Phospho-regulation of Cdc14A by polo-like kinase 1 is involved in β-cell function and cell cycle regulation

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Abstract. The objective of the present study was to investigate the effects of polo-like kinase 1 (PLK1) and the phosphorylation of human cell division cycle protein 14A (Cdc14A) by PLK1 on β-cell function and cell cycle regulation. Mouse β-TC3 cells were incubated with small interfering RNA (siRNA) to knock down the expression of PLK1. Cell cycle analysis was performed using flow cytometry, and cell proliferation and apoptosis was determined. Insulin secretion was evaluated by a radioimmunoassay under both low and high glucose conditions. Mouse β-TC3 cells were transfected with a wild type or a non-phosphorylatable Cdc14A mutant ($Cdc14A^{S351A/363A}$; $Cdc14A^{AA}$) to investigate whether the phosphorylation of Cdc14A is involved in cellular regulation of PLK1 under high glucose conditions. It was found that PLK1 siRNA significantly promoted cellular apoptosis, inhibited cell proliferation, decreased insulin secretion and reduced Cdc14A expression under both low and high glucose conditions. Cdc14A overexpression promoted β-TC3 cell proliferation and insulin secretion, while $Cdc14A^{AA}$ overexpression inhibited cell proliferation and insulin secretion under high glucose conditions. PLK1 siRNA partially reversed the proliferation-promoting effects of Cdc14A and further intensified the inhibition of proliferation by $Cdc14A^{AA}$ under high glucose conditions. Similarly, Cdc14A overexpression partially reversed the insulin-inhibiting effects of PLK1 siRNA, while $Cdc14A^{AA}$ overexpression showed a synergistic inhibitory effect on insulin secretion with PLK1 siRNA under high glucose conditions. In conclusion, PLK1 promoted cell proliferation and insulin secretion while inhibiting cellular apoptosis in β-TC3 cell lines under both low and high glucose conditions. In addition, the phospho-regulation of Cdc14A by PLK1 may be involved in β-TC3 cell cycle regulation and insulin secretion under high glucose conditions.

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Introduction

Inadequate production of insulin and insulin resistance are the main causes of type 2 diabetes (1). β-cells, the insulin producing cells of the pancreas, maintain glucose homeostasis (2). Both a decrease in the number of β-cells and their functional impairment play key roles in the pathogenesis of diabetes (2). Similar to other cells in the human body, β-cells are tightly regulated by cell cycle progression, a process that is not well understood (2). Improving pancreatic islet function and increasing the number of β-cells are potential strategies in the treatment of diabetes mellitus (2).

A number of studies have shown that cell cycle regulating factors are pivotal in β-cell function, proliferation and apoptosis (3,4). Nyblom *et al* (3) found that several pancreatic islet proteins controlling islet cell regeneration and proliferation changed significantly in patients with type 2 diabetes, including activation of polo-like kinase 1 (PLK1). In another study, Misfeldt *et al* (4) discovered an increase in PLK1 expression and islet cell hyperplasia after a 60% resection of rat pancreatic islet cells.

PLKs are a family of serine/threonine protein kinases that orchestrate a plethora of cellular processes in eukaryotic organisms (5,6). PLKs have an N-terminal catalytic kinase domain and one or more C-terminal polo boxes that are well conserved from budding yeast to *Drosophila*, *Xenopus* and mammals. The most well studied and universally expressed mammalian subfamily member, PLK1, plays a critical role in mitotic spatiotemporal regulation (7,8). PLK1 knockdown by RNA interference or inhibition with small molecules results in a failure to establish a bipolar spindle or to properly attach kinetochores to microtubules (9,10). Defective development is observed in PLK1-null mice due to massive mitotic arrest (11). PLK1 is also widely recognized as an oncogene, as high PLK1 expression in cancer correlates with a poor prognosis (12,13).

In eukaryotic cells, cell division cycle protein 14 (Cdc14) is essential for faithful cell cycle progression. As a member of the dual-specificity phosphatase family, Cdc14 has a conserved phosphatase domain located at the N-terminal. The function of human Cdc14A has not yet been fully elucidated; however,

Cdc14A has many critical functions, including in DNA damage checkpoint control, DNA repair, centrosome maturation and separation, and the regulation of cytokinesis (14-16). Cdc14A also plays a role in cell migration and adhesion, and may regulate tumor metastasis (17). In a previous study, the interrelationship between PLK1 and Cdc14A was identified and characterized (18). The C-terminal domain of Cdc14A auto-inhibits the phosphatase activity when bound to the N-terminal, which illustrates a self-inhibitory association (18). When phosphorylated by PLK1, the inhibitory self-association of Cdc14A, as judged by its phosphatase activity, was found to be disrupted in both *in vitro* and *in vivo* studies (18). The co-localization of PLK1 and Cdc14A in the centrosome were also revealed (19). In addition, faithful chromosome segregation during mitosis relies on the spatiotemporal interaction between PLK1 and Cdc14A (18). Therefore, it is of great interest to elucidate the physiological effects of the interrelationship between these two important proteins.

