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Angiotensin (1–7) reverses glucose-induced islet β cell dedifferentiation by Wnt/ β -catenin/FoxO1 signalling pathway

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Abstract

Introduction: Recent studies have shown that a decline in islet β cells quality is due to β -cell dedifferentiation, not only β -cell apoptosis. Angiotensin (1–7) [Ang(1-7)] could attenuate high glucose-induced apoptosis and dedifferentiation of pancreatic β cells by combining with MAS receptors. However, the mechanism of such action has not been elucidated. Recent studies have revealed that Wnt/ β -catenin and forkhead box transcription factor O1 (FoxO1) are associated with β -cell dedifferentiation. Our study aims to explore whether the effects of Ang(1-7) on islet β cell dedifferentiation are mediated through the Wnt/ β -catenin/FoxO1 pathway.

Material and methods: Islet β cells were divided into 6 groups: a control group, a high-glucose group, high glucose with Ang(1-7) group, high-glucose with Ang(1-7) and A779 group, high-glucose with angiotensin(1-7) and CHIR99021 group, and high-glucose with CHIR99021 group. A779 is a kind of MAS receptor antagonist that blocks the action of Ang(1-7), and CHIR99021 is a Wnt pathway activator. The morphology of pancreatic β cells was observed in each group after 48 hours of intervention. β -cell insulin secretory function and expressions of relevant factors were measured.

Results: Compared with the control group, the cell morphology became degraded in the high-glucose group and the capability of insulin secretion was reduced. Meanwhile, the expressions of mature β cells markers [pancreatic and duodenal homeobox 1 (Pdx1) and MAF BZIP transcription factor A (MafA)] were reduced, while the expressions of endocrine progenitor cells makers [octamer-binding transcription factor 4 (Oct4) and Nanog] were increased. The addition of CHIR99021 resulted in profound deep destruction of β cells compared with the high-glucose group. However, such changes were dramatically reversed following the treatment of Ang(1-7). The addition of A779 significantly inhibited the improvement caused by Ang(1-7).

Conclusion: Ang(1-7) can effectively reverse β cell dedifferentiation through Wnt/ β -catenin/FoxO1 pathway. It might be a new strategy for preventing and treating diabetes. (*Endokrynol Pol* 2023; 74 (5): 544–552)

Key words: diabetes; dedifferentiation of islet β cells; insulin secretion; angiotensin (1–7); Wnt/ β -catenin/FoxO1 pathway

Introduction

Type 2 diabetes mellitus (T2DM), which affects more than 450 million people worldwide, is one of the most significant global health problems [1]. T2DM is mainly characterized by pancreatic β cell dysfunction and insulin resistance (IR). The conventional view is that islet β cell apoptosis is the leading cause of islet β cell dysfunction and plays a vital role in the development of T2DM in humans [2]. However, exogenous insulin injection, especially short-term intensive insulin therapy for new-onset diabetes, allows β cells to rest and regain function [3]. It can be speculated that islet β -cell apoptosis is not the only contributor to β -cell dysfunction. Some studies demonstrate that the decline of β cell quality is due to β cell dedifferentiation. Dedifferentiated β cells can revert to progenitor-like cells expressing Neurogenin 3 (Ngn3), octamer-binding transcription factor 4 (Oct4), and Nanog. Meanwhile, markers of mature

β cells [pancreatic and duodenal homeobox 1 (Pdx1), Nk6 homeobox protein 1 (Nkx6.1), neuronal differentiation 1 (NeuroD1), and MAF BZIP transcription factor A (MafA)] were also decreased. Such findings provide a potential explanation for the insulin secretory defects of β cells observed in vivo [4]. However, the mechanism of pancreatic β -cell dedifferentiation is still unclear.

Wingless-type MMTV integration site family (Wnt) signalling pathway is an essential regulator of organ growth and cells fates. Genes encoding Wnt signalling factors are expressed in the pancreas. Wnt/ β -catenin signalling is necessary and sufficient for islet cells to proliferate and differentiate [5]. Prior studies provide have shown that Wnt signalling regulates the adipose tissue microenvironment and participates in essential steps of insulin utilization in the cells, resulting in the development of IR [6]. However, research on Wnt-related pathways regulating β -cell dedifferentiation is relatively rare. Activation of the Wnt/ β -catenin canonical pathway



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occurs by increasing the accumulation and nuclear translocation of β -catenin. β -catenin is phosphorylated by the glycogen synthase kinase 3 (GSK3 β) complex and degraded by the ubiquitin proteasome pathway. The inhibition of GSK3 β phosphokinase activity results in the non-degradation of β -catenin and a large quantity of free β -catenin aggregated in the cytoplasm [7, 8]. Non-phosphorylated β -catenin accumulates within the cells and is translocated to the nucleus, which regulates downstream gene expressions. CHIR99021, a selective inhibitor of GSK-3 β , increases the cytosolic level of β -catenin, further allowing nuclear translocation of active β -catenin. In mammalian cells, binding of β -catenin to forkhead box transcription factor O1 (FoxO1) enhances the transcriptional activity of FoxO1. More importantly, the association between β -catenin and FoxO1 is enhanced in cells exposed to oxidative stress [9]. Posttranslational modifications, including phosphorylation, ubiquitination, O-GlcNacylation, and acetylation, modulate FoxO1 subcellular localization and activity [10]. A growing body of studies demonstrates that FoxO1 regulates fundamental cellular processes, including cell differentiation, metabolism, and cell cycle arrest [10–12]. Findings in the last few years have revealed that the loss of FoxO1 under severe hyperglycaemia is implicated in β cell dedifferentiation, and other research also indicates a complex role of this transcription factor in β cell pathophysiology [4, 13, 14]. FoxO1 somatic deletions in β -cells resulted in β -cell dedifferentiation in ageing male mice and multiparous female mice in parallel with the up-regulation of progenitor and pluripotency markers [4]. This current study aimed to investigate the role of Wnt/ β -catenin/FoxO1 signalling pathway in the dedifferentiation of pancreatic β cells.

