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Prostatic Androgen-Repressed Message-1 as a Regulator of Adipocyte Differentiation in the Mouse

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Adipocyte differentiation is an important aspect in energy homeostasis. Although the regulation of adipocyte differentiation is relatively well understood, the underlying molecular mechanism remains unclear. In this study, subcutaneous and epididymal adipose tissues were used to study the differential expression of associated genes. We found that the expression level of mouse homologue of rat prostatic androgen-repressed message-1 (mPARM-1) gene was higher in subcutaneous, perirenal and mesenteric adipose tissues than in epididymal adipose tissue. In mouse subcutaneous, perirenal, and mesenteric adipose tissues, the expression level of this gene was higher in adipocytes than in non-adipocyte cells, i.e. stromal-vascular cells. Furthermore, mPARM-1 mRNA expression was up-regulated in subcutaneous, mesenteric, and epididymal adipose tissues of mice fed a high-fat diet compared to those fed a normal-fat diet. Expression level of mPARM-1 mRNA increased in the early stage of the chemically induced adipocyte differentiation, preceding the increase in peroxisome proliferator-activated receptor-gamma 2 (PPAR-γ2) mRNA. Tumor necrosis factor-alpha (TNF-α), an inhibitor of adipocyte differentiation, reduced the expression of mPARM-1 mRNA in differentiated 3T3-L1 cells and subsequently down-regulated the expression of adipogenic genes, including PPAR-γ2, leptin and adipogenin. Moreover, knockdown of mPARM-1 expression with siRNA reduced lipid accumulation and the expression levels of PPAR-γ2 and adipocyte protein 2 mRNAs, which suggest that the degree of adipocyte differentiation of 3T3-L1 cells has been reduced. These results indicate that mPARM-1 might be involved in the regulation of fat accumulation and adipocyte differentiation. ——— adipogenesis; mPARM-1; adipose tissue; si RNA; 3T3-L1.

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Adipocytes are essential for the regulation of energy homeostasis; they not only serve as a massive energy reserve but also secrete hormones and cytokines that affect food intake and metabolic regulation (Roh et al. 2006; Rosen and Spiegelman 2006). Since mature adipocytes do not undergo cell division, the number of these cells is thought to increase as a result of the differentiation of pre-adipocytes into mature adipocytes (Nagayama et al. 2007). Studies of cultured cell lines that can be induced to differentiate into adipocytes have shed light on the regulation of adipocyte differentiation, especially at the transcriptional level.

A cascade of key transcriptional factors, including peroxisome proliferator-activated receptor-gamma (PPARγ), CAAAT/enhancer binding protein (C/EBP) family proteins (Christy et al. 1989), and adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein 1 (ADD1/SREBP1), combine to regulate each other (Tontonoz et al. 1994) and to induce the transactivation of adipocyte-specific genes. These factors coordinate the expression of genes, such as those encoding insulin-sensitive glucose transporter 4, aP2 (adipocyte protein 2), adipsin, and adipogenin, which contribute to the creation and maintenance of the adipocyte phenotype (Hong et al. 2005a; Smas et al. 1997). In addition to the abovementioned transcriptional regulation mechanisms, posttranscriptional regulation mechanisms may exist, which partially determine the amounts of various proteins produced during adipocyte differentiation. However, the regulatory events that occur after the sequential gene transcription during adipogenesis remain largely unknown.

We carried out mRNA differential display to compare mRNA expressions in mouse subcutaneous and epididymal adipose tissues. The mouse homologue of rat prostatic androgen-repressed message-1 (mPARM-1) was isolated and identified as a highly expressed gene in subcutaneous

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adipose tissue compared to epididymal adipose tissue. Rat PARM-1 is known to be cloned from the rat prostate, codes for a 298-amino acid protein with a molecular weight of 33,000 (Cornet et al. 2003). Rat PARM-1 gene products have been identified as markers for the acquired androgen-independence of tumor cells (Bruyninx et al. 1999, 2000). In addition, rat PARM-1 enables certain prostatic cells to increase their telomerase activity, there by resisting apoptosis (Cornet et al. 2003). Nonetheless, the physiological functions of PARM-1 in adipocytes have yet to be reported. Therefore, in this study, we report the functions of mPARM-1 in adipogenesis and adipocyte development.

