Original Article

MOLECULAR METABOLISM

Mitochondrial fission is associated with UCP1 activity in human brite/beige adipocytes

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ABSTRACT

Objective: Thermogenic adipocytes (*i.e.* brown or brite/beige adipocytes) are able to burn large amounts of lipids and carbohydrates as a result of highly active mitochondria and enhanced uncoupled respiration, due to UCP1 activity. Although mitochondria are the key organelles for this thermogenic function, limited human data are available.

Methods/results: We characterized changes in the mitochondrial function of human brite adipocytes, using hMADS cells as a model of white- to brite-adipocyte conversion. We found that profound molecular modifications were associated with morphological changes in mitochondria. The fission process was partly driven by the DRP1 protein, which also promoted mitochondrial uncoupling.

Conclusion: Our data demonstrate that white-to-brite conversion of human adipocytes relies on molecular, morphological and functional changes in mitochondria, which enable brite/beige cells to carry out thermogenesis.

Keywords hMADS; UCP1; DRP1; Brite/beige adipocyte; Mitochondria

1. INTRODUCTION

Overweight and obesity are a consequence of an energy imbalance, which leads to an increase in white fat mass. Brown adipocytes and their activation are promising targets for the treatment of human obesity [1,2], because these cells show high metabolic activity under stimulated conditions in both rodents and adult humans [3–7]. Thermogenic adipocytes are found in discrete brown adipose tissue (BAT) depots as brown adipocytes and are interspersed in white fat depots as 'brite/beige' adipocytes [8,9]. Depending on their anatomical location, thermogenic adipocytes found in humans display a molecular signature of either classical brown or brite/beige fat cells [10–17].

Given the abundance of white adipose tissue (WAT) in humans, there is growing clinical interest in understanding how brite adipocytes develop, especially since their emergence has been associated with protection against obesity and metabolic dysfunctions in rodents [18–21]. Brite adipocytes are derived either from progenitors, or, for a large proportion of cells, through direct conversion of mature white adipocytes [22–24]. This mechanism of conversion highlights the plasticity of adipocytes in response to specific physiological situations [25,26]. Compared to white adipocytes, brown and brite adipocytes possess a higher mitochondrial content and express the uncoupling protein 1 (UCP1), which facilitates a proton leak and the uncoupling of the respiratory chain [27,28]. This phenomenon results in a high oxidative capacity and increased energy expenditure, which leads to thermogenesis. Conversely, white adipocytes contain fewer mitochondria, but their function is essential for adipocyte differentiation and function [29]. Mitochondria are dynamic organelles that display morphological changes, such as fusion/fission events, which represent an adaptation to the needs of the cell [30]. Mitochondria continuously undergo fusion and fission events: conditions requiring high mitochondrial ATP synthesis are associated with mitochondrial elongation [30,31], whereas, bioenergetic stress induces mitochondrial fragmentation, which may lead to apoptosis [30,32]. However, mitochondrial fission may not be deleterious *per se* in brown adipocytes. Indeed, adrenergic stimulation of rodent brown adipocytes induces substantial changes in the mitochondrial architecture, including a high rate of fragmentation [33,34]. This phenomenon favors enhanced mitochondrial uncoupling and

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energy expenditure. Taken together, these observations highlight the importance of mitochondrial biogenesis and dynamics in the function of brite and brown adipocytes.

The *in vivo* discrepancies between mice and humans in terms of the amount and location of brite fat cells, as well as the difficulty in routinely obtaining fresh human samples, underline the need to decipher the mechanisms regulating brite adipocyte formation and activity in human cells [35]. Herein, we have characterized the properties of the mitochondria during the conversion of human white to brite adipocytes using the human Multipotent Adipose Derived Stem Cell (hMADS) model [36]. We found that human brite adipocyte mitochondria had an enhanced oxidative capacity and sustained fission, which was driven by DRP1.

2. MATERIALS AND METHODS

2.1. Reagents

Culture media and buffer solutions were purchased from Lonza Verviers (Verviers, Belgium); fetal bovine serum, insulin, and trypsin from Invitrogen (Cergy Pontoise, France), hFGF2 from Peprotech (Neuilly sur Seine, France). Other reagents were from Sigma–Aldrich Chimie (Saint-Quentin Fallavier, France).

2.2. hMADS cell culture

The establishment and characterization of hMADS cells has been described [36–39]. Cells were used between passages 14 and 25; all experiments were performed at least 3 times and cells were free of viruses and mycoplasma. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 15 mM Hepes, and 2.5 ng/ml hFGF2. Cells were triggered for differentiation on the second day post-confluence (designated as day 0) in DMEM/Ham's F12 media supplemented with 10 μg/ml transferrin, 10 nM insulin, 0.2 nM triiodothyronine, 1 μM dexamethasone, and 500 μM isobutyl-methylxanthine. Two days later, the medium was changed (dexamethasone and isobutyl-methylxanthine removed), and 100 nM rosiglitazone were added. At day 9, rosiglitazone was withdrawn to enable white adipocyte differentiation. Rosiglitazone (100 nM) or GW7647 (300 nM) was added at day 14 to promote white to brite adipocyte conversion and cells were used at day 18.

