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Original Article

Exendin-4 protects β-cells against interleukin-1β-induced apoptosis via upregulating GMRP-1

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Abstract: Objective: To elucidate whether Exendin-4 (Ex-4) protects β cells against interleukin-1β (IL-1β)-induced apoptosis by regulating glucose metabolism-related protein-1 (GMRP-1) and suppressing the Jun N-terminal kinase (JNK) signaling pathway. Methods: Pancreatic β cells were treated with Ex-4 either in the presence or absence of IL-1β. Alterations in the expression of GMRP-1 and JNK signaling pathway-related proteins were examined by quantitative PCR, western blotting, and immunofluorescence. The anti-apoptotic efficacy of Ex-4 against IL-1β-induced apoptosis was assessed using flow cytometry. *In vivo*, a non-obese diabetic (NOD) mouse model was established, and lentivirus-mediated transfection was employed to knock down GMRP-1 expression. Results: Ex-4 inhibited IL-1β-induced activation of the JNK pathway and subsequent β-cell apoptosis. This inhibitory effect was associated with the upregulation of the level of GMRP-1. Mechanistically, Ex-4 upregulated GMRP-1 expression in a time- and dose-dependent manner and reversed the IL-1β-induced suppression of GMRP-1. Both *in vivo* and *in vitro* experiments confirmed that RNA interference-mediated inhibition of GMRP-1 abolished the protective effect of Ex-4 on JNK pathway activation and apoptosis. Conclusion: GMRP-1 serves as an essential mediator of Ex-4's cytoprotective effects. Ex-4 safeguards β cells against IL-1β-induced apoptosis primarily by upregulating GMRP-1, which in turn suppresses the pro-apoptotic JNK pathway. These results provide new insights into the molecular mechanisms underlying diabetes pathogenesis and identify GMRP-1 as a potential therapeutic target for the treatment of diabetes.

Keywords: GMRP-1, Exendin-4, β cells, diabetes

Introduction

With the improvement of living standards and the influence of environmental factors, the incidence of new-onset type 1 diabetes mellitus (T1DM) has markedly increased in recent years [1], seriously diminishing patients' quality of life and imposing a substantial burden on families and society. T1DM is a clinical syndrome characterized by impaired glucose metabolism, resulting from the interplay of genetic and environmental factors. Apoptosis of pancreatic islet β cells is a major contributor to disease onset [2, 3]. Interleukin-1β (IL-1β) is a key mediator in pancreatic β cell destruction and inflammatory responses; it promotes β cell apoptosis, inhibits insulin secretion, and triggers local inflammation [4]. Therefore, protecting and slowing islet β-cell apoptosis is crucial for the prevention and treatment of T1DM [5-7].

Exendin-4 (Ex-4) is a synthetic analogue of glucagon-like peptide-1 (GLP-1). It demonstrates a high affinity for the pancreatic GLP-1 receptor, functioning as a GLP-1 receptor agonist [8]. By binding to the GLP-1 receptor, Ex-4 regulates glucose homeostasis and is widely used in the management of T2DM, where it effectively lowers blood glucose levels. Studies have shown that Ex-4 reduces inflammatory infiltration in pancreatic islets of T1DM animal models and exerts protective effects on islet β cells [9]. Additional studies indicate that Ex-4 modulates the immune microenvironment of islet cells [10, 11], alters the expression of C-X-C motif chemokine ligand 10 (CXCL10) and its receptor [12], and preserves islet β cell function in T1DM

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models. Investigations into the kelch-like ECH-associated protein 1-NF-E2-related factor 2 (Keap1-Nrf2)/antioxidant response element (ARE) signaling pathway have demonstrated that the expression of Keap-1, Nrf2, and ARE proteins correlates with islet β-cell proliferation in non-obese diabetic (NOD) mice treated with Ex-4 [13, 14]. In addition, antioxidant stress is another mechanism underlying the cytoprotective effects of Ex-4. Therefore, these findings suggest that the protective action of Ex-4 on pancreatic β cells in T1DM likely involves multiple, interconnected molecular mechanisms.

Glucose metabolism related protein-1 (GMRP-1) belongs to the Bric à brac, Tramtrack, Broad complex/Poxvirus and Zinc finger (BTB/POZ) protein family. Through its BTB/POZ domain, GMRP-1 forms dimers, interacts with nuclear proteins, and regulates cellular activities. It plays an important role in controlling development, differentiation, and chromosomal reorganization, and is implicated in pathogenesis of several major diseases [15]. Wang et al. reported that GMRP-1 is predominantly expressed in the pancreatic islets of both human and mouse pancreatic tissues, where it promotes islet β cell proliferation and inhibits apoptosis by enhancing the phosphorylation of Akt and BAD [16]. It has been further proposed that a "glucose/insulin-GMRP1-Akt signal pathway" operates within islet β cells. Mackenzie et al. also demonstrated that GMRP-1 regulates islet β cell proliferation and function through the Akt-mediated pathway [17]. Recently, in vivo studies have suggested that GMRP-1 may promote the proliferation of islet β cells and inhibit their apoptosis by activating c-Myc transcription, although this mechanism remains to be fully validated.

Although the protective effects of Ex-4 on β cells have been well documented [18], its precise intracellular mechanisms, particularly in the context of countering cytokine-induced apoptosis, remain incompletely elucidated. Conversely, GMRP-1 has been established as a critical regulator of β-cell survival and proliferation, primarily through the potent Akt signaling pathway [13, 14]. Notably, Ex-4 is also known to influence multiple signaling cascades, including those converging on Akt activation [19]. Based on these observations, we hypothesized that Ex-4 exerts its protective effect on β cells by upregulating GMRP-1, which in turn suppresses the pro-apoptotic Jun N-terminal kinase (JNK) signaling pathway activated by IL-1β. Therefore, this study aimed to verify whether Ex-4 inhibits IL-1β-induced JNK activation and apoptosis, and to determine whether it regulates GMRP-1 expression. Most critically, we sought to establish whether GMRP-1 is functionally indispensable for E-4-mediated protection both in vitro and in a non-obese diabetic (NOD) mouse model of T1DM. Elucidating the role and underlying mechanism of GMRP-1 in Ex-4-mediated β cell protection will provide experimental evidence for its application in T1DM, and aid in the development of novel anti-diabetic drugs.