In this study, the roles of PLK1 in cell proliferation, apoptosis and insulin secretion regulation were investigated in the β-TC3 cell line under both low and high glucose conditions. Additionally, whether the phosphorylation of Cdc14A by PLK1 is involved in these processes was investigated. The data presented here reveal that the phospho-regulation of Cdc14A by PLK1 is involved in β-cell function and cell cycle regulation under high glucose conditions.

Materials and methods

cDNA construction. The cDNA of Cdc14A (NM_033312) was kindly donated by Jiri Lukas. To generate the plasmid encoding GFP-fused Cdc14A, the respective open reading frame was amplified by PCR using Taq Plus DNA polymerase (cat. no. ET105; Tiangen Biotech Co., Ltd.), and inserted into pEGFP-C1 vector (Clontech Laboratories, Inc.) by ligating E*co*RI-S*al*I sites in Cdc14A cDNA. The following PCR cycling conditions were used: 94˚C for 5 min; 35 cycles consisting of 94˚C for 30 sec, 55˚C for 30 sec and 72˚C for 2 min; 72˚C for 5 min; and finally, cooling to 16˚C. The Cdc14A primers were 5'-CATGAATTCATGGCAGCGGAGTCAGGGGA-3' (forward) and 5'-CGGGTCGACTCAGAAGGCTTCCTT GGCAC-3' (reverse). A green fluorescent protein-tagged non-phosphorylatable Cdc14A$^{S351A/363A}$ (Cdc14A^{AA}) mutant was constructed using a QuikChange Site-directed Mutagenesis kit (cat. no. 210518; Stratagene; Agilent Technologies Inc.).

Cell lines and culture conditions. The mouse β-TC3 cell line was purchased from CHI Scientific Inc. The cells were cultured in DMEM (HyClone; GE Healthcare Life Sciences) supplemented with 10% heat-inactivated FBS (cat. no. 16000-044; Gibco; Thermo Fisher Scientific Inc.) and 5% CO_2 at 37˚C. In addition, 1% L-glutamine and 1% antibiotics were also added to the culture medium. The culture medium was refreshed every 3 days.

Cell transfections. Cdc14A plasmids (2 μg/ml) and PLK1 siRNA (50 nM/l) were transfected into β-TC3 cells at 80% confluency using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. PLK1 siRNA (siRNA329, siRNA1566 and siRNA1568) and a control siRNA were designed and synthesized by Invitrogen (Thermo Fisher Scientific, Inc.). The sense strand of PLK1 siRNA329 was 5'-GAUUGUGCCUAAGUC UCUGTT-3', and its antisense strand was 5'-CAGAGACUU AGGCACAAUCTT-3'. The sense strand of PLK1 siRNA1566 was 5'-UGAAGAUCUGGAGGUGAAATT-3', and its antisense strand was 5'-UUUCACCUCCAGAUCUUCATT-3'. The sense strand of PLK1 siRNA1568 was 5'-AUUGUGCUU GGCUGCCAGUTT-3', and its antisense strand was 5'-ACU GGCAGCCAAGCACAAUTT-3'. The sense strand of the control siRNA was 5'-UUCUCCGAACGUGUCACGUTT-3', and its antisense strand was 5'-ACGUGACACGUUCGGAGA ATTT-3'. After transfection of PLK1 siRNA for 72 h, the cells were either collected or transfected with Cdc14A wild type or mutant plasmids for a further 48 h. The efficiency of PLK1 siRNA transfection was determined by reverse transcription quantitative (RT-q)PCR.

Isolation of RNA and RT-qPCR. Total RNA was extracted from β-TC3 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions, and was reverse-transcribed using a PrimeScript RT-PCR kit (Thermo Fisher Scientific, Inc.). RT was performed at 37˚C for 15 min, followed by 85˚C for 5 sec. The following PCR cycling conditions were used: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec, 60˚C for 40 sec and 95˚C for 15 sec. The PLK1 primers were 5'-GAGTGCCACCTT AGTGACTTGCT-3' and 5'-CTTGTCCGAATAGTCCACC CAC-3'. The GAPDH primers were 5'-AGAGGGAAATCG TGCGTGAC-3' and 5'-CCAAGAAGGAAGGCTGGA AAA-3'. qPCR was performed using an SYBR® *Premix Ex Taq*™ II kit [(Tli RNaseH Plus), ROX plus; cat. no. RR82LR; Takara Biotechnology Co., Ltd.) and a 7300 Real-Time PCR system (Thermo Fisher Scientific, Inc.). The data were analyzed by the relative standard curve method and normalized to GAPDH expression. Relative RNA expression was calculated using the $2^{-\Delta\Delta Cq}$ method (20). All experiments were performed in triplicate.

