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Investigation of adipocyte proteome during the differentiation of brown preadipocytes

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ABSTRACT

Brown adipocytes oxidize fatty acids to produce heat in response to cold or caloric overfeeding. The motivation and function of the development of brown fat may thus counteract obesity, though this remains uncertain. We investigated the brown adipocyte proteome by two-dimensional gel electrophoresis followed by mass spectrometry. Comparative analyses of proteins focused on total protein spots to filter differentially expressed proteins during the differentiation of mouse primary brown preadipocytes. A Western blot analysis was performed to verify the target proteins. The results indicated that 10 protein spots were differentially expressed with significant changes, including the three up-regulated proteins of prohibitin, hypoxanthine–guanine phosphoribosyltransferase, and enoyl-CoA hydratase protein; the 5 down-regulated proteins of triosephosphate isomerase, elongation factor 2, α-tropomyosin slow, endophilin-B1, and cofilin-1 (CFL1); and the two unequivocally expressed proteins of peroxiredoxin-1 and collagen α-1(i) chain precursor. We found that during brown adipogenesis, CFL1 has an inhibitory effect on brown adipocyte differentiation. The overexpression of CFL1 inhibited the brown fat deposition and repressed the brown marker genes UCP1, PRDM16, PGC-1α and PPARγ via actin dynamics and polymerization. These observations may be novel findings that bring new insight into the detailed mechanisms of brown adipogenesis and identify possible therapeutic targets for anti-obesity.

Biological significance
We use 2-DE to compare the proteomes of adipocytes during the brown adipogenesis of primary mouse preadipocytes. We identified 10 proteins that are differentially expressed. Among these, we found that the actin binding protein CFL1 inhibits the differentiation of brown preadipocytes. CFL1 overexpressing cells showed lower deposition of brown fat droplets, and the brown marker genes of UCP1, PRDM16, PGC-1α and PPARγ were decreased through actin dynamics and polymerization.

Abbreviations: AIP1, actin-interacting protein-1; APRT, adenine phosphoribosyltransferase; BAT, brown adipose tissue; CAP, cyclase-associated protein; CFL-1, cofilin-1; COL1A1, collagen alpha-1(i) chain; ECHS1, enoyl-CoA hydratase; EEF-2, elongation factor 2; EndoB1, endophilin B1; HPRT, hypoxanthine–guanine phosphoribosyltransferase; LIMK, LIM kinase; PHB, prohibitin; PRX-1, peroxiredoxin-1; TESK, testicular protein kinase; TPI, triosephosphate isomerase; TPM3, α-tropomyosin slow.

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1. Introduction

Obesity is a condition of increasing incidence, affecting more than 200 million men and 300 million women in 2008, as well as more than 40 million children under the age of five who were overweight worldwide in 2011 according to the World Health Organization (WHO) [1]. Obesity reduces one's average life expectancy and is associated with increased incidences of insulin resistance, diabetes, hypertension, lipid disturbances, cancer, osteoarthritis, work disability and sleep apnea. Obesity has a more pronounced impact on morbidity than on mortality [2], but the molecular bases of these associations are still not fully known. There are two types of adipose tissue, white and brown, which are primarily composed of white and brown adipocytes, respectively [3]. White adipocytes function primarily to store triglycerides as energy reservoirs [4,5], while brown adipocytes generate heat energy at the expense of ATP generation due to their high expression of the uncoupling protein UCP1 [6]. Mesenchymal stem cells give rise to distinct white and brown adipocyte precursors [7]. In addition, inducible brown-like adipocytes (beige or brite cells) arise from a non-Myf5 cell lineage and possess the molecular and morphological characteristics of classical brown adipocytes, including multi-locular lipid droplets [8]. Whereas brown adipose tissue (BAT) is present at distinct anatomical sites such as the interscapular, perirenal and axillary depots, brite adipocytes exist as pockets of UCP1-positive adipocytes in subcutaneous white adipocytes [9]. Recent evidence suggests that an increased level of PRDM16 expression can drive the differentiation of both myogenic and white fat precursors to brown adipocytes [10].

To date, several proteomic studies have been conducted in an effort to reveal differential proteome in protein expression patterns in white adipocytes [11], adipose tissue [12], lean and obese rats [13], obesity-susceptible and -resistant rats [14] and a bovine subcutaneous model [15]. However, to the best of our knowledge, no comparative analysis of the differentiation of brown preadipocytes from interscapular tissue has been conducted.

