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Research Paper

Weight Loss Upregulates the Small GTPase DIRAS3 in Human White Adipose Progenitor Cells, Which Negatively Regulates Adipogenesis and Activates Autophagy via Akt–mTOR Inhibition

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ABSTRACT

Long-term weight-loss (WL) interventions reduce insulin serum levels, protect from obesity, and postpone age-associated diseases. The impact of long-term WL on adipose-derived stromal/progenitor cells (ASCs) is unknown. We identified DIRAS3 and IGF-1 as long-term WL target genes up-regulated in ASCs in subcutaneous white adipose tissue of formerly obese donors (WLDs). We show that DIRAS3 negatively regulates Akt, mTOR and ERK1/2 signaling in ASCs undergoing adipogenesis and acts as a negative regulator of this pathway and an activator of autophagy. Studying the IGF-1–DIRAS3 interaction in ASCs of WLDs, we demonstrate that IGF-1, although strongly up-regulated in these cells, hardly activates Akt, while ERK1/2 and S6K1 phosphorylation is activated by IGF-1. Overexpression of DIRAS3 in WLD ASCs completely inhibits Akt phosphorylation also in the presence of IGF-1. Phosphorylation of ERK1/2 and S6K1 is lesser reduced under these conditions. In conclusion, our key findings are that DIRAS3 down-regulates Akt–mTOR signaling in ASCs of WLDs. Moreover, DIRAS3 inhibits adipogenesis and activates autophagy in these cells.

1. Introduction

Long-term weight-loss (WL) interventions, such as prolonged hypocaloric diets and bariatric surgeries, lead to reduced insulin levels, improvement in insulin sensitivity and glycemic homeostasis in formerly obese people and improve glycemic control in individuals with diabetes mellitus type 2 (T2DM) (Klein et al., 2004; Dixon et al., 2012). Although the underlying mechanisms are not precisely understood, one common key effect of these interventions is a long-term caloric restriction (CR) (Klein et al., 2004; Sjöström et al., 2004; Bradley et al., 2012; Knop and Taylor, 2013). Long-term CR, also referred to as dietary restriction (DR), defined as lessening caloric intake (typically by about 30% in rodents and monkeys) without malnutrition is the most robust intervention to extend health and maximum lifespan in most, but not all, laboratory animal models (Speakman and Mitchell, 2011; de Cabo et al., 2014). It is widely accepted that CR protects cells against oxidative damage (López-Lluch et al., 2008) and induces DNA-repair (López-Otín et al., 2013) and recycling processes such as autophagy (de Cabo et al.,

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2014). The underlying mechanisms are however not precisely understood. Increasing evidence suggests that reduced growth factor- and nutrient-responsive protein kinase signaling mediate beneficial effects of CR. Conserved CR-responses are reduced growth hormone (GH)/insulin-like growth factor-1 (IGF-1) and insulin signaling (Bartke et al., 2013; Kenyon, 2010). In mammals, GH produced by the pituitary gland induces production and secretion of IGF-1 in the liver, which acts as endocrine regulator. IGF-1 is also produced in peripheral organs by GH-dependent and -independent pathways, which acts locally in paracrine or autocrine fashion (Sonntag et al., 2012; Bartke et al., 2013). The impact of CR on IGF-1 signaling in the periphery is little understood. Another conserved CR-response is reduced activity of the nutrient-responsive protein kinase, mechanistic target of rapamycin (mTOR), associated with lifespan extension in invertebrates and mice (Kapahi et al., 2004; Selman et al., 2009). mTOR forms a network with insulin/IGF-1 signaling, regulating a wide range of cellular processes, such as autophagy, growth, differentiation and metabolism, which are thought to mediate effects of CR (Laplante and Sabatini, 2012). The mechanisms on how CR employs the insulin/IGF-1–mTOR signaling network to influence cellular downstream pathways are the current focus of obesity and aging research.

