

Original Article

Pyruvate kinase, muscle isoform 2 promotes proliferation and insulin secretion of pancreatic β -cells via activating Wnt/CTNNB1 signaling

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Abstract: Failure of pancreatic β -cells is closely associated with type 2 diabetes mellitus (T2DM), an intractable disease affecting numerous patients. Pyruvate kinase, muscle isoform 2 (PKM2) is a potential modulator of insulin secretion in β -cells. This study aims at revealing roles and possible mechanisms of PKM2 in pancreatic β -cells. Mouse pancreatic β -cell line NIT-1 was used for high glucose treatment and PKM2 overexpression by its specific expression vector. Cell proliferation by Thiazolyl blue assay, cell apoptosis by annexin V-fluorescein isothiocyanate/prodium iodide staining and insulin secretion assay by ELISA were performed in each group. The mRNA and protein levels of related factors were analyzed by real-time quantitative PCR and western blot. Results showed that Pkm2 was inhibited under high glucose conditions compared to the untreated cells ($P < 0.01$). Its overexpression significantly suppressed NIT-1 cell apoptosis ($P < 0.01$), and induced cell proliferation ($P < 0.05$) and insulin secretion ($P < 0.05$). Related factors showed consistent mRNA expression changes. Protein levels of β -catenin (CTNNB1), insulin receptor substrate 1 (IRS1) and IRS2 were all promoted by PKM2 overexpression ($P < 0.01$), indicating the activated Wnt/CTNNB1 signaling. These results indicated the inductive roles of PKM2 in pancreatic β -cell NIT-1, including promoting cell proliferation and insulin secretion, and inhibiting cell apoptosis, which might be achieved via activating the Wnt/CTNNB1 signaling and downstream factors. This study offers basic information on the role and mechanism of PKM2 in pancreatic β -cells, and lays the foundation for using PKM2 as a potential therapeutic target in T2DM.

Keywords: PKM2, pancreatic β -cell, insulin secretion, type 2 diabetes mellitus, Wnt/CTNNB1

Introduction

Diabetes mellitus is a troublesome disease which can cause a series of complications including heart disease, diabetic retinopathy and kidney failure, affecting numerous patients worldwide. Type 2 diabetes mellitus (T2DM) is a major form of diabetes mellitus, occupying about 95% of the diabetes population [1]. While type 1 diabetes mellitus is definitely due to the lack of insulin caused by failure of pancreatic islet cells, T2DM is characterized by its insulin resistance superimposed on the lack of insulin [2]. But the proportion of the two causes may vary among individuals, with some primarily showing insulin resistance and others the lack of insulin. Pharmacological research has dis-

covered many agents, such as metformin, the most commonly used agent for overweight patients, and glyburide, to control T2DM [3]. Insulin and insulin analogue therapy have been applied to T2DM treatment and acquired good results [4, 5]. Another promising treatment option is stem cell therapy, with a study showing the safety and efficacy of autologous stem cell implantation in most tested T2DM patients [6]. However, efforts are still needed in the exploration for better solutions and the explanation for elaborate mechanisms of T2DM.

The failure of pancreatic β -cells is involved in the pathological mechanism of T2DM. β -cells are sensitive to all kinds of cellular stresses, especially the glucose concentration under dia-

betic conditions. Chronic hyperglycemia induces β -cell apoptosis and decreases β -cell mass, and it handicaps insulin secretion via inhibiting insulin gene transcription factors like v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A and pancreatic and duodenal homeobox 1 (PDX1) [7]. Dysfunctional β -cells result in the low insulin secretion stimulated by glucose and the accelerated development of hyperglycemia and T2DM [8]. In this manner, it is of great significance to maintain a normal status of β -cells and improve the production of insulin in T2DM to an effective level.

Pyruvate kinase, muscle (PKM) is encoded by Pkm gene, whose alternative splicing generates two transcripts, Pkm1 and Pkm2 [9]. PKM-2 is increased in tumor cells [10] and its dimer acts as an active protein kinase, phosphorylating STAT3 and thus increasing transcription of MEK5, to promote tumor growth [11]. Enlightened by a former study showing that L-cysteine decreases insulin secretion via dissociating PKM2 subunits [12], this study focused on PKM2, aiming at revealing its influences on β -cells and insulin secretion. Mouse pancreatic β -cell line NIT-1 was used in the experiments and treated with high glucose and PKM2 overexpression by the specific vector. Changes in cell proliferation, apoptosis and insulin secretion were detected. Related factors of these processes were also monitored to reflect the possible regulatory mechanisms of PKM2. This study would increase the likelihood for using PKM2 in T2DM treatment, and provide rudimentary information for its regulatory mechanisms.