Cell cycle analysis. β-TC3 cells were fixed in ice-cold 70% ethanol for 1 h. Following this, 1 mg/ml RNase (Beyotime Institute of Biotechnology) was added to the culture medium and the cells were incubated at 37˚C for 30 min. Cells were analyzed using FACScan cytofluorometry equipment (Becton-Dickinson and Company) and FlowJo version 7.6.5 software (FlowJo LLC) after staining with propidium iodide (0.5 mg/ml) at 4˚C for 30 min.

Apoptosis analysis. Annexin V/PI double staining was used to determine the number of dead cells. Transfected β-TC3 cells were harvested as described earlier and washed with ice-cold PBS. The cells were stained with FITC-conjugated Annexin V (BD Pharmingen; BD Biosciences) in binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4), followed by FACScan cytofluorometry (BD Biosciences) and CellQuest Pro version 5.1 software (BD Biosciences) analysis. Each experiment was conducted in triplicate.

Cell proliferation assay. Cell proliferation was analyzed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular

Technologies, Inc.) assay. β-TC3 cells ($3x10^3$) were transferred into each well of a 96-well plate in DMEM. Each group of cells was plated into five wells and the test was repeated three times. A total of 10 μl of CCK-8 reagent was added to each well at each time-point followed by incubation for 4 h. The optical density was measured using a microplate reader (Bio-Rad Laboratories, Inc.) at a wavelength of 450 nm. The cell proliferation rate was plotted as a curve for each group of cells.

Western blot analysis. Total cell extracts were denatured in RIPA lysis buffer (Beyotime Institute of Biotechnology). The protein concentration was quantified using a BCA assay. Protein (30 μg/lane) was loaded and separated on 15% polyacrylamide gels. The proteins were then transferred to nitrocellulose filter membranes, blocked in blocking buffer [1% BSA (Sigma-Aldrich; Merck KGaA), 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween-20] for 1 h at 37˚C, and incubated with primary antibodies overnight at 4˚C. The primary antibodies for western blotting included anti-Cdc14A (1:500; cat. no. 13660-1-AP; ProteinTech Group, Inc.) and anti-GAPDH (1:10,000; cat. no. MAB374; EMD Millipore). The membrane was incubated with horseradish peroxidase-conjugated secondary antibody (1:5,000; cat. no. BA1050; Wuhan Boster Biological Technology, Ltd.) for 1 h at room temperature. Antigen-antibody complexes were visualized using ECL reagent (Pierce; Thermo Fisher Scientific, Inc.). Western blot bands were quantified by ImageJ Version 1.50 software (National Institutes of Health).

Insulin secretion assay. First, β-TC3 cells were incubated in DMEM at a low glucose concentration (5.5 mmol/l) for 24 h. Then, cells were cultivated for 30 min under high glucose conditions (25 mmol/l) before being transfected with PLK1 siRNA and/or Cdc14A plasmids. At 3-5 days following transfection, aliquots of the incubation media were collected for the insulin assays. Insulin levels were determined using a mouse insulin radioimmunoassay (RIA) kit (cat. no. K4271; BioVision, Inc.) according to the manufacturer's instructions (CIS Bio International).

Statistical analysis. Each experiment was performed in triplicate, and the results are expressed as the mean ± SD. One-way ANOVA and the least significant difference post hoc test was applied when comparing normally distributed data with homogeneity of variance among three or more groups. Kruskal-Wallis and Dunn's pairwise post hoc tests were used when making multiple comparisons among non-normally distributed measurement data. χ^2 test followed by Bonferroni tests were applied when comparing cell cycle distributions among different groups. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using SPSS version 23 (SPSS, Inc.).

Results

PLK1 promotes cell proliferation while inhibiting cellular apoptosis in β-TC3 cell lines under high glucose conditions. At 72 h post transfection with siRNA targeting PLK1 (siRNA329, siRNA1566 and siRNA1568), whole cell lysates and culture media were collected. RT-qPCR analysis was performed in triplicate to detect the effect of the PLK1 siRNA. As shown in Fig. 1A, PLK1 levels were the lowest when its expression was depleted with siRNA329. Therefore, siRNA329 was utilized in the following experiments.

To determine the function of PLK1 in β-TC3 cells, the effects of PLK1 siRNA on the cell cycle, cell proliferation and cellular apoptosis under high glucose conditions were first examined. Compared with low glucose, high glucose significantly promoted cell proliferation (P<0.05). PLK1 depletion significantly inhibited cell proliferation compared with the control siRNA under both low and high glucose conditions (P<0.05). The results of the CCK-8 assay indicate that PLK1 promotes β-TC3 cell proliferation under both low and high glucose conditions, with the maximum effect observed on day 4(Fig. 1B and C).

Cell cycle distribution was also determined on day 4; the results are shown in Fig. 2 and Table I. The results showed that PLK1 siRNA increased the population of cells in the G_1phase and decreased the population of cells in the G_2 and S phases under low glucose conditions (P<0.05). Under high glucose conditions, PLK1 siRNA also decreased the number of cells in the G_2 phase, but had no significant effect on the number of cells in the G_1 and S phases. High glucose conditions significantly decreased the population of cells in the G_1 phase and increased the number of cells in the G_2 and S phases (P<0.05). These results indicated that PLK1 controls the cell cycle progression of β-TC3 cells.

