Biochemical and Biophysical Research Communications 399 (2010) 55–59

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Transcriptional activation of peroxisome proliferator-activated receptor-γ requires activation of both protein kinase A and Akt during adipocyte differentiation

Sang-pil Kim [a,1], Jung Min Ha [b,1], Sung Ji Yun [b], Eun Kyoung Kim [b], Sung Woon Chung [a], Ki Whan Hong [b], Chi Dae Kim [b], Sun Sik Bae [b,*]

[a] Department of Thoracic & Cardiovascular Surgery, Pusan National University School of Medicine, Republic of Korea
[b] MRC for Ischemic Tissue Regeneration, Medical Research Institute, and Department of Pharmacology, Pusan National University School of Medicine, Republic of Korea

ARTICLE INFO

Article history:
Received 8 July 2010
Available online 16 July 2010

Keywords:
Akt
Adipocyte differentiation
PPAR-γ
cAMP

ABSTRACT

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is required for the conversion of pre-adipocytes. However, the mechanism underlying activation of PPAR-γ is unclear. Here we showed that cAMP-induced activation of protein kinase A (PKA) and Akt is essential for the transcriptional activation of PPAR-γ. Hormonal induction of adipogenesis was blocked by a phosphatidylinositol 3-kinase (PI3K) inhibitor (LY294002), by a protein kinase A (PKA) inhibitor (H89), and by a Rap1 inhibitor (GGTI-298). Transcriptional activity of PPAR-γ was markedly enhanced by 3-isobutyl-1-methylxanthine (IBMX), but not insulin and dexamethasone. In addition, IBMX-induced PPAR-γ transcriptional activity was blocked by PI3K/Akt, PKA, or Rap1 inhibitors. 8-(4-Chlorophenylthio)-2′-O-methyl-cAMP (8-pCPT-2′-O-Me-cAMP) which is a specific agonist for exchanger protein directly activated by cAMP (Epac) significantly induced the activation of Akt. Furthermore, knock-down of Akt1 markedly attenuated PPAR-γ transcriptional activity. These results indicate that both PKA and Akt signaling pathways are required for transcriptional activation of PPAR-γ, suggesting post-translational activation of PPAR-γ might be critical step for adipogenic gene expression.

1. Introduction

Stimulation of 3T3-L1 pre-adipocytes with various hormones evokes sequential events such as mitotic clonal expansion, cell cycle arrest, and terminal differentiation into adipocytes. Induction of adipocyte differentiation is generally achieved by incubation with 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin, collectively called a differentiation cocktail (DMI). During adipocyte differentiation, two transcriptional factors, CCAAT/enhancer-binding protein-β (C/EBP-β) and C/EBP-δ, are rapidly induced, leading to expression of the key adipogenic transcription factors such as C/EBP-α and peroxisome proliferator-activated receptor-γ (PPAR-γ) [1].

PPAR-γ has been implicated in modulating adipocyte differentiation and is believed to be a master regulator of adipocyte differentiation. Although hormonal induction of PPAR-γ gene expression is critical step for PPAR-γ-dependent adipocyte differentiation, several lines of evidence show that transcriptional activity of PPAR-γ is also regulated by synthetic agonists or by post-translational modification of PPAR-γ itself. For example, transcriptional activity of PPAR-γ is markedly enhanced by thiazolidinedione compounds such as troglitazone and rosiglitazone [2]. In addition, it has been reported that PPAR-γ is phosphorylated and inactivated by both mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling pathways [3–5]. However, the exact molecular mechanism underlying PPAR-γ activation during adipocyte differentiation is still ambiguous.