Materials and methods

Cell culture and high glucose treatment

Mouse pancreatic β -cell line NIT-1 (Meilian Shengwu, Shanghai, China) in Roswell Park Memorial Institute 1640 medium (RPMI-1640, Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin and 0.1 mg/mL streptomycin was cultured in humid atmosphere with 5% CO₂ at 37°C. During the culturing process, the medium was changed every other day. The cells were grouped into Control group (without high glucose treatment) and HG group (treated with 25 mM high glucose, Sigma-Aldrich, Shanghai, China). In each group, the cells were further divided for overexpressing PKM2 or not.

Plasmid and transfection

At 72 h post high glucose treatment, cells of difference groups (Control, HG, Control + PKM-2, and HG + PKM2) were transfected with the empty vector (pcDNA3.1(+), Invitrogen, Carlsbad, CA) or the recombinant vector constructed by sub-cloning the coding sequence of Pkm2 into pcDNA3.1(+). The transfection process was performed using Lipofectamine 2000 reagent (Invitrogen) according to the manuals, and stable transfectants were screened using G418 (Gibco) selection.

Cell proliferation assay

Thiazolyl blue (MTT) method was used to analyze proliferation of NIT-1 cells after treatments. Before the MTT assay, cells at logarithmic growth phase were adjusted to concentration of 5×10^4 /mL. Cell suspension of 20 μ L was added to each well of the 96-well plate and cultured for another 24 h. Cell Proliferation Kit I (Roche, Basel, Switzerland) was used in the experiment according to the manuals. Briefly, 20 μ L RPMI-1640 medium containing 0.5 mg/mL MTT was added to each well and the cells were incubated for 4 h before 200 μ L of dimethyl sulfoxide was applied to terminate the reaction. Then the optical density was measured at 492 nm using SpectraMax i3x (Molecular Devices, Silicon Valley, CA).

Cell apoptosis assay

Cell apoptosis assay was conducted using dual staining by annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI). Before the analysis, 2×10^5 cells were seeded in each well of the 6-well plate. Annexin V: FITC Apoptosis Detection Kit II (BD Biosciences, San Jose, CA) was used in the experiment according to the manuals. Then cells were analyzed using BD FACSCanto II flow cytometer (BD Biosciences). The total of apoptotic cells were defined as the annexin V positive/PI negative cells.

Insulin secretion analysis

The insulin secretion was detected using Rat/Mouse Insulin ELISA Kit (Millipore, Billerica, MA) according to the manuals. The medium supernatant of each group was added to antibody-covered 96-well plate and incubated for insulin capture. Then the uncaptured insulin was washed out and house reddish peroxidase (HRP) was added, using tetramethylbenzidine

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Table 1. Primer used in qPCR

Primer	Sequence (5' to 3')
Pkm2-Fw	TGCCGTGACTCGAAATCCC
Pkm2-Rv	GGCCAAGTTTACACGAAGGTC
Ctnnb1-Fw	ATGGATCCGGACAGAAAAGC
Ctnnb1-Rv	CTTGCCACTCAGGGAAGGA
Ccnd1-Fw	CATCAAGTGTGACCCGGACTG
Ccnd1-Rv	CCTCCTCCTCAGTGGCCTTG
Bcl2-Fw	TAGAGAGATGCGAGGAACCGATG
Bcl2-Rv	ATAAGCAATCCCAGGGTCTGTCC
Pdx1-Fw	GCTAACCCCTGCGTGCCTGT
Pdx1-Rv	TTTCCTCGGGTCCGCTGTGTA
Slc2a2-Fw	GGTGGGTGGCTCGGGGACAAC
Slc2a2-Rv	CACGAGGATGGGCTGTCGGTAA
Gck-Fw	GTGTAAAGATGTTGCCACCTA
Gck-Rv	GGAGAAGTCCCACGATGTTGTT
Irs1-Fw	GCGCAGGCACCATCTCAACAACC
Irs1-Rv	GCACGCACCCGGAAGGAACC
Irs2-Fw	CTCTGACTATATGAACCTG
Irs2-Rv	ACCTTCTGGCTTTGGAGGTG
Gapdh-Fw	TCAACAGCAACTCCCACTTCCA
Gapdh-Rv	ACCCTGTTGCTGTAGCCGTATTCA

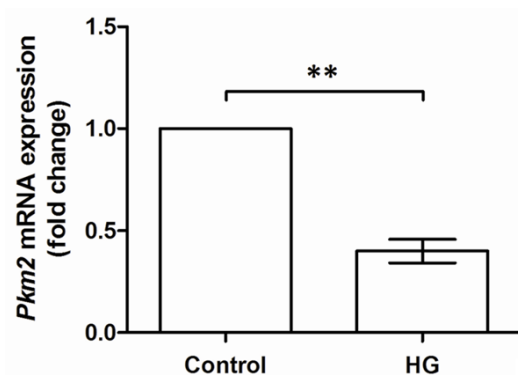


Figure 1. Transcription of *Pkm2* is inhibited in NIT-1 under high glucose conditions. Control, NIT-1 without high glucose treatment. HG, NIT-1 with high glucose (HG) treatment. ** $P < 0.01$. *Pkm2*, pyruvate kinase, muscle splicing variant 2.

as the substrate. After the reaction was terminated by 0.3 M HCl, the absorption peak at 450 nm, which was in direct proportion to the concentration of insulin, was measured and assessed based on the standard curve.

