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Fibroblast growth factor 21 regulates glucose metabolism in part by reducing renal glucose reabsorption

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ABSTRACT

Although previous studies have shown the potential of FGF21 to regulate blood glucose in animal and humans, the precise mechanisms of the action have not been well explored. The kidney plays a crucial role for glucose homeostasis. The purpose of this study is to explore the effect of FGF21 on renal glucose reabsorption. Administration of type 2 and type 1 diabetic mice with FGF21 reduced the transport maximum of glucose in the kidney and enhanced urinary glucose excretion in a dose-dependent manner. The inhibition of glucose reabsorption results showed little change in diabetic mice treated with Insulin. In physiological state, both FGF21 and insulin had no effect on glucose reabsorption and urinary glucose excretion. Next, we examined the expression of SGLT2 in the kidney, which is an important molecule for renal glucose reabsorption. SGLT2 was highly expressed in the kidneys of diabetic mice. Administration of FGF21 reduced SGLT2 expression in the kidney of diabetic mice. In contrast, the expression of SGLT2 had little change in diabetic mice treated with Insulin. FGF21 and Insulin did not promote SGLT2 expression in physiological state. To explore the mechanism which drives these changes, we detected the expression of PPARδ in mice and HK-2 cells, which plays a major role in regulating SGLT2 expression. Treatment with FGF21 promoted PPARδ expression in diabetic mice, whereas Insulin had no effect on PPARδ expression. At dose of 2 mg/kg FGF21 treatment promoted PPARδ expression in physiological state, whereas at dose of 1 mg/kg FGF21 did not. In HK-2 cells, treatment with FGF21 enhanced PPARδ expression, whereas Insulin treatment had no effect on PPARδ expression. Importantly, the expression of SGLT2 and PPARδ showed little change in HK-2 cells when β-klotho was knocked down. In conclusion, we discovered for the first time that FGF21 ameliorates hyperglycemia in part via reducing renal glucose reabsorption through PPARδ mediated SGLT2 pathway.

1. Introduction

The prevalence of diabetes has been increasing and becoming a global problem in recent years. According to the International Diabetes Federation, 592 million people worldwide will have diabetes by 2035 [1,2]. The kidney plays a key role in controlling glucose level via regulating the reabsorption of glucose back into the blood, and this is a crucial process for glucose homeostasis [3,4]. A key factor linking glucose transport is the sodium-glucose cotransporter 2 (SGLT2) in the early S1 segment of proximal tubular. SGLT2 is a low-affinity and high capacity to reabsorb 80–90% of glucose from renal tubule into the blood [3]. SGLT2 catalyses the active transport of glucose against a concentration gradient across the luminal membrance by coupling it with the transport of sodium [5–7]. The inward sodium gradient across the luminal epithelium is maintained by ATP-driven active extrusion of sodium across the anti-luminal surface into the blood [8,9]. In hyperglycemic status, the renal proximal tubule raises its capacity to reabsorb glucose from the proximal tubule in response to hyperglycemia because of increased SGLT2 activity [10,11]. Therefore, inhibiting SGLT2 can improve glycemic control in diabetic patients [12]. SGLT2 inhibitor has been proposed as a novel therapeutic strategy for diabetes. So far, seven types of SGLT2 inhibitors have been approved for clinical use.

Fibroblast growth factor 21 (FGF21) is a potent regulator of metabolism and circulates in the blood [13,14]. FGF21 acts through FGF receptors together with the co-receptor β-klotho. Systemic administration of FGF21 reduces plasma glucose, triglycerides and insulin levels, and improves insulin sensitivity and energy expenditure in a variety of animal models [15]. The effect of FGF21 on regulating blood glucose is completed through the coordination of various target organs, such as the liver, adipose tissue, pancreas and skeletal muscle. The liver

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critically regulates glucose homeostasis via maintenance of a balance between glycogenesis and glucose output [16,17]. Dysregulation of hepatic glucose output is associated with hyperglycemia in diabetes mellitus. It has been reported that FGF21 inhibits hepatic glucose output through downregulation of gluconeogenic genes, such as glucoe-6-phosphatase (G6pase) and phosphoenolpyruvate carboxykinase (PEPCK) [18,19]. Administration of FGF21 not only reduces hepatic glucose output but also stimulates gluconeogenesis during fasting, which indicates that FGF21 differentially regulates gluconeogenesis under normal and fasting conditions [16]. FGF21 has been demonstrated to stimulate glucose uptake and utilization by inducting glucose transporter-1 (GLUT1) expression in both white adipose tissue (WAT) and brown adipose tissue (BAT) and browning WAT [15]. In the pancreatic islets, FGF21 regulates insulin secretion and islet cell growth, thus improves glucose homeostasis under diabetic conditions. The skeletal muscle is an important glucose-utilizing tissue, local FGF21 in muscle cells may help to regulate metabolic adaptations in response to internal energetic status. Based upon these studies, it was proposed that FGF21 appears to exert antidiabetic effects by promoting functions in various organs. However, it has not been evaluated whether FGF21 controls glycemic level via reabsorbing glucose from the kidney. In this study, we investigated the ability of FGF21 to regulate renal glucose reabsorption and the mechanism of its action.

