

Roux-en-Y Gastric Bypass Improves Hepatic Glucose Metabolism Involving Upregulation of Sirt1 in Type 2 Diabetes Mellitus

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Background: Roux-en-Y gastric bypass (RYGB) is the most effective treatment for type 2 diabetes mellitus (T2DM). Previous studies have reported that silent information regulator 1 (Sirt1) closely relates to many pathological processes of glucose metabolism and insulin resistance (IR). However, it is unclear whether Sirt1 is involved in the hepatic glucose metabolism of T2DM after RYGB.

Methods: T2DM rats were randomly divided into four groups: Control, DM, Diet and RYGB. Normal rats were served as the control group. Hematoxylin and eosin (H&E) staining and Masson staining assays were performed to explore the changes of liver fibrous tissue after RYGB. The effect of RYGB on the protein expression of Sirt1 was detected by the Western blotting assay and immunohistochemical assay. Next, we built the insulin resistance model of human hepatocyte cell lines (FL62891 and HHL5) using the human recombinant insulin. Western blotting assay was applied to determine the expression of Sirt1 and the expression change of IRS1/mTOR2/PKB pathway-related proteins in FL62891 and HHL5 cells. Additionally, the effects of Sirt1 on the expression of PTP1B and FGF-21 in insulin-resistant FL62891 and HHL5 cells were investigated using Western blotting and immunofluorescence assay.

Results: Our results showed that following RYGB improved the pathological changes of liver and increased the expression of Sirt1 in rats with T2DM compared with the diabetic rats. In experiments in vitro, the expression of Sirt1 was downregulated in insulin-resistance FL62891 and HHL5 cells. Moreover, overexpression of Sirt1 significantly increased the expression of FGF-21 whereas decreased the expression of PTP1B in insulin-resistance FL62891 and HHL5 cells. These above changes were alleviated in RYGB and Diet groups. Furthermore, RYGB could improve the glucose metabolism through activating IRS1/mTOR2/PKB pathways by regulating Sirt1 in rats with T2DM.

Conclusion: RYGB could significantly improve hepatic glucose metabolism and increase the expression of Sirt1 in T2DM rats, which is related to the IRS1/mTOR2/PKB pathway.

Keywords: Roux-en-Y gastric bypass, RYGB, hepatic glucose metabolism, insulin resistance, Sirt1, IRS1, mTOR2, PKB

Introduction

Diabetes mellitus (DM) is a global and national health concern with high significance, which is also the major challenge for patients with DM.¹ It is the world's ninth leading cause of death.² Noticeably, type 2 diabetes mellitus (T2DM) accounts for about 90% of diabetes mellitus, for its complexity.^{3,4} T2DM is usually caused by insulin resistance (IR), which contributed to promoting glucose

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production in the liver and reduced glucose uptake in muscle and adipose tissue at a set insulin level.⁵ The development of T2DM involves many stages, including glucose tolerance impairment, insulin resistance, hyperinsulinemia, hyperglycemia, and insulin deficiency in most patients.⁶ However, insulin resistance and relative insulin deficiency have been considered essential to the etiology of T2DM. Therefore, insulin resistance has become the chief objective of the T2DM treatment.

Sirt1 is a histone deacetylase. As a multifunctional transcriptional regulator, it is closely related to cell survival, metabolic process and life activities including proliferation, senescence and apoptosis. It is widely involved in regulating multiple signal pathways of mammalian cell lifespan and multiple metabolic pathways such as glucose and lipid metabolism and insulin secretion to maintain the stability of glucose and lipid metabolism.^{7,8} It has been found that Sirt1 is expressed in liver, brain, kidney, fat and muscle tissues, and relatively uniformly expressed only in islet cells.⁸ And the expression of Sirt1 in the pancreas of mammals is significantly upregulated under the condition of calorie restriction (CR).⁹ When insulin resistance occurs in the early stage of T2DM, with the damage of β cells, the Sirt1 in the cells is significantly decreased. In addition, in the obese mice model of insulin resistance induced by high-fat (HF) or high-sucrose, the expression of Sirt1 in the pancreas decrease significantly.¹⁰ Therefore, Sirt1 may be a novel therapeutic target for the treatment of insulin resistance and T2DM.

Roux-en-Y gastric bypass (RYGB) is the most effective treatment to reduce body weight and improve glycaemic status including T2DM.^{11,12} The weight loss and improvement of glycaemic status after RYGB caused by RYGB induced rerouting of nutrients and improved the secretion of gastrointestinal hormones, such as ghrelin, peptide YY and glucagon-like peptide-1.¹³ RYGB helps to hyperinsulinemia and early normalization of glucose levels by promoting the hypersecretion of GLP-1 in T2DM.¹⁴ However, the role and molecular mechanism of RGYB on liver glucose metabolism are unclear. In conclusion, we speculate that Sirt1 may be involved in the therapeutic effect of RGYB on liver glucose metabolism and play a promoting role.

The objective of this study was to assess the pathological changes of liver, insulin resistance and the glucose metabolism after RYGB in rats. Further, we aimed to

investigate the molecular mechanism of RGYB on liver glucose metabolism.

Materials and Methods

Cell Culture

Human hepatic immortalized cell lines (FL62891 and HHL5) were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences, China. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS, Hyclone) and incubated in an incubator with 5% CO₂ at 37°C. The cells were passaged once every 3 days. Cells in logarithmic phase were used for the next study.

The insulin resistance models of liver cells FL62891 and HHL5 were treated with 10⁻⁷ mol/L human recombinant insulin and cultured for 24 h in an incubator with 5% CO₂ at 37°C. The insulin-resistant FL62891 and HHL5 cells have biological effects of insulin resistance.