Methods

Cell culture and transfection

RPMI 1640 medium was prepared with 10% fetal bovine serum, 1% penicillin and streptomycin, and 50 μmol dash-mercaptoethanol. Cells were cultured at 37°C in a humidified incubator with 5% CO_2 and 70%-80% relative humidity. For cell resuscitation, a cryovial containing 1 mL frozen cell suspension was rapidly thawed in a 37°C water bath. The contents were transferred into a centrifuge tube containing 4 mL of complete medium and gently mixed. At 800 RPM/min, the supernatant cells were centrifuged for 5 min. The supernatant was discarded, and the pellet was resuspended in 1-2 ml of medium. The cell suspension was transferred into a T25 culture flask and supplemented with medium to a total volume of 6 ml. When cell density reached 80%-90%, subculture was performed using standard trypsinization. Cell cryopreservation was carried out when cells were in optimal growth conditions. Transfections were carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Primary culture of rat Islet β cells

After intraperitoneal anesthesia with sodium pentobarbital, the pancreas was aseptically removed and placed in ice-cold Hanks' solution at 4°C. The pancreas was minced into ~1 mm^3 tissue fragments with fine ophthalmic scissors and digested with 0.5 g/L collagenase at 37°C for 15 min. The digested tissue was aspirated and digestion was terminated with a large volume of 4°C Hanks solutions. The remaining undigested tissue was further incubated in a small volume of 37°C Hanks solutions and gen-

tly triturated with a wide-bore pipette until most of the tissue was digested into fine granules. Digestion was again stopped using 4°C Hanks solutions. The two digestion fractions were mixed and washed three times with Hanks' solution at low speed. The resulting tissue suspension was resuspended in complete RPMI 1640 containing 100 U/ml penicillin, 100 mg/L-streptomycin, 11.1 mmol/L glucose, 10 mmol/L Hepes and 7% Fetal calf serum. The mixture was filtered through 108 μm pore nylon mesh to remove undigested and connective tissue residue. The isolated islet cells and clusters were cultured in flasks containing the above medium at 37°C in 5% CO_2. After 24 h, unattached cells were collected by gentle aspiration and low-speed centrifugation. The collected cells were counted under a microscope and seeded into 24-well cell culture plates at an appropriate density. Cultures were maintained for 48 h before subsequent experiments.

MTT assay for cell proliferation

Cells in the logarithmic growth phase were harvested and adjusted to a concentration of 10^5 cells/ml. A total of 100 μL of the cell suspension was seeded into each well of a 96-well plate and incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2. Before the experiment, cells were serum-starved in DMEM medium containing 2% fetal bovine serum for 2 h. Cells were then processed according to the conditions of each experimental group, with five replicate wells per dose. After a 24-hour incubation period, 20 μL of MTT solution (5 g/L) was added to each well, followed by a 4 h incubation at 37°C. The supernatant was then carefully removed, and 150 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Subsequently, the optical density (OD) at 490 nm was measured using a microplate reader. Cell inhibition rate (%) = (average value of A490 in control wells - average value of A490 in each treatment well)/ mean value of A490 in control wells × 100%. The experiment was performed in triplicate to ensure reproducibility.

Western blot

Following treatment, cells from each group were lysed using RIPA buffer. Equal amounts of protein (20 μg) were separated on 10% SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked with 5% non-fat milk containing 0.1% Tween-20 for 1 h at room temperature and then incubated overnight at 4°C with the following primary antibodies: p-JNK (1:1000, #4668, Cell Signaling Technology), p-c-Jun (1:1000, #3270, Cell Signaling Technology), GMRP-1 (1:1000, sc-377183, Santa Cruz Biotechnology), GAPDH (1:2500, ab9485, Abcam). After washing with TBST, membranes were incubated with diluted secondary antibodies for 1 h at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents, and band intensities were quantified using densitometric analysis.

Quantitative PCR

Total RNA was extracted from each cell group using the Aurum Total RNA Mini Kit (Bio-Rad) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from total RNA using a reverse transcription kit following established protocols. Quantitative PCR was conducted on a Bio-Rad MyiQ real-time PCR system using iQ SYBR Green Supermix (Bio-Rad). Each 20 μL reaction comprised 100 nmol/L primers and 1.5 μL cDNA template. The thermal cycling conditions included initial denaturation, followed by amplification with an annealing temperature of 59°C. The C-Jun primer sequences: forward 5'-AGTCTCAGGAGCGGATCAAG-3', reverse 5'-CTCTGTCGCAACCAGTCAAG-3'. Relative gene expression levels were calculated using the 2^-ΔΔCt method, with GAPDH serving as the internal control.

Detection of apoptosis by flow cytometry

Cells in the logarithmic growth phase were harvested, adjusted to 2.5 × 10^5 cells/ml, and inoculate into 6-well plates at 2 ml per well. After overnight incubation (approximately 16 h) at 37°C in a humidified 5% CO_2 atmosphere, cells were treated according to the experimental grouping and further cultured being incubated for 24 h under the same conditions. Following treatment, cells were collected by centrifugation and resuspended in 150 μL of AnnexinV-FITC binding buffer. Annexin V-FITC (3 μL) and propidium iodide (PI, 2 μL) were added according to the manufacturer's protocol. The cells were incubated at room temperature for 15 min in the dark, followed by addition of 500

μL PBS. The suspension was gently passed through a 300-mesh filter. Flow cytometric analysis was performed within 30 min using a BD flow cytometer, and apoptotic rates were quantified with CellQuest software.

