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Dynamic changes in lipid droplet-associated proteins in the "browning" of white adipose tissues☆

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ABSTRACT

The morphological and functional differences between lipid droplets (LDs) in brown (BAT) and white (WAT) adipose tissues will largely be determined by their associated proteins. Analysing mRNA expression in mice fat depots we have found that most LD protein genes are expressed at higher levels in BAT, with the greatest differences observed for Cidea and Plin5. Prolonged cold exposure, which induces the appearance of brown-like adipocytes in mice WAT depots, was accompanied with the potentiation of the lipolytic machinery, with changes in ATGL, CGI-58 and G0S2 gene expression. However the major change detected in WAT was the enhancement of Cidea mRNA. Together with the increase in Cidec, it indicates that LD enlargement through LD–LD transference of fat is an important process during WAT browning. To study the dynamics of this phenotypic change, we have applied 4D confocal microscopy in differentiated 3T3-L1 cells under sustained β-adrenergic stimulation. Under these conditions the cells experienced a LD remodelling cycle, with progressive reduction on the LD size by lipolysis, followed by the formation of new LDs, which were subjected to an enlargement process, likely to be CIDE-triggered, until the cell returned to the basal state. This transformation would be triggered by the activation of a thermogenic futile cycle of lipolysis/lipogenesis and could facilitate the molecular mechanism for the unilocular to multilocular transformation during WAT browning. This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

1. Introduction

Intracellular lipid droplets (LDs) are ubiquitous organelles present in all types of eukaryotic cells such as plants, mammals, algae and yeast [1–3]. Although they have a simple structure composed of a neutral lipid core surrounded by a phospholipid monolayer, the presence of LD-associated proteins provides a level of complexity and the ability to modulate key parameters such as their size, stability, inter-droplet interactions as well as regulatable lipid storage. Once considered passive accumulators of fat, the growing knowledge of the complexity of LD-based processes means that LDs are now emerging as dynamic organelles playing a central role in the regulation of lipid metabolism [4].

Adipocytes are specialized in the accumulation, storage and mobilization of neutral lipids in mammals and their LDs have specific properties not found in other cell types. While non-adipocyte cells usually contain tiny LDs (typically <1 μm), adipocytes accumulate massive amounts of TAG in supersized LDs, which can be in the 100 μm range [5]. White adipose tissue (WAT) acts as an energy depot, which stores lipids that are released into circulation when they are required. In contrast, brown adipose tissue (BAT) uses its stored fat to generate heat by oxidation of fatty acids to maintain the body temperature [6]. Their different functions are reflected in their morphology, as white adipocytes usually contain a single giant LD occupying most of the cytoplasm (unilocular), while brown adipocytes, rich in mitochondria, are filled with a number of smaller LDs (multilocular). Large LDs offer the more efficient form of fat storage, whereas smaller LDs, with higher surface/volume ratio, will facilitate the release of their stored lipids given the extensive surface accessible to lipases.

The recent identification of BAT in adult humans has stimulated the exploration of its potential role in obesity or type 2 diabetes therapies [7,8]. An especially promising strategy is based on the promotion of brown fat properties in WAT, in other words, the browning of white adipocytes. Following prolonged cold exposure, multilocular UCP1-positive adipocytes appear in WAT depots [9,10]. It has been suggested that they represent a third kind of adipocyte, named

Abbreviations: LD, lipid droplet; BAT, brown adipose tissue; scWAT, subcutaneous white adipose tissue; gonWAT, periovarian white adipose tissue; mesWAT, mesenteric white adipose tissue; FAs, fatty acids; TAG, triacylglycerol; IBMX, isobutylmethylxanthine; PKA, protein kinase A; NLSD-I, neutral lipid storage disease with ichthyosis; ER, endoplasmic reticulum; ROS, reactive oxygen species

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beige or BRITE (brown in white) cells, which resemble white cells in basal conditions but acquire a brown phenotype during cold acclimatization [11,12]. An alternative to the presence of a specific subset of switchable BRITE cells within white depots is that brown-like cells could originate by the transdifferentiation of classic white adipocytes, which would have the inherent ability to acquire brown features [13–15]. In any case, the phenotypic transformation experienced by WAT cells at cold temperatures will imply profound changes in the proteins controlling the LDs.

In this article we discuss the role of the main LD proteins in adipocytes and show their adipose tissue depot-specific expression pattern and regulation after the acclimatization of mice to cold temperatures. In addition, using 4D confocal microscopy, we have monitored the adipocyte LD dynamics during sustained β-adrenergic stimulation, modelling the phenotypic transformation involved in white to brown adipocyte transdifferentiation.

2. Material and methods

2.1. Mice treatment and gene expression analysis

Sv129 female mice (10 week old) were maintained at 28 °C, 22 °C, or 6 °C for 10 days. Adipose tissues were dissected and either snap frozen or fixed in neutral buffered formalin. RNA was prepared from the snap frozen tissue using Tri Reagent (Invitrogen) and reverse transcribed to cDNA using M-MLV reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed on an ABI 7900HT system (Applied Biosystems) using the JumpStart Taq polymerase kit (Sigma-Aldrich). Gene expression levels were normalized relative to β-tubulin mRNA. The primers used for each gene are listed in Supplemental Table 1.

