Biochemical and Biophysical Research Communications 454 (2014) 58–64

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

ATF3 represses PPARγ expression and inhibits adipocyte differentiation

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ARTICLE INFO

Article history:
Received 26 September 2014
Available online 14 October 2014

Keywords:
Adipocyte differentiation
Activating transcription factor 3 (ATF3)
Peroxisome proliferator activated receptor γ (PPARγ)
Promoter activity
ATF/CREB responsive element (ATF/CRE)

ABSTRACT

Activating transcription factor 3 (ATF3) is a stress-adaptive transcription factor that mediates cellular stress response signaling. We previously reported that ATF3 represses CCAAT/enhancer binding protein α (C/EBPα) expression and inhibits 3T3-L1 adipocyte differentiation. In this study, we explored potential role of ATF3 in negatively regulating peroxisome proliferator activated receptor-γ (PPARγ). ATF3 decreased the expression of PPARγ and its target gene in 3T3-L1 adipocytes. ATF3 also repressed the activity of −2.6 Kb promoter of mouse PPARγ2. Overexpression of PPARγ significantly prevented the ATF3-mediated inhibition of 3T3-L1 differentiation. Transfection studies with 5′ deleted-reporters showed that ATF3 repressed the activity of −2037 bp promoter, whereas it did not affect the activity of −1458 bp promoter, suggesting that ATF3 responsive element is located between the −2037 and −1458. An electrophoretic mobility shift assay and chromatin immunoprecipitation assay demonstrated that ATF3 binds to ATF/CRE site (5′-TGACGTTT-3′) between −1537 and −1530. Mutation of the ATF/CRE site abrogated ATF3-mediated transrepression of the PPARγ2 promoter. Treatment with thapsigargin, endoplasmic reticulum (ER) stress inducer, increased ATF3 expression, whereas it decreased PPARγ expression. ATF3 knockdown significantly blocked the thapsigargin-mediated downregulation of PPARγ expression. Furthermore, overexpression of PPARγ prevented inhibition of 3T3-L1 differentiation by thapsigargin. Collectively, these results suggest that ATF3-mediated inhibition of PPARγ expression may contribute to inhibition of adipocyte differentiation during cellular stress including ER stress.

1. Introduction

Adipose tissue plays a major role in regulating metabolism by storing excess energy and mobilizing the stored lipid for energy supply. Furthermore, adipose also functions as an endocrine organ, secreting various adipokines and cytokines, such as leptin and adiponectin, to influence metabolism [1]. The number of adipocytes present in an organism is determined to a large degree by the adipocyte differentiation process (adipogenesis). Therefore, adipocyte differentiation has many implications for human diseases including obesity and its metabolic complications. Adipocyte differentiation is controlled by a tightly regulated transcriptional cascade in which the transcription factors activate or repress each other's expression in a sequential manner [2]. Very early during adipogenesis, the expression of several transcription factors including CCAAT/enhancer binding protein (C/EBP) *β/δ*, glucocorticoid (GR), Krüppel-like factor 5 (KLFs), cAMP response element binding protein (CREB), early growth response2 (Krox20), and sterol regulatory element-binding protein 1c (SREBP-1c) is induced. These transcription factors in turn induce expression of PPARγ and C/EBPα, key adipogenic factors that induce expression of the genes for the mature adipocyte phenotype including aP2, CD36 and adiponectin. In contrast to activator of adipocyte differentiation, the Wnt/β-catenin pathway negatively regulates adipocyte differentiation.

Peroxisome proliferator-activated receptor (PPARγ) is a subfamily of nuclear hormone receptors that function as ligand-activated transcription factors to regulate various biological processes including adipogenesis, glucose homeostasis, and lipid metabolism [3]. PPARγ forms a heterodimer complex with retinoid X receptor-α (RXRα), which then binds to a specific DR-1 motif (a direct repeat AGGTCA separated by a single nucleotide). There are two isoforms of PPARγ, which are driven by alternative promoters but differ in the presence of 30 extra amino acids in the N-terminal region of PPARγ1 and have similar transcriptional activities [4]. While PPARγ1 is found in numerous tissues, PPARγ2 expression is mostly restricted to white and brown adipose tissues. The 30

Abbreviations: ATF3, activation transcription factor 3; ATF/CRE, ATF/CREB responsive element; C/EBP, CCAAT/enhancer-binding proteins; ChIP, chromatin immunoprecipitation; EMSA, electrophoretic mobility shift assay; ER, endoplasmic reticulum; aP2, adipocyte fatty acid binding protein; FAS, fatty acid synthase; PPARγ, peroxisome proliferator activated receptor γ.

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http://dx.doi.org/10.1016/j.bbrc.2014.10.028
0006-291X/

additional amino acids in PPARγ2 render the PPARγ aminoterminal transactivation domain more active. Thus, while both PPARγ isoforms can induce adipogenesis, PPARγ2 is thought to play a dominant role in this process.

