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Original Article

Divergent effects of lycopene on pancreatic alpha and beta cells

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Abstract: Lycopene is an antioxidant which has potential anti-diabetic activity, but the cellular mechanisms have not been clarified. In this study, different concentrations of lycopene were used to treat pancreatic alpha and beta cell lines, and the changes of cell growth, cell apoptosis, cell cycle, reactive oxygen species (ROS), ATP levels and expression of related cytokines were determined. The results exhibited that lycopene did not affect cell growth, cell apoptosis, cell cycle, ROS and ATP levels of alpha cells, while it promoted the growth of beta cells, increased the ratio of S phase, reduced the ROS levels and increased the ATP levels of beta cells. At the same time, lycopene treatment elevated the mRNA expression levels of *tnfα*, *tgfβ* and *hif1α* in beta cells. These findings suggest that lycopene plays cell-specific role and activates pancreatic beta cells, supporting its application in diabetes therapy.

Key words: lycopene; pancreatic alpha cell; pancreatic beta cell; cell cycle; reactive oxygen species

番茄红素对胰腺alpha和beta细胞的差异作用

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摘 要：番茄红素是一种强抗氧化剂，在糖尿病的治疗实验中显示出对机体的保护作用，但是其细胞机制尚不明确。本研究采用不同浓度梯度的番茄红素处理胰腺alpha和beta细胞系，检测细胞的生长、凋亡、周期、活性氧、ATP水平和相关细胞因子的表达变化。结果显示，番茄红素不影响alpha细胞的生长、凋亡、周期、活性氧和ATP水平，但番茄红素可以促进beta细胞的生长、上调其S期比例、降低活性氧水平并提升ATP水平。与此同时，番茄红素可以提升beta细胞*tnfα*、*tgfβ*和*hif1α* mRNA的表达。这些结果表明番茄红素的作用具有细胞特异性，可以活化胰腺beta细胞，为其在糖尿病防治的临床应用提供数据支撑。

关键词：番茄红素；胰腺alpha细胞；胰腺beta细胞； 细胞周期；活性氧
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Lycopene is a potent antioxidant and primarily found in red fruits and vegetables such as tomatoes [1]. Lycopene is considered non-toxic and safe supplement which has potential anti-diabetic effects [2]. Lycopene could ameliorate diabetes in streptozotocin (STZ)-induced pancreatic beta cell injury models [3–5]. Lycopene could also reverse the harmful effects of type 2 diabetes mellitus (T2DM) in rat models [6–8]. However, the cellular functions and mechanisms of lycopene in diabetes need to be further clarified. Pancreatic beta cell dysfunction was a key factor of diabetic animal models [9]. At the same time, pancreatic alpha cells also play important roles during diabetes [10]. Therefore, this study was designed to study the effects of lycopene on pancreatic alpha and beta cells.

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1 MATERIALS AND METHODS

1.1 *Cell culture*

AlphaTC1 and Beta-TC-6 cells were obtained from American Type Culture Collection (Rockville, MD, USA). AlphaTC1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 000 mg/L glucose and supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotic-antimycotic solution (100 U/mL penicillin and 100 μg/mL streptomycin). Beta-TC-6 cells were maintained in DMEM containing 4 500 mg/L glucose and supplemented with 15% FBS and antibiotic-antimycotic solution at 37 °C in a humidified atmosphere of 5% CO_2.

1.2 *Determination of cell viability*

Cell proliferation was monitored in real time by Real-Time Cell Analyzer (RTCA, xCELLigence, Roche). Briefly, 2×10^4 cells/well were seeded in E-plates 96. Lycopene and DMSO as vehicle control were added after cell attachment. Lycopene was kindly provided by Dr. Tian-Gang LIU (School of Pharmaceutical Sciences, Wuhan University, China). The final concentrations of lycopene were 0, 2.5, 5, 7.5, and 10 μmol/L. The E-plates 96 were monitored every 30 min for 72 h. Data analysis was performed using Real-Time Cell Analyzer software supplied with the instrument.

