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Ifi27 is indispensable for mitochondrial function and browning in adipocytes

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ABSTRACT

Brown adipose tissue (BAT) is specialized for energy expenditure, but the signaling pathways that regulate BAT metabolism and activity are incompletely understood. Interferon (IFN) signaling is a sophisticated defense mechanism to counteract viral infection. IFNs and interferon-stimulated genes (ISGs) are reported to exert profound effects on adipocytes. IFN-α inducible protein 27 (Ifi27/ISG12a) is a BAT-enriched gene, yet no any studies on its roles in BAT have been reported. Here, we show that Ifi27 protein localizes to mitochondria and the expression of Ifi27 can be induced by β3-adrenergic activation in adipose tissues. Knockdown of Ifi27 leads to reduced expression of key enzymes of tricarboxylic acid cycle (TCA), the subunits of electron transport chain (ETC) and uncoupling protein 1 (Ucp1) in brown and beige adipocytes. Moreover, the browning of subcutaneous white fat induced by β3-adrenergic agonist is also dramatically blocked. Ectopic expression of Ifi27 in brown adipocytes has the opposite effects. Together, these data indicate that Ifi27 regulates mitochondrial function and browning in adipocytes.

1. Introduction

Obesity is a serious chronic disease that is prevalent worldwide [1]. White adipose tissue (WAT) plays an important role in regulating energy storage and systemic insulin sensitivity, that can store excess energy in the form of triglycerides in order to prevent the accumulation of lipid in other organs [2]. In contrast, the main function of brown adipose tissue (BAT) is to consume energy and produce heat under cold stimulation to maintain normal body temperature [3]. The adaptive thermogenesis confers BAT the potential to combat obesity. However, much remains to clarify on how brown fat thermogenesis is regulated.

Interferon-stimulated gene 12 (ISG12) family is a group of type I interferon-induced genes, which includes many family members, but they all contain conservative sequences of about 80 amino acid residues called ISG motif [4,5]. There are four ISG12 family members in human beings, including IFI6, IFI27 (ISG12A), IFI27L2 (ISG12B) and IFI27L1 (ISG12C). And the mouse ISG12 family comprises Ifi27 (ISG12a), Ifi27l2a (ISG12b1) and Ifi27l2b (ISG12b2) [5,6].

It has been reported that human IFI27 (ISG12A) and mice Ifi27 (ISG12a) are orthologues [4]. Human IFI27 (ISG12A) inhibits the replication of hepatitis C virus and enhances the antiviral effect of IFN-α through the Jak/STAT signaling pathway [6]. Human IFI27 is localized to mitochondria which is implicated to promote cell apoptosis induced by DNA damage [7]. The three mouse ISG12 genes cluster on chromosome 12. Ifi27l2a (ISG12b1) is highly expressed in the mouse adipose tissue, especially the subcutaneous white fat, and can be induced by IFN-α [8]. Ifi27l2a (ISG12b1) is also localized in mitochondria, that can repress the adipogenic differentiation of white adipocytes and decrease mitochondrial biogenesis [9]. To date, the expression profile, regulation, and function of Ifi27 (ISG12a) have not been fully elucidated.

In this study, we identify that Ifi27 (ISG12a) gene is selectively expressed in brown adipose tissue in mice. Ifi27 protein is localized in mitochondria and knockdown of Ifi27 leads to a systematic decreased expression of genes involved in adaptive thermogenesis, tricarboxylic acid cycle (TCA) and mitochondrial electron transport chain (ETC) in brown and beige adipocytes. Ifi27 may represent a future target to modulate energy metabolism in brown and beige adipocytes.

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2. Materials and methods

2.1. Animal studies

Male C57BL6 mice were purchased from the Super-B&K laboratory animal Corp. Ltd (Shanghai, China) and maintained on a normal chow diet (NCD). To generate diet-induced obesity mouse model, 6-week-old C57BL6 mice were fed a high-fat diet (HFD) (60% kcal from fat, Research Diets #D12492) for 9 weeks. For cold exposure, 12-week-old male mice were kept at 4 °C for indicated time. β3-adrenergic agonist Cl316,243 (Sigma, Cat#C5976) was administered by daily intraperitoneal injection at a dose of 1 μg/g body weight. Nine-week-old male C57BL6 mice were used for adenoviral shRNA injection into inguinal fat pads. The adenoviruses expressing Ifi27 shRNA or scrambled shRNA (1.5×10^{10} pfu/side/mouse) were injected into the left inguinal WAT and right inguinal WAT of the same animal for 5 consecutive days (day 1 to day 5). Cl316,243 was intraperitoneally injected into mice from day 5 to day 7. The mice were sacrificed on day 8. All studies involving animal experimentation were approved by the Fudan University Shanghai Medical College Animal Care and Use Committee and followed the National Institutes of Health guidelines on the care and use of animals.

