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ATF3 inhibits PPARγ-stimulated transactivation in adipocyte cells

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ABSTRACT

Previously, we reported that activating transcription factor 3 (ATF3) downregulates peroxisome proliferator activated receptor (PPARγ) gene expression and inhibits adipocyte differentiation in 3T3-L1 cells. Here, we investigated another role of ATF3 on the regulation of PPARγ activity. ATF3 inhibited PPARγ-stimulated transactivation of PPARγ responsive element (PPRE)-containing reporter or GAL4/PPARγ chimeric reporter. Thus, ATF3 effectively repressed rosiglitazone-stimulated expression of adipocyte fatty acid binding protein (aP2), PPARγ target gene, in 3T3-L1 cells. Coimmunoprecipitation and GST pulldown assay demonstrated that ATF3 interacted with PPARγ. Accordingly, ATF3 prevented PPARγ from binding to PPRE on the aP2 promoter. Furthermore, ATF3 suppressed p300-mediated transcriptional coactivation of PPRE-containing reporter. Chromatin immunoprecipitation assay showed that overexpression of ATF3 blocked both binding of PPARγ and recruitment of p300 to PPRE on aP2 promoter induced by rosiglitazone treatment in 3T3-L1 cells. Taken together, these results suggest that ATF3 interacts with PPARγ and represses PPARγ-mediated transactivation through suppression of p300-stimulated coactivation in 3T3-L1 cells, which may play a role in inhibition of adipocyte differentiation.

1. Introduction

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 [1]. 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) on the promoters of PPARγ target genes including adipocyte fatty acid binding protein (aP2), fatty acid transporter protein-1 (FATP-1), or lipoprotein lipase (LPL) [2]. PPARγ activity is regulated by ligand including fatty acid derivatives or antidiabetic drugs thiazolinediones. Ligand binding to PPARγ induces conformational changes and recruits some coactivator such as p300, resulting chromatin remodeling followed by target gene transcription [3]. Aside from p300, the cofactors including steroid receptor co activator (SRC-1) family, the PPARγ binding protein PBP, PPARγ co-activator (PGC-1), CREB binding protein (CBP) and receptor interacting protein (RIP)-140 interacts with PPARγ and regulates PPARγ-mediated transcriptional activation [3]. Posttranscriptional modifications such as phosphorylation also regulates PPARγ activity. MAPK phosphorylation inhibits PPARγ activity [4,5], whereas cyclin-dependent kinases Cdk7 and Cdk9 increases PPARγ activity [6,7].

Activating transcription factor 3 (ATF3) is a stress-inducible transcription factor that is a member of the ATF/CREB family of transcription factors [8]. 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 [9]. Previously, we demonstrated that ATF3 negatively regulates the expression of adiponectin and its receptors in adipocyte cells [10–12] and expression of PDX-1 in pancreatic β-cells [13]. Furthermore, we also reported that ATF3 represses the expression of PPARγ and C/EBPα, resulting in inhibition of adipocyte differentiation [14,15]. Although ATF3 can bind to promoters and repress expression of some genes while activating expression of other genes, ATF3 also regulates cellular functions through interacting with other transcription factors via bZIP domain independent of ATF3 transcriptional activity [16–18]. We reported previously that ATF3 binds to PDX-1 and

Abbreviations: aP2, adipocyte fatty acid binding protein; ATF3, activation transcription factor 3; ATF/CRE, ATF/CREB responsive element; ChIP, chromatin immunoprecipitation; ER, endoplasmic reticulum; LBD, ligand-binding domain; PPARγ, peroxisome proliferator activated receptor γ; PPRE, PPARγ responsive element.

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represses PDX-1-mediated transactivation through inhibition of p300-stimulated coactivation in pancreatic β-cells [19].

In this study, to further study the role of ATF3 in PPARγ signaling in addition to direct downregulation of PPARγ expression, we examined the effects of ATF3 on PPARγ-mediated transactivation, and characterized the mechanism involved in these effects. Here, we demonstrated that ATF3 interacts with PPARγ and represses PPARγ-mediated transactivation through suppression of p300-stimulated coactivation, which may also play a role in ATF3-mediated inhibition of adipocyte differentiation.

