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Transcriptional activation of melanocortin 2 receptor accessory protein by PPARγ in adipocytes

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ABSTRACT

Adrenocorticotropic hormone (ACTH) in rodents decreases lipid accumulation and body weight. Melanocortin receptor 2 (MC2R) and MC2R accessory protein (MRAP) are specific receptors for ACTH in adipocytes. Peroxisome proliferator-activated receptor γ (PPARγ) plays a role in the transcriptional regulation of metabolic pathways such as adipogenesis and β-oxidation of fatty acids. In this study we investigated the transcriptional regulation of MRAP expression during differentiation of 3T3-L1 cells. Stimulation with ACTH affected lipolysis in murine mature adipocytes via MRAP. Putative peroxisome proliferator response element (PPRE) was identified in the MRAP promoter region. In chromatin immunoprecipitation and reporter assays, we observed binding of PPARγ to the MRAP promoter. The mutagenesis experiments showed that the −1209/−1198 region of the MRAP promoter could function as a PPRE site. These results suggest that PPARγ is required for transcriptional activation of the MRAP gene during adipogenesis, which contributes to understanding of the molecular mechanism of lipolysis in adipocytes.

1. Introduction

The adipose tissue regulates energy homeostasis and stores excess energy in the form of triglycerides to be released when necessary [1]. Adipokines are secreted by adipose tissue for metabolic regulation. The abnormal regulation of the energy metabolism leads to metabolic disorders such as diabetes, hypertension, and hyperlipidemia. Peroxisome proliferator-activated receptor γ (PPARγ) is a critical transcription factor in regulation of adipocyte differentiation, lipid metabolism and insulin sensitivity [2]. PPARγ activates target genes by binding to promoter regions after forming a complex with retinoid X receptor α (RXRα). The PPARγ-binding promoter regions consist of direct repeats of the nuclear receptor half-site motifs (AGGTCA) spaced by a single nucleotide, are referred to as peroxisome proliferator response elements (PPREs).

Adrenocorticotropic hormone (ACTH) regulates food intake, energy supply, and fat deposition in the body. It was shown that ACTH improved glucose intolerance and induced loss of body weight in mice [3]. Although ACTH reduces triglyceride content in adipocytes, the mechanism of the ACTH lipolytic activity is not completely understood. The ACTH activity is mediated by a G protein-coupled receptor known as the melanocortin 2 receptor (MC2R). MC2R is expressed primarily in the adrenal cortex, although it is also found in low levels in adipose tissue, pituitary, skin, and sympathetic ganglia [4–6]. MC2R is activated exclusively by ACTH, which results in increase in cAMP and protein kinase A (PKA) activity essential for promoting expression of steroidogenic enzymes [7]. The melanocortin 2 receptor accessory protein (MRAP) is a small single transmembrane protein that facilitates MC2R trafficking from the endoplasmic reticulum to the cell surface [7,8]. Although MRAP expression is induced in mature 3T3-L1 adipocytes [9], the transcriptional regulation of this gene has not been studied and the MRAP promoter has not yet been characterized.

The present study was conducted to investigate the effect of PPARγ on the MRAP expression in murine adipocytes. We report here that PPARγ regulates transcriptional activation of the MRAP gene to stimulate lipolysis induced by ACTH in mature adipocytes.

2. Materials and methods

2.1. Cell lines and cultures

3T3-L1 cell line was obtained from American Type Culture Collection (Rockville, MD, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2. When confluence was reached (day 0), 3T3-L1 cells were cultured into a differentiation medium (DMEM with 10% FBS) supplemented with 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 10 μg/

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ml insulin. After 48 h (day 2), the medium was changed to post-differentiation medium (DMEM, 10% FBS supplemented with 5 μg/ml insulin) and replaced every other day. For the ACTH treatment experiments, the medium was changed to serum free medium. For Oil Red O staining, cells were fixed with 4% formaldehyde for 15 min and stained with Oil Red O solution (Sigma–Aldrich, St. Louis, MO, USA) at room temperature for 5 min, rinsed with isopropanol, and overlaid with PBS. Cos7 cells were maintained in DMEM containing 10% FBS and 100 U/ml penicillin–streptomycin.