Cell Transfection

The insulin-resistant FL62891 and HHL5 cells were seeded at a density of 2×10⁵ cells/mL in 6-well plates. The plasmid of Sirt1 was used for Sirt1 overexpression. Then, the overexpression plasmid of Sirt1 (pcDNA-Sirt1, 500ng/ μ l, GenePharma, Shanghai, China) and negative control (pcDNA-NC, GenePharma) were incubated in Opti-MEM (Invitrogen, Carlsbad, USA) using LipofectamineTM 2000 (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. The inhibitor NT157 of IRS1 (3 μ M, Beijing Bio Lebo Technology Co., Ltd, Beijing, China) was added in FL62891 and HHL5 cells.¹⁵

Hematoxylin and Eosin (H&E) and Masson Staining

Liver tissues were fixed with 10% buffered formalin at room temperature for 48 h. They are then dehydrated and embedded in paraffin. The sections were stained with hematoxylin and eosin H&E Masson's trichrome for histological evaluation. Blinded analysis of liver tissues was performed using a light microscope (Leica DM 500, Leica light microscope, Wetzlar, Germany). Moreover, tumor tissues pathological alterations were examined by a board-certified pathologist.

Immunohistochemistry Assay

For immunohistochemistry analysis, the fresh tumor tissue samples were fixed in formalin for 48 h. Then, the tissue

block was put into paraffin and cut into slides for the desired thickness in a microtome. Next, it was fixed into a slide. After washing, the samples were prepared for blocking and incubating with antibody which were diluted in 5% horse serum with chilled PBS at 4°C overnight. Isotype-matched IgG was used instead of primary antibody against Sirt1 (1:1000; ab189494; Abcam) as a negative control of the staining. Sections were then incubated with diluted streptavidin-peroxidase HRP at room temperature with a staining kit, according to the manufacturer's instructions. The sections were then stained with hematoxylin for 5 min and were mounted and observed using a phase-contrast microscope.

Immunofluorescence

After treatment, the cells were fixed with acetone:methanol (1:1). The slides were washed with PBS/0.05% Tween 20 at room temperature (RT), and blocked with serum-free DAKO-Block (DAKO Deutschland GmbH, Hamburg, Germany). Then, it was washed again and was incubated with 1:1000 primary antibodies against FGF-21 (ab171941; Abcam) and PTP1B (ab244207; Abcam). After washing, the slides were incubated with second antibody, and they were washed again and were incubated with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen). Finally, the slides were washed, mounted with fluorescence mounting medium (DAKO) and analyzed using a Zeiss Axiovert 200 microscope (Zeiss) with the VisiView software (Visitron Systems, Puchheim, Germany).

In vivo Mouse Experiments

SD rats of SPF grade, aged 5–6 weeks, were purchased from Beijing HFK BIOSCIENCE CO., LTD (Beijing, China). Rats were kept in an SPF environment at 20–24°C with 50–60% humidity in cycle of light 12h and dark 12h. After adaptation to the environment and being fed for 2 weeks. Twenty-four SD rats were randomly divided into four groups: control, DM, Diet, RYGB. The 12 rats in DM and RYGB were free access to food and water. They were subjected to intraperitoneal injection of STZ 30 mg/kg 4 weeks later, and continued to be fed with high-fat diet. The blood glucose level was recorded weekly. When the random blood glucose was 16.7 mmol/L and remained stable for 4 weeks, the rats were identified as Type 2 diabetes.¹⁶ The rats were fasted for 12 h before surgery, and were anesthetized with isoflurane inhalation, then supine on the operating table.

Rats in the RYGB operation group were made a longitudinal incision along the midabdominal line of 3 cm, and the proximal end of the stomach was cut off with an arc under the cardia to form 20% gastric sac, and the distal residual stomach was closed with 6-0 suture line. The jejunum was dissected about 15 cm from the distal end of the Treitz ligament, and the distal jejunum was anastomosed to the gastric sac. Proximal and distal jejunum anastomoses were performed at 10 cm from the anastomosis. The rats were given 10% glucose solution for 3 days. At the end of the experiment, the liver tissues were harvested and stored in RNAlater™ stabilization solution for downstream studies. All the above experimental operations have been approved by the ethics committee of Yixing People's Hospital.

qRT-PCR Analysis

Total RNAs were extracted from cells by TRIzol reagent (Thermo Fisher Scientific, Inc.). RNAs were synthesized into cDNA using a reverse transcription kit (Promega). PCR was performed using SYBR green Supermix (ABI) in ABI 7500 PCR system. PCR conditions were as follows: 94 °C for 30 sec, 55 °C for 30 sec and 72 °C for 30 sec (32 cycles). The relative expressions of target genes were calculated by $2^{-\Delta\Delta Ct}$ method and normalized to the housekeeping gene GAPDH. The sequences of PCR primers were as follows: Sirt1 forward, 5'-TGACCGATGGACTCCTCACT-3' and reverse, 5'-ATTGTTTCGAGGATCGGTGCC-3'; GAPDH forward, 5'-TGACCTCAACTACATGGTCTACA-3' and reverse, 5'-CTTCCCATTCTCGGCCTTG-3'.

Western Blotting Assay

Total protein was extracted from cells and liver tissues using RIPA buffer. Protein concentrations were determined with a Pierce BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL). Next, 20 ug protein was loaded and separated with 12% SDS gels and transferred to polyvinylidene difluoride membrane (Millipore, Bedford, MA). Following transfer, membranes were blocked with 10% skimmed milk and incubated with the appropriate 1:1000 primary antibodies against Sirt1 (ab189494, Abcam), FGF-21 (ab171941, Abcam), PTP1B (ab244207, Abcam), p-PKB (ab175349, Abcam), p-mTOR2 (ab134903, Abcam), IRS1 (ab40777, Abcam), GLUT4 (ab654, Abcam) and GAPDH (ab9485, Abcam) at 4°C overnight. Furthermore, the corresponding HRP-conjugated secondary antibodies were used to detect the protein expression. Chemiluminescent film (Roche) was applied for assessment of protein expression with ImageJ software (NIH).

Statistical Analysis

The SPSS13.0 statistical software was used to analyze the results, and the measurement data were expressed as the mean \pm standard deviation. The single-factor analysis of variance was applied to compare the intergroup difference. $P < 0.05$ was considered statistically significance difference.