2. Materials and methods

2.1. Adipocyte 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

Expression vectors for HA-ATF3 and pCDNA-PPARγ were generous gifts from Dr. T. Hai (Ohio State University). Expression vectors for GST-PPARγ full length, GST-PPARγ LBD were gifts from Dr. L. Fajas (Institut de Recherche en Cancerologie de Montpellier, France). Flag-PPARγ expression vector and PPRE-Luc reporter were purchased from Addgene (Cambridge, MA). pFA-PPARγ LBD (Gal4-PPARγ) and pFR-Gal4 (UAS-Gal4-luciferase) were gifts from Dr. Y.H. Kim (Chungnam National University, South Korea).

2.3. Transient transfection

To maximize the transfection efficiency, microliter volume electroporation of 3T3-L1 adipocytes was performed with MicroPorator MP-100 (Digital Biotechnology, Suwon, South Korea). 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. Briefly, HEK293 cells were cultured at a density of 2.5×10^5 cells/6-well in DMEM. The next day, cells were transfected with the indicated luciferase reporter plasmids using jetPRIME® transfection reagent according to the manufacturer's instructions. After 4-h incubation, the cell medium was replaced with fresh complete medium. After 48-h incubation, the cells were washed with PBS and harvested in 200 μl of passive lysis buffer (Promega, Madison, WI). The cells were mixed vigorously for 15 s and centrifuged at 12,000*g* for 10 min at 4 °C. The supernatants were transferred into a fresh tube, and 5-μl aliquots of the cleared whole-cell lysate were assayed for luciferase activity using a Luciferase Reporter Assay System (Promega). Each transfection experiment was performed in triplicate. PPARγ ligand-binding activity was measured using a GAL4/PPARγ transactivation assay.

2.4. 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: mouse adipocyte fatty acid binding protein (aP2) F (5′-ACACCGAGATTTCC TTCAAACTG-3′) and R (5′-CCATCTAGGGTTATGATGCTCTTCA-3′). As an internal reference control, 18s ribosomal RNA gene was amplified using the sense primer 5′-CGGCTACCACATCCAAGGAA-3′ and the antisense 5′-GCTGGAATTACCGCGGCT-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 $2\Delta^{CT}$ method. Student's *t*-test was performed to assess statistical significance.

2.5. 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 [13]. 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 PPRE region on aP2 promoter, sense primer: 5′-GAGCCATGCGGATTCTTG-3′, antisense primer: 5′-CCAG-GAGCGGCTTGATTGTTA-3′; and a non-PPRE region in the aP2 promoter: sense primer 5′-CCTCCCGGTAGGCAAACTGGA-3′, antisense primer 5′-CCACTGCACAGCTGTTTAAGTGACTGG-3′.

2.6. Coimmunoprecipitation

3T3-L1 cells were lysed in Pierce IP lysis/wash buffer [0.025 mol/L Tris (pH 7.4), 1% Nonidet P40, 0.15 mol/L NaCl, 0.001 mol/L EDTA, 5% glycerol] at 4 °C, then vortexed and centrifuged at 14,000 rpm for 10 min at 4 °C. The concentration of total protein was determined by a modified Bradford method (Bio-Rad, Hercules, CA). For co-immunoprecipitation assays, the Pierce™ classic magnetic IP/Co-IP kit (Thermo scientific, Rockford, IL) was used. Briefly, lysates were precleared by incubation with the control magnetically separate beads component, and then added to antibody. After incubation at 4 °C overnight, immunoprecipitates were washed two times with Pierce IP lysis/wash buffer and once with elution buffer. For protein detection, samples were separated by 10–12% gradient SDS–PAGE and then transferred to a PVDF membrane (Millipore, Bedford, MA). Western blot analysis was performed.

2.7. In vitro translation and GST-pull-down assay

pCDNA-PPARγ was translated *in vitro* in the presence of [35^S] methionine using T7-TNT™ Quick Coupled Transcription/Translation System (Promega). GST fusion or GST alone was expressed in *Escherichia coli* BL21 (DE3) strain and affinity-purified by glutathione-Sepharose (Promega). GST-fusion proteins were purified, according to the manufacturer's protocol (MagneGST™ Pull-Down System, Promega). *In vitro*-translated ATF3 was incubated with GST or fusion GST-PPARγ proteins coupled to glutathione Sepharose beads in 200 μl of lysis buffer at 4 °C for 4 h, and the bound fraction was purified through glutathione-Sepharose beads at 4 °C for 2 h. The pull-down complexes were separated by SDS 12% PAGE and examined by Western blot analysis.

