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Annexin A6 regulates adipocyte lipid storage and adiponectin release

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ABSTRACT

Lipid storage and adipokine secretion are critical features of adipocytes. Annexin A6 (AnxA6) is a lipid-binding protein regulating secretory pathways and its role in adiponectin release was examined. The siRNA-mediated AnxA6 knock-down in 3T3-L1 preadipocytes impaired proliferation, and differentiation of AnxA6-depleted cells to mature adipocytes was associated with higher soluble adiponectin and increased triglyceride storage. The latter was partly attributed to reduced lipolysis. Accordingly, AnxA6 overexpression in 3T3-L1 adipocytes lowered cellular triglycerides and adiponectin secretion. Indeed, serum adiponectin was increased in AnxA6 deficient mice. Expression analysis identified AnxA6 protein to be more abundant in intra-abdominal compared to subcutaneous adipose tissues of mice and men. AnxA6 protein levels increased in white adipose tissues of obese mice and here, levels were highest in subcutaneous fat. AnxA6 protein in adipocytes was upregulated by oxidative stress which might trigger AnxA6 induction in adipose tissues and contribute to impaired fat storage and adiponectin release.

1. Introduction

Adipocytes are highly specialized cells and store enormous amounts of neutral lipids in lipid droplets (Ducharme and Bickel, 2008). These lipid droplets are composed of a core of triglycerides surrounded by a phospholipid and cholesterol monolayer at the cytoplasmic interphase (Le Lay et al., 2004). Due to the excessive accumulation of neutral lipids in prevalent human diseases such as diabetes and obesity, this has prompted increased efforts to identify the players that contribute to the de-regulation of lipid pathways in adipose tissues (Buechler et al., 2015; Ducharme and Bickel, 2008; Fruhbeck et al., 2014; Kloting et al., 2007).

Annexins are calcium-dependent membrane-binding proteins and preferentially associate with phosphatidylserine and phosphatidylethanolamine of the intracellular leaflet (Raynal et al., 1996). Out of the annexin family, AnxA6 has been implicated in lipid homeostasis and consequently membrane transport pathways possibly relevant for adipocytes (Enrich et al., 2011; Grewal et al., 2010). Overexpression of AnxA6 perturbs the cellular distribution of cholesterol, leading to strongly reduced cholesterol levels at the Golgi and plasma membrane (Cubells et al., 2007). This intracellular imbalance of cholesterol triggers dysfunction of membrane trafficking, including an accumulation of caveolin-1 in the Golgi complex, which is associated with a reduced number of cell surface caveolae (Cubells et al., 2007). Caveolae are found abundantly in adipocytes with multiple roles in endocytosis, store-operated Ca^{2+} channels, as well as cholesterol and fatty acid transport, lipid droplet formation and signal transduction (Murphy et al., 2009; Pani and Singh, 2009).

Other de-regulated membrane trafficking events upon AnxA6 upregulation include Soluble NSF Attachment Protein Receptor (SNARE) dependent secretory pathways, such as syntaxin4/SNAP23 mediated fibronectin secretion (Reverter et al., 2011) and syntaxin-6 regulated cell surface delivery of integrins (Reverter et al., 2014).

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All of these SNARE proteins play fundamental roles in the metabolic response and regulate translocation of the glucose transporter GLUT4 to the cell surface in adipocytes (Kioumourtzoglou et al., 2014).

Other observations further implicate a role of AnxA6 in adipokine secretion. This includes the fact that calcium ions and low pH induce the translocation of cytosolic AnxA6 to secretory granules (Podszywalow-Bartnicka et al., 2007). In neuron-like PC12 cells AnxA6 overexpression and depletion blocks or enhances exocytosis of catecholamines, respectively (Podszywalow-Bartnicka et al., 2010). White adipocyte exocytosis is stimulated by cyclic AMP whilst Ca^{2+} has a modulatory role herein (El Hachmane et al., 2015; Komai et al., 2014). Adiponectin is the most abundant protein released by adipocytes via this secretory route (Komai et al., 2014).

Adiponectin is exclusively secreted from white adipocytes and high levels are found in the circulation. This multifunctional hormone exerts beneficial effects in glucose and lipid homeostasis. Systemic adiponectin is decreased in obesity because of impaired synthesis and release. Hypoadiponectinemia contributes to metabolic abnormalities in human and rodent obesity (Arita et al., 1999; Buechler et al., 2011; Hoffstedt et al., 2004; Turer and Scherer, 2012).

In previous studies we showed that adiponectin reduces AnxA6 expression in monocytes and thereby may improve cholesterol homeostasis (Stogbauer et al., 2009). Monocyte AnxA6 protein levels are increased in obesity and type 2 diabetes, suggesting a role in lipid metabolism (Stogbauer et al., 2009). Fatty acids and lipopolysaccharide that are elevated in obesity do not affect AnxA6 levels in the immune cells (Buechler et al., 2011; Stogbauer et al., 2009). The effect of reactive oxygen species generated by hyperoxia has not been tested in immune cells but is shown to induce AnxA6 levels in rat lung (Konsavage et al., 2013). Oxidative stress is increased in the adipose tissues of the obese and one of its consequences is reduced production of adiponectin. Subsequently, inflammatory and fibrotic pathways are activated and contribute to insulin resistance (Buechler et al., 2015; Netzer et al., 2015).