Immunofluorescence (IF) staining

After treatment, β cells grown on glass coverslips were fixed with 4% paraformaldehyde for 15 min at room temperature and then blocked with 5% normal goat serum for 1 h. Cells were subsequently incubated overnight at 4°C with primary antibodies specific to phosphorylated JNK, or GMRP-1. Following three washes with PBS, the cells were incubated with an Alexa Fluor 594-conjugated goat anti-rabbit IgG secondary antibody (ab150080, Abcam) for 1 h at room temperature in the dark. Nuclei were subsequently counterstained with DAPI (Life Technologies), and coverslips were mounted using an anti-fade mounting medium. Image acquisition was performed on a confocal laser scanning microscope (Olympus FV1000).

Establishment of T1DM in NOD mice

Sixty female NOD mice, aged 4 weeks, were obtained from the Institute of Experimental Animals, Chinese Academy of Medical Sciences. All animals were housed under a specific pathogen-free environment with free access to sterilized food and water. T1DM was induced by intraperitoneal injection of streptozotocin (STZ, 100 mg/kg). Beginning at 5 weeks of age, body weight and non-fasting blood glucose levels were monitored weekly. Mice that exhibited weight loss and maintained non-fasting blood glucose levels ≥ 13.8 mmol/L on two separate measurements within one week were considered successfully modeled. At the end of the experiment, mice were euthanized via intraperitoneal injection of an overdose of sodium pentobarbital (150 mg/kg). The absence of a heartbeat and respiration, along with the loss of toe-pinch reflex, was used to confirm death prior to tissue collection. This study was approved by the Animal Ethics Committee of Nantong First People's Hospital.

Treatment of NOD mice

An adeno-associated virus serotype 8 (AAV8) vector carrying the mouse insulin promoter (mIP) promoter and miR30-sh-GMRP-1 (AAV8-mIP-miR30-sh-GMRP-1) was constructed to achieve β-cell- specific knockdown of GMRP-1, as previously described [20, 21]. All viruses were constructed, packaged, and purified by Hefei Bixin Biotechnology (Hefei, China). After 5 weeks of STZ induction, NOD mice were treated with Ex-4 (10 μg/kg) or received subcutaneous PBS injections as controls [22]. Then, model mice were administered AV8-mIP-miR30-sh-GMRP-1 via intraperitoneal injection at a dose of 4×10^{11} viral genomes per mouse [20].

Detection of apoptosis in pancreatic tissues by TUNEL assay

Apoptosis in pancreatic tissue was assessed using TUNEL assay. Paraffin-embedded sections were deparaffinized twice in xylene (15-20 min each), rehydrated through a graded ethanol series (100% × 2, 95%, 90%, 80%, 70%; 5-10 min each), and rinsed in distilled water. Antigen retrieval was performed by incubating sections with proteinase K working solution at 37°C for 30 min. Sections were permeabilized with membrane-permeabilization buffer at room temperature for 20 min. After PBS washes (3 × 5 min), TUNEL reagent (TdT:dUTP = 2:29) was applied to each section, followed by incubation in a humidified chamber at 37°C for 2 h. Sections were washed again with PBS (3 × 5 min) and mounted with anti-fade mounting medium. Fluorescence images were captured using an inverted fluorescence microscope (Nikon, Japan).

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software. The protein expression level was expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons between the two groups were performed using independent-samples t-test, while comparisons among multiple groups were analyzed by one-way analysis of variance (ANOVA). A P-value < 0.05 was considered statistically significant.

Results

Ex-4 inhibited IL-1β-induced activation of the JNK pathway

Ex-4, as an upstream regulator of JNK, suppresses the activation of the JNK pathway [23, 24], which is known to promote β-cell apoptosis