2.2. Brown adipocytes differentiation

Immortalized BAT preadipocyte cell line was a gift from Dr. Yong-Xu Wang, University of Massachusetts Medical School, USA. The brown preadipocytes (Pparδ f/f) were generated as described previously [10]. The preadipocytes are differentiated into mature adipocytes followed the standard protocol. Briefly, preadipocytes were grown to confluence (day 0) in differentiation medium (culture medium supplemented with 20 nM insulin and 1 nM 3,3′,5-triiodo-L-thyronine), then changed to differentiation medium further supplemented with 0.5 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 0.125 mM indomethacin for 48 hr. After that, cells were kept in differentiation medium (day 2) which was replenished every other day. The cells on day 6 exhibited a fully differentiated phenotype with massive accumulation of multilocular fat droplets and were used for experiment.

To obtain stromal vascular fractions (SVFs) from BAT, interscapular BATs were dissected from C57BL6 mice and digested in BAT isolation buffer [123 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 5 mM Glucose, 100 mM HEPES (pH7.4), 4% BSA and 1 mg/ml collagenase B] for 30 min at 37 °C. Then centrifuge at 200 g for 5min to collect supernatant adipocytes and SVF pellets.

2.3. Plasmids and viruses

The coding sequence of mouse Ifi27 gene (NM_026790.2) is PCR amplified from BAT cDNA of C57BL6 mice and has been verified by DNA sequencing. Ifi27 cDNA was subcloned into pENTR-1a vector (Addgene) and recombined with pLenti-CMV/puro (Addgene) to generate lentiviral constructs as described [11]. Ifi27 shRNA targeting sequences are: shRNA-1, 5′-GATGACATCTTCAGCAGCCAT-3'; shRNA-2, 5′-GCTAAGATGATGTCCTTGTCA-3'. Pgc-1α shRNA targeting sequence is: 5′-GGTGGATTGAAGTGGTGTAGA-3' [10]. Short hairpin RNA oligos were subcloned into Psp-108 (Addgene) or pAdTrack-U6 vectors that were used for packaging lentivirus or adenovirus. Lentiviruses and adenovirus were produced and purified as described previously [12].

2.4. Real-time qPCR and western blot

Total RNA from cells and tissues was extracted by TRIzol (Invitrogen). cDNA was synthesized from total RNA with SuperScript III and random primers (Invitrogen). Quantitative RT-PCR was performed with Power SYBRgreen PCR master mix (Applied Biosystems, Carlsbad, CA) and a Prism 7500 instrument (Applied Biosystems), with U36 as an internal control. To measure mitochondrial DNA content, the mitochondrial DNA level relative to nuclear DNA level was assessed. Total DNA was extracted from mature brown adipocytes. Mitochondria-encoded gene Cox2 was detected by Real-time qPCR, with Cebpα as a nuclear genome as control. For western blot analysis, cells were harvested with lysis buffer containing 100 mM NaCl, 0.5% Triton-X-100, 5% glycerol, 50 mM Tris-HCl (pH 7.5), 1 mM PMSF and protease inhibitor mixture (Roche). All the samples were quantified for protein concentration, separated by SDS-PAGE and immunoblotted with antibodies.

2.5. Oil red O staining

Cultured adipocytes (day 6) in 6-well plates were washed once with PBS and subsequently fixed for 30 min in 4% buffered formaldehyde at room temperature. Cells were then stained with Oil Red O working solution for 1 h. The excess staining was washed out with PBS prior to visualization.

2.6. Immunofluorescence and histology

Immortalized brown preadipocytes with lentiviral Ifi27-HA expression were seeded onto sterile coverslips, then differentiated into mature adipocytes. Mature adipocytes were loaded with mitochondria-selective dye Mitotracker Red (Thermo, Cat#M7512) for 15 min under normal culture conditions. After washing with PBS, cells were fixed using 4% formaldehyde. For immunofluorescence, cells were permeabilized with 0.5% Triton-X 100 in PBS and blocked using 5% goat serum for 30 min. Coverslips were incubated with anti-HA for 2 h at room temperature, washed three times with PBS and incubated for 1 h in the dark with goat anti-mouse FITC-conjugated secondary antibody. The coverslips were washed with PBS, incubated with DAPI for 15 min and mounted in 60% glycerol and examined for fluorescence on Confocal Laser Scanning Microscopy (Leica) using filters to visualize red, green and blue fluorescence.

2.7. Antibodies

Primary antibodies including Anti-Ucp1 (ab10983) was purchased from Abcam; Anti-α-Tubulin (66031-1-Ig), Anti-Ogdh (15212-1-AP), Anti-Idh3a (15909-1-AP), Anti-Cidea (13170-1-AP), Anti-Cs (16131-1-AP), Anti-Ndufa9 (20312-1-AP) were purchased from Proteintech; Anti-HA(sc-7392) was purchased from SantaCruz.

2.8. Statistics

All experiments were independently repeated at least three times. Results are presented as mean ± SEM. Differences between two groups were assessed using the unpaired two-tailed Student's t-test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Ifi27 is highly expressed in brown adipose tissue and localized to mitochondria

Ifi27 is identified as a BAT-enriched gene based on our previous RNA-seq data in BAT, WAT and soleus muscle [13]. But little is

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