The aim of the current study was to assess the role of AnxA6 in adipocyte biology. This was evaluated upon AnxA6 overexpression or knockdown in 3T3-L1 adipocytes, a commonly used cell model to study adipocyte biology (Poulos et al., 2010). Adiponectin levels in AnxA6-deficient mice were also analyzed. Interestingly, we found an association of AnxA6 levels with lipolysis and adiponectin secretion and a more abundant AnxA6 protein expression in intra-abdominal compared to subcutaneous adipose tissues of mice and men. Given that AnxA6 protein expression in adipocytes was upregulated by high fat diet as well as oxidative stress, we conclude that differential AnxA6 expression may contribute to modulate fat storage and adiponectin release in health and disease.

2. Experimental procedures

2.1. Material

The AnxA6 antibody has been described elsewhere (Grewal et al., 2000). Antibodies for beta-actin, Akt, pAkt, ATGL, β-actin, caveolin-1, cyclophilin A, ERK1/2, pERK1/2, FABP4, FAS, GAPDH, HSL, pHSL and PPARγ were from New England Biolabs GmbH (Frankfurt am Main, Germany). Antibodies against SREBP1c and MnSOD and Bodipy were from Thermo Fisher Scientific (Schwerte, Germany). The SREBP2 antibody was from Cayman Chemicals (IBL International GmbH, Hamburg, Germany) and the GFP and ABCA1 antibodies were from Abcam (Cambridge, UK). Lipopolysaccharide (LPS), lovastatin, metformin and arsenic trioxide were from Sigma (Deisenhofen, Germany). The CellTiter-Blue Cell Viability Assay was from Promega (Mannheim, Germany) and LDH assay was from Roche (Mannheim, Germany). Intracellular triglyceride concentrations were measured using the GPO-PAP micro-test (purchased from Roche). Glycerol was determined by an assay from BioCat (Heidelberg, Germany). Adiponectin antibody, recombinant adiponectin and ELISA kits for adiponectin, interleukin-6 (IL-6) and chemerin were from R&D Systems (Wiesbaden-Nordenstadt, Germany).

2.2. Fatty acid treatment

Palmitate (PA), oleate (OA) and linoleate (LA) were from Sigma (Deisenhofen, Germany). The 200 mM fatty acid stock solutions were prepared in ethanol by heating at 70 °C, and 100 µl of 200 mM PA, OA and LA stock solution was added to 900 µl of a 10% fatty acid-free BSA solution (Roche) to obtain a 20 mM stock solution and incubated at 55 °C. The BSA-bound FFA stock solutions or equal amounts of BSA were added to the cells at day 0 and medium was changed at day 3, 6, 7 and 8.

2.3. Human adipocytes and adipose tissues

Purified human preadipocytes from subcutaneous and visceral fat were from BioCat (Heidelberg, Germany) and differentiated as suggested by the manufacturer. Paired samples of subcutaneous and visceral adipose tissues of eleven patients undergoing surgery for different diseases were obtained as described (Weigert et al., 2008). The study protocol was approved by the local ethics committee and carried out in accordance with the Helsinki guidelines. All patients gave written informed consent.

2.4. Animals

The male mice were from The Jackson Laboratory (Bar Harbor, USA). Fourteen weeks old C57Bl/6 animals (n = 6 per group) were fed *ad libitum* a control diet (ssniff® EF acc. D12450B (I) mod.) or a high fat diet (ssniff® EF R/M, D12451, 42% of energy from fat) for 14 weeks. Procedures were approved by the University of Regensburg Laboratory Animal Committee and complied with the German Law on Animal Protection and the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals, 1999. Experiments were conducted according to institutional and governmental regulations for animal use (Government of the Oberpfalz).

Serum of eight to nine months old male AnxA6 knock-out mice (6 animals) and C57Bl6/J controls (5 animals) was collected. Further, subcutaneous adipose tissue of seven to ten months old male AnxA6 (5 mice) and C57Bl6/J controls (5 animals) was analyzed. These mice have been already described (Hawkins et al., 1999). For GTT, mice were fasted for 5 h and injected with 2 g/kg of glucose intraperitoneally. For ITT, fed mice were injected with 0.75 U/kg of insulin intraperitoneally. Blood glucose levels were determined from blood obtained by a small incision in the mouse tail using a glucometer (Glucocard G+ meter set, Arkray). For plasma analysis, blood was collected by intracardial punction into BD Microtainer tubes. Plasma was prepared and free cholesterol, HDL, LDL, triacylglycerides and free fatty acids were measured by colorimetric and spectrometric techniques. Insulin (US Mouse Insulin ELISA, Mercodia) plasma levels were determined by ELISA according to manufacturer's instructions.

Experimental procedures were approved by the Local Ethical Committee of the University of Barcelona following European (2010/63/UE) and Spanish (RD 53/2013) regulations for the care