2.2. Construction of lentiviral vector for the 3T3-L1 cells infection

Short hairpin RNA (shRNA) lentiviral vector (pLKO.1) containing the MRAP target sequence was constructed. Firstly, pLKO.1 was digested with *AgeI* and *EcoRI*, and oligonucleotides targeting the murine shRNA-MRAP were annealed. Sequences of shRNA-MRAP are as follows:

sense: 5′-CCGGACCTTCGTGGTGCTCCTCTTTCTCGAGAAAGAGGA GCACCACGAAG GTTTTTTG-3′ and antisense: 5′-AATTCAAAAAACCT TCGTGGTGCTCCTCTTTCTCGAGAAAGAGGAGCA CCACGAAGGT-3′.

The hybridized oligonucleotides were ligated into the pLKO.1 vector, yielding shRNA MRAP pLKO.1 vector. HEK 293T cells were co-transfected with shRNA MRAP vector and packaging vectors (pCMV-dR8 and pCMV-VSVG). At 72 h post-transfection viral supernatants were collected to infect 3T3-L1 cells.

2.3. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 3T3-L1 cells using Trizol reagent (Life Technologies, Grand Island, NY, USA), and 1 μg of total RNA was subjected to reverse transcription with random oligomers using the GeneAmp RNA PCR kit (Life Technologies, Grand Island, NY, USA) according to the manufacturer's protocol. The cDNA was amplified by PCR using following cycling protocol: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension step of 72 °C for 10 min. PCR products were separated by gel electrophoresis in 0.7% agarose and stained with ethidium bromide for visualization under ultraviolet light. Amplified cDNA was quantified by real-time PCR using the iCycler iQ Multi-color real-time PCR detection system (Bio-Rad, Hercules, CA, USA). PCR conditions were 95 °C for 3 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 10 s, and 72 °C for 30 s. Changes in fluorescence were monitored with SYBR Green PCR Supermix (Bio-Rad, Hercules, CA, USA) after each cycle, and melting curve analysis was performed at the end of 40 cycles to verify PCR product identity. The GAPDH expression was used for normalization.

2.4. Construction of reporter vectors and mutagenesis

Firefly luciferase reporter pGL3-based vectors containing different fragments of the 2 kb MRAP promoter region (starting from the transcription initiation site) were constructed: pGL3-mMRAP-Luc −148 (−148/+20), −406 (−406/+20), −763 (−763/+20), −1122 (−1122/+20), −1738 (−1738/+20), and −2050 (−2050/+20). The fragments were generated by PCR using the following primers:

position −2050 5′-GGGGTACCCTGGCAGTGGTGGATTTC-3′,
position −1738 5′-GGGGTACCGAGGAGGGGATGGACC-3′,
position −1122 5′-GG GGTACCTAGCAACAGCTTCCGTCT-3′,
position −763 5′-GG GGTACCAGGCGGATTTCTGAGTT-3′,
position −406 5′-GGGGTACCCCCTCGGTGTCAGCTAAG-3′,
position −148 5′-GGGGTACCCCCTTGCGATTGCATTAA-3′,
position +20 5′-CCCTCGAGCCAAAGGCTACTAGAGACGC-3′.

The PCR products were digested with the restriction enzymes *KpnI* and *XhoI*, and subcloned into the promoterless luciferase vector pGL3 (Promega, Madison, WI, USA).

Site-directed mutagenesis of the MRAP promoter (pGL3-MRAP-1738) was performed using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. The following mutagenic primers were used:

F1 5′- GCCTTTACCCTTTGCGGCACACAGACTCTGCT G-3′,
R1 5′- CAGCAGAG TCTGTGTGCCGCAAAGGGTAAAGGC-3′,
F2 5′-GCCTTTGGCCTTTGCGGCACACAGA CTCTGCTG-3′, R2 5′- CAGCAGAGTCTGTGTGCCGCAAAGGCCAAAGGC-3′.

Successful mutagenesis was confirmed by sequence analysis.

