Experimental Cell Research 345 (2016) 100–107

Contents lists available at ScienceDirect

Experimental Cell Research

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

Research article

# The adaptor protein alpha-syntrophin regulates adipocyte lipid droplet growth

Kristina Eisinger, Lisa Rein-Fischboeck, Rebekka Pohl, Elisabeth M. Meier, Sabrina Krautbauer, Christa Buechler*

*Department of Internal Medicine I, University Hospital of Regensburg, Regensburg, Germany*

ARTICLE INFO

*Article history:*
Received 8 January 2016
Received in revised form
4 May 2016
Accepted 25 May 2016
Available online 27 May 2016

*Keywords:*
Adipogenesis
Triglycerides
Lipolysis
Insulin

ABSTRACT

The scaffold protein alpha-syntrophin (SNTA) regulates lipolysis indicating a role in lipid homeostasis. Adipocytes are the main lipid storage cells in the body, and here, the function of SNTA has been analyzed in 3T3-L1 cells. SNTA is expressed in preadipocytes and is induced early during adipogenesis. Knock-down of SNTA in preadipocytes increases their proliferation. Proteins which are induced during adipogenesis like adiponectin and caveolin-1, and the inflammatory cytokine IL-6 are at normal levels in the mature cells differentiated from preadipocytes with low SNTA. This suggests that SNTA does neither affect differentiation nor inflammation. Expression of proteins with a role in cholesterol and triglyceride homeostasis is unchanged. Consequently, basal and epinephrine induced lipolysis as well as insulin stimulated phosphorylation of Akt and ERK1/2 are normal. Importantly, adipocytes with low SNTA form smaller lipid droplets and store less triglycerides. Stearoyl-CoA reductase and MnSOD are reduced upon SNTA knock-down but do not contribute to lower lipid levels. Oleate uptake is even increased in cells with SNTA knock-down. In summary, current data show that SNTA is involved in the expansion of lipid droplets independent of adipogenesis. Enhanced preadipocyte proliferation and capacity to store surplus fatty acids may protect adipocytes with low SNTA from lipotoxicity in obesity.



## 1. Introduction

Obesity presents a growing health problem and a major risk factor for metabolic diseases. Inappropriate fat storage in adipose tissues causes triglyceride deposition in the liver and muscle which contributes to insulin resistance [1–4]. A feature of insulin resistant patients is prolonged postprandial hyperglycemia due to a decreased glucose uptake by muscle and fat tissues [5]. Muscle mass and strength is reduced in diabetic patients further contributing to obesity and metabolic inflexibility [6]. Consequently, most skeletal muscle pathologies including muscular dystrophies are associated with metabolic abnormalities [7].

The dystrophin-associated protein complex (DAPC) is essential for skeletal muscle integrity. The DAPC contributes to the structural stability of the plasma membrane and further is a platform for the formation of signaling complexes [8,9]. DAPC components are dystrophin, the dystroglycans, sarcoglycans, sarcospan, dystrobrevin and the syntrophins [9]. The domain organization of the five syntrophin family members is the same: a pleckstrin homology (PH) domain at the N-terminus with an embedded PDZ domain, a second PH domain and a C-terminal syntrophin unique domain [8]. The split PH domain of alpha-syntrophin (SNTA) binds inositol phospholipids while the second PH domain interacts with dystrophin. The PDZ domain is involved in lipid binding and further interacts with proteins like neuronal nitric oxide synthase [8,10,11].

SNTA is diminished in skeletal muscle of diabetic rats and patients with gestational diabetes and it is suggested that this protein is involved in the insulin response of skeletal muscle [12,13].

Members of the DAPC are all expressed in adipocytes [14] indicating a role in fat cell biology. Beta-sarcoglycan deficient mice do not form a sarcoglycan complex in adipocytes and skeletal muscle cells. These mice are insulin resistant and glucose intolerant. Interestingly, weight of intraabdominal adipose tissues is reduced in beta-sarcoglycan null mice [15].

Catecholaminergic stimulation, subsequent raise of cAMP levels and protein kinase A mediated phosphorylation of hormone sensitive lipase (HSL) and perilipin stimulates triglyceride breakdown in adipocytes in the fasting state [16]. This is a crucial pathway regulating the energy homeostasis of the whole organism [16]. SNTA binds guanine nucleotide-binding protein alpha-subunits which are downstream of various G-protein coupled receptors including β-adrenergic receptors [17]. Knock-down of SNTA in COS-7 cells enhances beta-adrenergic agonist induced cAMP

* Corresponding author.
*E-mail address:* christa.buechler@klinik.uni-regensburg.de (C. Buechler).

http://dx.doi.org/10.1016/j.yexcr.2016.05.020
0014-4827/

production showing that SNTA inhibits lipolysis in these cells [17]. SNTA also binds to the C-terminal amino acids of the ATP-binding cassette transporter A1 (ABCA1) to stabilize the protein [18]. Adipocyte specific deletion of ABCA1 is linked to increased lipid storage and enlarged fat pad weights in rodents [19]. ABCA1 is, however, normally expressed in the liver of mice deficient in SNTA and beta 2 syntrophin (SNTB2) arguing against a role of SNTA in stabilizing hepatic ABCA1 [20]. Further, ABCA1 mediated cholesterol and phosphatidylcholine efflux is unchanged in macrophages with deleted SNTA and SNTB2 [21].

The cellular function of SNTA depends on its association with different binding partners [8] suggesting tissue specific roles of this adaptor protein. In the current study the function of SNTA in adipocytes was analyzed to evaluate whether this adaptor protein affects adipogenesis and lipid homeostasis.