Real-time quantitative PCR (qPCR)

Treated cells of each group were collected and washed, then lysed in TRIzol (Invitrogen) for RNA extraction. The quality and quantity of extract-

ed RNAs were measured using agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific, Carlsbad, CA). RNAs of 1 μ g were applied to the synthesis of first strand complementary DNAs (cDNAs) using PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China). cDNAs of 20 ng and the gene-specific primers (**Table 1**) were included in each reaction system, which was conducted on LightCycler 480 (Roche). *Gapdh* was used as the internal reference. Experiments were conducted in triplicate and data were analyzed with the $2^{-\Delta\Delta Ct}$ method.

Western blot

Proteins were extracted from the treated cell of each group using protein lysis buffer (Beyotime, Shanghai, China). The same amounts of protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, after which the protein bands were transferred from the gel to a polyvinylidene difluoride membrane (Roche). The membrane was incubated in 5% skim milk for blocking at room temperature for 2 h. Then it was incubated in the specific primary antibodies (Abcam, Cambridge, UK) at 4°C overnight. GAPDH was used in this step as an internal reference. After washed, the membrane was incubated in HRP-conjugated secondary antibodies and ECL Plus Western Blotting Substrate (Thermo Scientific) was used to develop the signals. The density of bands was analyzed using ChemiDoc XRS System (Bio-Rad, Hercules, CA).

Statistical analysis

All experiments were performed at least in triplicate. The results were indicated as mean \pm standard deviation. Data were analyzed using one-way analysis of variance in SPSS 19.0 (IBM, New York, USA). Differences were considered significant if $P < 0.05$.

Results

PKM2 is inhibited under high glucose conditions

PKM2 is related to insulin secretion as aforementioned, thus in this study, its expression pattern in high glucose-treated pancreatic β -cell line NIT-1 was the first to be examined. qPCR was used to reflect the transcription level of *Pkm2* (**Figure 1**). In high glucose-treated NIT-1, the expression of *Pkm2* mRNA was lower compared to the untreated NIT-1, with significant differ-

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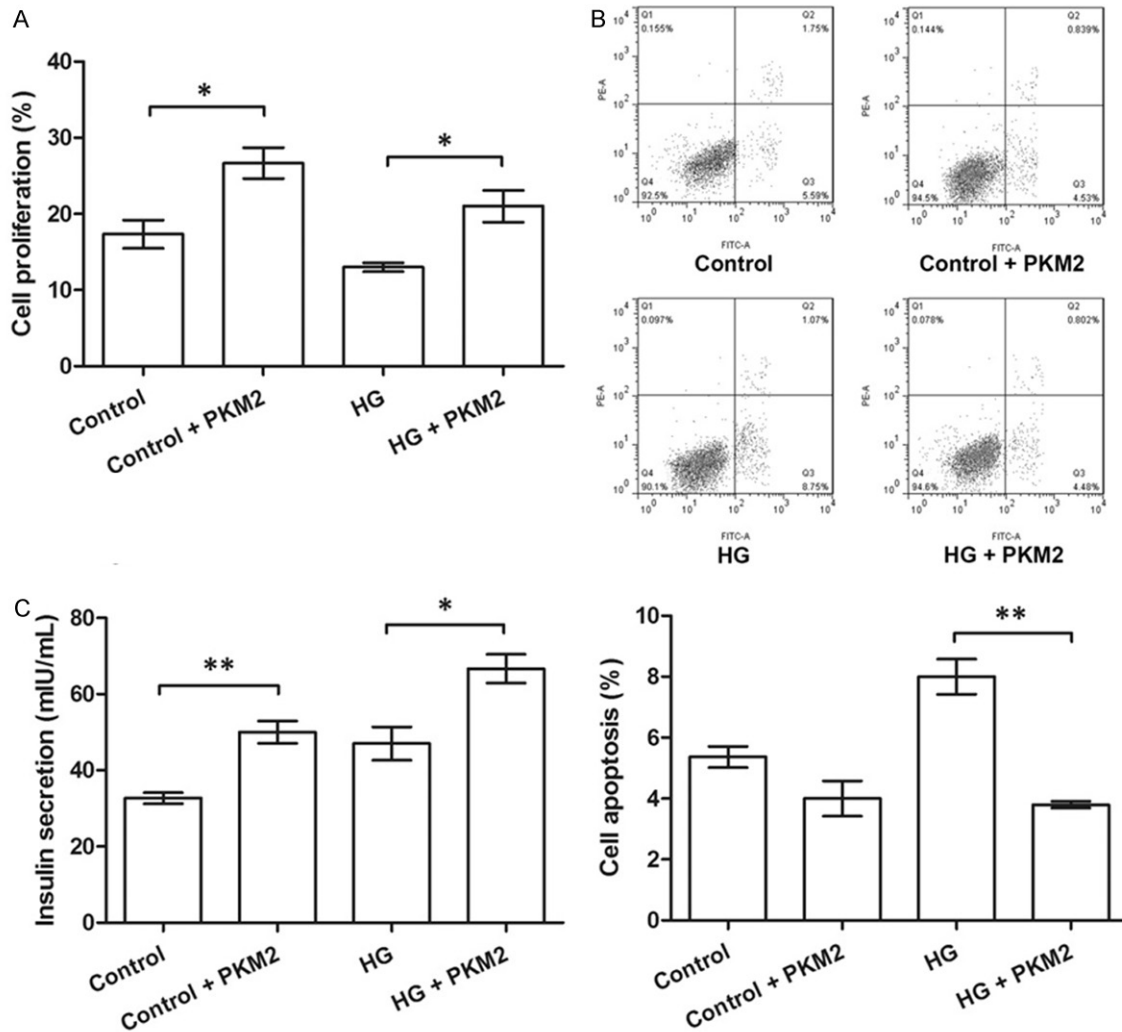


