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cAMP-response element binding protein (CREB) positively regulates mouse adiponectin gene expression in 3T3-L1 adipocytes

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ABSTRACT

Adiponectin is expressed in adipose tissue by adipogenic transcription factors including PPARγ, C/EBPα, and ADD1/SREBP1c. Because cAMP-response element binding protein (CREB) is also a central transcriptional activator of adipocyte differentiation, we evaluated CREB to determine if it stimulates adiponectin gene expression. To accomplish this, we evaluated the effects of activated CREB on the promoter activity of the mouse adiponectin gene, and identified the cAMP-response element (CRE) in the promoter. The constitutively active form of CREB increased the promoter activity of the mouse adiponectin gene. In addition, transfection studies using 5′ serial deleted promoters revealed the presence of a putative CRE located between the −1250 and −1000 bp region. Furthermore, an electrophoresis mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) analysis demonstrated that CREB bound to the region between −1022 and −995 in the adiponectin promoter. Insulin-like growth factor (IGF-1), which activate CREB, increased the adiponectin promoter activity. However, this stimulation was prevented by the dominant negative form of CREB (ACREB) and pretreatment with PD098059, indicating that IGF-1 stimulate adiponectin expression through CREB phosphorylation via the ERK pathway. Importantly, the transactivation of adiponectin expression by CREB was inhibited by ATF3. Coimmunoprecipitation and GST pull-down assay revealed that ATF3 bound to CREB and prevented CREB phosphorylation induced during differentiation of 3T3-L1 adipocytes. Collectively, these findings demonstrate that CREB is a positive regulator of mouse adiponectin gene expression in adipocytes, which play an important role in the regulation of adiponectin expression in response to growth factor.

Introduction

Adipose tissue is considered to be an endocrine organ that secretes central and peripheral metabolic signaling molecules known as adipokines. Adipokines include tumor necrosis factor α (TNF-α), resistin, interleukine-6 (IL-6), IL-8, acylation-stimulating protein (ASP), angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), leptin, and adiponectin [1,2]. These proteins play important regulatory roles in a variety of complex processes, such as fat metabolism, feeding behavior, homeostasis, vascular tone, energy balance, and insulin sensitivity. Among these proteins, adiponectin is the most abundant circulating adipokine with a serum concentration 1000 times greater than other hormones and 10 times greater than that of other inflammatory cytokines [1]. Adiponectin exerts biological effects on antidiabetic, antiatherogenic, antiinflammatory, antiangiogenic and antitumor functions [1]. Therefore, overexpression of adiponectin improves insulin sensitivity [3]. The plasma levels of adiponectin correlate closely with systemic insulin sensitivity, and decreased adiponectin concentrations are often observed in individuals with insulin resistance and type 2 diabetes [4,5].

Adiponectin is expressed during adipocyte differentiation and secreted only by differentiated adipocytes [6]. Therefore, the most important regulators of adiponectin expression are transcription factors involved in adipogenesis, such as peroxisome proliferator activated receptor γ (PPARγ) [7], CCAAT-enhancer binding protein α (C/EBPα) [8,9], and adipocyte determination-differentiation factor 1c (ADD1/SREBP1c) [10]. A promoter analysis study revealed that PPARγ, C/EBPα, and ADD1/SREBP1c stimulate adiponectin expression in adipocytes. In contrast, three transcription factors, nuclear factor of activated T cells (NFATc4), activating transcription factor 3 (ATF3) and activator protein 2 (AP-2), have been shown to act as negative regulators of adiponectin transcription and are likely related to obesity-induced adiponectin suppression [11,12]. Furthermore, adiponectin expression is controlled under

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conditions of metabolic stress [13], as well as by a number of hormones [14] and factors involved in the regulation of metabolic and immune function [15,16].

cAMP-response element binding protein (CREB) belongs to the bZIP superfamily of transcription factors [17]. Like all bZIP transcription factors, CREB family members contain a C-terminal basic domain that mediates DNA binding, as well as a leucine zipper domain that facilitates dimerization. CREB can form both homo- and heterodimers with related factors, and each can bind to the same cis-the regulatory element, the cAMP-response element (CRE), which consists of the palindromic consensus sequence TGACGTCA [17]. The key steps involved in CRE-mediated gene expression include dimerization of CREB, binding to the CRE element, and phosphorylation. In turn, phosphorylation of CREB recruits associated proteins such as CREB-binding protein (CBP) and RNA polymerase, which results in the formation of a large transcription complex [17]. Recently, CREB has been shown to play a crucial role in adipocyte differentiation such as PPARγ2, CEBPα, and ADD1/SREBP1c [18,19]. CREB binds to putative CREs in several adipocyte-specific gene promoters and regulates the transcription of adipocyte-specific genes for C/EBPβ, phosphoenolpyruvate carboxykinase (PEPCK), fatty acid binding protein (FABP [aP2/422]), and fatty acid synthetase (FAS).