2.8. 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 inhibits PPARγ-mediated transcriptional activation

Previously, we reported that ATF3 inhibits adipocyte differentiation through repression of PPARγ expression [14]. In this study, we determined whether ATF3 could also modulate PPARγ-mediated transcriptional activation. To accomplish this, we investigated the effects of ATF3 on PPARγ-stimulated transactivation of a reporter containing three PPARγ responsive elements (PPRE) (PPRE-Luc). We cotransfected HEK293 cells with PPRE-Luc and expression vectors for PPARγ and ATF3. As shown in Fig. 1A, PPARγ increased the promoter activity of PPRE-Luc, which was further enhanced by treatment with the PPARγ agonist rosiglitazone. However, the transcriptional activation was markedly diminished by ATF3. To further demonstrate that ATF3 negatively influences the transcriptional activation of PPARγ, we used the one-hybrid system, in which a chimeric molecule, Gal4 DNA binding domain-PPARγ ligand binding domain (Gal4-PPARγ), interacts with the Gal4 response element-driven promoter (pFR-Luc). Cotransfection of Gal4-PPARγ with pFR-Luc resulted in increase in luciferase activity, which was further enhanced by rosiglitazone. However, ATF3 coexpression significantly decreased the transcriptional activation by PPARγ (Fig. 2B). Taken together, these results demonstrated that ATF3 can inhibit PPARγ-mediated transactivation. We then examined the effect of ATF3 on the expression of endogenous PPARγ target genes stimulated by rosiglitazone in differentiated 3T3-L1 cells. As shown in Fig. 2C, the expression of aP2 was stimulated by treatment with rosiglitazone. However, overexpression of ATF3 effectively repressed rosiglitazone-stimulated expression of aP2, indicating that ATF3 suppresses the transactivation of PPARγ and represses the expression of its target gene in adipocyte cells.

3.2. ATF3 interacts with PPARγ

To examine mechanisms of ATF3-mediated suppression of PPARγ-induced transactivation, we examined the interaction of ATF3 with PPARγ using coimmunoprecipitation. 3T3-L1 cells were cotransfected with expression vectors for HA-ATF3 and flag-PPARγ. The cell lysates were then analyzed by immunoprecipitation using HA antibody, followed by Western blot with flag or HA antibody. As shown in Fig. 2A, HA-immunoprecipitate was only readily blotted by flag antibody in the cells cotransfected with HA-ATF3 and flag-PPARγ-expressing vectors, not in the cells transfected with only HA-ATF3-expression vector, demonstrating that ATF3 can interact with PPARγ *in vivo*. As a more direct method to observe the interaction of ATF3 with PPARγ *in vitro*, we conducted a glutathione S-transferase (GST)-pulldown assay using GST-PPARγ expression vectors. As shown in Fig. 2B, we observed ATF3 interactions with ligand binding domain (LBD) as well as full-length of PPARγ (full). In contrast, ATF3 did not bind GST alone in the absence of PPARγ. Taken together, these results demonstrated that ATF3 can interact with PPARγ *in vivo* and *in vitro*.

Ligand binding to PPARγ facilitates the heterodimerization of PPARγ with RXR and increases the binding of the PPARγ/RXR heterodimers to PPRE. The ligand-activated heterodimer subsequently stimulates transcription of the target gene [1]. Therefore, we

