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# Cysteine dioxygenase type 1 promotes adipogenesis via interaction with peroxisome proliferator-activated receptor gamma

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ABSTRACT

Mammalian cysteine dioxygenase type 1 (CDO1) is an essential enzyme for taurine biosynthesis and the biodegradation of toxic cysteine. As previously suggested, Cdo1 may be a marker of liposarcoma progression and adipogenic differentiation, but the role of Cdo1 in adipogenesis has yet been reported. In this study, we found that the expression of Cdo1 is dramatically elevated during adipogenic differentiation of 3T3-L1 pre-adipocytes and mouse bone marrow-derived mesenchymal stem cells (mBMSCs). Conversely, knockdown of Cdo1 inhibited expression of adipogenic specific genes and lipid droplet formation in 3T3-L1 cells and mBMSCs. Mechanistically, we found Cdo1 interacted with Pparγ in response to adipogenic stimulus. Further, depletion of Cdo1 reduced the recruitment of Pparγ to the promoters of C/EBPα and Fabp4. Collectively, our finding indicates that Cdo1 may be a co-activator of Pparγ in adipogenesis, and may contribute to the development of disease associated with excessive adipose tissue.

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## 1. Introduction

Adipogenesis refers to the process whereby mesenchymal stem cells (MSCs) or preadipocytes differentiate into lipid-laden and insulin-responsive adipocytes, which may involve several stages as defined previously, including mesenchymal precursors, committed pre-adipocytes, growth-arrested pre-adipocytes, mitotic clonal expansion, terminal differentiation and mature adipocytes [1,2]. Adipocytes play an important role in energy balance by storing or mobilizing triacylglycerol according to energy level in body [3]. In addition, adipocytes can synthesize and secrete numerous cytokines and hormones that are involved in overall energy homeostasis [4]. However, accumulation of adipose tissue is associated with various diseases, such as obesity [5], osteoporosis [6], and cardiac steatosis [7].

Adipogenesis is a highly controlled process involving several positive and negative regulators, such as a cascade of transcription factors, cell-cycle proteins, and Wingless and INT-1 proteins (Wnts) [8,9]. Among these, peroxisome proliferator-activated receptor gamma (Pparg) is considered as a master transcription factor of adipogenesis, which has been shown to directly promote expression of the adipocyte gene program. In particular, no factor has been discovered that can rescue adipogenesis in the absence of Pparg [9]. To initial transcription of target genes, Pparγ with retinoid X receptor (RXR) binds the promoter as a heterodimer [2]. Co-activators may also be recruited with ligand stimulation. For example, PPARg is constitutively associated with steroid receptor co-activators (SRCs) in the promoter of fatty acid binding protein 4 (Fabp4) in adipocytes [10,11]. In addition, it is recently reported that CCAAT/enhancer binding protein α (C/EBPα) is required for the binding of Pparγ to the promoters of several key metabolic adipocyte genes [12].

Cysteine dioxygenase type 1 (Cdo1) is a non-heme structured, iron-containing metalloenzym, which plays a vital role in taurine biosynthesis by catalyzing the oxidation of cysteine to cysteine sulfinic acid [13,14]. Previous studies have shown that CDO1 is expressed in adipose tissue, liver, brain, and kidney [15]. Specifically, Well-diferentiated liposarcomas (WDLSs) has a significantly higher CDO1 expression level than dedifferentiated liposarcomas (DDLSs) [16]. Further, expression of CDO1 is up-regulated during adipogenic differentiation of human bone marrow-derived MSCs and adipose tissue-derived pre-adipocytes [16,17], which suggests that CDO1 may play a role in regulation of adipogenesis.

In this study, we found up-regulation of Cdo1 expression during adipogenic differentiation of 3T3-L1 pre-adipocytes and mBMSCs in vitro. Further, siRNA-mediated depletion of Cdo1 significantly

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suppressed adipogenesis of 3T3-L1 and mBMSCs. Through IP and ChIP assay, we found that Cdo1 is required for Pparγ binding to the target gene promoters.

## 2. Materials and methods

### 2.1. Cell culture

3T3-L1 (murine embryonic fibroblast) cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-Glutamine, plus 100 U/ml of K-Penicillin G and 100 mg/ml of Streptomycin sulfate at 37 °C with a humidified atmosphere of 5% $CO_2$ (all from Gibco). Primary MSCs from mouse bone marrow (mBMSCs) were isolated and cultured as described previously [18]. All animal procedures were conducted in accordance with the guidelines for the care and use of laboratory animals of State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University.

### 2.2. Induction of adipogenic differentiation

Adipogenic induction (AI) medium was comprised of 90% DMEM high glucose (Gibco), 10% FBS, 1 μM dexamethasone, 10 μg/mL insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 0.2 mM indomethacin (all from Sigma). After 7–10 days differentiation, Oil Red O staining was performed to detect the lipid droplet formation according to the manufacturer's instructions (Diagnostic Biosystems). Then, the plates were photographed, and the stained cultures were destained by isopropanol. The absorbance of the solution was read at 450 nM using a microplate reader (Bio-Rad).

### 2.3. Small interfering RNA (siRNA) and transfection

All the siRNAs were purchased from Invitrogen (Carlsbad, CA, USA), including a scrambled siRNA (Scr) as a control, and two siRNAs: si1 (5′-AUGCCAAAUUCGAUCAAUAUU-3′), and si2 (5′-CUGCAAAGGGUGUGU CCUAUU-3′), targeting mRNA of Cdo1. Transfection was performed using Lipofectamine RNAiMAX reagent (Invitrogen) according to manufacturer's instructions. The cells were transfected with 30–60 pmol siRNA and incubated for 48 h, and the knockdown efficiency was determined by RT-PCR and western blot.

