Tacrolimus inhibits insulin release and promotes apoptosis of Min6 cells through the inhibition of the PI3K/Akt/mTOR pathway

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Abstract. As a calcineurin inhibitor, tacrolimus is commonly used as a first-line immunosuppressant in organ transplant recipients. Post-transplantation diabetes mellitus (PTDM) is a common complication following kidney transplantation and is associated with immunosuppressant drugs, such as tacrolimus. PTDM caused by tacrolimus may be related to its influence on insulin secretion and insulin resistance. However, the specific mechanism has not been fully elucidated. The aim of the present study was to investigate whether the PI3K/Akt/mTOR signaling pathway served an important role in the pathogenesis of PTDM induced by tacrolimus. In the present study, the Cell Counting Kit-8 assay was used to measure the effect of tacrolimus on the viability of Min6 mouse insulinoma cells. The effects of tacrolimus on the insulin secretion and the activity of caspase-3 of Min6 cells stimulated by glucose exposure were measured by ELISA. Superoxide dismutase (SOD) and malondialdehyde (MDA) levels were measured using WST-8 and thiobarbituric acid assays, respectively. The effects of tacrolimus on the mRNA expression levels of PI3K, Akt and mTOR were detected by reverse transcription-quantitative PCR (RT-qPCR), whereas the protein expression levels of PI3K, Akt, mTOR, phosphorylated (p)-AKT and p-mTOR in Min6 cells were assessed using western blotting. The present data indicated that, compared with the control group, 5, 25 and 50 ng/ml tacrolimus treatment could inhibit the insulin secretion of Min6 cells stimulated by glucose solution, and 50 ng/ml tacrolimus could notably decrease the stimulation index ($P<0.05$). Moreover, 50 ng/ml tacrolimus markedly increased the activity of caspase-3 by 175.1% ($P<0.05$), it also decreased the SOD activity ($P<0.01$) and increased MDA levels ($P<0.05$). The RT-qPCR results demonstrated that the mRNA expression levels of PI3K, Akt and mTOR were downregulated by 25 and 50 ng/ml tacrolimus ($P<0.01$). Furthermore, the western blotting results suggested that tacrolimus had no significant effects on the expression levels of total PI3K, Akt and mTOR proteins ($P>0.05$), but 25 and 50 ng/ml tacrolimus could significantly inhibit the expression levels of p-Akt and p-mTOR ($P<0.01$). In conclusion, tacrolimus decreased the activity and insulin secretion of pancreatic β cells and induced the apoptosis of islet β cells by inhibiting the mRNA expression levels of PI3K, Akt and mTOR and reducing the phosphorylation of Akt and mTOR proteins in the PI3K/Akt/mTOR signaling pathway, which may ultimately lead to the occurrence of diabetes mellitus, and may be considered as one of the specific mechanisms of PTDM caused by tacrolimus.

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Introduction

As a calcineurin inhibitor, tacrolimus is currently used as the first-line immunosuppressant by organ transplant recipients in the clinical setting. Post-transplantation diabetes mellitus (PTDM), also known as post-transplantation new-onset diabetes, is a common complication following kidney transplantation. The incidence of PTDM after solid organ transplantation is 2-53% (1), whereas the incidence of PTDM after renal transplantation is 10-40% (2). PTDM can increase the risk of cardiovascular disease in transplant recipients (3), as well as reduce the survival time of the graft and the transplant recipients (4). The United Renal Data System analyzed 11,659 kidney transplant recipients and reported that PTDM was significantly correlated with increased graft failure, death-censored graft failure and mortality (5). Multiple studies have shown that the use of tacrolimus was a risk factor for PTDM after transplantation (6,7) The Chinese Guidelines for Diagnosis and Treatment of Diabetes After Organ Transplantation (2019 edition) stated that the risk of PTDM caused by tacrolimus was five times than that caused by cyclosporine (8). Studies have also shown that PTDM caused by tacrolimus may be associated with its influence on insulin secretion and insulin resistance (9,10), but the specific mechanism of action is yet to be fully determined. It is well known that the pathophysiological characteristics of PTDM are similar to those of type 2 diabetes (11). Min6 mouse insulinoma cells are established from islet tumors in transgenic non-obese diabetic mice expressing the 40 large T antigen of the simian virus. Their endocrine function is similar to that of normal pancreatic β cells, and thus, can be

used as an ideal model for studying the function of pancreatic β cells (12).