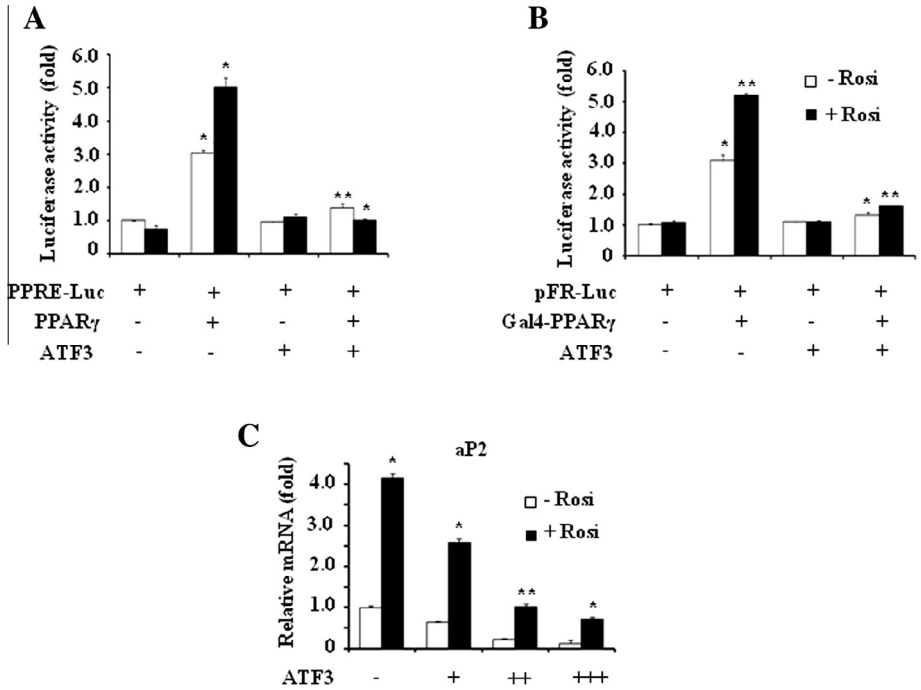

Fig. 1. ATF3 inhibits PPARγ-mediated transcriptional activation. (A) HEK293 cells were cotransfected with PPRE-Luc (0.2 μg) together with PPARγ (0.2 μg) and ATF3 (0.6 μg) expressing vectors in the presence or absence of rosiglitazone. Luciferase activities are presented relative to the observable activity from control vector without PPARγ and ATF3. All values represent the mean ± SE from three independent experiments ($^{*}P < 0.05$; $^{**}P < 0.001$). (B) HEK293 cells were cotransfected with Gal4-PPARγ (0.2 μg) and pFR-Luc (0.2 μg) together with PPARγ (0.2 μg) and ATF3 (0.6 μg) expressing vectors and incubated in the presence of rosiglitazone or in the absence of rosiglitazone. Luciferase activities are presented relative to the observable activity from control vector without Gal4-PPARγ and ATF3. All values represent the mean ± SE from three independent experiments ($^{*}P < 0.05$; $^{**}P < 0.001$). (C) Fully differentiated 3T3-L1 cells were transfected with ATF3 expression vector and incubated in the presence or absence of rosiglitazone. Expression of aP2 was measured by q-PCR. 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 ($^{*}P < 0.05$; $^{**}P < 0.001$).

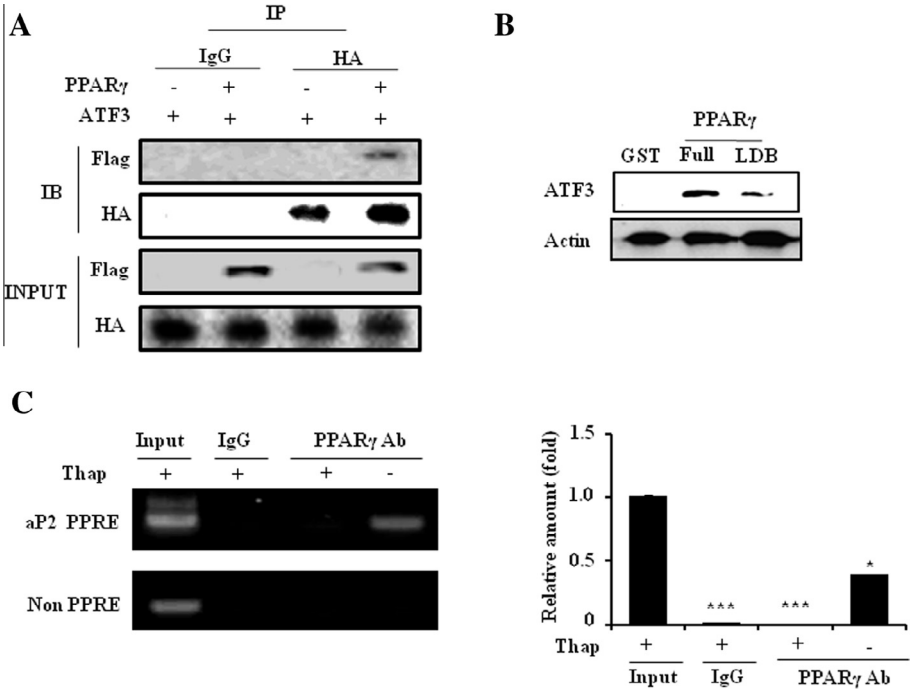

Fig. 2. ATF3 interacts with PPARγ and inhibits the recruitment of PPARγ to PPRE on aP2 promoter. (A) 3T3-L1 cells transfected with flag-PPARγ or HA-ATF3 expressing vector were immunoprecipitated with HA antibody, after which Western blots were conducted using flag or HA antibody. (B) The lysates from 3T3-L1 cells were incubated with glutathione-Sepharose beads containing bacterially expressed GST alone, GST-PPARγ (full), GST-PPARγ (LBD) fusion proteins and then analyzed by SDS–PAGE using ATF3 antibody. (C) Fully differentiated 3T3-L1 cells were incubated in the presence of thapsigargin for 24 h, after which ChIP was performed with PPARγ antibody, and PCR was done using primers covering the PPRE on the mouse aP2 promoter. The data represent the average ± SE from three independent experiments ($^{*}P < 0.05$, $^{***}P < 0.0001$).