Results

Following RYGB Improved the Pathological Changes of Liver in Rats with Type 2 Diabetes Mellitus

To investigate the potential roles of RYGB in rats with type 2 diabetes mellitus, H&E staining assay was performed to explore the pathological changes of liver. As shown in [Figure 1A](#), the rat hepatocytes were arranged in a cord-like and orderly manner with the central vein as the center. In DM group, hepatocytes have steatoid degeneration. The cells around the central vein are filled with fat droplets of different sizes. The cord-like structure is irregular. In Diet group, hepatocyte steatosis was slightly improved. After RYGB treatment, the hepatocytes showed mild steatosis and the cord-like structure of sinus hepaticus was regular. Additionally, Masson staining assay was employed to explore the changes of liver fibrous tissue. As shown in [Figure 1B](#), no obvious staining of fibrous tissue was observed in NC group.

The staining of green stained fibrous tissue was increased in the porta hepatis of DM rats. The green staining area in Diet group was decreased and the high-dose group was the most significant. Therefore, following RYGB improved the pathological changes of liver in rats with type 2 diabetes mellitus.

The Expression of Sirt1 Was Upregulated in Following RYGB in Rat Liver with Type 2 Diabetes Mellitus

To further explore the molecular mechanism of following RYGB improved the pathological changes of liver in rats with type 2 diabetes mellitus, we validated the protein level expression of Sirt1 in rat liver with type 2 diabetes mellitus. The protein expression of Sirt1 was measured in Control, DM, Diet and RYGB groups by Western blotting. As shown in [Figure 2A](#), the protein expression of Sirt1 was reduced in the DM group, whereas the protein expression of Sirt1 was increased in Diet and RYGB groups. Moreover, the protein expression of Sirt1 in RYGB group was higher than that of Diet group, showing statistically significant difference, compared to the Control group. Equally, the protein expression of Sirt1 revealed by immunohistochemical assay was similar to the result of Western blotting ([Figure 2B](#)). Thus, the expression of Sirt1 was upregulated in following RYGB in rat liver with type 2 diabetes mellitus.

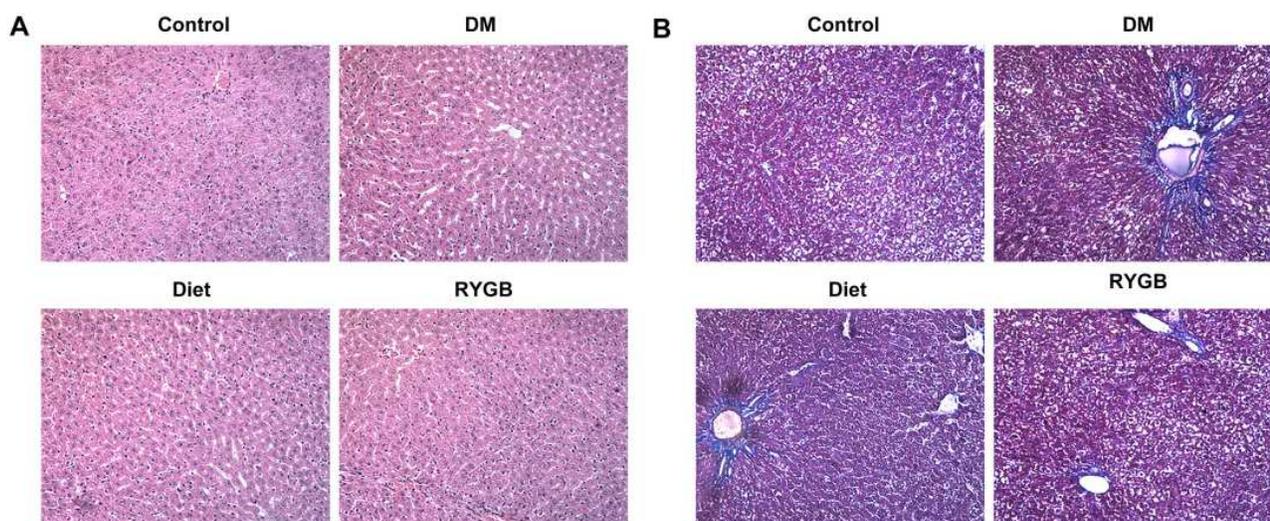


Figure 1 Following RYGB improved the pathological changes of liver in rats with type 2 diabetes mellitus. **(A)** H&E staining assay was explored to the changes of liver fibrous tissue. **(B)** Masson staining assay was explored to the changes of liver fibrous tissue.

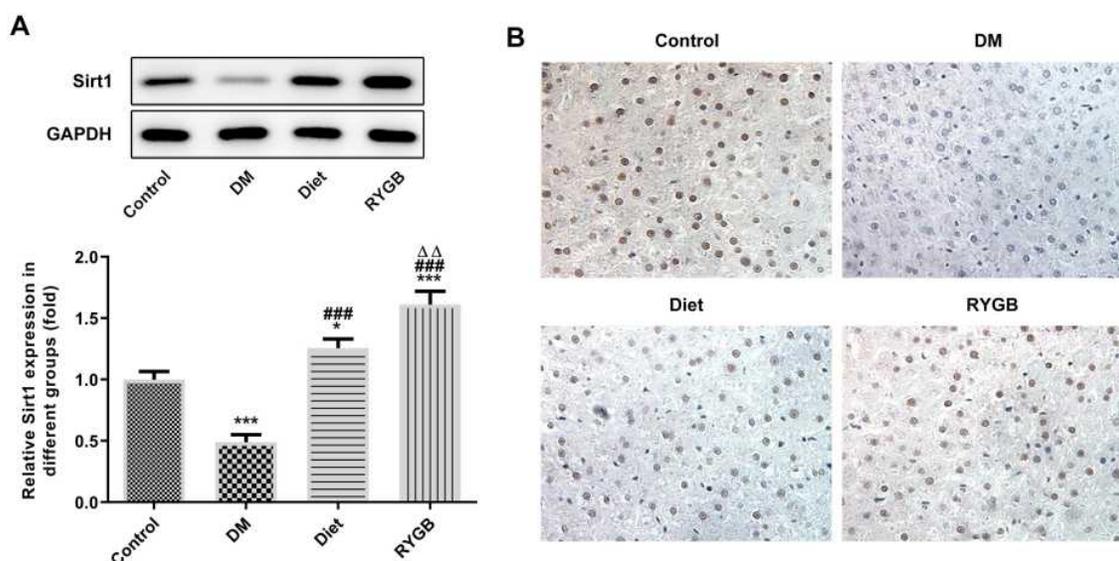


Figure 2 The expression of Sirt1 was upregulated in following RYGB in rat liver with type 2 diabetes mellitus. **(A)** The protein expression of Sirt1 was detected in Control, DM, Diet and RYGB groups by Western blotting. **(B)** Immunohistochemical assay was performed to detect the expression of Sirt1. * $p < 0.05$ and *** $p < 0.001$ vs Control; ### $p < 0.001$ vs DM; $\Delta\Delta p < 0.01$ vs Diet.