Activating transcription factor 3 (ATF3) is a stress-inducible transcription factor that is a member of the ATF/CREB family of transcription factors [5]. ATF3 has the basic region-leucine zipper (bZip) DNA binding motif and binds to the consensus sequence TGACGTCA *in vitro*. ATF3 is induced by cellular stress such DNA damage, cell injury, oxidative stress and ER stress and plays important roles in multiple biological processes including differentiation, proliferation, inflammation and apoptosis [6]. We previously reported that ATF3 negatively regulates the expression of adiponectin and its receptors in adipocyte cells [7–9]. Furthermore, we demonstrated that ATF3 represses the expression of C/EBPα, resulting in inhibition of adipocyte differentiation [10].

In this study, to further study the role of ATF3 in ATF3-mediated inhibition of adipocyte differentiation, we investigated the negative effect of ATF3 on PPARγ expression. To accomplish this, we investigated the effect of ATF3 on PPARγ expression in 3T3-L1 cell, and conducted promoter analysis of the mouse PPARγ2 to characterize the ATF3-responsive elementin the promoter. We show that ATF3 downregulates PPARγ expression by repressing its promoter activity, which leads to inhibition of adipocyte differentiation.

2. Material and methods

2.1. Adipocytes differentiation

3T3-L1 cells (American Type Culture Collection, Manassas, VA) were maintained in DMEM containing 10% fetal calf serum (FCS, Invitrogen, Carlsbad, CA). For differentiation, confluent cells were cultured in DMEM supplemented with 10% FBS containing 0.5 mM IBMX, 125 μM Indomethacin, 2 μg/ml dexamethasone, 1 nM T3 and 20 nM insulin. 48 h later, the induction medium was replaced with maintenance medium (DMEM supplemented with 10% FBS, 1 nM T3 and 20 nM insulin). Fresh maintenance medium was added every 2 days until day 8.

2.2. Plasmids and oligonucleotides

Expression vectors for pCDNA3-ATF3 and pCDNA3-PPARγ was a generous gift from Dr. T. Hai (Ohio State University) and purchased from Addgene (Cambridge, MA), respectively. The region of the mouse PPARγ2 promoter corresponding to nucleotides −2618 to +35 was amplified by PCR from mouse genomic DNA and inserted into the *SacI*/*Mlu*I sites in pGL3 basic (Promega, Madison, WI) using sense primer 5′-GCGAGCTCCCTAGGAGCAGATGCTACCAGA-3′ and antisense primer 5′-CCCTCGAGA ACAGCATAAAACAGAGATT-3′ [for P(−2618)/Luc]. Deletions in the 5′-flanking regions of the promoter were constructed by PCR using pairwise combinations of the sense primers 5′-GCGAGCTCCAAGGGGCCTCCATTTCTCATC-3′ [for P(−2037)/Luc], 5’-GCGAGCTCCACAGTGATCCCCCATCATTTG-3′ [for P(−1458)/Luc], 5’-CGCGAGCTCGCAAGAAGCCAGAGTTTTCCTG-3′ [for P(−1023)/Luc], 5′-GCGAGCTCGACA TAGCACTTATCACTTAAA CA-3′ [for P(−901)/Luc] and the antisense primer 5′-CCCTCGAGA ACAGCATAAAACAGAGATT-3′. Mutations to 5′-PPARγ2 promoter [P(−2037)/Luc] were made using the Quikchange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. PCR was performed using 25 ng of P(−2037)/Luc as a template with primer 5′-TCCTTAAGTAGTC TGGTTTCTAAGCATTGAG-3′. After digestion with *Dpn*I, the resulting plasmids containing the mutated P(−2037)/Luc were transformed into *Escherichia coli* XL10-Gold ultracompetent cells (Stratagene). The transformed cells were plated on LB ampicillin (100 μg/ml) agar plates and incubated at 37 °C overnight. The mutated P(−2037)/Luc was confirmed by sequencing analyses.

2.3. Transient transfection and luciferase assay

For luciferase assay, HepG2 cells grown in six-well plates were transiently transfected with mouse PPARγ2 promoters and ATF3 expression vector using Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. After transfection for 24 h, the cells were lysed in reporter lysis buffer (Promega) and the luciferase activity was measured using a Luciferase Assay System (Promega). To normalize the transfection efficiency, pCMV-β gal was included in each transfection as an internal control plasmid and the luciferase activity was normalized against the β-galactosidase activity. For expression of pCDNA3-ATF3 and ATF3siRNA in 3T3-L1 adipocytes, microliter volume electroporation of 3T3-L1 adipocytes was performed with MicroPorator MP-100 (Digital Biotechnology, Suwon, South Korea) according to the manufacturer's instructions. Briefly, the cells were trypsinized, washed with 1× PBS, and finally resuspended in 12 μl of resuspension buffer R and 2 μg of plasmid at a concentration of 500,000 cells per pipette. The cells were then microporated at 1400 V, with a 30 ms pulse width, 1 pulse. Following microporation, the cells were seeded in 6-well cell culture dishes and placed at 37 °C in a 10% CO_2-humidified atmosphere. For luciferase assays of the promoter constructs, jetPRIME® transfection reagent (Polyplus-transfection, Illkirch, France) was used.