Increasing evidence shows that the pathological stimulation of the renin-angiotensin system (RAS) is associated with type 2 diabetes. Some findings prove that angiotensin II (AngII)-induced RAS activation is a major contributor to islet β -cell dedifferentiation; they also confirm that irbesartan, an inhibitor of AngII receptor, can markedly reverse impaired glucose-stimulated insulin secretion, and downregulate dedifferentiated cells markers in a high glucose environment, resulting in restoration of β -cell function [15]. Angiotensin-converting enzyme 2 (ACE2) is able to efficiently degrade AngII to form angiotensin (1–7) [Ang(1–7)], and Ang(1–7) exerts actions opposite to those of AngII [16]. A recent study has revealed that systemic Ang(1–7) treatment can improve β -cell dysfunction in a T2DM rat model by improving pancreatic microcirculation [17]. Such effects of Ang(1–7) could be attenuated by its receptor Mas antagonist A779, indicating that the islet endothelial function mediated by ACE2/Ang(1–7)/Mas axis exerts effects on β -cell function [17]. Studies have reported

that activation of ACE2/Ang(1–7) improves pancreatic β -cell dedifferentiation in a high-fat-diet mouse model [18]. There are few studies on the role of Ang(1–7) in islet β -cell dedifferentiation, and its mechanism of action is unclear. Other experiments have shown that AngII can further activate the glomerular Wnt/ β -catenin signalling pathway in diabetic mice and accelerate the progression of podocyte damage and glomerular sclerosis, and Ang(1–7) can partially block the effect of AngII by inhibiting the activity of Wnt/ β -catenin [19]. We hypothesized that Ang(1–7) could exert a protective effect on islet β cells through this signalling pathway.

The purpose of this study is to investigate the role of Wnt/ β -catenin/FoxO1 on the dedifferentiation of pancreatic β cells under high-glucose conditions and explore whether Ang(1–7) can reverse the dedifferentiation of pancreatic β cells through the Wnt/ β -catenin signalling pathway. Our findings could provide new ideas and targets for the prevention and treatment of diabetes.

Material and methods

Reagents and antibodies

The mouse insulinoma cell line, MIN6 cell, was purchased from Fuheng Biology (Shanghai, China). Ang(1–7) was purchased from Med Chem Express. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Jiangsu Meimian Industrial Co. to detect insulin. Anti-FoxO1, anti-Pdx1, anti-Nanog, and anti- β -catenin antibodies were purchased from the Proteintech Group (Wuhan, China). BCA Protein Assay Kit, RIPA Lysis Buffer, anti-MafA, anti-Oct4, and horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Boster Bioengineering (Wuhan, China). The polymerase chain reaction (PCR) and electrophoresis system instrument were purchased from Bio-Rad Laboratories (Hercules, CA, United States). The high-pressure steam steriliser was purchased from ZEALWAY (USA).

Methods

Culture and passage of islet β cells

MIN6 cells were cultured in RPMI-1640 (Cat No. SH30809.01, HyClone) with 10% foetal bovine serum (FBS), 1% penicillin-streptomycin, and 50 μ M mercaptoethanol in 5% CO₂ at 37°C. When the cells were grown to 80–90% fusion, they were digested with 0.25% trypsin and passaged in a 1:3 ratio. The cells were used in 3/4 passages in all experiments. This study was approved by the Ethics Committee of the Second Hospital of Shanxi Medical University (Approval No. DW2022041).

Cells were seeded onto 6-well plates at a density of 5×10^4 cells/well and were randomized into 6 groups. The grouping was as follows: Group A, normal control (11.1 mM d-glucose); Group B, high glucose (HG) control (33.3 mM d-glucose) [15]; Group C, HG with Ang(1–7) (10^{-6} mmol/L); Group D, HG with Ang(1–7) and A779 (10^{-5} mmol/L); Group E, HG with Ang(1–7) and CHIR99021 (5 μ mol/L); and Group F, HG with CHIR99021. Three replicates were obtained for each group. Pancreatic β -cell morphology was observed in each group after 48 h of intervention, and then islet secretory function and expressions of protein and gene were measured.

Glucose-stimulated insulin secretion (GSIS) analysis

After 48 h of intervention, the culture medium was collected from each group. The islet β cells were then cultured in Krebs-Ringer bicarbonate HEPES buffer (KRBH) without glucose for 1 h to nor-

malize the basal insulin secretion status. The islet β cells were then incubated in KRBH containing 3.3 mM d-glucose for 1 h. The buffer was collected for assaying of basal insulin secretion (BIS) levels. The cells were then incubated for an additional hour with fresh KRBH buffer containing 27.8 mM d-glucose to measure the glucose stimulated insulin secretion level [20]. Cells were washed 3 times with phosphate-buffered saline (PBS) before treatment with different media. The media were collected for ultra-sensitive enzyme-linked immunosorbent assay (ELISA) analysis.

