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Expression of human and mouse adenine nucleotide translocase (ANT) isoform genes in adipogenesis

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ABSTRACT

Adenine nucleotide translocases (ANTs) are mitochondrial proteins encoded by nuclear DNA that catalyze the exchange of ATP generated in the mitochondria for ADP produced in cytosol. There are four ANT isoforms in humans (hANT1-4) and three in mice (mANT1, mANT2 and mANT4), all encoded by distinct genes. The aim of this study was to quantify expression of ANT isoform genes during the adipogenesis of mouse 3T3-L1 and human Simpson–Golabi–Behmel syndrome (SGBS)-derived preadipocytes. We also studied the effects of the adipogenesis regulators, insulin and rosiglitazone, on ANT isoform expression in differentiated adipocytes and examined the expression of ANT isoforms in subcutaneous and visceral white adipose tissue (WAT) from mice and humans. We found that adipogenesis was associated with an increase in the expression of ANT isoforms, specifically mANT2 in mouse 3T3-L1 cells and hANT3 in human SGBS cells. These changes could be involved in the increases in oxidative metabolism and decreases in lactate production observed during differentiation. Insulin and rosiglitazone induced mANT2 gene expression in mature 3T3-L1 cells and hANT2 and hANT3 gene expression in SGBS adipocytes. Furthermore, human WAT expressed greater amounts of hANT3 than hANT2, and the expression of both of these isoforms was greater in subcutaneous WAT than in visceral WAT. Finally, inhibition of ANT activity by atractyloside or bongkrekic acid impaired proper adipocyte differentiation. These results suggest that changes in the expression of ANT isoforms may be involved in adipogenesis in both human and mouse WAT.

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1. Introduction

Adenine nucleotide translocase (ANT) is an integral inner mitochondrial membrane protein that exchanges newly synthesized ATP inside the mitochondrial matrix for cytosolic ADP, thus allowing the proper function of oxidative phosphorylation (OXPHOS) (Klingenberg, 1989). There are four ANT isoforms in humans and three ANT isoforms in mice, all encoded by distinct genes. Human ANT1 (SLC25A4) and mouse ANT1 (Slc25a4), referred to here as hANT1 and mANT1, respectively, are expressed predominantly in highly oxidative cells of the muscular lineage, such as skeletal muscle and heart-tissues where OXPHOS is most active (Stepien et al., 1992; Dolce et al., 2005; Doerner et al., 1997). Consequently, ANT1 is the isoform classically associated with oxidative phosphorylation. hANT2 (SLC25A5), first cloned from human fibroblasts and identified as an early-response gene (Battini et al., 1987), has since been described as a marker of cell proliferation and shown to be upregulated in many cancer cell types characterized by high glycolytic activity (Barath et al., 1999b; Giraud et al., 1998). These properties could suggest that this isoform serves functions other than classical OXPHOS since, generally speaking, higher hANT2 expression is correlated with lower OXPHOS activity. However, mANT2 (Slc25a5) is expressed in most tissues other than skeletal muscle, where it is substituted for by mANT1 (Levy et al., 2000). The ANT3 isoform is unique to humans and is ubiquitously expressed (Stepien et al., 1992; Dolce et al., 2005; Doerner et al., 1997); in

Abbreviations: ADIPOQ, adiponectin; ANT, adenine nucleotide translocase; MT-CO1, mitochondrially encoded cytochrome c oxidase subunit I; COX4I1, cytochrome c oxidase, subunit IV, isoform 1; GLUT4, facilitated glucose transporter, member 4; GPR81, G protein-coupled receptor 81; OXPHOS, oxidative phosphorylation; PPARγ, peroxisome proliferator-activated receptor-γ; PGC-1α, PPARγ coactivator 1α; RT-qPCR, quantitative real-time PCR; TZDs, thiazolidinediones; WAT, white adipose tissue.

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tissues other than heart and skeletal muscle, hANT3 (SLC25A6) is expected to serve OXPHOS-related functions. We also recently demonstrated that hANT3 is upregulated during cell differentiation through a mechanism that depends on PGC-1α (peroxisome proliferative activated receptor-γ, coactivator 1α) (Gavalda-Navarro et al., 2014). Expression of hANT4 (SLC25A31) and mouse mANT4 (Slc25a31) is essentially restricted to the testis (Dolce et al., 2005; Rodic et al., 2005).

Although there is some information about the expression of ANT isoforms, there are no reports describing the expression of ANT isoform genes during adipocyte differentiation. Considering that changes in mitochondrial biogenesis and remodeling are inherent features of adipocyte differentiation and appear to be influenced by the actions of insulin and insulin sensitizers, such as rosiglitazone (Wilson-Fritch et al., 2003), it is reasonable to suppose that the expression of ANT isoforms would be relevant in this context.

Visceral adipose tissue activity is closely associated with cardiovascular disease risk factors and more strongly correlates with insulin resistance than does subcutaneous adipose tissue activity (Wajchenberg, 2000). Furthermore, visceral adipocytes are more metabolically active and sensitive to lipolysis than subcutaneous adipocytes; on the other hand, subcutaneous adipose tissue is more active in synthesizing triglycerides than visceral adipose tissue (Ibrahim, 2010). However, the ANT isoform expression profile in different white adipose tissue (WAT) depots has not been described.