Effect on the Expressions of FGF-21 and PTP1B, and the Activation of IRS1/ mTOR2/PKB Pathway in Following RYGB in Rat Liver with Type 2 Diabetes Mellitus

To evaluate the effects of following RYGB on the expressions of metabolic regulatory molecules FGF-21 and PTP1B in rat liver with type 2 diabetes mellitus, the expressions of FGF-21 and PTP1B were detected by Western blotting and immunofluorescence. As shown in [Figure 3A](#), results of Western blot showed that the expression of FGF-21 was decreased in DM group and increased in Diet group, and RYGB significantly promoted the expression of FGF-21 compared with Control group, while the expression of FGF-21 was the highest in RYGB group than that in Diet group with statistical difference compared to the Control group. Meanwhile, the expression of PTP1B was increased in DM group and was decreased in Diet group and RYGB group compared with Control group, whereas the expression of FGF-21 was lower in RYGB group than Diet group, showing statistically significant difference compared with the Control group. Next, results of immunofluorescence assay showed that RYGB and Diet significantly increased the expression of FGF-21 and decreased the expression of PTP1B in rats with type 2 diabetes mellitus, there was a statistical difference between DM group and RYGB group ([Figure 3B](#) and [C](#)). These phenomenon indicated that following RYGB

had effects on the expression of FGF-21 and PTP1B. Next, the phosphorylation levels of IRS1/mTOR2/PKB pathway-related proteins (PKB, mTOR2, IRS1 and GLUT4) were detected by Western blotting. GLUT4 in liver tissue of DM rats showed low expression, which was involved in promoting glucose utilization.¹⁷

As shown in [Figure 3D](#), RYGB and Diet significantly increased the expressions of p-PKB, p-mTOR2, IRS1 and GLUT4 compared to the DM group, and the expressions of p-PKB, p-mTOR2, IRS1 and GLUT4 was the highest in RYGB group. Thus, following RYGB could regulate the glucose metabolism through IRS1/mTOR2 /PKB pathway in rats with type 2 diabetes mellitus.

The Expression of Sirt1 Was Downregulated and the Effect of Sirt1 Overexpression on the Expressions of FGF-21 and PTP1B in Insulin-Resistant FL62891 and HHL5 Cells

Subsequently, we detected the expression of Sirt1 in insulin-resistant cells. The insulin resistance model of liver cells FL62891 and HHL5 was built using human recombinant insulin, as. As shown in [Figure 4A](#), compared with the untreated FL62891 cells, Sirt1 was downregulated in insulin-resistant FL62891 cells. The expression of Sirt1 in insulin-resistant FL62891 cells