Transfection experiments were performed using HiPerfect (QIAGEN, France) at day 14 of differentiation. Cells were incubated with a mixture containing HiPerfect and siRNA (50 nM) in DMEM. Four hours later, the mixture was supplemented with F12 medium containing 20 μg/ml transferrin, 20 nM insulin, and 0.4 nM triiodothyronine. siRNA against human DRP1 was purchased from Ambion (Life Technologies, Courtaboeuf, France) and validated to specifically target DRP1 (ID #: s19560).

2.3. Western blot analysis

Proteins were extracted and blotted as previously described [40]. Primary antibody incubation was performed overnight at 4 °C (anti-UCP1, Calbiochem, #662045, dilution 1:750; and anti-β-tubulin, Sigma #T5201, dilution 1:2000; anti-DRP1, Cell Signaling #5391, dilution 1:1000; anti-phosphoDRP1(Ser616), Cell Signaling #4494, dilution 1:1000; anti-citrate synthase, Abcam #ab96600, dilution 1:10,000) and then detected with HRP-conjugated anti-rabbit or anti-mouse immunoglobulins (Promega, Charbonnières-les-Bains, France). Detection was performed using Chemiluminescent HRP Substrate (Millipore, Molsheim, France). OD band intensities were evaluated using PCBas Software.

For mitochondrial complex quantitation, equal amounts of cell proteins were separated using gradient SDS-PAGE (10–20%) and blotted onto nitrocellulose membranes. Saturated membranes were incubated overnight with a 1:1000 dilution of total OXPHOS human Western Blot Antibody Cocktail (#MS601, Mitosciences) followed by 60 min incubation with HRP-conjugated anti-mouse immunoglobulins. Chemiluminescence obtained after addition of Clarity Western ECL Substrate (BioRad, Marnes-la-Coquette, France) was detected using a ChemiDoc MP Imaging System (Bio-Rad) and quantified with Image Lab 5.0 software (Bio-Rad).

2.4. Immunostaining analysis

Cells were fixed with PAF 4% for 10 min, permeabilized with 0.1% Triton X-100 for 10 min, and then sequentially incubated with primary antibody overnight at 4 °C (anti-UCP1, Calbiochem, #662045, dilution 1:100; anti-TIMM23, BD Biosciences #611222, dilution 1:500; anti-cytochrome C, SantaCruzBT #sc-13560, dilution 1:100) and with the relevant secondary antibody coupled to Alexa-488 or Alexa-594 (Invitrogen, dilution 1:500) for 30 min at RT. Cells were finally mounted and visualized with an Axiovert microscope (Carl Zeiss, Le Pecq, France) under oil immersion, and pictures were captured and treated with AxioVision software (Carl Zeiss). The mitochondrial network was analyzed using Fiji software [41].

2.5. Isolation and analysis of RNA

These procedures were carried out according to MIQE recommendations [42]. Total RNA was extracted using TRI-Reagent (Euromedex, Souffelweyersheim, France) according to the manufacturer's instructions. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was conducted as described previously [43]. The expression of selected genes was normalized to that of TATA-box binding protein (TBP) and 36B4 housekeeping genes and then quantified using the comparative-ΔCt method. Primer sequences are available upon request.

2.6. Mitochondrial DNA quantification

DNA was extracted using a DNA extraction kit (Macherey–Nagel EURL, France). 2 ng of the total DNA were used for qPCR analysis, and the mitochondrial DNA content was calculated from the ratio of the DNA of the NADH dehydrogenase subunit 1 gene (mitochondrial gene) to that of lipoprotein lipase gene (a nuclear gene) as previously described [44].

2.7. Oxygen consumption analysis

For respiration analysis, hMADS cells were seeded in a 24 multi-well plate (Seahorse) and differentiated as described previously [45]. The oxygen consumption rate (OCR) of 18-day-old differentiated cells was determined using an XF24 Extracellular Flux Analyzer (Seahorse Bioscience). Uncoupled and maximal OCR were determined using oligomycin (1.2 μM) and FCCP (1 μM). Rotenone and Antimycin-A (2 μM each) were used to inhibit mitochondrial respiration. All parameters were calculated as described previously [46].

2.8. Electron microscopy

hMADS adipocytes were fixed in 1.6% glutaraldehyde in 0.1 M phosphate buffer. They were rinsed with cacodylate buffer, then post-fixed in osmium tetroxide (1%), and reduced with potassium ferricyanide for 1 h. After a water wash, cells were dehydrated with several incubations in increasing concentrations of ethanol and embedded in Epon resin. Eighty nanometer sections were contrasted with uranyl acetate and lead citrate and then observed with an electron