In general, stimulation of pre-adipocytes with a cocktail of differentiation inducers such as insulin, IBMX, and dexamethasone results in the initiation of the differentiation program. It has been reported that phosphatidylinositol 3-kinase (PI3K) and Akt/protein kinase B (PKB) signaling pathways play crucial roles in adipocyte differentiation. For instance, addition of PI3K inhibitors completely blocks the differentiation process [6]. Expression of a constitutively active version of Akt1 causes spontaneous adipocyte differentiation of 3T3-L1 cells [7]. In addition, cells lacking Akt1 displayed defects in adipocyte differentiation even after forced expression of

Abbreviations: PPAR-γ, peroxisome proliferator-activated receptor-γ; PKA, protein kinase A; PI3K, phosphatidylinositol 3-kinase; IBMX, 3-isobutyl-1-methylxanthine; C/EBP-β, CCAAT/enhancer-binding protein-β; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; cAMP, cyclic adenosine monophosphate; Epac, exchanger protein directly activated by cAMP; PPRE, PPAR-γ responsive element; 6-MB-cAMP, N^6-monobutyryl-cAMP; 8-pCPT-2′-O-Me-cAMP, 8-(4-chlorophenylthio)-2′-O-methyl-cAMP.

* Corresponding author. Address: Department of Pharmacology, Pusan National University School of Medicine, Bumeo-ri, Mulgeum-eup, Yangsan si, Kyungnam 626-770, Republic of Korea. Fax: +82 51 5108068.
E-mail address: sunsik@pusan.ac.kr (S.S. Bae).

[1] These two authors contributed equally.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.07.038

PPAR-γ [8]. IBMX, one of the differentiation inducers in the cocktail, is an inhibitor of phosphodiesterase (PDE) that decreases intracellular cyclic AMP (cAMP) levels [1]. It has been reported that cAMP plays an important role in adipocyte differentiation through exchange protein directly activated by cAMP (Epac) and protein kinase A (PKA) [9]. Although both PI3K/Akt and cAMP signaling pathways are necessary for the stimulation of the adipocyte differentiation program, the significance of these pathways for the activation of PPAR-γ transcriptional activity is still unclear. In the present study, we provide evidence that transcriptional activity of PPAR-γ is modulated by both cAMP and Akt signaling pathways.

2. Materials and methods

2.1. Materials

All culture media were purchased from Hyclone Inc. Rabbit polyclonal antibodies against Akt1 and pan Akt were obtained from Upstate Biotechnology Inc. Anti-PPAR-γ antibody was purchased from Santa Cruz Biotech. Inc. The luciferase reporter gene assay system for the PPAR-γ promoter and the PPAR-γ responsive element (PPRE) were a generous gift from JB Seo of Seoul National University, Korea. LY294002 (a PI3K inhibitor), SH-5 (an Akt inhibitor) and H89 (a PKA inhibitor) were obtained from Calbiochem. GGTI-298 (a Rap1 inhibitor) was purchased from Sigma–Aldrich Inc. Selective agonists for PKA (N^6-monobutyryl-cAMP, 6-MB-cAMP) or Epac (8-(4-chlorophenylthio)-2′-O-methyl-cAMP, 8-pCPT-2′-O-Me-cAMP) were purchased from Biolog Life Science Inst. IRDye700- or IRDye800-conjugated rabbit or mouse secondary antibodies were obtained from Li-COR Bioscience.

2.2. Promoter assay

3T3-L1 cells in 12-well plates were co-transfected with a firefly luciferase gene tagged with pGL3-PPRE and renilla luciferase using Lipofectamine 2000 (Invitrogen). Medium was replaced with fresh medium after 10 h. Twenty-four hours post-transfection, cells were stimulated with either differentiation cocktail or IBMX alone in the presence or absence of various inhibitors. Luciferase activity was assayed 12 h later using a dual-luciferase reporter assay system (Promega).

2.3. Cell culture and adipocyte differentiation

Cells (3T3-L1 pre-adipocytes) were maintained in Dulbecco′s modified Eagle medium (DMEM) containing 10% bovine calf serum and 1% penicillin–streptomycin at 37 °C in a humidified 5% CO_2 incubator. To initiate differentiation of 3T3-L1 cells into adipocytes, cells were grown until confluence for 2 days. The media was changed to differentiation cocktail containing DMEM, 10% fetal bovine serum (FBS), 2 μg/ml insulin, 0.4 μg/ml dexamethasone, and 0.5 mM IBMX. After 2 days, cells were fed fresh differentiation cocktail for an additional 2 days. Cells were replaced with normal culture medium and medium was changed every 2 days for 4 days.