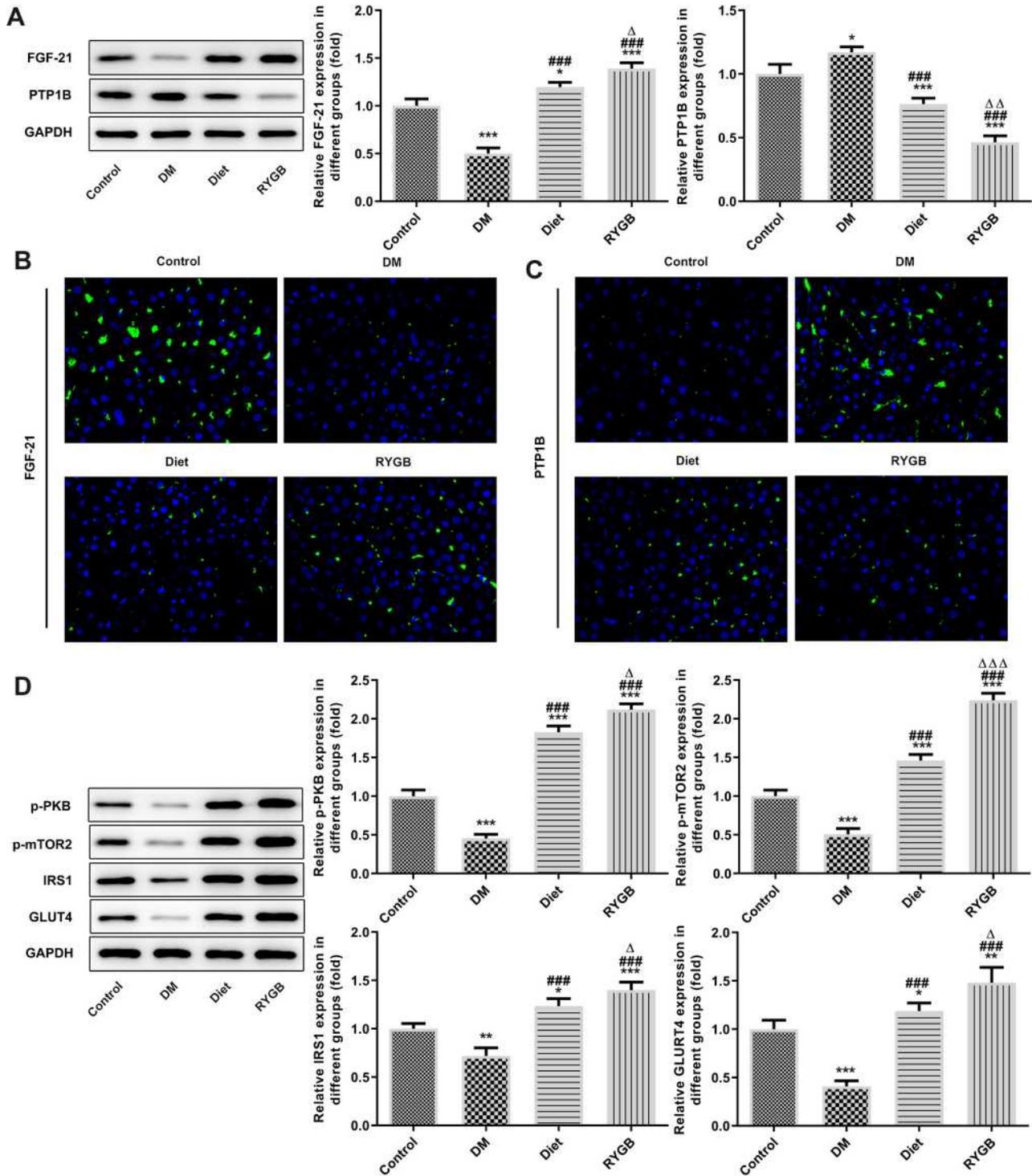


Figure 3 The effect on the expressions of FGF-21 and PTP1B, and the activation of IRS1/mTOR2 /PKB pathway in following RYGB in rat liver with type 2 diabetes mellitus. **(A)** The effect on the expressions of FGF-21 and PTP1B in following RYGB in rat liver with type 2 diabetes mellitus was detected by Western blotting. **(B)** The effect on the expressions of FGF-21 in following RYGB in rat liver with type 2 diabetes mellitus was detected by immunofluorescence analysis. **(C)** The effect on the expressions of PTP1B in following RYGB in rat liver with type 2 diabetes mellitus was detected by immunofluorescence analysis. **(D)** The effect on the expressions of IRS1/mTOR2 /PKB pathway-related proteins (p-PKB, p-mTOR2, IRS1 and GLUT4) in rat liver with type 2 diabetes mellitus was detected by Western blotting. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs Control; ####*p* < 0.001 vs DM; Δ*p* < 0.05, ΔΔ*p* < 0.01 and ΔΔΔ*p* < 0.001 vs Diet.