2.5. Luciferase reporter assay

Cos7 cells grown in 24-well plates were co-transfected with pGL3 luciferase vector constructs and pRL-TK *Renilla* luciferase reporter vector. Cells were cultured in DMEM medium and incubated with or without both PPARγ and RXRα for 24 h. For transfection with the PPARγ and RXRα expression vectors, the ratio of the reporter and expression vectors was kept constant by addition of the pCMV empty vector. Cells were collected and treated with lysis buffer (50 mM Tris–HCl, pH 8.1, 10 mM EDTA, and 1% SDS). The firefly and *Renilla* luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instruction with the TD-20/20 luminometer. The firefly luciferase activity was normalized to the *Renilla* luciferase activity.

2.6. Glycerol release assay

After treatment of differentiated adipocytes with ACTH in the serum-free medium, glycerol released into the cell supernatant was quantified using the Free Glycerol Measuring kit according to the manufacturer's instruction (Sigma–Aldrich, St. Louis, MO, USA). Glycerol was measured at 535/590 nm (Ex/Em) using a plate reader.

2.7. Chromatin immunoprecipitation (ChIP) assay

Cells were cross-linked with 0.5% formaldehyde at 37 °C for 5 min. Cells were washed in ice-cold PBS, suspended in the lysis buffer supplemented with a protease inhibitor cocktail, and incubated for 10 min on ice. Chromatin samples were sonicated, precleared with blocked Staph A cells, and immunoprecipitated with anti-PPARγ (Abcam, Cambridge, MA, USA) or anti-IgG (Jackson ImmunoResearch, West Grove, PA, USA) antibodies. After incubation at 4 °C overnight, the DNA–protein complexes were immunoprecipitated with Staph A cells. After washing, DNA was recovered by column purification (Bio-Rad, Hercules, CA, USA). For PCR analysis, aliquots of the samples were saved prior to immunoprecipitation (input). PCR was performed to analyze the presence of DNA precipitated by a specific antibody. The primers used for PCR were: sense 5′-GGTAACCCCTTTGACGTGTG-3′, antisense 5′- TCCAAAGGTT GGCATGCTC-3′. The PCR was performed as follows: denaturation at 95 °C for 5 min; 30 cycles of 1 min denaturation at 95 °C, 1 min annealing at 58 °C, and 1 min extension at 72 °C, a final step of 72 °C for 5 min. PCR products resulting from the input or precipitated DNAs were separated by ethidium bromide-stained 1.5% agarose gels. Antibody-free negative control was included in each experiment.

2.8. Statistical analysis

Each experiment was performed in triplicates and repeated independently 3 times. Values were expressed as means ± SD. Data

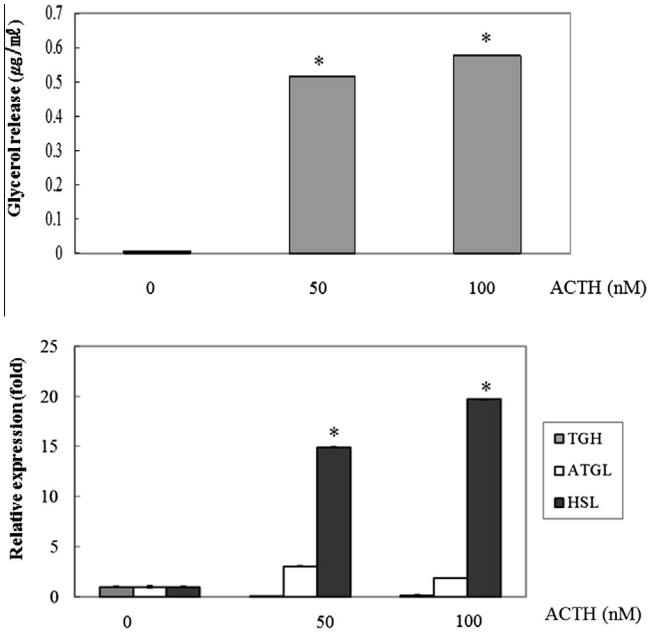

Fig. 1. Induction of lipolysis in 3T3-L1 cells exposed to ACTH. Mature 3T3-L1 cells were incubated with ACTH in serum free medium. (A) Glycerol content in cell supernatants was measured by spectrophotometry using free glycerol reagent. Results are expressed as means ± SD, $^{*}P < 0.05$. (B) Expression of TGH, ATGH, and HSL mRNA in 3T3-L1 cells treated with different concentrations of ACTH was quantified by real-time PCR. Expression levels were normalized to GAPDH. The data are presented as means ± SD of 3 independent experiments, $^{*}P < 0.05$.

