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Original Research

Mesenchymal stromal cells ameliorate mitochondrial dysfunction in α cells and hyperglucagonemia in type 2 diabetes via SIRT1/FoxO3a signaling

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Abstract

Dysregulation of α cells results in hyperglycemia and hyperglucagonemia in type 2 diabetes mellitus (T2DM). Mesenchymal stromal cell (MSC)-based therapy increases oxygen consumption of islets and enhances insulin secretion. However, the underlying mechanism for the protective role of MSCs in α-cell mitochondrial dysfunction remains unclear. Here, human umbilical cord MSCs (hucMSCs) were used to treat 2 kinds of T2DM mice and αTC1-6 cells to explore the role of hucMSCs in improving α-cell mitochondrial dysfunction and hyperglucagonemia. Plasma and supernatant glucagon were detected by enzyme-linked immunosorbent assay (ELISA). Mitochondrial function of α cells was assessed by the Seahorse Analyzer. To investigate the underlying mechanisms, Sirtuin 1 (SIRT1), Forkhead box O3a (FoxO3a), glucose transporter type1 (GLUT1), and glucokinase (GCK) were assessed by Western blotting analysis. In vivo, hucMSC infusion improved glucose and insulin tolerance, as well as hyperglycemia and hyperglucagonemia in T2DM mice. Meanwhile, hucMSC intervention rescued the islet structure and decreased α- to β-cell ratio. Glucagon secretion from αTC1-6 cells was consistently inhibited by hucMSCs in vitro. Meanwhile, hucMSC treatment activated intracellular SIRT1/FoxO3a signaling, promoted glucose uptake and activation, alleviated mitochondrial dysfunction, and enhanced ATP production. However, transfection of SIRT1 small interfering RNA (siRNA) or the application of SIRT1 inhibitor EX-527 weakened the therapeutic effects of hucMSCs on mitochondrial function and glucagon secretion. Our observations indicate that hucMSCs mitigate mitochondrial dysfunction and glucagon hypersecretion of α cells in T2DM via SIRT1/FoxO3a signaling, which provides novel evidence demonstrating the potential for hucMSCs in treating T2DM.

Key words: α cells; hucMSCs; glucagon secretion; mitochondrial function; SIRT1/FoxO3a.

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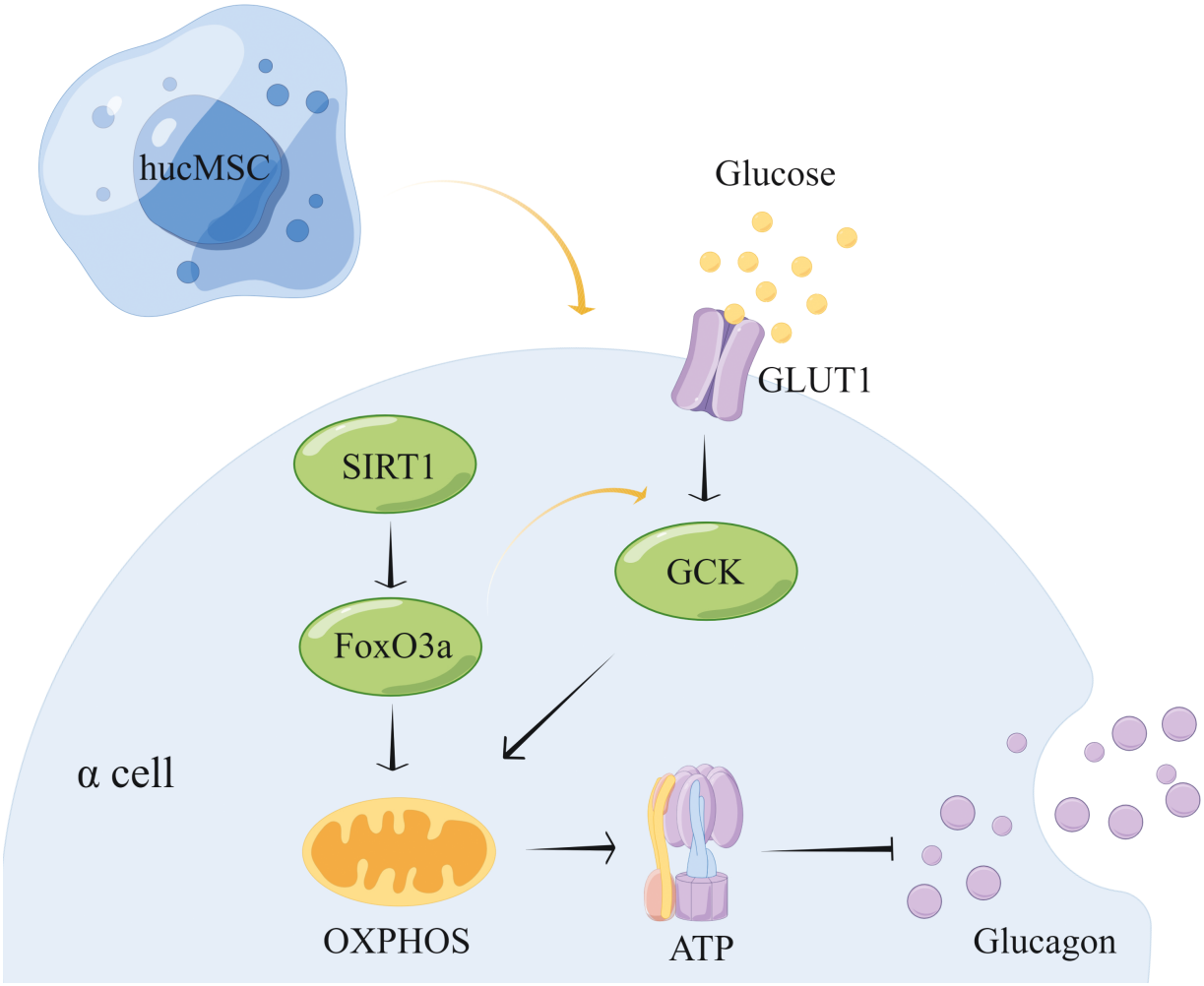
Graphical Abstract



Significance statement

Relative hyperglucagonemia caused by α-cell dysregulation contributes to hyperglycemia in patients with type 2 diabetes mellitus (T2DM), in which mitochondrial dysfunction and subsequent abnormal ATP production play an important role. Mesenchymal stromal cell (MSC)-based therapy is one of the most promising and effective approaches for treating diabetes. However, studies showing the role of hucMSCs in pancreatic α-cell dysfunction are few. Our observations indicate that hucMSCs mitigate mitochondrial dysfunction and glucagon hypersecretion of α-cells in T2DM via SIRT1/FoxO3a signaling, which provides novel evidence demonstrating the potential for hucMSCs in the regenerative treatment of diabetes.

Introduction

Pancreatic α cells release glucagon and promote glucose output from the liver.[1] Relative hyperglucagonemia caused by α-cell dysregulation contributes to fasting and postprandial hyperglycemia in patients with type 2 diabetes mellitus (T2DM).[2] Single-cell transcriptomes from cultured pancreatic islets of T2DM donors have revealed that genes responsible for energy metabolism in mitochondria are significantly downregulated in α cells.[3] Defective mitochondrial metabolism in α-cells accounts for dysregulated glucagon secretion in T2DM.[4]

Sirtuins regulate metabolism by controlling mitochondrial function, which is closely related to T2DM, particularly Sirtuin 1 (SIRT1).[5] SIRT1, a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase, participates in mitochondrial biogenesis and oxidative metabolism.[6,7] It is reported that insulin resistance and metabolic syndrome are associated with low SIRT1 gene and protein expression.[8] A mutation in the SIRT1 gene could cause the development of type 1 diabetes mellitus (T1DM).[9] In pancreatic β-cells, SIRT1 enhances glucose-stimulated insulin secretion.[10-12] The deletion of SIRT1 in mouse β-cells impairs insulin secretion by disrupting glucose sensing.[13] However, studies showing the role of SIRT1 in pancreatic α-cell mitochondrial function are limited.

Mesenchymal stromal cell (MSC)-based therapy is one of the most promising and effective approaches for treating diabetes.[14] Human umbilical cord MSCs (hucMSCs) are easier to obtain and have high proliferative potential and lower immunogenicity compared with other MSCs, and this makes hucMSCs an ideal choice in diabetes treatment.[15,16] Studies

have shown that MSC intervention is effective in many diseases by regulating mitochondrial function such as myocardial infarction and diabetic endothelial dysfunction.[17-19] Interestingly, MSCs increase oxygen consumption in islets and enhance insulin secretion.[20] Our previous study demonstrated that MSC-conditioned medium alleviates high-fat diet (HFD)-induced hyperglucagonemia.[21] However, whether MSCs regulate α-cell mitochondrial function in T2DM remains unclear.

Therefore, in this study, we used 2 kinds of T2DM mice and αTC1-6 cells pre-exposed to palmitate (PA) to explore the effects and underlying mechanism of hucMSCs in improving α-cell mitochondrial dysfunction and hyperglucagonemia.

Materials and methods

Cell culture and treatments

With the approval of the Ethics Committee of Qilu Hospital of Shandong University, we obtained fresh human umbilical cords from healthy newborns. All of the participants gave informed consent for the use of umbilical cords in this study. After washing the umbilical cords thrice in normal saline, we removed blood vessels and cut Wharton's jelly into small pieces with sterile scissors. These pieces were then spread in cell culture flasks with α-MEM medium (Gibco) containing 10% fetal bovine serum (FBS; Gibco) and 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air. We changed the culture medium every 3 days until the cells reached 80% confluence. The third to the fifth passage of cells were used for identification, administration, and Transwell co-culturing.

Human embryo lung fibroblasts (HELFs) were obtained from the China Cell Culture Center and cultured in Dulbecco's modified Eagle's medium (DMEM)/high-glucose medium (Gibco) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C and in a 5% CO_2 incubator.

αTC1-6 cells were obtained from the American Type Culture Collection (ATCC Number: CRL-2934) and cultured in DMEM/high-glucose medium (Gibco) supplemented with 10% FBS and antibiotics. In the experiments, 1×10^6 αTC1-6 cells were seeded onto 6-well plates, and 0.5-mM PA was added 1 day later to stimulate the cells for 48 hours. The αTC1-6 cells were then cocultured with 1×10^5 hucMSCs or HELFs for 24 hours via a Transwell system (Cat. No. 3450; Corning) in DMEM/high-glucose medium containing FBS and antibiotics. To further explore the mechanism of hucMSCs, α cells were pre-treated with SIRT1 inhibitor (EX-527; 20 μM, Selleck), siR-SIRT1, and siR-FoxO3 before treatment with hucMSCs.