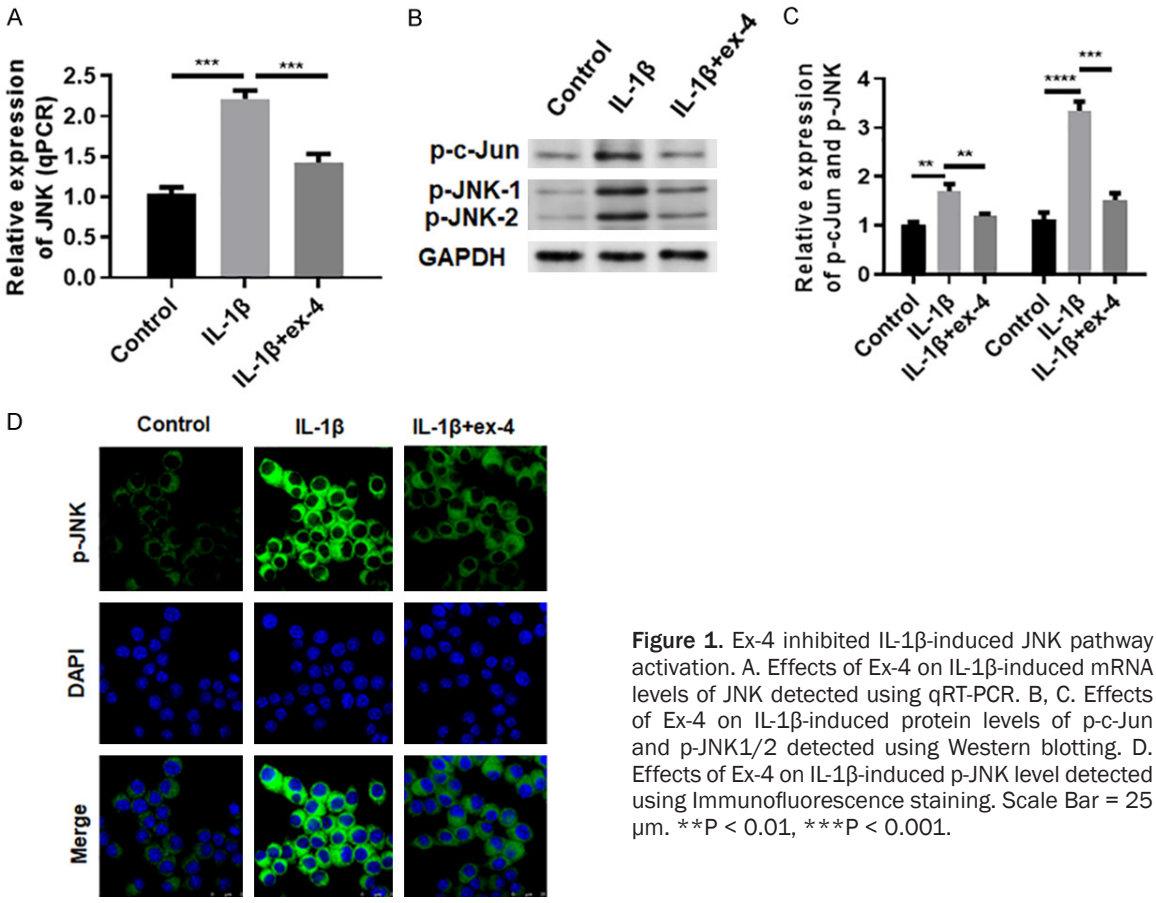

Figure 1. Ex-4 inhibited IL-1β-induced JNK pathway activation. A. Effects of Ex-4 on IL-1β-induced mRNA levels of JNK detected using qRT-PCR. B, C. Effects of Ex-4 on IL-1β-induced protein levels of p-c-Jun and p-JNK1/2 detected using Western blotting. D. Effects of Ex-4 on IL-1β-induced p-JNK level detected using Immunofluorescence staining. Scale Bar = 25 μm. **P < 0.01, ***P < 0.001.

upon activation [16]. We hypothesized that Ex-4 modulates JNK signaling and thereby attenuates apoptosis in IL-1β-stimulated β cells. qRT-PCR analysis revealed that IL-1β stimulation markedly increased JNK mRNA expression compared with the control group, whereas Ex-4 treatment significantly suppressed this upregulation (**Figure 1A**). Western blot experimental results further demonstrated that IL-1β induction could up-regulate p-c-Jun and p-JNK expression, both of which were inhibited by Ex-4 treatment (**Figure 1B**, **1C**). Immunofluorescence staining corroborated that IL-1β enhanced p-JNK expression in β cells, and Ex-4 counteracted this effect (**Figure 1D**).

Ex-4 inhibited IL-1β-induced apoptosis in β cells

To further verify the cytoprotective role of Ex-4, cell proliferation and apoptosis assays were performed. The results showed that IL-1β treatment significantly reduced cell proliferation, while Ex-4 treatment restored proliferative activity (**Figure 2A**). Flow cytometry analysis demonstrated that IL-1β markedly increased apoptotic cell death, which was effectively inhibited by Ex-4 (**Figure 2B**, **2C**). WB analysis of apoptosis-related proteins revealed that IL-1β treatment up-regulated the expression of caspase-3 and caspase-9, which was suppressed by Ex-4 treatment (**Figure 2D-F**).

Ex-4 stimulated GMRP-1 expression

To further elucidate the molecular mechanism underlying Ex-4's protective effect, we further examined its influence on GMRP-1 expression. The experimental results showed that Ex-4 up-regulated GMRP-1 expression in a time-dependent manner (**Figure 3A**). Similarly, Ex-4 enhanced GMRP-1 expression in a dose-dependent manner, as higher concentrations of Ex-4 led to a progressive increase in GMRP-1 levels (**Figure 3B**).

Ex-4 reversed IL-1β - mediated suppression of GMRP-1 expression

To determine whether Ex-4 could modulate IL-1β - induced changes in GMRP-1, we ass-