2.4. Oil Red O staining

To stain triacylglycerol in lipid droplets, differentiated cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 1 h. Fixed cells were washed with PBS three times followed by incubation with Oil Red O solution (0.3%) for 2 h. Cells were washed three times with water. Pictures were taken using a bright field microscope (20× magnification). For quantitation of lipid accumulation, deposited Oil Red O was extracted with isopropyl alcohol and optical density at 510 nm was measured.

2.5. Constructs

To silence Akt1, oligonucleotides tagged with 5′-end *AgeI* site and 3′-end *EcoRI* site were designed (5′-*CCG GTA ACT TCT CAG TGG CAC AAT GCC TCG AGG CAT TGT GCC ACT GAG AAG TTT TTT TG*-3′), and both sense and anti-sense oligonucleotides were synthesized. Both complementary oligonucleotides were mixed and heated at 98 °C for 5 min and cooled to room temperature. Annealed nucleotides were subcloned into the AgeI/EcoRI site of a pLKO.1 lentiviral vector.

2.6. Lentiviral knock-down

For gene silencing, HEK293-FT packaging cells were grown to ~70% confluence in six-well plates. Cells were triple transfected with 5 μg of pLKO.1 lentiviral construct, 1 μg of Δ8.9, and 1 μg of pVSV-G using a calcium phosphate method. Medium was replaced with fresh medium 8 h post-transfection. Lentiviral supernatants were harvested 24 h post-transfection and passed through 0.45 μm filters. Cell-free viral culture supernatants were used to infect 3T3-L1 pre-adipocytes in the presence of 8 μg/ml of polybrene. An additional round of infection was done at 48 h and 72 h post-transfection. Infected cells were isolated by selection with 10 μg/ml puromycin for 2 days.

2.7. Western blotting

Western blotting was done essentially as described in a previous report [8].

2.8. Statistical analysis

Results are expressed as the mean ± SD. of two independent experiments ($n = 3$ for each experiment). When comparing two groups, an unpaired Student's *t*-test was used to assess differences. *P*-values less than 0.05 were considered significant.

3. Results and discussion

3.1. Adipocyte differentiation requires multiple signaling pathways

It has been reported that simultaneous addition of dexamethasone (D), IBMX (M), and insulin (I) as a DMI cocktail promotes the differentiation program [1]. Missing one of these components resulted in the abrogation of adipocyte differentiation [10]. Likewise, our results showed that missing one of the components in the differentiation cocktail did not induce the differentiation of 3T3-L1 pre-adipocytes (Fig. 1A). These results indicate that hormonal activation and generation of cAMP are required for full differentiation. Since there is evidence that activation of PI3K/Akt and cAMP-dependent activation of PKA and Epac/Rap1 signaling pathways are important for adipocyte differentiation [6–9], we next examined the effect of specific inhibitors of signaling pathways on adipocyte differentiation. As shown in Fig. 1B, induction of adipocyte differentiation by the DMI cocktail was significantly blocked by an inhibitor for one of the signaling molecules such as PI3K (LY294002), PKA (H89), or Rap1 (GGTI-298). These results indicate simultaneous necessity of multiple signaling pathways for the induction of adipocyte differentiation.

3.2. Transcriptional activity of PPAR-γ is directly activated by cAMP signaling pathways

Transcriptional activation of PPAR-γ is a critical step for the induction of adipocyte differentiation [11]. In particular, sequential