Because CREB is an adipogenic transcription factor, we conducted this study to determine if CREB positively regulates adiponectin in adipocyte. The results of this study showed that CREB stimulates the promoter activity of the mouse adiponectin gene through binding to the promoter, which plays an important role in IGF-1-stimulated adiponectin expression.

Materials and methods

Cell culture and treatments. The 3T3-L1 pre-adipocytes (ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium with high glucose (Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal calf serum (GibcoBRL, Gaithersburg, MD, USA). 3T3-L1 pre-adipocytes were differentiated as described previously [10]. To investigate the effects of IGF-1 on the adiponectin promoter, 3T3-L1 adipocytes transfected with promoter reporters were treated with 5–20 nM IGF-1 (Calbiochem, San Diego, CA, USA) for 24 h.

Plasmid constructs. The mouse adiponectin promoter region spanning −3000 to +50 bp was amplified by PCR using mouse genomic DNA and then inserted into the KpnI/BglII restriction sites of the pGL3 basic vector (Promega, Madison, WI, USA). Deletions in the 5′ flanking regions of the adiponectin promoter were constructed by PCR using a pairwise combination of the following sense primers: 5′-AGT TGG CTG TTA GCC CAG A-3′ [for P(−1000)/Luc], 5′-AGT TGG AGG AAG CAG AT-3′ [for P(−1250)/Luc], 5′-ATAC AGT TTG CTT GGG A-3′ [for P(−1500)/Luc], 5′-ATA GTT AGA ATC TGC TGA-3′ [for P(−2000)/Luc], and 5′-AGC CAG GGC TAC ACA GA-3′ [for P(−3000)/Luc], with the antisense primer, 5′-AGA TCT CTT TTG GTG TCG TCA GAT CC-3′. All primers were designed to contain the KpnI or BglII restriction enzyme recognition sites at the 5′ ends. A deletion-mutant plasmid lacking the putative CRE was constructed in the pGL3-adiponectin luciferase plasmid using the two-step PCR method. Expression vectors for the constitutively active CREB (VP-16 CREB) and the dominant negative CREB (ACREB) were provided by Richard Goodman of Oregon Health Sciences University (Portland, OR, USA).

Transient transfection and luciferase activity. The fully differentiated 3T3-L1 adipocytes were grown in 6-well plates and then subjected to transient transfection using Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. After transfection for 24 h, the cells were lysed in lysis buffer (Promega), after which the luciferase activity was measured using the Luciferase Assay System (Promega). To normalize the transfection efficiency, a pCMV-β gal was included in each transfection as internal control plasmid.

Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA). Nuclear extracts from 3T3-L1 adipocytes were prepared using the method described by Crabtree [20]. Protein concentrations were determined using the Bradford method. EMSAs were performed using probe corresponding to each promoter binding region as previously described [8].

Chromatin immunoprecipitation (ChIP). ChIP assays were performed in fully differentiated 3T3-L1 cells as previously described [21]. Immunoprecipitated DNA was amplified by PCR using the following primers, which are specific for the adiponectin promoter: sense (−1085 to −1070), 5′-TGT GAG TCC GCC GAG A-3′, antisense (−940 to −925), 5′-TAA ACC TCA GTT TCC A-3′.

Western blot analysis and coimmunoprecipitation. Total proteins were extracted using PRO-PREP reagent (Intron Biotechnology, Daejeon, Korea) and the immune complexes were identified using the enhanced chemiluminescence detection system (ECL; Amersham Biosciences, Uppsala, Sweden). For coimmunoprecipitation assay, the lysates from the 3T3-L1 cells transfected with GFP or GFP-ATF3 were immunoprecipitated with GFP or CREB, and then immunoblotted with 2 μg of specific antibodies, CREB and GFP (Santa Cruz).

Glutathione S-transferase pull-down assay. For in vitro-binding assay of ATF3 and CREB, 500 μg lysate of 3T3-L1 cells were incubated with 3.0 μg glutathione S-transferase (GST) or fusion GST-ATF3 proteins coupled to glutathione-Sepharose beads in 300 μl lysis buffer overnight at 4 °C. After three times of intensive washing, the bound proteins were eluted and separated by SDS–PAGE followed by Western blotting using CREB antibody.

Statistical analysis. All experiments were performed at least three times. The results are expressed as means ± SE. Statistical analysis was performed using Student's *t*-test. The data were considered statistically significant at $P < 0.001$.

Results

CREB stimulates the promoter activity of the mouse adiponectin gene

Adipogenic transcription factors including PPARγ, C/EBPα, and ADD1/SREBP1c activate adiponectin gene expression in adipocyte [8–10]. Because CREB is also a central transcriptional activator of adipocyte differentiation, we evaluated whether CREB stimulates adiponectin gene expression in adipocyte. To this end, we examined the effect of activated CREB on the promoter activity of the mouse adiponectin gene. To accomplish this, the −3000/+10 of the 5′ flanking regions of the mouse adiponectin gene were isolated by PCR and then inserted into a pGL3 basic vector, designated P(−3000)/Luc. Next, this promoter reporter was transiently transfected into 3T3-L1 adipocyte cells with constitutive active VP-16 CREB expression vector, after which the luciferase activity was examined. As shown in Fig. 1A, expression of VP-16 CREB increased the promoter activity of P(−3000)/Luc reporter. However, the dominant mutant CREB (ACREB) did not increase the promoter activity of adiponectin, demonstrating that active CREB stimulates adiponectin gene expression.