Materials and Methods

Animals

Three-week-old male C57BL/6J mice were obtained from Charles River Japan (Yokohama, Japan). They were housed individually in cages with wire-mesh bottoms at a constant temperature of 20-22°C, at a humidity of 50-60%, under a 12h-light /12h-dark cycle. The animals had free access to water and chow (Oriental Yeast, Chiba, Japan) containing 8.5% (w/w) fat, 43.7% carbohydrate, and 29.7% protein (energy content, 3.69 kcal/g) for an acclimatization period of 1 week. The mice were then weighed and divided into two groups of six, with each group having an approximately equal mean body weight. One group was fed the standard diet and the other received a high-fat diet for 11 weeks (4 to 15 weeks of age). The high-fat diet was obtained from Research Diet (New Brunswick, NJ) and contained 41% fat, 36% carbohydrate, and 23% protein (energy content, 4.33 kcal/g); its fat source was the same as that of the standard diet and it contained the same absolute amounts of protein and fiber as did the standard diet. The animals were weighed every week. At the end of the experimental period, the mice were sacrificed by decapitation. White adipose tissues were separated from subcutaneous, perirenal, mesenteric, epididymal, and parametrial fat sites, immediately frozen in liquid nitrogen, and stored at –80°C until RNA extraction. A range of non-adipose tissues were also collected and stored: brain, pituitary, heart, kidney, liver, lung, stomach, muscle, colon, spleen, small intestine, and pancreas. All experiments were conducted in accordance with the Shinshu University Guide for the Care and Use of Experimental Animals.

Differential display reverse transcription-polymerase chain reaction

Differential display reverse transcription polymerase chain reaction (DDRT-PCR) was performed as described by Hishikawa et al. (2005). Total RNA was extracted from subcutaneous and epididymal adipose tissues of 15 week-old- male mice. DDRT-PCR was performed on both subcutaneous and visceral adipose tissues using a Differential Display Kit (Takara Co., LTD, Tokyo, Japan). We used 9 forward primers and 24 reverse primers for DDRT-PCR amplification. In total, we used 216 forward and reverse primer combinations to screen for genes differentially expressed in subcutaneous and epididymal mouse adipose tissues. The amplified PCR products (10 μl) were separated on an 8% polyacrylamide gel under non-denaturing conditions in TBE buffer for ~3.5 h at 40 W. Gels were stained with ethidium bromide and exposed to UV light, then scanned to compare changes in gene expression between the subcutaneous and epididymal adipose tissues. Differentially displayed PCR bands were excised from the gel, washed twice with 100 μl of RNase-free water, and boiled for 5 min in a water bath. Extracted DNA fragments were subjected to re-amplification in a 40 μl reaction mixture under the same conditions as in the initial PCR. The resulting products were then cloned into the pGEM-T vector (Promega, Madison, WI, USA). Recombinant plasmids containing cDNA inserts were purified and the nucleotide sequences of the inserts were determined with an automated sequencer (ABI 310, Applied Biosystems, CA, USA) and a Dye Terminator reaction kit (Perkin Elmer, Norwalk, CT, USA).

Cultures and differentiation of 3T3-L1 cells

3T3-L1 cells were purchased from ATCC (Manassas, VA, USA). The production of 3T3-L1 cell cultures and the induction of differentiation were performed as described by Hong et al. (2005b). For the portion of our experiments examining the effects of hormonal stimulation on mPARM-1 expression, confluent preadipocytes were treated with 1.7 μM insulin, 1 μM dexamethasone (DEX), and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) for 2 days post-confluence. In a separate portion of our experiments, troglitazone (5 μM) was added to the differentiation medium on day 0, and this treatment was continued throughout the experimental period. On day 10, tumor necrosis factor-α (TNF-α, 10 ng/ml) in differentiated adipocytes was treated for 24 hr.

Oil red O staining

Cytoplasmic lipid droplets were stained with oil red O as described by Gotoh et al. (2007). Briefly, cells were rinsed three times in phosphate-buffered saline (PBS) and then fixed in 10% (v/v) formaldehyde for 10 min. After washing twice with PBS, cells were stained for 30 min at 37°C in freshly diluted oil red O (Sigma Chemical Co., St Louis, MO, USA) solution (six parts oil red O stock and four parts H_2O; oil red O stock solution is 0.5% oil red O in isopropanol), followed by further washing with PBS.