Figure 2. Influences of PKM2 on NIT-1. A. Overexpression of PKM2 promotes NIT-1 cell proliferation. B. Flow cytometry indicates overexpression of PKM2 inhibits NIT-1 cell apoptosis. The lower right quadrant indicates the percent of apoptotic cells. C. Insulin secretion is promoted by PKM2 overexpression. mIU, million International Unit. * $P < 0.05$. ** $P < 0.01$. HG, high glucose. PKM2, pyruvate kinase, muscle isoform 2.

ences ($P < 0.01$). This result inferred the aberrant expression of PKM2 in NIT-1 under high glucose conditions, suggesting the involvement of PKM2 in the function of pancreatic β -cells.

PKM2 suppresses apoptosis, induces proliferation and insulin secretion of NIT-1

Next, this study investigated the influence of PKM2 on NIT-1 by overexpressing PKM2 using its expression vector. Cell proliferation changes were detected by MTT assay (Figure 2A). Results showed that PKM2 could increase cell proliferation significantly ($P < 0.05$), regardless of the high glucose treatment, which also reflected the successful overexpression of PKM2. Consistently, the percent of apoptotic cells indi-

cated by annexin V-FITC and PI staining was decreased significantly by PKM2 overexpression in the high glucose-treated NIT-1 ($P < 0.01$), though no significant difference was detected in untreated NIT-1 ($P > 0.05$, Figure 2B). Insulin secretion detected by ELISA showed that high glucose could induce insulin secretion, comparing the untreated with the high glucose-treated NIT-1 (Figure 2C). Overexpression of PKM2 significantly increased the secretion of insulin in both high glucose-treated and untreated NIT-1 ($P < 0.05$ and $P < 0.01$).

The indices of cell growth, insulin secretion and β -cell proliferation were also examined from their transcription levels to verify these changes. Cell growth markers, including β -catenin

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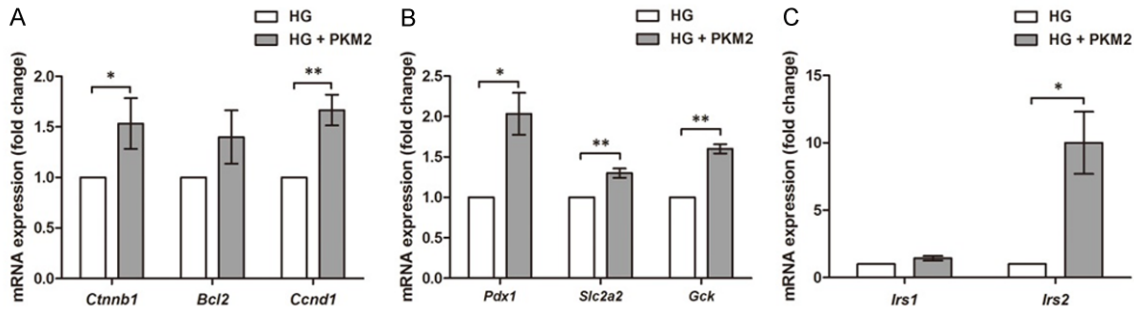