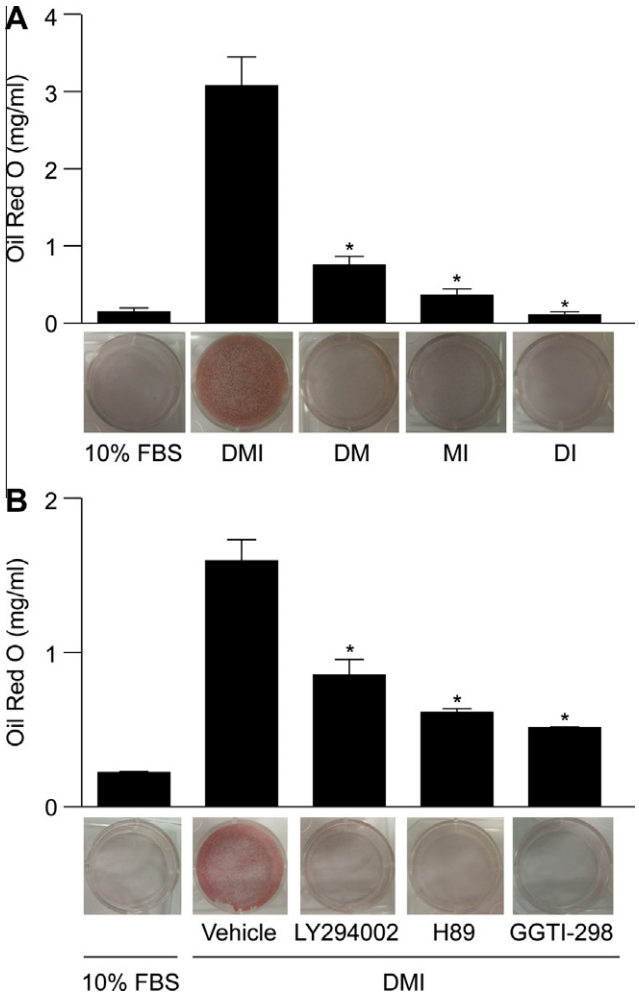

Fig. 1. Multiple signaling pathways are required for adipocyte differentiation. (A) Adipocyte differentiation of 3T3-L1 cells was induced by differentiation cocktail (DMI), DM (missing insulin), MI (missing dexamethasone), or DI (missing IBMX). Differentiation was assessed by staining neutral lipids with Oil Red O solution. Images were captured with a digital camera, and the degree of differentiation was quantified by measuring absorbance of isopropyl alcohol extracts at 510 nm. Data are the means ± SD. of three independent experiments ($n = 3$ for each experiment). *Significantly different from values of DMI ($p < 0.05$). (B) Experiments were done essentially as in (A) in the presence of various inhibitors such as LY294002 (10 μM, PI3K inhibitor), H89 (10 μM, PKA inhibitor), or GGTI-298 (10 μM, Rap1 inhibitor). Data are the means ± SD. of three independent experiments ($n = 3$ for each experiment). *Significantly different from values of vehicle treated cells ($p < 0.05$).