The PI3K/Akt signaling pathway is the main downstream molecular pathway of insulin (13). PI3K activates Akt by activating the binding of Akt and phosphoinositide dependent kinase (PDK)-1. Activated Akt can activate or inhibit its downstream target protein through phosphorylation, which serves an important role in cell proliferation and metabolism (14). The PI3K/Akt signaling pathway can promote the proliferation and survival of islet β cells. mTOR is a serine/threonine protein kinase that is activated by the PI3K/Akt signaling pathway coupled with the tryptophan kinase, and it serves a key role in sensing nutritional signals and regulating cell proliferation (15). Tacrolimus exerts its immunosuppressive effect by interfering with Ca^{2+}/calmodulin calcineurin signaling pathways. It has been reported that tacrolimus has direct effects to reversibly inhibit insulin gene transcription, leading to a decline in insulin mRNA levels, insulin synthesis and ultimately insulin secretion (16). Tacrolimus can upregulate the expression and activity of caspase-3 and induce the apoptosis of islet cells after treatment with tacrolimus for 24 h, which may be associated with the decreased levels of Akt phosphorylation caused by tacrolimus (17). Therefore, the aim of the present study was to investigate whether the PI3K/Akt/mTOR signaling pathway served an important role in the pathogenesis of PTDM induced by tacrolimus.

Materials and methods

Reagents and antibodies. Tacrolimus (cat. no. 104987-11-3; purity, ≥99%) was purchased from Wuhan Xinxin Jiali Biological Technology Co., Ltd. The mouse insulin ELISA kit (cat. no. 71584) was purchased from Abbkine Scientific Co., Ltd. The Cell Counting Kit (CCK)-8 kit (cat. no. C0038), Bradford protein assay kit (cat. no. P0006), BCA protein assay kit (cat. no. P0012S), caspase-3 activity assay kit (cat. no. C1116), total superoxide dismutase (SOD) assay kit with WST-8 (cat. no. S0101) and lipid peroxidation malondialdehyde (MDA) assay kit (cat. no. S0131) were purchased from Beyotime Institute of Biotechnology. The primary antibody against PI3K (cat. no. 60225-1-Ig) was purchased from ProteinTech Group, Inc., specific primary antibodies against Akt (cat. no. 4691), mTOR (cat. no. 2983), phosphorylated (p)-Akt (Ser473; cat. no. 4060) and p-mTOR (Ser2448; cat. no. 5536) were purchased from Cell Signaling Technology, Inc., and β-actin (cat. no. BM0627) was purchased from Wuhan Boster Biological Technology Co., Ltd. The HRP-conjugated goat anti-mouse (cat. no. BA1051) and the goat anti-rabbit (cat. no. BA1054) secondary antibodies were purchased from Wuhan Boster Biological Technology Co., Ltd.

Cell culture. Min6 mouse insulinoma cells were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences. Cells were cultured with DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 15% FBS (PAN-Biotech GmbH) under 5% CO_2 at 37˚C and grown to 70-80% confluence.

CCK-8 assay. Min6 cells were seeded in 96-well plates (100 µl/well). After treating with different concentrations of tacrolimus (100, 50, 30, 25, 20, 15, 10, 5, 3, 2 and 0 ng/ml) at 37˚C for 48 h, 10 µl CCK-8 reagent was added into each well for incubation at 37˚C for 30 min, according to the manufacturer's instructions. The absorbance of each well was measured at 450 nm using a microplate reader (Shenzhen Leidu Technology Co., Ltd.). Cell viability rate=(OD in the experimental group/OD in the control group) x100; where OD is the optical density.

Glucose-stimulated insulin release. Min6 cells ($1x10^5$/well) were cultured in 24-well culture plates with DMEM containing 15% FBS or different concentrations of tacrolimus at 37˚C for 48 h. To each well 2.8 or 16.7 mmol/l glucose solution (Biofroxx; neoFroxx GmbH) was added for 30 min at 37˚C. Subsequently, the supernatant was collected by centrifugation at 1,000 x g for 20 min at room temperature, and the mouse insulin ELISA kit was used to determine the insulin content, according to the manufacturer's protocols. Finally, the stimulation index (SI), which approximately reflects the function of the islets (18), was calculated. SI=(the insulin content stimulated by high glucose solution) / (the insulin content stimulated by low glucose solution).