Isolation of adipocytes and S-V cells from adipose tissues

White adipose tissue from the mesenteric, perirenal, and parametrial fat regions of 26-week-old female mice was separated from connective tissue and blood vessels. The adipose tissue was then divided into adipocyte and S-V cell fractions as described by Hong et al. (2005b). Briefly, freshly excised fat pads from 26-week-old female mice were rinsed in Krebs-Ringer bicarbonate (KRB) medium, minced, and digested with 3.5% bovine serum albumin and 0.5 mg/ml type I collagenase (Worthington, Freehold, NJ, USA) for 40 min at 37 °C in KRB medium (pH 7.4). The digested tissue was filtered through a 250-μm nylon mesh to remove undigested tissue and centrifuged at 500 *g* for 5 min. The floating adipocyte fraction was removed, and the remaining tissue was washed in buffer and re-centrifuged to isolate free adipocytes. The S-V pellets were resuspended in an erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$), filtered through a 28-μm nylon mesh to remove endothelial cells, and pelleted at 1,500 *g* for 5 min. Isolated adipocytes and S-V cells were used for measuring mPARM-1 mRNA.

Total RNA extraction and SQ RT-PCR

Total RNA was extracted from non-adipose tissues, confluent 3T3-L1 cells, and differentiated adipocytes using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was extracted from the four adipose tissues, adipocytes, and S-V cells using RNeasy Lipid Tissue Mini Kit (Qiagen KK, Tokyo, Japan). Following the method described by Gotoh et al. (2007), semiquantitative RT-PCR (SQ RT-

Table 1. Primers used for semiquantitative reverse transcription polymerase chain reaction (RT-PCR).

Gene			Length (bp)	Cycle	Tm[1] (°C)
mPARM-1	forward	5´-TGAAGCAACTGTGCCAGCTA-3´	420	28	55
	reverse	5´-TATGCTGCACCTCCAAACAC-3´			
PPAR-2	forward	5´-TGGGTGAAACTCTGGGAGAT-3´	454	28	58
	reverse	5´-CCATAGTGGAAGCCTGATGC-3´			
Leptin	forward	5´-TGACACCAAAACCCTCATCA-3´	357	30	55
	reverse	5´-CTCAAAGCCACCACCTCTGT-3´			
aP2	forward	5´-TCTCCAGTGAAAACTTCGAT-3´	366	28	60
	reverse	5´-CTCATGCCCTTTCATAAACT-3´			
adipogenin	forward	5´-TATGCGATGAAGTACCCTCT-3´	482	33	55
	reverse	5´-CCCCCATTTCTTTAATAGGT-3´			
β-actin	forward	5´-AGGTCATCACTATTGGCAAC-3´	363	28	55
	reverse	5´-ACTCATCGTACTCCTGCTTG-3´			

[1]Tm, Melting Temperature.

PCR) was used to measure levels of mPARM-1, PPAR-γ2, leptin, aP2, adipogenin and β-actin mRNA. The gene-specific primers are shown in Table 1. PCR products were purified in a 1.2% agarose gel. The DNA was visualized by ethidium bromide staining and analyzed using NIH image software. The mRNA levels of mPARM-1 were corrected using the transcription level of the β-actin gene as an internal standard. The amplified cDNAs were subcloned into the pGEM-T easy vector (Promega, Madison, WI, USA), and the sequences were confirmed using an automated DNA sequencer (ABI 310, Applied Biosystems, CA, USA).

siRNA construction and transfection

Using the siRNA design tool provided by Dharmacon Research (Lafayette, CO, USA), we designed siRNA oligonucleotides to interact in mPARM-1 mRNA. Oligonucleotide sequences were: 5´-AAC CUGAGGAACACAGUCUUG-3´ (mPARM-1 siRNA), and 5´-AAU CAACUGACUCGACCACUA-3´ (scrambled siRNA). The scrambled siRNA was used as a control. The siRNAs were constructed employing the Silencer™ siRNA construction kit (Ambion, Austin, TX, USA) according to the protocol provided by the manufacturer. We mixed mPARM-1 and scrambled siRNAs (50 nM) with siPORT™ Lipid transfection agent (Ambion, Austin, TX, USA), then incubated the mixture for 20 min at room temperature to allow transfection complexes to form. Upon confluence, the 3T3-L1 cell media were changed to growth media, without the use of antibiotics. Two days later, cells were transfected with the transfection complexes and then incubated for 4 h at 37°C, with 5% CO_2. 3 ml of differentiation medium without antibiotics were added 4 h after transfection, and the cells were maintained in differentiation media and induced to differentiate as described above. RT-PCR was used to assess down-regulation of siRNA-targeted mPARM-1.