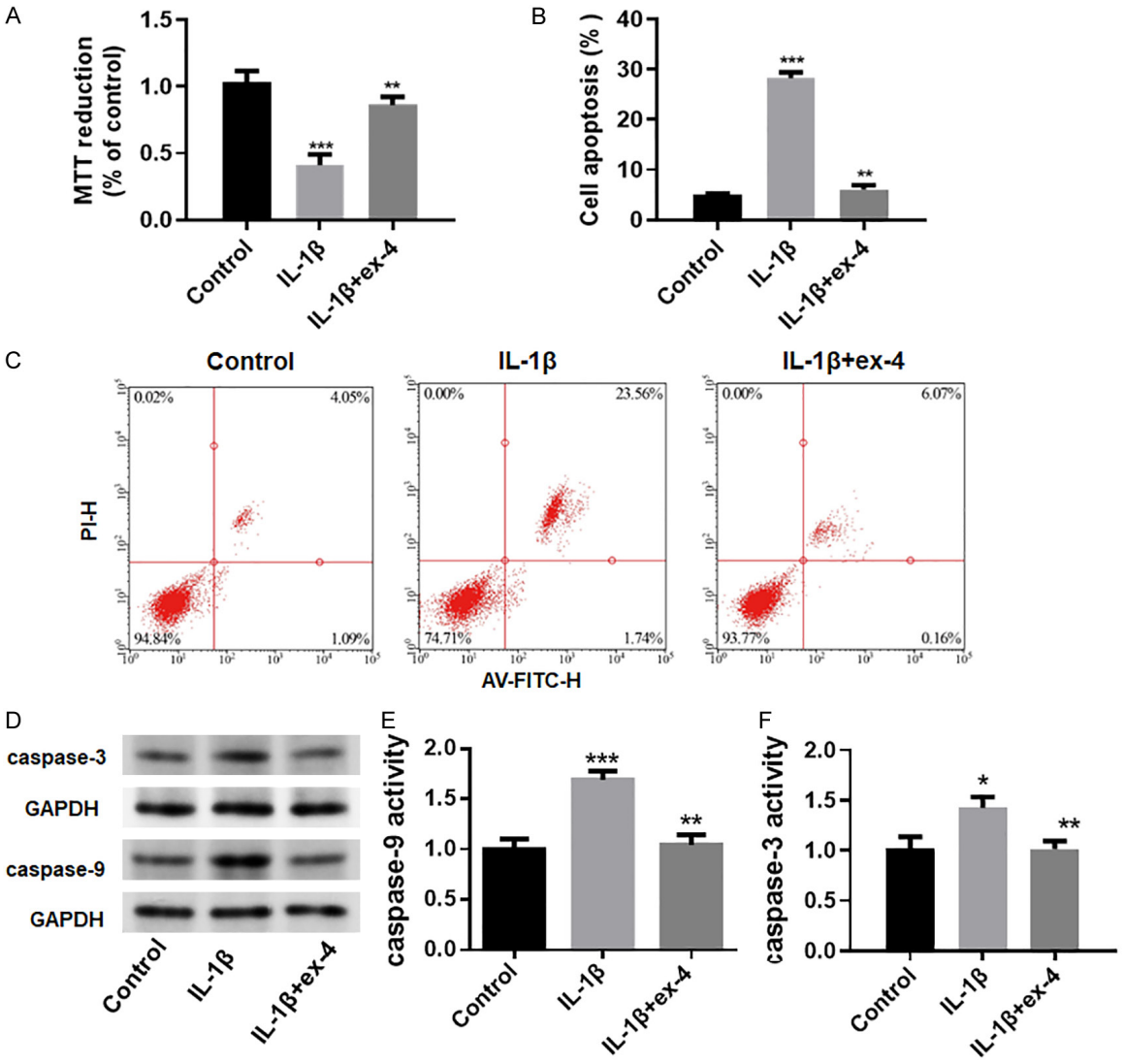

Figure 2. Ex-4 inhibited IL-1β-induced apoptosis in β cells. A. Effects of Ex-4 on IL-1β-induced cell viability detected using MTT assay; B, C. Effects of Ex-4 on IL-1β-induced cell apoptosis detected using Flow cytometry; D. Effects of Ex-4 on IL-1β-induced protein levels of Caspase-3 and Caspase-9 detected using Western blotting; E, F. Quantitative results for protein levels of Caspase-9 and Caspase-3. *P < 0.05, **P < 0.01, ***P < 0.001.

essed GMRP-1 expression following IL-1β stimulation. The results demonstrated that IL-1β markedly suppressed GMRP-1 expression, whereas Ex-4 treatment effectively reversed this inhibition (**Figure 4A**). WB experiments corroborated those obtained, demonstrating that IL-1β reduced GMRP-1 protein levels, which were restored by Ex-4 treatment. Consistent with these observations, IF staining showed decreased GMRP-1 expression in IL-1β - treated β cells, while Ex-4 counteracted this effect (**Figure 4B-D**).

GMRP-1 silencing reversed the inhibitory effect of Ex-4 on apoptosis

To investigate the role of GMRP-1 in mediating the protective effects of Ex-4, we examined pancreatic β-cell responses following GMRP-1 knockdown. siRNA-mediated silencing of GMRP-1 was confirmed by validation assays (**Figure 5A**, **5B**). Subsequent experiments demonstrated that the inhibitory effects of Ex-4 were abolished upon GMRP-1 silencing. Specifically, IL-1β significantly elevated the levels of

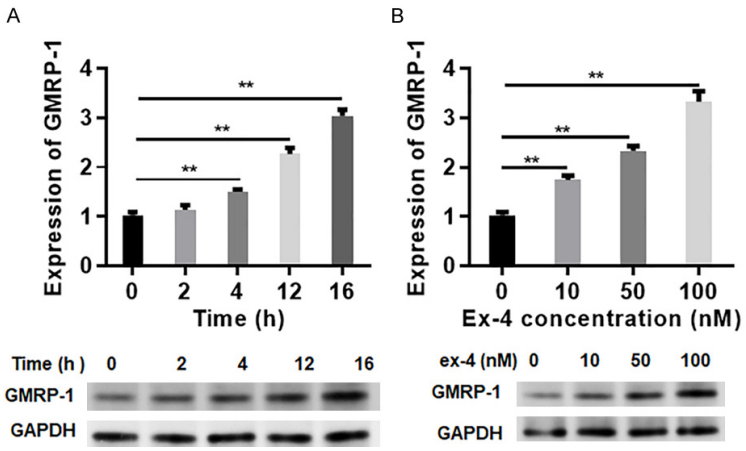

Figure 3. Ex-4 upregulated GMRP-1 expression. A. Ex-4 time-dependently up-regulated GMRP-1 expression. B. Ex-4 dose-dependently up-regulated GMRP-1 expression. **P < 0.01.

p-c-Jun and p-JNK, while Ex-4 effectively reduced the expression of these proteins, which was reversed by GMRP-1 knockdown (**Figure 5C-E**). Flow cytometry of apoptosis rates confirmed that IL-1β induced significant β-cell apoptosis. While Ex-4 effectively inhibited apoptosis under normal conditions, this protective effect was lost upon GMRP-1 silencing (**Figure 5F**, **5G**).

Inhibition of GMRP-1 abolished the protective effect of Ex-4 in a diabetic animal model

To further investigate the role of GMRP-1 in mediating the protective effects of Ex-4 in diabetes, we constructed a diabetic model in NOD mice. Lentivirus-mediated transfection technology was used to knock down GMRP-1 expression in NOD mice. Expression analysis showed that GMRP-1 expression was down-regulated in diabetic mice, while Ex-4 treatment enhanced GMRP-1 levels (**Figure 6A**). TUNEL staining was performed to assess islet β-cell apoptosis in different groups of mice. The results showed that the islet β-cell apoptosis was significantly increased in the diabetes model group, which was ameliorated by Ex-4 administration (**Figure 6B**). Additionally, MTT assay demonstrated that the proliferative capacity of islet β-cells was diminished in diabetic mice, but was restored upon Ex-4 treatment (**Figure 6C**). Furthermore, we applied lentivirus-mediated shRNA to silence GMRP-1 expression in the diabetic model. The results showed that after GMRP-1 knockdown, the protective effect of Ex-4 on islet cells were abolished, with no significant improvement in islet cell proliferation or apoptosis (**Figure 6D**, **6E**).