Identification of hucMSCs

The third passage of hucMSCs was used for flow cytometry analysis according to characteristics that they are positive for CD73 (Cat. No. 05811-60; BioGems) and CD105 (Cat. No. 17111-80; BioGems), and negative for CD34 (Cat. No. 343505; BioLegend) and HLA-DR (Cat. No. 327021; BioLegend). To detect the differentiation potential of hucMSCs, cells were cultured in adipogenic differentiation medium (Cat. No. HUXUC-90031; OriCell) and osteogenic differentiation medium (Cat. No. HUXUC-90021; OriCell), respectively. Oil Red O (Cat. No. G1262; Solarbio) and Alizarin Red S (Cat. No. G1450; Solarbio) were used for staining of lipid droplets and calcium nodes.

Animal models and hucMSC administration

We used 2 types of T2DM mouse models in the present study. All animal experiments were approved by the Animal Ethics Committee of Qilu Hospital of Shandong University. The first model was an HFD- and STZ-induced T2DM model. Six-week-old male C57BL/6J mice were purchased from the Model Animal Research Center of Shandong University. The mice were housed with a 12-hours light/dark cycle at a temperature (22 °C-25 °C)- and humidity (55 ± 5%)-controlled environment. T2DM was established by a continuous HFD for 16 weeks followed by a single dose of streptozotocin (STZ, 100 mg/kg, Cat. No. S0130; Sigma-Aldrich) intraperitoneally after fasting for 12 hours. The second model was genetically obese, leptin receptor-deficient db/db mice, which is a spontaneous T2DM model. Four-week-old male db/db mice and their matching control db/m mice were purchased from Changzhou Cavens Experimental Animal Co., Ltd. and were fed a normal chow diet. T2DM was identified as fasting glucose ≥ 16.7 mmol/L on 2 different days after fasting for 6 hours. Then, hucMSCs (1×10^6 cells/mouse) were suspended in 200 μL PBS and injected into 2 types of T2DM mice via the tail vein every 7 days for 6 cycles. The control or db/m group and T2DM group were infused with 200 μL PBS at the same time.

To evaluate the mechanism of hucMSCs in vivo, HFD- and STZ-induced T2DM mice were also administered intraperitoneally with EX-527 (10 mg/kg) every 3 days for 6 weeks during hucMSC intervention, while the other groups were given the same amount of dimethyl sulfoxide (DMSO) as the control.

Intraperitoneal glucose tolerance test (IPGTT)

The intraperitoneal glucose tolerance test (IPGTT) was performed 1 week after the last hucMSC administration. Each group contained 4-6 mice selected via a randomization procedure. During IPGTT, after fasting for 12 hours, the mice were injected intraperitoneally with 2 g/kg body weight of glucose. Tail vein blood glucose levels were measured by Accu-Chek Performa (Roche Life Science) at 0, 15, 30, 60, 90, 120, and 180 minutes. The area under the curve (AUC) was calculated using the trapezoidal rule.

Blood and tissue collection

One week after IPGTT, the mice were anaesthetized with isoflurane and the blood of them were collected from the tip of the heart. The sera were then stored at −80 °C for glucagon enzyme-linked immunosorbent assay (ELISA; Cloud-Clone) and insulin ELISA (ALPCO) test. The mice were subsequently sacrificed and the pancreas was removed and fixed in 4% paraformaldehyde for immunofluorescence staining.

hucMSCs tracking

hucMSCs were labeled using the PKH67 Green Fluorescent Cell Linker Kit (PKH67, Sigma-Aldrich) and injected into T2DM mice via tail vein. After 24 h, mice were sacrificed and their pancreas, heart, liver, spleen, lungs, kidneys, and intestines were fixed in 4% paraformaldehyde for frozen section and immunofluorescence staining. The slides of pancreas were incubated with anti-mouse glucagon antibody

(Proteintech, Cat. No. 67286-1-Ig, 1:200) and goat anti-mouse TRITC (Zhongshan, Cat. No. ZF-0313, 1:200). The fluorescence signal was detected by fluorescence microscope.

Immunofluorescence staining of the pancreas

The pancreatic tissues that were fixed in 4% paraformaldehyde were embedded in paraffin, sectioned at 5-μm thickness, and mounted on glass slides. The slides were dewaxed, after which antigen retrieval was performed using an antigen unmasking buffer. After blocking for 30 minutes at room temperature in a protein-blocking solution (10% normal goat serum), the slides were incubated with anti-mouse insulin antibody (Proteintech, Cat. No. 66198-1-Ig, 1:1,000) and anti-rabbit glucagon antibody (Proteintech, Cat. No.15954-1-AP, 1:200) overnight at 4 °C. The slides were then incubated with goat anti-rabbit FITC (Zhongshan, Cat. No. ZF-0311, 1:200) and goat anti-mouse TRITC (Zhongshan, Cat. No. ZF-0313, 1:200) for 60 minutes at room temperature and then stained for DAPI for 5 minutes. Fluorescence was observed and captured using a fluorescence microscope (Olympus BX53, Japan). The areas of α cells and β cells were analyzed by Image-Pro Plus software. The α- or β-cell ratio in islets was measured by the glucagon- or insulin-positive area divided by the total islet area.

Isolation of primary islets

Primary islets were isolated from normal C57BL/6J mice as reported previously.[21] Pancreas were digested using 1.5 mg/mL collagenase V (Solarbio; Cat. No. C8170) and 62.5 U/mL DNase I (Thermo Fisher; Cat. No. EN0521) followed by hand picking under a stereoscopic microscope. After incubation overnight, the islets were exposed to 0.5-mM PA for 48 hours and then cocultured with hucMSCs for 24 hours.

Glucagon secretion

After different kinds of intervention, supernatants were removed and αTC1-6 cells and isolated islets were balanced in Krebs-Ringer bicarbonate HEPES (KRBH) buffer (120 mM NaCl, 0.75 mM $CaCl_2{\cdot}2H_2O$, 4 mM KH_2PO_4, 10 mM $NaHCO_3$, 1 mM $MgSO_4{\cdot}7H_2O$, 30 mM HEPES, 1% BSA) containing no glucose for 0.5 hours in 37 °C, followed by incubation in KRBH buffer containing 25 mmol/L glucose for 2 hours. Subsequently, supernatants were collected and kept at −80 °C for glucagon ELISA (Cloud-Clone).

Real-time quantitative PCR analysis

Total RNA from αTC1-6 cells and isolated islets was extracted using an E.Z.N.A. MicroElute Total RNA Kit (Cat. No. R6831-01; Omega BioTek) following the manufacturer's instructions. Next, 1 μg RNA was reverse-transcribed into cDNA using the Prime Script RT Reagent Kit (Cat. No. RR047A; Takara). Primers were chemically synthesized by GenePharma Co., Ltd.. The primer sequences of *Proglucagon* were sense 5′-GCACATTCACCAGCGACTAC-3′ and antisense 5′-CTGGTGGCAAGATTGTCCAG-3′. The primer sequences of *β-actin* were sense 5′-AAGAGCTATGAGCTGCCTGA-3′ and antisense 5′-TACGGATGTCAACGTCACAC-3′. Real-time PCR was conducted with the SYBR Green PCR Kit (Cat. No. RR420A; Takara), gene expression changes were determined with the comparative CT ($2^{-\Delta\Delta Ct}$) method, and quantification was achieved by normalization using *β-actin* as control.

Preparation of cell lysate and Western blotting analysis

After being washed with ice-cold PBS, cells and isolated islets were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer (P0013B, Beyotime, Shanghai, China) for approximately 30 minutes. Then, the samples were centrifuged at 15 000 g at 4 °C for 20 minutes. Protein concentration was determined using the bicinchoninic acid (BCA) method (Beyotime). Subsequently, proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (EpiZyme) and transferred onto polyvinylidene difluoride (PVDF) membranes (IPVH00010 0.45 μm, Millipore). Then, the membranes were blocked with 5% skim milk in Tris-buffered saline solution containing Tween-20 (Sigma-Aldrich) for 1 hour at room temperature and incubated with specific primary antibodies at 4 °C overnight. After detection by horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature, the proteins were visualized using enhanced chemiluminescence.

The primary antibodies were as follows: glucagon (Abcam, Cat. No. ab92517, 1:1000), sirtuin 1 (SIRT1, Proteintech, Cat. No. 13161-1-AP, 1:1000), forkhead box O3a (FoxO3a, CST, Cat. No. 2497S, 1:1000), glucose transporter type 1 (GLUT1, Bioss, Cat. No. bs0472R, 1:1000), glucokinase (GCK, Abcam, Cat. No. ab88056, 1:1000), NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8 (NDUFB8, Proteintech, Cat. No. 14794-1-AP, 1:1000), succinate dehydrogenase complex, subunit B (SDHB, Proteintech, Cat. No. 10620-1-AP, 1:5000), ubiquinol-cytochrome c reductase core protein II (UQCRC2, Proteintech, Cat. No. 14742-1-AP, 1:1000), cytochrome c oxidase II (MTCO2, Proteintech, Cat. No. 55070-1-AP, 1:1000), ATP synthase, H^+ transporting, mitochondrial F1 complex, alpha subunit 1 (ATP5A1, Proteintech, Cat. No. 14676-1-AP, 1:2000), heat shock protein 90 (HSP90, Santa Cruz, Cat. No. sc13119, 1:500), β-actin (CST, Cat. No. 3700S, 1:1000).

Transmission electron microscopy

αTC1-6 cells were exposed to PA, followed by treatment with hucMSCs or HELFs, and then collected by trypsinization. Afterward, cells were fixed with 2.5% glutaraldehyde and post-fixed in 1% phosphate-buffered osmium tetroxide. After being embedded, sectioned, and double-stained with uranyl acetate and lead citrate, electron photomicrographs of αTC1-6 cell ultrastructure were taken via TEM (JEM-1200EX II, JEOL).

Glucose uptake assay

Glucose uptake ability of αTC1-6 cells was evaluated using fluorescent glucose 2-NBDG (Maokang Bio, Cat. No. MX4511) following the manufacturer's instructions. After intervention, αTC1-6 cells were gently washed with KRBH and incubated with 50 μM 2-NBDG at 37 °C for 30 minutes. The fluorescent intensity of the cells was detected on a microplate reader (excitation wavelength: 465 nm; emission wavelength: 540 nm).

Seahorse analysis

The Mito Stress Test Kit (Agilent, Cat. No. 103015-100) was used to detect the oxygen consumption rate (OCR) according to the procedure provided by the manufacturer. Cells were seeded onto each well of an XF96 cell culture microplate at 2×10^4 cells/well. The OCR was assessed in Seahorse XF DMEM Medium (pH 7.4) containing 10-mM glucose,

2-mM pyruvate glutamine, and 1-mM pyruvate according to the manufacturer's instructions. The concentrations of oligomycin, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and antimycin A/rotenone were 1.5, 2, and 0.5 μM, respectively. OCR was determined and analyzed on the Agilent Seahorse Bioscience XF96 Extracellular Flux Analyzer (Agilent Technologies).