investigated whether ATF3 binding to PPARγ LBD affects the recruitment of PPARγ to PPRE on target gene promoter. To this end, we performed ChIP experiment on the PPRE of aP2 promoter in 3T3-L1 cells treated with thapsigargin, ATF3 inducer. As shown in Fig. 2C, the chromatin immunoprecipitated using PPARγ antibody was amplified by PCR using primers specific for aP2 promoter region containing a PPRE, located between −5329 and −5341. However, the amplification of the aP2 promoter was significantly reduced when ATF3 was increased with thapsigargin treatment (Fig. 2C). No amplification of aP2 promoter was observed when nonspecific IgG was used to immunoprecipitate the chromatin. These results suggest that interaction of ATF3 with PPARγ inhibits the binding of PPARγ to PPRE on the promoter of PPARγ target genes, suppressing the activation of PPARγ target genes.

3.3. ATF3 suppresses p300-enhanced transactivity of PPARγ

Furthermore, ligand-activated PPARγ recruits p300 to PPRE on the PPARγ target gene promoter, which is critical for the activation of PPARγ target genes [20]. To further examine the mechanism of ATF3-mediated suppression of PPARγ transcriptional activity, we investigated the effects of ATF3 on the p300-enhanced transcriptional activity of PPARγ using a PPRE-containing reporter. HepG2 cells were cotransfected with PPRE-Luc reporter plasmid and expression plasmids for PPARγ, p300 and ATF3, after which the luciferase activities were measured. As shown in Fig. 3, p300 synergistically enhanced the PPARγ-stimulated promoter activity of the PPRE-Luc reporter, which was further increased by rosiglitazone treatment. However, coexpression of ATF3 significantly repressed the p300-enhanced transcriptional activity of PPARγ (Fig. 3) in a dose-dependent manner, suggesting that ATF3 can interfere with the coactivation function of p300 for PPARγ.

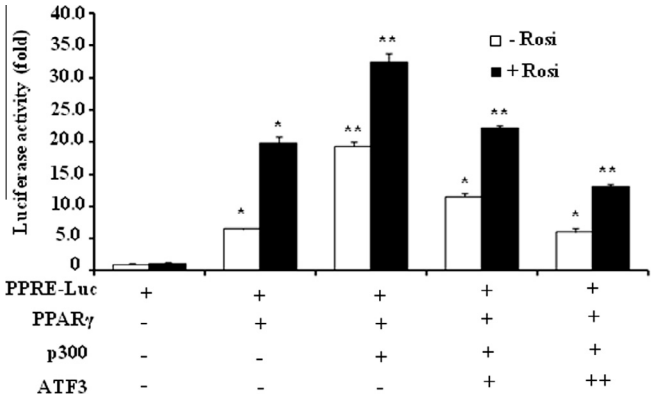

Fig. 3. ATF3 inhibits p300-enhanced transactivation of PPARγ. HEK293 cells were cotransfected with PPRE-Luc with or without expression vectors for PPARγ, p300 and ATF3, and incubated with or without rosiglitazone. Luciferase activities are measured and presented relative to the observable activity from control vector without PPARγ, p300 and ATF3. All values represent the mean ± SE from three independent experiments ($^{*}P < 0.05$; $^{**}P < 0.001$).

3.4. ATF3 decreases the recruitment of p300 to PPRE on aP2 promoter

We subsequently examined the interaction of p300 with PPARγ on the PPRE site of aP2 *in vivo*. 3T3-L1 cells were transfected with ATF3 expression vector, and ChIP experiments were performed on the PPRE site of aP2 with PPARγ or p300 antibody. Consistent with PPARγ antibody (Fig. 4A), the amplification of immunoprecipitated chromatin using p300 antibody was increased with rosiglitazone treatment (Fig. 4B). However, ATF3 significantly decreased the amplification (Fig. 4B), suggesting that ATF3 binds to PPARγ LBD and thus decreases the recruitment of p300 to PPARγ on the PPRE site of PPARγ-target gene promoters.