Discussion

T1DM is an autoimmune disease primarily mediated by T lymphocytes [25]. The destruction of islet β cells leads to inflammation, insufficient insulin secretion, and the onset of the disease, which requires lifelong insulin therapy [26]. Inadequate or improper treatment can result in serious complications, potentially leading to death, thus significantly affecting patients' quality of life and imposing heavy financial burdens on their families. T1DM is considered a T cell-mediated autoimmune disease, where genetic predisposition, combined with environmental factors (e.g., microorganisms, chemicals, and food components), induces an autoimmune response against islet β cells. This response is characterized by pancreatitis and damage to the insulin-producing β cells, resulting in glucose metabolism disorders. Its complex pathogenesis remains a research hotspot [27-29].

Dysfunction of pancreatic islet β cells leads to a relative or absolute loss of insulin secretion, resulting in hyperglycemia, which greatly contributes to pathogenesis of diabetes. Recent studies have highlighted the role of inflammation in the onset of diabetes. IL-1β activates multiple signaling pathways such as mitogen-activated protein kinase (MAPK), nuclear factor kB (NF-kB), protein kinase C (PKC), all of which contribute to β-cell dysfunction and the development of diabetes [30-32]. IL-1β plays a central role in mediating β-cell dysfunction, making it a critical factor in the pathogenesis of diabetes. In this study, IL-1β was selected to induce apoptosis in β cells.

Glucagon-like peptide (GLP-1), secreted by the human body, promotes insulin secretion at high blood glucose concentrations but does not affect insulin secretion when blood glucose levels are normal. However, GLP-1 is easily degraded by dipeptidases in the body, resulting in a half-life of less than 2 min, which limits its clinical use. Subsequently, a 39-amino acid-con-

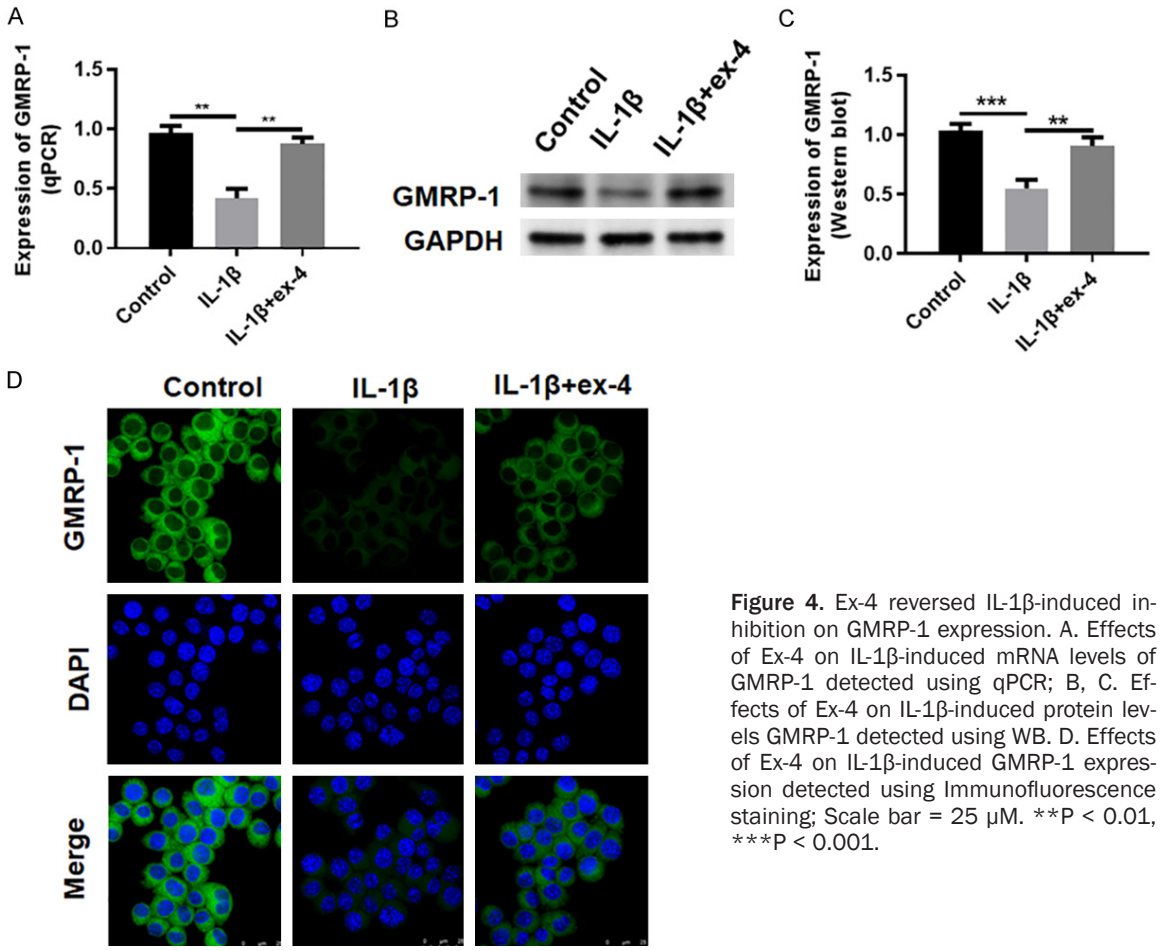

Figure 4. Ex-4 reversed IL-1β-induced inhibition on GMRP-1 expression. A. Effects of Ex-4 on IL-1β-induced mRNA levels of GMRP-1 detected using qPCR; B, C. Effects of Ex-4 on IL-1β-induced protein levels GMRP-1 detected using WB. D. Effects of Ex-4 on IL-1β-induced GMRP-1 expression detected using Immunofluorescence staining; Scale bar = 25 μM. **P < 0.01, ***P < 0.001.

