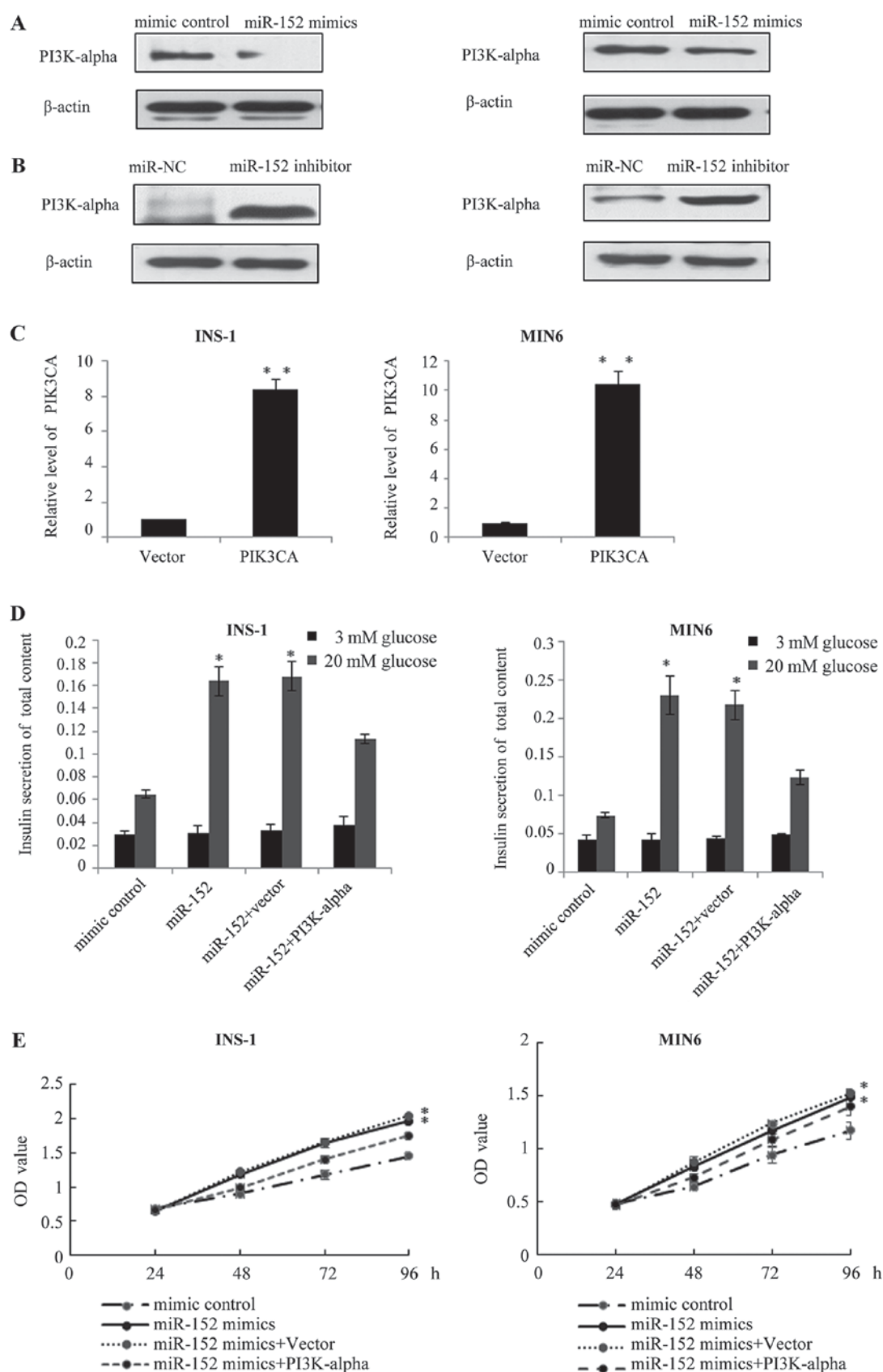
Figure 4. PI3Kα is involved in miR-152-mediated pancreatic β cell proliferation and insulin secretion. Western blot analysis was performed to measure the protein expression of PI3Kα in INS-1 and MIN6 cells transfected with (A) miR-152 mimic or miRNA mimic control, or transfected with (B) miR-152 inhibitor or miR-NC inhibitor control, n=3. (C) Reverse transcription-quantitative polymerase chain reaction was performed to measure the mRNA expression of PI3Kα in INS-1 and MIN6 cells transfected with PI3Kα overexpression plasmid or vector, n=3. **P<0.01 vs. vector group. (D) Glucose-stimulated insulin secretion was measured in INS-1 and MIN6 cells transfected with miR-152 mimic, miRNA mimic control, miR-152 mimic + vector or miR-152 mimic + PI3Kα overexpression plasmid. Transfected cells were treated with 3 or 20 mM glucose. *P<0.05 vs. mimic control, n=5. (E) MTT assay was performed in INS-1 and MIN6 cells transfected with miR-152 mimic, miRNA mimic control, miR-152 mimic + vector or miR-152 mimic + PI3Kα overexpression plasmid. *P<0.05. n=3. PI3Kα, phosphatidylinositol 3-kinase catalytic subunit α; miR, microRNA; miRNA, microRNA; NC, negative control; OD, optical density.