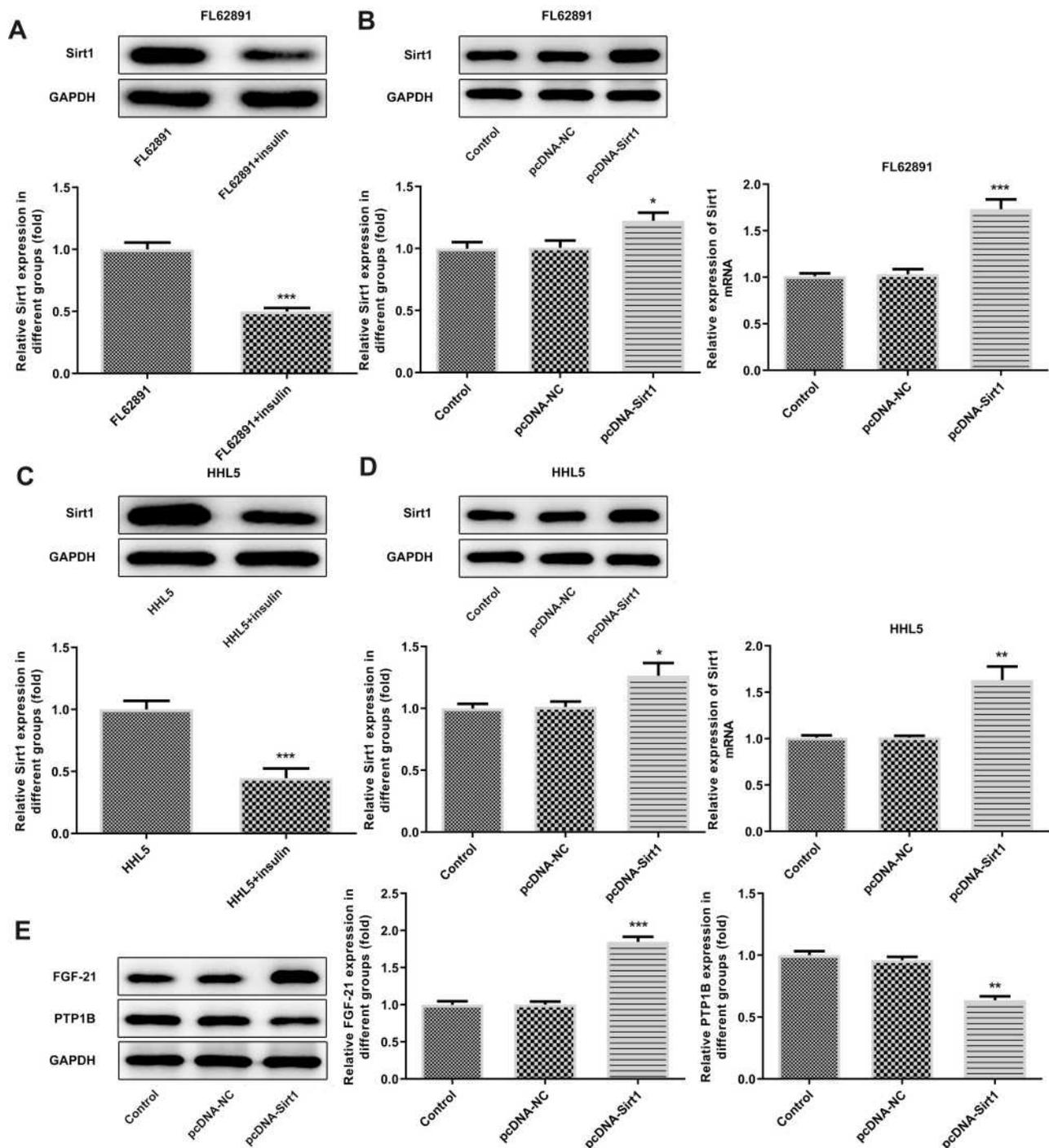


Figure 4 The expression of Sirt1 was downregulated and the effect of Sirt1 overexpression on the expressions of FGF-21 and PTP1B in insulin resistance FL62891 and HHL5 cells. **(A)** The expressions of Sirt1 in the untreated FL62891 cells and insulin resistance FL62891 cells were detected by Western blotting. *** $p < 0.001$ vs FL62891. **(B)** The efficiency of Sirt1 overexpression was examined by qRT-PCR and Western blotting in transfected insulin resistance FL62891 cells. * $p < 0.05$ and *** $p < 0.001$ vs pcDNA-NC. **(C)** The expressions of Sirt1 in the untreated HHL5 cells and insulin resistance HHL5 cells were detected by Western blotting. *** $p < 0.001$ vs HHL5. **(D)** The efficiency of Sirt1 overexpression was examined by qRT-PCR and Western blotting in transfected insulin resistance HHL5 cells. * $p < 0.05$ and *** $p < 0.001$ vs pcDNA-NC. **(E)** Effects of Sirt1 overexpression on the expressions of FGF-21 and PTP1B in insulin resistance FL62891 and HHL5 cells. ** $p < 0.01$ and *** $p < 0.001$ vs pcDNA-NC.

was increased by the transfection with pcDNA-Sirt1. As shown in Figure 4B, the results of Western blot and qRT-PCR showed that the expression of Sirt1 in pcDNA-Sirt1 group was significantly higher than that

in pcDNA-NC group. Similar results were observed in insulin-resistant HHL5 cells. As shown in Figure 4C, compared with the untreated HHL5 cells, Sirt1 was downregulated in insulin-resistant HHL5 cells. The

expression of Sirt1 in insulin-resistant HHL5 cells was increased by the transfection with pcDNA-Sirt1. As shown in Figure 4D, the results of Western blot and qRT-PCR showed that the expression of Sirt1 in pcDNA-Sirt1 group was significantly higher than that in pcDNA-NC group. Finally, the effects of Sirt1 overexpression on the expressions of FGF-21 and PTP1B in FL62891 and HHL5 cells were detected by Western blotting. As shown in Figure 4E, Sirt1 overexpression significantly increased the expression of FCF-21, whereas it decreased the expression of PTP1B compared with the pcDNA-NC group. Taken together, the expression of Sirt1 was downregulated and sirt1 exerted its effects on the expression of FGF-21 and PTP1B in insulin-resistant FL62891 and HHL5 cells.

Effect of Sirt1 Overexpression on the Expression of FGF-21 and PTP1B in Insulin-Resistant FL62891 and HHL5 Cells

In order to detect the effect of Sirt1 overexpression on the expressions of FGF-1 and PTP1B again, this effect was verified by immunofluorescence. As shown in Figure 5A and B, Sirt1 overexpression promoted the expression of FCF-21 and reduced the expression of PTP1B in insulin-resistant FL62891 cells. In addition, in insulin-resistant HHL5 cells, Sirt1 overexpression could also promote the expression of FCF-21 and suppressed the expression of PTP1B (Figure 5C and D). These results demonstrated that Sirt1 exerted its effects on the expression of FGF-21 and PTP1B in insulin-resistant FL62891 and HHL5 cells.