To define the CREB-responsive region of the adiponectin promoter, we constructed 5′ serial deleted adiponectin promoters and performed transfection experiments in 3T3-L1 cells (Fig. 1B). As shown in Fig. 1B, deletion of the sequence from −3000 bp to −1250 bp of the promoter did not effect the transactivation of the adiponectin promoter by CREB. However, a further deletion to −1000 bp resulted in almost complete loss of induction by CREB, suggesting that the CREB-responsive region is located in the region between −1250 and −1000 bp.

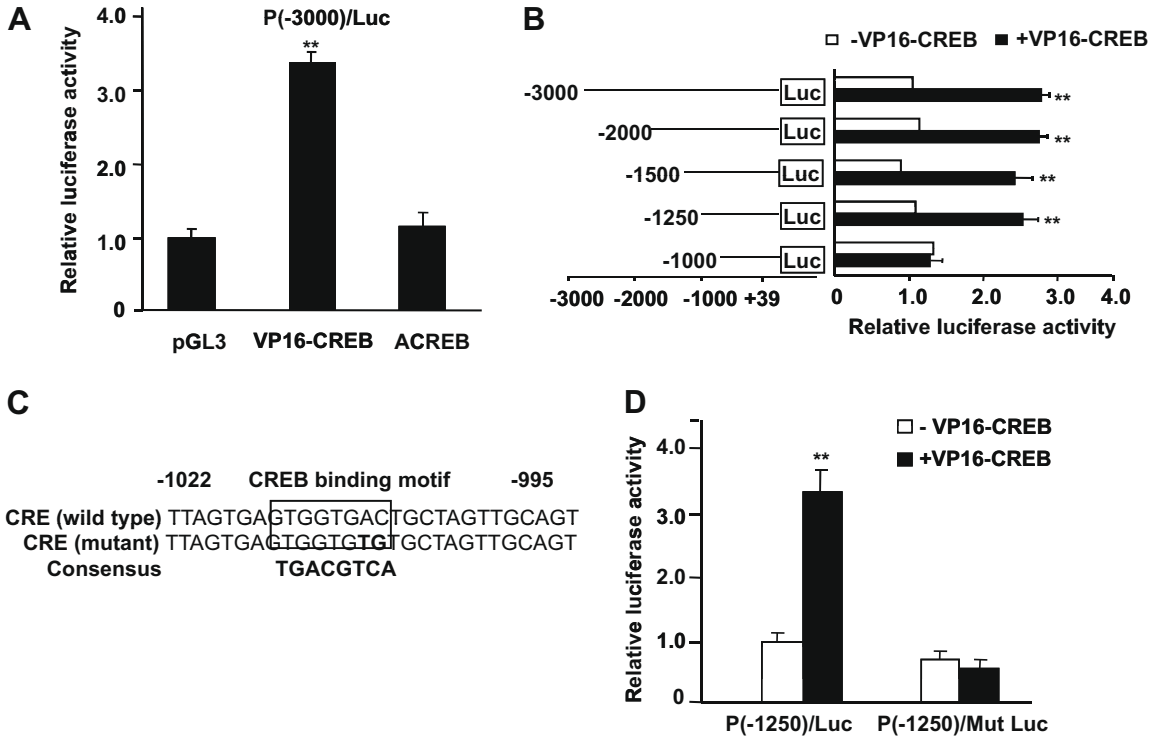

Fig. 1. CREB stimulates the promoter activity of the mouse adiponectin gene which is dependent on CRE located between −1250 and −1000. (A) The 3.0 kb mouse adiponectin promoter was transfected into 3T3-L1 adipocytes with VP-16 CREB or ACREB, after which the luciferase activity was measured. The data are presented as means ± SD of three independent experiments ($^{**}P < 0.001$). (B) 5′ serial deletions of the mouse adiponectin promoters were constructed and transfected with VP-16 CREB; after which the levels of promoter activity were measured in 3T3-L1 adipocytes. (C) Sequence comparison of the putative CRE between −1022 and −995 in the mouse adiponectin promoter with the canonical CRE. The CREB core binding site is boxed. Bold letters indicate mutated sites. (D) A wild-type or a CRE-deleted mutant version of the mouse adiponectin promoter was transfected into 3T3-L1 adipocytes. The data are presented as means ± SD of three independent experiments ($^{**}P < 0.001$).

A computer-assisted search of this 250 bp region using TFSEARCH revealed the presence of the putative cAMP-response element (CRE) between −1011 and −1004 bp (Fig. 1C). To verify that the putative CRE is crucial for CREB-mediated transactivation of the adiponectin promoter, we deleted the putative CRE from P(−1250)/Luc reporter and then transformed 3T3-L1 cells with the CRE-deleted mutant promoter. Transfection with the CRE-deleted mutant abolished the CREB-stimulated promoter activity of adiponectin (Fig. 1D), indicating that the putative CRE is a crucial site for transactivation of the adiponectin gene by CREB.