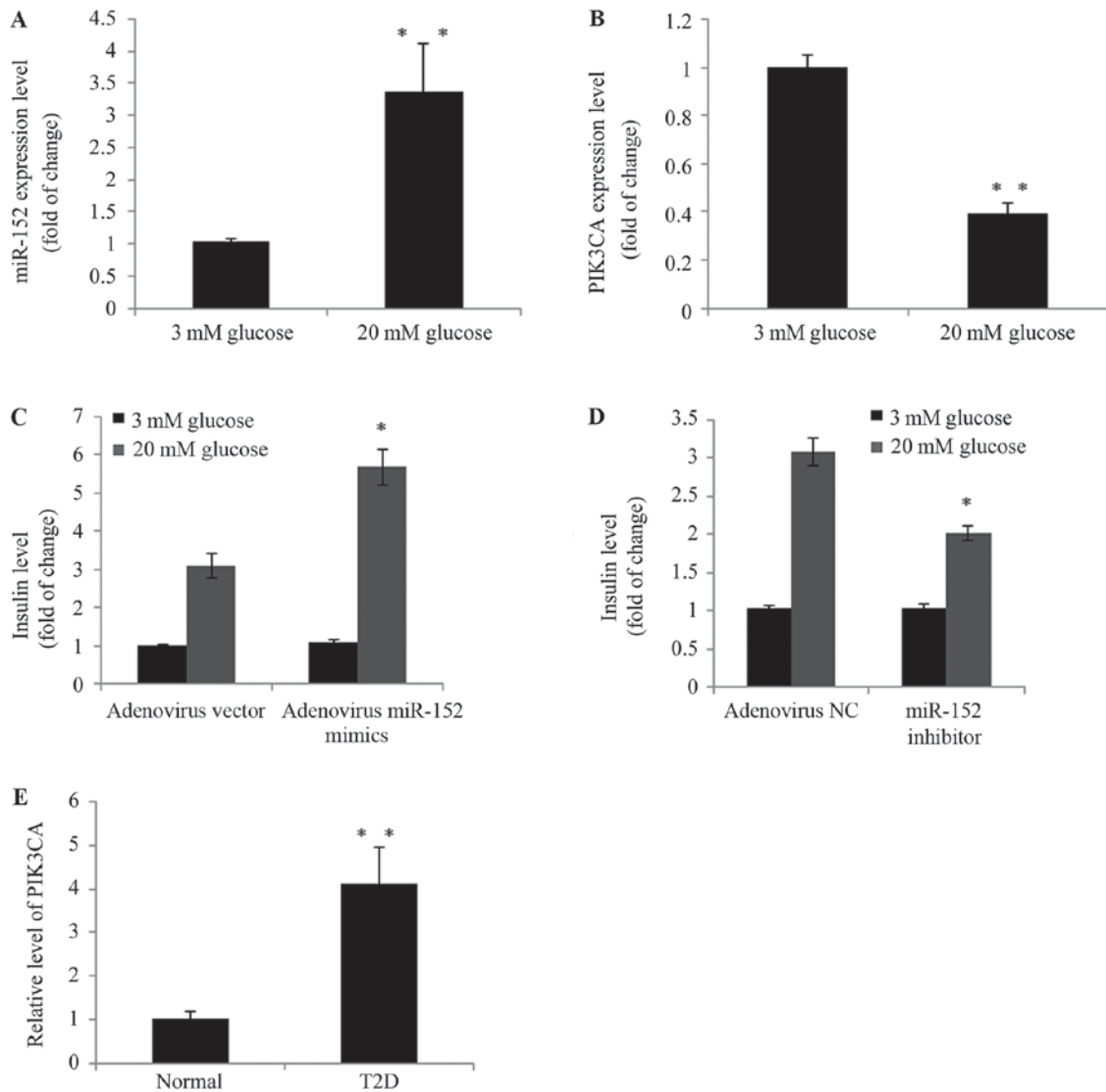
Figure 5. The miR-152/PI3Kα axis regulated glucose-stimulated insulin secretion in murine pancreatic islets and patients with T2D. Freshly isolated mouse pancreatic islets were isolated and incubated with 3 or 20 mM glucose for 24 h. The expression of (A) miR-152 and (B) PI3Kα was analyzed using RT-qPCR, n=3. Pancreatic islets were transduced with (C) adenovirus vector or adenovirus miR-152, or transduced with (D) adenovirus NC and adenovirus miR-152 inhibitor. Cells were subsequently incubated with 3 or 20 mM glucose for 2 h and insulin secretion was measured, n=3. (E) RT-qPCR was performed to measure the PI3Kα levels in blood samples from patients with T2D (n=50) and healthy volunteers (n=20). For parts A and B, **$P<0.01$ vs. 3 mM glucose. For parts C and D, *$P<0.05$ vs. adenovirus vector or adenovirus NC. For part E, **$P<0.01$ vs. normal group. miR, microRNA; PI3Kα, phosphatidylinositol 3-kinase catalytic subunit α; T2D, type 2 diabetes; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; NC, negative control; normal group, healthy volunteers.