Small interfering RNA transfections

Small interfering RNA (siRNA) oligonucleotides were synthesized by Shanghai GenePharma Co., Ltd. The sequences of negative control siRNA were as follows: sense 5′-UUCUCCGAACGUGUCACGUTT-3′ and antisense 5′-ACGUGACACGUUCGGAGAATT-3′. The sequences for the SIRT1 siRNA were sense 5′-GGGAUCAAGAGGUUGUUAATT-3′ and antisense 5′-UUAACAACCUCUUGAUCCCTT-3′. The sequences for the FoxO3 siRNA were as follows: sense 5′-GGAGCUUGGAAUGUGACAUTT-3′ and antisense 5′-AUGUCACAUUCCAAGCUCCTT-3′.

αTC1-6 cells were transfected with siRNA for SIRT1 and FoxO3 using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's instructions. Briefly, the αTC1-6 cells were plated in 6-well plates until they reached 80%-90% confluence. The medium was then removed and replaced by Opti-MEM I reduced serum medium (Gibco) mixed with siRNA for SIRT1 and FoxO3 (125 nM) for 6 hours. Subsequently, the culture medium was replaced by DMEM medium with 10% FBS, and the cells were treated with hucMSCs. After 24 hours, the cells and supernatant were collected for the next experiments.

Statistical analysis

All of the data were presented as the mean ± SEM. Differences between 2 groups were evaluated using unpaired Student's t test; for 3 groups or more, a one-way ANOVA was performed followed by Bonferroni's test for multiple comparisons. $P < .05$ was considered to be statistically significant. GraphPad Prism 8 was used to conduct all of the statistical analyses and to create the graphs.

Results

hucMSC infusion ameliorates hyperglycemia and hyperglucagonemia in HFD and STZ-induced T2DM mice

To investigate whether hucMSCs exert therapeutic effects in T2DM mice, primary hucMSCs were first isolated and identified by their specific surface markers and adipogenic and osteogenic differentiation. Fluorescence-activated cell sorter analysis showed that hucMSCs were positive for stem cell markers CD105 and CD73, and negative for CD34 and HLA-DR (Supplementary Figure S1A). Oil Red O staining and Alizarin Red S staining suggested that hucMSCs could differentiate into adipocytes (Supplmentary Figure S1B) and osteoblasts (Supplementary Figure S1C). To track hucMSCs in vivo, hucMSCs were labeled with PKH67 and injected into T2DM mice. After hucMSC infusion, a fluorescence signal could be detected in the islets (Figure 1A), liver, lung, spleen, and lymph nodes of the intestine, while there was no signal in the heart or kidney (Supplementary Figure S2).

Subsequently, to evaluate the effects of hucMSCs on pancreatic α cells in vivo, we established a T2DM mouse model by HFD and STZ injections and administered hucMSCs into mice via the tail vein. The results of IPGTT showed that hucMSC infusion significantly reduced fasting glucose levels and improved glucose tolerance. The AUC supported the above findings (Figure 1B). The body weight of mice was unaffected during hucMSC intervention (Supplementary Figure S3A). Circulatory glucagon levels markedly increased in HFD and STZ-induced T2DM mice and decreased after hucMSC intervention (Figure 1C), whereas insulin levels were elevated in hucMSCs group compared to HFD + STZ group (Figure 1D). Meanwhile, in HFD and STZ-induced T2DM mice, the normal structure of islets was changed (Figure 1E) and there was a higher α-cell ratio (Figure 1F) and a lower β-cell ratio (Figure 1G). hucMSC administration partially rescued the islet structure and reversed α- and β-cell imbalance (Figure 1E-G).

hucMSC infusion alleviates hyperglycemia and hyperglucagonemia in db/db mice

To further verify the effects of hucMSCs in T2DM mice, we also injected hucMSCs into db/db mice. Similarly, we found that hucMSC infusion reduced fasting glucose levels and improved the glucose tolerance during IPGTT. The AUC also supported the above findings (Figure 2A). The body weight of db/db mice was unaffected by hucMSCs (Supplementary Figure S3B). Circulatory glucagon and insulin levels of db/db mice were higher than db/m mice, whereas hucMSCs intervention ameliorated hyperglucagonemia and hyperinsulinemia (Figure 2B, C). Moreover, hucMSCs infusion also improved islet structure (Figure 2D) and reduced α-cell ratio (Figure 2E) while elevating β-cell ratio (Figure 2F). These results suggest that hucMSCs alleviate hyperglycemia and hyperglucagonemia in T2DM mice.

hucMSCs decrease glucagon secretion in αTC1-6 cells

To observe the effect of hucMSCs on α cells in vitro, we cocultured αTC1-6 cells with hucMSCs or HELF via a Transwell system after PA stimulation. ELISA, RT-qPCR, and Western blotting were performed for the detection of glucagon levels in supernatants and intracellular glucagon mRNA and protein content. We found that hucMSC treatment remarkably reduced glucagon secretion (Figure 3A) while promoting intracellular glucagon protein expression (Figure 3B). There were no significant changes in *Proglucagon* mRNA levels (Supplementary Figure S4A). Meanwhile, TEM images showed that there were fewer glucagon granules in the PA group and more granules in the hucMSC group (Figure 3C, D), which coincided with intracellular glucagon protein levels. In addition, in isolated primary islets, hucMSCs also decreased glucagon secretion (Supplementary Figure S5A) and increased intracellular glucagon protein expression (Supplementary Figure S5B), while having no significant influence on *Proglucagon* mRNA levels (Supplementary Figure S5C). These results demonstrate that hucMSCs regulate the glucagon secretion process.

hucMSCs regulate SIRT1/FoxO3a signaling and improve mitochondrial function in αTC1-6 cells

To investigate the specific mechanism involved in the regulation of α-cell function by hucMSCs, we detected changes in SIRT1 signaling and mitochondrial function in αTC1-6 cells. The results showed that PA decreased SIRT1 and its downstream FoxO3a expression (Figure 4A) and was

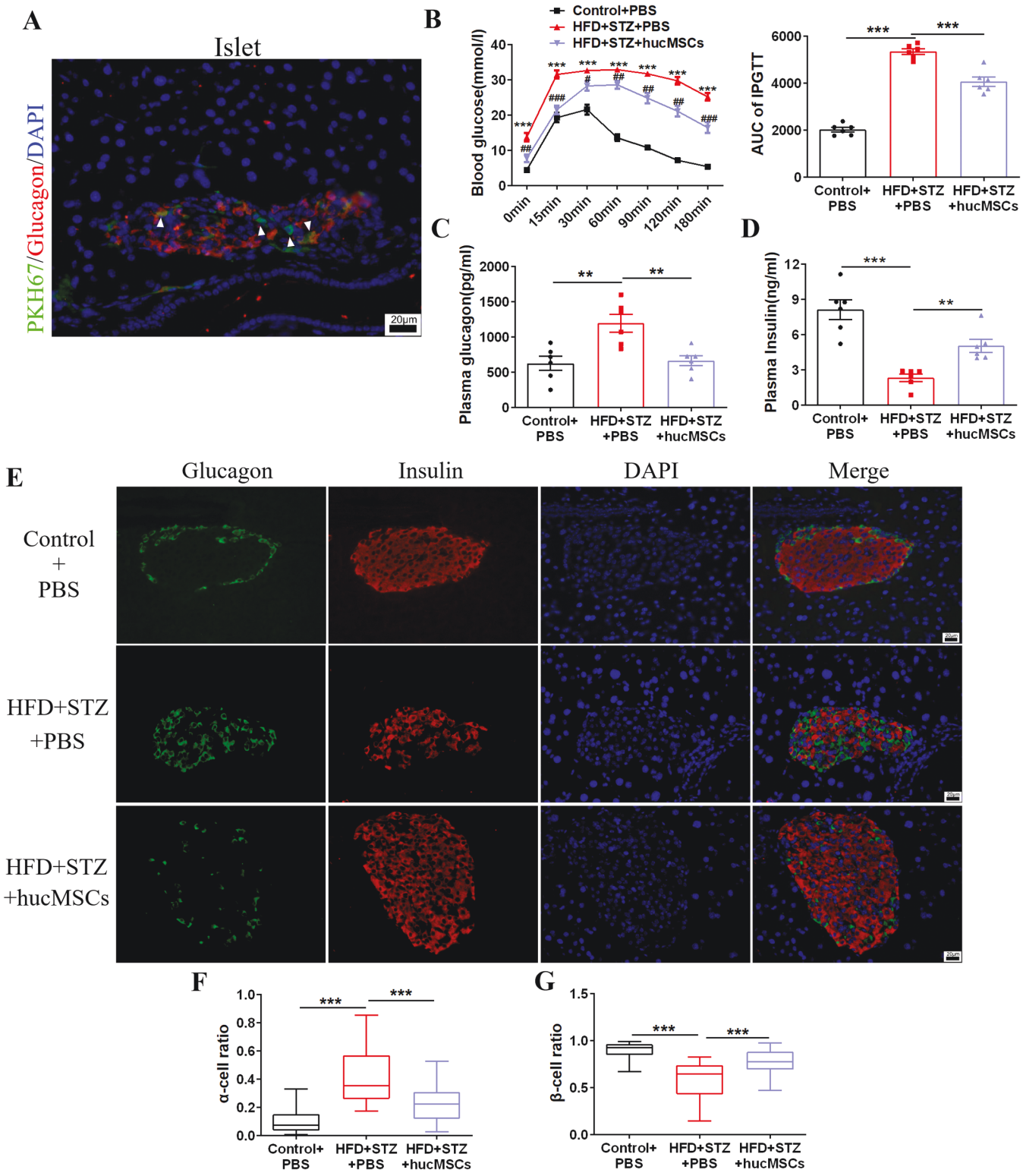


Figure 1. hucMSC infusion ameliorates hyperglycemia and hyperglucagonemia in HFD- and STZ-induced T2DM mice. A. PKH67-labeled hucMSCs could be detected in islets. B. IPGTT showed that hucMSC infusion reduced fasting glucose levels and improved glucose tolerance. The AUC supported the above findings (n = 6 mice per group; *T2DM + PBS group vs control + PBS group; #T2DM + hucMSCs group vs T2DM + PBS group). C. hucMSC intervention decreased circulatory glucagon levels in T2DM mice. D. hucMSC intervention increased circulatory insulin levels in T2DM mice. E. Immunofluorescence staining showed that hucMSC administration partially rescued the islet structure (scale bar = 20 μm). F, G. hucMSC infusion reduced α-cell ratio and elevated β-cell ratio, as analyzed by Image-Pro Plus software (n = 30-50 islets per group). The results are expressed as the mean ± SEM. (*P < .05; **P < .01; ***P < .001).