Adipose tissue is a main organ implicated in regulation of healthspan induced by reduced insulin/IGF-1–mTOR signaling (Broughton and Partridge, 2009). Decreased insulin sensitivity in subcutaneous white adipose tissue (sWAT) due to an age-related deterioration of sWAT is a hallmark of aging (Borkan et al., 1983). Long-term CR leads to reduced adipocyte size and remodeling of body fat composition away from visceral (v) WAT to sWAT (Huffman and Barzilai, 2010; Speakman and Mitchell, 2011). Since sWAT has rather beneficial and vWAT detrimental effects in aging and obesity this contributes to extension of healthspan. While sWAT adipocytes seem to be particularly beneficial for insulin action due to their crucial role in maintaining whole body glucose homeostasis and lipid metabolism, increasing evidence suggests that health benefits of CR exceed those directly associated with weight-loss. Adipocytes arise from adipose-derived stromal/progenitor cells (ASCs), which constitute a large pool of precursors, crucial for adipose tissue renewal, homeostasis, expansion and hence function (Berry et al., 2013; Zwierzina et al., 2015). Upon stimulation by insulin, glucocorticoids, cAMP inducers, and additional serum components ASCs enter a differentiation program, referred to as adipogenesis, to acquire their specific functions as adipocytes (Rosen and MacDougald, 2006). According to the current model adipogenesis involves growth arrest, early and terminal differentiation, including morphological changes, lipid accumulation and the expression of fat cell specific genes, such as fatty acid binding protein-4 (FABP4), perilipin and adipokines. The stages of adipogenesis are orchestrated by a transcriptional cascade involving the adipogenic key factor nuclear receptor peroxisome proliferator-activated receptor-γ2 (PPARγ2) and members of the CCAAT/enhancer-binding protein (C/EBP) family.

The impact of WL on ASCs is unknown. By comparing ASCs from abdominal sWAT of normal weight (NWD), obese (OD) and long-term weight-losing formerly obese donors (WLDs) we showed that long-term WL amongst others reduced the adipogenic activity in these cells (Mitterberger et al., 2014b). To better understand the impact of long-term WL on human ASCs, we compared gene expression in a well characterized ASC population (Mitterberger et al., 2012; Zwierzina et al., 2015) isolated from sWAT of age-matched NWDs, ODs, and WLDs using microarray gene expression analysis. Intriguingly, two strongly induced WL target genes were insulin-like growth factor 1 (IGF-1), the activator of signaling from the IGF-1-receptor, and GTP-binding RAS-like 3 (DIRAS3) (Yu et al., 1999), an imprinted tumor suppressor gene. DIRAS3 encodes a small GTPase which was shown to inhibit signaling through phosphatidylinositol-3-kinase (PI3K) and Ras/Mitogen-activated protein kinase (MAPK) in tumor cells (Luo et al., 2003) and induces a dwarf phenotype in transgenic mice (Xu et al., 2000).

2. Material and methods

2.1. Donors

Human sWAT samples were taken from persons undergoing routine abdominoplasty at the Institute for Plastic and Reconstructive Surgery (Medical University Innsbruck) (Mitterberger et al., 2010, 2011, 2012, 2014a, 2014b). The patients gave their informed written consent and had been approved by the ethical committee of Innsbruck Medical University, Austria, according to the Declaration of Helsinki. All sWAT samples were obtained from the lower abdomen. Obesity and normal weight were defined according to the World Health Organization criteria on the basis of the body mass index (BMI = weight [kg]/height [m^2]). Female donors were divided into three groups according to their BMI, obese (OD) (BMI ≥ 30 kg/m^2), normal weight (NWD) (BMI 19–25 kg/m^2), and long-term weight losing initially obese (WLD) (former BMI ≥ 30 kg/m^2 and current BMI ≤ 25 kg/m^2). None of the women had diabetes, liver, renal, or other severe metabolic diseases. Long-term WL was achieved either by hypocaloric diet or laparoscopic adjustable gastric banding (LAGB). The detailed WL regimens are described in Mitterberger et al. (2010). The women were age matched. The clinical and anthropometric parameters are indicated in Table S1.

2.2. Mouse xenograft studies

Mice were treated in accordance with the guidelines of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” and the Austrian law. Animal experiments were approved by the ethics committee of the Austrian Federal Ministry of Science and Research (Application No. Zl. 188809/13). Further details are explained in the supplementary experimental procedure.

2.3. Isolation of ASC from human subcutaneous adipose tissue

ASCs were isolated as described (Mitterberger et al., 2012).

2.4. Cell culture

ASCs were cultivated as described (Mitterberger et al., 2012).

2.5. Adipogenic differentiation

Adipogenic differentiation was conducted as described (Mitterberger et al., 2012).

2.6. Retroviral gene expression system

See supplementary experimental procedures.

2.7. Laser scanning confocal indirect immunofluorescence microscopy (IF-CLSM)

IF-CLSM was performed as described (Mitterberger et al., 2012).

2.8. Affymetrix microarray gene expression analysis

See supplementary experimental procedures.

2.9. Quantitative RT-PCR analysis

Expression analysis with q-RT-PCR was performed as described (Mitterberger et al., 2012). β-actin was used for normalization. Primer sequences are listed in Table S2.