cells, the protein expression of PI3Kα was reduced, compared with transfection control cells (Fig. 4A). Conversely, the protein levels of PI3Kα were markedly increased following the transfection of miR-152 inhibitors, compared with the transfection control group (Fig. 4B). Based on these results, we hypothesized that PI3Kα may function as a downstream effector of miR-152. To verify this hypothesis, the pcDNA3.1-PI3Kα plasmid was constructed to restore the expression of PI3Kα in INS-1 and MIN6 cells. At 48 h following transfection, RT-qPCR was performed to analyze the mRNA levels of PI3Kα. As demonstrated in Fig. 4C, PI3Kα levels were significantly higher in the INS-1 and MIN6 cells transfected with PI3Kα overexpression plasmid, compared with the vector group. Subsequently, the effect of PI3Kα overexpression on miR-152-mediated insulin secretion and cell proliferation was investigated. As demonstrated in Fig. 4D, compared with the cells transfected with

miR-152 mimic alone, transfection of INS-1 and MIN6 cells with the miR-152 mimic and PI3Kα overexpression plasmid abolished the effect of miR-152 mimics on glucose-stimulated insulin secretion. Similarly, the MTT assay revealed that PI3Kα overexpression reduced the effect of the miR-152 on the proliferation of INS-1 and MIN6 cells (Fig. 4E).