Caspase-3 activity assay. Min6 cells ($1.3x10^6$/well) were inoculated into a 6-well plate and cultured with 5, 25 and 50 ng/ml tacrolimus at 37˚C for 48 h. Cells were collected by centrifugation at 600 x g for 5 min at 4˚C, lysed on ice for 15 min with lysis buffer (Beyotime Institute of Biotechnology), followed by centrifugation at 16,000 x g for 15 min at 4˚C. Finally, the protein supernatant was collected and the Bradford method was used to determine the protein concentration in each well. The standard curve of *p*-nitroaniline (pNA) was made and the reaction system was established according to the manufacturer's protocols of the caspase-3 activity assay kit. The absorbance at 405 nm of each well was determined, and the activity of caspase-3 was normalized to the protein content of each sample.

Detection of SOD and MDA activities. Min6 cells ($2x10^6$/well) were seeded in a 6-well plate and incubated for 12 h with DMEM containing 15% FBS at 37˚C; subsequently, the cells were treated with 5, 25 and 50 ng/ml tacrolimus at 37˚C for 48 h. After collecting the supernatant by centrifugation at 12,000 x g for 5 min at 4˚C, the protein concentration in each well was determined using the BCA method, and the SOD and MDA activities were determined using the SOD assay kit with WST-8 and the MDA assay kit, respectively. The activities of SOD and MDA were then normalized to the protein content of each sample.

Reverse transcription-quantitative PCR (RT-qPCR) assay. Min6 cells ($2x10^6$/well) were seeded in a 6-well plate and treated with 5, 25 and 50 ng/ml tacrolimus at 37˚C for 48 h. The total RNA from cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and the quality of the RNA was evaluated according to the A260/A280 ratio. cDNA was synthesized using 3.2 µg total RNA from each sample, 2 µl Oligo$(dT)_{18}$ (10 µM), 4 µl dNTP (2.5 mM), 4 µl 5X HiScript buffer, 1 µl HiScript reverse transcriptase, 0.5 µl ribonuclease inhibitor and RNase-free ddH_2O up to a total

volume of 20 μl at 25˚C for 5 min, 50˚C for 15 min, 85˚C for 5 min and 4˚C for 10 min. qPCR was performed using an ABI QuantStudio 6 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR Green Master mix (Vazyme Biotech Co., Ltd.). The total volume (20 μl) of each PCR reaction consisted of 10 μl SYBR Green Master Mix, 4 μl cDNA, 0.4 μl 50X ROX Reference Dye 2, 4.8 μl ddH_2O and 0.4 μl each of forward and reverse primers (10 μM). qPCR was performed using the following thermocycling conditions: Initial denaturation at 50˚C for 2 min and 95˚C for 10 min, followed by 40 cycles of 95˚C for 30 sec and 60˚C for 30 sec. β-actin was used as the internal control. The murine primer sequences were as follows: β-actin forward, 5'-CACGAT GGAGGGGCCGGACTCATC-3' and reverse, 5'-TAAAGA CCTCTATGCCAACACAGT-3'; PI3K forward, 5'-ACCTGG ACTTAGAGTGTGCC-3' and reverse, 5'-TCAGCAGTGTCT CGGAGTTT-3'; Akt forward, 5'-CTGCCCTTCTACAACCA GGA-3' and reverse, 5'-CATACACATCCTGCCACACG-3'; and mTOR forward, 5'-CGCTACTGTGTCTTGGCATC-3' and reverse, 5'-GGTTCATGCTGCTTAGTCGG-3'. The relative expression levels of PI3K, Akt and mTOR genes were expressed as the difference of the quantitation cycle number value (ΔCq) between the target genes and the β-actin gene. The $2^{-\Delta\Delta Cq}$ method was used to determine the relative gene expression (19). The experiments were performed in triplicate.