taining polypeptide, Ex-4, isolated from the venom of the Mexican lizard, was discovered to exhibit homology to GLP-1. Ex-4 not only promotes insulin secretion in a manner similar to GLP-1 but also has better stability, offering promising potential for clinical application in the treatment of T2DM. Studies have shown that the biological effects of Ex-4 and GLP-1 are almost identical. Ex-4 increases intracellular cAMP concentration [33], stimulates glucose-dependent insulin secretion, (i.e., promoting insulin secretion at high blood glucose levels while having little effect at normal levels) [34], inhibits programmed cell apoptosis, stimulates cell proliferation and regeneration, and also counteracts cytokine-induced apoptosis [35, 36]. In addition, Ex-4 can reduce hunger and postprandial blood glucose levels, lower glucagon concentrations, slow gastric emptying, inhibit food absorption, and regulate glucose transport in peripheral tissues [37-39].

Despite these known effects, the protective mechanism of Ex-4 on β cells needs further study. Specifically, the regulatory mechanisms and functions of GMRP-1, which is involved in the interaction between the hypothalamus and the pancreas, and is closely linked to glucose metabolism and insulin secretion, remain poorly understood. Wang et al. demonstrated that GMRP1 knockout exacerbates β-cell apoptosis [16]. In this study, GMRP-1 knockdown reversed the protective effects of Ex-4, particularly its inhibition of IL-1β-mediated c-Jun/JNK pathway activation and reduction in apoptosis. These results indicate that GMRP-1 is essential for Ex-4's ability to inhibit the JNK pathway and provide protection to β cells.

Studying the function of glucose-regulated proteins in β cells is of great significance. The glucose-regulated genes in β cells include various transcription factors, genes related to glucose metabolism, insulin signaling pathway genes, and genes involved in cell proliferation, growth, and atrophy. Therefore, glucose can not only change the phenotype of β cells, thereby affecting insulin secretion but also affect the number

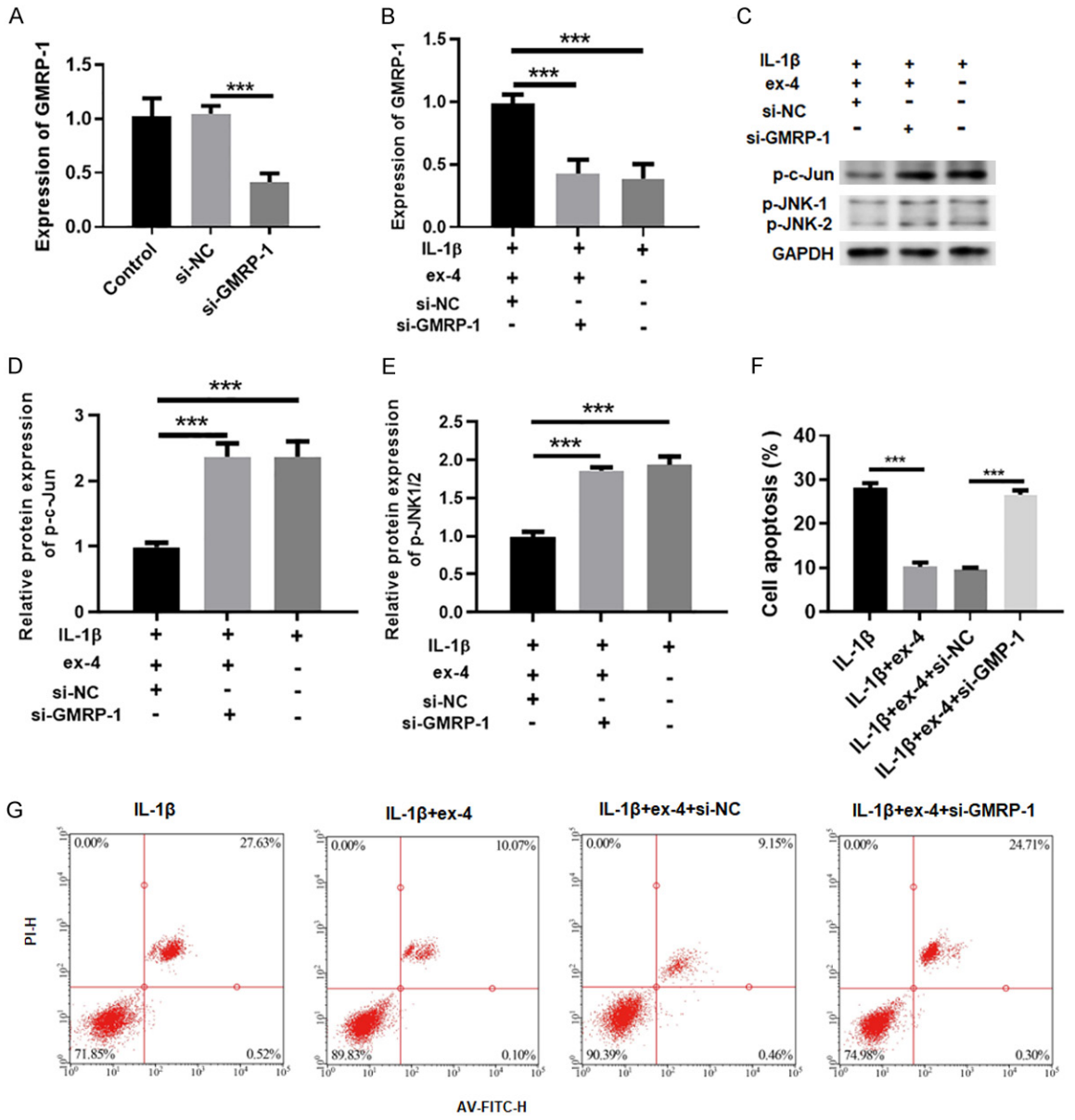

Figure 5. GMRP-1 knockdown abolished the inhibitory effect of Ex-4 on β-cell apoptosis. A. Verification of GMRP-1 knockdown efficiency by qPCR; B. Effects of GMRP-1 knockdown on Ex-4-mediated regulation of on GMRP-1 expression; C. Western blot analysis of p-c-Jun and p-JNK levels under different treatments; D, E. Quantitative analysis of relative protein expression levels of p-c-Jun and p-JNK1/2. F. Flow cytometric quantification of apoptotic β cells under different treatments. G. Representative flow cytometry scatter plots showing apoptosis rates for each group. ***P < 0.001.