2.4. Construction of recombinant lentivirus containing ATF cDNA and infection

Construction of recombinant lentivirus containing ATF3 cDNA and infection into 3T3-L1 preadipocytes were described previously [10].

2.5. Oil red O staining

Cells were washed three times with PBS and then fixed for 2 min with 3.7% formaldehyde. Oil red O (0.5% in isopropanol, Sigma) was diluted with water (3:2), filtered through a 0.45-μm filter, and incubated with the fixed cells for 1 h at room temperature. Cells were washed with water, and the stained fat droplets in the cells were visualized by light microscopy and photographed.

2.6. Quantitative real time PCR (Q-PCR)

Total RNA was extracted using Trizol reagent (Invitrogen). The mRNA in the samples was reverse-transcribed using a GoScript™ Reverse Transcription System (Promega). The resulting cDNA was amplified by PCR using the following primer pairs: Activating transcription factor (ATF3) F (5′-AGTGAGTGCTTCTGCCATCG-3′) and R (5′-GCAGAGGTGCTTGTTCTGGA-3′), mouse peroxisome proliferator-activated receptor γ (PPARγ) F (5′-GTGCCAGTTTCGATCCGTA GA-3′) and R (5′-GGCCAGCATCGTGTAGATGA-3′), adipocyte fatty acid binding protein (aP2) F (5′-ACACCGAGATTTCCTTCAAACTG-3′) and R (5′-CCATCTAGGGTTATGATGCTCTTCA-3′), fatty acid synthase (FAS) F (5′-AGGTGGTGATAGCCGGTATGT-3′) and R (5′-TGGGTAATCCATAGAGCCCAG-3′). As an internal reference control, 18s ribosomal RNA gene was amplified using the sense primer 5′-CGGCTACCACATCCAAGGAA-3′ and the antisense 5′-GCTGGAAT-TACCGCGGCT-3′. Real-time PCRs were carried out on a Roche LightCycler®96 (Roche Diagnostics, Swiss) with TOPreal™ SYBR Green (Enzynomics, South Korea) in 96-well plates. Results were analyzed using the comparative critical threshold (ΔΔCT) method in which the amount of the target RNA is adjusted to an internal reference (18r RNA). The fold changes were calculated using the

$2^{\Delta C_T}$ method. Student's *t*-test was performed to assess statistical significance.

2.7. Electrophoretic mobility shift assay (EMSA)

3T3-L1 cells were treated with thapsigargin for 24 h. Nuclear extract from the cells was prepared using Nuclear and cytoplasmic extraction reagent (Pierce, Rockford, IL) according to the manufacturer's instructions. A DNA probe for PPARγ was generated by annealing biotin-labeled sense oligonucleotide (5′-TTAAGTAGTGACGTTTCTAAGCAT-3′) (−1545/−1522) and cold antisense oligonucleotide. For competition of probe binding, unlabeled oligonucleotides were added in 100-fold molecular excess relative to the respective probe. DNA–protein binding reactions were carried out as previously described [10].

2.8. Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation was conducted using a ChIP assay kit (Millipore, Temecula, CA) according to the manufacturer's protocol, modified as previously described. 3T3-L1 cells were incubated in the presence of thapsigargin for 24 h or left unstimulated. Immunoprecipitated DNA was amplified by PCR using primers specific for the PPARγ2 promoter, sense primer: 5′-TGCTTTTGACCTGTAAGTTCCAC-3′ (−1594/−1572), antisense primer: 5′-GATTAGGTTGTGTCACTAGGGG-3′ (+1467/+1446).