## 2. Materials and methods

### 2.1. ELISA

Adiponectin ELISA and IL-6 ELISA were from R&D Systems (Wiesbaden, Germany).

### 2.2. Immunoblot

Immunoblot was performed as recently published [22]. The annexin A6 antibody has been described [23]. SNTA antibody was kindly provided by Prof. Adams and was described recently. The antibody recognizes a peptide sequence in the PH1b domain of SNTA and was raised in rabbits [24,25]. Antibodies for detection of ACC, pACC, Akt, pAkt, AMPK, pAMPK, ATGL, β-actin, caveolin-1, Cox IV, ERK1/2, pERK1/2, FABP4, FAS, GAPDH, HSL, pHSL, PARP1, perilipin, PPARγ, Rab5 and SCD1 were from New England Biolabs GmbH (Frankfurt am Main, Germany). Heme oxygenase 1 antibody was from Novus Biologicals (Cambridge, UK). SREBP2 antibody was from Cayman Chemicals (IBL International GmbH, Hamburg, Germany). ABCA1, adipophilin, alpha 1 adrenergic receptor and PGC1α antibodies were from Abcam (Cambridge, UK). Antibodies to eNOS and phosphorylated eNOS were from Merck Millipore (Schwalbach, Germany). Chemerin antibody was from R&D Systems (Wiesbaden, Germany). MnSOD antibody was from Thermo Fisher Scientific (Schwerte, Germany).

### 2.3. Transfection with siRNAs

3T3-L1 preadipocytes were from the ATCC (Manassas, VA, USA) and differentiated as described [22]. Transfection of preadipocytes with siRNAs was performed with XtremeGene transfection reagent (Roche, Mannheim, Germany) or Endoporter (Gene Tools LLC, Philomath, Oregon, USA). Silencer® Select Pre-Designed siRNAs and Negative Control siRNA were from Applied Biosystems (Darmstadt, Germany). The siRNA s74114 (CGAUGGUCUUUAUCAUCCAtt) was used to knock down SNTA. SCD1 was knocked down with s73339 (GGGAUUUUCUACUACAUGAtt) and s73340 (CCGCGCAUCUCUAUGGAUAtt).

### 2.4. Fatty acid treatment

Oleate, linoleate and palmitate were ordered from Sigma (Deisenhofen, Germany). Fatty acids were complexed to fatty acid-free bovine serum albumin (Roche, Mannheim, Germany) with a molar ratio of 1:1. Equal amounts of bovine serum albumin were added to control cells. Fatty acids were added to the culture medium during differentiation or for 24 h.

### 2.5. Immunofluorescence

3T3-L1 adipocytes cultivated on cover slips (VWR, Darmstadt, Germany) were fixed with methanol at −20 °C for 15 min. After incubation with blocking solution (3% BSA, 0.05% Tween 20), and washing with PBS and PBS Tween (0.1%), cells were incubated with SNTA antibody (1:100-fold diluted) and adipophilin antibody (1:10-fold diluted) at 4 °C overnight. After washing, cells were incubated with secondary antibodies and DAPI (Roche) for 1 h at 37 °C. Slides were mounted with fluorescent mounting medium (DAKO, Glostrup, Denmark).

### 2.6. Cell viability

CellTiter-Blue Cell Viability Assay was from Promega (Mannheim, Germany). LDH assay was from Roche (Mannheim, Germany).

### 2.7. Quantification of lipids

Triglycerides were measured using GPO-PAP micro-test (Roche, Mannheim, Germany). Glycerol and free fatty acids were determined by an assay from BioCat (Berlin, Germany) and cholesterol by an assay from Diaglobal (Berlin, Germany).

### 2.8. Statistical analysis

Data are given as box plots showing the median, lower and upper quartiles and range of the values (SPSS Statistics 21.0 program). Statistical differences were analyzed by Student's *t*-test (Ms Excel). A value of $p < 0.05$ was regarded as significant.

## 3. Results

### 3.1. SNTA increases during adipogenesis

SNTA was expressed in 3T3-L1 preadipocytes and increased two days after initiation of adipogenesis. Levels were not further changed during adipocyte maturation (Fig. 1A and B). Immunofluorescence revealed cytoplasmic localization of SNTA (Fig. 1C). SNTA did not colocalize with adipophilin in mature adipocytes excluding a close association of the adaptor protein with this lipid droplet associated protein. Differentiation of the cells in medium supplemented with oleate increased lipid storage (data not shown) and fatty acid binding protein 4 (FABP4) but did not affect SNTA levels (Fig. 1D). Similarly, palmitate and linoleate added during differentiation had no effect on SNTA protein (Fig. 1E). Chemerin is shown as positive control and is induced in adipocytes differentiated in the presence of the fatty acids as described [22] (Fig. 1E). The peroxisome proliferator activated receptor (PPAR) γ agonist pioglitazone did not upregulate SNTA in adipocytes (data not shown). This suggests that induction of SNTA early during adipogenesis is not regulated by the main adipogenic transcription factor PPARγ.

### 3.2. SNTA knock-down increases proliferation of preadipocytes and hinders formation of large lipid droplets in mature cells

Knock-down of SNTA in preadipocytes increased the number of viable cells suggesting enhanced proliferation (Fig. 2A). When the 3T3-L1 siRNA transfected preadipocytes were differentiated to adipocytes the cells with low SNTA formed only small lipid droplets (Fig. 2B). The number of lipid droplets with a diameter below 6 μM tended to be increased while the number of very large lipid droplets was significantly reduced (Fig. 2C). Adipocytes with low