Figure 3. mRNA expression changes induced by PKM2. A. Cell growth indices Ctnnb1, Bcl2 and Ccnd1 are promoted by PKM2. B. Insulin secretion indices Pdx1, Slc2a2 and Gck are promoted by PKM2. C. Pancreatic β -cell proliferation indices Irs1 was slightly promoted and Irs2 was significantly promoted by PKM2. * $P < 0.05$. ** $P < 0.01$. HG, high glucose. PKM2, pyruvate kinase, muscle isoform 2. Ctnnb1, catenin (cadherin-associated protein), beta 1. Bcl2, B-cell CLL/lymphoma 2. Ccnd1, cyclin D1. Pdx1, pancreatic and duodenal homeobox 1. Slc2a2, solute carrier family 2, member 2. Gck, glucokinase. Irs, insulin receptor substrate.

(C-tnnb1), B-cell CLL/lymphoma 2 (Bcl2) and cyclin D1 (Ccnd1), were all promoted by PKM2 under high glucose conditions, with Ctnnb1 and Ccnd1 showing significantly increase ($P < 0.05$ and $P < 0.01$, **Figure 3A**). Insulin secretion indices, such as pancreatic and duodenal homeobox 1 (Pdx1), solute carrier family 2, member 2 (Slc2a2, previously named Glut2) and glucokinase (Gck) were also significantly promoted by PKM2 ($P < 0.05$ and $P < 0.01$, **Figure 3B**). Besides, insulin receptor substrate 1 (Irs1) and Irs2, two factors involving in β -cell proliferation, were examined, and results indicated that Irs1 expression was slightly changed with no significance ($P > 0.05$), while Irs2 expression was significantly promoted by PKM2 ($P < 0.01$, **Figure 3C**). The expression changes in these indices were in accordance with the observed cell proliferation and apoptosis, and insulin secretion changes, further confirming the influences of PKM2 on NIT-1. Taken together, PKM2 overexpression brought about great influences on NIT-1, especially under high glucose conditions, including the promoted cell proliferation, the suppressed cell apoptosis and the induced insulin secretion, implying PKM2 might benefit pancreatic β -cells from these aspects.

PKM2 functions via the Wnt/CTNNB1 signaling

Based on the research that PKM2 participates in the migration of colon cancer cells via Wnt/CTNNB1 pathway [13], it was of great possibility that PKM2 could modulate pancreatic β -cells via the similar mechanism. Thus protein expressions of CTNNB1 its downstream factors IRS1

and IRS2 were analyzed (**Figure 4A and 4B**). Results showed that CTNNB1 expression was significantly promoted by PKM2 ($P < 0.01$), consistent with its mRNA expression changes, indicating CTNNB1 was activated by PKM2. Protein levels of IRS1 and IRS2 were both promoted by PKM2 ($P < 0.01$ and $P < 0.001$), suggesting the activated CTNNB1 might further induce the activation of IRS1 and IRS2 translation. Taken together, CTNNB1 and its downstream factors were activated by PKM2, which implied that PKM2 was likely to execute its functions in NIT-1 via activating the Wnt/CTNNB1 signaling.

Discussion

In this study, PKM2 was found to be down-regulated in high glucose-treated pancreatic β -cells NIT-1. Its overexpression leads to the promotion of proliferation, the inhibition of apoptosis and the induction of insulin secretion of NIT-1. Related factor analysis revealed that roles of PKM2 were possibly achieved via the Wnt/CTNNB1 signaling.

PKM2 is a kind of pyruvate kinase, the key regulatory glycolytic enzyme dephosphorylating phosphoenol pyruvate into pyruvate, pivotal in the final step of glycolysis. PKM2 can be regulated by pantothenate kinase 4, which is up-regulated by high glucose conditions, thus possibly modulating the glucose metabolism [14]. The down-regulation of PKM2 found in this study in the high glucose-treated NIT-1 might imply that its roles were to some extent blocked in these cells, on the base of which the investigation on roles of PKMs was necessary.

