The International Journal of Biochemistry & Cell Biology 78 (2016) 149–155

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Short communication

Inositol hexakisphosphate kinase-1 interacts with perilipin1 to modulate lipolysis

Sarbani Ghoshal [a], Richa Tyagi [b], Qingzhang Zhu [a], Anutosh Chakraborty [a,*]

[a] Department of Metabolism and Aging, The Scripps Research Institute, Jupiter, FL 33458, USA
[b] The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 20 May 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
IP6K
Lipolysis
Perilipin
Obesity
Diabetes

ABSTRACT

Lipolysis leads to the breakdown of stored triglycerides (TAG) to release free fatty acids (FFA) and glycerol which is utilized by energy expenditure pathways to generate energy. Therefore, a decrease in lipolysis augments fat accumulation in adipocytes which promotes weight gain. Conversely, if lipolysis is not complemented by energy expenditure, it leads to FFA induced insulin resistance and type-2 diabetes. Thus, lipolysis is under stringent physiological regulation, although the precise mechanism of the regulation is not known. Deletion of inositol hexakisphosphate kinase-1 (IP6K1), the major inositol pyrophosphate biosynthetic enzyme, protects mice from high fat diet (HFD) induced obesity and insulin resistance. IP6K1-KO mice are lean due to enhanced energy expenditure. Therefore, IP6K1 is a target in obesity and type-2 diabetes. However, the mechanism/s by which IP6K1 regulates adipose tissue lipid metabolism is yet to be understood. Here, we demonstrate that IP6K1-KO mice display enhanced basal lipolysis. IP6K1 modulates lipolysis *via* its interaction with the lipolytic regulator protein perilipin1 (PLIN1). Furthermore, phosphorylation of IP6K1 at a PKC/PKA motif modulates its interaction with PLIN1 and lipolysis. Thus, IP6K1 is a novel regulator of PLIN1 mediated lipolysis.

1. Introduction

A coordinated stimulation of lipolysis and energy expenditure is beneficial in reducing adipose mass without causing insulin resistance (Ahmadian et al., 2009; Boden, 2008; Langin, 2006). Insulin inhibits lipolysis whereas catecholamines, like adrenaline, stimulate the process by β-AR/PKA (β-adrenergic receptor-protein kinase A) mediated regulation of lipolytic enzymes and regulators (Langin, 2006). Various enzymes are involved in hydrolyzing TAG to FFA *via* intermediate production of DAG (diglycerides) and MAG (monoglycerides). ATGL (adipose TAG lipase) converts TAG to DAG; HSL (hormone sensitive lipase) converts TAG to DAG and DAG to MAG; and MGL (monoacyl glycerol lipase) works on the last step to release FFA and glycerol (Young and Rudolf, 2013). In addition, proteins such as perilipin (PLIN) and CGI-58 regulates lipolysis by modulating activities of the lipases (Young and Rudolf, 2013). CGI-58 stimulates lipolysis by enhancing ATGL activity (Young and Rudolf, 2013). PLINs are a family of proteins of which PLIN1 is the most studied (Brasaemle, 2007). PLIN1 binds and inhibits CGI-58′s stimulatory action on ATGL and thus, inhibits basal lipolysis. β-AR stimulation/PKA activation phosphorylates PLIN1 to disrupt its association with CGI-58. Phosphorylated PLIN1 binds and stimulates HSL mediated lipolysis. PLIN1-KO mice are resistant to obesity and they display high basal but impaired β-AR induced lipolysis (Tansey et al., 2001). These mice also exhibit enhanced β-oxidation and UCP1 expression (Saha et al., 2004). Moreover, PLIN1 deletion reverses obesity in ob/ob mice (Martinez-Botas et al., 2000). PLIN2-KO mice are also protected against diet induced obesity, fatty liver and inflammation presumably *via* increased browning and decreased food intake (McManaman et al., 2013). On the other hand, PLIN1 loss of function mutation causes increased basal lipolysis, lipodystrophy and insulin resistance in humans (Kozusko et al., 2013) which is explainable as the balance between lipolysis and energy expenditure is disturbed. Thus, a complete understanding of PLIN1 regulation in healthy and metabolically sick conditions is critical. However, *in vivo* regulation of PLIN1 is not well understood. It is also not known whether a common regulator exists that coordinates PLIN1/lipolysis and energy expenditure *in vivo*.

Inositol hexakisphosphate kinases (IP6Ks) primarily generate the signaling molecule, inositol pyrophosphate, commonly known as 5-IP7 (Saiardi et al., 1999). Functions of three mammalian IP6K isoforms (IP6K1-3), of which IP6K1 is the major isoform (Chakraborty et al., 2011; Wilson et al., 2013; Thota and Bhandari, 2015), are currently being explored. Although IP6Ks display con-

* Corresponding author.
E-mail address: achakrab@scripps.edu (A. Chakraborty).

http://dx.doi.org/10.1016/j.biocel.2016.06.018
1357-2725/

served active sites, they possess unique regulatory regions which mediate specific protein–protein interactions (Barker et al., 2009; Chakraborty et al., 2011). Accordingly, IP6Ks exhibit different cellular functions (Chakraborty et al.,, 2011; Morrison et al., 2009). For example, IP6K1 generated 5-IP7 facilitates insulin secretion (Illies et al., 2007), IP6K2 regulates apoptosis and cancer metastasis (Chakraborty et al., 2011; Koldobskiy et al., 2010; Morrison et al., 2009; Rao et al., 2015) whereas IP6K3 modulates synapse formation in cerebellar Purkinje cells (Fu et al., 2015).

Mice deleted of IP6K1 are insulin hypersensitive on chow-diet (Bhandari et al., 2008). In addition, IP6K1-KO mice are protected against high fat diet (HFD) induced obesity, insulin resistance, hyperinsulinemia, hypertriglyceridemia and fatty liver (Chakraborty et al., 2010). Aged and HFD-fed IP6K1-KO mice maintain insulin sensitivity due to the loss of 5-IP7′s inhibitory action on the insulin sensitizing protein kinase Akt (Chakraborty et al., 2010). 5-IP7 inhibits Akt *in vivo* and *in vitro* (Chakraborty et al., 2010; Gokhale et al., 2013; Prasad et al., 2011; Wu et al., 2013; Xu et al., 2013; Zhang et al., 2014a,b). Moreover, IP6K1-KO mice are lean due to enhanced energy expenditure as energy intake is unaltered in these mice (Chakraborty et al., 2010). Therefore, IP6K1 is a target in obesity/T2D (Boucher et al., 2014; Chakraborty et al., 2011; Mackenzie and Elliott, 2014; Shears, 2016). However, the precise mechanism/s by which IP6K1 regulates lipid metabolism in not known. Here, we demonstrate that IP6K1 is a novel regulator of lipolysis. Furthermore, IP6K1 modulates lipolysis by phosphorylation mediated interaction with PLIN1.

2. Materials and methods

2.1. Materials

Antibodies: pPKA and pPKC substrate specific antibodies; PLIN1 and pHSL (S660): from Cell Signaling Technology. CGI-58, HSL, GST and β-Actin: from Santa Cruz Biotechnology. Myc: from Roche. IP6K1: from Genetex. **Plasmid construct:** pT7T3D-PacI-PLIN1 construct was purchased from GE Healthcare/Open Biosystems. **Reagents and Kits:** Glycerol, FFA and TAG assay kits: from Cayman Chemicals; insulin Elisa kit from Crystal Chem; BCA protein assay kit: Pierce Biotechnology; Jetprime transfection reagent from Polypus; RetroX concentrator: from Clontech; Cyclic AMP from Cell Signaling; protein A/G beads from EMD Millipore. **Pharmacologic modulators**: Forskolin/FSK (Cell Signaling), Isoproterenol (Sigma), CL316243 (Sigma); Rottlerin, LY333531 and GF109203X (Tocris). Protease plus phosphatase inhibitor tablets; Thermo Scientific, Waltham, MA. Unless otherwise stated, all chemicals are purchased from Sigma Aldrich.

2.2. Animals

Six to eight weeks old male WT and IP6K1-KO mice were housed under barrier conditions with standard chow diet (CD) (Harlan Laboratories # 2018SX) and water provided *ad libitum*. Animals were maintained at 12 h light–dark cycle at ambient temperature of 23 °C. All protocols were approved by the Scripps Florida, Institutional Animal Care and Use Committee.

2.3. Tissue collection

Mice were euthanized by carbon dioxide asphyxiation (ARC Facility, Scripps Florida). For end point studies, blood was collected by cardiac puncture for plasma preparation. Fat pads were snap-frozen in liquid nitrogen and stored at −80 °C for measuring protein expression by immunoblotting or for immunoprecipitation studies.

2.4. Lipolysis studies

Isoproterenol induced: Isoproterenol treatment was done following a standard protocol (Qiao et al., 2011). Briefly, isoproterenol was injected intraperitoneally to overnight fasted mice at 5 mg/kg body weight. Blood samples were collected 20 min before (basal) and after (stimulated) injection in vacutainer plasma preparation tubes. Blood was spun at 1500 g for 10 min at 4 °C and plasma was aliquoted and stored at 80 °C. Plasma was analyzed for Glycerol and FFA using kits from Cayman Chemicals for each parameter. **CL316243 induced:** CL316243 was injected in *ad libitium* mice intraperitoneally (0.5 mg/kg body weight). Blood was collected immediately before and 30 min after injection (Roth Flach et al., 2013). **In adipose tissue explants:** To measure lipolysis in explants *in vitro*, standard procedure was followed (Taschler et al., 2011) with slight modifications suggested by the Zechner laboratory. Briefly, equal weights of EWAT, IWAT and RWAT were taken into a 96-well plate with 200 μl media. The plate was incubated at 37 °C for 2 h with gentle shaking (basal lipolysis). Thereafter, samples were transferred to a fresh plate containing media with 10 μM isoproterenol and incubated for 30 min. Finally, fat pads were transferred to another fresh plate having media and isoproterenol and was incubated for 1 h (stimulated lipolysis). **In 3T3L1 adipocytes:** Fully differentiated 3T3L1 adipocytes were treated with DMEM containing 0.5% Fatty Acid Free BSA (Sigma) for four hours, and this media was collected for basal lipolysis measurement. Thereafter, cells were treated with 10 μM isoproterenol for 1 h and the media was collected for measurement of stimulated lipolysis (Gauthier et al., 2008).

2.5. Measurement of plasma insulin following CL316243 treatment

CL316243 treated plasma was obtained as described in the lipolysis section. Plasma was used to measure insulin levels as per manufacturer's instructions.

2.6. Plasma triglyceride analysis

Plasma was isolated from WT and IP6K1-KO mice and triglyceride was measured as per manufacturer's instructions.

2.7. Mass spectrometry to identify IP6K1 interacting proteins in 3T3L1 adipocytes

3T3L1 preadipocytes overexpressing Myc–IP6K1 and control vector were differentiated for 8 days. Afterwards, cells were lysed using lysis buffer (containing protease and phosphatase inhibitors). One mg of the lysate was pre-cleared with IgG and Protein A/G beads. Thereafter, the supernatant was incubated with IgG or Myc-antibody and Protein A/G beads overnight. Samples were spun down, supernatant was discarded and beads were washed 3X with wash buffer. Immunoprecipiated IgG control and Myc-IP6K1 interactors were separated in a SDS-PAGE gel. Bands that were specifically co-immunopripitated with Myc-IP6K1 were cut and submitted for mass spectrometry based identification. Corresponding bands from IgG control were also sequenced. Proteins identified only in Myc-IP6K1 samples were confirmed as IP6K1 interactors.

2.8. Mass spectrometry to identify IP6K1 interactome in the IWAT

For immunoprecipitation of endogenous IP6K1 from IWAT, total protein was extracted from the tissue using lysis buffer. Immunoprecipitation was performed following the same procedure as described above. IgG and IP6K1 antibodies were used for this experiment. The washed beads were processed at the mass spectrometry