2.2. Light microscopy and immunohistochemistry

Immediately after removal, adipose tissues were fixed overnight by immersion at 4 °C in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). They were then dehydrated, cleared, and paraffin-embedded. Sections from 3 different levels (100 μm apart) were hematoxylin and eosin stained to assess morphology and for immunohistochemistry. UCP1 or CIDEA immunoreactivity was examined as follows. For each section level, 3 μm thick, dewaxed sections were incubated with rabbit anti-UCP1 (1:500 v/v, Abcam, cat # 10983) or rabbit anti-CIDEA (1:200 v/v; Sigma-Aldrich, cat # C7977), according to the avidin-biotin complex (ABC) method. Briefly, endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol and sections incubated in normal goat serum (1:75) for 20 min to reduce nonspecific background. Sections were then incubated with primary antibodies against UCP1 or CIDEA overnight at 4 °C followed with goat anti-rabbit IgG biotin conjugated (1:200; Vector Labs) and detection with ABC kit (Vector Labs) enzymatic reaction to reveal peroxidase with Sigma Fast 3,3′-diaminobenzidine (Sigma-Aldrich) as substrate. Finally, sections were counterstained with hematoxylin and mounted in Eukitt (Fluka). All observations were performed with a Nikon Eclipse 80i light microscope. The images were stored as TIFF files. Brightness and contrast of the final images were adjusted using Adobe Photoshop.

2.3. Cell culture

3T3-L1 cells were cultured in glass bottom dishes (MatTek Corp.) previously coated with gelatin. The undifferentiated cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l glucose and L-glutamine (Invitrogen) supplemented with 10% newborn calf serum (Invitrogen) and antibiotics at 37 °C and 5% CO_2. Two days after confluence, cell differentiation was initiated with DMEM with 10% fetal bovine serum (FBS, Invitrogen), 500 μM isobutylmethylxanthine (IBMX) (Sigma-Aldrich), 0.25 μM dexamethasone (Sigma-Aldrich) and 170 nM insulin (Sigma-Aldrich). After 48 h medium was changed to DMEM with 10% FBS and 170 nM insulin, and replenished every two days for 8–10 days.

2.4. 4D confocal imaging

The differentiated 3T3-L1 cells were washed with DMEM and incubated in DMEM with HEPES (Invitrogen) containing 0.1 μg/ml BODIPY 493/503 (Invitrogen). After 10 min at 37 °C, FBS was added to a final concentration of 10% and the dish was positioned in a Leica SP5 confocal microscope with a 63× Plan-APO, 1.4 NA oil immersion objective and equilibrated at 37 °C for 30 min. The imaging was initiated after the addition of IBMX (500 μM) and isoproterenol (10 μM). The cells were recorded during 15 h on a xyzt configuration, using 5% of the argon laser power on the 488 nm excitation channel. Z-stack confocal images were acquired every 1 or 2 min, and the time-lapse series were represented as their maximum projection and managed with ImageJ (National Institutes of Health) and Adobe Photoshop.

3. Results and discussion

3.1. LD protein gene expression in mouse white and brown adipose tissue depots

To investigate the importance of the main LD proteins (Table 1) in white and brown adipose tissues, we analysed their gene expression in interscapular BAT and three WAT depots: subcutaneous (scWAT), gonadal (gonWAT) and mesenteric (mesWAT) (Fig. 1). Our results clearly show that for most of the LD proteins, their mRNA levels are much higher in BAT than in WAT. This suggests a quantitatively greater involvement of LD-associated processes in the brown adipocytes, as well as a major complexity in LD dynamics of these cells. The adipose tissues are composed of lipid-filled mature adipocytes and other non-adipocyte cells, which form the stromal vascular fraction that consists of various types of cells, including immune cells, fibroblasts, pericytes, endothelial cells, adipocyte progenitors, and stromal cells, as well as undefined pool of stem cells. Although all these cells will contain LDs and associated proteins, adipocytes will be the predominant site of LD-associated protein gene expression in the adipose tissue. Considering that these proteins are coating the LDs, and that the total LD surface is higher in multilocular adipocytes, it is not surprising that brown fat cells will require higher levels of LD proteins. Among the studied genes, only Plin4, Cidec and Cav1 exhibit similar expression levels in BAT, scWAT and gonWAT depots, indicating that they are particularly important for WAT function. Mettl7b results should be interpreted with caution, as it is poorly expressed in adipose tissue (Expression profiling of mouse tissues using Affymetrix Mouse MOE430 2.0 chips, data available at http://biogps.org [61,62]). In contrast, mesWAT expressed the lowest levels of all LD protein genes, indicating a more passive role of LDs in the physiology of mesenteric adipocytes.

Despite most of the analysed genes showing higher mRNA levels in BAT than WAT, two factors stand out as expressed at much greater levels in BAT compared to WAT: Cidea and Plin5. This BAT-specific expression highlights them as markers for brown adipocytes. CIDEA is a multifunctional protein that is involved in apoptosis and transcriptional regulation but its most clearly defined role in adipocytes is the promotion of LD enlargement [33,35]. Its molecular mechanism is the same as for CIDEC, inducing LD growth through a slow transference of fat between LDs [37], and both proteins seem to be responsible for the formation of the large LDs found in brown and white adipocytes. Despite the similarity in the sequence and function of CIDEA and CIDEC, the specific expression of Cidea in BAT indicates a differential role of these proteins. During adipocyte differentiation, LD enlargement is likely to be produced by the CIDE-triggered