Immunofluorescence analysis

After the cell intervention was completed, the cells were washed 3 times with PBS. The cells slides were fixed with 4% (w/v) paraformaldehyde for 30 min at room temperature. These slides were permeabilized with a solution containing 0.5% Triton X-100 for 10 min and then blocked with 3% BSA for 30 min at room temperature. The cell slides were then incubated with primary antibody at 4°C overnight. (MafA: 1:200, Pdx1: 1:100, Nanog: 1:100, Oct4: 1:50). Following 3 washes with PBS, the slides were incubated for 1 h at room temperature with fluorophore-labelled secondary antibodies (1:100). DAPI was applied after the secondary antibody to counterstain the nuclei. After 3 washes with PBS, the slides were fixed with anti-fluorescence quenching PVP mounting liquid. Imaging was done by confocal microscopy.

Real-time polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from islet β cells using the TRIzol reagent, and cDNA was synthesised by reverse transcription. The mRNA levels of Pdx1, MafA, Oct4, and Nanog were determined by real-time PCR. PCR primer sequences (5'-3') are as follows (Tab. 1). Shanghai Shengggong Bioengineering Technology Service completed the primer design and synthesis. The cycling conditions were as follows: initial denaturation at 95°C for 3 min, then 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 30 s. Relative mRNA levels were calculated by normalizing them to β -actin mRNA levels using the comparative cycle threshold (2^{-C_t}) method [21].

Western Blot analysis

Islet β cells were lysed in RIPA buffer for 30 min on ice, then they were pelleted by centrifugation of 12,000 rpm for 10 min at 4°C. A BCA Protein Assay Kit determined the protein concentration, and aliquots of total protein were separated by SDS-polyacryl-

amide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was incubated overnight at 4°C, with a proper ratio (FoxO1:1:4000, p-FoxO1:1:750, β -catenin:1:5000, Pdx1:1:5000, MafA:1:1000, Oct4:1:1000, Nanog:1:5000, β -actin:1:4500) of diluted primary antibodies in the case of the blocking buffer. The membrane was then washed and incubated for 2 h with an HRP-conjugated secondary antibody. After washing, the protein was visualized with an enhanced chemiluminescence detection kit. The bands were subjected to greyscale analysis using Image J software.

Statistical analysis

All statistical analyses were performed with SPSS 26 or GraphPad Prism 8. All data were expressed as mean \pm standard deviation (SD). Statistical differences between groups were analysed by one-way ANOVA. $P < 0.05$ was accepted as a significant difference.

Results

Changes in the morphology of islet β cells

We performed microscopic analysis of the islet cell morphology of all groups after 48 h of intervention. Compared with the control group, the cells of HG showed significant morphological changes, with the cells becoming shuttle-shaped and sparse. After Ang(1-7) treatment, the cell morphology was significantly improved and some cells reverted to normal morphology. The addition of A779 significantly inhibited the Ang(1-7)-mediated improvement of cell morphology. After 48 h of intervention in the HG with CHIR99021 group, the number of visible cells decreased, the morphology changed to the spindle, and even floating cells were visible. The effect of CHIR99021 was significantly reversed after Ang(1-7) treatment (Fig. 1).

High glucose-stimulated insulin secretion

To investigate the secretory function of islet β cells, insulin secretion was stimulated with low (3.3 mM) and high (27.8 mM) concentrations of glucose. Compared with control group, the BIS and GSIS of MIN6 cells were reduced considerably in the high-glucose group. However, the capability of insulin secretion of β cells was noticeably improved after Ang(1-7) treatment. The addition of A779 significantly inhibited the improvement of Ang(1-7)-mediated GSIS, but there was no significant change in BIS. The results showed that both basal insulin secretion and glucose-stimulated insulin secretion were significantly reduced in the CHIR99021 group compared to the high-glucose group, and the effect of CHIR99021 was significantly reversed after the addition of Ang(1-7) (Fig. 2).

The protein expression levels of Pdx1, MafA, Oct4, and Nanog

Western blot analysis (Fig. 4) and immunofluorescence detection (Fig. 3) shows that expressions of Pdx1 and MafA in the HG group were significantly

Table 1. Real-time polymerase chain reaction (RT-PCR) primers

Gene	Primer sequence
Pdx1	5'-GAC ATC TCC CCA TAC GAA GTG-3' (Forward)
	5'-GTG AGC TTT GGT GGA TTT CAT C-3' (Reverse)
MafA	5'-GCT TCA GCA AGG AGG AGG TCA TC-3' (Forward)
	5'-GGC ACTTCTCGCTCTCCAGAATG-3' (Reverse)
Nanog	5'-CAT AAC TTC GGG GAG GAC TTT C-3' (Forward)
	5'-GCT TCC AAA TTC ACC TCC AAA T-3' (Reverse)
Oct4	5'-TTA AGA ACA TGT GTA AGC TGC G-3' (Forward)
	5'-GCA TAT CTC CTG AAG GTT CTC A-3' (Reverse)
β -actin	5'-GGC TGT ATT CCC CTC CAT CG -3' (Forward)
	5'-CCA GTT GGT AAC AAT GCC ATG T -3' (Reverse)