Western blot analysis. Min6 cells were seeded in a 6-well plate and cultured with different concentrations of tacrolimus for 48 h, washed three times with PBS, and then 80 μl pre-cooled RIPA lysis buffer (Beyotime Institute of Biotechnology) containing PMSF was added and lysed on ice for 30 min. The cellular proteins were collected by centrifugation at 10,000 x g for 5 min at 4˚C and the BCA protein assay kit was used for protein quantification. The protein supernatant and the loading buffer were mixed at a volume ratio of 4:1, incubated in a boiling water bath for 10 min and 40 μg protein from each group was separated by 10% SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with Tris-HCl buffered salt solution (TBS containing 0.05% Tween-20) containing 5% skim milk for 2 h at room temperature. The membranes were incubated with different primary antibodies against PI3K (1:5,000), Akt (1:1,000), mTOR (1:500), p-Akt (1:2,000), p-mTOR (1:1,000) and β-actin (1:500) overnight at 4˚C in a shaker, after which they were washed five times with TBST and incubated with an appropriate HRP-conjugated secondary antibody (1:50,000) at 37˚C for 2 h. After washing five times with TBST, ECL solution (Beijing Applygen Technologies, Inc.) was added and reacted for 5 min at room temperature. The blots were then imaged using the Bio-Rad chemiluminescence imaging system (Bio-Rad Laboratories, Inc.) and the optical density value of each color band was measured with Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.). The gray ratio of target proteins (PI3K, Akt, mTOR)/β-actin, p-Akt/Akt and p-mTOR/mTOR was used to determine the relative protein expression levels in each group.

Statistical analysis. Statistical analysis was conducted on GraphPad Prism 8.0.1 (GraphPad Software, Inc.). All data are presented as the mean ± SD of three independent experiments.

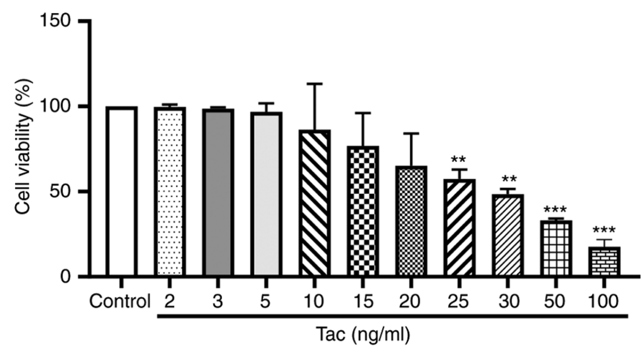

Figure 1. Effects of Tac on the viability of Min6 cells. The viability of Min6 cells was measured using a Cell Counting Kit-8 assay. Data are presented as the mean ± SD of three replicates. **P<0.01, ***P<0.001 vs. Control. Tac, tacrolimus.

Significant differences were performed using one-way ANOVA, followed by the Tukey-Kramer post-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Tacrolimus inhibits the viability of Min6 cells. The viability of Min6 cells after treatment with different concentrations of tacrolimus is shown in Fig. 1. The IC_{50} value was calculated as 30.44 ng/ml. Thus, 5, 25 and 50 ng/ml were selected as the low, moderate and high concentrations of tacrolimus, respectively, in the following experiments.

Tacrolimus inhibits glucose-stimulated insulin release of Min6 cells. The results demonstrated that, compared with the control group, the insulin secretion contents stimulated by high glucose solution (16.7 mmol/l) were decreased after treatment with 5, 25 and 50 ng/ml tacrolimus (Fig. 2A), although no significant differences were identified (P>0.05), and the insulin secretion contents in the low glucose (2.8 mmol/l) treatment groups showed no obvious decrease (P>0.05; Fig. 2B); the SI in the 50 ng/ml tacrolimus group showed a significant decrease compared with the control group (P<0.05; Fig. 2C), which suggested that tacrolimus could inhibit the secretion function of islet cells.

Tacrolimus induces the apoptosis of Min6 cells. As shown in Fig. 3, after treatment with 5, 25 and 50 ng/ml tacrolimus for 48 h, the caspase-3 activities were notably increased compared with the control group. Treatment with 25 ng/ml tacrolimus could enhance the activity of caspase-3 by 51.7%, whereas 50 ng/ml tacrolimus could significantly increase the activity of caspase-3 by 175.1% (P<0.05). These results suggested that tacrolimus may induce the apoptosis of islet β cells.

Tacrolimus decreases SOD activity and increases the MDA level. The activity of SOD in Min6 cells was significantly inhibited following treatment with 25 and 50 ng/ml tacrolimus for 48 h (P<0.05 and P<0.01; Fig. 4A), especially in the 50 ng/ml tacrolimus group. Moreover, it was found that 50 ng/ml tacrolimus significantly increase the level of MDA in Min6 cells treated with tacrolimus for 48 h (P<0.05; Fig. 4B). These results suggested that tacrolimus may cause oxidative stress in pancreatic β cells.