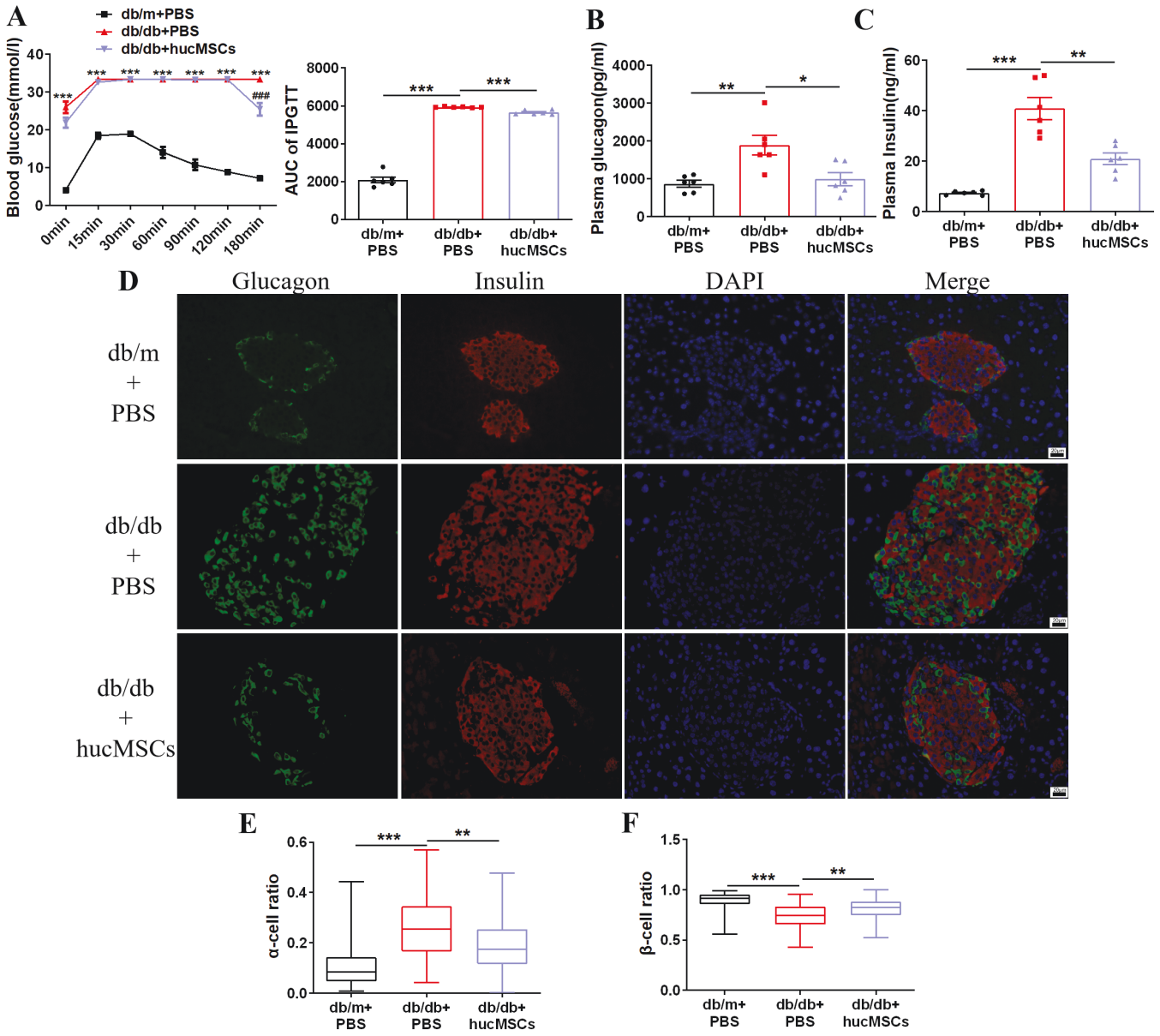


Figure 2. hucMSC infusion alleviates hyperglycemia and hyperglucagonemia in db/db mice. A. IPGTT showed that hucMSC infusion reduced fasting glucose levels and improved glucose tolerance. The AUC supported the above findings (n = 6 mice per group; *db/db + PBS group vs db/m + PBS group; #db/db + hucMSCs group vs db/db + PBS group). B, C. hucMSC intervention decreased circulatory glucagon and insulin levels in db/db mice. D. Immunofluorescence staining showed that hucMSC administration partially rescued islet structure (scale bar = 20 μm). E, F. hucMSC infusion reduced α-cell ratio and elevated β-cell ratio, as analyzed by Image-Pro Plus software (n = 50-80 islets per group). The results are expressed as the mean ± SEM (*P < .05; **P < .01; ***P < .001).

accompanied by a downregulation of GLUT1 and GCK (Figure 4B). hucMSC treatment promoted SIRT1 and FoxO3a expression and upregulated the expression of GLUT1 and GCK. Meanwhile, we used fluorescent glucose 2-NBDG to further confirm that hucMSCs increase glucose uptake by α cells (Figure 4C). Subsequently, to study the effects of hucMSCs on mitochondrial function, we first detected protein levels of the mitochondrial complex and found that hucMSCs elevated the expression of SDHB and MTCO2 (Figure 4D). The results from OCR directly proved that hucMSCs enhance mitochondrial oxidative phosphorylation (OXPHOS), as evidenced by increased basal respiration, maximal respiration, spare respiratory capacity, and ATP production (Figure 4E). These results indicate that hucMSCs promote SIRT1 signaling and improve mitochondrial function in α cells.

hucMSCs mitigate mitochondrial dysfunction and glucagon hypersecretion by activating SIRT1

To assess whether hucMSCs regulate mitochondrial function and glucagon secretion through SIRT1, SIRT1 siRNA, and the specific inhibitor EX-527 were used to pretreat αTC1-6 cells. In SIRT1 knockdown cells, hucMSC treatment weakly increased SIRT1 and FoxO3a expression (Figure 5A). Moreover, the effects of hucMSCs on the upregulation of GLUT1, GCK, and glucose uptake were partially blocked (Figure 5B, C). In addition, hucMSC therapy did not sufficiently restore mitochondrial OXPHOS and ATP production

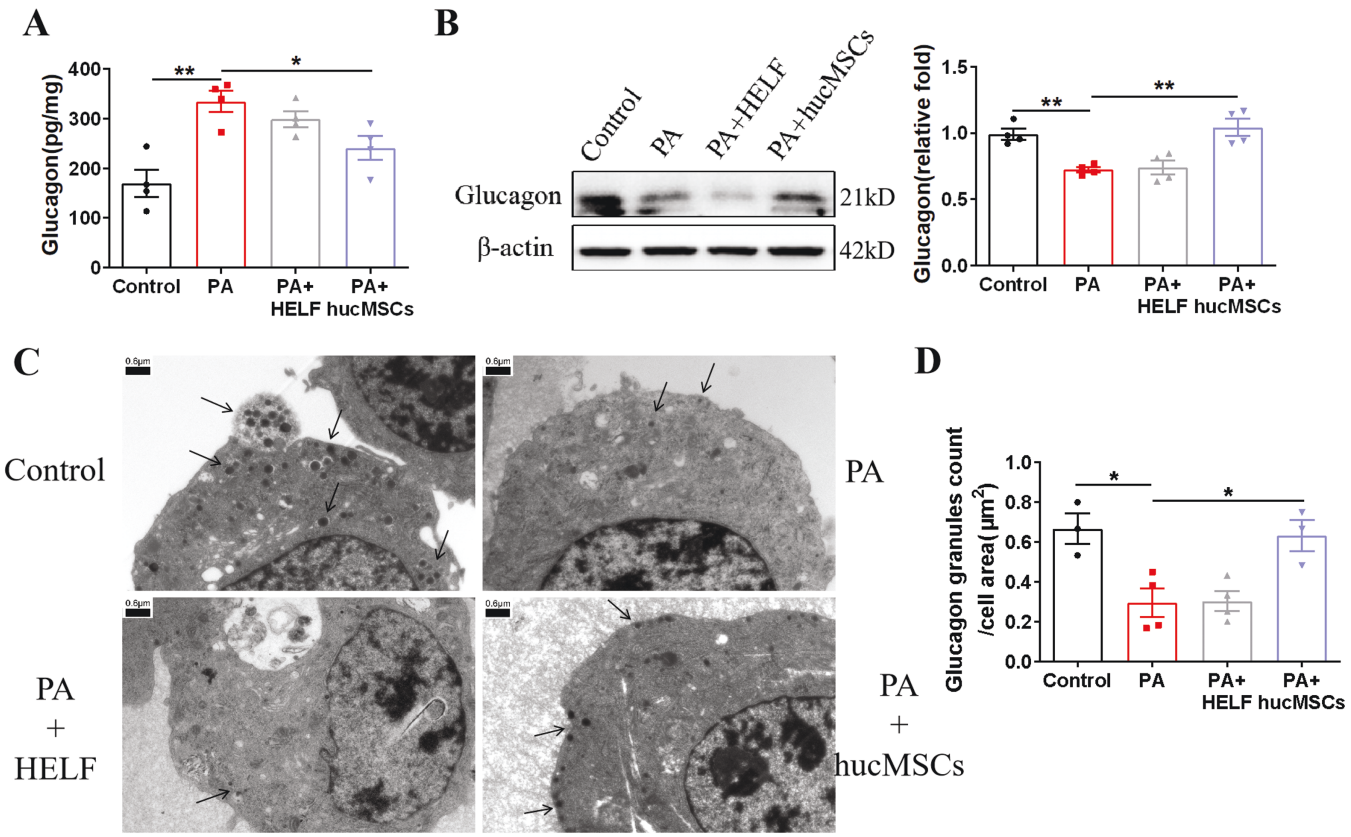


Figure 3. hucMSCs decrease glucagon secretion in αTC1-6 cells. A. ELISA showed that hucMSC treatment remarkably reduced glucagon levels in the supernatant. B. Western blotting demonstrated that hucMSCs promoted intracellular glucagon expression. Quantification of bands was performed using ImageJ software. C, D. TEM images showed fewer glucagon granules in the PA group, whereas more granules were observed in the hucMSC group, which were concordant to intracellular glucagon protein levels (scale bar = 0.6 μm). All these experiments were repeated 3-4 times, and the results are presented as the mean ± SEM (*$P < .05$; **$P < .01$).

(Figure 5D, E) when SIRT1 was knocked down. Finally, glucagon secretion was not effectively inhibited by hucMSCs (Figure 5F, G; Supplementary Figure S4B).