Pdx1 — pancreatic and duodenal homeobox 1; MafA — MAF BZIP transcription factor A; Oct4 — octamer-binding transcription factor 4

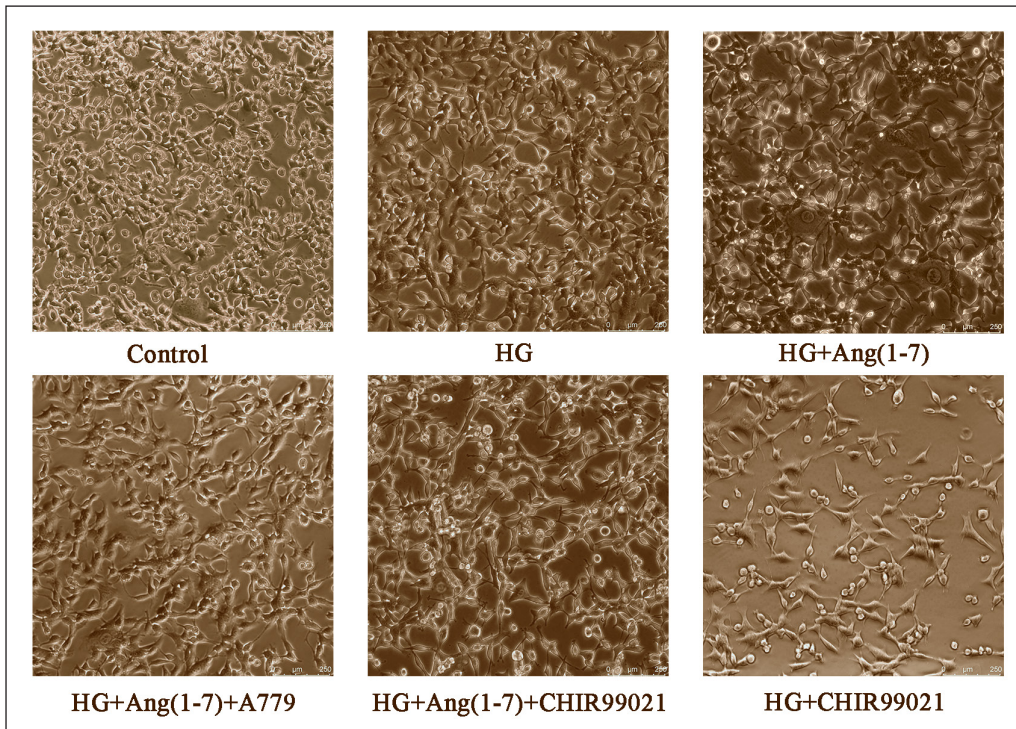


Figure 1. Morphology changes of the pancreatic cells from each group. MIN6 cells were divided into 5 groups: control group (Control), high-glucose group (HG), high-glucose with angiotensin(1-7) group [HG + Ang(1-7)], high-glucose with angiotensin(1-7) and A779 group (HG + Ang(1-7) + A779), high-glucose with angiotensin(1-7) and CHIR99021 group (HG + Ang(1-7) + CHIR99021), and high-glucose with CHIR99021 group (HG + CHIR99021). Pancreatic beta cell morphology was observed in each group after 48 h