were analyzed by one-way analysis of variance and unpaired Student's *t*-test. Statistical analysis was performed using SAS (v6.12, SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Induction of ACTH-mediated lipolysis in 3T3-L1 cells

First, we analyzed the lipolysis in the ACTH-stimulated 3T3-L1 cells. For this, glycerol content was measured in the supernatant of mature adipocytes exposed to ACTH. As shown in Fig. 1A, the glycerol release increased when cells were treated with 50 or 100 nM ACTH. In addition, expression of the lipolysis-related genes was determined by real-time PCR in cells exposed to different doses of ACTH. The level of the hormone sensitive lipase (HSL) mRNA in 3T3-L1 cells was significantly increased by the ACTH treatment, whereas the expression of adipose triglyceride lipase (ATGL) and triacylglycerol hydrolase (TGH) genes was not affected by the ACTH presence (Fig. 1B). These results suggest that ACTH activates HSL causing to induction of lipolysis in differentiated 3T3-L1 cells.

3.2. Repression of lipolysis by knockdown of MRAP gene

Since MRAP as an accessory protein mediates the ACTH function in 3T3-L1 cells, the level of glycerol release in the supernatant of the MRAP knockdown cells was measured at 6 h following ACTH exposure. The glycerol release to the cell supernatant decreased by threefold in the MRAP-depleted cells compared to the control cells (Fig. 2A). By Oil Red O staining, we observed that formation of fat droplets in the MRAP knockdown cells significantly increased at day 10 of differentiation (Fig. 2B). In addition, the HSL mRNA in the MRAP knockdown cells decreased below detection level, whereas PPARγ and ATGL expression did not change (Fig. 2C). These results indicate that the lipolytic activity of ACTH in adipocytes is mediated by MRAP.

3.3. The expression of MRAP in mature adipocyte and the putative PPAR binding site of its promoter

Next, we analyzed MRAP expression in 3T3-L1 cells during adipogenesis. As shown in Fig. 3A, the expression of MRAP and MC2R, similarly to PPARγ, was increased after the adipocyte differentiation. Since transcription of MC2R in adipocytes is regulated by PPARγ [10], the association of PPARγ with the MRAP promoter was also analyzed. A putative PPAR binding site located between −1209 and −1198 bp was found in the MRAP promoter sequence (Fig. 3B). By ChIP assay, we investigated whether PPARγ bound to the PPRE region of the MRAP promoter. As shown in Fig. 3C, a PCR band corresponding to PPRE was amplified from 3T3-L1 cells. These results demonstrate that PPARγ binds to the PPRE region of the MRAP gene promoter.

3.4. Transcriptional activation of MRAP gene by PPARγ

Finally, the effect of PPARγ on transcription of the MRAP gene was examined by using a reporter assay. We generated 6 constructs containing different upstream region of the transcription initiation site in the MRAP promoter. These fragments (−148, −406, −763, −1122, −1738, and −2050) were cloned into a luciferase reporter vector and used to transiently transfect Cos7 cells. Both PPARγ and RXRα plasmids were also used to co-transfect the cells in order to determine whether PPARγ activity depended on the MRAP promoter. In the absence of the PPARγ/RXRα heterodimers, the luciferase activity in the cells transfected with pGL3 MRAP-1738 and pGL3 MRAP-2050 vectors was low, but cells expressing exogenous PPARγ/RXRα proteins showed increased reporter activity (Fig. 4A). However, the luciferase activity in the rest of the pGL3 vectors was low regardless of the PPARγ/RXRα expression. To confirm the presence of the PPARγ binding site in the MRAP promoter, we conducted site-directed mutagenesis by modifying the PPRE conserved sites. As shown in Fig 4B, the activity of PPRE mutant was significantly downregulated, whereas the intact PPRE exhibited a high promoter activity. These results suggest that the PPRE region (−1209/−1198) in the MRAP promoter is important for the transcriptional activation of MRAP induced by PPARγ.