1.3 *Cell apoptosis detection*

Annexin V-FITC/propidium iodide (PI) apoptosis kit was used to detect cell apoptosis. Cells in 6-well plate were treated with various concentrations of lycopene (0, 2.5, 5, 7.5, 10 μmol/L) and DMSO as vehicle control for 24 h. Cells were detached by trypsin and harvested. After washing with PBS, the cells were stained with Annexin V-FITC and PI at 20 °C for 10 min according to manufacturer's instructions. The cell suspensions were immediately analysed by a flow cytometer (FACSAriaIII, Becton-Dickinson, San Jose, CA, USA).

1.4 *Cell cycle analysis*

Cells in 6-well plate were washed with PBS, and then cultured in 0.1% FBS medium with various concentrations of lycopene (0, 2.5, 5, 7.5, 10 μmol/L) and DMSO as vehicle control for 24 h. Cells were harvested and fixed in 70% ethanol at −20 °C overnight. After washing with PBS, the cells were incubated with PI (50 μg/mL) and RNase A (100 μg/mL) at 4 °C for 30 min. The cell suspensions were analysed by the flow cytometer, and the results were analyzed using Modifit software.

1.5 *Measurement of intracellular reactive oxygen species (ROS) levels*

Flow cytometry was used to assess the intracellular amounts of ROS with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Briefly, cells were washed with PBS and incubated with 10 μmol/L DCFH-DA at 37 °C for 30 min. After washed thrice with serum-free medium, cells were divided into 6 groups and treated with different concentrations of lycopene. Intracellular production of ROS was measured by flow cytometer with an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The DCF fluorescence intensity indicates the amount of intracellular ROS and the results were analyzed by mean fluorescent intensity (MFI).

1.6 *ATP detection assay*

Luminescent ATP Detection Assay Kit (Abcam) was used to measure the level of ATP within the cell. Cells in 6-well plate were treated with various concentrations of lycopene (0, 2.5, 5, 7.5, 10 μmol/L) and DMSO as vehicle control for 30 min. Cells were collected, and the levels of ATP were determined according to manufacturer's instructions.

1.7 *Quantitative RT-PCR*

RNA was extracted using TRIzol method and reversely transcribed to cDNA using RT kit (Invitrogen). Quantitative PCR was performed on Bio-Rad CFX96 System using the SYBR Green PCR Master Mix (TaKaRa). The relative RNA levels of each gene were normalized to *β-actin*. Primers used were as follows: *nfκb*: 5'-CGA GGC AGC ACA TAG ATG AAC-3' and 5'-CTG AGT TTG CGG AAG GAT GTC-3'; *tnfα*: 5'-CCT CAC ACT CAG ATC ATC-3' and 5'-AAC CTG GGA GTA GAC AAG-3'; *tgfβ*: 5'-GAA CCA AGG AGA CGG AAT AC-3' and 5'-CCA TGA GGA GCA GGA AGG-3'; *hif1α*: 5'-AAC GTG GAA GGT GCT TCA CT-3' and 5'-GAG CGG CCC AAA AGT TCT TC-3'; *glucagon*: 5'-AGA CAG AAG CGC ATG AGG AC-3' and 5'-TGG CAA TGT TGT TCC GGT TC-3'; *insulin*: 5'-ATG TCC CGC CGT GAA GTG-3' and 5'-AGT GCC AAG GTC TGA AGG TC-3'; *β-actin*: 5'-TGA AGA TCA AGA TCA TTG CTC CTC-3' and 5'-CCT GCT TGC TGA TCC ACA TC-3'.

1.8 *Statistical analysis*

Data were expressed as the mean ± standard error of the mean (SEM). Group results were compared by one-way ANOVA test. $P < 0.05$ was considered statistically significant.

2 RESULTS

2.1 Lycopene promoted the growth of beta cells

To investigate the effects of lycopene on cell growth of AlphaTC1 and Beta-TC-6 cells, cells were cultured in RTCA system after optimization of cell seeding density. Lycopene did not affect the growth curve of AlphaTC1 cells (Fig. 1*A*). However, the growth curve of Beta-TC-6 cells showed lycopene dosage dependent elevation (Fig. 1*B*). Beta-TC-6 cells treated with 5, 7.5, and 10 μmol/L lycopene exhibited significant higher growth than untreated blank control and DMSO vehicle control. Altogether, lycopene could promote the growth of beta cells, while had no effect on the growth of alpha cells. In order to explain the increased growth index, cell apoptosis was further determined by Annexin V and PI staining. No significant differences of Annexin V^+ PI^- (early apoptotic) and Annexin V^+ PI^+ (late apoptotic/necrotic) cell ratios among groups were observed in both alpha and beta cells (Fig. 1*C*, *D*). Therefore, lycopene could promote the growth of beta cells by increasing cell proliferation without affecting apoptosis.