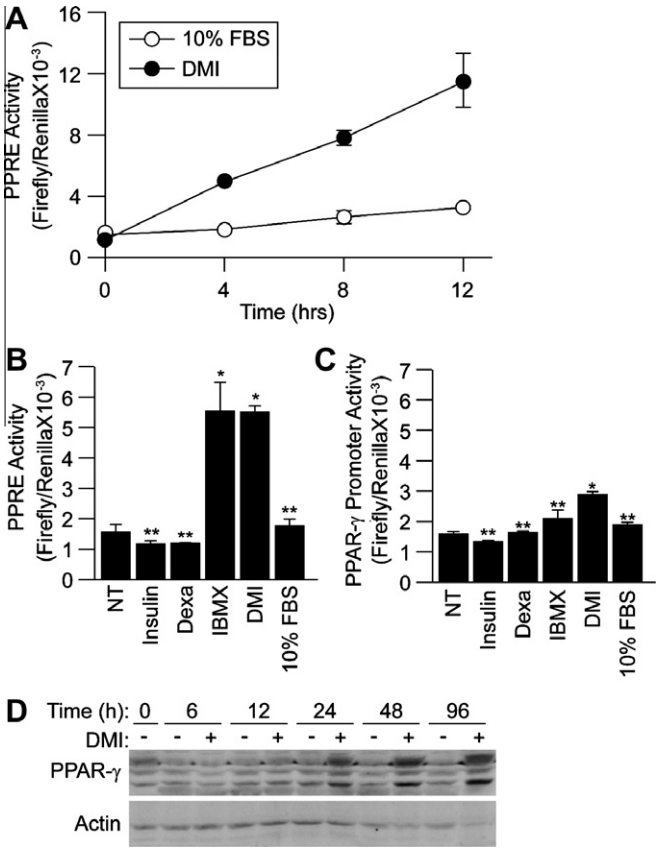

Fig. 2. cAMP stimulates transcriptional activation of PPAR-γ in the absence of PPAR-γ expression. (A) 3T3-L1 cells were co-transfected with PPRE tagged with a firefly luciferase reporter gene and with a plasmid expressing renilla luciferase as an internal control. One day later, cells were incubated with normal culture medium or differentiation cocktail for the indicated times. PPRE activity was expressed as a ratio of firefly/renilla luciferase activity. Cells were either co-transfected with either pGL3-PPRE (B) or pGL3-PPAR-γ promoter (C), and promoter activity was measured in the presence of each component of the differentiation cocktail. Data are the means ± SD. of three independent experiments ($n = 3$ for each experiment). *Significantly different from values of NT ($p < 0.05$). **Significantly indifferent from values of NT ($p > 0.05$), (D) Expression of PPAR-γ during adipocyte differentiation was detected by Western blot analysis.

expression of C/EBPα, C/EBPβ, and PPAR-γ has been implicated in transcriptional regulation during adipocyte differentiation [1]. However, the issue on the level of PPAR-γ and the induction of adipocyte differentiation has been challenged by post-translational control of PPAR-γ transcriptional activity. For example, PPAR-γ is selectively activated by thiazolidinedione compounds [12]. Furthermore, transcriptional activity of PPAR-γ is markedly reduced by MAPK- and JNK-dependent phosphorylation [3,5]. These results indicate that transcriptional activity of PPAR-γ might be modulated by signaling-mediated modification. In line with this, stimulation of pre-adipocytes with differentiation inducers significantly induced transcriptional activity of PPAR-γ within 12 h (Fig. 2A). Therefore, it is reasonable to conclude that one of the components in the differentiation cocktail mediates transcriptional activation of PPAR-γ. Indeed, treatment of cells with IBMX, which elevates intracellular cAMP *via* inhibition of PDE, significantly enhanced PPAR-γ transcriptional activity, while neither insulin nor dexamethasone had any effect on PPAR-γ transcriptional activity (Fig. 2B). Therefore, cAMP signaling pathway might be a pathway responsible for the transcriptional activation of PPAR-γ. In addition, it has been reported that cAMP stimulates adipocyte differentiation *via* dual activation of PKA and Epac signaling pathways [9]. Regarding the mechanism of enhanced PPAR-γ responsive element (PPRE) activity, there are two possible mechanisms such as direct activation of PPAR-γ and induction of PPAR-γ expression itself. The latter seems not to be the possible mechanism since PPAR-γ promoter activity was not affected either by DMI or IBMX (Fig. 2C). In addition, expression of PPAR-γ was initiated after 24 h of induction with DMI (Fig. 2D). It is noteworthy that PPRE activity was initiated within 12 h of DMI stimulation (Fig. 2A). Therefore, cAMP signaling pathways might modulate PPAR-γ transcriptional activity rather than expression of PPAR-γ itself.

3.3. Activation of PPAR-γ by cAMP requires both PKA and Akt signaling pathways

Since transcriptional activation of PPAR-γ was achieved by cAMP generation, we next examined downstream signaling pathways responsible for cAMP-mediated activation of PPAR-γ. As shown in Fig. 3A, transcriptional activation of PPAR-γ by the DMI cocktail was completely blocked by inhibitors of PI3K (LY294002), Akt (SH-5), Rap1 (GGTI-298), or PKA (H89). Since IBMX was major component in the DMI cocktail that activates