4. Discussion

In the present study, we investigated whether the ACTH-dependent lipolysis is mediated by MRAP. It has been reported that the ACTH receptor MC2R modulates lipid composition in adipocytes [11]. The activation of lipolysis reduces lipid accumulation in adipocytes, which is regulated by cAMP-dependent PKA induced by ACTH [12]. The activation of AMPK is also induced by ACTH [13]. The lipolysis-related PKA and AMPK induce phosphorylation of HSL at Ser^{565} which results in the enzyme activation [14]. HSL is a rate-limiting enzyme in hydrolysis of diacylglycerols and is involved in lipolysis [15,16]. ACTH via the PKA pathway also stimulates expression of steroidogenic factor-1, which activates HSL transcription presumably by interaction with putative binding sequences within the HSL promoter [17]. In our study, treatment of the adipocytes with ACTH resulted in the glycerol release into the cell supernatant via HSL activation. MRAP promoted HSL expression in the presence of ACTH. The knockdown of MRAP suppressed the ACTH activity and consequently lipid accumulation in the adipocytes. In the MRAP-depleted cells, the MC2R protein could not be transferred to the cell surface and associated with

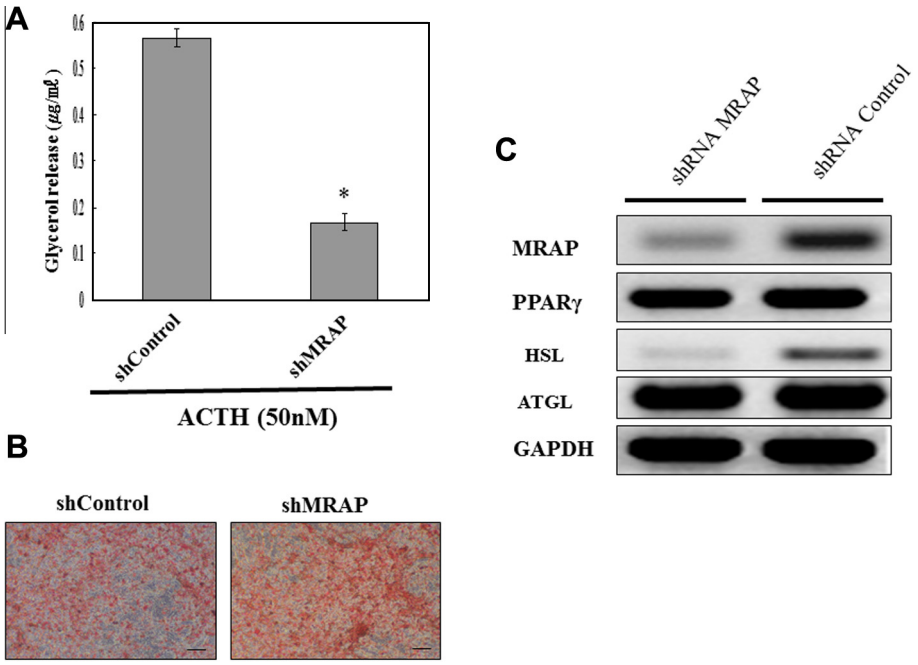

Fig. 2. Inhibition of lipolysis in the MRAP depleted cells. 3T3-L1 cells were infected with the control shRNA (shControl) or shMRAP lentiviruses in the presence of ACTH. (A) Glycerol release in 3T3-L1 cells was measured to estimate the rate of lipolysis. All data represent means ± SD of 3 independent experiments, $^{*}P < 0.05$. (B) Oil Red O staining was performed in the MRAP knockdown cells after induction of differentiation. Representative microscopic images are shown. Magnification, 20×. (C) Expression of MRAP, PPARγ, HSL, and ATGL mRNAs was assessed by RT-PCR. GAPDH was used as an internal control.

