Biochemical and Biophysical Research Communications xxx (2017) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Suppression of CD36 attenuates adipogenesis with a reduction of P2X7 expression in 3T3-L1 cells

Huanqing Gao [a, c], Danyang Li [a], Ping Yang [a], Lei Zhao [a], Li Wei [a], Yaxi Chen [a, *], Xiong Z. Ruan [a, b, c, **]

[a] Centre for Lipid Research & Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, The Second Affiliated Hospital, Chongqing Medical University, 400016 Chongqing, China
[b] The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases (CCID), Zhejiang University, 310058 Hangzhou, China
[c] John Moorhead Research Laboratory, Centre for Nephrology, University College London Medical School, Royal Free Campus, University College London, London NW3 2PF, United Kingdom

ARTICLE INFO

Article history:
Received 10 July 2017
Accepted 13 July 2017
Available online xxx

Keywords:
CD36
Adipogenesis
ATP
P2X7

ABSTRACT

Adipogenesis is a process of differentiation from preadipocyte into adipocyte, and is regulated by several transcription factors, including the peroxisome proliferator-activated receptor gamma (PPARγ) and the CCAAT-enhancer-binding protein alpha (C/EBPα). CD36 is a membrane protein which contributes to the metabolic disorders such as obesity. Although the previous study demonstrated CD36 participated in the progression of adipogenesis, the mechanism is still unclear. We report here that knockdown of CD36 expression by CD36 small interfering RNA (siRNA) resulted in a reduction of adipocyte differentiation and adipogenic protein expression. In addition, purinergic receptor P2X, ligand-gated ion channel 7 (P2X7) was downregulated in CD36-knockdown 3T3-L1 cells, suggesting that the suppression of CD36 attenuates adipogenesis via the P2X7 pathway in 3T3-L1 cells.

1. Introduction

Obesity is a risk factor for several diseases, including type II diabetes, cancer, and cardiovascular disease [1]. In humans, white adipose tissue (WAT) is the main fat mass, therefore, figuring out the molecular mechanisms of the development of WAT is important. Adipogenesis is the primary process of conversion of preadipocyte into adipocyte [2], which is still not well understood. It is well-described that the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors and Peroxisome proliferator-activated receptor-γ (PPARγ) act as key regulators in the transcriptional process of adipogenesis [3,4]. Mitochondrial biogenesis was also involved in the progress of adipogenesis [5,6]. At the early stage of adipogenesis, mitochondrial biogenesis and metabolism are crucial for the initiation and promotion of adipocyte differentiation [7]. The generation of adenosine 5'-triphosphate (ATP) by mitochondria is to ensure the normal metabolism of lipid synthesis during the progress of adipogenesis [8].

CD36 is a receptor for long-chain free fatty acids (FFAs) that has been shown to facilitate FFA uptake into the adipose tissue and muscle of rodents and humans. CD36 also has been recently implicated in several other aspects related to inflammation, immune responses, atherogenesis and thrombosis [9,10]. Several groups have found that adipocyte bind oxLDL in a CD36-dependent manner that results in insulin resistance in adipose tissue [11,12]. The previous study has demonstrated that CD36 plays a critical role in adipose tissue biology [13]. However, the molecule mechanism of CD36 in adipogenesis remains unclear.

P2X7 receptor (described as P2X7 in the text) is a ligand-gated cation channel encoded by P2RX7 gene (purinergic receptor P2X, ligand-gated ion channel 7), belonging to the P2X receptor family. Activation of P2X7 by extracellular ATP causes the movement of Ca^{2+}, Na^{+} and K^{+} across the plasma membrane. P2X7 is predominately expressed on cells of the hematopoietic, epithelial, mesenchymal and neural lineages [14]. Subsequently, P2X7 plays important roles in inflammation, immunity, bone homoeostasis,

* Corresponding author.
** Corresponding author. Centre for Lipid Research & Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, The Second Affiliated Hospital, Chongqing Medical University, 400016 Chongqing, China.
E-mail addresses: chenyaxi@cqmu.edu.cn (Y. Chen), x.ruan@ucl.ac.uk (X.Z. Ruan).

http://dx.doi.org/10.1016/j.bbrc.2017.07.077
0006-291X/

neurological function and neoplasia. P2X7 has also been reported to connect with lipid metabolism. For instance, male mice lacking functional P2X7 develop ectopic lipid accumulations as they age, and P2X7 plays a generalised role in regulating lipid storage and metabolism in vivo [15]. The progression of adipogenesis is associated with the generation and consumption of ATP. Activation of P2X7 by extracellular ATP causes the downstream events, including pro-inflammatory mediators. However, the effect of P2X7 on adipogenesis is unknown.

In this study, we demonstrated that CD36 was increased after induction of differentiation in 3T3-L1 cells, while knocking down CD36 suppressed adipogenesis by both decreasing the delivery of fatty acids to mitochondria and inhibiting mitochondrial biogenesis. This may suppress adipogenesis by inhibiting P2X7.