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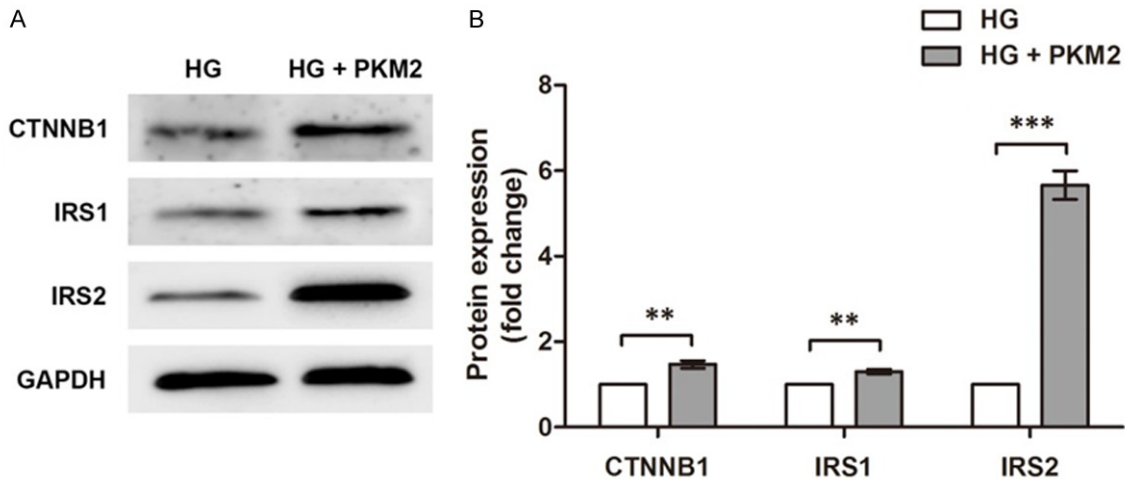


Figure 4. PKM2 functions via Wnt/CTNNB1 signaling. A. Western blot showing the protein expression changes of CTNNB1, IRS1 and IRS2. GAPDH was used as the internal reference. B. Histogram showing the relative intensity of bands in western blot. ** $P < 0.01$. *** $P < 0.001$. HG, high glucose. PKM2, pyruvate kinase, muscle isoform 2. CTNNB1, catenin (cadherin-associated protein), beta 1. IRS, insulin receptor substrate.

In high glucose-treated NIT-1, overexpression of PKM2 was able to increase cell proliferation, inhibit cell apoptosis and induce insulin secretion by NIT-1. Besides changes in cellular activities, key factors associated with these activities were also examined. Ctnnb1 is a gene encoding β -catenin, a member of Wnt signaling, participating in cadherin-mediated intercellular adhesion, which is also found to be related to cell growth by many studies, with its down-regulation inhibiting growth and inducing apoptosis of various kinds of cells [15, 16]. Bcl-2 and Ccnd1 possess similar functions involving regulating apoptosis and cell cycle, which have been proved in malignant glioma [17], pancreatic cancer [18], colon cancer [16], and squamous cell esophageal cancer [19]. Up-regulation of these factors is usually accompanied by induced cell growth and inhibited cell apoptosis, which is consistent with what was found in this study. Inhibition of Pdx1, Slc2a2 and Gck was related to suppressed insulin secretion, as is proven in studies of pancreatic β -cells [20-22]. The promoted expression of the three factors discovered in this study verified the induced insulin secretion by PKM2 in NIT-1. In addition, Irs1 and Irs2, two factors capable of increasing pancreatic β -cell proliferation and decreasing β -cell apoptosis [23], were also examined. The two factors are also vital regulators of glucose transport through activating their downstream phosphoinositide 3-kinases [24]. Consistent with the promoted proliferation

and inhibited apoptosis found by MTT assay and annexin V-FITC/PI staining, the two indices of cell proliferation and apoptosis were both induced by PKM2. Taken together, based on the analyses of cell activity and related factors, the roles of PKM2 in regulating pancreatic β -cells were confirmed: PKM2 could induce pancreatic β -cell proliferation and insulin secretion, and reduce cell apoptosis, suggesting its potential application to T2DM treatment.

In addition to the roles of PKM2 in pancreatic β -cells, the possible functioning mechanism of PKM2 was further investigated. Albeit the multiple factors examined in this study, CTNNB1 was chosen to be further analyzed from its protein expression changes due to its significant position in Wnt signaling [25, 26]. Ccnd1 is a target gene of Wnt signaling, transcriptionally activated by CTNNB1 [27]. Results found the protein level of CTNNB1 was also increased by PKM2 overexpression, which might lead to the promoted transcription of Ccnd1 observed by qPCR, implying the activation of Wnt/CTNNB1 signaling pathway. Protein levels of IRS1 and IRS2 were analyzed, since their transcription levels induced by PKM2 generated dubious results, with Irs1 showing no obvious changes, while Irs2 possessing significant increase. Their protein level changes were similar to their transcription patterns, but with more evident increases. So it could be considered that both IRS1 and IRS2 were up-regulated by PKM2 overexpression. As the important downstream

target genes of Wnt/CTNNB1 signaling [28, 29], their up-regulation further confirmed that the Wnt/CTNNB1 signaling was activated by PKM2 in NIT-1, suggesting the PKM2-activated Wnt/CTNNB1 signaling in high glucose-treated NIT-1.