Consistently, upregulations of SIRT1 and FoxO3a were partially abolished in αTC1-6 cells after hucMSC therapy when simultaneously treated with SIRT1 inhibitor EX-527 (Figure 5H). Meanwhile, hucMSCs weakly elevated GLUT1 and GCK expression and glucose uptake when co-treated with EX-527 (Figure 5I, J). Furthermore, improvement of mitochondrial OXPHOS and ATP production was also weaker in hucMSCs and EX-527 co-treated group compared with the hucMSC-treated group (Figure 5K, L), which resulted in the restoration of glucagon secretion (Figure 5M, N; Supplementary Figure S4C). These results demonstrate that hucMSCs mitigate mitochondrial dysfunction and glucagon hypersecretion by activating SIRT1.

FoxO3a is involved in the improvement of mitochondrial dysfunction and glucagon hypersecretion by hucMSCs

To verify whether SIRT1 downstream factor FoxO3a is involved in the regulation of mitochondrial function and glucagon secretion by hucMSCs, FoxO3 siRNA was transfected into αTC1-6 cells. When FoxO3 was knocked down, the hucMSCs were not able to efficiently promote the expression of FoxO3a (Figure 6A), GLUT1, and GCK (Figure 6B), and increase glucose uptake (Figure 6C). At the same time, the improvement of mitochondrial OXPHOS and ATP production were partially weakened in FoxO3 knockdown cells (Figure 6D, E) and was accompanied by restoration of glucagon secretion (Figure 6F, G; Supplementary Figure S4D). These findings suggest that FoxO3a participates in the effects of hucMSCs on α cells.

hucMSCs improve hyperglycemia and hyperglucagonemia in vivo via SIRT1

To further illustrate the role of hucMSCs in vivo through SIRT1, we injected EX-527 into T2DM mice, together with hucMSCs. Our results showed that when co-treated with EX-527, the effect of hucMSCs on improving glucose tolerance was obviously weakened (Figure 7A). Meanwhile, circulatory glucagon levels in hucMSCs and the EX-527-treated group were higher than the hucMSC-treated group, while insulin levels were lower (Figure 7B, C). Additionally, immunofluorescence staining showed that when co-treated with EX-527, hucMSCs did not effectively rescue islet structure and α- and β-cell imbalance (Figure 7D–F). The body weight of mice was unaffected during hucMSC and EX-527 intervention (Supplementary Figure S3C). Taken together, these results indicate that hucMSCs mitigate hyperglycemia and hyperglucagonemia in vivo via SIRT1.

Discussion

In this study, we explored the underlying mechanisms by which hucMSCs alleviated pancreatic α-cell dysfunction in T2DM.

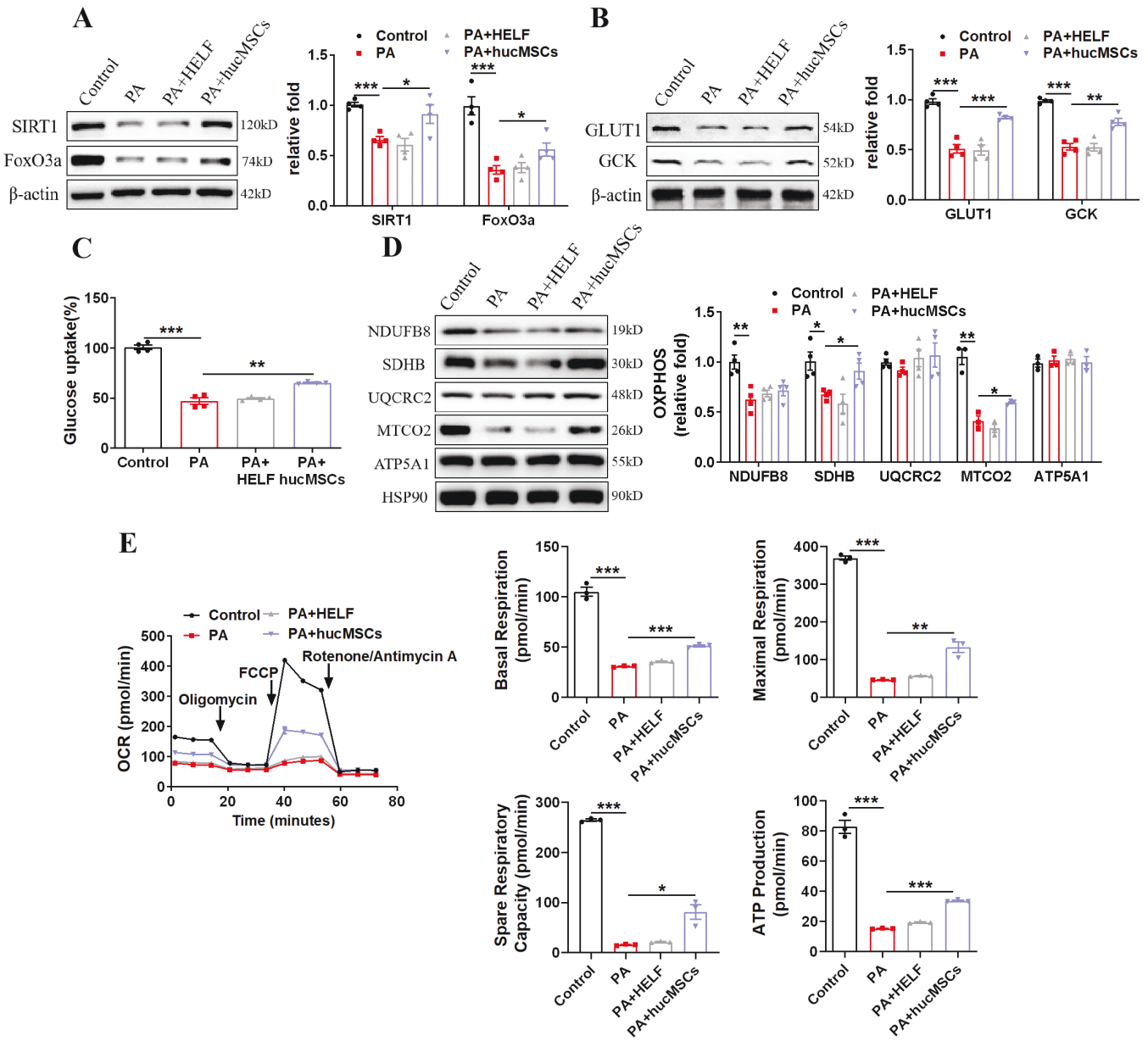


Figure 4. hucMSCs regulate SIRT1/FoxO3a signaling and improve mitochondrial function in αTC1-6 cells. A. Western blotting showed that PA downregulated the expression of SIRT1 and its downstream FoxO3a, while hucMSCs promoted SIRT1/FoxO3a signaling. Quantification of bands was performed using ImageJ software. B. hucMSCs upregulated glucose transporter GLUT1 and glucokinase (GCK) levels. C. hucMSCs increased glucose uptake, as detected by fluorescent glucose 2-NBDG. D. Western blotting indicated that hucMSC therapy elevated the expression of mitochondrial complex SDHB and MTCO2. E. Seahorse analysis showed that hucMSCs enhanced mitochondrial OXPHOS, as evidenced by increased basal respiration, maximal respiration, spare respiratory capacity, and ATP production. All of these experiments were repeated 3-4 times, and the data are presented as the mean ± SEM (*$P < .05$; **$P < .01$; ***$P < .001$).

By activating SIRT1/FoxO3a signaling, hucMSCs increased glucose uptake and activation, enhanced mitochondrial OXPHOS, and promoted ATP production, which eventually reduced glucagon secretion and mitigated hyperglucagonemia in T2DM.

T2DM is characterized by pancreatic β-cell dysfunction and insulin resistance. α Cells also play a vital role in maintaining glucose homeostasis by secreting glucagon.[1] T2DM is associated with higher glucagon-to-insulin ratios and augmentation of the α- to β-cell area ratios, which result in hyperglucagonemia and hyperglycemia.[2,22,23] In addition, enhanced glucagon concentrations are also observed in the prediabetes population.[24] In the treatment of diabetes, MSC-based therapy has been considered to be the most effective and promising.[14] There are multiple sources for MSCs acquisition such as bone marrow, adipose tissue, and umbilical cord.[25,26] Our previous studies reported that bone marrow-derived MSCs (bmMSCs) ameliorate islet endothelium apoptosis and functional impairment,[27] and bmMSC-conditioned medium alleviated high-fat-induced hyperglucagonemia.[21] However, compared with other MSCs, hucMSCs are more readily available and possess greater proliferative capacity and reduced immunogenicity, which makes hucMSCs a better choice.[15,16] Previous studies showed that hucMSCs reversed β-cell dedifferentiation.[28] However, studies showing the role of hucMSCs in pancreatic α-cell dysfunction are few. In this

