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Dapper1 attenuates hepatic gluconeogenesis and lipogenesis by activating PI3K/Akt signaling

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ABSTRACT

Studies have shown that hepatic insulin resistance, a disorder of glucose and lipid metabolism, plays a vital role in type 2 diabetes (T2D). To clarify the function of Dapper1 in glucose and lipid metabolism in the liver, we investigated the relationships between Dapper1 and adenosine triphosphate (ATP)- and Ca^{2+}-mediated activation of PI3K/Akt. We observed a reduction in hepatic Dapper1 in db/db (mice that are homozygous for a spontaneous diabetes mutation) and HFD-induced diabetic mice with T2D. Hepatic overexpression of Dapper1 improved hyperglycemia, insulin resistance, and fatty liver. It also increased Akt (pAkt) signaling and repressed both gluconeogenesis and lipogenesis. Conversely, Ad-shDapper1-induced knockdown of hepatic Dapper1 promoted gluconeogenesis and lipogenesis. Furthermore, Dapper1 activated PI3K p110α/Akt in an insulin-independent manner by inducing ATP production and secretion in vitro. Blockade of P2 ATP receptors, the downstream phospholipase C (PLC), or the inositol triphosphate receptor (IP3R all reduced the Dapper1-induced increase in cytosolic free calcium and Dapper1-mediated PI3K/Akt activation, as did removal of calcium in the medium. In conclusion, Dapper1 attenuates hepatic gluconeogenesis and lipogenesis in T2D.

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1. Introduction

Abundant epidemiological and experimental evidence has demonstrated that hepatic insulin resistance and fatty liver play important roles in the development and progression of type 2 diabetes (T2D), obesity, cardiovascular disease, and hepatic steatosis (Cornier et al., 2008).

Although the cause of T2D remains unclear, data have demonstrated its two main pathophysiological features (Henry, 1998): insulin resistance (IR) in several tissues and impaired glucose-stimulated insulin secretion (GSIS) in the pancreatic β-cells. IR is classically defined as the relative incapacity of insulin to stimulate glucose removal from circulation, while impaired GSIS is necessary for the development of T2D because a normal β-cell response to glucose is able to compensate for IR and maintain normal blood glucose levels (Henry, 1998). Recent studies suggest that hyperglycemia in T2D is due to the excessive release of glucose mainly by the liver as the result of increased hepatic gluconeogenesis (Magnusson et al., 1992, Boden et al., 2001, Gastaldelli et al., 2001).

Furthermore, T2D correlates with disorders of lipid metabolism (Ryysy et al., 2000). Preclinical and experimental studies in humans indicate that both insulin sensitivity and GSIS are markedly affected by lipotoxicity, which refers to the processes leading to endorgan damage or dysfunction following the exposure of tissues to excess levels of fatty acids. Thus, lipotoxicity plays a key role not only in fat-induced IR and impaired GSIS, which lead to T2D (Unger, 1995), but also in the progression of dyslipidemia (Gaggini et al., 2013, DeFronzo and Tripathy, 2009, Lomonaco et al., 2012, Lewis et al., 2002). Some antihyperglycemic drugs such as peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (thiazolidinediones) that are used to treat T2D are also effective in reducing liver fat and improving liver histology (Chalasani et al., 2012, Gastaldelli et al., 2006, Miyazaki et al., 2002, Belfort et al., 2006). Decreasing liver fat content is associated with improvements in insulin suppression of glucose production and consequently with improvements in fasting plasma glucose levels (Petersen et al., 2005, Ravikumar et al., 2008).

Therefore, the liver, as one of the major organs regulating glucose, triglyceride (TAG or TG) and cholesterol (CHO) metabolism (Biddinger and Kahn, 2006), is closely associated with T2D. Hepatic

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insulin resistance is characterized by the failure of insulin to repress the expression of gluconeogenic genes mainly via the PI3K/Akt signaling pathway and is closely associated with the dysregulation of glucose and lipid metabolism in the liver (Biddinger and Kahn, 2006). In addition, it has been reported that a reduction in hepatic adenosine triphosphate (ATP) synthesis is related to the progression of hepatic insulin resistance, T2D, and steatosis in humans and mice (Turner and Heilbronn, 2008).

Dapper1 was first identified as a protein that interacts with Dvl, a central mediator in Wnt signaling (Cheyette et al., 2002, Gloy et al., 2002). Existing studies have indicated that Dapper1 shuttles between the nucleus and the cytoplasm. Additionally, Dapper1 interacts with Dishevelled and has been suggested to inhibit Wnt signaling by promoting Dishevelled degradation in lysosomes (Zhang et al., 2006). At the same time, Dapper1 also enhances the Atg14L-Beclin1-Vps34 complex formation to drive autophagy (Ma et al., 2014).