The entry point of PKM2 into the Wnt/CTNNB1 signaling might be CTNNB1, since studies show that as the coactivator of CTNNB1, nucleus PKM2 interacts with phosphorylated CTNNB1 and activate epidermal growth factor receptor, thus promoting CCND1 [30]. Furthermore, c-Myc/hnRNPA1 signaling, hypoxia inducible factor 1 alpha, and insulin-like growth factor 1, amongst others, are upstream regulators of PKM2[31-33]. With all the intricate regulatory relationships, it is necessary to ascertain and integrate these associating pathways before the application of PKM2 in T2DM treatment.

In summary, this study uncovers the roles of PKM2 in pancreatic β -cell NIT-1. PKM2 inhibits NIT-1 cell apoptosis, promotes cell proliferation, as well as insulin secretion, which are achieved by its activation of Wnt/CTNNB1 signaling. This study reveals the possible mechanism of PKM2 in modulating pancreatic β -cells, providing the fundamental evidences for using PKM2 as a potential therapeutic target in T2DM treatment.

Disclosure of conflict of interest

None.

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References

- [1] Olokola AB, Obeateru OA and Olokola LB. Type 2 diabetes mellitus: A review of current trends. *Oman Med J* 2012; 27: 269-273.
- [2] Kahn CR. Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 1994; 43: 1066-1085.
- [3] Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill C, Zinman B and Viberti G. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; 355: 2427-2443.
- [4] Barnett AH. Complementing insulin therapy to achieve glycemic control. *Adv Ther* 2013; 30: 557-576.
- [5] Cameron CG and Bennett HA. Cost-effectiveness of insulin analogues for diabetes mellitus. *CMAJ* 2009; 180: 400-407.
- [6] Wang ZX, Cao JX, Li D, Zhang XY, Liu JL, Li JL, Wang M, Liu Y, Xu BL and Wang HB. Clinical efficacy of autologous stem cell transplantation for the treatment of patients with type 2 diabetes mellitus: a meta-analysis. *Cytotherapy* 2015; 17: 956-968.
- [7] Robertson RP. Beta-cell deterioration during diabetes: what's in the gun? *Trends Endocrinol Metab* 2009; 20: 388-393.
- [8] Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 2015; 6: 456-480.
- [9] Noguchi T, Inoue H and Tanaka T. The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *J Biol Chem* 1986; 261: 13807-13812.
- [10] Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL and Cantley LC. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 2008; 452: 230-233.
- [11] Gao X, Wang H, Yang JJ, Liu X and Liu ZR. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol Cell* 2012; 45: 598-609.
- [12] Nakatsu D, Horiuchi Y, Kano F, Noguchi Y, Sugawara T, Takamoto I, Kubota N, Kadowaki T and Murata M. L-cysteine reversibly inhibits glucose-induced biphasic insulin secretion and ATP production by inactivating PKM2. *Proc Natl Acad Sci U S A* 2015; 112: E1067-1076.
- [13] Yang P, Li Z, Wang Y, Zhang L, Wu H and Li Z. Secreted pyruvate kinase M2 facilitates cell migration via PI3K/Akt and Wnt/beta-catenin pathway in colon cancer cells. *Biochem Biophys Res Commun* 2015; 459: 327-332.
- [14] Li Y, Chang Y, Zhang L, Feng Q, Liu Z, Zhang Y, Zuo J, Meng Y and Fang F. High glucose up-regulates pantothenate kinase 4 (PanK4) and thus affects M2-type pyruvate kinase (Pkm2). *Mol Cell Biochem* 2005; 277: 117-125.
- [15] Xia H, Ng SS, Jiang S, Cheung WK, Sze J, Bian XW, Kung HF and Lin MC. miR-200a-mediated downregulation of ZEB2 and CTNNB1 differentially inhibits nasopharyngeal carcinoma cell growth, migration and invasion. *Biochem Biophys Res Commun* 2010; 391: 535-541.
- [16] Yoo JH, Lee HJ, Kang K, Jho EH, Kim CY, Baturen D, Tunsag J and Nho CW. Lignans inhibit cell growth via regulation of Wnt/beta-catenin signaling. *Food Chem Toxicol* 2010; 48: 2247-2252.