### 2.4. RNA isolation and reverse transcription-PCR (RT-PCR)

Total RNA was extracted using the Trizol reagent (Invitrogen), and complementary DNA was then synthesized from 1 μg of total RNA, using PrimeScript RT Reagent Kit (Takara). Quantitative real-time PCR was performed using SYBR Premix Ex Taq (Takara). The real-time PCR conditions were 40 cycles at 94 °C for 10 s and 60 °C for 60 s. The primer sequences used for this analysis were: 5′-AATG ATTCCATTGGCTTACACCG-3' (forward) and 5′-GGCATGTATCGA AGGGTGGAC-3' (reverse) for Cdo1; 5′-AACTGGATGACAGTGACAT TTCCC-3' (forward) and 5′-CCCCTCCTGCAACTTCTCAAT-3' (reverse) for Pparγ; 5′-GTCACTGGTCAACTCCAGCA-3' (forward) and 5′-T GGACAAGAACAGCAACGAG-3' (reverse) for C/EBPα; 5′-AAGGTGAA-GAGCATCATAACCCT-3' (forward) and 5′-TCACGCCTTTCATAACACA TTCC-3' (reverse) for Fabp4; 5′-ACAACTTTGGCATTGTGGAA-3' (forward) and 5′-GATGCAGGGATGATGTTCTG-3' (reverse) for GAPDH.

### 2.5. Western blot

Cells were harvested and lysed on ice for 30 min in CelLytic MT solution (Sigma), supplemented with protease inhibitor cocktail (Pierce Biotechnology), and centrifuged at 18,000 g for 15 min at 4 °C. Aliquots of the lysates were electrophoresed on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. The resolved proteins were then transferred onto nitrocellulose membranes (Bio-Rad), which were subsequently incubated with primary antibodies followed by a horseradish peroxidase-conjugated secondary antibody (Boster, Wuhan, China). Protein bands were detected using an enhanced chemiluminescence western blotting detection kit (Thermo). Antibodies for western blot were purchased from the following commercial sources: mouse polyclonal anti-Pparγ (Santa Cruz), rabbit monoclonal anti-Cdo1 (Abcam).

### 2.6. Immunoprecipitation (IP) assay

Protein was collected after 3 day of adipogenic differentiation. Aliquots of the protein sample were incubated with Pparγ antibodies or Cdo1 antibody overnight at 4 °C with gentle orbital rotation. Then 30 μl of protein G-Sepharose beads (Pierce Biotechnology) were added and the incubation was extended for another 2 h. The antibody/antigen complexes were eluted from the beads and subjected to 12.5% SDS-PAGE followed by western blot procedures.

### 2.7. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was performed using a Simple ChIP Assay Kits (Cell Signaling Technology) according to the manufacturer's protocol. All precipitated DNA samples were quantified with Real-time PCR. Data are expressed as the percentage of input DNA. Same antibodies for western blot are used for ChIP assay. The primer sets were designed around the Pparγ binding elements in the mouse C/EBPα and Fabp4 gene: 5′-CTGAGCTACACCCTCGGCTC-3' (forward) and 5′-TCCCCACCG GAGGGCATGAG-3' (reverse) for C/EBPα; 5′-AAATGCACATTT

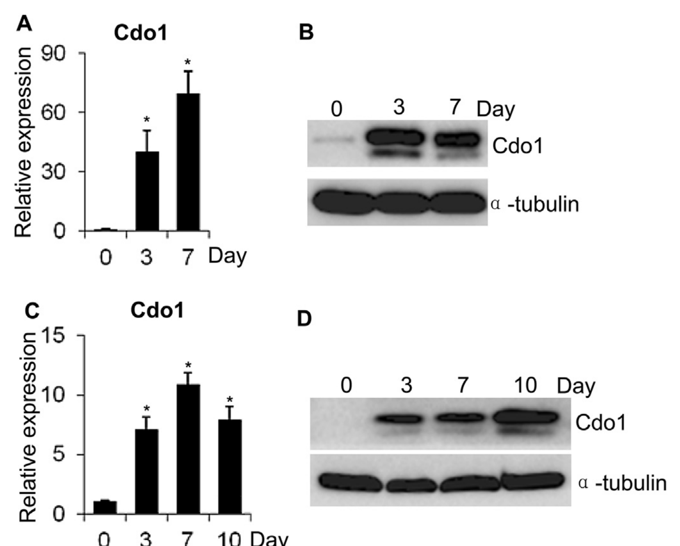


**Fig. 1.** Cdo1 is up-regulated during adipogenesis. (A) The mRNA expression levels of Cdo1 during adipogenesis of 3T3-L1 pre-adipocytes at 0, 3, 7 days. (B) The protein expression levels of Cdo1 during adipogenesis of 3T3-L1 pre-adipocytes at 0, 3, 7 days. (C) The mRNA expression levels of Cdo1 during adipogenesis of mBMSCs at 0, 3, 7, 10 days. (D) The protein expression levels of Cdo1 during adipogenesis of mBMSCs at 0, 3, 7, 10 days. (E) H&E staining of paraffin sections of femurs from 2- to 24-month-old mice. (F) The mRNA expression levels of Cdo1 in bone morrow of 2- and 24-month-old mice (n = 4). *$p < 0.05$.

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