CREB binds to the putative CRE site on the mouse adiponectin promoter

To determine if CREB is capable of binding to the putative CRE sites, we performed EMSA using a labeled oligonucleotide probe covering −1022/−995 on the nuclear extracts from 3T3-L1 cells and in vitro translated CREB. When both proteins were incubated with ^{32}P radiolabeled CRE oligonucleotides, binding complexes that formed with the labeled 28 bp oligonucleotides were observed (Fig. 2A). However, incubation with a 100-fold molar excess of unlabeled oligonucleotides for homologous adiponectin promoter and consensus CRE nearly abolished the binding bands, whereas unlabeled mutant oligonucleotides did not exert a significant effect on binding, which suggests that the binding complex is specific. To determine if the complex consisted of bound CREB, a supershift assay was conducted using antibody against CREB. As shown in Fig. 2A, treatment with anti-CREB shifted the binding complex, whereas treatment with anti-C/EBP did not. Taken together, these data demonstrate that CREB specifically binds to the CRE site between −1022 and −995 bp of the adiponectin promoter in vitro.

Next to evaluate the binding of CREB to the adiponectin promoter in vivo, chromatin immunoprecipitation (ChIP) was performed in differentiated 3T3-L1 adipocytes. The chromatins that were immunoprecipitated with CREB antibody were amplified by PCR using adiponectin promoter-specific primers covering the CRE site from −1022 and −995 bp. As shown in Fig. 2B, a 160 bp PCR product was generated with the CREB antibody in 3T3-L1 cells, whereas no PCR product was observed when primers for sequences in exon 3 were used. Taken together, these findings indicate that CREB formed a specific complex with the adiponectin promoter in vivo. Overall, these results indicate that CREB activates adiponectin gene expression by binding to the CRE in −1022/−995 bp of the promoter.

IGF-1 increases adiponectin gene expression through activation of CREB

It has been reported that IGF-1 increase the expression and secretion of adiponectin [15]. However, the signaling molecules required for these effects have not yet been identified. Because CREB is activated by IGF-1 through phosphorylation via several signaling pathways [22], we evaluated that CREB played a role in IGF-1-stimulated expression of the adiponectin gene. To accomplish this, we examined the effects of IGF-1 on promoter activity of the adiponectin gene in 3T3-L1 adipocytes. As shown in Fig. 3A, IGF-1 increased the promoter activity of the P(−3000)/Luc reporter. However, ACREB abolished this IGF-1-induced promoter activity (Fig. 3A). Furthermore, we determined if the stimulation is dependent on CRE in the adiponectin promoter. As shown in Fig. 3B, IGF-1 did not increase the promoter activity of the CRE-deleted reporter construct. These findings suggest that IGF-1 increases adiponectin