In this study, we used a proteomic approach to identify novel factors involved in the regulation of brown adipocyte differentiation. We identified several uncharacterized proteins using high-resolution two-dimensional electrophoresis (2-DE) couple-to-mass spectrometric analysis. From this analysis, we identified 10 proteins showing differential expression patterns. After validation by Western blot analysis, we selected the cofilin-1 (*Cfl1*) gene to characterize and understand the active mechanisms during the differentiation of brown preadipocytes. CFL1 is an actin-binding protein that has an important role in the regulation of actin dynamics [16,17]. CFL-1 regulates actin severing, stabilization and nucleation in a concentration-dependent fashion. The CFL1-mediated severing of actin filaments at the leading edge of motile cells controls the formation of lamellipodia, which is essential for T-cell migration and cancer cell metastasis [18,19]. CFL1 activity is spatially regulated within the cell through the activity of kinases, including LIM kinase (LIMK), which inactivates it by the phosphorylation of Ser3, resulting in the stabilization of actin filaments [20–22]. CFL1 reactivation by specific (slingshot and chronophin) and general phosphatases (PP2A) causes F-actin severing and depolymerization [23]. We found that CFL1 increased the level of actin polymerization and inhibited brown preadipocyte differentiation.

2. Experimental procedures

2.1. Cell culturing and brown adipogenic differentiation

Brown preadipocytes were isolated from the interscapular BAT of mice (age: late fetal to post-natal 2–3 days) by collagenase diffusion as described previously [24,25]. Isolated cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) containing 20% fetal bovine serum (FBS) and 1% antibiotic solution at 37 °C with 5% CO_2. For differentiation, brown preadipocytes were stimulated to differentiate into mature adipocytes, as described previously [26,27]. After 100% confluence at 2 days (Day 0), cells were placed in differentiation medium consisting of DMEM with 10% FBS and 1% antibiotics and a differentiation cocktail [0.5 mM isobutylmethylxanthine (IBMX), 0.5 mM dexamethasone, 0.12 mM indomethacin], after which they were changed to a maintenance medium at 4 days (Day 2) which consisted of DMEM with 10% FBS and 1% antibiotic, 1 nM T_3 and 20 nM insulin. The cells were then maintained for 8 days (Day 6). The medium was changed every other day. For the treatment of cold exposure, mice were kept in a climate chamber at 25 °C (RT) and 8 °C (cold) for 7 days. Then, BAT was obtained from the interscapular region of mice.

2.2. Protein sample preparation and 2-DE

An IPG strip (pH 3–10, 7 cm) (GE Healthcare, Boston, MA) was rehydrated at RT for 12 h with 65 μg of protein in 150 μl of rehydration buffer. One-dimensional isoelectric focusing was carried out at 20 °C at 200 V for 1 Vhr, 3500 V for 2800 Vhr, and 3500 V for 12,000 Vhr by a Multiphor II IEF System (GE Healthcare, Boston, MA). The focused strip was equilibrated with an equilibration buffer consisting of 50 mM Tris–Cl (pH 8.8), 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS and a trace amount of Bromophenol Blue (BPB). Just prior to equilibration, fresh chemicals of 0.1% (w/v) DTT and 0.25% (w/v) iodoacetamide (IAA) were added to the equilibration buffer and were allowed to incubate at room temperature for 15 min. The isoelectrofocused proteins on the strip gel were separated on 12% (w/v) SDS-PAGE gel on Mini-PROTEAN 3 (Bio-RAD) until the BPB migrated off the bottom of the gel [28,29].

2.3. Protein visualization and image analysis

After the electrophoresis process, the 2-DE analytical gels (n = 9) were stained with a PlusOne Silver Staining Kit (GE Healthcare, Boston, MA). The stained gels were imaged using an Epson scanner (ver.2.20E, Epson America, Inc.). Raw scans were processed by the 2-D gel analysis software Progenesis SameSpots version 3.0 (Nonlinear Dynamics Ltd.). To validate the automated spot detection and matching process, the images were edited manually and streaks, speckles, and artifacts were removed. Spot patterns of all gels were matched to each other to quantify each spot after normalization using the local regression model available in Progenesis SameSpots. The average intensities of