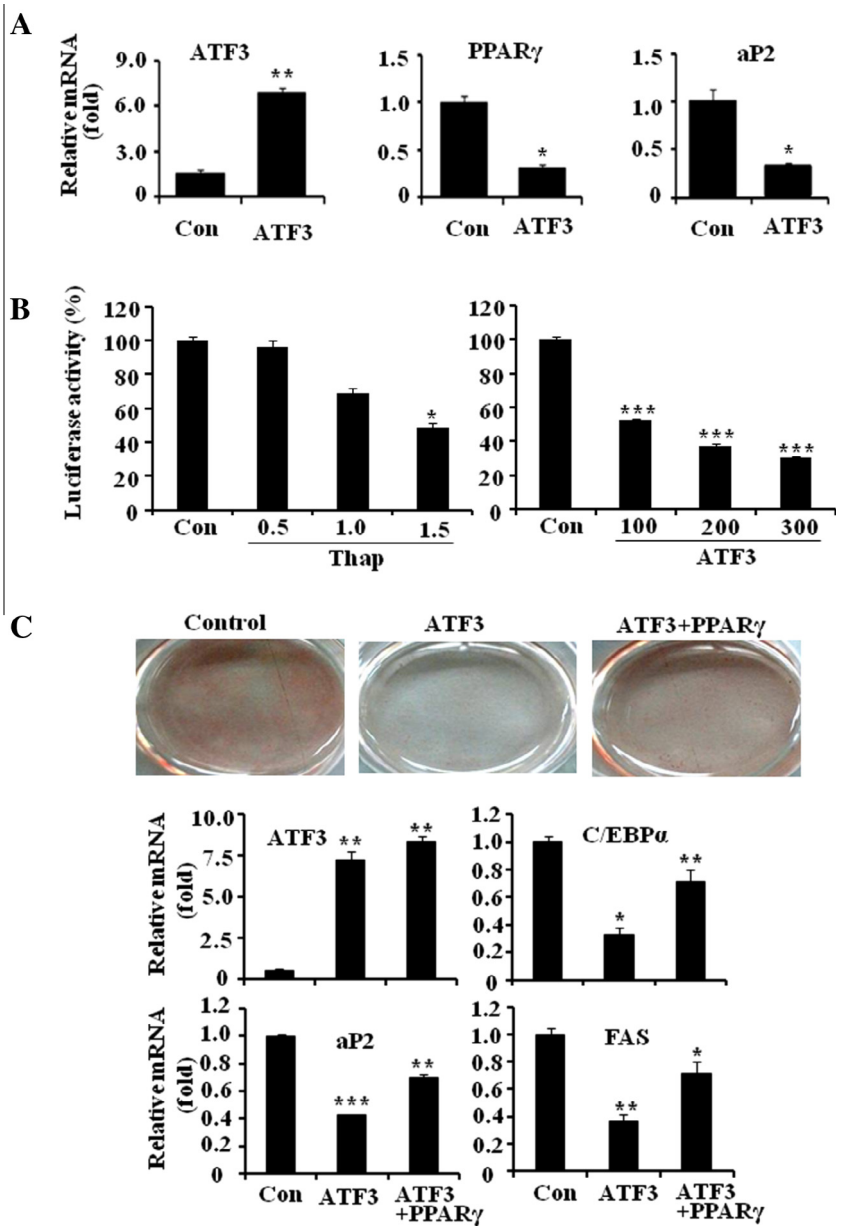

Fig. 1. ATF3 downregulates PPARγ expression. (A) 3T3-L1 adipocytes were transfected with pcDNA3-ATF3 and subjected to q-PCR for the expression of ATF3, PPARγ and its target genes. The amounts of mRNA are presented relative to the observable mRNA from control vector without ATF3. All values represent the mean ± SE from three independent experiments (*$P < 0.05$; **$P < 0.001$). (B) HepG2 cells were transfected with reporter P(−2681)/Luc containing the 2.6 Kb mouse PPARγ2 promoter and incubated with thapsigargin (0.5, 1.0, 1.5 μM) for 24 h. HepG2 cells were transfected with P(−2681)/Luc (0.2 μg) and ATF3 expressing vector (0.1, 0.2, 0.3 μg) for 24 h. Then, luciferase activities were measured and presented relative to no thapsigargin treatment or control vector without ATF3. All values represent the mean ± SE from three independent experiments (*$P < 0.05$; ***$P < 0.0001$). (C) 3T3-L1 preadipocytes were infected with lentivirus containing ATF3 and then transfected with PPARγ expression vector, and were differentiated as described. Oil red O staining was performed for measurement of adipocytes differentiation. Expression of adipogenic genes was measured by q-PCR using specific primers. The amounts of mRNA are presented relative to the observable mRNA from control vector without ATF3. All values represent the mean ± SE from three independent experiments (*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$).

2.9. Statistical analysis

All experiments were performed at least three times. The results are expressed as the mean ± SE. Statistical analysis was performed using Student's *t*-test. The data were considered statistically significant at $P < 0.05$.

3. Results

3.1. ATF3 represses PPARγ expression in 3T3-L1 adipocytes

Previously, we reported that ATF3 inhibits 3T3-L1 differentiation through repression of C/EBPα [10]. To see if ATF3 may also negatively regulate PPARγ expression and inhibit 3T3-L1 differentiation, we first investigate the effect of ATF3 on PPARγ expression in 3T3-L1 adipocytes. As shown in Fig. 1A, ATF3 repressed the expression of PPARγ and its target gene, aP2, in 3T3-L1 cells, indicating that ATF3 represses PPARγ expression. Next, we examined the effect of ATF3 on the promoter activity of PPARγ gene to see whether repression of PPARγ occurred at the transcriptional level. We isolated the 2.6 kb promoter of the mouse PPARγ2 gene and cloned it into pGL3, designated P(−2618)/Luc. Then, the effect of ATF3 on its promoter activity was examined. As shown in Fig. 1B, both treatment with ATF3 activator thapsigargin and ATF3 overexpression inhibited promoter activity of P(−2618)/Luc in a dose-dependent manner, suggesting that ATF3 represses PPARγ expression through inhibiting promoter activity of PPARγ. Then, we investigated whether overexpression of PPARγ blocks ATF3-mediated inhibition of 3T3-L1 differentiation. As shown in Fig. 1C, the overexpression of ATF3 inhibited differentiation of 3T3-L1 preadipocytes concomitant with decreased expression of adipogenic genes. However, overexpression of PPARγ significantly prevented ATF3-mediated inhibition of 3T3-L1 differentiation and recovered the decrease in the expression of adipogenic genes (Fig. 1C). These results suggest that ATF3 downregulates PPARγ expression, which may contribute to inhibition of adipocyte differentiation.