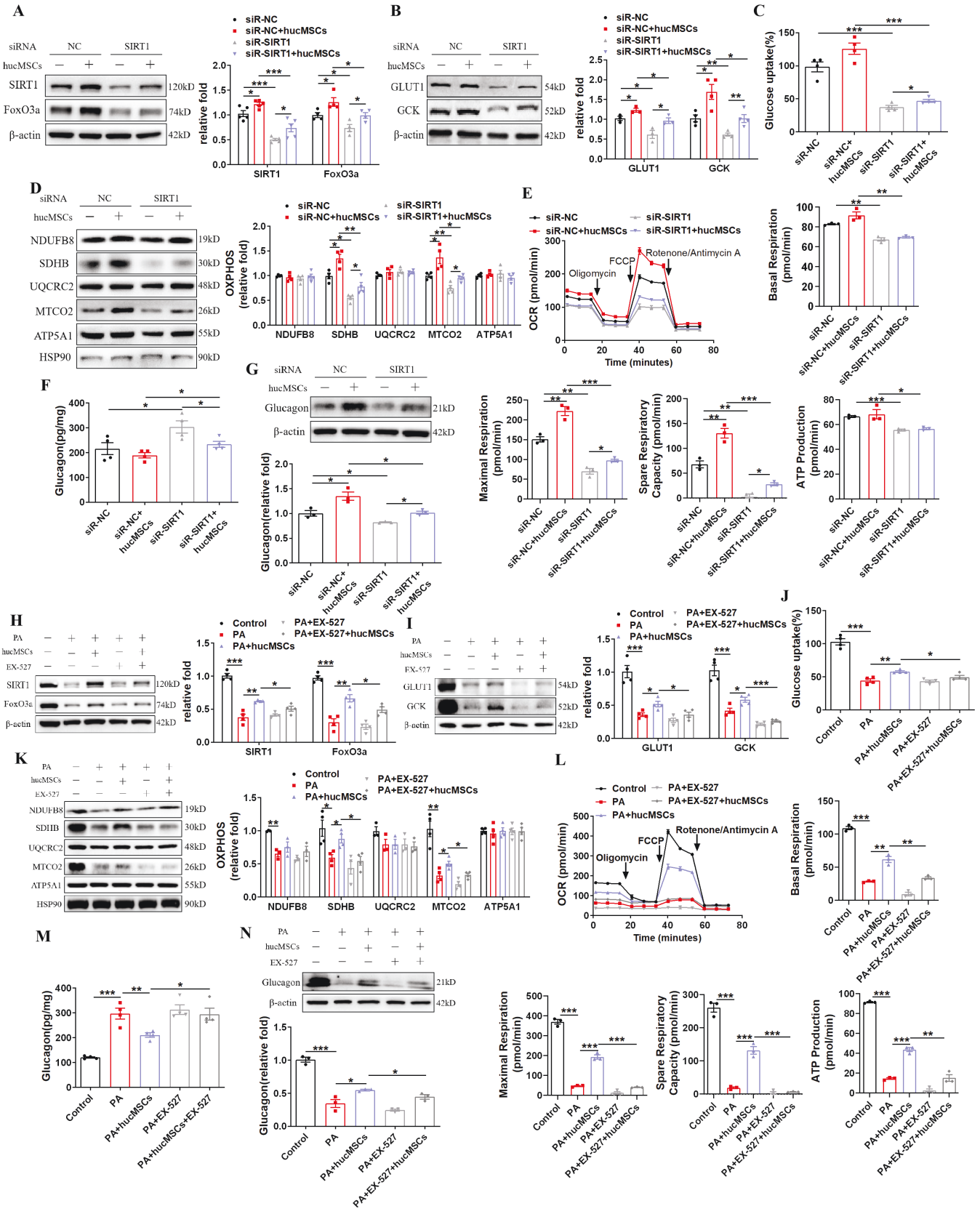


Figure 5. hucMSCs mitigate mitochondrial dysfunction and glucagon hypersecretion by activating SIRT1. A. αTC1-6 cells were transfected with SIRT1 siRNA. Western blotting showed that in SIRT1 knockdown cells, hucMSC treatment weakly increased SIRT1 and FoxO3a expression. Quantification of bands was performed using ImageJ software. B, C. The effects of hucMSCs on the upregulation of GLUT1, GCK, and glucose uptake were partially blocked when SIRT1 was knocked down. D. Western blotting indicated that the effects of hucMSCs on the elevation of mitochondrial complex SDHB and MTCO2 were partially blocked when SIRT1 was knocked down. E. Seahorse analysis showed that the role of hucMSCs in enhancing mitochondrial OXPHOS was partially weakened in SIRT1 knockdown cells, as evidenced by basal respiration, maximal respiration, spare respiratory

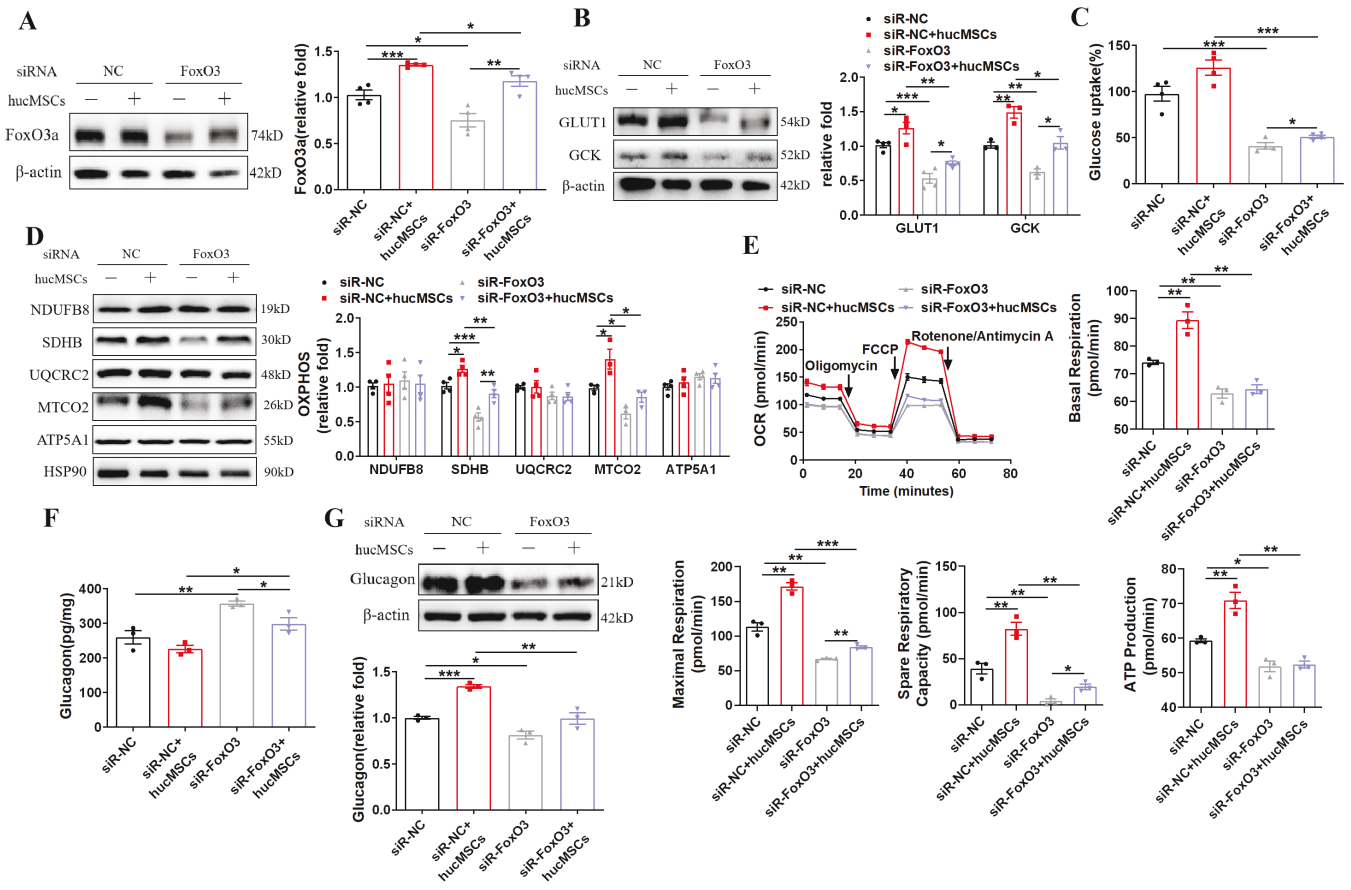


Figure 6. FoxO3a is involved in the improvement of mitochondrial dysfunction and glucagon hypersecretion by hucMSCs. A. αTC1-6 cells were transfected with FoxO3 siRNA. Western blotting showed that when FoxO3 was knocked down, hucMSCs did not efficiently promote FoxO3a expression. Quantification of bands was performed using ImageJ software. B, C. The effects of hucMSCs on the upregulation of GLUT1, GCK, and glucose uptake were partially blocked when FoxO3 was knocked down. D. Western blotting indicated that the effects of hucMSCs on enhancing mitochondrial complex SDHB and MTCO2 expression were partially blocked when FoxO3 was knocked down. E. Seahorse analysis showed that the role of hucMSCs in enhancing mitochondrial OXPHOS was partially blocked in FoxO3 knockdown cells, as evidenced by basal respiration, maximal respiration, spare respiratory capacity, and ATP production. F. ELISA showed that glucagon secretion was not effectively inhibited by hucMSCs when FoxO3 was knocked down. G. Western blotting showed that intracellular glucagon protein levels were not efficiently elevated by hucMSCs. All experiments were repeated 3-4 times, and the data are presented as the mean ± SEM (*$P < .05$; **$P < .01$; ***$P < .001$).

study, we found that both in HFD and STZ-induced T2DM mice and db/db mice, hucMSC infusion could rescue islet structure and decrease α- to β-cell ratio, which contributes to amelioration of hyperglycemia and hyperglucagonemia. Consistently, in αTC1-6 cells, hucMSC intervention decreased glucagon levels in the supernatant, whereas upregulated intracellular glucagon protein levels, which indicates that the glucagon secretion process was inhibited by hucMSCs in vitro. It is worth noting that plasma insulin levels (Figure 1D vs Figure 2C) changed in opposite directions between the 2 T2DM mice models. In the HFD- and STZ-induced T2DM model, a large number of β cells were destroyed and underwent apoptosis or dedifferentiation, resulting in decreased insulin secretion and islet dysfunction. MSC intervention could restore islet function, promote β cell proliferation, and reduce β cell dedifferentiation, thereby promoting insulin secretion.[28-30] However, in db/db mice, severe insulin resistance led to elevated blood insulin levels. It has been reported that MSC intervention ameliorated insulin resistance[26,31] and reduced plasma insulin levels.[32-34]

Glucagon secretion by α cells is mainly under the control of glucose and insulin.[2] Upon uptake via GLUTs, glucose is phosphorylated by GCK and participates in mitochondrial oxidation, which results in the accumulation of intracellular ATP and closure of K_{ATP}-channels and voltage-gated calcium channels (VGGCs), thereby inhibiting glucagon release.[35-39] Therefore, the effects of mitochondrial function and ATP content in regulating glucagon secretion should not

capacity, and ATP production. F. ELISA showed that glucagon secretion was not effectively inhibited by hucMSCs when SIRT1 was knocked down. G. Western blotting showed that intracellular glucagon protein levels were not effectively elevated by hucMSCs. H. Upregulation of SIRT1 and FoxO3a was weakened in αTC1-6 cells after hucMSC therapy when simultaneously treated with SIRT1 inhibitor EX-527. I, J. hucMSCs weakly elevated GLUT1 and GCK expression and glucose uptake when co-treated with EX-527. K, L. Improvement of mitochondrial OXPHOS and ATP production was also weakened in hucMSCs and EX-527 co-treated group compared with hucMSCs-treated group. M. ELISA showed that the role of hucMSCs in decreasing glucagon secretion was blocked when co-treated with EX-527. N. Western blotting indicated that the effects of hucMSCs on elevation of intracellular glucagon levels were partially blocked when co-treated with EX-527. All of the experiments were repeated 3-4 times, and the data are presented as the mean ± SEM (*$P < .05$; **$P < .01$; ***$P < .001$).