To determine the effects of lycopene on AlphaTC1 and Beta-TC-6 cell cycle shift, cells were cultured in

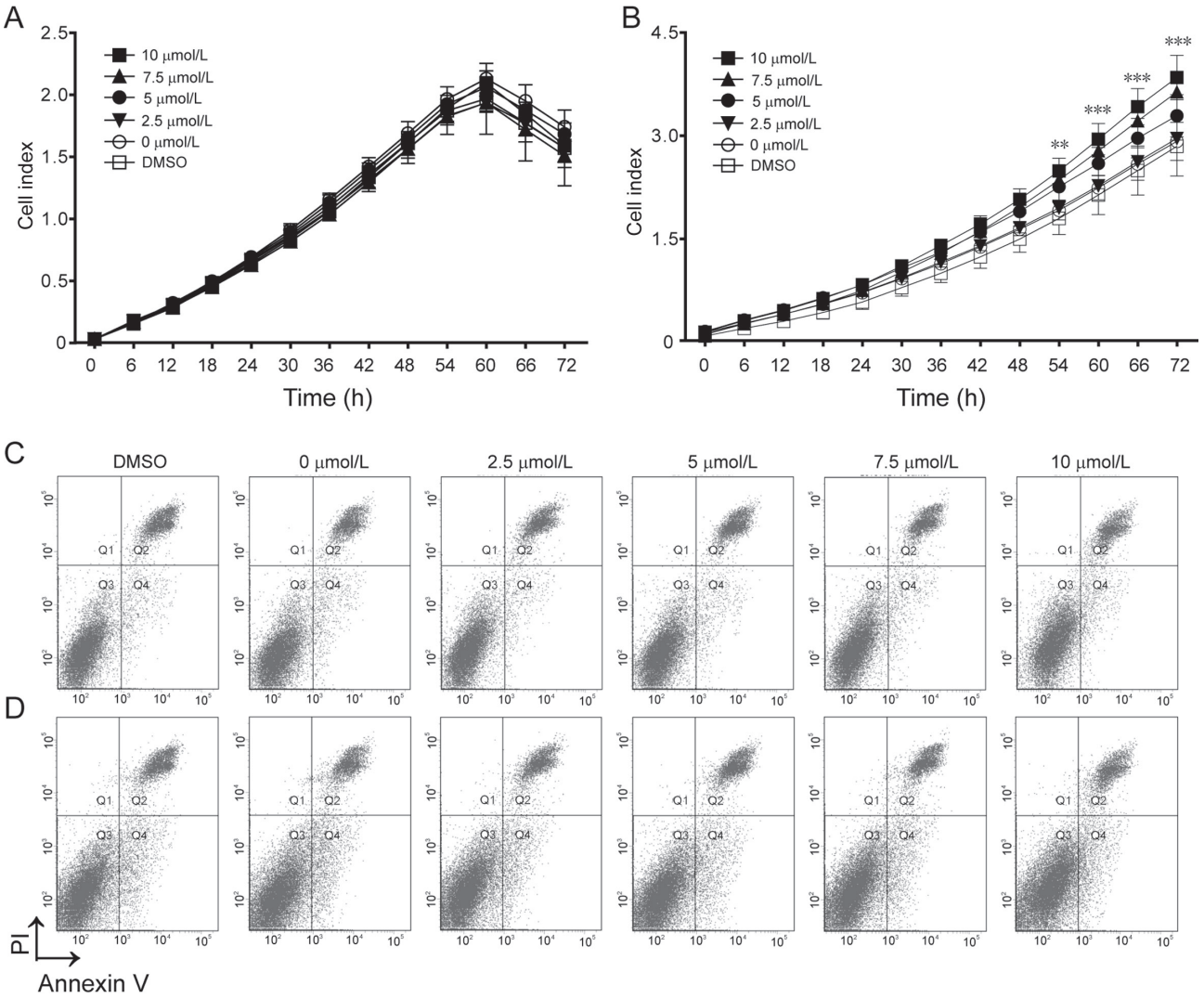

Fig. 1. Real-Time Cell Analyzer (RTCA) and apoptosis analysis of lycopene treated AlphaTC1 and Beta-TC-6 cells. AlphaTC1 (*A* & *C*) and Beta-TC-6 (*B* & *D*) cells were treated with different concentrations of lycopene and DMSO as vehicle control. Cell index detected by RTCA system was shown in *A* & *B*. The statistical differences were analyzed between lycopene and control (vehicle and blank controls) groups. The cell index in 5, 7.5, and 10 μmol/L lycopene groups exhibited significant differences compared with that in control groups in Beta-TC-6 cells. Mean ± SEM, $n = 3$. $^{**}P < 0.01$; $^{***}P < 0.001$ *vs* vehicle and blank controls. Cell apoptosis detected by flow cytometry was shown in *C* & *D*.

the presence of increasing concentrations of lycopene for 24 h. (61.1 ± 0.1)% AlphaTC1 cells were in G0/G1 phase, (10.8 ± 0.3)% in S and (28.1 ± 0.5)% in G2/M phase in DMSO group (Fig. 2*A*). Following exposure to lycopene, AlphaTC1 cells showed no significant shift of the phases. At the same time, Beta-TC-6 cells exhibited lycopene dosage dependent increase of S phase, while the portions of cells in G0/G1 and G2/M phases decreased (Fig. 2*B*). (67.6 ± 1.5)% Beta-TC-6 cells were in G0/G1 phase, (2.5 ± 0.4)% in S and (29.9 ± 1.9)% in G2/M phase in DMSO group. Beta-TC-6 cells in S phase increased from (6.6 ± 0.2)% to (16.5 ± 0.3)% when the concentration of lycopene increased from 2.5 μmol/L to 10 μmol/L. Meanwhile, Beta-TC-6 cells in G0/G1 phase decreased from (66.9 ± 0.1)% to (60.9 ± 1.3)% and cells in G2/M phase decreased from (26.5 ± 0.2)% to (22.6 ± 1.6)% (Fig. 2*B*) when the concentration of lycopene increased from 2.5 μmol/L to 10 μmol/L. Therefore, our data demonstrated that lycopene could promote entry into S phase of beta cells and did not affect cell cycle of alpha cells.