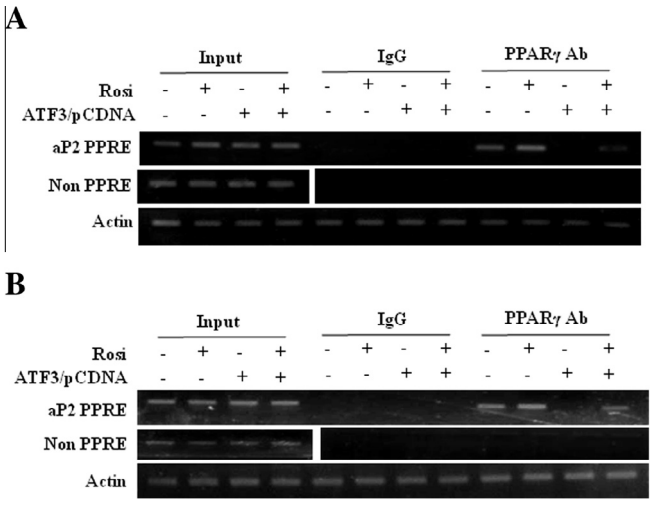

Fig. 4. ATF3 decreases the recruitment of p300 to PPRE on aP2 promoter. 3T3-L1 cells were transfected with ATF3 expression vector and incubated in the presence of rosiglitazone for 24 h, after which ChIP was performed with PPARγ (A) or p300 antibody (B), and PCR was done using primers covering the PPRE on the mouse aP2 promoter. The data represent the average ± SE from three independent experiments.

4. Discussion

Previously, we reported that ATF3 downregulates PPARγ expression by repressing its promoter activity in adipocyte cells, which leads to inhibition of adipocyte differentiation [14]. Here, we explored the negative 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 inhibits the transactivation activity of PPARγ through interaction with PPARγ. Therefore, our finding suggests that ATF3-mediated inhibition of PPARγ signaling may contribute to inhibition of adipocyte differentiation during cellular stress including ER stress and hypoxia.

We studied the effect of ATF3 on PPARγ-mediated transactivation using PPRE-Luc or Gal4-PPARγ LBD/pFR-Luc reporter. Our current study showed that PPARγ expression activated the promoter activity of PPRE-Luc or Gal4-PPARγ LBD/pFR-Luc, which was further stimulated by treatment with rosiglitazone. However, ATF3 expression significantly suppressed PPARγ-stimulated promoter activities of both reporters, demonstrating that ATF3 inhibits PPARγ-mediated transactivation. To gain insight into the mechanism by which ATF3 inhibits PPAR-γ-mediated transactivation, we investigated the interaction of ATF3 with PPARγ. We found that ATF3 directly bound to LBD of PPARγ. Then, we investigated whether the binding of ATF3 to PPAR-γ affects the PPARγ binding to PPRE on the promoter of its target genes. ChIP assay revealed that ATF3 blocks the binding of PPARγ to PPRE on aP2 promoter. The blocking of binding of PPARγ to PPRE is due to the interaction of ATF3 with LBD of PPARγ because ligand binding to PPAR-γ facilitates formation of a heterodimer complex with RXRα and increases the binding to PPREs within the promoters of PPAR-γ-targeted genes [1]. These results suggest that interaction of ATF3 with PPARγ prevents PPARγ from binding to PPRE on its target genes and then downregulates expression of PPARγ target genes.

Furthermore, the inactivated PPARγ is associated with a corepressor that silences its transcriptional activity by the recruitment of histone deacetylases [2]. The activation of PPARγ by ligand binding causes dissociation of the corepressor proteins followed by recruitment of coactivators such as PPARγ coactivator (PGC-1), the histone acetyltransferase p300, CREB binding protein (CBP), and steroid receptor coactivator (SRC)-1, resulting in transcriptional activation of the target gene. Therefore, we examined the effects of ATF3 on p300-stimulated PPARγ transcription activity on rosiglitazone treatment. p300 synergistically increased the promoter activity of PPRE-Luc, which was further enhanced by rosiglitazone treatment. However, ATF3 expression suppressed the p300-enhanced promoter activity, suggesting that ATF3 inhibits p300-mediated transcoactivation in PPARγ signaling. Then, we assessed whether the interference of p300-coactivation of PPARγ by ATF3 was associated with decreased interaction of PPARγ with p300 on PPRE of target gene promoter. We found that treatment with rosiglitazone increased recruitment of p300 to PPARγ on the PPRE of aP2 promoter, but ATF3 overexpression efficiently prevented the increased recruitment of p300 to PPARγ on the PPRE, suggesting that ATF3 binding to PPARγ LBD may block the p300-enhanced transcriptional activity of PPARγ by interfering with p300 recruitment to PPARγ in PPARγ target gene promoter. Previously, we also reported that ATF3 inhibits pancreatic and duodenal homeobox-1 (PDX-1)-mediated transactivation through the inhibition of p300-stimulated coactivation, which may lead to β-cell dysfunction by ER stress [19]. Collectively, it appears that the interaction of ATF3 with PPARγ inhibits both the binding of PPARγ to PPRE and the attraction of p300 coactivation of PPARγ-responsive promoter.