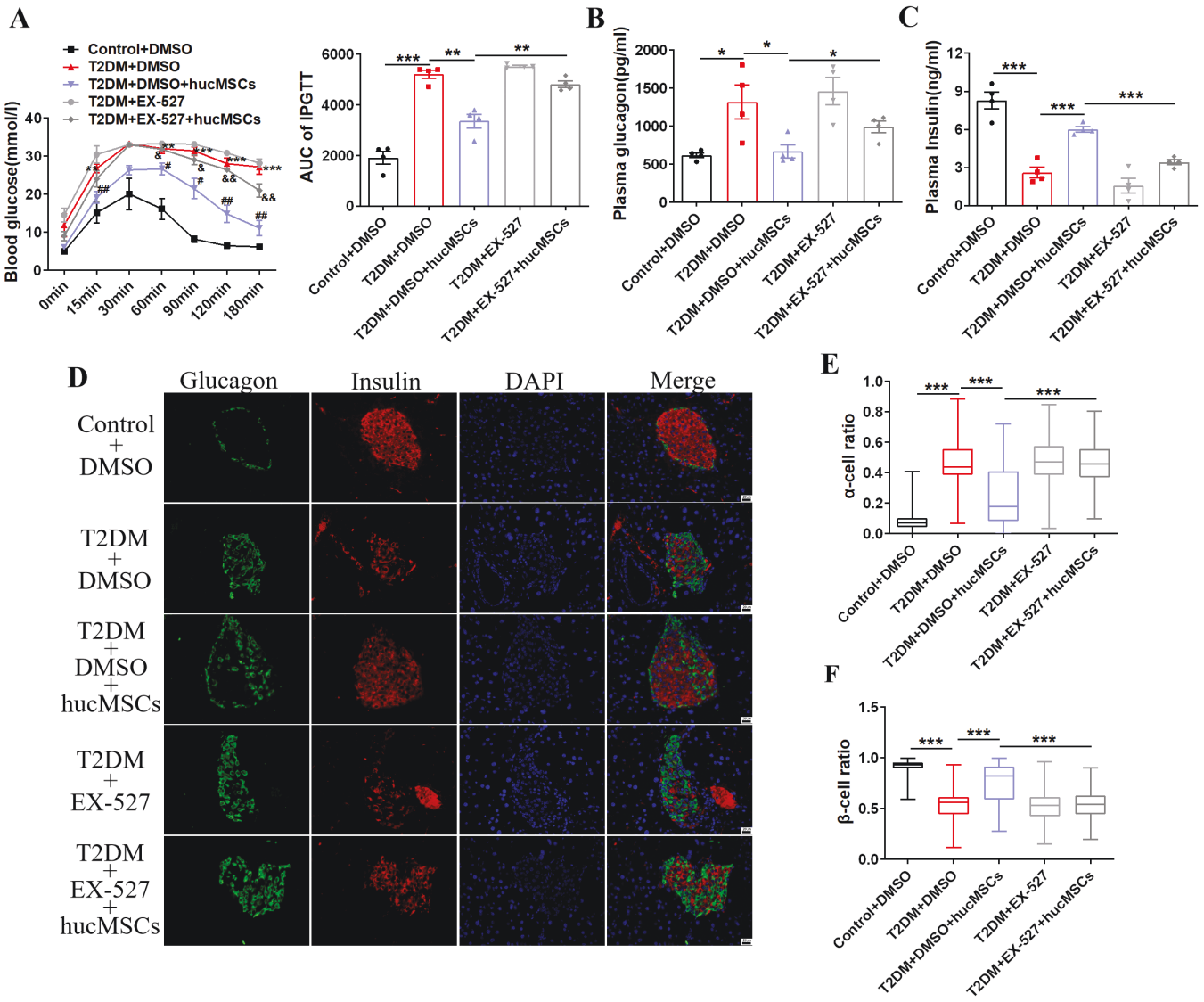


Figure 7. hucMSCs improve hyperglycemia and hyperglucagonemia in vivo via SIRT1. A. HFD- and STZ-induced T2DM mice were simultaneously injected with hucMSCs and EX-527. IPGTT showed that the effects of hucMSCs on improving glucose tolerance and insulin tolerance were obviously weakened. The AUC supported the above findings (n = 4 mice per group. *T2DM + DMSO group vs. control + DMSO group; #T2DM + DMSO + hucMSCs group vs T2DM + DMSO group; &T2DM + EX-527 + hucMSCs group vs T2DM + DMSO + hucMSCs group). B. Circulatory glucagon levels in the hucMSC and EX-527-treated group were higher than sthose in the hucMSC-treated group. C. Insulin levels in hucMSC- and EX-527-treated group were lower than the hucMSC-treated group. D. Immunofluorescence staining showed that when co-treated with EX-527, hucMSCs did not rescue islet structure (scale bar = 20 μm). E, F. hucMSCs could not effectively restore α- and β-cell imbalance when co-treated with EX-527, as analyzed by Image-Pro Plus software (n = 20-50 islets per group). The results are expressed as the mean ± SEM (*P < .05; **P < .01; ***P < .001).

be underestimated. Studies have shown that individuals with T2DM incur functional and morphological alterations in the mitochondria of different tissues and organs.[40-43] Segerstolpe et al sequenced the transcriptomes of thousands of human islet cells from healthy and type 2 diabetic donors and found that genes responsible for mitochondrial metabolism are significantly downregulated in most cell types in T2D individuals.[3] Pancreatic α cells extracted from T2DM mice showed a lower density of mitochondria, a less expressed matrix, and a lower number of cristae. These deformities in mitochondrial ultrastructure imply decreased efficiency in mitochondrial ATP production, which accounts for dysregulated glucagon secretion in T2DM.[4] In addition, the regulation of mitochondrial function is also involved in the effects of MSCs on target organs.[17-19] Rackham et al recently found that human adipose MSCs could transfer mitochondria to β-cells and promote insulin secretory function.[20] However, the role of MSCs in α-cell mitochondrial function remains unclear. In our study, glucose uptake and mitochondrial OXPHOS were impaired in α cells after PA exposure, which resulted in reduced ATP production. However, hucMSC treatment promoted glucose uptake and activation alleviated mitochondrial dysfunction, and enhanced intracellular ATP production. This is the first study that illustrates the effects of MSCs on α-cell mitochondrial function; however, the findings require confirmation using in vivo experiments. In fact, we also observed the effects of hucMSCs on mitochondrial function in β cells. Our results showed that hucMSCs also enhanced mitochondrial

OXPHOS in MIN6 cells (Supplementary Figure S6), which suggests that hucMSCs do not specifically improve the mitochondrial function of α cells, but could also improve the function of other cells. It may also involve the interaction among all kinds of cells in the islet, which is what we are deeply exploring currently.

Sirtuins participate in regulating metabolism through their regulation of inflammation, oxidative stress, and mitochondrial function via multiple mechanisms, which contribute to the improvement of T2DM.[5] Sirtuins include 7 family members, among which SIRT1 plays an important role in mitochondrial biogenesis and oxidative metabolism.[6,7] It has been reported that insulin resistance and metabolic syndrome are associated with low SIRT1 expression,[8] and a mutation in the SIRT1 gene contributes to T1DM.[9] Loss of SIRT1 in mouse β-cells impairs glucose disposal due to insufficient insulin secretion. SIRT1 regulates the expression of specific mitochondria-related genes that control metabolic coupling.[13] Bordone et al found that SIRT1 knockout mice display constitutively high UCP2 expression, which is associated with a failure of cells to increase ATP levels and insulin secretion after glucose stimulation.[12] Additionally, Zhang et al reported that SIRT1 activation attenuated α cell hyperplasia, hyperglucagonemia, and hyperglycemia in STZ-induced diabetic mice.[44] However, studies illustrating the role of SIRT1 in α-cell mitochondrial function are limited. In this study, we first investigated the effects of SIRT1 on glucose uptake and activation, mitochondrial function, and glucagon secretion in α cells. Moreover, our previous study has suggested that MSC-CM improved mitochondrial function in the liver via SIRT1, which is beneficial for diabetes-associated non-alcoholic fatty liver disease.[45] Meanwhile, other studies showed the effects of MSCs or extracellular vesicles on target tissues via SIRT1.[19,46] Therefore, we explored whether hucMSCs regulate SIRT1 signaling in α cells. In αTC1-6 cells, the upregulation of SIRT1 signaling and mitochondrial OXPHOS were partially abolished after hucMSC intervention when simultaneously treated with SIRT1 inhibitor EX-527 or when SIRT1 was knocked down, which resulted in restoration of glucagon secretion. Consistently, EX-527 infusion also weakened the effects of hucMSCs on hyperglycemia and hyperglucagonemia in T2DM mice, which indicates that hucMSCs regulate glucagon secretion via SIRT1 signaling.

Conclusions

In summary, our observations indicate that by activating SIRT1/FoxO3a signaling, hucMSCs can increase glucose uptake and activation, enhance mitochondrial OXPHOS, and promote ATP production, which eventually reduces glucagon secretion and mitigates hyperglucagonemia in T2DM. To the best of our knowledge, this is the first study that shows that hucMSCs alleviate mitochondrial dysfunction and glucagon hypersecretion of α cells in T2DM, which provides novel evidence demonstrating the potential for hucMSCs in treating T2DM.

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Author contributions

Jia Song: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing—original draft. Lingshu Wang: Conceptualization (Supporting), Funding acquisition (Supporting). Liming Wang: Data curation (Supporting), Software (Supporting). Xinghong Guo, Qin He, Chuan Wang, and Fuqiang Liu: Investigation (Supporting), Methodology (Supporting). Chen Cui: Data curation (Supporting), Investigation (Supporting). Huiqing Hu: Formal analysis (Supporting), Project administration (Supporting). Nan Zang and Mengmeng Yang: Data curation (Supporting), Project administration (Supporting). Fei Yan: Funding acquisition (Supporting), Investigation (Supporting). Kai Liang: Formal analysis (Supporting), Software (Supporting). Yujing Sun: Methodology (Supporting), Validation (Supporting). Zheng Sun: Methodology (Supporting), Supervision (Supporting). Hong Lai: Funding acquisition (Supporting), Supervision (Supporting). Xinguo Hou: Conceptualization, Supervision, Writing—review & editing. Li Chen: Conceptualization, Funding acquisition, Supervision, Writing—review & editing. All authors read and approved the final version of the manuscript

Conflicts of interest

All authors declare that they have no competing interests.

Consent for publication

Not applicable.

Data availability

All data generated or analyzed during this study are included in this published article.

Supplementary material

Supplementary material is available at *Stem Cells Translational Medicine* online.