2. Material and methods

2.1. Cell culture and adipocyte differentiation

3T3-L1 preadipocytes were provided by Prof Fiona M. Watt (Kings College London) and were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf serum (Sigma, UK). The cells were cultured for 2 days after confluence (day 0), differentiation was induced in DMEM containing 10% fetal bovine serum (Labtech, UK), 5 μg/ml insulin (Sigma), 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (Sigma) for 2 days. After 2 days, the medium was changed to growth medium containing 5 μg/ml insulin for another 2 days. Then the medium was replaced with growth medium and replaced every other day until day 8.

2.2. Oil Red O staining

In brief, following fixation of the mature adipocyte in 10% (v/v) formaldehyde solution for 30 min, the cells were stained with 0.5% Oil Red O for 30 min at room temperature and were washed three times with PBS. Cells were visualised by a microscopy. Oil red O was extracted with 100% isopropanol and concentration were determined by measuring absorbance at 510 nm.

2.3. Small interfering RNA

Small interfering RNA (siRNA) transfections were performed with Lipofectamine RNAiMAX (Invitrogen, UK), using siRNA targeting murine CD36 (catalogue number AM16708 160083; Invitrogen), or a nontargeting control (catalogue number 12935300; Invitrogen) at a final concentration of 50 nM. At the day 4, we used another transfection for persistent inhibition of CD36 protein synthesis.

2.4. Protein isolation and Western blot analysis

3T3-L1 cells were lysed in RIPA buffer supplied with protease inhibitor cocktail (Sigma). 20–30 μg protein was separated by SDS-PAGE and then transferred to the PVDF membrane, blocked with 5% BSA 1 h at room temperature and incubated the primary antibody overnight at the cold room. Antibodies against PPARγ, FABP4, cebpα, were purchased from Cell Signaling Technology (USA). The antibody for β-actin was obtained from Sigma. The antibody against CD36 (MAB1955) was purchased from R & D company (UK). The P2x7 antibody was purchased from Abcam (UK). After incubated with second antibody 1 h at room temperature, washed the membrane with TBST three times, then, the membrane was exposed to ECL. The membrane was stripped by stripping buffer (Sigma) and re-probed for actin as loading control.

2.5. Real-time RT-PCR analysis

RNA was extracted using TRIzol Reagent (Invitrogen) according to manufacturers' instructions. The total RNA (2 μg) per sample was made into cDNA using reverse transcriptase (Applied Biosystems, UK). Prepared cDNA was amplified using the LightCycler 96 system (Roche) and analyzed using the SYBR Green PCR Master Mix (Lab science, UK). Cycle threshold (Ct) values were normalized for amplification using β actin, and the data were analyzed using the ΔCt method. The primer sequences used in this study are shown in Table S1.

2.6. Statistical analysis

Data is presented as the mean ± SEM of three independent experiments. Statistical significance was analyzed by an unpaired *t*-test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. CD36 is increased during the adipogenic differentiation

To clarify the precise function of CD36 during adipogenesis, we first observed the different time course of CD36 expression during the 3T3-L1 adipocyte differentiation into mature adipocyte. As shown in Fig. 1A and B, the CD36 protein level was increased after adipogenic induction and maintained a high-level of expression in the process of adipocyte differentiation. We also detected the expression of several key transcription factors in adipogenesis, such as PPARγ, cebpα and FABP4, which were increased appreciably in the progress of adipogenesis. The mRNA levels of CD36 and adipogenic factors exhibited a similar expression with protein levels (Fig. 1C). The genes, such as SREBP1 which is involved in lipogenesis were also increased during the adipogenic differentiation (Fig. 1C).

3.2. Knockdown of CD36 decreased lipid accumulation

To figure out whether CD36 participates in the adipogenic process, we knockdown CD36 by transfecting CD36 siRNA into 3T3-L1 preadipocyte during the differentiation induction. CD36 knockdown resulted in the markedly decreased numbers of mature adipocyte by Oil Red O staining (Fig. 2).

3.3. Suppression of CD36 reduced the adipogenic gene expression

The suppression of CD36 significantly suppressed the expression of adipogenic marker genes and proteins, such as FABP4, cebpα, PPARγ (Fig. 3A, B). Moreover, mitochondrial development is associated with adipocyte differentiation; we tested the mRNA expression of the mitochondrial gene including peroxisome proliferator activated receptor (PPAR) coactivator-1α (PGC-1α) and mitochondrial transcription factor A (mtTFA) which were significantly decreased along with knockdown of CD36 (Fig. 3C).

3.4. P2X7 was downregulated in CD36-knockdown 3T3-L1 cells

We demonstrated that suppression of CD36 decreased the expression of P2X7 (Fig. 4A, B, C). To assess whether the effect of CD36 is mediated by PPARγ, we added rosiglitazone into the differentiation progress. We found that rosiglitazone could reverse the

Please cite this article in press as: H. Gao, et al., Suppression of CD36 attenuates adipogenesis with a reduction of P2X7 expression in 3T3-L1 cells, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.07.077