Several factors are reported to regulate PPARγ activity and adipocyte differentiation in adipocyte cells. The retinoblastoma protein (RB) forms complex with PPARγ and this PPARγ–RB complex contains the histone deacetylase HDAC3, thereby attenuating PPARγ's capacity to drive gene expression and adipocyte differentiation [21]. The HIV-1 accessory protein viral protein R (Vpr) also interacts with the ligand-binding domain of PPARγ and suppresses PPARγ-induced transactivation, which inhibits PPARγ agonist-induced adipocyte differentiation [22]. In contrast, cyclin G2, cell cycle regulator, positively regulate the transcriptional activity of PPARγ through PPARγ interaction and stimulate adipocyte differentiation [23]. Lipin1 physically interacts with PPARγ and activates PPARγ activity by releasing co-repressors, NcoR1 (nuclear receptor co-repressor 1) and SMRT (silencing mediator of retinoid and thyroid hormone receptor), from PPARγ in the absence of ligand and enhance adipocyte differentiation [24]. In current study, we propose that the negative regulation of PPARγ transcriptional activity by ATF3 is also an important regulator in adipocyte differentiation.

ATF3 is a transcription factor induced by endoplasmic reticulum (ER) stress. ER stress has been reported to induce insulin resistance and cause apoptosis of pancreatic β-cells, which leads to type 2 diabetes [25,26]. Our current work suggests that ATF3 may represent the molecular link between PPARγ signaling and ER stress-induced type 2 diabetes. Since ER stress-inducible ATF3 functions as a negative regulator of PPARγ activity, which is reported to critically influence peripheral tissue insulin sensitivity, ATF3 may play an important role in ER stress-mediated insulin resistance and type 2 diabetes.

In conclusion, the stress-inducible ATF3 interacts with PPARγ, and inhibits recruitment of both PPARγ and p300 to PPRE, which suppresses p300-coactivation of PPARγ. Therefore, the present study provides new insight into the mechanism by which ATF3 inhibits adipocyte differentiation and causes insulin resistance.