Additionally, flow cytometry analysis revealed that, when transfected with control siRNA, high glucose conditions inhibited β-TC3 cell apoptosis compared with the rate of apoptosis in the low glucose conditions (P<0.05). PLK1 siRNA promoted cellular apoptosis compared with control siRNA under both low and high glucose conditions (P<0.05). These results indicated that PLK1 inhibits β-TC3 cell apoptosis under both low or high glucose conditions (Fig. 3A and B).

PLK1 promotes insulin secretion in β-TC3 cell lines under high glucose conditions. To determine insulin release in response to glucose stimulation and PLK1 depletion, aliquots of the culture media of different groups of cells 3-5 days after transfection were taken to be used to assess insulin levels using a mouse insulin RIA kit. Exposure of β-TC3 cells to a high concentration of glucose promoted insulin secretion compared to exposure to a low concentration of glucose when

Table I. Cell cycle distributions of different groups.

Group	Cell cycle phase		
	G_1 (%)	S (%)	G_2 (%)
Control siRNA+low-glucose	82.79	9.64	7.55
PLK1 siRNA+low-glucose	89.52	5.86	4.59
Control siRNA+high-glucose	79.28	9.87	10.82
PLK1 siRNA+high-glucose	82.00	9.07	8.90

PLK1, polo-like kinase 1; siRNA, small interfering RNA.

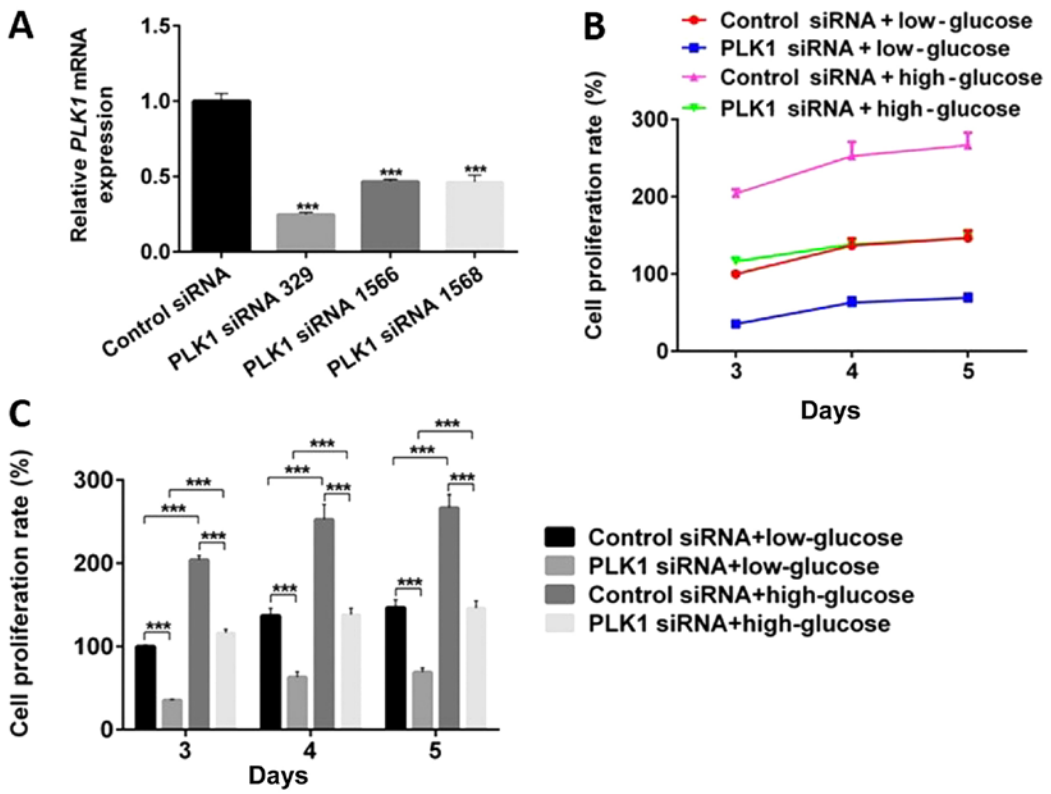

Figure 1. Knockdown of PLK1 inhibits β-TC3 cell proliferation. (A) PLK1 expression was detected by reverse transcription quantitative PCR in β-TC3 cells following transfection with PLK1 or control siRNA for 72 h. ***P<0.001 vs. control siRNA. Cell proliferation was (B) detected and (C) quantified in β-TC3 cells using the CCK-8 assay on days 3, 4 and 5 following transfection with PLK1 or control siRNA in low and high glucose conditions. ***P<0.001. PLK1, polo-like kinase 1; siRNA, small interfering RNA.