However, the physiological role of Dapper1 in glucose and lipid metabolism in the liver which is connected closely with T2D remains completely unknown. In this study, we report that Dapper1 expression is significantly decreased in db/db mice (homozygous for a spontaneous diabetes mutation in the leptin receptor gene), HFD-induced diabetic mice, and ob/ob mice (homozygous for a spontaneous obesity mutation in the leptin gene). Hepatic overexpression of Dapper1 significantly attenuates hyperglycemia, insulin resistance, and fatty liver in those mice by means of insulin-independent Ca^{2+}/CaM-mediated activation of PI3K p110α/Akt. Overall, we have determined that Dapper1 contributes to the regulation of glucose and lipid metabolism in the liver and that dysregulation of Dapper1 can lead to T2D in mice.

2. Materials and methods

2.1. Experimental animals

The use of eight-week-old male C57BL/6 mice and eight-to-twelve-week-old male db/db and ob/ob mice in this study was approved by the Model Animal Research Center of Nanjing University. Eight-week-old male db/db mice were forced to swim in a warm (approximately 36 °C) water bath for 30 min per day for 7 weeks. Eight-week-old C57BL/6 mice were fed a 45% high fat diet (HFD) (Research Diets, New Brunswick, NJ) for 12 weeks prior to the experimental assays (Zhou et al., 2012).

2.2. Western blot analysis

Cells were harvested and total protein was prepared by gently lysing cells for 40 min with lysis buffer (20 mM sucrose, 1 mM EDTA, 20 μM Tris-Cl pH7.2, 1 mM DTT, 10 mM KCl 1.5 mM $MgCl_2$, 5 μg/mL pepstatin A, 10 μg/mL leupeptin, and 2 μg/mL aprotinin) and supernatants were collected. For western blot analysis, an equal amount of protein was subjected to electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels and the gels were transferred to a PVDF membrane (Merk Millipore, Germany). Blots were incubated overnight at 4 °C with desired antibodies, and then with the peroxidase-labeled goat anti-rabbit or goat anti-mouse immunoglobulin (Cell Signaling Technology, Danvers, MA) before visualizing by enhanced chemiluminescence (ECL) (Cell Signaling Technology, Danvers, MA). Antibodies against DAPPER1, pAkt, Akt, pGSK3α/β, pGSK3, G6pase, PEPCK were purchased from Abcam (Cambridge, FL). Antibodies against AdipoR1, AdipoR2, pAMPK, AMPK, UCP2 and FAS, alpha-Tubulin, eIF5 and other antibodies were purchased from Cell Signaling Technology. All the other regeats above were from sigma (Sigma, St. Louis, MO). The results were representative of three independent experiments.

2.3. H & E staining and immunohistochemistry (IHC)

Livers were excised and fixed in 4% formalin buffer. The fixed specimens were processed to paraffin blocks, sectioned, and subject to hematoxylin-eosin (H & E) staining and IHC staining which has been detailed previously (Zhou et al., 2012). The regeats for H & E and IHC were bought from Abcam (Cambridge, FL).

2.4. RNA isolation, cDNA synthesis, and real-time Q-PCR analysis

Total RNA was isolated from the mouse livers with TRIzol (Invitrogen, Carlsbad, CA). First-strand cDNA synthesis was performed using the Superscript III RT kit and oligo-dT primers (Invitrogen, Carlsbad, CA). Quantitative real-time PCR (Q-PCR) reactions were performed with the SYBR Green PCR system (Applied Biosystems) in an ABI 7500 thermal cycler (Applied Biosystems). β-actin was used as a reference gene. The primer sequences used are listed in Supporting Table 1.

2.5. Liver and serum triglyceride content

Triglyceride content in the liver and serum was determined in duplicate using the Triglyceride (GPO-Trinder) kit as described by the manufacturer (Sigma, St. Louis, MO).

2.6. Oral glucose tolerance test (OGTT)

After being fasted for 6 h, the mice were then gavaged with 3 g/kg of glucose. Blood glucose levels were then measured in blood collected from the tail vein using a Free Style brand glucometer (Roche, Switzerland) at the following time points: 0, 15, 30, 60, 90, and 120 min following gavage.

2.7. Determination of ATP content

Cultured cells or frozen liver tissues were lysed in a lysis buffer provided with the ATP Assay kit (Roche, Switzerland). The medium of the cultured cells was also collected for ATP determination. The ATP content was measured (nmol) and normalized by protein concentration (nmol/mg protein) in the same sample and presented as a percentage of the control.

2.8. Statistical analysis

Results are expressed as the mean ± SEM. Statistical significance of differences between groups was analyzed by using the unpaired *t*-test or by one-way analysis of variance (ANOVA) when more than two groups were compared. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Expression of Dapper1 is reduced in livers of db/db, HFD-induced diabetic, and ob/ob mice

To study the potential role of Dapper1 in glucose and lipid metabolism, its expression in the liver (the primary glucose- and lipid-metabolizing tissue of db/db, HFD-fed and ob/ob mice with T2D)was determined. Dapper1 protein expression was found to be lower in db/db mouse livers and in the livers of patients with lipid metabolism disorder by immunohistochemical analyses (IHC) (Fig. 1A and Supporting Fig. 1). Both the mRNA and protein levels of Dapper1 were significantly reduced in the livers of db/db mice (Fig. 1B), HFD-fed mice (Fig. 1C), and ob/ob mice (Fig. 1D), compared to healthy control mice. Interestingly, hepatic Dapper1 expression

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