2.2 Lycopene changed ROS and ATP levels of beta cells

Since lycopene is an antioxidant, the ROS levels of AlphaTC1 and Beta-TC-6 cells after lycopene treatment were assessed. The MFI showed no difference among lycopene and control groups in AlphaTC1 cells (Fig. 3*A*). At the same time, the MFI exhibited lycopene dosage dependent decrease in Beta-TC-6 cells after 15 min treatment (Fig. 3*B*). When the lycopene treatment last longer, the MFI decreased in all groups and the differences between lycopene groups and control groups became smaller. Nevertheless, the data revealed lycopene could efficiently reduce the ROS levels of beta cells. At the same time, the ATP levels in AlphaTC1 cells maintained unaltered, while those in Beta-TC-6 cells increased after lycopene treatment (Fig. 3*C*, *D*).

2.3 Lycopene increased cell cytokine production in beta cells

To investigate the mechanism for the divergent effects of lycopene on alpha and beta cells, the mRNA expression levels of some critical factors were detected. The mRNA levels of *nfκb* showed no significant change after lycopene treatment in both alpha and beta cells. The mRNA levels of *glucagon* in AlphaTC1 cells and *insulin* in Beta-TC-6 cells also had no significant change after lycopene treatment. At the same time, the mRNA levels of *tnfα*, *tgfβ* and *hif1α* increased in Beta-TC-6 cells after lycopene treatment. In the 5, 7.5 and 10 μmol/L lycopene groups, the levels of *tnfα*, *tgfβ* and *hif1α* were significantly higher than those in blank and DMSO vehicle controls (Fig. 4*B*). Moreover, the mRNA levels of *hif1α* showed lycopene dosage dependent increase in Beta-TC-6 cells. However, the mRNA levels of *tnfα*, *tgfβ* and *hif1α* exhibited no significant change after lycopene treatment in AlphaTC1 cells (Fig. 4*A*). Together, these data showed that lycopene could promote *tnfα*, *tgfβ* and *hif1α* mRNA expression in beta cells.