3.2. ATF/CRE site between −1537 and −1530 is critical for ATF3-mediated downregulation of PPARγ

A search for putative ATF/CRE sites revealed several ATF/CRE elements in the 2.6 kb promoter region of PPARγ2. To assess which ATF/CRE element is critical for repression of PPARγ2 expression, we constructed several 5′-deleted promoters and measured the

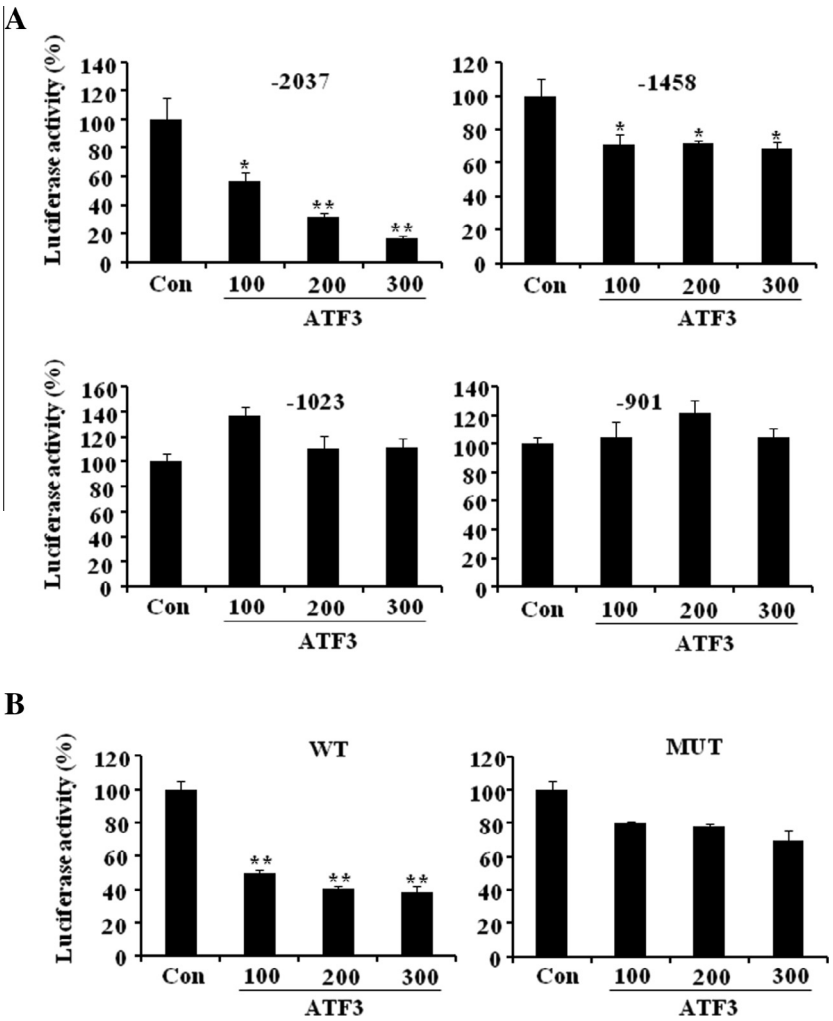

Fig. 2. ATF/CRE site between −1537 and −1530 is critical for ATF3-mediated downregulation of PPARγ. (A) 5′ serial deletion reporters of mouse PPARγ2 (0.2 μg) were transfected into HepG2 cells with ATF3 expression vector (0.1, 0.2, 0.3 μg), and then luciferase activities were measured. Luciferase activities are presented relative to the observable activity from control vector without ATF3. All values represent the mean ± SE from three independent experiments (*$P < 0.05$; **$P < 0.001$). (B) The mutant reporter (0.2 μg) was transfected into HepG2 cells with ATF3 expression vector (0.1, 0.2, 0.3 μg) and then luciferase activity was measured. Luciferase activities are presented relative to the observable activity from control vector without ATF3. All values represent the mean ± SE from three independent experiments (**$P < 0.001$).

effects of ATF3 on their promoter activities. As shown in Fig. 2A, deletions between −2618 and −2037 in the promoter retained the ATF3-mediated repressive effect on promoter activity. However, deletions between −2037 bp and −1458 bp in the promoter significantly abolished the repressive effect by ATF3, suggesting that the ATF3-responsive region is located in the region between −2037 and −1458. Within this region, a candidate ATF/CRE site (5′-TGACGTTT-3′) is present between −1537 and −1530, and could be involved in ATF3-mediated repression of PPARγ expression. To verify that this site is critical in ATF3-mediated repression, we mutated the ATF/CRE site (5′-TGACGTTT-3′) to (5′-TCTGGTTT-3′) within the context of P(−2037)/Luc. As shown in Fig. 2B, mutation of this site abolished the inhibitory effect of ATF3, suggesting that the ATF/CRE site (5′-TGACGTTT-3′) between −1537 and −1530 is critical for ATF3-mediated inhibition of PPARγ2 expression.