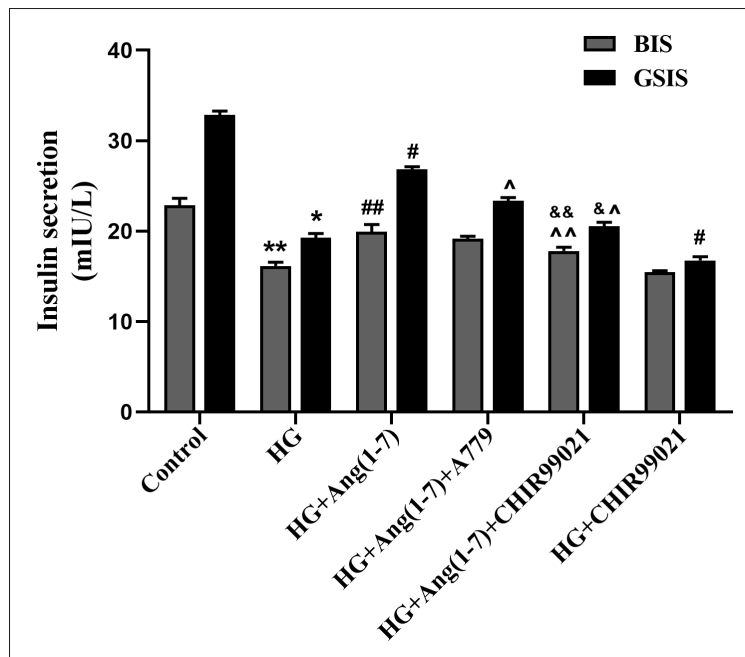


Figure 2. Results of glucose-stimulated insulin secretion in each group. MIN6 cells were divided into 6 groups: control group (Control), high-glucose group (HG), high-glucose with angiotensin(1-7) group [HG + Ang(1-7)], high-glucose with angiotensin(1-7) and A779 group (HG + Ang(1-7) + A779), high-glucose with angiotensin(1-7) and CHIR99021 group (HG + Ang(1-7) + CHIR99021), and high-glucose with CHIR99021 group (HG + CHIR99021). Insulin levels were measured after low (3.3 mmol/L, grey column, basal insulin secretion [BIS])- and high (27.8 mmol/L, black column, GSIS)-concentration glucose stimulation in each group. * $p < 0.05$ vs. GSIS of Control group, ** $p < 0.05$ vs. BIS of Control group, # $p < 0.05$ vs. GSIS of HG group, ## $p < 0.05$ vs. BIS of HG group, ^ $p < 0.05$ vs. GSIS of HG + Ang(1-7) group, ^^ $p < 0.05$ vs. BIS of HG + Ang(1-7) group, & $p < 0.05$ vs. GSIS of HG+CHIR99021 group, && $p < 0.05$ vs. BIS of HG + CHIR99021 group