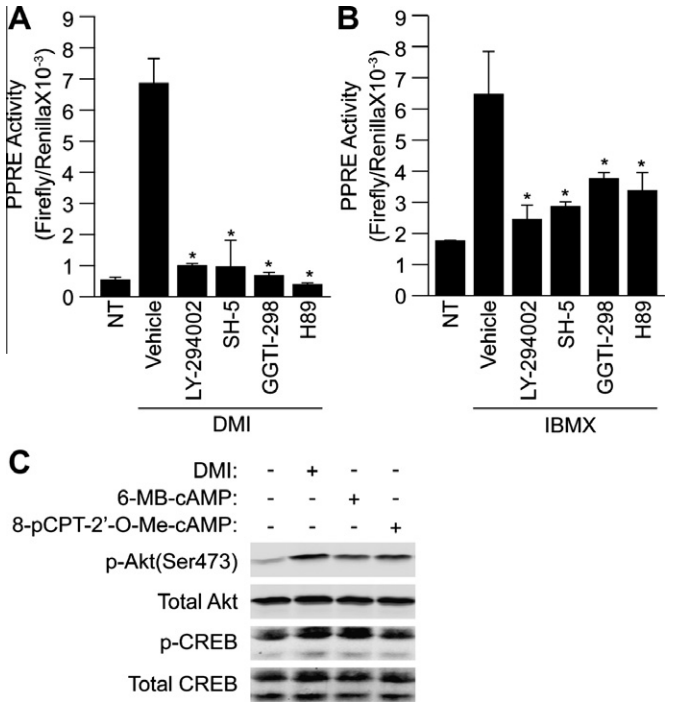

Fig. 3. cAMP-dependent activation of PPAR-γ transcriptional activity requires PKA, Rap1, PI3K, and Akt signaling. DMI-induced (A) or IBMX (0.5 mM)-induced (B) PPRE activity was measured in the presence or absence of LY294002 (10 μM, PI3K inhibitor), SH-5 (10 μM, Akt inhibitor), GGTI-298 (10 μM, Rap1 inhibitor), and H89 (10 μM, PKA inhibitor). Data are the means ± SD. of three independent experiments ($n = 3$ for each experiment). *Significantly different from values of vehicle treated cells ($p < 0.05$). (**C**) 3T3-L1 cells were stimulated with DMI, PKA-specific agonist (100 μM, 6-MB-cAMP), or Epac-specific agonist (100 μM, 8-pCPT-2′-O-Me-cAMP) for 10 min. Activation of Akt and CREB was verified by Western blotting with phospho-specific antibodies.

PPAR-γ transcriptional activity (Fig. 2B), IBMX-dependent activation of PPAR-γ should be suppressed by PI3K, Akt, Rap1, or PKA inhibitors. Indeed, IBMX-induced activation of PPAR-γ was blocked by PI3K, Akt, Rap1, or PKA inhibitors (Fig. 3B). Therefore, it is possible that elevation of intracellular cAMP leads to the activation of PI3K/Akt, PKA, and Rap1 signaling. In general, cAMP activates not only PKA but also Epac which is a guanine nucleotide exchange factor for Rap1 small G protein. Likewise, Epac-specific cAMP analog (8-pCPT-2′-O-Me-cAMP) stimulated Akt activation (Fig. 3C). Recently, it has been demonstrated that Epac-dependent activation of Akt is mediated by Rap1. For example, activated Epac forms molecular complex of Rap1/PI3K/Akt at specific subcellular compartment [13,14]. In these regards, cAMP could activate both PKA and Akt signaling pathways. It is notable that both signaling pathways are required for the induction of PPAR-γ activity since blocking one of these pathways blunted PPRE activities (Fig. 3B).

3.4. Silencing of Akt1 abrogates cAMP-induced transcriptional activation of PPAR-γ

It has been reported that PI3K/Akt signaling pathways play crucial roles in adipocyte differentiation. For instance, over-expression of the constitutively active form of Akt1 evoked spontaneous differentiation of 3T3-L1 cells [7]. In addition, silencing of Akt1 abrogated 3T3-L1 differentiation [10]. The requirement for Akt1 was further confirmed by experiments showing that mouse embryo fibroblast cells derived from mice lacking Akt1 displayed defects in adipocyte differentiation [8]. It is notable that over-expression of PPAR-γ did not enhance adipocyte differentiation in the absence of Akt1 indicating that Akt is necessary for the induction of PPAR-γ transcriptional activity. In correlation with this idea, DMI- or IBMX-dependent transcriptional activation of PPAR-γ was abolished by silencing of Akt1 (Fig. 4). Therefore, it is evident that Akt1 is necessary for both transcriptional activation of PPAR-γ and adipocyte differentiation.