3 DISCUSSION

In this study, the proliferation of pancreatic alpha cells

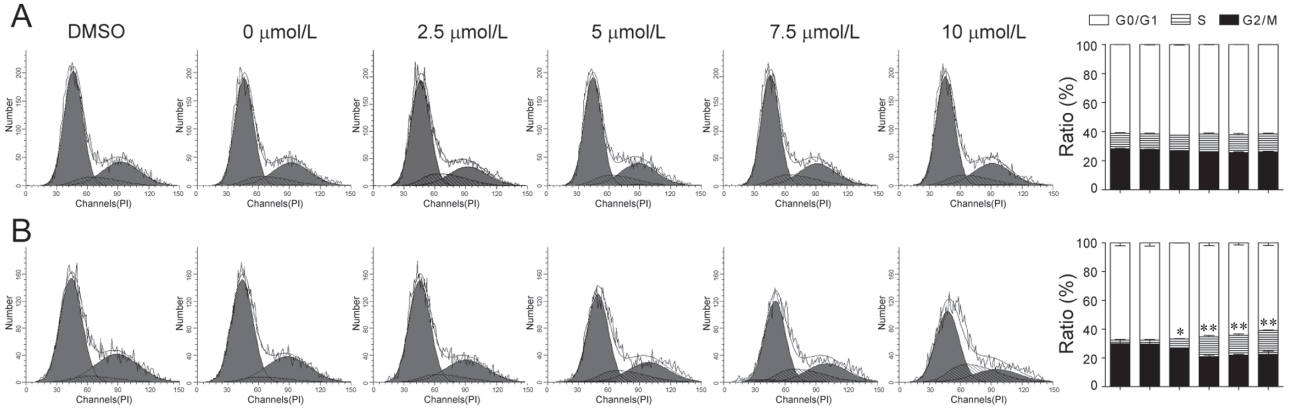

Fig. 2. Cell cycle analysis of lycopene treated AlphaTC1 and Beta-TC-6 cells. AlphaTC1 (*A*) and Beta-TC-6 (*B*) cells were treated with different concentrations of lycopene and DMSO as vehicle control for 24 h. Representative graphs of DNA contents analyzed by FACS and percentages of each cell cycle phase were shown. Mean ± SEM, $n = 3$. $^{*}P < 0.05$; $^{**}P < 0.01$ *vs* vehicle and blank controls.

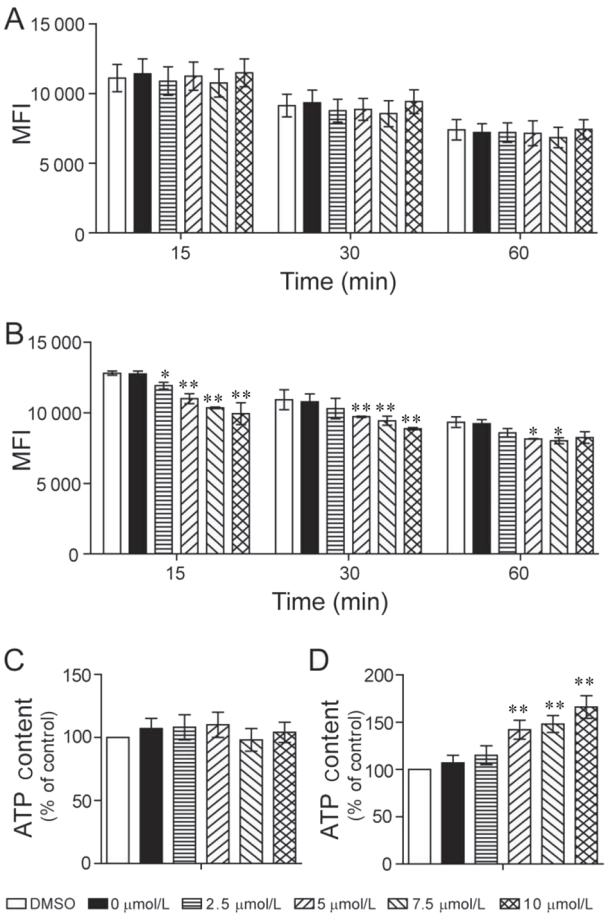

Fig. 3. ROS and ATP levels of lycopene treated AlphaTC1 and Beta-TC-6 cells. AlphaTC1 (*A*) and Beta-TC-6 (*B*) cells were treated with different concentrations of lycopene and DMSO as vehicle control for 15, 30 and 60 min, and the mean fluorescent intensity (MFI) indicating the amount of intracellular ROS detected by FACS was shown. ATP levels of AlphaTC1 (*C*) and Beta-TC-6 cells (*D*) treated with different concentrations of lycopene and DMSO for 30 min were shown. The statistical differences were analyzed between lycopene and control (vehicle and blank controls) groups. Mean ± SEM, $n = 3$. $^{*}P < 0.05$; $^{**}P < 0.01$ *vs* vehicle and blank controls.