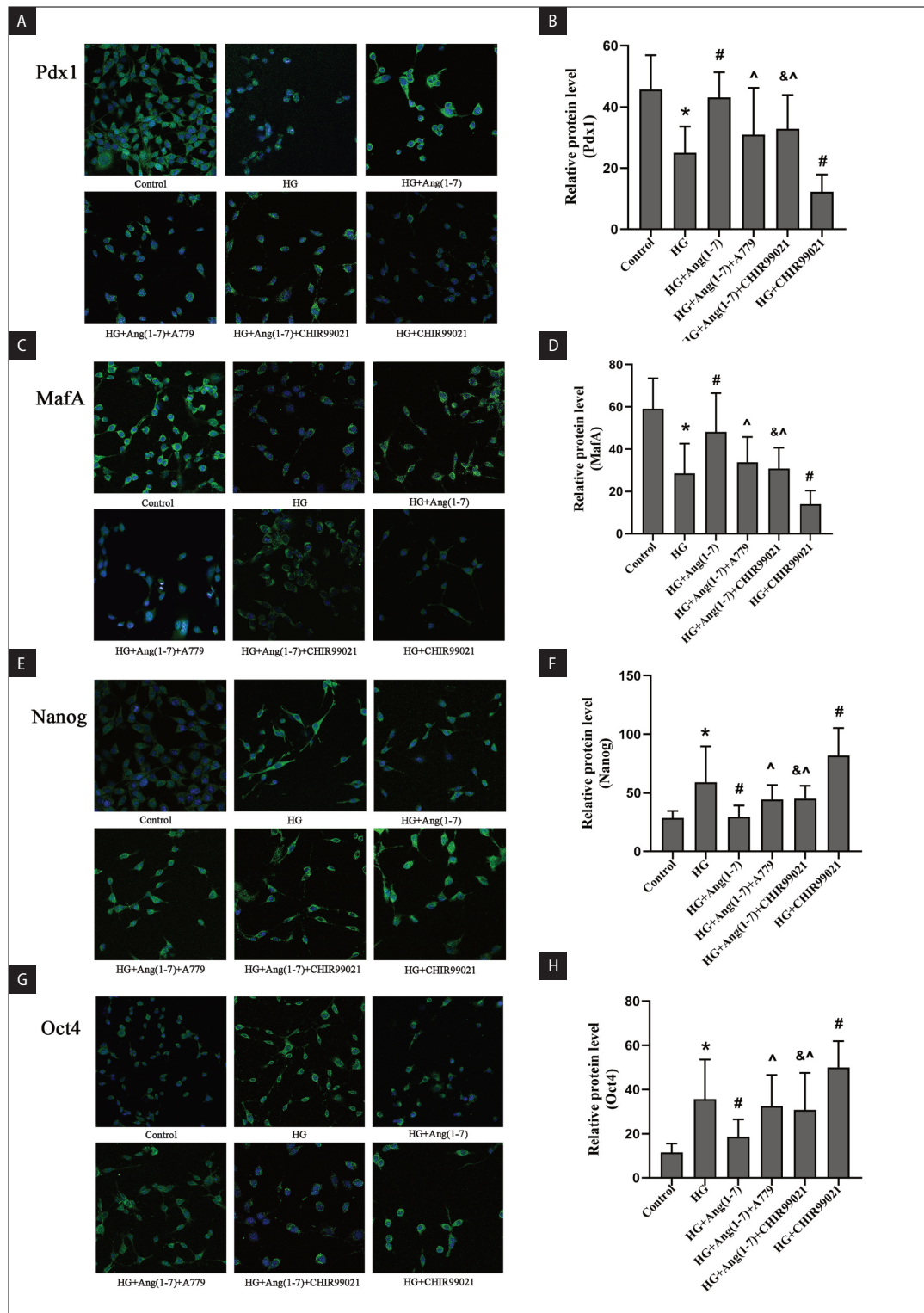


Figure 3. Immunofluorescence analysis of the protein expression levels of pancreatic and duodenal homeobox 1 (Pdx1), MAF BZIP transcription factor A (MafA), Nanog and octamer-binding transcription factor 4 (Oct4) in each group. MIN6 cells were divided into 6 groups: a control group (Control), a high-glucose group (HG), high-glucose with angiotensin(1-7) group [HG + Ang(1-7)], high-glucose with angiotensin(1-7) and A779 group [HG + Ang(1-7) + A779], high-glucose with angiotensin(1-7) and CHIR99021 group [HG + Ang(1-7) + CHIR99021], and high-glucose with CHIR99021 group (HG+ CHIR99021). After 48 h, the expressions of protein were measured. Pdx1 and MafA are pancreatic β -cell markers. Nanog and Oct4 are endocrine progenitor cell markers. **A.** Protein expression of Pdx1; **B.** Quantification of A results; **C.** Protein expression of MafA; **D.** Quantification of C results; **E.** Protein expression of Nanog; **F.** Quantification of E results; **G.** Protein expression of Oct4; **H.** Quantification of G results. Representative immunofluorescence analysis images (400 \times) are shown. * $p < 0.05$ vs. Control group, # $p < 0.05$ vs. HG group, ^ $p < 0.05$ vs. HG + Ang (1-7) group, & $p < 0.05$ vs. HG+CHIR99021 group