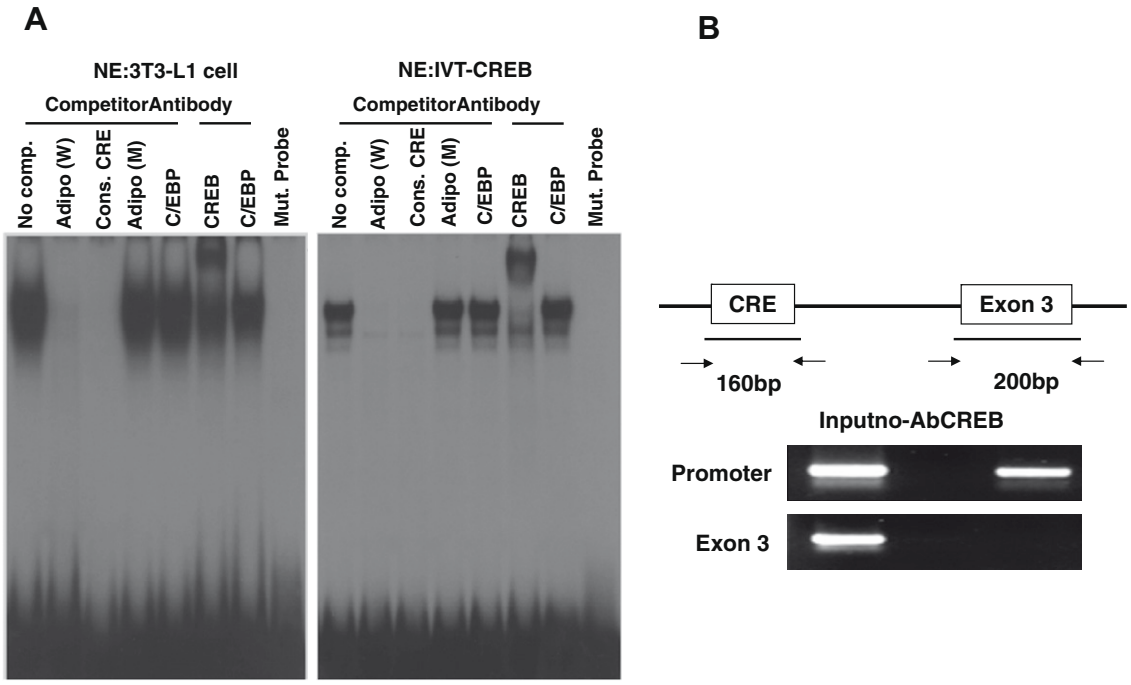

Fig. 2. CREB binds to the mouse adiponectin promoter. (A) EMSAs were performed on the CRE site (−1022 to −995) using nuclear extracts from 3T3-L1 adipocytes (left panel), and in vitro translated CREB (right panel). For the oligonucleotide competition experiments, a 100-fold excess of oligonucleotides was used (lane 1: no competition; lane 2: adiponectin wild CRE oligonucleotides; lane 3: Consense CRE oligonucleotides; lane 4: adiponectin mutant CRE oligonucleotides; lane 5: C/EBP oligonucleotides). The protein–DNA complex was supershifted by CREB antibody (lane 6), but not by the nonspecific antibody (lane 7). (B) Chromatin immunoprecipitation was performed in fully differentiated 3T3-L1 cells. DNA that was immunoprecipitated with anti-CREB was amplified by PCR using primers specific for adiponectin promoter or exon 3.

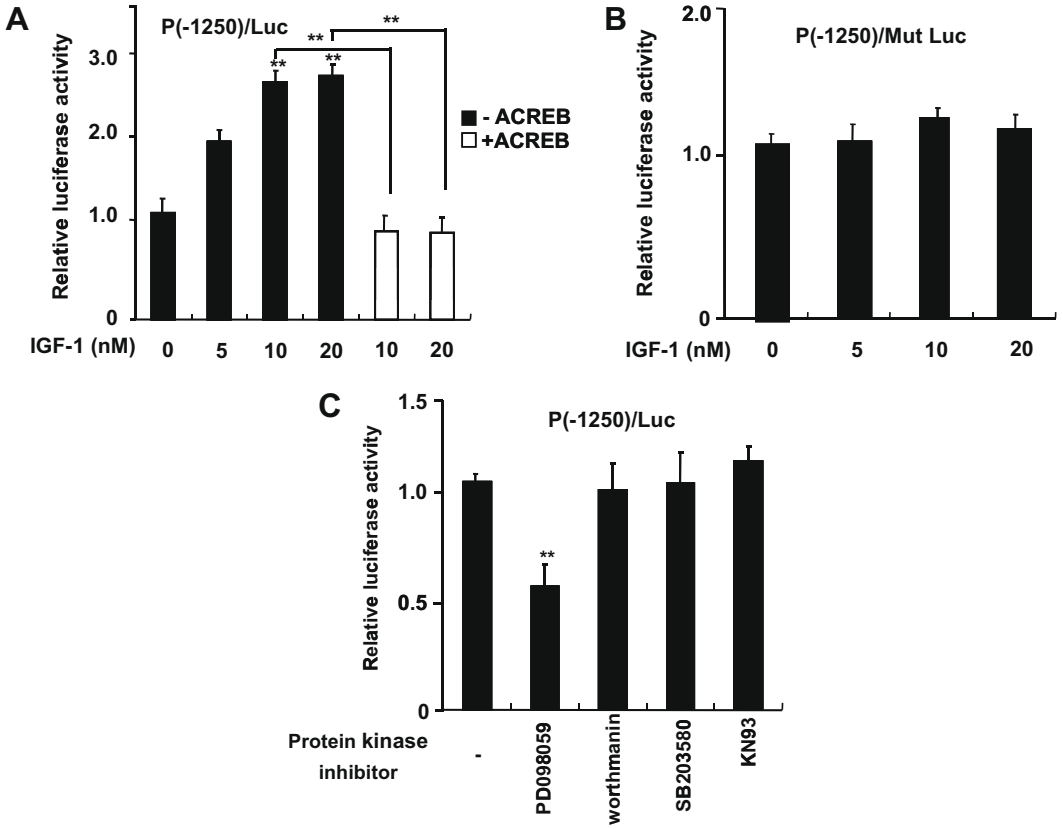

Fig. 3. CREB is associated with IGF-1-stimulated adiponectin expression. (A) 3T3-L1 adipocytes transfected with P(−1250)/Luc reporter were stimulated with IGF-1 for 24 h with or without ACREB. (B) 3T3-L1 adipocytes were transfected with P(−1250)/mut Luc and then treated with various concentrations of IGF-1. (C) 3T3-L1 adipocytes were transfected with P(−1250)/Luc reporter, preincubated in the presence of various kinase inhibitors and then incubated with IGF-1 for 24 h. The data are presented as means ± SD of three independent experiments ($^{**}P < 0.001$).

expression through the activation of CREB, which is dependent on CRE in the adiponectin promoter. Next, we identified the signaling involved in IGF-1-induced stimulation of adiponectin expression. To accomplish this, we evaluated the effects of several kinase inhibitors on the IGF-1-stimulated promoter activity of adiponectin. Specifically, 3T3-L1 adipocytes were pretreated with either the ERK inhibitor, PD98059, the PI3 kinase inhibitor, worthmanin, the p38 MAPK inhibitor, SB-203580, or the CalMK inhibitor, KN93, after which they were treated with IGF-1 for 24 h. As shown in Fig. 3C, PD98059 prevented the stimulation of adiponectin promoter activity by IGF-1, which indicates that IGF-1 stimulates adiponectin expression through CREB phosphorylation via ERK.