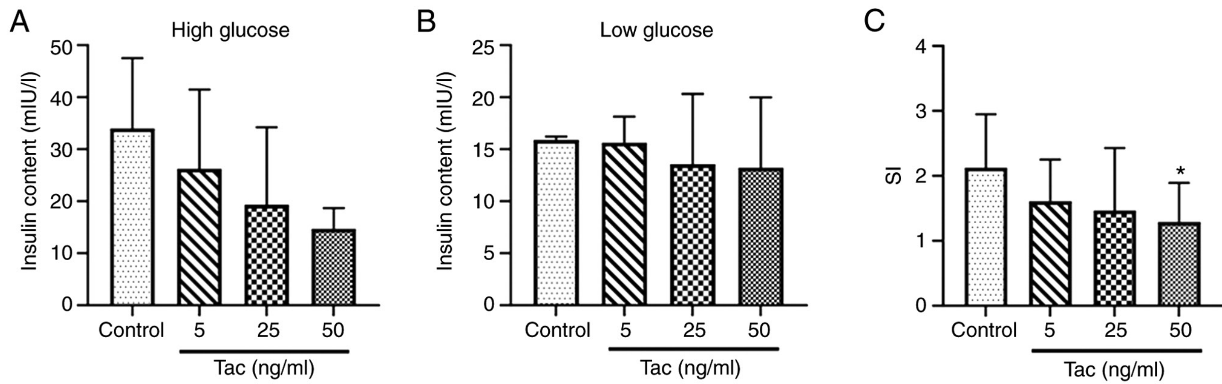

Figure 2. Effects of Tac on the insulin release of glucose-stimulated Min6 cells. The insulin contents stimulated by (A) high glucose and (B) low glucose were determined with the mouse insulin ELISA kit. (C) The SI was calculated according to the following formula, SI=(the insulin content stimulated by high glucose solution)/(the insulin content stimulated by low glucose solution). Data are presented as the mean ± SD of three replicates. *P<0.05 vs. Control. SI, stimulation index; Tac, tacrolimus.

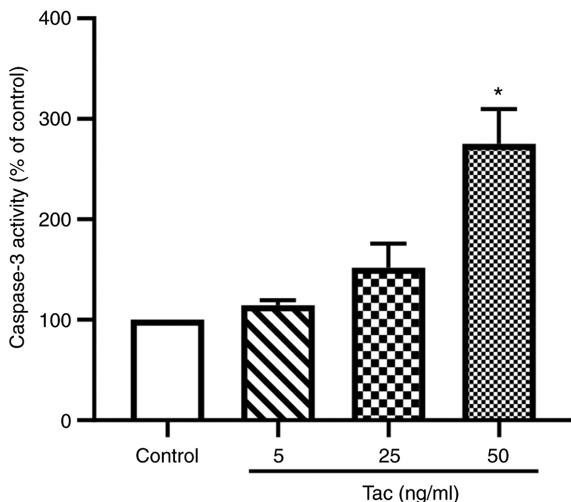

Figure 3. Effects of Tac on the caspase-3 activities in Min6 cells. The level of caspase-3 was measured using a caspase-3 activity assay kit. Data are presented as the mean ± SD of three replicates. *P<0.05 vs. Control. Tac, tacrolimus.

Tacrolimus decreases the mRNA expression levels of PI3K, Akt and mTOR. As presented in Fig. 5, compared with the control group, 5, 25 and 50 ng/ml tacrolimus treatments significantly downregulated the mRNA expression levels of PI3K and mTOR (P<0.01). Tacrolimus concentrations of 25 ng/ml (P<0.05) and 50 ng/ml (P<0.01), but not 5 ng/ml (P>0.05), also significantly reduce the expression level of Akt mRNA. These results indicated that tacrolimus decreased the mRNA expression levels of components of the PI3K/Akt/mTOR pathway.

Tacrolimus inhibits the expression levels of p-Akt and p-mTOR but not PI3K, Akt and mTOR. Compared with the control group, the expression levels of total PI3K, Akt and mTOR proteins showed no significant difference when treated with different concentrations of tacrolimus (P>0.05; Fig. 6A-D). However, after 48 h treatment with 25 and 50 ng/ml tacrolimus, the expression levels of p-Akt and p-mTOR in Min6 cells were significantly decreased compared with the control group (P<0.01; Fig. 6A, E and F). Furthermore, 5 ng/ml tacrolimus significantly decreased the expression level of p-mTOR protein (P<0.05), but had no significant effect on the expression level of p-Akt protein (P>0.05).