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- [17] Ito H, Kanzawa T, Kondo S and Kondo Y. Microtubule inhibitor D-24851 induces p53-independent apoptotic cell death in malignant glioma cells through Bcl-2 phosphorylation and Bax translocation. *Int J Oncol* 2005; 26: 589-596.
- [18] Wang Z, Azmi AS, Ahmad A, Banerjee S, Wang S, Sarkar FH and Mohammad RM. TW-37, a small-molecule inhibitor of Bcl-2, inhibits cell growth and induces apoptosis in pancreatic cancer: involvement of Notch-1 signaling pathway. *Cancer Res* 2009; 69: 2757-2765.
- [19] Jain M, Kumar S, Upadhyay R, Lal P, Tiwari A, Ghoshal UC and Mittal B. Influence of apoptosis (BCL2, FAS), cell cycle (CCND1) and growth factor (EGF, EGFR) genetic polymorphisms on survival outcome: an exploratory study in squamous cell esophageal cancer. *Cancer Biol Ther* 2007; 6: 1553-1558.
- [20] Iguchi H, Ikeda Y, Okamura M, Tanaka T, Urashima Y, Ohguchi H, Takayasu S, Kojima N, Iwasaki S, Ohashi R, Jiang S, Hasegawa G, Ioka RX, Magoori K, Sumi K, Maejima T, Uchida A, Naito M, Osborne TF, Yanagisawa M, Yamamoto TT, Kodama T and Sakai J. SOX6 attenuates glucose-stimulated insulin secretion by repressing PDX1 transcriptional activity and is down-regulated in hyperinsulinemic obese mice. *J Biol Chem* 2005; 280: 37669-37680.
- [21] Roccisana J, Reddy V, Vasavada RC, Gonzalez-Pertusa JA, Magnuson MA and Garcia-Ocaña A. Targeted inactivation of hepatocyte growth factor receptor c-met in beta-cells leads to defective insulin secretion and GLUT-2 downregulation without alteration of beta-cell mass. *Diabetes* 2005; 54: 2090-2102.
- [22] Li X, Shu YH, Xiang AH, Trigo E, Kuusisto J, Hartiala J, Swift AJ, Kawakubo M, Stringham HM, Bonnycastle LL, Lawrence JM, Laakso M, Allayee H, Buchanan TA and Watanabe RM. Additive effects of genetic variation in GCK and G6PC2 on insulin secretion and fasting glucose. *Diabetes* 2009; 58: 2946-2953.
- [23] Kahn SE, Hull RL and Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; 444: 840-846.
- [24] Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000; 106: 171-176.
- [25] Lague MN, Paquet M, Fan HY, Kaartinen MJ, Chu S, Jamin SP, Behringer RR, Fuller PJ, Mitchell A, Dore M, Huneault LM, Richards JS and Boerboom D. Synergistic effects of Pten loss and WNT/CTNNB1 signaling pathway activation in ovarian granulosa cell tumor development and progression. *Carcinogenesis* 2008; 29: 2062-2072.
- [26] Song J, Du Z, Ravasz M, Dong B, Wang Z and Ewing RM. A Protein Interaction between beta-Catenin and Dnmt1 Regulates Wnt Signaling and DNA Methylation in Colorectal Cancer Cells. *Mol Cancer Res* 2015; 13: 969-981.
- [27] Suriano G, Vrcelj N, Senz J, Ferreira P, Masoudi H, Cox K, Nabais S, Lopes C, Machado JC, Seruca R, Carneiro F and Huntsman DG. beta-catenin (CTNNB1) gene amplification: a new mechanism of protein overexpression in cancer. *Genes Chromosomes Cancer* 2005; 42: 238-246.
- [28] Bommer GT, Feng Y, Iura A, Giordano TJ, Kuick R, Kadikoy H, Sikorski D, Wu R, Cho KR and Fearon ER. IRS1 regulation by Wnt/beta-catenin signaling and varied contribution of IRS1 to the neoplastic phenotype. *J Biol Chem* 2010; 285: 1928-1938.
- [29] Gui S, Yuan G, Wang L, Zhou L, Xue Y, Yu Y, Zhang J, Zhang M, Yang Y and Wang DW. Wnt3a regulates proliferation, apoptosis and function of pancreatic NIT-1 beta cells via activation of IRS2/PI3K signaling. *J Cell Biochem* 2013; 114: 1488-1497.
- [30] Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W, Gao X, Aldape K and Lu Z. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature* 2011; 480: 118-122.
- [31] Luan W, Wang Y, Chen X, Shi Y, Wang J, Zhang J, Qian J, Li R, Tao T, Wei W, Hu Q, Liu N and You Y. PKM2 promotes glucose metabolism and cell growth in gliomas through a mechanism involving a let-7a/c-Myc/hnRNPA1 feedback loop. *Oncotarget* 2015; 6: 13006-13018.
- [32] Li W, Wang J, Chen QD, Qian X, Li Q, Yin Y, Shi ZM, Wang L, Lin J, Liu LZ and Jiang BH. Insulin promotes glucose consumption via regulation of miR-99a/mTOR/PKM2 pathway. *PLoS One* 2013; 8: e64924.
- [33] Salani B, Ravera S, Amaro A, Salis A, Passalacqua M, Millo E, Damonte G, Marini C, Pfeffer U, Sambucetti G, Cordera R and Maggi D. IGF1 regulates PKM2 function through Akt phosphorylation. *Cell Cycle* 2015; 14: 1559-1567.