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Hepatic NPC1L1 Overexpression Ameliorates Glucose Metabolism in Diabetic Mice Via Suppression of Gluconeogenesis

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ABSTRACT

Objective. Inhibition of intestinal NPC1L1 by ezetimibe has been demonstrated to improve glucose metabolism in rodent models; however, the role of hepatic NPC1L1 in glucose metabolism has not been elucidated. In this study, we analyzed the effects of hepatic NPC1L1 on glucose metabolism.

Material and Methods. We overexpressed NPC1L1 in the livers of lean wild type mice, diet-induced obesity mice and db/db mice with adenoviral gene transfer.

Results. We found that in all three mouse models, hepatic NPC1L1 overexpression lowered fasting blood glucose levels as well as blood glucose levels on ad libitum; in db/db mice, hepatic NPC1L1 overexpression improved blood glucose levels to almost the same as those found in lean wild type mice. A pyruvate tolerance test revealed that gluconeogenesis was suppressed by hepatic NPC1L1 overexpression. Further analyses revealed that hepatic NPC1L1 overexpression decreased the expression of FoxO1, resulting in the reduced expression of G6Pase and PEPCK, key enzymes in gluconeogenesis.

Conclusions. These results indicate that hepatic NPC1L1 might have distinct properties of suppressing gluconeogenesis via inhibition of FoxO1 pathways.

1. Introduction

Diabetes and dyslipidemia are both worldwide spreading diseases threatening human health. Several studies have shown that some agents for dyslipidemia modulate glucose metabolism. Among them, ezetimibe, an inhibitor of Niemann-Pick C1 like 1 protein (NPC1L1), has been reported to improve insulin resistance and fatty liver in rodents [1]; deletion of NPC1L1 gene in rodent has also been shown to ameliorate insulin resistance [2]. In contrast to the remarkable improvement of glucose metabolism in these rodent models by ezetimibe, only a few reports have demonstrated

Abbreviations: Ad-L1, adenovirus coding NPC1L1; Ad-Null, blank adenovirus; DIO, diet-induced obesity; FoxO1, forkhead box O 1; G6Pase, glucose 6-phosphatase; L1-mice, mice infected with Ad-L1; NPC1L1, Niemann-Pick C1 like 1 protein; Null-mice, mice infected with Ad-Null; PEPCK, phosphoenolpyruvate carboxykinase.

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that treatment with ezetimibe in humans ameliorates glucose metabolism; in addition, the degrees of improvement observed in these reports were less than those expected from animal studies [3,4]. Furthermore, a recently reported randomized controlled trial has shown that treatment with ezetimibe deteriorated glucose tolerance in subjects with non-alcoholic fatty liver disease [5]. One possible explanation for this discrepancy is that in rodents the level of NPC1L1 expression in the liver is very low compared with that in the intestine, while in humans NPC1L1 is expressed in the liver at almost the same level as that in the intestine [6]. Although the established roles of intestinal and hepatic NPC1L1 are to absorb free cholesterol from the intestinal lumen and bile duct canaliculi, respectively, the influences of NPC1L1 on glucose metabolism would be different between in the intestine and in the liver. We have recently reported that hepatic NPC1L1 overexpression had distinct properties such as the emergence of apoE rich lipoproteins and the suppression of forkhead box O 1 (FoxO1) [7].

FoxO1 has been demonstrated to regulate glucose metabolism in the pancreas [8], liver [9], skeletal muscle [10], and adipose tissue [11]. Regarding glucose metabolism in the liver, FoxO1 has been established to be a positive regulator of key enzymes involved in gluconeogenesis; glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) [9]. Observations from our previous report therefore suggested that hepatic NPC1L1 overexpression might modulate glucose metabolism via suppression of FoxO1-gluconeogenesis pathway.

In this study, we overexpressed NPC1L1 in diabetic mice and demonstrated that hepatic NPC1L1 overexpression ameliorated glucose metabolism in diabetic mice models through the inhibition of liver FoxO1 at the transcription level, which resulted in the suppression of gluconeogenesis in the liver.