Statistical analysis

Data in each figure are presented as the mean ± SEM. The data were analyzed using Student's *t*-test. Significance was set at $P < 0.05$. These tests were performed using StatView software (SAS Institute, Inc., Cary, NC, USA).

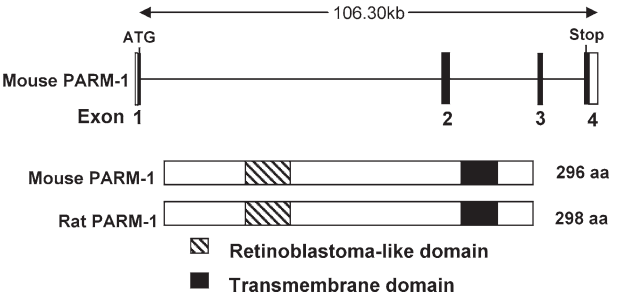

Fig. 1. Structure of the mouse mPARM-1 gene and the predicted mouse mPARM-1 protein. The exon-intron structure of mouse mPARM-1 (GenBank Accession No. EF_564364) was predicted from the Mouse Genome Database. The retinoblastoma-like domain and single transmembrane domain are shown for both mouse and rat PARM-1 mRNA.

Results

Sequence analyses of mPARM-1

mPARM-1 contains an 891bp open reading frame, starting with an ATG initiation codon at position 216, and ending with a TAA termination codon at position 1106 (Fig. 1). We submitted its sequence to GenBank, with Accession No. EF564364. The putative encoded protein has a predicted molecular mass of 38 kDa, comprised of 296 amino acids, and its gene is located on mouse chromosome 5. Comparison of the mPARM-1 sequence with other GenBank entries revealed a 91% correlation with rat PARM-1 (NM_173114). Pfam analysis showed that mPARM-1 contains both a retinoblastoma-like domain and a transmembrane domain.

The expression levels of mPARM-1 mRNA in various mouse tissues, adipocytes, and S-V cells

To examine its tissue distribution, SQ RT-PCR was performed for mPARM-1 against on tissues in the brain,