2. Materials and methods

2.1. FGF21 preparation

The mouse FGF21 was cloned into a commercial *Escherichia. coli (E. coil)* expression vector, pSUMO (LifeSeusors Inc). The recombinant plasmid was transformed into host bacterium Rossetta (DE3). Single colony was inoculated to LB media containing ampicillin (100ug/ml). When the OD_{600} reached 0.4-0.6, 0.25 mmol/L IPTG was added into the medium. The recombinant FGF21 protein was purified by a Ni Sepharose of 6 Fast Flow column in AKTA purifier (GE Healthcare). The concentration of purified protein was measured by BCA protein assay (BCA Protein Assay Kit, Thermo, USA) and stored at -80℃ until use.

2.2. Animals

Male db/db mice and C57BL/6 mice (6–8 weeks) were purchased from Shanghai Silaike Experimental Animal Co. Ltd (Shanghai, China; animal quality certification number SCK [Shanghai] 2017-0005). All animals were housed in a controlled temperature of 22-25℃ and a relative humidity of 55%–65% with a 12 h light/dark cycle and free access to food and water. All the animal experiments were carried out in strict accordance with the protocols of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by Harbin Veterinary Research Institute Animal Care and Use Committee. T1DM was induced by consecutive intraperitoneal injections of streptozotocin (STZ) (50 mg/kg, 3 times). After the third injection, blood glucose was measured, and the blood glucose level of mice > 16 mmol/L were considered to have diabetes [20].

2.3. Measuring the antidiabetic effects of FGF21 in db/db and T1DM mice

Db/db, STZ-induced diabetic mice or normal mice were administrated with FGF21 (the dose of FGF21 in FL and FH groups were 1 mg/kg and 2 mg/kg, respectively) or saline (n = 10) once a day for 15 days. Blood glucose was measured by tail incision using a Ruidien YD-588 A glucometer.

OGTT was examined at the end of the treatment period. The mice were fasted for 6 h (with access to water), and the mice were given glucose (2 g/kg body weight) by gavage. The blood glucose was measured at 0, 30, 60, 90 and 120 min. The kidney from each mouse was obtained immediately after sacrifice.

2.4. Evaluation of hypoglycemic risk

Male C57BL/6 mice were fasted for 6 h, and then treated with FGF21. Measurement of plasma glucose was performed as described above.

2.5. Transport maximum for glucose

Transport maximum for glucose test was performed according to methods described previously [21].

2.6. Glucose uptake assay in HK-2 cells

HK-2 cells, human proximal tubule epithelial cells, were purchased from the American Type Culture Collection (ATCC), were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) containing 10% FBS and 1%antibiotics (penicillin-streptomycin) in a CO_2 incubator (5% CO_2) at 37℃. The cells were starved for 12 h in serum-free medium and then stimulated with or without FGF21 for another 24 h. To explore the effect of FGF21 on glucose uptake, we transfected siRNA targeting β-klotho (Sangon Biotech, Shanghai) by using Lipofectamine 3000 transfection reagent (Invitrogen). The sense and antisense strands of siRNA were as follows: CAUCCACACACACCUUAAATT (sense) and UUUAAGGUGUGUGUGGAAUGTT (antisense). FGF21 was added after siRNA transfection for 6 h. In control, cells were treated with vehicle. Glucose uptake was measured by glucose assay kit (Beijing Kingkawk Pharmaceutical CO., LTD) according to the manufacturer's protocol.

2.7. Real-time PCR

Total RNA from the kidney or HK-2 cells were extracted with Trizol-reagent (Invitrogen, USA) according to the manufacturer's instructions. Five micrograms of total RNA were reverse transcribed into cDNA using MMLV first stand cDNA synthesis kit (Promega, USA).The cDNA templates were used for real-time by using SYBR green (ABI 7500, Applied Biosystems, Carlsbad, CA, USA). The following primer sequences were used: human β-actin forward 5`-CATGTACGTTGCTATCCAGGC-3`; reverse 5`-CTCCTTAATGTCACGCACGAT-3`; human SGLT2 forward 5`-TCCTGCTGACATCCTAGTCATT-3`; reverse 5`-GAAGAGCGCATTCCAC TCG-3`; human PPAR δ forward 5`-CAGGGCTGACTGCAAACGA-3`; reverse 5`-CTGCCACAATGTCTCGATGTC-3`; mice β-actin forward 5`-GGC TGTATTCCCCTCCATCG-3`; reverse 5`－CCAGTTGGTAACAATGCCA TGT-3`; mice SGLT2 forward 5`-ATGGAGCAACACGTAGAGGC-3`; reverse 5`-ATGACCAGCAGGAAATAGGCA-3`; mice PPAR δ forward 5`-TCCATC GTCAACAAAGACGGG-3`; reverse 5`-ACTTGGGCTCAATGATGTCAC-3`.

2.8. Western blot analysis

The kidney and cell samples were prepared by using radio immunoprecipitation assay (RIPA) buffer (Beyotime Institute of Biotechnology) according to the manufacturer`s instructions. The protein concentration was measured by using the pierce BCA Protein Assay Kit. We used 30–40 u g protein on a Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred it to nitrocellulose membrane (GE Healthcare, USA). We then blocked with 5% skim milk in phosphate buffer saline (PBS) and probed with the following antibodies overnight at 4℃: SGLT2, PPARδ and**β**-actin (1:1000, Abcam, USA), and then followed by a horseradish peroxidase-conjugated secondary antibodies to IgG (1:7500, R&D, USA) 1 h at 37℃. Blots were visualized using an enhanced chemiluminescence kit (ECL; Thermo Scientific, USA).

2.9. Statistical analysis

All analyses were performed with SPSS 16.0. All data were performed using one-way analysis of variance (ANOVA), followed by the