Discussion

PTDM is a known side effect in transplant recipients; it is associated with the use of immunosuppressant drugs, such as tacrolimus (20). Previous studies have suggested that the possible mechanisms of PTDM caused by tacrolimus include direct β cell toxicity, reducing the utility of glucose, inhibition of insulin secretion and increasing insulin resistance (21,22). However, the specific mechanism of action has not been fully elucidated. Direct β cell toxicity is manifested by swelling of the cytoplasm, formation of vacuoles and induction of cell damage and apoptosis (23). Tacrolimus can quickly and directly inhibit insulin exocytosis and affect glucose-, glucagon-like peptide-1- and potassium chloride-induced insulin release, as well as increase the caspase-3 activity of human islet cells after treatment with tacrolimus for 24 h (17). Soleimanpour *et al* (24) reported that tacrolimus could inhibit mouse pancreatic β cell proliferation and induce human pancreatic β cell apoptosis, both of which are accompanied by a decrease of intracellular phosphorylation of Akt. Tacrolimus can reduce the expression of neuronal PAS domain protein 4 (Npas4) by inhibiting the activity of calcineurin, thereby causing toxic effects on β cells. In addition, overexpression of Npas4 can inhibit the tacrolimus-induced apoptosis of Min6 cells, and the molecular mechanism may be associated with Akt, Ca^{2+}/calmodulin-dependent protein kinase and the downstream signaling molecules of calcineurin (25). Results from the present study demonstrated that tacrolimus reduced the relative viability of Min6 cells, decreased insulin secretion stimulated by glucose solution and enhanced the activity of caspase-3 in Min6 cells, which suggested that tacrolimus may inhibit the viability and the insulin secretion function of Min6 cells, as well as induce the apoptosis of Min6 cells.

In previous studies, oxidative stress has been linked with both the physiological response to insulin and the pathophysiological mechanisms of diabetes mellitus (26-28); it is also known to be enhanced in renal transplant recipients compared

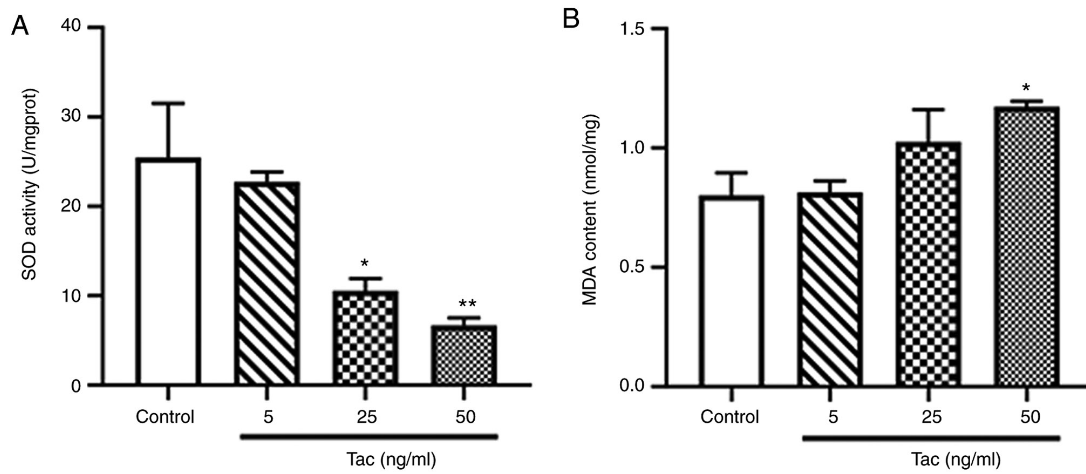

Figure 4. Effects of Tac on SOD and MDA activity in Min6 cells. (A) SOD activity was determined using a SOD assay kit with WST-8. (B) MDA content was measured using a lipid peroxidation MDA assay kit. Data are presented as the mean ± SD of three replicates. *P<0.05, **P<0.01 vs. Control. MDA, malondialdehyde; SOD, superoxide dismutase; Tac, tacrolimus.