ATF3 represses CREB-stimulated adiponectin promoter activity

Then, we investigated the effect of ATF3 in CREB-mediated activation of adiponectin promoter because ATF3 inhibits adiponectin gene expression and acts as important negative regulator in down-regulation of adiponectin expression under obesity or type 2 diabetes condition [11]. As shown in Fig. 4A, expression of ATF3 inhibited the promoter activity of adiponectin stimulated by CREB. To clarify whether the inhibition is due to the functional interaction of ATF3 with CREB, we investigated the interaction of ATF3 with CREB. To this end, 3T3-L1 preadipocyte cells were transfected with GFP or GFP-ATF3 expression vector and then differentiated for 6 days. After immunoprecipitation with GFP or CREB antibody, the Western blots were performed with CREB or GFP antibody, respectively. When GFP-immunoprecitates were immunoblotted with CREB, the immunoblotted bands were observed in both non differentiated and differentiated 3T3-L1 cells transfected with GFP-ATF3. Similarly, the immunoblotted band with GFP in CREB-immunoprecipitates was also observed in the cells transfected with GFP-ATF3 suggesting that ATF3 interacts with CREB in 3T3-L1 cells (Fig. 4B). Importantly, the phosphorylated CREB significantly decreased in differentiated cells transfected with GFP-ATF3 compared with the cells transfected with GFP, suggesting that binding of ATF3 with CREB may inhibit CREB phosphorylation during differentiation of 3T3-L1 adipocytes cells (Fig. 4B). To further demonstrate the direct binding of CREB with ATF3, the GST pull-down assay was conducted using purified GST or GST-ATF3. As shown in Fig. 4C, CREB also bound to GST-ATF3 in both non differentiated and differentiated 3T3-L1 cells, but not to control GST beads.

Discussion

Many aspects of adipogenesis can be described as a cascade of gene expression regulated by a small set of transcription factors, namely adipogenic transcription factors including PPARγ2, CEBPα, and ADD1/SREBP1c [4]. These transcription factors have been shown to stimulate adiponectin expression, as well as adipocyte-specific genes [7–10]. Because CREB is now considered to be a primary inducer of adipogenesis, we determined in this study that CREB also plays a role in the positive regulation of mouse adiponectin expression in adipocytes. Constitutively active CREB stimulates the promoter activity of mouse adiponectin gene, which occurs as a result of CREB binding to the −1022 and −995 region (CRE) of the adiponectin promoter. The CRE-deleted adiponectin promoter was not stimulated by CREB, indicating that CREB positively regulates adiponectin expression in adipocyte through direct binding to the −1022 and −995 region of the mouse adiponectin promoter.