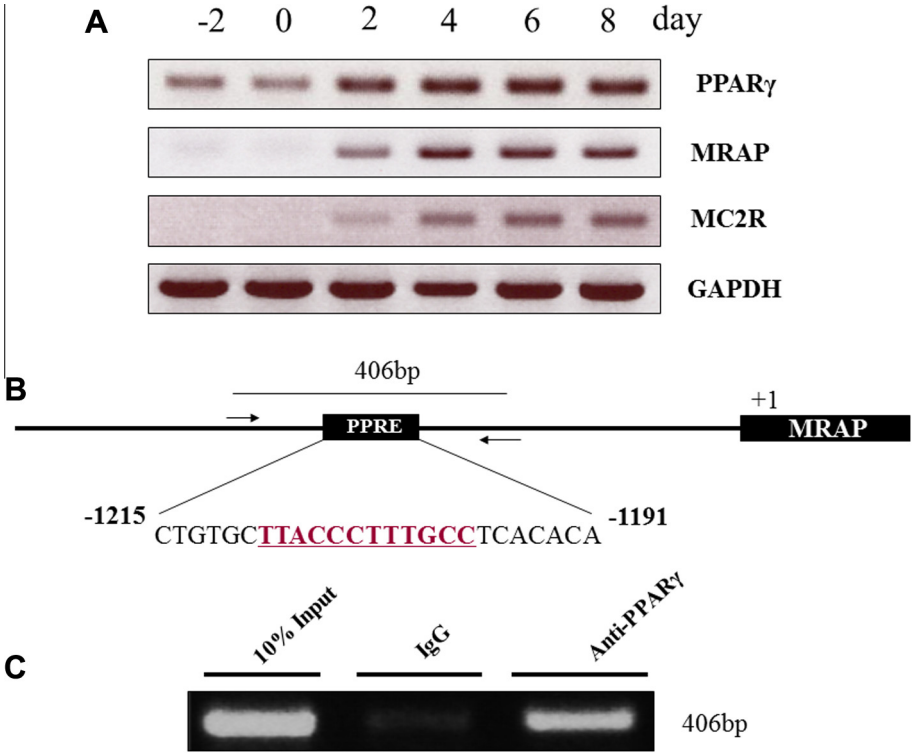

Fig. 3. Expression of PPARγ and MRAP in mature adipocytes and association of PPARγ with a putative PPRE region in the MRAP promoter. (A) The levels of PPARγ, MRAP and MC2R mRNA were determined by RT-PCR after extraction of total RNA from differentiating 3T3-L1 cells. (B) Location and sequence of a putative PPARγ-binding site in the MRAP promoter. Arrows indicate the position of primers designed for PCR amplification. (C) The binding of PPARγ to PPRE regions in the MRAP promoter was analyzed by ChIP assays. After immunoprecipitation, PPRE regions were amplified using PCR. Total chromatin input is indicated as 'input'. Pre-immune IgG was used as a negative control.