3.3. ATF3 binds to promoter region of PPARγ2 containing ATF/CRE site between −1537 and −1530

Next, to determine whether ATF3 is able to bind this site directly, EMSA was performed with biotin-labeled oligonucleotide probe (−1545/−1522) and nuclear extract from 3T3-L1 cells treated with thapsigargin, ATF3 activator. As shown in Fig. 3A, the probe produced a binding complex. A 100-fold excess of cold homologous oligonucleotide (PPARγ ATF/CRE) and ATF/CRE-consensus oligonucleotide competed for the binding, but the binding was not abolished by heterogeneous oligonucleotide (SP1), suggesting that the binding complex is specific. An antibody supershift experiment was also conducted with ATF3 antibody. Co-incubation of ATF3 antibody abolished the binding band (Fig. 3A), suggesting that ATF3 binds to the DNA region spanning −1545/−1522. Furthermore, ChIP analysis was performed to confirm ATF3 binding on the ATF/CRE site *in vivo*. The fully differentiated 3T3-L1 cells were treated with thapsigargin for 6 h and then with formaldehyde to cross-link DNA protein complexes. After sonication, chromatin fragments were immunoprecipitated with ATF3 antibody, and the immunoprecipitated fragmented DNA was subjected to PCR to amplify PPARγ promoter DNA (between −1594 and −1446) containing the ATF/CRE site between −1537 and −1530. As illustrated in Fig. 3B, ATF3 antibody immunoprecipitated ATF/CRE site-containing promoter fragments from 3T3-L1 cells. Treatment with thapsigargin increased the ATF3 binding. There was no amplified band when PCR was performed with the primers which does not contain ATF/CRE site. These results indicate a specific association of ATF3 with PPARγ2 gene promoter. Taken together, these findings demonstrate that ATF3 interacts with an ATF/CRE site between −1537 and −1530 in the PPARγ2 promoter.

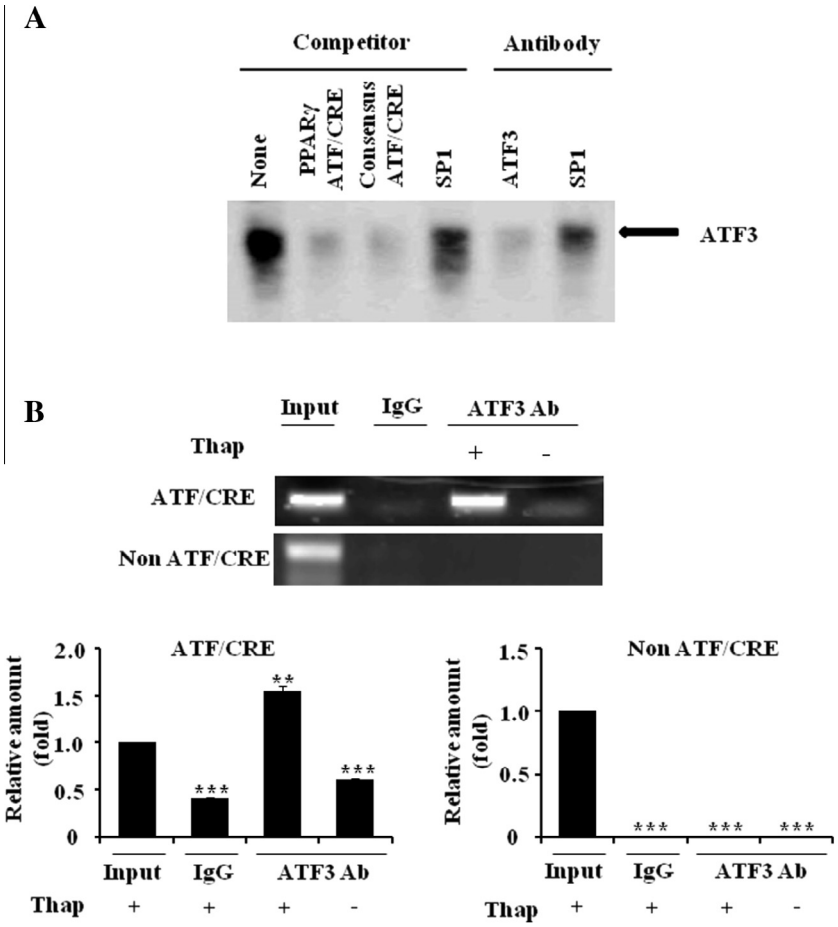

Fig. 3. ATF3 binds to the promoter region containing ATF/CRE element between −1537 and −1530. (A) EMSA was performed on oligonucleotide probe (−1545/−1522) using nuclear extract from 3T3-L1 cells. For the oligonucleotide competition experiments, a 100-fold excess of oligonucleotides was used (lane 1: no competition; lane 2: ATF/CRE oligonucleotides of PPARγ2; lane 3: consensus ATF/CRE oligonucleotides; lane 4: SP1 oligonucleotides. The protein–DNA complex was supershifted by ATF antibody (lane 5), but not by SP1 antibody (lane 6). (B) Fully differentiated 3T3-L1 cells were incubated with 1 μM thapsigargin for 6 h, and ChIP was performed with ATF3 antibody. DNA that was immunoprecipitated with anti-ATF3 was amplified by PCR using primers covering ATF/CRE of PPARγ2 promoter. All values represent the mean ± SE from three independent experiments (**$P < 0.001$; ***$P < 0.0001$).