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References

[1] E.D. Rosen, B.M. Spiegelman, PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth, J. Biol. Chem. 276 (2001) 37731–37734.
[2] E.D. Rosen, O.A. MacDougald, Adipocyte differentiation from the inside out, Nat. Rev. Mol. Cell Biol. 7 (2006) 263–273.
[3] N.J. McKenna, J. Xu, Z. Nawaz, S.Y. Tsai, M.J. Tsai, B.W. O'Malley, Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions, J. Steroid Biochem. 69 (1999) 3–12.
[4] E. Hu, J.B. Kim, P. Sarraf, B.M. Spiegelman, Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ, Science 274 (1996) 2100–2103.
[5] H.S. Camp, S.R. Tafuri, Regulation of peroxisome proliferator-activated receptor γ activity by mitogen-activated protein kinase, J. Biol. Chem. 272 (1997) 10811–10816.
[6] I. Iankova, Peroxisome proliferator-activated receptor γ recruits the positive transcription elongation factor b complex to activate transcription and promote adipogenesis, Mol. Endocrinol. 20 (2006) 1494–1505.
[7] E. Compe, Dysregulation of the peroxisome proliferator-activated receptor target genes by XPD mutations, Mol. Cell Biol. 25 (2005) 6065–6076.
[8] T. Hai, M.G. Hartman, The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis, Gene 273 (2001) 1–11.
[9] T. Hai, C.C. Wolford, Y.S. Chang, ATF3, a hub of the cellular adaptive-response network, in the pathogenesis of diseases: is modulation of inflammation a unifying component, Gene Expr. 15 (2010) 1–11.
[10] H.B. Kim, M. Kong, T.M. Kim, Y.H. Suh, W.H. Kim, J.H. Lim, J.H. Song, M.H. Jung, NFATc4 and ATF3 negatively regulate adiponectin gene expression in 3T3-L1 adipocytes, Diabetes 55 (2006) 1342–1352.
[11] H.J. Park, Y.M. Kang, C.H. Kim, M.H. Jung, ATF3 negatively regulates adiponectin receptor 1 expression, Biochem. Biophys. Res. Commun. 400 (2010) 72–77.
[12] I.U. Koh, J.H. Lim, M.K. Joe, W.H. Kim, M.H. Jung, J.B. Yoon, J. Song, AdipoR2 is transcriptionally regulated by ER stress-inducible ATF3 in HepG2 human hepatocyte cells, FEBS J. 277 (2010) 2304–2317.
[13] M.K. Jang, H.J. Park, M.H. Jung, ATF3 represses PDX-1 expression in pancreatic β-cells, Biochem. Biophys. Res. Commun. 412 (2011) 385–390.
[14] M.K. Jang, M.H. Jung, ATF3 represses PPARγ expression and inhibits adipocyte differentiation, Biochem. Biophys. Res. Commun. 454 (2014) 58–64.
[15] M.K. Jang, C.H. Kim, J.K. Seong, M.H. Jung, ATF3 inhibits adipocyte differentiation of 3T3-L1 cells, Biochem. Biophys. Res. Commun. 421 (2012) 38–43.
[16] Y. Kang, C. Chen, J. Massague, A self-enabling TGFβ response coupled to stress signaling: Smad engages stress response factor ATF3 for Id1 repression in epithelial Cells, Mol. Cell 11 (2003) 915–926.
[17] H. Wang, P. Mo, S. Ren, C. Yan, Activating transcription factor 3 activates p53 by preventing E6-associated protein from binding to E6, J. Biol. Chem. 285 (2010) 13201–13210.
[18] W. Liu, M. Iiizumi-Gairani, H. OkudaH, A. Kobayashi, M. Watabe, S.K. Pai, P.R. Pandey, F. Xing, K. Fukuda, V. Modur, S. Hirota, K. Suzuki, T. Chiba, M. Endo, T. Sugai, K. Watabe, KAI1 gene is engaged in NDRG1 gene-mediated metastasis suppression through the ATF3–NFB complex in human prostate cancer, J. Biol. Chem. 286 (2011) 18949–18959.
[19] W.H. Kim, M.K. Jang, C.H. Kim, H.K. Shin, M.H. Jung, ATF3 inhibits PDX-1-stimulated transactivation, Biochem. Biophys. Res. Commun. 414 (2011) 681–687.
[20] L. Gelman, G. Zhou, L.E. Raspé, J.C. Fruchart, J. Auwerx, P300 interacts with the N- and C-terminal part of PPARgamma2 in a ligand-independent and -dependent manner, respectively, J. Biol. Chem. 274 (1999) 7681–7688.
[21] L. Fajas, V. Egler, R. Reiter, J. Hansen, K. Kristiansen, M.B. Debril, S. Miard, J. Auwerx, The retinoblastoma-histone deacetylase 3 complex inhibits PPARgamma and adipocyte differentiation, Dev. Cell 3 (2002) 903–910.
[22] S. Shrivastav, T. Kino, T. Cunningham, T. Ichijo, U. Schubert, P. Heinklein, G.P. Chrousos, J.B. Kopp, Human immunodeficiency virus (HIV)-1 viral protein R suppresses transcriptional activity of peroxisome proliferator-activated receptor gamma and inhibits adipocyte differentiation: implications for HIV-associated lipodystrophy, Mol. Endocrinol. 22 (2008) 234–247.
[23] V. Aguilar, J.S. Annicotte, X. Escote, J. Vendrell, D. Langin, L. Fajas, Cyclin G2 regulates adipogenesis through PPAR gamma coactivation, Endocrinology 151 (2010) 5247–5254.
[24] H.E. Kim, E. Bae, D.Y. Jeong, M.J. Kim, W.J. Jin, S.W. Park, G.S. Han, G.M. Carman, E. Koh, K.S. Kim, Lipin1 regulates PPARγ transcriptional activity, Biochem. J. 453 (2013) 49–60.
[25] U. Ozcan, Q. Cao, E. Yilmaz, A.H. Lee, N.N. Iwakoshi, E. Ozdelen, G. Tuncman, C. Görgün, L.H. Glimcher, G.S. Hotamisligil, Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes, Science 306 (2004) 457–461.
[26] R.J. Kaufman, S.H. Back, B. Song, J. Han, J. Hassler, The unfolded protein response is required to maintain the integrity of the endoplasmic reticulum, prevent oxidative stress and preserve differentiation in β-cells, Diabetes Obes. Metab. 12 (2010) 99–107.