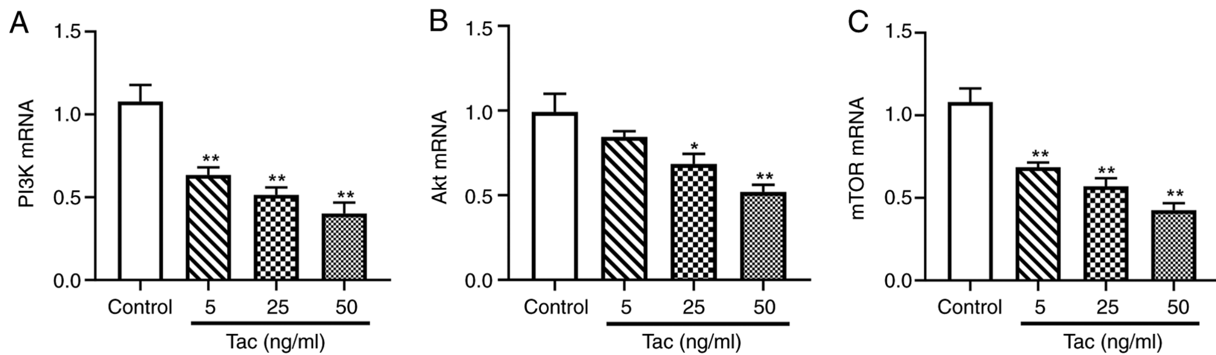

Figure 5. Effects of Tac on the mRNA expression levels of PI3K, Akt and mTOR in Min6 cells. mRNA expression levels of (A) PI3K, (B) Akt and (C) mTOR in Min6 cells were determined by reverse transcription-quantitative PCR. Data are presented as the mean ± SD of three replicates. *P<0.05, **P<0.01 vs. Control. Tac, tacrolimus.

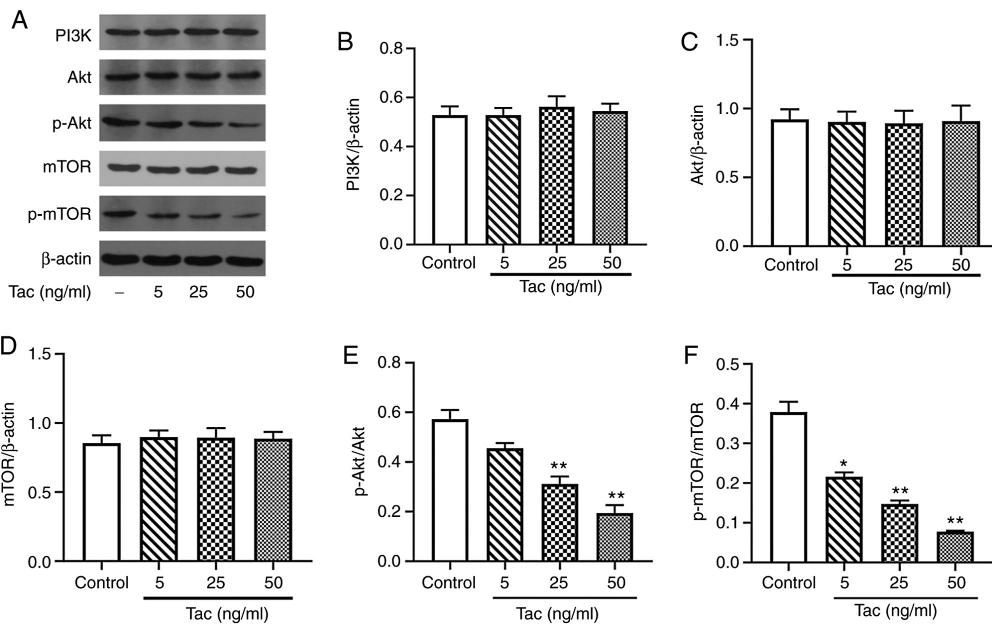

Figure 6. Tac suppresses the viability and insulin secretion of Min6 cells by inhibiting the PI3K/Akt/mTOR signaling pathway. (A) Protein expression levels of total PI3K, Akt and mTOR, as well as p-Akt and p-mTOR in Min6 cells were measured by western blotting. (B) PI3K, (C) Akt and (D) mTOR relative expression levels, and the (E) p-Akt/Akt and (F) p-mTOR/mTOR ratios were calculated using the densitometric values of the protein bands. Data are presented as the mean ± SD of three replicates. *P<0.05, **P<0.01 vs. Control. p-, phosphorylated; Tac, tacrolimus.

with the general population (29). The level of MDA, an oxidative stress biomarker, is higher in patients with established diabetes mellitus compared with healthy controls (30,31). Yepes-Calderón *et al* (32) reported that plasma MDA concentration was inversely and independently associated with long-term risk of PTDM in renal transplant recipients, and these findings support a potential underrecognized role of oxidative stress in post-transplantation glucose homeostasis. Moreover, Jin *et al* (33) observed that tacrolimus decreased cell viability and increased reactive oxygen species production in both insulin-secreting β-cell derived (INS-1) and human kidney-2 (HK-2) cell lines. SOD is an important peroxidase that can eliminate the possible oxygen free radicals, whereas MDA is a lipid peroxidation product mediated by oxygen free radicals, and it is also an important indicator of tissue and cell damage caused by oxygen free radicals (34). In the present study, it was found that tacrolimus inhibited SOD activity and increased cellular MDA levels, suggesting that it may reduce the ability of Min6 cells to scavenge oxygen free radicals and leads to oxidative stress, thereby causing the damage of islet β cells.

