Molecular and Cellular Endocrinology 413 (2015) 217–227

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Yin Yang 1 is a multi-functional regulator of adipocyte differentiation in 3T3-L1 cells

Younho Han [a], You Hee Choi [a], Sung Ho Lee [a], Yun-Hye Jin [a], Heesun Cheong [b, **], Kwang Youl Lee [a, *]

[a] College of Pharmacy & Research Institute of Drug Development, Chonnam National University, Gwangju, South Korea
[b] Research Institute, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-769, South Korea

ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form
21 June 2015
Accepted 29 June 2015
Available online 6 July 2015

Keywords:
3T3-L1
Adipocyte differentiation
C/EBPβ
C/EBPα
PPARγ
YY1

ABSTRACT

Yin Yang 1 (YY1) is an ubiquitously distributed transcription factor that belongs to the GLI-Kruppel class of zinc finger proteins. The mechanism by which YY1 regulates adipocyte differentiation remains unclear. In this study, we investigated the functional role of YY1 during adipocyte differentiation. During the early stage, YY1 gene and protein expression was transiently downregulated upon the induction of differentiation, however, it was consistently induced during the later stage. YY1 overexpression decreased adipocyte differentiation and blocked cell differentiation at the preadipocyte stage, while YY1 knockdown by RNA interference increased adipocyte differentiation. YY1 physically interacted with PPARγ (Peroxisome proliferator-activated receptor gamma) and C/EBPβ (CCAAT/enhancer-binding protein beta) respectively in 3T3-L1 cells. Through its interaction with PPARγ, YY1 directly decreased PPARγ transcriptional activity. YY1 ectopic expression prevented C/EBPβ from binding to the PPARγ promoter, resulting in the downregulation of PPARγ transcriptional activity. These results indicate that YY1 repressed adipocyte differentiation by repressing the activity of adipogenic transcriptional factors in 3T3-L1 cells.

1. Introduction

Adipogenesis, the differentiation of fibroblast-like mesenchymal stem cells into adipocytes, plays a central role in the regulation of the whole-body energy metabolism. Adipocyte differentiation is attributed to the activation of the expression of adipocyte-specific genes. It is now well-accepted that both CCAAT/enhancer binding proteins (C/EBPs) and peroxisome proliferator-activated receptor γ (PPARγ) function as critical regulators of adipocyte differentiation in a complex transcriptional cascade (Ramji and Foka, 2002; Rosen et al., 1999). As a master regulator of adipocyte differentiation, PPARγ induces the expression of adipocyte-specific genes through the interacting with retinoid X receptor-α (RXRα) heterodimers to a PPAR-response element (PPRE) (Mukherjee et al., 1997; Tontonoz et al., 1994a), resulting in the accumulation of intracellular fat. The 3T3-L1 cell line is a widely accepted adipogenic model, which can be conveniently differentiated into mature adipocytes upon hormonal stimulation (Green and Kehinde, 1975). Temporary exposure of 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (Dex), and insulin (MDI) triggers adipocyte differentiation, changing the expression of hundreds of genes, including that of various transcription factors that regulate one another as well as structural genes (Farmer, 2006).

Yin Yang 1 (YY1) is a zinc-finger protein with the ability to bind DNA (Seto et al., 1991), which functions as a chromatin modifier (Thomas and Seto, 1999). YY1 is a ubiquitously expressed transcription factor, which establishes and maintains transcriptional silencing by recruiting histone deacetylase (HDAC) (Gordon et al., 2006). This intrinsic factor is important in cell differentiation by regulating gene expression. It regulates chondrocyte differentiation from mesenchymal stem cells by regulating the expression of cartilage-specific genes (Aoyama et al., 2010b). Additionally, YY1 represses myoblast differentiation by blocking muscle-specific genes such as those for smooth muscle, skeletal, and cardiac α-actin (MacLellan et al., 1994). Recently, our group elucidated that YY1 suppresses BMP2-induced osteogenic differentiation by inhibiting the transcriptional activity of Runx2 (Jeong et al., 2014).

* Corresponding author.
** Corresponding author.
E-mail addresses: heesunch@ncc.re.kr (H. Cheong), kwanglee@chonnam.ac.kr (K.Y. Lee).

http://dx.doi.org/10.1016/j.mce.2015.06.035
0303-7207/

However, the function of YY1 in adipocyte differentiation has not been elucidated.

Transcriptional regulation in specific cells involves gene-specific (DNA-binding) regulatory factors and a variety of coregulatory factors such as histone acetyltransferases and methyltransferases (Roeder, 2005). Several cofactors have been investigated that regulate the action of PPARγ through the interaction (Cho et al., 2009; Gelman et al., 1999). Interestingly, they work as constitutive factors to permit PPARγ-dependent transcription without particular change of the expression level during differentiation. As this constitutive role, increasing the expression of these coactivators above the basal state does not affect adipocyte differentiation. Therefore, we first analyzed the expression pattern of YY1 during adipocyte differentiation to examine the role of YY1 in adipocyte differentiation. YY1 was decreased during the early stage and continuously increased during the later differentiation stage. It is an unprecedented pattern, which differs from that of other transcriptional regulators known as repressor or activator of PPARγ or C/EBPs. YY1 overexpression decreased adipocyte differentiation in 3T3-L1 cells, while YY1 knockdown increased adipocyte differentiation. Furthermore, YY1 interacted with both PPARγ and C/EBPβ and regulated each transcription factor. YY1 repressed PPARγ-induced transcription activity as shown by luciferase assay. These results suggest that YY1 is a critical regulator governing the whole stage of adipocyte differentiation.

