Sci. Bull.
DOI 10.1007/s11434-016-1200-1

www.scibull.com
www.springer.com/scp

Article

Life & Medical Sciences

Activating transcription factor 5 regulates lipid metabolism in adipocytes

Jing-Hui Jiang · Yue Zhao · Liu-Ling Xiao · Cui-Song Zhu · Shu-Fen Li · Xi Li

Received: 25 August 2016 / Revised: 10 October 2016 / Accepted: 26 October 2016

Abstract Activating transcription factor 5 (ATF5) is a member of the activating transcription factor/cAMP response element binding protein (ATF/CREB) family, and is highly expressed in liver and adipose tissue. Previous reports have shown that ATF5 promoted 3T3-L1 preadipocytes differentiation. In this study, we found that ATF5 was highly expressed in mature adipocytes, suggesting a potential role of ATF5 in mature adipocytes, which has not been reported previously. To understand the function of ATF5 in mature adipocytes, we knocked down the expression of ATF5 in 3T3-L1 mature adipocytes and observed decreased lipid droplets. Consistent with the in vitro experiment, the knockdown of ATF5 in white adipose tissue led to less adipose tissue and smaller adipocytes size. Further research revealed that the inhibition of ATF5 diminished the adipocytes size via the inhibition of fatty acid synthetase, stearyl coenzyme A desaturation enzyme 1, and the induction of carnitine palmitoyl transferase 1, one key enzyme of lipid metabolism. In addition, ATF5 knockdown in inguinal white adipose tissue improved whole body insulin sensitivity. Our work provides a new understanding of ATF5 function in mature adipocytes and a potential therapeutic target of diabetes.

J.-H. Jiang · Y. Zhao · L.-L. Xiao · C.-S. Zhu · S.-F. Li · X. Li (✉)
Key Laboratory of Metabolic Molecular Medicine, the Ministry of Education, Department of Biochemistry and Molecular Biology, Fudan University Basic Medical School, Shanghai 200032, China
e-mail: lixi@shmu.edu.cn

Present Address:
Y. Zhao
Jiangsu Key Laboratory of Molecular Medicine, School of Medicine, Nanjing University, Nanjing 210093, China

X. Li
Chongqing Medical University, Chongqing 400016, China

Keywords Activating transcription factor 5 · Adipocytes · Lipid metabolism · Fatty acid synthetase · Stearyl coenzyme A desaturation enzyme 1 · Carnitine palmitoyl transferase 1

1 Introduction

White adipose tissue (WAT) plays a central role in energy homeostasis [1, 2]. In response to over nutrition, adipose tissue is rapidly and dynamically remodeled through two mechanisms: hyperplasia (increase of cell number) and hypertrophy (increase of cell size) [1]. Enlarged adipocytes provoke insulin resistance, a growing health problem in the obese human population [3–5]. According to a prospective study, enlarged mean subcutaneous abdominal adipocytes size but not the high percentage of body fat is an independent predictor of diabetes, in addition to low insulin sensitivity [6]. Adipocytes volume is determined by the balance of lipid metabolism including lipogenesis, lipolysis, and fatty acid oxidation. Thus, genes encoding the key enzymes for lipid metabolism may underlie obesity and related metabolic disorders. FAS, a key enzyme in the process of the de novo fatty acid synthesis, catalyzes all of the reaction steps for synthesizing saturated fatty acids from acetyl-CoA and malonyl-CoA [7]. SCD1 catalyzes the Δ-9-cis desaturation of saturated fatty acyl-CoA substrates (SFAs) to monounsaturated fatty acids (MUFAs) [8]. CPT1 is the rate-limiting enzyme of the β-oxidation [9]. These key enzymes that involved in lipid metabolism are under the tight control of the sympathetic nervous system and hormones [10].

ATF5 is a member of the ATF/CREB family, which contain a leucine zipper domain, a basic DNA binding domain, and a proline rich domain [11, 12]. ATF5 is ubiquitously expressed in various tissues, particularly in

Published online: 01 December 2016

liver and adipose tissue [13]. ATF5 plays particularly important roles in a variety of cell functions, such as cell differentiation, proliferation, survival and apoptosis, and cancer development [14–17]. The overexpression of ATF5 stimulates asparagine synthetase promoter/reporter gene transcription via the nutrient-sensing response unit [18]. Other studies suggested that ATF5 might regulate hepatic gluconeogenic metabolism and lipid metabolism during fasting through PGC1α-associated mechanisms [13, 19, 20]. The homologue analysis of amino acid sequences of ATF5 and ATF4 showed that ATF5 may also take part in the lipid metabolism of white adipose tissue (WAT) [12, 21]. We previously reported that ATF5 expression is positively correlated with body mass index in human beings and promotes adipogenesis of 3T3-L1 preadipocytes [22]. In this study, we knocked down ATF5 in mice to explore the role of ATF5 in mature adipocytes.