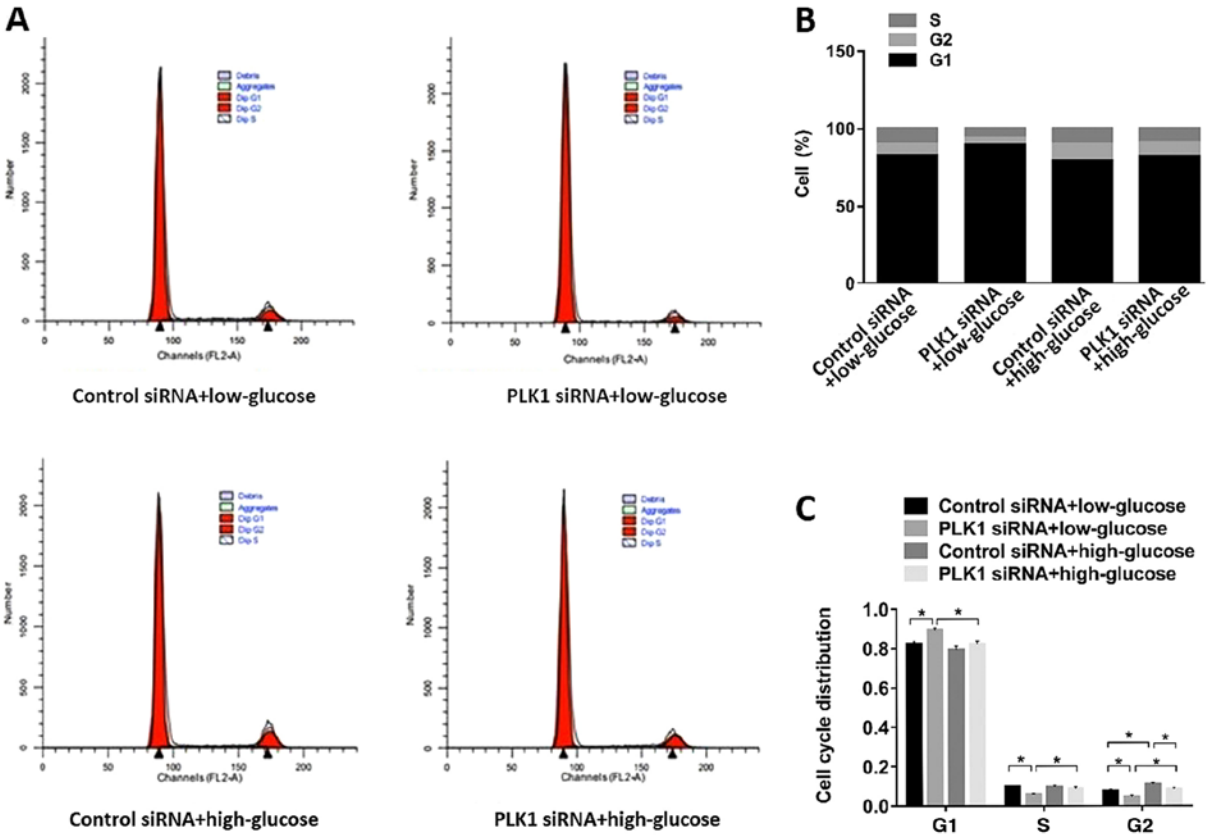

Figure 2. PLK1 siRNA regulates the cell cycle. (A) The cell cycle distribution was investigated by flow cytometry on the day 4 following transfection with PLK1 or control siRNA in low and high glucose conditions. (B) Quantification and (C) analysis of the cell cycle distribution. *P<0.05. PLK1, polo-like kinase 1; siRNA, small interfering RNA.