The miR-152/PI3Kα axis regulates glucose-stimulated insulin secretion in murine pancreatic islets, and in patients with T2DM. The present study further determined the role of the miR-152/PI3Kα axis in glucose-stimulated insulin secretion in islets isolated from mice. As demonstrated in Fig. 5A and B, high-glucose treatment upregulated the expression of miR-152 and downregulated PI3Kα expression in murine pancreatic islets. Furthermore, in miR-152 overexpression islets, insulin secretion was markedly increased compared with

mimic control-transfected islets following treatment with 20 mM glucose (Fig. 5C). By contrast, insulin secretion was suppressed when miR-152 was inhibited in islets, compared with control-transfected cells, following treatment with 20 mM glucose (Fig. 5D). In addition, the plasma PI3Kα levels were determined in patients with T2D and healthy controls using ELISA. As demonstrated in Fig. 5E, in the blood samples of patients with T2D, PI3Kα levels were significantly increased compared with healthy individuals (Fig. 5E). These observations strongly indicated that dysregulation of the miR-152 and PI3Kα axis may be involved in the development of T2D.

Discussion

Diabetes is characterized by high levels of blood glucose, and a lack of insulin-producing pancreatic β cells is the major cause of type 1 diabetes, while increased insulin resistance leads to the development of T2D and metabolic syndrome (2,16). Increasing evidence has indicated that various types of miRNA may be involved in the pathogenesis of diabetes (17,18). It was previously reported that miR-9 controlled the expression of granuphilin-a and the secretory response of insulin-producing cells (19). In addition, by negatively regulating hepatic gluconeogenesis, miR-29a-c reduced fasting blood glucose levels (20), and miR-301a mediated the effect of interleukin (IL)-6 on the AKT/glycogen synthase kinase pathway and hepatic glycogenesis through the regulation of phosphatase and tensin homolog (PTEN) expression (21). Furthermore, miR-200s contributed to IL-6-induced insulin resistance in hepatocytes (22). However, reports concerning the function of miR-152 were different depending on the cancer and tissue type. For example, Huang *et al* (23) reported that miR-152 targeted PTEN to inhibit cell apoptosis and promote cancer cell proliferation in nasopharyngeal carcinoma cells. However, in hepatocellular carcinoma, miR-152 inhibited tumor cell growth by directly targeting rhotekin (24). These different functions of miR-152 may be observed as miR-152 has multiple target genes. The results of the present study demonstrated that miR-152 promoted insulin secretion and pancreatic β proliferation, and insulin is a critical factor in the pathogenesis of diabetes. According to the bioinformatics, although several potential targets for miR-152 were identified, the binding of PI3Kα was of significant importance. As PI3Kα was previously reported to be implicated in the development and progression of T2D (6), the present study focused on PI3Kα as a target of miR-152. Overexpression of miR-152 resulted in a marked decrease in the protein expression of PI3Kα in MIN6 and INS-1 cells, while inhibition of miR-152 led to increased PI3Kα expression. Therefore, miR-152 may be involved in the regulation of insulin secretion and pancreatic β cell proliferation via PI3Kα. Consistently, the expression of miR-152 was lower, while the expression of PI3Kα mRNA was higher, in patients with T2D compared with healthy controls. However, further research is required to validate these findings and compare PI3Kα protein expression in blood samples of patients T2D and healthy volunteers. The expression of miR-152 in the blood samples may be due to the secretion of circulating miRNAs. Investigation of the plasma miR-152 levels in the present study was performed with the aim of identifying novel blood-based biomarkers for T2D diagnosis and prognosis. In addition, miR-152 expression was also analyzed in murine pancreatic islets. The results demonstrated that high-glucose treatment upregulated the expression of miR-152 and downregulated PI3Kα expression. Furthermore, in miRNA-152 overexpression islets, compared with mimic control-transfected islets, insulin secretion was markedly increased following treatment with high-glucose.

To the best of our knowledge, the present study provides novel experimental evidence demonstrating that miR-152 may function as a potential candidate for treating diabetes. However, further investigation is required in future studies.

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