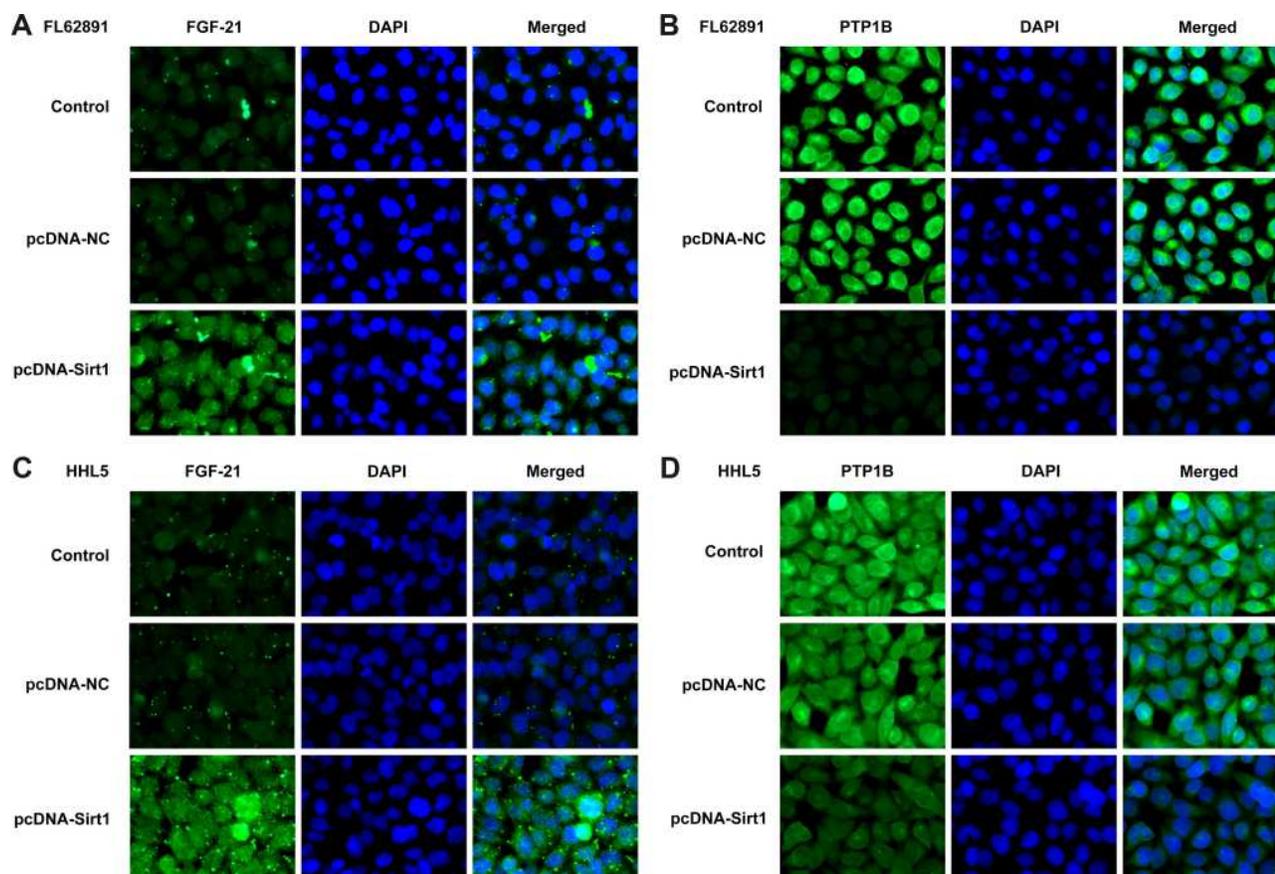


Figure 5 The effect of Sirt1 overexpression on the expression of FGF-21 and PTP1B in insulin resistance FL62891 and HHL5 cells. (A) The effect of Sirt1 overexpression on the expression of FGF-21 in insulin resistance FL62891 cells was detected by immunofluorescence. (B) The effect of Sirt1 overexpression on the expression of PTP1B in insulin resistance FL62891 cells was detected by immunofluorescence. (C) The effect of Sirt1 overexpression on the expression of FCF-21 in insulin resistance HHL5 cells was detected by immunofluorescence. (D) The effect of Sirt1 overexpression on the expression of PTP1B in insulin resistance HHL5 cells was detected by immunofluorescence.

Sirt1 Regulated the Glucose Metabolism Through IRS1/mTOR2 /PKB Pathway in Insulin-Resistant FL62891 and HHL5 Cells

In the end, effects of Sirt1 overexpression on the activation of IRS1/mTOR2 /PKB pathway were detected by Western blotting. As shown in Figure 6A and B, Sirt1 overexpression significantly increased the expressions of IRS1 and GLUT4, and the phosphorylation levels of PKB and mTOR2 compared with the Control groups in insulin-resistant FL62891 and HHL5 cells. To investigate the molecular mechanism of Sirt1 on the glucose metabolism, we added the inhibitor NT157 of IRS1 (3 μ M)¹⁸ in insulin-resistant FL62891 and HHL5 cells, and divided the experimental groups into Control+NT157, pcDNA-Sirt1+NT157 and pcDNA-Sirt1+NT157. As shown in Figure 6C and D, results of Western blotting revealed that the Sirt1 overexpression decreased the expressions of IRS1 and GLUT4, the phosphorylation levels of PKB and mTOR2. These above changes could be reversed by the NT157. Therefore, Sirt1

could regulate the glucose metabolism via IRS1/mTOR2 /PKB pathway in insulin-resistant FL62891 and HHL5 cells.

Discussion

A large number of clinical researches confirmed that RYGB is an effective surgical treatment for T2DM.^{19,20} The typical RYGB improved the glucose tolerance and insulin sensitivity, along with weight loss and decreased food intake in T2DM.²¹ RYGB decreased visceral fat area correlated with improved arterial stiffness in Chinese obese patients with T2DM.^{22,23} In the present study, the expression of Sirt1 was upregulated in following RYGB in rat liver with T2DM and the overexpression of Sirt1 significantly increased the expression of FCF-21 whereas decreased the expression of PTPB1 in insulin-resistant FL62891 and HHL5 cells, which may relate to the IRS1/mTOR2 /PKB pathway.

Silent information regulator 1 (Sirt1) is a class of NAD⁺-dependent protein deacetylase. Previous studies showed that Sirt1 is closely related to multiple pathological links of insulin resistance and the activation of Sirt1 can directly enhance the insulin sensitivity in the liver.²⁵ On the one hand, overexpression of Sirt1 regulates the