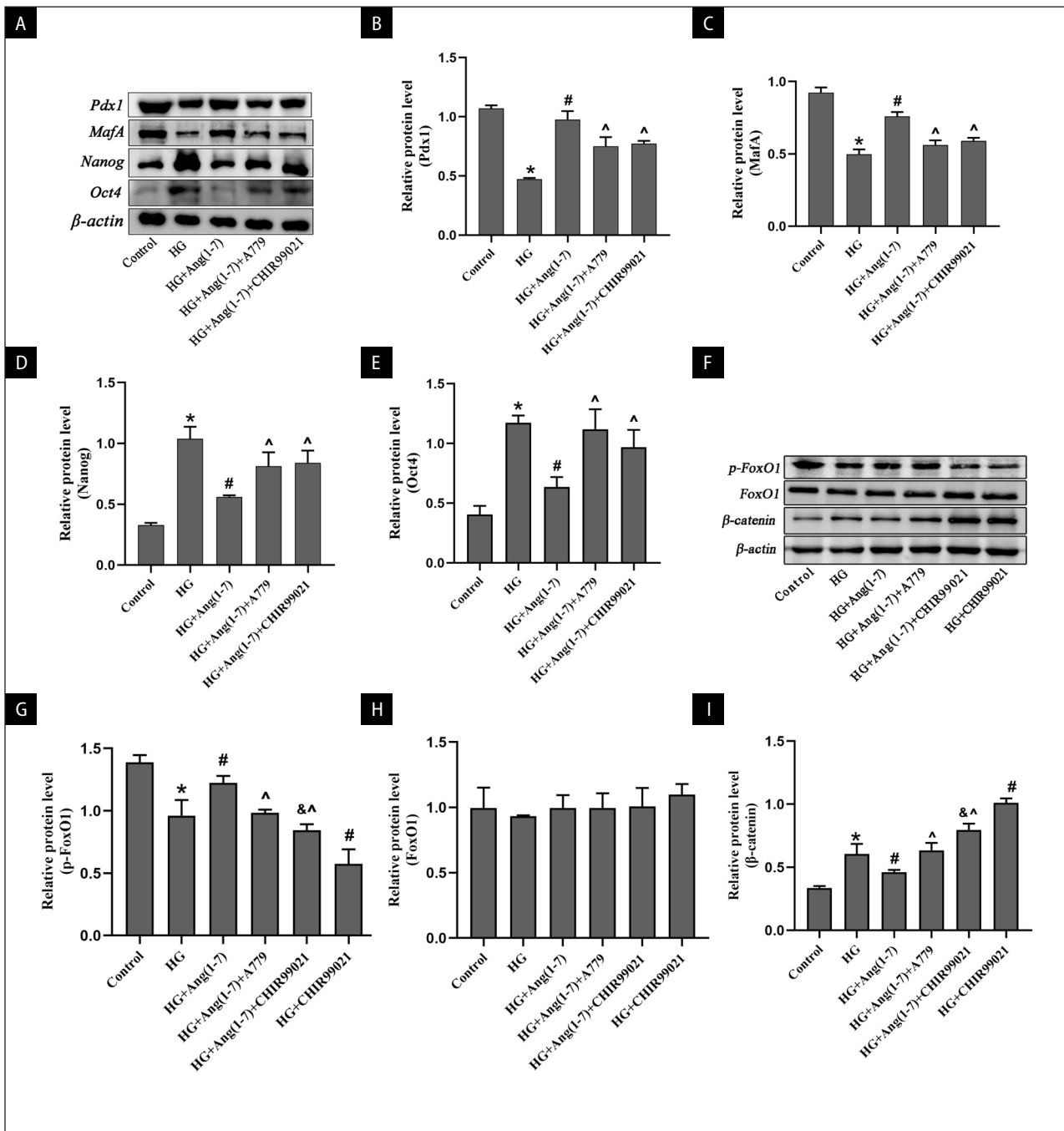


Figure 4. Western blot analyses of pancreatic and duodenal homeobox 1 (*Pdx1*), MAF BZIP transcription factor A (*MafA*), Nanog, octamer-binding transcription factor 4 (*Oct4*), forkhead box transcription factor O1 (*FoxO1*), phosphorylated FOXO1 (*p-FoxO1*), and β -catenin protein expressions. MIN6 cells were divided into 6 groups: control group (Control), high-glucose group (HG), high-glucose with angiotensin(1-7) group [HG + Ang(1-7)], high-glucose with angiotensin(1-7) and A779 group [HG + Ang(1-7) + A779], high-glucose with angiotensin(1-7) and CHIR99021 group [HG + Ang(1-7) + CHIR99021], and high-glucose with CHIR99021 group (HG + CHIR99021). The protein expressions of *Pdx1*, *MafA*, *Nanog*, *Oct4*, *FoxO1*, *p-FoxO1*, and β -catenin were measured. **A.** Western blot presented protein expressions of *Pdx1*, *MafA*, *Nanog* and *Oct4* in MIN6 cells; **B–E.** Quantification of (**A**) results; **F.** Western blot presented protein expressions of *p-FoxO1*, *FoxO1* and β -catenin; **G–I.** Quantification of (**F**) results. * $p < 0.05$ vs. Control group, # $p < 0.05$ vs. HG group, ^ $p < 0.05$ vs. HG + Ang(1-7) group, & $p < 0.05$ vs. HG + CHIR99021 group

lower than those in the control group, whereas *Oct4* and *Nanog* expressions increased. After Ang(1-7) treatment, expressions of *Pdx1* and *MafA* were increased to different degrees compared with the HG

group. The protein levels of *Oct4* and *Nanog* significantly declined, albeit without reaching normal levels. The addition of A779 significantly inhibited the Ang(1-7)-mediated improvement of insulin se-

Table 2. The mRNA expression levels of indicated genes (mean \pm SD)

Group	Pdx1	MafA	Nanog	Oct4
Control	1.007 \pm 0.148	1.004 \pm 0.117	1.006 \pm 0.136	1.013 \pm 0.199
HG	0.596 \pm 0.059*	0.444 \pm 0.042*	2.614 \pm 0.015*	2.389 \pm 0.072*
HG + Ang(1-7)	0.780 \pm 0.020#	0.832 \pm 0.017#	1.698 \pm 0.163#	1.637 \pm 0.112#
HG + Ang(1-7) + A779	0.561 \pm 0.03 [^]	0.636 \pm 0.058 [^]	2.018 \pm 0.072 [^]	1.998 \pm 0.170 [^]
HG + CHIR99021 + Ang(1-7)	0.549 \pm 0.017 [^]	0.563 \pm 0.099 [^]	1.879 \pm 0.039 [^]	2.038 \pm 0.085 [^]
HG + CHIR99021	0.362 \pm 0.048#	0.319 \pm 0.025#	2.917 \pm 0.048#	2.891 \pm 0.116#

MIN6 cells were divided into 6 groups: control group (Control), high-glucose group (HG), high-glucose with angiotensin(1-7) group [HG + Ang(1-7)], high-glucose with angiotensin(1-7) and A779 group (HG + Ang(1-7) + A779), high-glucose with angiotensin(1-7) and CHIR99021 group (HG + Ang(1-7) + CHIR99021), and high-glucose with CHIR99021 group (HG + CHIR99021). Pancreatic and duodenal homeobox 1 (Pdx1) and MAF BZIP transcription factor A (MafA) are pancreatic β -cell marker proteins, and Nanog and octamer-binding transcription factor 4 (Oct4) are endocrine progenitor cell markers. *p < 0.05 vs. Control group, #p < 0.05 vs. HG group, [^]p < 0.05 vs. HG + Ang(1-7) group, [^]p < 0.05 vs. HG + CHIR99021 group

cretion. After 48 h of intervention in the HG with CHIR99021, the protein expressions of Pdx1 and MafA were decreased compared with the high-glucose group, while expressions of Oct4 and Nanog were increased, and they were significantly reversed by Ang(1-7) treatment.

The mRNA expression levels of indicated genes

Compared with the control group, mRNA expressions of Pdx1 and MafA in the HG group were decreased. Meanwhile, expressions of Oct4 and Nanog were elevated. Subsequent administration of Ang(1-7) dramatically reversed the expressions of these genes, and the addition of A779 significantly inhibited the Ang(1-7)-mediated improvement of cellular phenotype factors. After 48 h of intervention in the HG with CHIR99021, the mRNA expressions of Pdx1 and MafA were decreased compared with the high-glucose group, while the expressions of Oct4 and Nanog were increased, and they were significantly reversed after Ang(1-7) treatment (Tab. 2).

The protein expression levels of FoxO1, p-FoxO1, and β -catenin

Western blot analysis showed that the protein levels of β -catenin in a high-glucose environment were significantly increased compared with the control group. There were no significant changes in FoxO1 protein levels in MIN6 cells incubated with high glucose, but high glucose stimulated the dedifferentiation of MIN6 cells with a low synthesis of phosphorylated FOXO1 (p-FoxO1) protein. After the CHIR99021 intervention, the protein expression of p-FoxO1 was lower. However, all of the above conditions improved after Ang(1-7) treatment but have not resumed normal levels. The accession of A779 significantly inhibited the improvement of Ang(1-7)-mediated alterations in pathway proteins (Fig. 4).