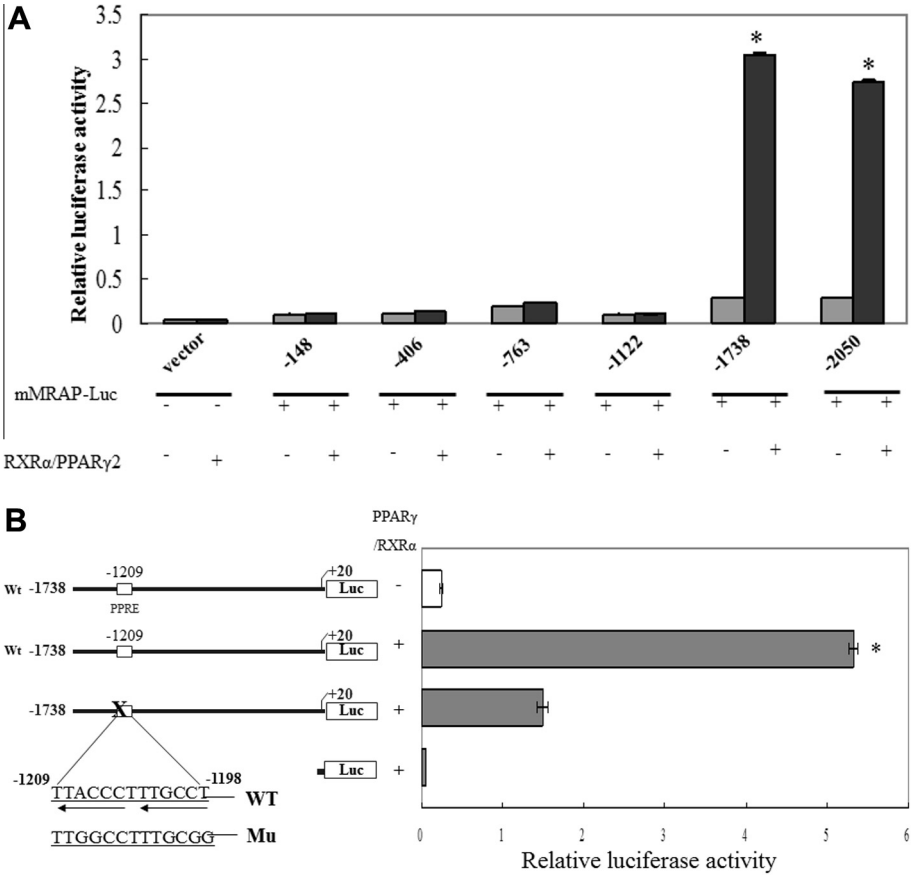

Fig. 4. Identification of the functional PPRE region in the MRAP promoter. (A) The deleted mutants for the MRAP promoter were designed as pGL3 mMRAP-Luc −148 (−148/+20), −406 (−406/+20), −763 (−763/+20), −1122 (−1122/+20), −1738 (−1738/+20) and −2050 (−2050/+20). The pGL3 empty vector was used as a control. The truncated mutants were transfected with or without PPARγ/RXRα vectors. After 24 h, the luciferase reporter activity was measured. The data are presented as means ± SD of 3 independent experiments, $^{*}P < 0.05$. (B) The PPRE domain of MRAP promoter was mutated as indicated. The luciferase reporter vectors were used to transfect cells with or without the PPARγ/RXRα expression vectors. The luciferase activity was measured 48 h later. The data are presented as means ± SD of 3 independent experiments, $^{*}P < 0.05$.

ACTH [8]. The HSL activation induced by ACTH-mediated MRAP may be regulated by the PKA/AMPK pathways, leading to the critical function of MRAP for the ACTH lipolysis activity.

Lipid metabolism is important for adipocytes during adipogenesis [18]. PPARγ, a key regulator of adipogenesis, is also involved in the regulation of lipid metabolism. The ablation of PPARγ in 3T3-L1 cells reduces basal and stimulated lipolysis, and enhances the insulin-mediated anti-lipolytic activity [19]. PPARγ positively regulates the HSL gene expression, which requires the presence of the specificity protein-1 [20–22]. In our study, we found that MRAP mRNA expression was regulated by PPARγ, whereas PPARγ expression was independent of MRAP, indicating that the MRAP-dependent HSL activation is regulated by PPARγ. Therefore, transcription of the HSL gene is regulated by the PPARγ/MRAP-mediated signaling pathway. Taken together, our data provide evidence for direct multilevel effects of PPARγ on adipogenesis. These results suggest that PPARγ is a component of the principal signaling pathway implicated in the ACTH-induced lipolysis via regulation of HSL in 3T3-L1 cells. These findings may have important implications for understanding the molecular mechanisms of the PPARγ signaling in the ACTH-mediated lipolysis.

Acknowledgments

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References

[1] G. Fruhbeck, J. Gomez-Ambrosi, F.J. Muruzabal, M.A. Burrell, The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation, Am. J. Physiol. Endocrinol. Metab. 280 (2001) 827–847.
[2] P. Tontonoz, E. Hu, B.M. Spiegelman, Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor, Cell 79 (1994) 1147–1156.
[3] B.A. Boston, The role of melanocortins in adipocyte function, Ann. N.Y. Acad. Sci. 885 (1999) 75–84.
[4] I. Gantz, T.M. Fong, The melanocortin system, Am. J. Physiol. Endocrinol. Metab. 284 (2003) 468–474.
[5] F.M. Cammas, G.D. Pullinger, S. Barker, A.J.L. Clark, The mouse adrenocorticotropin receptor gene: cloning and characterization of its promoter and evidence for a role for the orphan nuclear receptor steroidogenic factor 1, Mol. Endocrinol. 11 (1997) 867–876.
[6] B.A. Boston, R.D. Cone, Characterization of melanocortin receptor subtype expression in murine adipose tissue and in the 3T3-L1 cell line, Endocrinology 137 (1996) 2043–2050.
[7] S.N. Cooray, A.J.L. Clark, Melanocortin receptors and their accessory proteins, Mol. Cell. Endocrinol. 331 (2011) 215–221.
[8] L.A. Metherell, J.P. Chapple, S. Cooray, A. David, C. Becker, F. Rüschendorf, D. Naville, M. Begeot, B. Khoo, P. Nürnberg, A. Huebner, M.E. Cheetham, A.J. Clark, Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2, Nat. Genet. 37 (2005) 166–170.
[9] A. Xu, K.L. Choi, Y. Wang, P.A. Permana, L.Y. Xu, C. Bogardus, G.J. Cooper, Identification of novel putative membrane proteins selectively expressed during adipose conversion of 3T3-L1 cells, Biochem. Biophys. Res. Commun. 293 (2002) 1161–1167.
[10] L.A. Noon, A.J.L. Clark, P.J. King, A peroxisome proliferator-response element in the murine *mc2-r* promoter regulates its transcriptional activation during differentiation of 3T3-L1 adipocytes, J. Biol. Chem. 279 (2004) 22803–22808.