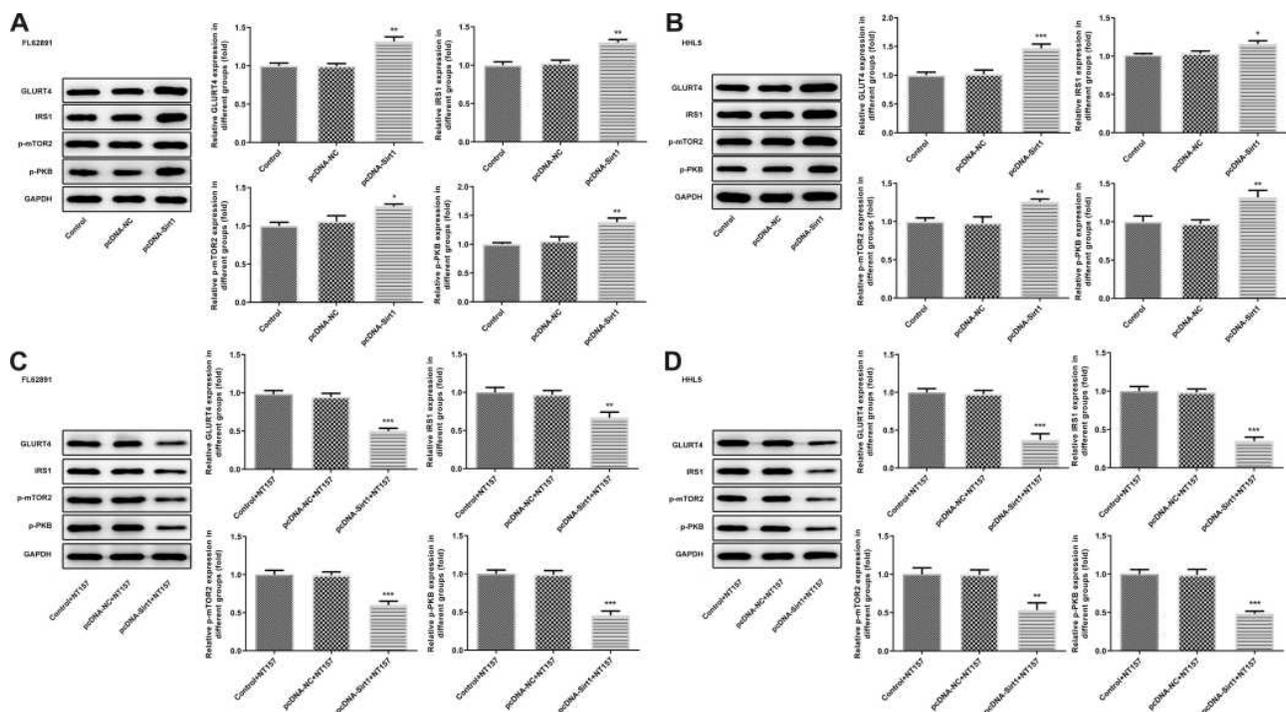


Figure 6 Sirt1 regulated the glucose metabolism through IRS1/mTOR2 /PKB pathway in insulin resistance FL62891 and HHL5 cells. **(A)** Effects of Sirt1 overexpression on the phosphorylation levels of PKB and mTOR2, and the expressions of IRS1 and GLUT4 in insulin resistance FL62891 cells were measured by Western blotting. * $p < 0.05$ and ** $p < 0.01$ vs pcDNA-NC. **(B)** Effects of Sirt1 overexpression on the phosphorylation levels of PKB and mTOR2, and the expressions of IRS1 and GLUT4 in insulin resistance HHL5 cells were measured by Western blotting. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs pcDNA-NC. **(C)** Effects of Sirt1 overexpression together with NT157 on the phosphorylation levels of PKB and mTOR2, and the expressions of IRS1 and GLUT4 in insulin resistance FL62891 cells were measured by Western blotting. ** $p < 0.01$ and *** $p < 0.001$ vs pcDNA-NC+NT157. **(D)** Effects of Sirt1 overexpression together with NT157 on the phosphorylation levels of PKB and mTOR2, and the expressions of IRS1 and GLUT4 in insulin resistance HHL5 cells were measured by Western blotting. ** $p < 0.01$ and *** $p < 0.001$ vs pcDNA-NC+NT157.

insulin secretion by inhibiting the expression of mitochondrial uncoupling protein 2 and regulating the sensitivity of K^+ ions in ATP channel. One previous study showed that Sirt1 can regulate the activity of Sirt1 through deacetylation and then affect the balance of both glucose and lipids in the body.²⁴ In addition, the overexpression of Sirt1 improves the insulin resistance in the mouse liver.²⁵ Sirt1 knockdown can lead to the oxidative stress and hyperglycemia caused by the overproduction of glucose in the liver.²⁶ We found that IRS1 inhibitor could significantly interfere the effects of SIRT1 overexpression. IRS1 is one of the most critical molecules in insulin signaling pathway, and its under-expression or abnormal phosphorylation would affect insulin sensitivity,²⁷ which indicated that SIRT1 mediated IR. In general, Sirt1 is expected to be a potential therapeutic target for the improvement of insulin resistance and the treatment in T2DM. In the present study, the expression of Sirt1 is upregulated following RYGB in rat liver with T2DM, whereas is downregulated in insulin-resistant FL62891 and HHL5 cells. This result indicates that Sirt1 may be involved in the treatment of T2DM by RYGB.

Fibroblast growth factor-21 (FGF-21) is a new type of metabolic regulatory molecule, which could bind to the coreceptor β -Klotho and FGF receptor (FGFRs) to form a relatively stable complex to exert its biological effects. Previous study has shown that FGF21 is a safe, effective and insulin-independent metabolic regulator, which plays an important role in regulating glucose, lipid metabolism and maintaining energy balance in the body.²⁸ Smith et al reported that monkeys were injected with different doses of FGF-21 after 6 weeks, their body weight was decreased significantly in a dose-dependent manner.²⁹ In addition, FGF-21 could alleviate IR to some degree.³⁰ PTP1B is one of the main members in the protein tyrosine phosphatase family, which participates in intracellular signal regulation in multiple tissues and cells, dephosphorylating tyrosine. Protein tyrosine phosphatase-1B (PTP1B) is related to insulin and leptin signal transduction pathway and plays a role in glucose metabolism and energy regulation, which is closely related to the occurrence of T2DM and obesity-related metabolic disorders. It is noteworthy that PTP1B is a negative regulator of insulin signal transduction.^{31,32} An experimental study showed that the blood glucose returned to normal and the weight lost after the obese diabetic mouse model was treated with PTP1B inhibitor.³³ In the present study, the expression of FGF-21 was upregulated while the expression of PTP1B was

downregulated in rat liver with type 2 diabetes mellitus following RYGB. And overexpression of Sirt1 significantly increased the expression of FCF-21 and decreased the expression of PTP1B in insulin-resistant FL62891 and HHL5 cells. Therefore, upregulation of Sirt1 could participate in the process of RYGB treatment of T2DM by promoting FGF-21 and inhibiting PTP1B.

Insulin function is synergistically coordinated by molecular events associated with IR.³⁵ PKB/mTOR signaling pathway was the major pathway of insulin signaling transduction.³⁴ Mammalian target of rapamycin (mTOR) is an important nutrient sensor in T2DM.³⁵ In this study, the expression of IRS1 and GLUT4 and the phosphorylation levels of PKB and mTOR2 were increased in RYGB groups compared with the DM group and also exhibited high expression in the pcDNA-Sirt1 group compared with the pcDNA-NC group. Overall, following RYGB could regulate the glucose metabolism through IRS1/mTOR2 /PKB pathways in rats with type 2 diabetes mellitus.

In summary, our studies demonstrated that RYGB improved the pathological changes of liver in rats with T2DM. The expression of Sirt1 was upregulated in following RYGB in rat liver with T2DM. Moreover, Sirt1 overexpression could regulate the glucose metabolism through modulating IRS1/mTOR2 /PKB pathway, and the expressions of FCF-21 and PTP1B in insulin-resistant FL62891 and HHL5 cells. Thus, RYGB improves hepatic glucose metabolism involving in upregulation of Sirt1 in T2DM. This research may provide novel perspective for the treatment of Diabetes mellitus, showing new insight to the effect of RYGB on hepatic glucose metabolism.

Data Sharing Statement

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

Ethics Approval and Consent

All animal experiments were approved by the Ethics Medical University and conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

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Disclosure

The authors declare that they have no conflicts of interest for this work.

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