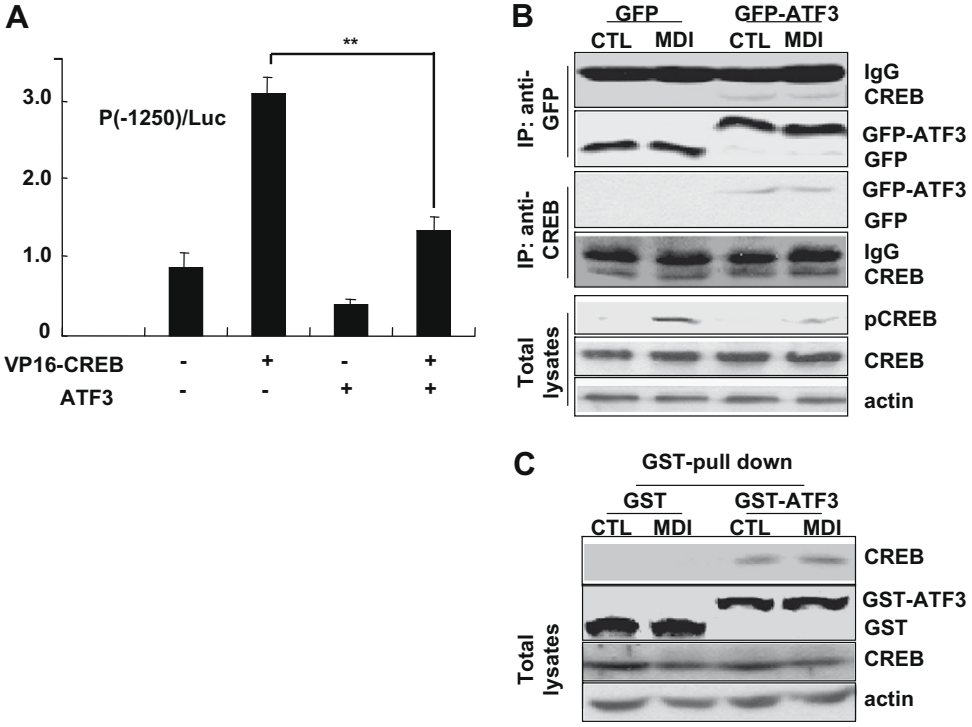

Fig. 4. CREB binds to ATF3 and decreases CREB phosphorylation during adipocyte differentiation. (A) P(−1250)/Luc reporter was cotransfected with VP-16 CREB and ATF3 expression vector in the 3T3-L1 adipocytes, after which the activity of the promoter was measured. The data are presented as means ± SD of three independent experiments ($^{**}P < 0.001$). (B) 3T3-L1 preadipocyte cells were transfected with GFP or GFP-ATF3 expression vector and then differentiated for 6 days. The lysates from 3T3-L1 adipocytes were immunoprecipitated with GFP or CREB antibody, and the immunoprecipitates were subjected to the Western blots with CREB or GFP antibody, respectively. (C) The lysates of 3T3-L1 cells were incubated with GST or GST-ATF3 coupled with glutathione-sepharose beads. The bound proteins were eluted and subjected to Western blot using CREB antibody. CTL: undifferentiated 3T3-L1 cells, MDI: differentiated 3T3-L1 cells.

Adiponectin gene expression is regulated by several extracellular signals and hormones, including insulin, IGF-1, TNF-α and adrenergic agonists [14,15]. Among them, IGF-1 has been reported to increase adiponectin expression in adipocytes [15]. However, the link between the hormones and adiponectin gene expression has not been elucidated. There are numerous protein kinases that phosphorylate CREB. Protein kinase A (PKA), Ca^{2+}/calmodulin-dependent kinase (CaMK)IV and mitogen-activated protein kinase (MAPK) each phosphorylate CREB at serine 133, which leads to the activation of gene transcription [22]. Therefore, we determined that which signaling was associated with IGF-1-stimulated adiponectin gene expression. Among the inhibitors evaluated in this study, only PD98059 inhibited the stimulation of adiponectin promoter as well as the phosphorylation of CREB (data not shown). These results suggest that IGF-1 stimulates mouse adiponectin expression in adipocytes through activation of CREB via the ERK signaling pathway.

ATF3, stress-inducible transcriptional repressor, has been found to inhibit adiponectin gene expression in 3T3-L1 adipocytes by direct binding to adiponectin promoter and play an important role in ER stress or obesity-induced downregulation of adiponectin expression [11]. In current study, we demonstrated indirect role of ATF3 in negative regulation of adiponectin expression. ATF3 binds to CREB, which was revealed by both coimmunoprecipitation assay and GST pull-down, and inhibits the adiponectin promoter activity transactivated by CREB. Importantly, ATF3 expression decreases the phosphorylation of CREB activated during differentiation of 3T3-L1 adipocytes. Therefore, these results suggested that ATF3 may inhibit the expression of adiponectin by preventing the transactivation of CREB through interaction with CREB.

However, in contrast to our result, recent study reported that adipocyte CREB downregulates expression of adiponectin indirectly [23]. Because CREB binding over adiponectin promoter was not found by ChIP assay, the study suggested that CREB stimulated ATF3 which in turn repressed expression of adiponectin gene. However, the difference of two studies is that human chromatins were employed for the ChIP-chip assay in the previous study, whereas mouse adiponectin promoter was used in our study to see the effects of ATF3 on adiponectin gene expression. We identified CREB-bound CRE on the promoter of mouse adiponectin, and demonstrated that CRE is essential in the positive regulation of CREB in adiponectin expression. Searching of sequence homology revealed that the CRE in mouse adiponectin promoter was not found in human adiponectin promoter. Accordingly, CREB binding was not detectable on the human chromatin by the ChIP-chip assay. As the previous study did not show direct effect of CREB on human adiponectin promoter even though no binding of CREB to human chromatin, we do not have any explanation for this disparity. Whereas, another recent study on pig adiponectin gene showed that CREB binds to CRE spanned by nucleotides −1150 to −1130 of pig adiponectin promoter and stimulates pig adiponectin gene expression in consistent with our result [24]. Therefore, the study indicates that CREB is an essential regulatory factor for the transcriptional activity of pig adiponectin.

In summary, we have shown for the first time that activation of CREB transactivates mouse adiponectin gene expression in adipocytes, which plays an important role in the regulation of adiponectin expression in response to growth factor.