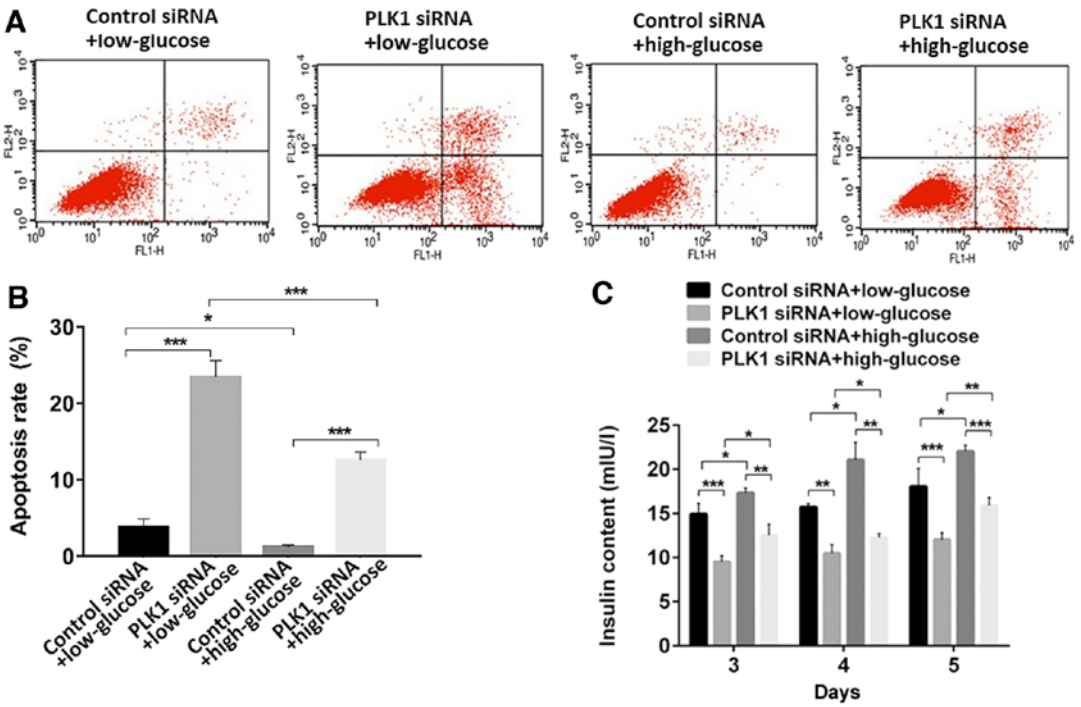

Figure 3. Downregulation of PLK1 affects β-TC3 cell apoptosis and insulin secretion. (A) Apoptosis was analyzed by flow cytometry and (B) quantified on day 4 following transfection with PLK1 or control siRNA in low and high glucose conditions. (C) Insulin secretion was determined by a radioimmunoassay on days 3, 4 and 5 following transfection with PLK1 or control siRNA in low and high glucose conditions. *P<0.05, **P<0.01, ***P<0.001. PLK1, polo-like kinase 1; siRNA, small interfering RNA.

transfected with control siRNA (P<0.05). Depletion of PLK1 inhibited insulin secretion under both low and high glucose conditions (P<0.05). These results indicated that PLK1 promotes insulin section in β-TC3 cells under both low and high glucose conditions (Fig. 3C).

Phospho-regulation of Cdc14A by PLK1 is involved in β-TC3 cell cycle regulation and insulin secretion under high glucose conditions. Cdc14A is a substrate of PLK1 (18). PLK1 phosphorylates Cdc14A, stimulating its phosphatase activity (18). In this study the interactions of these two proteins in β-TC3 cells were investigated. As shown in Fig. 4A and B, PLK1 silencing decreased Cdc14A expression in both low and high glucose conditions compared with control siRNA at 48 and 72 h after transfection (P<0.05). High glucose conditions promoted Cdc14A expression compared with low glucose conditions when transfected with control siRNA (P<0.05).

Serine351 and Serine 363 of Cdc14A are the phosphorylatable sites for PLK1 (18). Both serine residues were mutated to alanine in order to generate a GFP-tagged non-phosphorylatable Cdc14A, Cdc14A^{AA}, which exhibited significantly attenuated phosphatase activity (18). β-TC3 cell proliferation and insulin secretion was monitored under high glucose conditions on day 4 after transfection with the indicated siRNAs and plasmids. As shown in Fig. 4C, compared with the control siRNA and empty GFP-vector transfected groups, co-transfection with control siRNA and Cdc14A under high glucose conditions promoted β-TC3 cell proliferation, while transfection with Cdc14A^{AA} inhibited cell proliferation (P<0.05). When co-transfected with PLK1 siRNA, similar results were observed (P<0.05). Moreover, PLK1 siRNA partially reversed the proliferation-promoting effects of Cdc14A compared with those associated with the control siRNA + Cdc14A group or with the PLK1 siRNA + Cdc14A group (P<0.05). PLK1 silencing further intensified the proliferation inhibition of Cdc14A^{AA}-transfected cells compared with the control siRNA + Cdc14A^{AA} group or with the PLK1 siRNA + Cdc14A^{AA} group (P<0.05). These results indicated that the phospho-regulation of Cdc14A is involved in β-TC3 cell proliferation and that it is regulated by PLK1 under high glucose conditions.

As shown in Fig. 4D, PLK1 depletion or Cdc14A^{AA} overexpression inhibited β-TC3 cell insulin secretion compared with that in the control siRNA + GFP-vector group (P<0.05). Overexpression of wild type Cdc14A promoted insulin secretion compared to that in the control group under high glucose conditions and with that in the control siRNA + GFP-vector group (P<0.05). Cdc14A partially reversed the insulin inhibiting effect of PLK1 siRNA compared to that of the control siRNA + Cdc14A group and with that of the PLK1 siRNA + Cdc14A group (P<0.05). Cdc14A^{AA} showed a synergistic effect with PLK1 siRNA compared to that of the control siRNA + Cdc14A^{AA} group and with that of the PLK1 siRNA + Cdc14A^{AA} group (P<0.05). These results indicated that the phospho-regulation of Cdc14A by PLK1 is also involved in the regulation of β-TC3 cell insulin secretion under high glucose conditions.