2. Materials and methods

2.1. Cell culture and differentiation conditions

Mouse preadipocyte 3T3-L1 cells were maintained at 37 °C, 5% CO_2 in Dulbecco's modified Eagle medium (DMEM, Life Technologies, Carlsbad, CA, USA) supplemented with 1% fetal calf serum (FCS, Gibco Invitrogen, Carlsbad, CA, USA) (growth medium). For adipocyte differentiation, cells were grown to full confluence for 48 h in 24-well plates and the growth medium was subsequently replaced (day 0) with DMEM supplemented with 10% fetal bovine serum (FBS, Gibco Invitrogen), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, St. Louis, MO, USA), 1 μM dexamethasone (Sigma), and 10 μg/mL insulin (Sigma). The standard mixture composed 0.5 mM of 3-isobutyl-1-methylxantine, 1 μM dexamethasone, and 10 μg/mL insulin in 10% fetal bovine serum is stated as MDI. After 48 h, the differentiation medium was replaced (day 2) with DMEM + 10% FBS containing 10 μg/mL insulin and the cells were allowed to accumulate lipid droplets until used for further experiments.

2.2. Plasmids, antibodies, and reagents

N-terminal epitope-tagged mouse YY1, PPARγ, and C/EBPβ expression plasmids were constructed in a CMV promoter-derived mammalian expression vector (pCS4). Deletion of PPARγ was generated by PCR-based mutagenesis and confirmed by DNA sequencing. For YY1 knock-down experiments, oligonucleotides for small harpin RNA (shRNA) were generated by targeting a 19-base sequence CGACGACTACA TTGAACAA of the mouse YY1 gene. Sense and antisense oligonucleotides were annealed and ligated into pSuper-retro vector (Oligoengine). Antibodies against YY1 (sc-1703, Santa Cruz Biotechnology, Santa Cruz, CA, USA), C/EBPβ (04-1153, Upstate Biotechnologies, Lake Placid, NY, USA), C/EBPα (04-1104, Upstate Biotechnologies), PPARγ (MAB3872, Chemicon International Inc., Temecula, CA, USA), Myc (9E10, Santa Cruz Biotechnology), HA (12CA5, Roche Applied Science, Basel, Switzerland), and α-tubulin (sc-53646, Santa Cruz Biotechnology) were used. Insulin (I2643, Sigma), dexamethasone (D4902, Sigma), 3-isobutyl-1-methylxanthine (I5879, Sigma), and rosiglitazone (R2408, Sigma) were also used.

2.3. Luciferase reporter assays

3T3-L1 cells were transfected with a luciferase reporter plasmid, containing the promoter for the adipocyte fatty acid binding protein (aP2) gene or the PPAR Responsive Element (PPRE) of aP2, pCMV-β-gal, and combinations of PPARγ and YY1 expression plasmids for 36 h. Luciferase activities were measured using the Luciferase Reporter Assay Kit (Promega, Madison, WI, USA) and normalized to β-galactosidase activity for transfection efficiency. Experiments were performed in triplicate and repeated at least three times.

2.4. Oil Red O staining

At the end of the differentiation (day 8), cells were washed two times with phosphate-buffered saline (PBS), fixed with 10% formalin for 30 min, and stained with 0.5% Oil Red O in isopropanol for 30 min at room temperature on a shaker. After washing the cells three times with PBS, the stained lipid droplets in the cells were visualized by using a light microscope and photographed with a digital camera. To quantify lipid accumulation, the stained dye was dissolved in isopropanol and the absorbance was measured at 530 nm using a spectrophotometer.

2.5. RT-PCR (reverse transcription polymerase chain reaction) analysis

Total cellular RNA was prepared using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. Random primed cDNAs were synthesized from 1 mg of total RNA using Super-Script III First-Strand Synthesis System (Life Technologies). The following conditions were used for PCR: initial denaturation at 95 °C for 5 min; 25–30 cycles of denaturation at 95 °C for 1 min, annealing at a temperature optimized for each primer pair for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR primer sequences are listed in Table 1.

2.6. Immunoblotting (IB) and immunoprecipitation (IP)

For IB, 3T3-L1 cells were transfected for 48 h and lysed in an ice-cold lysis buffer [25 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 1 mM EDTA (Ethylenediaminetetraacetic acid), 1 mM Na_3VO_4, 250 μM PMSF (phenylmethanesulfonylfluoride), 10 μg/mL leupeptin and 10 μg/mL aprotinin]. After centrifugation, supernatants containing 30 μg of total protein were subjected to SDS–PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Proteins were

Table 1
Primer sequences for RT-PCR.

Gene	Primer sequence (5′ → 3′)
YY1	(F) 5′-AAG GGC GGC GGC AAG AAG AG-3′
	(R) 5′-CAG TTG CTT AGG GTC TGA GA-3′
C/EBPβ	(F) 5′-AGC CCC TAC CTG GAG CCG CT-3′
	(R) 5′-GCG CAG GGC GAA CGG GAA AC-3′
C/EBPα	(F) 5′- TGC TGG AGT TGA CCA GTG AC-3′
	(R) 5′-AAA CCA TCC TCT GGG TCT CC-3′
PPARγ	(F) 5′-ATC AGC TCT GTG GAC CTC TC-3′
	(R) 5′-ACC TGA TGG CAT TGT GAG AC-3′
GAPDH	(F) 5′-ACC ACA GTC CAT GCC ATC AC-3′
	(R) 5′-TCC ACC ACC CTG TTG CTG TA-3′