The aim of the present work was to determine whether genes for distinct ANT isoforms are differentially regulated during adipogenesis in mouse and human adipocytes using 3T3-L1 and SGBS adipocytes as model *in vitro* adipogenic differentiation systems for mice and humans respectively. 3T3-L1 fibroblasts are a continuous substrain of mouse 3T3 cells that have the capacity to differentiate into adipose cells, while SGBS cells are human preadipocytes (obtained from a Simpson–Golabi–Behmel syndrome patient) that maintain the capacity to undergo adipogenesis (Wabitsch et al., 2001). We also studied the effects of the adipogenesis regulators insulin and rosiglitazone on the expression of ANT isoform genes in differentiated adipocytes. Furthermore, we examined the expression of ANT isoforms *in vivo* in visceral and subcutaneous human and mice WAT depots, which are characterized by differences in metabolic activity. These studies demonstrated that mANT2 is the predominant isoform expressed in mouse adipocyte, whereas hANT3 is the main isoform expressed in human adipocytes. Furthermore, we found that adipogenesis is associated with an increase in the expression of genes for specific ANT isoforms, namely mANT2 in mouse 3T3-L1 cells and hANT3 in human SGBS cells, and that inhibition of ANT activity impairs adipocyte differentiation. These increases might be involved in the observed increase in oxidative metabolism and decrease in lactate production that accompanies differentiation. The adipogenic factors insulin and rosiglitazone increased mANT2 gene expression in 3T3-L1 cells and hANT2 and hANT3 in SGBS adipocytes. We also found that the pattern of ANT isoform expression differed between human visceral and subcutaneous WAT. Collectively, our results show that the expression of ANT isoforms is related to WAT adipogenesis in humans and mice.

2. Materials and methods

2.1. Cell culture

Mouse 3T3-L1 preadipocytes were cultured in Maintenance Medium (MM), composed of DMEM supplemented with 10% (vol/vol) FBS and 100 units/ml penicillin/streptomycin (P/S) (Gibco Life Technologies, Foster City, CA) at 37 °C in a humidified 95% air/5% CO_2 incubator. 3T3-L1 adipocyte differentiation was induced in post-confluent preadipocytes by changing the medium to MM supplemented with 0.5 mM isobutylmethylxanthine (IBMX), 0.25 μM dexamethasone, and 5 μg/ml insulin (Sigma–Aldrich, St. Louis, MO, USA). After 48 h, the medium was changed to MM supplemented with 5 μg/ml insulin, and cells were incubated for an additional 48 h. At this time, the medium was replaced with standard MM and cells were cultured until adipocytes differentiated. Human SGBS preadipocytes were grown in Medium A consisting of DMEM containing 10% FBS, 1% P/S, 33 mM biotin, and 17 mM pantothenic acid (Sigma–Aldrich) at 37 °C in a humidified 95% air/5% CO_2 incubator. Adipogenic differentiation was induced by first incubating in Medium A supplemented with 20 nM insulin, 100 nM cortisol, 200 pM triiodothyronine (T_3), 0.01 mg/ml transferrin, 500 μM IBMX, 25 nM dexamethasone (Sigma–Aldrich) and 2 μM rosiglitazone (Alexis; Enzo Life Sciences, Inc., Lausen, Switzerland) for 4 days, and then in Medium A supplemented with 20 nM insulin, 100 nM cortisol, 200 pM T_3, and 0.01 mg/ml transferrin for 10 days. For ANT activity inhibition experiments, 3T3-L1 and SGBS adipocytes were treated with 100 μM atractyloside or 10 μM bongkrekic acid (Sigma–Aldrich) throughout the differentiation process.

2.2. Adipose tissue samples

Briefly, 9-month-old mice were decapitated and visceral and subcutaneous samples were collected from epididymal and inguinal fat depots, respectively, according to a procedure approved by the Ethical Committee of Animal Experimentation of the University of Barcelona. Human biopsy samples of subcutaneous adipose tissue were taken from the abdominal area by needle aspiration under 1% lidocaine local anesthesia. Samples of visceral adipose tissue were obtained on occasion of elective laparoscopic cholecystectomy. Procedures were undertaken with the understanding and written consent of each subject and in accordance to the standards set by the Declaration of Helsinki, and the study was approved by the Hospital de la Santa Creu i Sant Pau ethics committee. All tissue samples were immediately frozen in liquid nitrogen.

2.3. RNA isolation, cDNA synthesis, and quantitative real-time PCR

Total RNA from cultured cells or tissue was isolated using a column-affinity–based method (NucleoSpin RNA II; Macherey-Nagel, Düren, Germany). Total RNA (500 ng) was then reverse transcribed into cDNA using Multiscribe reverse transcriptase and random-hexamer primers (TaqMan Reverse Transcription Reagents from Applied Biosystems; Life Technologies, Foster City, CA, USA). For quantitative analysis of mRNA expression, TaqMan quantitative real-time PCR reactions were performed on a 7500 Real-Time PCR System (Applied Biosystems) in a final volume of 20 μl using Platinum Quantitative PCR SuperMix-UDG with ROX reagent (Life Technologies) and the following specific primer pair probes (TaqMan Gene Expression Assays; Applied Biosystems): SLC25A4 (hANT1), Hs00154037_m1; SLC25A5 (hANT2), Hs00854499_g1; SLC25A6 (hANT3), Hs00745067_s1; PPARGC1A (PGC-1α), Hs00173304_m1; PPARG (PPARγ), Hs00234592_m1; ADIPOQ, Hs00605917_m1; SLC2A4 (GLUT4), Hs00168966_m1; MT-CO1, Hs02596864_g1; COX4I1, Hs00266371_m1; 18S, Hs99999901_s1; Slc25a4 (mANT1), Mm01207393_m1; Slc25a5 (mANT2), Mm00846873_g1; Ppargc1a (PGC-1α), Mm00447183_m1; Pparg (PPARγ), Mm00440945_m1; Adipoq, Mm00456425_m1; Slc2a4 (GLUT4), Mm00436615_m1; mt-Co1, *on-demand*; and Cox4i1, Mm00438289_g1. The relative

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