2 Materials and methods

2.1 Animals and treatment

Male C57BL/6J mice were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd. (SLAC, Shanghai, China). Five-week-old male mice were maintained under 12-h light/12-h dark cycles and provided free access to a normal chow diet (ND; 12% kcal in fat) and water. After 1 week of accommodation, mice were randomly assigned to three groups: the self-contrast group, control group, and experimental group. The left iWAT of the self-contrast group was injected with adenovirus expressing *shLacZ*, and the right iWAT was injected with adenovirus expressing *shATF5*. Bilateral iWAT of the control group was treated with adenovirus expressing *shLacZ*, and the experimental group was treated with adenovirus expressing *shATF5*. After 6 weeks of treatment (twice per week) mice were sacrificed and tissues collected. To produce high fat diet fed mice, 6-week-old C57BL/6J mice were fed a high fat diet (HFD; D12492i, 60 % fat; Research Diets, New Brunswick, NJ, USA), and mice fed a normal chow diet (ND) were used as the control. 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 Cell culture

3T3-L1 preadipocytes were maintained in DMEM containing 10 % (v/v) calf serum. Two days after preadipocytes reached confluence (differentiation Day 0, D0), the medium was replaced with DMEM containing 10 % (v/v) fetal bovine serum (FBS), 1 μg/mL insulin (I), 1 μmol/L dexamethasone (D), and 0.5 mmol/L 3-isobutyl-1-methylxanthine (M) until day 2. Cells were then fed with DMEM supplemented with 10% FBS and 1 μg/mL insulin for 2 d. After this, medium with 10 % FBS was changed every other day. Cells were treated with adenovirus expressing shLacZ or shATF5 on D8. Expression of adipocytes genes and adipocytes phenotype were detected on D10.

2.3 Construction of recombinant adenovirus

The sequence (5′–3′) for *ATF5* shRNA (*shATF5*) was: CCTGCTAATTGAGGTGTATAACTC; and that for *shLacZ* was: AATTTAACCGCCAGTCAGGCT. Recombinant adenovirus for ATF5 knockdown were constructed as described in BLOCK-iT^{TM}. Adenoviral RNAi Expression System with shLacZ as the control (Invitrogen, Carlsbad, CA, USA).

2.4 Isolation of stromal vascular fraction (SVF) and adipocytes

The iWAT was cut into small pieces and treated with collagenase (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 37 °C, and then was filtered through a 100-μm mesh filter and centrifuged for 8 min at 1,800 r/min. The supernatant cells were adipocytes and the precipitated material was SVF. The SVF was collected after treated with red blood cell lysis buffer for 5 min at room temperature. Next, 1 mL Trizol reagent (Invitrogen) was added to both SVF and adipocytes.

2.5 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from iWAT with Trizol reagent according to manufacturer's protocol, and the mRNA was reverse transcribed by using a RevertAid first-strand cDNA synthesis kit (Fermentas, Waltham, MA, USA). qRT-PCR reactions were performed using the SYBR®Green PCR Master Mix and the ABI Prism 7500 Real-Time PCR machine (Applied Biosystems, Foster City, CA, USA). All cDNA levels were normalized using 18S rRNA as an internal control. The following primer sequences were used: 18S forward 5′-CGCCGCTAGAGGTGAAATTCT-3′ and reverse 5′-CATTCTTGGCAAATGCTTTCG-3′; *ATF5* forward 5′-TGGGCTGGCTCGTAGACTAT-3′ and reverse 5′-GTCATCCAATCAGAGAAGCCG-3′; *FAS* forward 5′-CGTGTTGGCCTACACCCAGAGCT-3′ and 5′-GGCAGCAGGGCCTCCAGCACCTT-3′; *SCD1* forward 5′-GGTGATGTTCCAGAGGAGGTACT-3′ and reverse 5′-GGTGCTAACGAACAGGCT-3′; *SREBP1c* forward 5′-GCAGCCACCATCTAGCCTG-3′ and reverse 5′-CAGCAGTGAGTCTGCCTTGAT-3′; ATGL forward 5′-GGATGGCGGCATTTCAGACA-3′ and reverse