References

1. Baetens D, Malaisse-Lagae F, Perrelet A, Orci L. Endocrine pancreas: three-dimensional reconstruction shows two types of islets of Langerhans. *Science*. 1979;206(4424):1323-1325. https://doi.org/10.1126/science.390711
2. Dunning BE, Gerich JE. The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. *Endocr Rev*. 2007;28(3):253-283. https://doi.org/10.1210/er.2006-0026
3. Segerstolpe A, Palasantza A, Eliasson P, et al. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. *Cell Metab*. 2016;24(4):593-607. https://doi.org/10.1016/j.cmet.2016.08.020
4. Grubelnik V, Markovič R, Lipovšek S, et al. Modelling of dysregulated glucagon secretion in type 2 diabetes by considering

mitochondrial alterations in pancreatic α-cells. *R Soc Open Sci*. 2020;7(1):191171. https://doi.org/10.1098/rsos.191171
5. Kitada M, Ogura Y, Monno I, Koya D. Sirtuins and Type 2 diabetes: role in inflammation, oxidative stress, and mitochondrial function. *Front Endocrinol (Lausanne)*. 2019;10:187. https://doi.org/10.3389/fendo.2019.00187
6. Canto C, Gerhart-Hines Z, Feige JN, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature*. 2009;458(7241):1056-1060. https://doi.org/10.1038/nature07813
7. Price NL, Gomes AP, Ling AJ, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab*. 2012;15(5):675-690. https://doi.org/10.1016/j.cmet.2012.04.003
8. de Kreutzenberg SV, Ceolotto G, Papparella I, et al. Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms. *Diabetes*. 2010;59(4):1006-1015. https://doi.org/10.2337/db09-1187
9. Biason-Lauber A, Böni-Schnetzler M, Hubbard BP, et al. Identification of a SIRT1 mutation in a family with type 1 diabetes. *Cell Metab*. 2013;17(3):448-455. https://doi.org/10.1016/j.cmet.2013.02.001
10. Hsu YH, Chen YC, Chen YW, et al. Far-infrared radiation prevents decline in β-cell mass and function in diabetic mice via the mitochondria-mediated Sirtuin1 pathway. *Metabolism*. 2020;104:154143. https://doi.org/10.1016/j.metabol.2020.154143
11. Moynihan KA, Grimm AA, Plueger MM, et al. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab*. 2005;2(2):105-117. https://doi.org/10.1016/j.cmet.2005.07.001
12. Bordone L, Motta MC, Picard F, et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol*. 2006;4(2):e31. https://doi.org/10.1371/journal.pbio.0040031
13. Luu L, Dai FF, Prentice KJ, et al. The loss of Sirt1 in mouse pancreatic beta cells impairs insulin secretion by disrupting glucose sensing. *Diabetologia*. 2013;56(9):2010-2020. https://doi.org/10.1007/s00125-013-2946-5
14. Xv J, Ming Q, Wang X, et al. Mesenchymal stem cells moderate immune response of type 1 diabetes. *Cell Tissue Res*. 2017;368(2):239-248. https://doi.org/10.1007/s00441-016-2499-2
15. Chandravanshi B, Bhonde RR. Human umbilical cord-derived stem cells: isolation, characterization, differentiation, and application in treating diabetes. *Crit Rev Biomed Eng*. 2018;46(5):399-412. https://doi.org/10.1615/CritRevBiomedEng.2018027377
16. Xie Z, Hao H, Tong C, et al. Human umbilical cord derived mesenchymal stem cells elicit macrophages into an anti inflammatory phenotype to alleviate insulin resistance in type 2 diabetic rats. *Stem Cells*. 2016;34(3):627-639. https://doi.org/10.1002/stem.2238
17. Liu X, Li X, Zhu W, et al. Exosomes from mesenchymal stem cells overexpressing MIF enhance myocardial repair. *J Cell Physiol*. 2020;235(11):8010-8022. https://doi.org/10.1002/jcp.29456
18. Hughey CC, James FD, Ma L, et al. Diminishing impairments in glucose uptake, mitochondrial content, and ADP-stimulated oxygen flux by mesenchymal stem cell therapy in the infarcted heart. *Am J Physiol Cell Physiol*. 2014;306(1):CC19-CC27. https://doi.org/10.1152/ajpcell.00156.2013
19. Yuan Y, Shi M, Li L, et al. Mesenchymal stem cell-conditioned media ameliorate diabetic endothelial dysfunction by improving mitochondrial bioenergetics via the Sirt1/AMPK/PGC-1α pathway. *Clin Sci (Lond)*. 2016;130(23):2181-2198. https://doi.org/10.1042/CS20160235
20. Rackham CL, Hubber EL, Czajka A, et al. Optimizing beta cell function through mesenchymal stromal cell-mediated mitochondria transfer. *Stem Cells*. 2020;38(4):574-584. https://doi.org/10.1002/stem.3134
21. Song J, He Q, Guo X, et al. Mesenchymal stem cell-conditioned medium alleviates high fat-induced hyperglucagonemia via miR-181a-5p and its target PTEN/AKT signaling. *Mol Cell Endocrinol*. 2021;537:111445. https://doi.org/10.1016/j.mce.2021.111445
22. Fujita Y, Kozawa J, Iwahashi H, et al. Human pancreatic alpha- to beta-cell area ratio increases after type 2 diabetes onset. *J Diabetes Investig*. 2018;9(6):1270-1282. https://doi.org/10.1111/jdi.12841
23. Lee M, Kim M, Park JS, et al. Higher glucagon-to-insulin ratio is associated with elevated glycated hemoglobin levels in type 2 diabetes patients. *Korean J Intern Med*. 2019;34(5):1068-1077. https://doi.org/10.3904/kjim.2016.233
24. Sharma A, Varghese RT, Shah M, et al. Impaired insulin action is associated with increased glucagon concentrations in nondiabetic humans. *J Clin Endocrinol Metab*. 2018;103(1):314-319. https://doi.org/10.1210/jc.2017-01197
25. Shi H, Xu X, Zhang B, et al. 3,3′-Diindolylmethane stimulates exosomal Wnt11 autocrine signaling in human umbilical cord mesenchymal stem cells to enhance wound healing. *Theranostics*. 2017;7(6):1674-1688. https://doi.org/10.7150/thno.18082
26. Si Y, Zhao Y, Hao H, et al. Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: identification of a novel role in improving insulin sensitivity. *Diabetes*. 2012;61(6):1616-1625. https://doi.org/10.2337/db11-1141
27. Wang L, Qing L, Liu H, et al. Mesenchymal stromal cells ameliorate oxidative stress-induced islet endothelium apoptosis and functional impairment via Wnt4-beta-catenin signaling. *Stem Cell Res Ther*. 2017;8(1):188. https://doi.org/10.1186/s13287-017-0640-0
28. Wang L, Liu TL, Liang R, et al. Mesenchymal stem cells ameliorate beta cell dysfunction of human type 2 diabetic islets by reversing beta cell dedifferentiation. *EBioMedicine*. 2020;51:102615. https://doi.org/10.1016/j.ebiom.2019.102615
29. Yin Y, Hao H, Cheng Y, et al. Human umbilical cord-derived mesenchymal stem cells direct macrophage polarization to alleviate pancreatic islets dysfunction in type 2 diabetic mice. *Cell Death Dis*. 2018;9(7):760. https://doi.org/10.1038/s41419-018-0801-9
30. Gao LR, Zhang NK, Zhang Y, et al. Overexpression of apelin in Wharton' jelly mesenchymal stem cell reverses insulin resistance and promotes pancreatic beta cell proliferation in type 2 diabetic rats. *Stem Cell Res Ther*. 2018;9(1):339.doi. https://doi.org/10.1186/s13287-018-1084-x
31. Chen G, Fan XY, Zheng XP, et al. Human umbilical cord-derived mesenchymal stem cells ameliorate insulin resistance via PTEN-mediated crosstalk between the PI3K/Akt and Erk/MAPKs signaling pathways in the skeletal muscles of db/db mice. *Stem Cell Res Ther*. 2020;11(1):401. https://doi.org/10.1186/s13287-020-01865-7
32. Feng JX, Lu C, Dai Q, et al. SIRT3 Facilitates amniotic fluid stem cells to repair diabetic nephropathy through protecting mitochondrial homeostasis by modulation of mitophagy. *Cell Physiol Biochem*. 2018;46(4):1508-1524. https://doi.org/10.1159/000489194
33. Li M, Guo KQ, Taketani S, Adachi Y, Ikehara S. Stem cell replacement improves expression of SMP30 in db/db Mice. *Int J Mol Sci*. 2015;16(12):29971-29979. https://doi.org/10.3390/ijms161226217
34. Li M, Vanella L, Zhang YM, et al. Stem cell transplantation increases antioxidant effects in diabetic mice. *Int J Biol Sci*. 2012;8(10):1335-1344. https://doi.org/10.7150/ijbs.4654
35. Rorsman P, Salehi SA, Abdulkader F, Braun M, MacDonald PE. K(ATP)-channels and glucose-regulated glucagon secretion. *Trends Endocrinol Metab*. 2008;19(8):277-284. https://doi.org/10.1016/j.tem.2008.07.003
36. Leung YM, Ahmed I, Sheu L, et al. Insulin regulates islet α-cell function by reducing K_{ATP} channel sensitivity to adenosine 5-triphosphate inhibition. *Endocrinology*. 2006;147(5):2155-2162. https://doi.org/10.1210/en.2005-1249
37. Khan FA, Goforth PB, Zhang M, Satin LS. Insulin activates ATP-sensitive K(+) channels in pancreatic α-cells through a phosphatidylinositol 3-kinase-dependent pathway. *Diabetes*. 2001;50(10):2192-2198. https://doi.org/10.2337/diabetes.50.10.2192
38. Berger C, Zdzieblo D. Glucose transporters in pancreatic islets. *Pflugers Arch*. 2020;472(9):1249-1272. https://doi.org/10.1007/s00424-020-02383-4

39. Basco D, Zhang Q, Salehi A, et al. α-cell glucokinase suppresses glucose-regulated glucagon secretion. *Nat Commun*. 2018;9(1):546. https://doi.org/10.1038/s41467-018-03034-0
40. Anello M, Lupi R, Spampinato D, et al. Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia*. 2005;48(2):282-289. https://doi.org/10.1007/s00125-004-1627-9
41. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*. 2002;51(10):2944-2950. https://doi.org/10.2337/diabetes.51.10.2944
42. Heinonen S, Buzkova J, Muniandy M, et al. Impaired mitochondrial biogenesis in adipose tissue in acquired obesity. *Diabetes*. 2015;64(9):3135-3145. https://doi.org/10.2337/db14-1937
43. Pisano A, Cerbelli B, Perli E, et al. Impaired mitochondrial biogenesis is a common feature to myocardial hypertrophy and end-stage ischemic heart failure. *Cardiovasc Pathol*. 2016;25(2):103-112. https://doi.org/10.1016/j.carpath.2015.09.009
44. Zhang Y, Thai K, Jin T, Woo M, Gilbert RE. SIRT1 activation attenuates alpha cell hyperplasia, hyperglucagonaemia and hyperglycaemia in STZ-diabetic mice. *Sci Rep*. 2018;8(1):13972. https://doi.org/10.1038/s41598-018-32351-z
45. Yang M, Cui Y, Song J, et al. Mesenchymal stem cell-conditioned medium improved mitochondrial function and alleviated inflammation and apoptosis in non-alcoholic fatty liver disease by regulating SIRT1. *Biochem Biophys Res Commun*. 2021;546:74-82. https://doi.org/10.1016/j.bbrc.2021.01.098
46. Feng R, Ullah M, Chen K, et al. Stem cell-derived extracellular vesicles mitigate ageing-associated arterial stiffness and hypertension. *J Extracell Vesicles*. 2020;9(1):1783869. https://doi.org/10.1080/20013078.2020.1783869