of β cells. Our data demonstrates a novel signaling axis in which Ex-4 upregulates GMRP-1 expression, leading to the suppression of the c-Jun/JNK pathway, and ultimately protecting against IL-1β-induced apoptosis in pancreatic β-cells. It was clarified that GMRP-1 plays a crucial role in mediating the protective effect of Ex-4 on β cells. This study lays the foundation for identifying new therapeutic targets for diabetes in the future.

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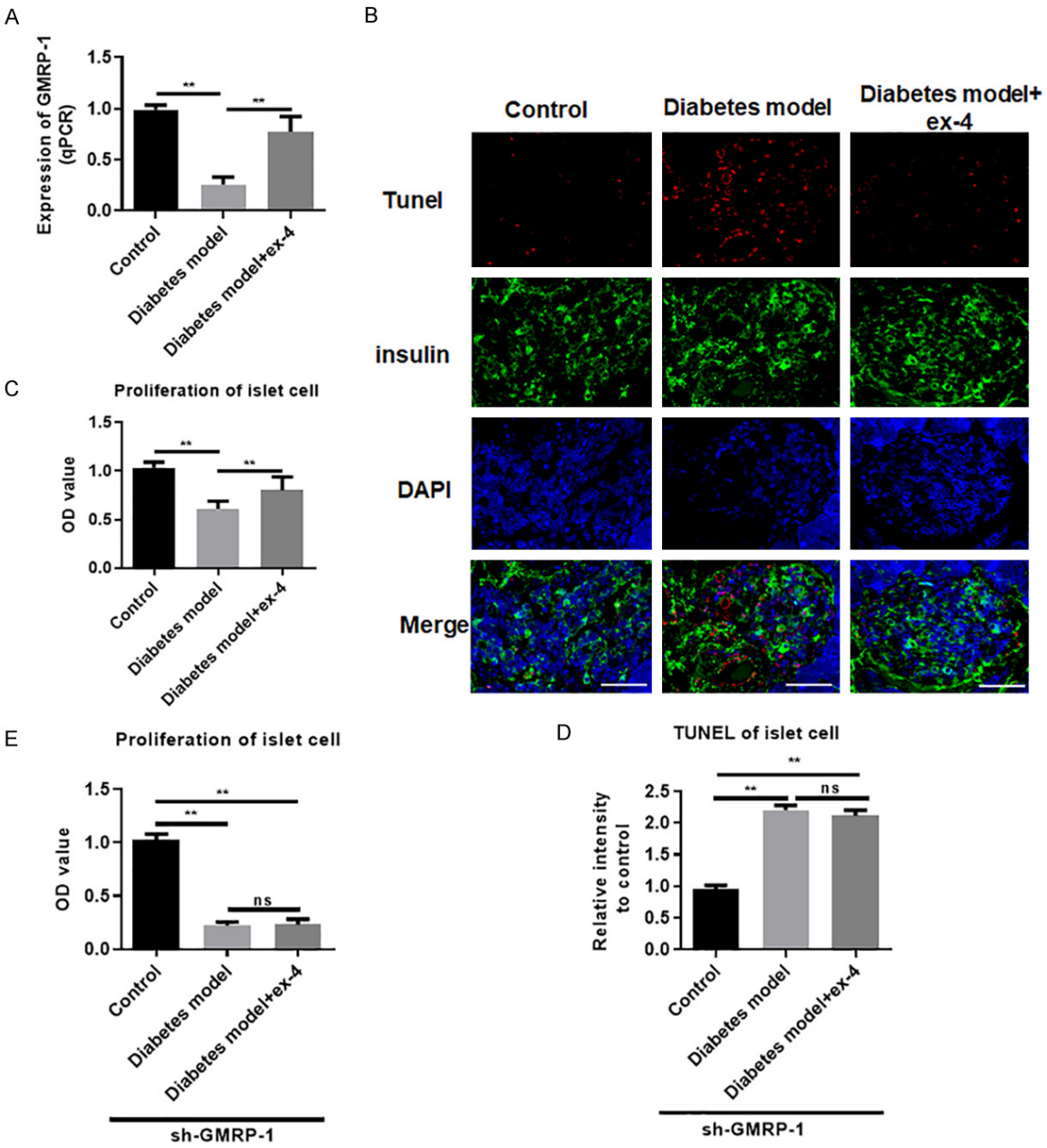

Figure 6. The protective effect of Ex-4 was abolished after GMRP-1 knockdown in T1DM mice. A. PCR detection of GMRP-1 expression in pancreatic tissues from control, diabetic model, and Ex-4-treated diabetic mice; B. Apoptosis of islet cells in mice detected using TUNEL staining; Scale Bar = 20 μm; C. MTT assay analysis of islet cell proliferation in control, diabetic model, and Ex-4-treated diabetic mice; D. Quantitative analysis of TUNEL fluorescence intensity in islet cells after lentivirus-mediated GMRP-1 knockdown; E. MTT assay showing islet cell proliferation after GMRP-1 silencing in diabetic and Ex-4-treated mice. **P < 0.01.

Disclosure of conflict of interest

None.

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