Discussion

Low islet β cell quality can lead to the development of T2DM [22]. Chronic exposure to high glucose and lipid concentration leads to β -cell dysfunction through various mechanisms, including oxidative stress, endoplasmic reticulum (ER) stress, and inflammation [23, 24]. The present study demonstrates that β -cell dedifferentiation occurs commonly in T2DM [25, 26]. Moreover, canonical β -cell transcription factor (Pdx1, MafA, and Nkx6.1) levels are insufficient to maintain β -cell function in an altered metabolic environment. Activation of endocrine progenitor markers has been detected in endocrine cells during metabolic stress. Our results also confirmed these findings. Compared with the control group, a high-glucose environment resulted in a sharp decrease in glucose-stimulated insulin secretion. Meanwhile, expression levels of Pdx1 and MafA were decreased and expression levels of Oct4 and Nanog were increased, which indicates that high glucose levels are involved in islet β -cell dedifferentiation. In a recent study, β -cell regeneration has been described as an efficient approach to significantly aid the health burden of diabetes [27]. Reversal of β -cell dedifferentiation may thus represent a novel therapeutic option for diabetic patients to protect their insulin-producing β -cell pool.

Some findings prove that AngII-induced RAS activation significantly contributes to β -cell dedifferentiation; they confirmed that irbesartan, a RAS inhibitor, markedly reversed impaired glucose-stimulated insulin secretion and downregulated dedifferentiated cell markers in a high-glucose environment, resulting in the restoration of β -cell function [17]. ACE2 can efficiently degrade AngII to form Ang(1-7), thus exerting the opposite effect to that of Ang II [16]. Activation of ACE2/Ang(1-7) attenuates pancreatic β -cell de-differentiation in a high-fat-diet mouse model [18]. Our results demonstrated that insulin secretion from β cells induced

by glucose in the Ang(1-7) treatment group was slightly improved compared with that in β cells cultured in the high-glucose environment. After Ang(1-7) treatment, the expressions of Pdx1 and MafA were increased in different degrees compared with the HG group, and the levels of Oct4 and Nanog significantly declined. The addition of A779 (Mas receptor antagonists) significantly inhibited the improvement caused by Ang(1-7). Thus, Ang(1-7) plays a protective role against the dedifferentiation of islet β cells. However, the mechanism of Ang(1-7) on pancreatic β -cell dedifferentiation is still unclear, thus requiring further in-depth study.

Growing evidence shows that early abnormalities of Wnt/ β -catenin signalling pathway can cause abnormal glucose metabolism and increase the risk of T2DM. Canonical Wnt activation by wnt3a-induce marked dedifferentiation of both 3T3-L1 and human adipocytes, and these cells also become insulin-resistant with impaired upstream insulin signalling and reduced glucose uptake [28]. Studies have revealed that lipotoxicity-induced dedifferentiation of pancreatic β -cell is associated with the Wnt/ β -catenin pathway. β -catenin overexpression could promote macrophage activation and cause β -cell dedifferentiation, resulting in insulin resistance [29]. Casteel et al. showed that short-term pharmacological FoxO1 inhibition could modulate β -cell dedifferentiation by downregulating the expressions of specific transcription factors in β cells, resulting in the aberrant expressions of progenitor genes and the α -cell marker glucagon [30]. It has been found that a high glucose concentration causes β -cell dedifferentiation and significantly reduces the expression of FoxO1 mRNA level and p-FoxO1 protein level [31]. These studies suggest the pathophysiological role of the Wnt/ β -catenin/FoxO1 pathway in β -cell dedifferentiation. In this study, we proved the role of the Wnt/ β -catenin/FoxO1 pathway in islet β -cell dedifferentiation. Our study found that the β -catenin level in the high-glucose group was significantly higher than that in the control group. There was no significant change in total FoxO1 protein levels, but high glucose stimulated the dedifferentiation of MIN6 cells with a low synthesis of p-FoxO1 protein. More importantly, compared with the HG group, the use of a selective inhibitor of GSK-3 β (CHIR99021), which activates the Wnt/ β -catenin pathway, resulted in a profound destruction of β cells. Protein expressions of p-FoxO1 were lower. Meanwhile, the expression levels of Pdx1 and MafA were decreased, while the expression levels of Oct4 and Nanog were increased. These findings suggest the adverse effects of Wnt/ β -catenin/FoxO1 signalling pathway on the dedifferentiation of islet β cells. Interestingly, supplementation of Ang(1-7) could alleviate such alterations in both conditions. Activation of RAS

signalling by Ang(1-7) treatment appeared to prevent the changes of β -catenin and p-FoxO1 and increased insulin levels, which results in a significant improvement in β -cell function. However, supplementation of A779 could antagonize these effects. Based on these findings, we conclude that activation of the Wnt/ β -catenin/FoxO1 pathway has a negative impact on the development of islet β -cell differentiation. However, Ang(1-7) can counteract this effect by increasing the expression of β -catenin and promoting insulin secretion, thereby improving β -cell function.

These results demonstrate that Ang(1-7) can reverse glucose-induced islet β -cell dedifferentiation by the Wnt/ β -catenin/FoxO1 signalling pathway, which might be a valid way to restore β -cell function in T2DM. Our research explores the mechanisms of β -cell dedifferentiation and provides valuable information for developing new strategies of treating T2DM. However, the molecular mechanisms responsible for the protection effects of Ang(1-7) on β -cell dedifferentiation should be further investigated.

Conclusion

Our research shows that Ang(1-7) can effectively reverse pancreatic β -cell dedifferentiation through Wnt/ β -catenin/FoxO1 signalling pathway.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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