2. Materials and Methods

2.1. Animal Experiments

C57BL/6 mice and db/db mice were purchased from CLEA Japan (Tokyo, Japan) and Sankyo Lab Service (Tokyo, Japan), respectively. Ezetimibe-containing chow (10 mg/100 g food) was prepared as described previously [7]. For lean wild type mice, 6-week-old mice were fed normal chow containing ezetimibe (EZ(+)) or vehicle (EZ(–)) until the end of the experiments; administration of adenoviruses was performed at the age of 9 weeks. For the experiment with diet-induced obesity (DIO) mice, C57BL/6 mice were fed a high-fat diet (60% fat diet, Oriental Yeast, Tokyo, Japan) for 12 weeks from the age of 6 weeks, and the administration of adenoviruses was performed. For the experiments with db/db mice, 11 week old db/db mice fed with a normal chow were utilized.

Each mouse was injected with adenoviral vectors coding NPC1L1 (Ad-L1, L1-mice) or control blank adenovirus (Ad-Null, Null-mice) [7] via the tail vein at a dose of 2.5×10^8 pfu/g body weight. The mice experiments were performed on the fifth day after viral administration.

All animal experiments were conducted in accordance with the guidelines for Animal Care and were approved by the animal committee within the University of Tokyo.

2.2. Analysis of Blood Glucose and Plasma Insulin Levels

Blood samples were obtained after 6 hour fasting and on ad libitum. Blood glucose levels were measured with a Glutest sensor (Sanwa Kagaku, Nagoya, Japan), and plasma insulin levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan).

2.3. Pyruvate Tolerance Test

Mice were fasted for 16 hours and loaded with 2 g/kg BW sodium pyruvate (Sigma-Aldrich, St. Louis, MO) intraperitoneally. Blood glucose levels were measured before and at 20, 40, 60, 90 and 120 minutes after administration.

2.4. Insulin Tolerance Test

Mice were fasted for 6 hours and challenged with 1 U/kg BW (for DIO mice) or 1.5 U/kg BW (for db/db mice) Humulin R (Ely Lilly, Indianapolis, IN) intraperitoneally. Blood glucose levels were measured before and at 20, 40, 60, 90 and 120 minutes after administration.

2.5. Western Blot Analyses of Liver or Muscle Homogenates and Cell Lysates

Whole cellular proteins, membranous proteins, and nuclear proteins were prepared as described previously [7,12]. Western blot analyses were performed using the following antibodies; anti-Akt (Ser473, A00959), anti-phospho Akt (Phospho-Ser473, A00965), anti-FoxO1 (FKHR) (Ser256, A00427), anti-phospho FoxO1 (FKHR) antibody (Ser256, A00395) (GenScript, Piskataway, NJ), anti-acetyl FoxO1 (D-19), anti-G6Pase (C-14), anti-PEPCK (H-300), anti-Lamin A/C antibody (H-110, Santa Cruz Biotechnology, Santa Cruz, CA), anti-β-actin antibody (PM053, MBL, Nagoya, Japan), anti-NPC1L1 antibody (10005385, Cayman Chemical, Ann Arbor, MI), and anti-pan cadherin antibody (RB-9036-PD, Thermo Fisher Scientific, Fremont, CA).

2.6. Real Time PCR

Total RNAs extracted from murine livers with the GenElute Mammalian Total RNA Miniprep kit (Sigma-Aldrich) were subjected to reverse transcription with Superscript II enzyme (Invitrogen, Carlsbad, CA). Quantitative PCR was performed using an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) for G6Pase (Mm00839363_m1), PEPCK (Mm01247058_m1), FoxO1 (Mm00490671_m1 [Figs. 2I, 4E, and 6E] and Mm00490672_m1 [Fig. 7]) and β-actin (Mm00607939_s1). The expression levels of the genes of interest were adjusted to those of the endogenous control β-actin mRNA.

2.7. Statistical Analysis

The results were expressed as mean ± SEM. Differences between two groups were evaluated with Student's t-test, and the differences among more than two groups were assessed using one-way ANOVA, followed by multiple comparison tests. P-values less than 0.05 were deemed as statistically significant.