The PI3K/Akt/mTOR signaling pathway serves an important role in cell differentiation, proliferation, cellular metabolism, cytoskeletal reorganization, apoptosis and survival (35,36). This pathway also serves a pivotal role in the metabolic and mitogenic actions of insulin and insulin-like growth factor1 (37,38). PI3K is closely associated with oxidative stress, and can inhibit apoptosis induced by oxidative stress. PI3K p110 α and p110 β serve important roles in promoting cellular proliferation and homeostasis, as well as opposing apoptosis caused by oxidative stress (39). The PI3K/Akt signaling pathway has important regulatory effects on the expression levels of genes involved in gluconeogenesis and fatty acid synthesis by regulating the activity of downstream molecules, in addition to having important regulatory effects on glucose transport (37,40). The blockade of this signaling pathway is one of the most basic mechanisms leading to type 2 diabetes and insulin resistance in peripheral tissues. mTOR consists of mTOR complex (mTORC)1 and mTORC2. Both mTORC1 and mTORC2 are activated by the PI3K signaling pathway coupled with tyrosine kinase. However, mTORC1 is a downstream molecule of Akt and is activated by p-Akt. As a PDK2, mTORC2 can fully activate Akt through phosphorylation of the Ser473 site of Akt (41).

A study published in 2019 reported that the mTOR inhibition may be a mechanism contributing to the diabetogenic effect of tacrolimus (42). In the present study, it was demonstrated that tacrolimus could markedly decrease the expression levels of PI3K, Akt and mTOR mRNA *in vitro*. Moreover, tacrolimus showed no obvious effects on the expression levels of total PI3K, Akt and mTOR proteins, but it could inhibit p-Akt and p-mTOR expression in Min6 cells in a dose-dependent manner, especially for p-mTOR. The possible reasons why the PCR data do not correlate with the western blotting data for PI3K, Akt and mTOR, include that the transcription and translation process of mRNA is not synchronized with protein expression, not all mRNA is expressed and mRNA extracted in PCR is mainly from the nucleus, whereas proteins extracted for western blotting are from the entire cell (43). Collectively, the present results suggested that tacrolimus may lead to diabetes mellitus through the inhibition of the PI3K/Akt/mTOR signaling pathway.

At present, the prevention and treatment measures for PTDM caused by tacrolimus mainly include blood glucose monitoring, replacement of immunosuppressive treatment options, such as replacing tacrolimus with cyclosporine, and adopting hypoglycemic programs similar to those for type 2 diabetes, including changing lifestyle, oral hypoglycemic drugs and insulin therapy (44). However, a large number of clinical studies are required to further verify the effectiveness and safety of long-term use of hypoglycemic drugs in the treatment of PTDM. The results of the present study suggested that it may be possible to develop drugs targeting the PI3K/Akt/mTOR signaling pathway for the prevention and treatment of PTDM in future.

In summary, results from the present study indicated that tacrolimus inhibited the viability and insulin secretion of pancreatic β cells and induced the apoptosis of islet β cells by inhibiting the expression levels of PI3K, Akt and mTOR genes and reducing the phosphorylation of Akt and mTOR proteins in the PI3K/Akt/mTOR signaling pathway. This may be considered as one of the specific mechanisms of PTDM caused by tacrolimus. However, the *in vivo* effects of tacrolimus on the PI3K/Akt/mTOR signaling pathway remain to be further investigated.

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Availability of data and materials

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

Authors' contributions

LT conceived and designed the experiments, performed the experiments, analyzed the data, wrote the manuscript and prepared figures. WL, YZhan, FZ, YZhao, LZ, JL, ZS, MY and CZ performed the experiments, analyzed the data and contributed reagents and analysis tools. LT and WL confirm the authenticity of all raw data. AY conceived the experiments and corrected the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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