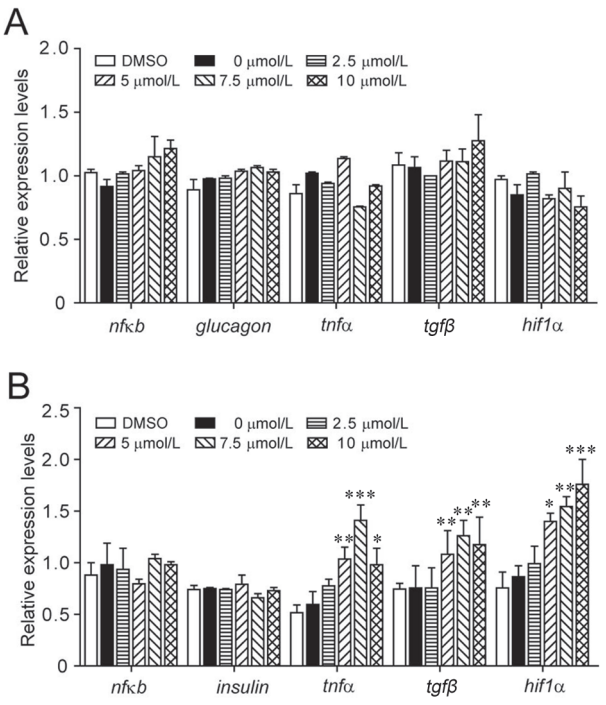

Fig. 4. Quantitative PCR analysis of some critical factors in lycopene treated AlphaTC1 and Beta-TC-6 cells. AlphaTC1 (*A*) and Beta-TC-6 (*B*) cells were treated with different concentrations of lycopene and DMSO as vehicle control for 60 min. Relative mRNA levels were shown. Mean ± SEM, $n = 3$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs* vehicle and blank controls.

was unresponsive to lycopene, while beta cell proliferation was elevated by lycopene. It has been well established that lycopene has anti-proliferation activities in various types of cancer cells including pancreatic cancer cell PANC-1, breast cancer, lung cancer, prostate and endometrial cancer [11–13]. However, it was also reported that 2 μmol/L lycopene did not affect the cell viability of pancreatic exocrine cells (AR42J), while 10 μmol/L lycopene could increase the cell viability [14]. Altogether, these data reveal the cell type specific effects of lycopene. Multiple targets have been reported for lycopene, such as platelet-derived growth factor receptor (PDGFR), insulin-like growth factor-IR (IGF-IR), and vascular endothelial growth factor receptor (VEGFR) mediated signal transduction pathways [15]. The different responses of alpha and beta cells to lycopene may be due to different activating statuses of these pathways. Since lycopene accounts for about 50% of carotenoids in human serum [16], our study also represents a step toward a better understanding of the role of lycopene in islet endocrine.

The NFκB pathway was reported critical in lycopene mediated signaling [15]. However, the mRNA levels of *nfκb* maintained unchanged after lycopene treatment in both alpha and beta cells. In this study, beta cells had increased mRNA levels of *tnfα* and *hif1a* after lycopene treatment. It is reported that lycopene could ameliorate oxidative stress and inflammation status [5]. Since *tnfα* plays critical roles in beta cell apoptosis [17, 18], other inflammatory related factors need to be further checked after lycopene treatment. It is reported that β cell specific *hif1a* knockout mice had unaltered β cell mass and insulin content, but decreased ATP concentrations

in islets [19]. Therefore, the increased ATP levels in beta cells shown in this study may be associated with increased *hif1a* mRNA levels after lycopene treatment. Although the signaling pathways of lycopene need to be further studied, our data suggested different alteration of signaling in pancreatic alpha and beta cells by lycopene treatment.

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