Discussion

Type 2 diabetes has become a common disease due to lifestyle factors and poses a serious threat to human health. A lack of functional β-cells ultimately results in both type 1 and type 2 diabetes. Scientists are searching for more sources of islet cells

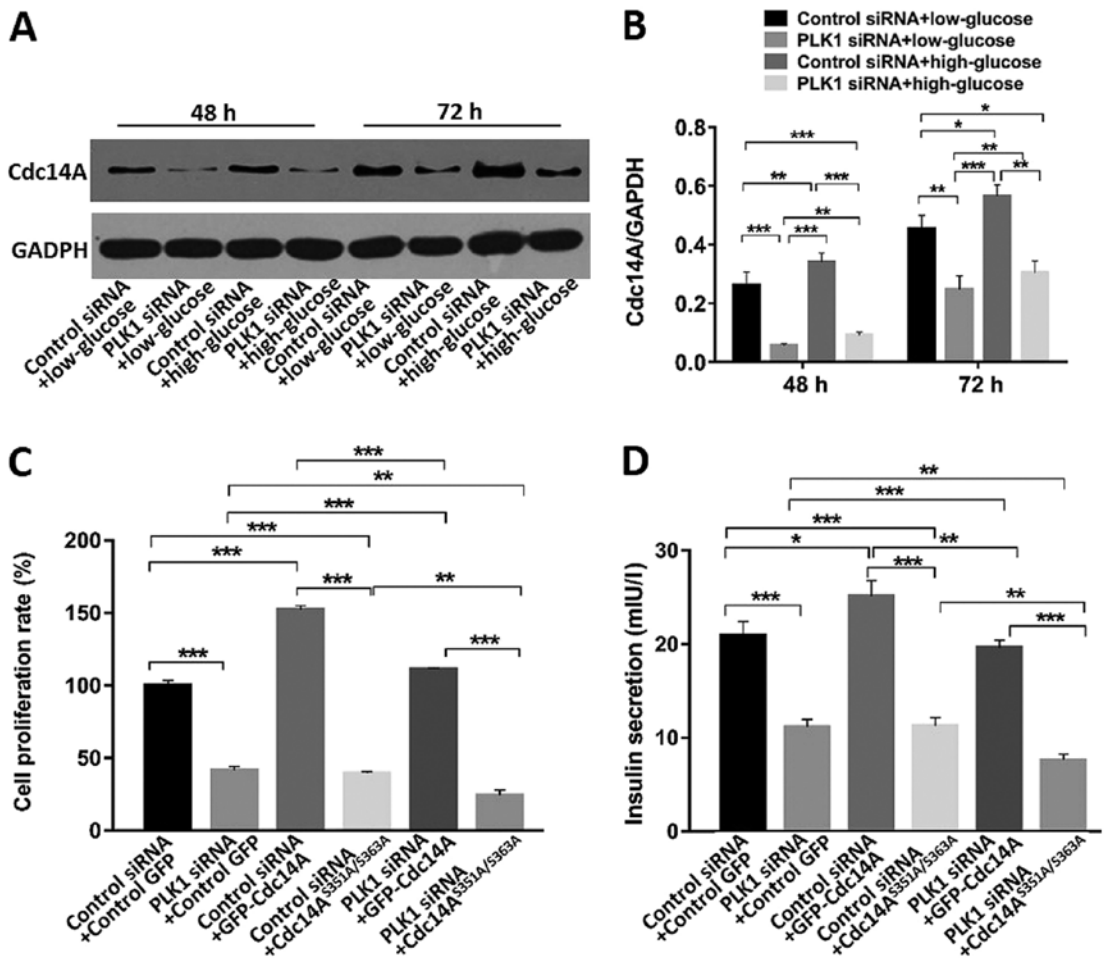

Figure 4. Phospho-regulation of Cdc14A by PLK1 is involved in β-TC3 cell cycle regulation and insulin secretion in high glucose conditions. Cdc14A expression was evaluated by (A) western blot analysis and (B) densitometry at 48 and 72 h after transfection with PLK1 or control short interfering RNA (siRNA) in low and high glucose conditions. GAPDH was used as an loading control. (C) Proliferation was determined on day 4 following transfection with the indicated siRNAs and plasmids under high glucose conditions. (D) Insulin secretion was analyzed on day 4 following transfection with the indicated siRNAs and plasmids under high glucose conditions. *P<0.05, **P<0.01, ***P<0.001. Cdc14A, cell division cycle 14A; Cdc14A$^{S351A/363A}$, non-phosphorylatable Cdc14A mutant; PLK1, polo-like kinase 1; siRNA, small interfering RNA.

or β-cells to treat diabetes; however, cadaveric islet cell transplantation remains promising (19). Although sources of newly formed islet cells remain elusive, it is clear that β-cells have the capacity to replicate themselves (21,22). Thus, it may be possible for diabetic patients to benefit from the manipulation of β-cell proliferation.