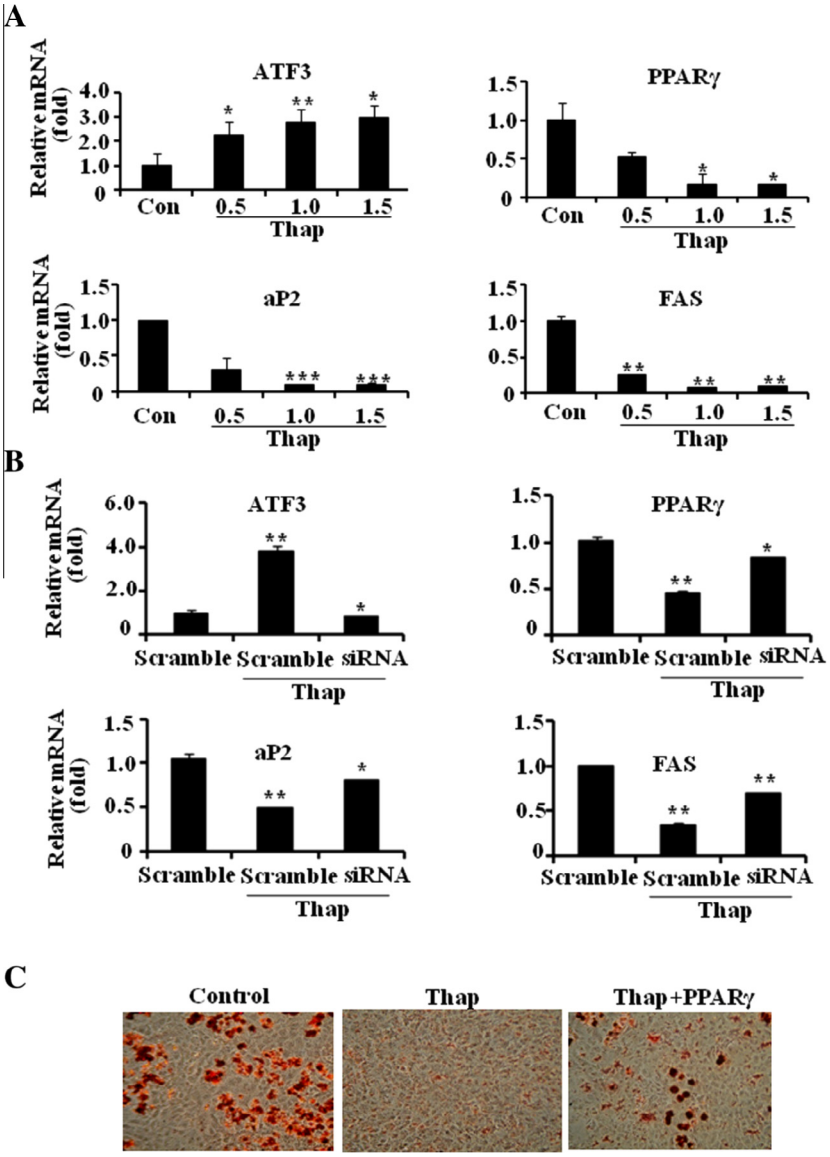

Fig. 4. ER stress-mediated inhibition of adipocyte differentiation is associated with downregulation of PPARγ by ATF3. (A) Fully differentiated 3T3-L1 cells were incubated with the indicated concentration of thapsigargin (0.5, 1.0, 1.5 μM) for 24 h and subjected to q-PCR for the expression of ATF3, PPARγ and its target genes. The amounts of mRNA are presented relative to the observable mRNA from control, no thapsigargin treatment. All values represent the mean ± SE from three independent experiments ($^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$). (B) Fully differentiated 3T3-L1 cells were transfected with ATF3 siRNA or scramble RNA, and then incubated with 0.5 μM thapsigargin for 24 h. Expression of ATF3, PPARγ and its target genes was measured by q-PCR. The amounts of mRNA are presented relative to the observable mRNA from scramble RNA transfection. All values represent the mean ± SE from three independent experiments ($^{*}P < 0.05$; $^{**}P < 0.001$). (C) 3T3-L1 preadipocytes were transfected with pcDNA3-PPARγ and differentiated in the presence of thapsigargin for 8 d. Oil red O staining was used to measure adipocytes differentiation.