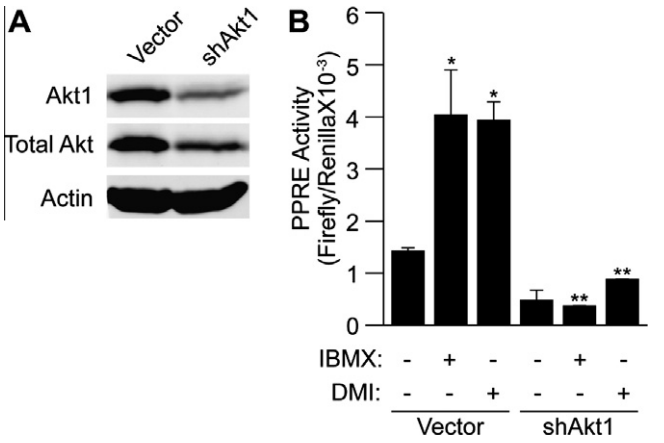

Fig. 4. DMI- and IBMX-induced transcriptional activation of PPAR-γ requires Akt1 (A) 3T3-L1 cells were infected with lentivirus harboring shRNA for Akt1. The expression level of Akt1 was verified by western blotting with specific antibodies against Akt1 or pan Akt. Expression of actin is shown as internal control. (B) DMI- or IBMX-dependent PPRE activation was measured after silencing of Akt1. Data are the means ± SD. of three independent experiments ($n = 3$ for each experiment). *Significantly different from values of vehicle treated cells ($p < 0.05$).

The downstream mechanism whereby Akt1 leads to the transcriptional activation of PPAR-γ is still not established. For the activation of PPRE, heterodimer formation of PPAR-γ with retinoid X receptor (RXR) is required [15]. In addition, recruiting co-activators such as the p160/CBP/p300/pCAF complex are involved in the modulation of PPRE activation [16]. A link in between Akt and PPRE activation has been established by the fact that Akt phosphorylates CBP/p300 and enhances its acetyl transferase activity [17]. These results implicate that acetylation/deacetylation processes may be involved in the activation of PPAR-γ. In line with this, expression of histone deacetylase 4 (HDAC4) significantly inhibited both adipocyte differentiation and the activation of PPAR-γ transcriptional activity (Supplementary Fig. 1). In this regard, PKA- and Akt-dependent signaling pathways are simultaneously required for PPAR-γ-dependent gene expression and adipocyte differentiation.

In conclusion, transcriptional activation of PPAR-γ requires activation of both PKA and Akt. Although cAMP signaling pathways are enough for the induction of PPAR-γ transcriptional activity, additional insulin and dexamethasone signaling pathways are required for adipocyte differentiation. Delineation of co-activator components that are acetylated in an Akt-dependent manner will provide the exact molecular mechanism for PPAR-γ-dependent gene expression and adipocyte differentiation.

Acknowledgment

This study was supported by Medical Research Institute Grant (2009-14) of Pusan National University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.07.038.

References

[1] S.R. Farmer, Transcriptional control of adipocyte formation, Cell Metab. 4 (2006) 263–273.