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References

[1] G.N. Chaldakov, I.S. Stankulov, M. Hristova, P.I. Ghenev, Adipobiology of disease: adipokines and adipokine-targeted pharmacology, Curr. Pharm. Des. 9 (2003) 1023–1031.
[2] C.J. Lyon, R.E. Law, W.A. Hsueh, Minireview: adiposity, inflammation, and atherogenesis, Endocrinology 144 (2003) 2195–2200.
[3] T.P. Combs, U.B. Pajvani, A.H. Berg, Y. Lin, L.A. Jelicks, M. Laplante, A.R. Nawrocki, M.W. Rajala, P.E. Scherer, A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity, Endocrinology 145 (2004) 367–383.
[4] K. Hotta, T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, Y. Matsuzawa, Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1595–1599.
[5] C. Weyer, T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R.E. Pratley, P.A. Tataranni, Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia, J. Clin. Endocrinol. Metab. 86 (2001) 1930–1935.
[6] K. Maeda, K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa, K. Matsubara, cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose most abundant gene transcript 1), Biochem. Biophys. Res. Commun. 16 (1996) 286–292.
[7] M. Iwaki, M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima, I. Shimomura, Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors, Diabetes 52 (2003) 1655–1663.
[8] S.K. Park, S.Y. Oh, M.Y. Lee, S. Yoon, K.S. Kim, J.W. Kim, CCAAT/enhancer binding protein and nuclear factor-Y regulate adiponectin gene expression in adipose tissue, Diabetes 53 (2004) 2757–2766.
[9] L. Qiao, P.S. Maclean, J. Schaack, D.J. Orlicky, C. Darimont, M. Pagliassotti, J.E. Friedman, J. Shao, C/EBPalpha regulates human adiponectin gene transcription through an intronic enhancer, Diabetes 54 (2005) 1744–1754.
[10] J.B. Seo, H.M. Moon, M.J. Noh, Y.S. Lee, H.W. Jeong, E.J. Yoo, W.S. Kim, J.J.B. Kim, Adipocyte determination- and differentiation-dependent factor 1/sterol regulatory element-binding protein 1c regulates mouse adiponectin expression, J. Biol. Chem. 279 (2004) 22108–22117.
[11] H.B. Kim, M. Kong, T.M. Kim, Y.H. Suh, W.H. Kim, J.H. Lim, J. Song, M.H. Jung, NFATc4 and ATF3 negatively regulate adiponectin gene expression in 3T3-L1 adipocytes, Diabetes 55 (2006) 1342–1352.
[12] K. Ikeda, H. Maegawa, S. Ugi, Y. Tao, Y. Nishio, S. Tsukada, S. Maeda, A. Kashiwagi, Transcription factor activating enhancer-binding protein-2beta, a negative regulator of adiponectin gene expression, J. Biol. Chem. 281 (2006) 31245–31253.
[13] M. Fasshauer, R. Paschke, M. Stumvoll, Adiponectin, obesity, and cardiovascular disease, Biochimie 86 (2004) 779–784.
[14] M. Fasshauer, J. Klein, S. Neumann, M. Eszlinger, R. Paschke, Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 290 (2002) 1084–1089.
[15] C.M. Halleux, M. Takahashi, M.L. Delporte, R. Detry, T. Funahashi, Y. Matsuzawa, S.M. Brichard, Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue, Biochem. Biophys. Res. Commun. 288 (2001) 1102–1107.
[16] J.M. Bruun, A.S. Lihn, C. Verdich, S.B. Pedersen, S. Toubro, A. Astrup, B. Richelsen, Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E527–E533.
[17] B. Mayr, M. Montminy, Transcriptional regulation by the phosphorylation-dependent factor CREB, Nat. Rev. Mol. Cell Biol. 2 (2001) 599–609.
[18] J.E. Reusch, L.A. Colton, D.J. Klemm, CREB activation induces adipogenesis in 3T3-L1 cells, Mol. Cell. Biol. 20 (2000) 1008–1020.
[19] D.J. Klemm, J.W. Leitner, P. Watson, A. Nesterova, J.E. Reusch, M.L. Goalstone, B. Draznin, Insulin-induced adipocyte differentiation. Activation of CREB rescues adipogenesis from the arrest caused by inhibition of prenylation, J. Biol. Chem. 276 (2001) 28430–28435.
[20] G.R. Crabtree, Calcium, calcineurin, and the control of transcription, J. Biol. Chem. 276 (2001) 2313–2316.
[21] M.J. Latasa, M.J. Griffin, Y.S. Moon, C. Kang, H.S. Sul, Occupancy and function of the −150 sterol regulatory element and −65 E-box in nutritional regulation of the fatty acid synthase gene in living animals, Mol. Cell. Biol. 23 (2003) 5896–5907.
[22] M. Johannessen, M.P. Delghandi, U. Moens, What turns CREB on?, Cell Signal. 16 (2004) 1211–1227.
[23] L. Qi, M. Saberi, E. Zmuda, Y. Wang, J. Altarejos, X. Zhang, R. Dentin, S. Hedrick, G. Bandyopadhyay, T. Hai, J. Olefsky, M. Montminy, Adipocyte CREB promotes insulin resistance in obesity, Cell Metab. 9 (2009) 277–286.
[24] F. Ling, J. Li, Y. Chen, H. Du, Y. Mei, D. Mo, C. Wang, Cloning and characterization of the 5′-flanking region of the pig adiponectin gene, Biochem. Biophys. Res. Commun. 381 (2009) 236–240.