[11] M.J. Betz, N. Hatiboglu, B. Mauracher, D. Hadaschik, A. Sauter, H. Demmelmair, B. Koletzko, F. Beuschlein, M. Slawik, Mc2 receptor knockdown modulates differentiation and lipid composition in adipocytes, Horm. Metab. Res. 44 (2012) 670–675.

[12] J.D. Corbin, E.M. Reimann, D.A. Walsh, E.G. Krebs, Activation of adipose tissue lipase by skeletal muscle cyclic adenosine 3′,5′-monophosphate-stimulated protein kinase, J. Biol. Chem. 245 (1970) 4849–4851.

[13] W. Yin, J. Mu, M.J. Birnbaum, Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes, J. Biol. Chem. 278 (2003) 43074–43080.

[14] C. Roepstorff, B. Vistisen, M. Donsmark, J.N. Nielsen, H. Galbo, K.A. Green, D.G. Hardie, J.F. Wojtaszewski, E.A. Richter, B. Kiens, Regulation of hormone-sensitive lipase activity and Ser563 and Ser565 phosphorylation in human skeletal muscle during exercise, J. Physiol. 560 (2004) 551–562.

[15] F.B. Kraemer, W.J. Shen, Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis, J. Lipid Res. 43 (2002) 1585–1594.

[16] C. Holm, Molecular mechanisms regulating hormone-sensitive lipase and lipolysis, Biochem. Soc. Trans. 31 (2003) 1120–1124.

[17] M. Hollysz, N. Derebecka-Holysz, W.H. Trzeciak, Transcription of LIPE gene encoding hormone-sensitive lipase/cholesteryl esterase is regulated by SF-1 in human adrenocortical cells: involvement of protein kinase A signal transduction pathway, J. Mol. Endocrinol. 46 (2011) 29–36.

[18] M. Lafontan, D. Langin, Lipolysis and lipid mobilization in human adipose tissue, Prog. Lipid Res. 48 (2009) 275–297.

[19] M. Watanabe, K. Inukai, H. Katagiri, T. Awata, Y. Oka, S. Katayama, Regulation of PPAR gamma transcriptional activity in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 300 (2003) 429–436.

[20] E.E. Kershaw, M. Schupp, H.P. Guan, N.P. Gardner, M.A. Lazar, J.S. Flier, PPAR gamma regulates adipose triglyceride lipase in adipocytes in vitro and in vivo, Am. J. Physiol. Endocrinol. Metab. 293 (2007) 1736–1745.

[21] S. Rodriguez-Cuenca, S. Carobbio, V.R. Velagapudi, N. Barbarroja, J.M. Moreno-Navarrete, F.J. Tinahones, J.M. Fernandez-Real, M. Oresic, A. Vidal-Puig, Peroxisome proliferator-activated receptor γ-dependent regulation of lipolytic nodes and metabolic flexibility, Mol. Cell. Biol. 32 (2012) 1555–1565.

[22] T. Deng, S. Shan, P.P. Li, Z.F. Shen, X.P. Lu, J. Cheng, Z.Q. Ning, Peroxisome proliferator-activated receptor-γ transcriptionally up-regulates hormone-sensitive lipase via the involvement of specificity protein-1, Endocrinology 147 (2006) 875–884.