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ORIGINAL ARTICLE

Rapamycin-ameliorated diabetic symptoms involved in increasing adiponectin expression in diabetic mice on a high-fat diet

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Abstract Recent studies showed that rapamycin improved diabetic complications. Here, we investigated the metabolic effects of rapamycin in type 2 diabetes model (T2DM) mice. Mice were treated with a daily intraperitoneal injection of rapamycin at 2 mg/kg or vehicle only for 3 weeks and were maintained on a high-fat diet. The treated diabetic mice exhibited decreased body weight, blood glucose levels, and fat mass. FGF21 expression was suppressed in C57B/L6 mice, but adiponectin expression increased both in FGF21 KO and C57B/L6 mice. These results suggest that rapamycin may alleviate FGF21 resistance in mice on a high-fat diet. The reduction of adipose tissue mass of the diabetic mice may be due to the increased adiponectin.

Introduction

Diabetes mellitus (DM) is one of the three most significant health problems worldwide. Previous studies proposed that insulin deficiency and (or) insulin resistance are the pathological basis of DM. β-cell dysfunction also inhibits the regulation of insulin [1,2]. The identification of a new drug that can counter insulin resistance or islet cell function and also prevent diabetic complications would be highly beneficial.

Fibroblast growth factor 21 (FGF21) is a member of the FGF family and functions as a regulator of energy metabolism. FGF21 is mainly expressed in the liver. High glucose inhibits FGF21 action, and the circulation level of FGF21 is significantly elevated in diabetic subjects [3–7]. Previous

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studies reported that treatment of db/db mice, a model of diabetes, with recombinant FGF21 protein reduced blood glucose and triglyceride levels to normal levels and improved both muscle and liver insulin resistance [7]. Recently, studies demonstrated that FGF21 improves lipid metabolism disorder, including effects to reduce the LDL level and increase the HDL level, biological effects that rely on action of FGF21-PPARγ-adiponectin [8—10]. Studies also reported mTORC1/S6K is a major FGF21-regulated signaling node [11] and long-term caloric restriction results in neuroprotection via the FGF21-induced AMPK/mTOR pathway in ApoE-deficient mice [12].

The mTOR protein is a serine and threonine protein kinase that regulates energy metabolism and nutrient availability, and controls cellular growth and metabolism [13,14]. Additionally, mTOR is the target of rapamycin. Rapamycin has important uses in oncology, cardiology, and transplantation medicine, but it is also used to treat metabolic disorders such as diabetes by targeting mTOR. The sustained activation of mTOR/S6K1 causes insulin resistance and inflammatory response [15,16]. Additionally, previous studies suggest that the activity of mTOR is significantly increased in type 2 diabetes model mice [17—19]. Several studies showed that administration of rapamycin can improve insulin sensitivity and reduce body weight in diabetic mice [14,20,21]. Additionally, the inhibition of mTOR by rapamycin improves diabetic nephropathy [22,23], cardiac function [20], and retinopathy in T2DM [24]. There is evidence of a role of rapamycin in the regulation of metabolic syndromes in type 2 diabetes mouse models [25]. Here, our results revealed the reduction by rapamycin of several complications of diabetes, including body weight, blood glucose, and fat mass. The mTOR protein integrates signals from growth factors, hormones, and cellular energy levels to regulate protein translation and cell growth, proliferation, and survival. The goal of this study was to examine the effect of rapamycin treatment on body weight, blood glucose, and fat accumulation on type 2 diabetic mice, and to determine how these changes relate to factors regulated by mTOR, particularly FGF21 and its downstream effector, adiponectin. Here, our results revealed that the effects of rapamycin to alleviate complications of diabetes are related to the increased expression level of adiponectin in high-fat fed diabetic mice.

Materials and methods

Animals

C57B/L6 mice (B6) were purchased from the Shanghai Laboratory Animal Co. (Shanghai, China) and FGF21 knockout mice (F21 KO) were obtained from The University of Hong Kong. All animals were kept under 12 h light—dark cycles at 22—24 °C. 8-week-old B6 and F21 KO mice were fed a high-fat diet (Research Diet) for 6 weeks, and then were intraperitoneally injected with streptozocin (STZ, Sigma, 80 mg/kg). The blood glucose was measured after administration of STZ for one week, and the blood glucose level over 22.2 mmol/L indicated the successful development of the diabetic model. B6 and F21 KO mice were randomly divided into two groups after 6 weeks of STZ administration. One group of B6 and F21 KO mice were treated with rapamycin (Selleck, 2 mg/kg/day) for 3 weeks and the control group mice were treated with DMSO only. The blood glucose and body weight were monitored throughout the experimental period.

Hematoxylin and eosin (H&E) staining

Fresh tissues were fixed in 10% neutral formalin for 24 h, dehydrated, and then embedded in paraffin. Paraffin sections (5 μm) were cut and mounted on glass slides and were stained with H&E via standard procedures. All slides were examined using a NIKON biological microscope.

RNA extraction and real-time PCR assay

Total RNA was isolated from liver and adipose tissues with the Trizol method (Takara). Next, 1 μg total RNA was reverse-transcribed to cDNA using the M-MLV first strand kit (Invitrogen). Quantitative real-time PCR was preformed using SYBR Green Q-PCR Mix with specific primers (Table 1). The relative expression levels were computed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All analyses were performed with GraphPad. The data were expressed as mean ± SEM. Statistical comparisons were performed using two-tailed Student's t test, and a P value < 0.05 was used considered a statistically significance difference.

Results

Rapamycin decreases the blood glucose and body weight of diabetic mice

In this study, high-fat diet-fed B6 mice were remarkably overweight relative to FGF21 KO mice on the same diet and maintained under the same conditions. Body weight was significantly reduced in rapamycin-treated B6 and FGF21 KO mice compared to the B6 mice that received the high fat

Table 1 Primers used for Q-PCR.

Gene (mouse)	Forward primer (5′-3′)	Reverse primer (5′-3′)
FGF21	CTGGGGGTCTACCAAGCATA	CACCCAGGATTTGAATGACC
Adiponectin	AGACCTGGCCACTTTCTCCTCATT	AGAGGAACAGGAGAGCTTGCAACA
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT