Brief communication

MOLECULAR METABOLISM

Raptor/mTORC1 loss in adipocytes causes progressive lipodystrophy and fatty liver disease

Peter L. Lee, Yuefeng Tang, Huawei Li, David A. Guertin*

ABSTRACT

Objective: Normal adipose tissue growth and function is critical to maintaining metabolic homeostasis and its excess (e.g. obesity) or absence (e.g. lipodystrophy) is associated with severe metabolic disease. The goal of this study was to understand the mechanisms maintaining healthy adipose tissue growth and function.

Methods: Adipose tissue senses and responds to systemic changes in growth factor and nutrient availability; in cells mTORC1 regulates metabolism in response to growth factors and nutrients. Thus, mTORC1 is poised to be a critical intracellular regulator of adipocyte metabolism. Here, we investigate the role of mTORC1 in mature adipocytes by generating and characterizing mice in which the *Adiponectin-Cre* driver is used to delete floxed alleles of *Raptor*, which encodes an essential regulatory subunit of mTORC1.

Results: $Raptor^{Adipoq\text{-}cre}$ mice have normal white adipose tissue (WAT) mass for the first few weeks of life, but soon thereafter develop lipodystrophy associated with hepatomegaly, hepatic steatosis, and insulin intolerance. $Raptor^{Adipoq\text{-}cre}$ mice are also resistant to becoming obese when consuming a high fat diet (HFD). Resistance to obesity does not appear to be due to increased energy expenditure, but rather from failed adipose tissue expansion resulting in severe hepatomegaly associated with hyperphagia and defective dietary lipid absorption. Deleting *Raptor* in WAT also decreases C/EBPα expression and the expression of its downstream target adiponectin, providing one possible mechanism of mTORC1 function in WAT.

Conclusions: mTORC1 activity in mature adipocytes is essential for maintaining normal adipose tissue growth and its selective loss in mature adipocytes leads to a progressive lipodystrophy disorder and systemic metabolic disease that shares many of the hallmarks of human congenital generalized lipodystrophy.

Keywords mTORC1; Raptor; Rapamycin; Lipodystrophy; Obesity; White Adipose Tissue (WAT)

1. INTRODUCTION

White adipose tissue (WAT) functions both as the body's major energy storage site, and as a critical endocrine tissue, and interest in understanding its biology has intensified with the obesity epidemic. Obesity (defined as a BMI $>$ 30) results from energy imbalance and can lead to ectopic lipid deposition in non-adipose tissues (e.g. the liver), type 2 diabetes, cardiovascular disease, and some cancers. Obesity now affects more than 1 in 3 adults in the United States, and between 8% and 25% of adults in countries of the European Union, making this a major international clinical problem of this era [1–3]. Lack of adipose tissue or lipodystrophy also associates with severe metabolic complications. For example, patients suffering from congenital generalized lipodystrophy (or Berardinelli-Seip Syndrome) also develop insulin resistance, hypertriglyceridemia, and fatty liver disease, which can lead to hepatomegaly and liver failure. Thus, normal adipose tissue growth and function is critical to maintaining metabolic homeostasis and understanding the mechanisms that promote healthy fat has broad clinical implications.

The mechanistic target of rapamycin complex 1 (mTORC1) integrates multiple upstream signals from nutrient availability to promote anabolic metabolism. For example, mTORC1 detects intracellular amino acid availability through multiple sensors that converge upon the Rag GTPases to control mTORC1 subcellular localization, and circulating glucose levels through the insulin-signaling pathway, which promotes mTORC1 activity through the TSC/Rheb pathway [4–6]. Thus, mTORC1 is poised to be a critical regulator of adipocyte function. To test this, we conditionally deleted the essential mTORC1 regulatory subunit *Raptor* in mature adipocytes with Adiponectin-Cre, which is reported to have greater efficiency and specificity for mature adipocytes than aP2-Cre [7–11]. We find that $Raptor^{Adipoq\text{-}Cre}$ mice have normal WAT mass for the first few weeks of life, but progressively develop a lipodystrophy disorder resembling human congenital generalized lipodystrophy including insulin intolerance and hepatic steatosis. These and several additional characteristics of the $Raptor^{Adipoq\text{-}Cre}$ mice differ significantly from those of mice in which *Raptor* was deleted with aP2-Cre [12]. Our results provide a new framework for understanding how mTORC1 signaling helps maintain healthy adipose tissue and could provide insight into human lipodystrophy disorders.

Program in Molecular Medicine, University of Massachusetts Medical School, 373 Plantation Street, Worcester, MA 01605, USA

*Corresponding author. E-mail: david.guertin@umassmed.edu (D.A. Guertin).

Received March 21, 2016 • Revision received March 29, 2016 • Accepted April 1, 2016 • Available online 11 April 2016

http://dx.doi.org/10.1016/j.molmet.2016.04.001

2. MATERIALS & METHODS

2.1. Mice

Raptor floxed mice are described in [13] and were backcrossed 10 generations to C57BL/6 and crossed to mice expressing the Adiponectin-Cre driver or the Ucp1-Cre driver (generous gifts of Evan Rosen). Floxed Cre-negative mice were used as controls. Mice were kept on a daily 12 h light/dark cycle and fed a normal chow diet (Prolab® Isopro® RMH 3000) from Lab Diet ad libitum at 22 °C. All animal experiments were approved by the University of Massachusetts Medical school animal care and use committee.

2.2. Antibodies and reagents

PPARγ antibody is from Santa Cruz (sc-7196). UCP1 antibody is from AbCam (ab-10983). All other antibodies were purchased from Cell Signaling Technologies: ACC (3676), ACLY (4332), AKT (9727), P-AKT-S473 (4058), ATGL (2439), FASN (3180), HSL (4107), P-HSL-S660 (4126), ULK1 (8054), P-ULK1-S757 (6888), 4EBP1 (9644), P-4EBP1-S65 (9456), P-4EBP1-T37/46. All other reagents were from Sigma–Aldrich.

2.3. Diet & metabolic studies

At 12 weeks of age, male mice were placed on a 60% high fat diet (HFD) (D12492 Harlan Laboratories) for 8 weeks. Body weight was recorded weekly. The analysis of blood metabolites was performed by the Joslin Diabetes Center and MMPC at the University of Cincinnati. For glucose tolerance tests (GTT) mice were fasted overnight (16 h) and then administered 2 g/kg of body weight of glucose or sodium pyruvate by intraperitoneal (i.p.) injection. For insulin tolerance tests (ITT), mice were fasted for 6 h before i.p. administration of 0.75 unit/kg of body weight of insulin. Blood glucose concentrations were measured before and after the injection at indicated time points.

2.4. Tissue harvest and histology

Adipose tissue depots were carefully dissected to avoid contamination from surrounding tissue. Samples for RNA or protein were snap frozen in liquid nitrogen and stored at −80 °C until analysis. For histology, tissue pieces were fixed in 10% formalin. Embedding, sectioning, and Hematoxylin & Eosin (HE) staining was done by the UMass Medical School Morphology Core. For Oil Red O staining, liver samples were embedded in OCT before sectioning and staining. For cell size measurements a minimum of 9–12 images were taken used per mouse ($n = 3$ wild type and 3 conditional knockouts). Image J was used to measure cell size and the distribution of cell size as percentage of total counted cells was analyzed.

2.5. Western blots

Tissue samples or cells were lysed in a buffer containing 50 mM Hepes, pH 7.4, 40 mM NaCl, 2 mM EDTA, 1.5 mM NaVO4, 50 mM NaF, 10 mM sodium pyrophosphate, 10 mM sodium β-glycerophosphate and 1% Triton X-100. Tissues were homogenized using a TissueLyser (Qiagen) in the same lysis buffer supplemented with 0.1% SDS, 1% sodium deoxycholate. Equal amounts of total protein were loaded into acrylamide/bis-acrylamide gels and transferred to PVDF membranes for detection with the indicated antibodies. Membranes were incubated with primary antibodies in 5% milk/PBST or 5% BSA/PBST overnight. Membranes were then incubated for 1hr with HRP-conjugated secondary antibodies. Western blots were developed by enhanced chemiluminescence (PerkinElmer) and detected by X-ray films. To obtain UCP1 signaling from WAT lysates, films were exposed for varying durations, up to 12 hrs for the indicated "long exposure (LE)".

2.6. Lipolysis

Excised pgWAT tissue was incubated in 12 well cell culture dishes in DMEM with or without isoproterenol at 10 μM for 4 h, respectively, before collecting medium to measure glycerol concentration using a commercial kit (Sigma). The glycerol level was normalized with mass of the excised tissue.

2.7. Fecal lipids extraction

Mice were housed individually for 48 h and 1 g of dried feces was collected from each mouse. Feces were powderized using a tissue grinder, and rehydrated in 5 ml of normal saline. A 2:1 chloroform:methanol solution was used to extract lipids from the suspension.

2.8. Body composition and metabolic cages

Mice were placed into metabolic cages for 3 days at 25 days into their HFD feeding. Lean mass and whole body fat mass were measured noninvasively using 1H-MRS (Echo Medical Systems, Houston TX), and a 3-day measurement of physical activity, energy expenditure, respiratory exchange ratio, and food intake were conducted using metabolic cages (TSE Systems, Bad Homburg, Germany) by the UMass Mouse Metabolic Phenotyping Center. Thereafter, mice were kept on HFD in normal housing until dissection.

2.9. Gene expression analysis

Cells or tissues were lysed with Qiazol (Invitrogen) and total RNA was isolated with the RNeasy kit (Invitrogen). Equal amounts of RNA were retro-transcribed to cDNA using a High capacity cDNA reverse transcription kit (#4368813, Applied Biosystems). Quantitative RT-PCR was performed in 10 μL reactions using a StepOnePlus real-time PCR machine from Applied Biosystems using SYBR Green PCR master mix (#4309156, Applied Biosystems) according to manufacturer instructions. Relative mRNA expression was determined by the ΔCt method, and Tbp (TATA sequence binding protein) expression was used as a normalization gene in all conventional RT-PCR experiments.

2.10. Statistics

Unless otherwise stated, values given are mean ± SEM. Two-way ANOVA was performed where indicated. For most experiments, unpaired two-tailed Student's *t* test was used to determine statistical significance among two groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3. RESULTS AND DISCUSSION

3.1. Adipocyte *Raptor* KO mice have normal WAT mass early in life

To investigate the role of mTORC1 in adipocytes, we generated *Adiponectin-Cre;Raptor*$^{fl/fl}$ mice (herein *Raptor*$^{Adipoq\text{-}cre}$ mice). *Raptor*$^{Adipoq\text{-}cre}$ mice are born at the expected Mendelian ratio and are grossly indistinguishable from their littermates at birth. At post-natal day 14 (P14), there is no difference in total body weight between control and *Raptor*$^{Adipoq\text{-}cre}$ mice (Figure 1A). The major subcutaneous and visceral perigonadal white adipose tissue depots (sWAT and pgWAT) are not significantly different in mass from controls at P14 (Figure 1B). Liver mass at P14 is also not significantly different though it is trending slightly larger in the KOs; the mass of other lean tissues such as the heart, kidney, and skeletal muscle is normal (Figure 1C). We confirmed *Raptor* loss by Western blots using whole sWAT and interscapular brown adipose tissue (iBAT) depots (Figure 1D); the residual signal reflects the non-adipocyte population (see Section 3.2). Inactivation of mTORC1 activity was confirmed by decreased phosphorylation of the