3.4. ER stress-mediated inhibition of adipocyte differentiation is associated with downregulation of PPARγ by ATF3

ER stress has been reported to inhibit the differentiation of 3T3-L1 preadipocytes [10]. To see if ER stress-mediated inhibition of 3T3-L1 preadipocyte occurs via downregulation of PPARγ by ATF3, we first examined the effect of thapsigargin, ER stress inducer, on the expression of PPARγ and its target genes in 3T3-L1 adipocytes. As shown in Fig. 4A, treatment with thapsigargin increased ATF3 expression, whereas it decreased the expression of PPARγ and its target genes including aP2 and FAS. Knockdown of ATF3 using ATF3siRNA significantly blocked the thapsigargin-mediated repression of PPARγ and its target genes (Fig. 4B), suggesting that ATF3 is involved in thapsigargin-mediated downregulation of PPARγ. Furthermore, we investigated whether PPARγ recovered thapsigargin-mediated inhibition of 3T3-L1 differentiation. As shown in Fig. 4C, overexpression of PPARγ significantly prevented thapsigargin-mediated inhibition of 3T3-L1 differentiation.

4. Discussion

The transcriptional cascade controlling adipogenesis has been well characterized [2]. The adipogenic cascade can be divided into at least two waves of transcription factors that drive the adipogenic program. The first wave is initiated by adipogenic stimuli that activate several early adipogenic transcription factors, which in turn induce expression of the second wave of transcription factors such as PPARγ and C/EBPα, the key adipogenic factors. In particular, PPARγ and C/EBPα drive the final adipocyte differentiation and/or modulate adipocyte metabolism. Therefore, understanding

the events associated with regulation of PPARγ and C/EBPα will provide novel insights into how adipogenic transcription factor networks orchestrate adipocyte differentiation. Previously, we demonstrated that ATF3 inhibits adipocyte differentiation by downregulating C/EBPα expression [10]. Here, we explored the potential role of ATF3 in regulating PPARγ-mediated signaling. We identified ATF3 as a novel repressor of PPARγ-mediated transaction in adipocyte cells that thus may play a role in the inhibition of adipocyte differentiation induced by cellular stress. ATF3 binds to the promoter of the PPARγ gene and represses its expression in adipocytes. Therefore, our finding suggests that ATF3-mediated inhibition of PPARγ expression may contribute to inhibition of adipocyte differentiation during cellular stress including ER stress and hypoxia.

Since ATF3 is a DNA binding transcription factor, we first thought that ATF3 would generally regulate PPARγ expression through binding to the promoter region of PPARγ in adipocytes [3]. We found that thapsigargin treatment or overexpression of ATF3 decreased PPARγ expression in both brown adipocytes and 3T3-L1 cells, and efficiently repressed the activity of the promoter from −2618 to +35 of PPARγ2, indicating that ATF3 downregulates PPARγ expression at the transcriptional level by repressing the promoter activity of PPARγ. Then we identified the ATF3 responsive element in the PPARγ2 promoter. A search for putative ATF/CRE sites using Transfec program revealed five putative ATF/CRE sites within the 2.6 kb promoter. 5′-Deletion mutants of the 2.6 kb promoter suggested that TGACGTTT between −1537 and −1530 was the ATF/CRE site responsible for ATF3-mediated repression of PPARγ2 promoter activity, on which ATF3 directly bound. Mutation of the TGACGTTT into TCCCGTTT abolished the repressive effect of ATF3 on the promoter activity, further indicating that the ATF/CRE site between −1537 and −1530 is biologically critical for ATF3-mediated repression of PPARγ2.

PPARγ is also reported to critically influence peripheral tissue insulin sensitivity as well as adipocyte differentiation [11]. Thiazolidinediones are pharmacological PPARγ ligands and have been used extensively in the treatment of type 2 diabetes and other disorders characterized by insulin resistance. We previously demonstrated that ATF3 repressed expression of adiponectin and its receptors in adipocyte and liver cells, resulting in attenuation of adiponectin signaling in obesity or diabetic conditions, leading to insulin resistance [7–9]. Furthermore, we also showed that AT3 negatively regulates the expression and transactivation of PDX-1, resulting in impaired insulin secretion in pancreatic β-cells [12]. As our current study revealed that ATF3 is a negative regulator of PPARγ, ATF3 may also contribute to insulin resistance by attenuating PPARγ signaling.

ER stress has been reported to induce insulin resistance through suppression of insulin receptor signaling by the hyperactivation of JNK and subsequent serine phosphorylation of insulin receptor substrate-1 (IRS-1) in the liver and adipocytes [13]. Furthermore, ER stress has been shown to cause apoptosis of pancreatic β-cells via transcriptional induction of CHOP, activation of JNK, and activation of the ER-localized cysteine protease, caspase-12 [14]. Therefore, chronic ER stress is a core mechanism involved in triggering insulin resistance and type 2 diabetes. Our current work suggests that ATF3 may represent the molecular link between PPARγ signaling and ER stress-induced insulin resistance. ER stress-inducible ATF3 functions as a negative regulator of PPARγ, which may contribute to insulin resistance in insulin sensitive tissues. Therefore, ATF3 may play an important role in ER stress-mediated insulin resistance and type 2 diabetes.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2012R1A1A4A01013659).

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