However, it is difficult to increase β-cell mass directly. The accumulation of double-stranded DNA damage may be observed when a substantial number of β-cells enter mitosis, resulting in apoptosis and not in functional β-cell expansion (23). More work is needed to control β-cell proliferation. New pathways and compounds have been identified by high-throughput screening methods and must be studied further. The G_1/S and G_2/M transitions are key cell cycle checkpoints that can be regulated. However, there is still an outstanding question as to whether these strategies affect other cell types within the body. Many pathways have been implicated, including the adenylyl cyclase/protein kinase A pathway, the mitogen-activated protein kinase pathway, the JAK-STAT pathway, the PI3-kinase-PKB/Akt pathway and the insulin receptor substrate-2 pathway. The basic cell cycle machinery of β-cells has been outlined in a previous review (24).

It is well known that acute high glucose stimulation promotes insulin secretion by β-cells. The compensatory increase in β-cell mass that is, in part, accounted for by β-cell replication corrects the transient hyperglycemia caused by sub-partial or partial pancreatectomy in rodents (25-27). Glucotoxicity is a phenomenon in which chronic hyperglycemia causes β-cell dysfunction and death (28,29). The precise molecular mechanisms underlying glucotoxicity are not completely understood.

Although PLK1 and Cdc14A are both essential for the cell cycle and are conserved in invertebrates and mammals, little is known about their crosstalk and mutual regulation in β-TC3 cells, especially under high glucose conditions.

The objective of this study was to provide a basis for understanding the mechanism underlying diabetes. The effects of PLK1 siRNA on cell proliferation, the cell cycle, apoptosis and insulin secretion in β-TC3 cells was investigated as was whether the phosphorylation of Cdc14A by PLK1 is involved in these processes. β-TC3 cells were treated in culture media with low or high concentrations of glucose. It was found that exposure of β-TC3 cells to high glucose for 3-5 days promoted cell proliferation, apoptosis and insulin release compared with exposure to a lower glucose

concentration. PLK1 siRNA inhibited β-TC3 cell proliferation, apoptosis and insulin release and decreased Cdc14A expression under both low and high glucose conditions. Cdc14A overexpression enhanced β-TC3 cell proliferation and insulin secretion under high glucose conditions. PLK1 silencing intensified the inhibition of proliferation and insulin secretion of $Cdc14A^{AA}$.

In this study, the effects of PLK1 siRNA on proliferation, apoptosis and insulin secretion were observed under both low and high glucose conditions. Therefore, the effects of PLK1 siRNA may only partially result from high glucose stimulation. Considering that high glucose alone stimulates β-TC3 cell proliferation and insulin secretion, the effect of PLK1 siRNA on insulin secretion may simply reflect effects on cell proliferation. More studies should be designed to test whether PLK1 is involved in glucose stimulation and insulin secretion. The results presented here are consistent with those of another study, in which PLK1 was significantly activated during β cell replication in individuals with type 2 diabetes and partial pancreatectomy-treated rats (4).

It was also found that PLK1 siRNA decreased the numbers of β-TC3 cells in the G_2- and S phases of the cell cycle while significantly increasing the G1 population under low glucose conditions. High glucose had the opposite effects, but only in G_2 cells. This finding may be because high glucose conditions have the opposite effect on β-TC3 cells. These results suggested that cell cycle regulation may be associated with the interaction of PLK1 siRNA and high glucose, which is a relationship that requires further study.

In this study, PLK1 silencing decreased Cdc14A expression. Cdc14A can be phosphorylated by PLK1, and this phosphorylation partially releases the self-inhibition of Cdc14A, thereby enhancing its phosphatase activity (18). Therefore, PLK1 silencing would change not only the expression but also the phosphorylation status of Cdc14A. More research is needed to investigate the crosstalk between Cdc14A and PLK1. It is possible that the phosphorylation of Cdc14A is involved in the stability or degradation of Cdc14A; alternatively, PLK1 may regulate the promoter activity of Cdc14A. The molecular mechanisms of the reduced expression of Cdc14A should be further explored and discussed.

The present study was limited due to the cell line type and techniques used in the experiments. Therefore, the isolation, purification and primary culture of human β-cells is required. Animal studies are also needed to test the crosstalk between Cdc14A and PLK1.

In conclusion, PLK1 and Cdc14A may play important roles in β-TC3 cell cycle regulation. PLK1 and Cdc14A promoted β-TC3 cell proliferation under high glucose conditions. Decreased insulin secretion was also observed when PLK1 was silenced. The phosphorylation of Cdc14A by PLK1 is involved in these processes. These findings may lead to new therapeutic strategies to understand how the number of β-cells is regulated and how this can be manipulated.

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Availability of data and materials

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

Authors' contributions

HH and JC designed the experiments. HH, LW, FH, XH, YL, XX, SZ and PZ performed the experiments. HH, DS, JL and JC analyzed the data. HH wrote the manuscript. All authors have read and approved the 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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