[2] H.S. Camp, O. Li, S.C. Wise, Y.H. Hong, C.L. Frankowski, X. Shen, R. Vanbogelen, T. Leff, Differential activation of peroxisome proliferator-activated receptor-γ by troglitazone and rosiglitazone, Diabetes 49 (2000) 539–547.
[3] H.S. Camp, S.R. Tafuri, T. Leff, C-Jun N-terminal kinase phosphorylates peroxisome proliferator-activated receptor-γ1 and negatively regulates its transcriptional activity, Endocrinology 140 (1999) 392–397.
[4] C. Diradourian, J. Girard, J.P. Pegorier, Phosphorylation of PPARs: from molecular characterization to physiological relevance, Biochimie 87 (2005) 33–38.
[5] 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.
[6] C.T. Christoffersen, H. Tornqvist, C.J. Vlahos, D. Bucchini, J. Jami, P. De Meyts, R.L. Joshi, Insulin and insulin-like growth factor-I receptor mediated differentiation of 3T3-F442A cells into adipocytes: effect of PI 3-kinase inhibition, Biochem. Biophys. Res. Commun. 246 (1998) 426–430.
[7] R. Magun, B.M. Burgering, P.J. Coffer, D. Pardasani, Y. Lin, J. Chabot, A. Sorisky, Expression of a constitutively activated form of protein kinase B (c-Akt) in 3T3-L1 preadipose cells causes spontaneous differentiation, Endocrinology 137 (1996) 3590–3593.
[8] S.J. Yun, E.K. Kim, D.F. Tucker, C.D. Kim, M.J. Birnbaum, S.S. Bae, Isoform-specific regulation of adipocyte differentiation by Akt1/protein kinase B, Biochem. Biophys. Res. Commun. 371 (2008) 138–143.
[9] R.K. Petersen, L. Madsen, L.M. Pedersen, P. Hallenborg, H. Hagland, K. Viste, S.O. Doskeland, K. Kristiansen, Cyclic AMP (cAMP)-mediated stimulation of adipocyte differentiation requires the synergistic action of Epac- and cAMP-dependent protein kinase-dependent processes, Mol. Cell. Biol. 28 (2008) 3804–3816.
[10] J. Xu, K. Liao, Protein kinase B/AKT 1 plays a pivotal role in insulin-like growth factor-1 receptor signaling induced 3T3-L1 adipocyte differentiation, J. Biol. Chem. 279 (2004) 35914–35922.
[11] E.D. Rosen, P. Sarraf, A.E. Troy, G. Bradwin, K. Moore, D.S. Milstone, B.M. Spiegelman, R.M. Mortensen, PPAR γ is required for the differentiation of adipose tissue *in vivo* and *in vitro*, Mol. Cell 4 (1999) 611–617.
[12] J.M. Lehmann, L.B. Moore, T.A. Smith-Oliver, W.O. Wilkison, T.M. Willson, S.A. Kliewer, An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPAR γ), J. Biol. Chem. 270 (1995) 12953–12956.
[13] F.C. Mei, J. Qiao, O.M. Tsygankova, J.L. Meinkoth, L.A. Quilliam, X. Cheng, Differential signaling of cyclic AMP: opposing effects of exchange protein directly activated by cyclic AMP and cAMP-dependent protein kinase on protein kinase B activation, J. Biol. Chem. 277 (2002) 11497–11504.
[14] U.K. Misra, S.V. Pizzo, Coordinate regulation of forskolin-induced cellular proliferation in macrophages by protein kinase A/cAMP-response element-binding protein (CREB) and Epac1–Rap1 signaling: effects of silencing CREB gene expression on Akt activation, J. Biol. Chem. 280 (2005) 38276–38289.
[15] I.G. Schulman, G. Shao, R.A. Heyman, Transactivation by retinoid X receptor-peroxisome proliferator-activated receptor γ (PPARγ) heterodimers: intermolecular synergy requires only the PPARγ hormone-dependent activation function, Mol. Cell. Biol. 18 (1998) 3483–3494.
[16] Y. Kamei, L. Xu, T. Heinzel, J. Torchia, R. Kurokawa, B. Gloss, S.C. Lin, R.A. Heyman, D.W. Rose, C.K. Glass, M.G. Rosenfeld, A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors, Cell 85 (1996) 403–414.
[17] W.C. Huang, C.C. Chen, Akt phosphorylation of p300 at Ser-1834 is essential for its histone acetyltransferase and transcriptional activity, Mol. Cell. Biol. 25 (2005) 6592–6602.