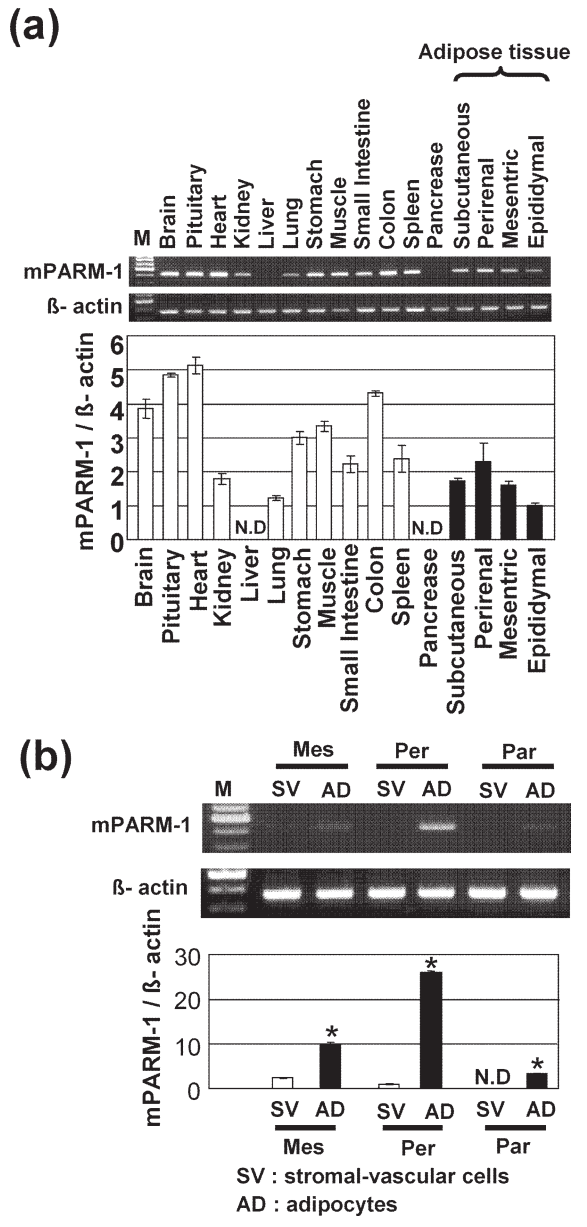

Fig. 2. The expression levels of mPARM-1 mRNA in various mouse tissues, adipocytes, and S-V cells.
(a) The expression levels of mPARM-1 mRNA in various tissues of six 15-week-old male mice. Upper panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1 and β-actin. M: 100-bp molecular size ladder. Lower panel: data normalized using β-actin mRNA and expressed as a fold of the value obtained for epididymal tissue. All data are represented as means ± SEM of six animals. N.D: None detected.
(b) The expression levels of mPARM-1 mRNA in adipocytes and S-V cells isolated from three adipose tissue types from female mice. Upper panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1 and β-actin. Lower panel: data normalized using β-actin mRNA and expressed as a fold of the value obtained for S-V cells. All data are represented as means ± SEM of three independent experiments. $^*P < 0.05$ vs. S-V cells. Mes: mesenteric; Per: perirenal; Par: parametrial; N.D: None detected.

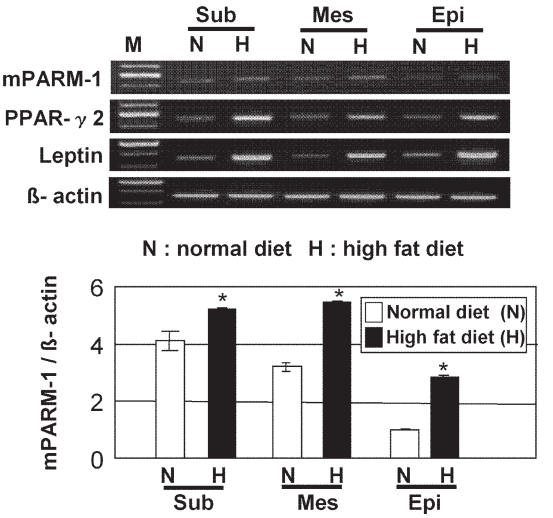

Fig. 3. The expression levels of mPARM-1 mRNA in adipose tissues of six male mice fed normal vs. high-fat diets. Upper panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1, PPAR-γ2, leptin, and β-actin. Lower panel: data normalized using β-actin mRNA and expressed as a fold of the value obtained for epididymal adipose tissue of mice fed the normal diet. All data are represented as the mean ± SEM of the six mice. $^*P < 0.05$ vs. mice fed normal diet. Sub: subcutaneous adipose; Mes: mesenteric adipose; Epi: epididymal adipose.

pituitary, heart, kidney, liver, lung, stomach, muscle, colon, spleen, small intestine, pancreas, and four different white adipose tissues (Fig. 2a). In terms of non-adipose tissues, mPARM-1 was expressed strongly in the heart, pituitary, brain, and colon, and was expressed to a lesser degree in the kidney, lung, stomach, muscle, small intestine, and spleen. Interestingly, mPARM-1 mRNA was higher in subcutaneous, perirenal, and mesenteric adipose tissues than in epididymal adipose tissue.

To investigate whether mPARM-1 was expressed in isolated adipocytes and S-V cells, we examined adipocytes prepared from adipose tissues of 26-week old female mice. As shown in Fig. 2b, the mPARM-1 mRNA was undetectable in S-V cells; it was expressed mainly in the adipocytes.

The expression levels of mPARM-1 mRNA in adipose tissue of mice fed normal and high fat diets

Understanding how the expression of a gene is regulated in varying nutritional states may provide valuable clues about its functional relevance in metabolic disease states. Accordingly, mPARM-1 gene expression was examined in adipose tissues from mice fed high-fat and normal diets. As previously reported (Hong et al. 2005b), after 11 weeks on a high-fat diet, the body weight of mice was 30% greater than that of mice that had been fed a normal diet. In addition, the weight of the three different fat pads of these animals was approximately 2-3 times greater than those of mice fed the normal diet. RT-PCR analysis showed that mPARM-1 was significantly up-regulated in the three adipose tissues of

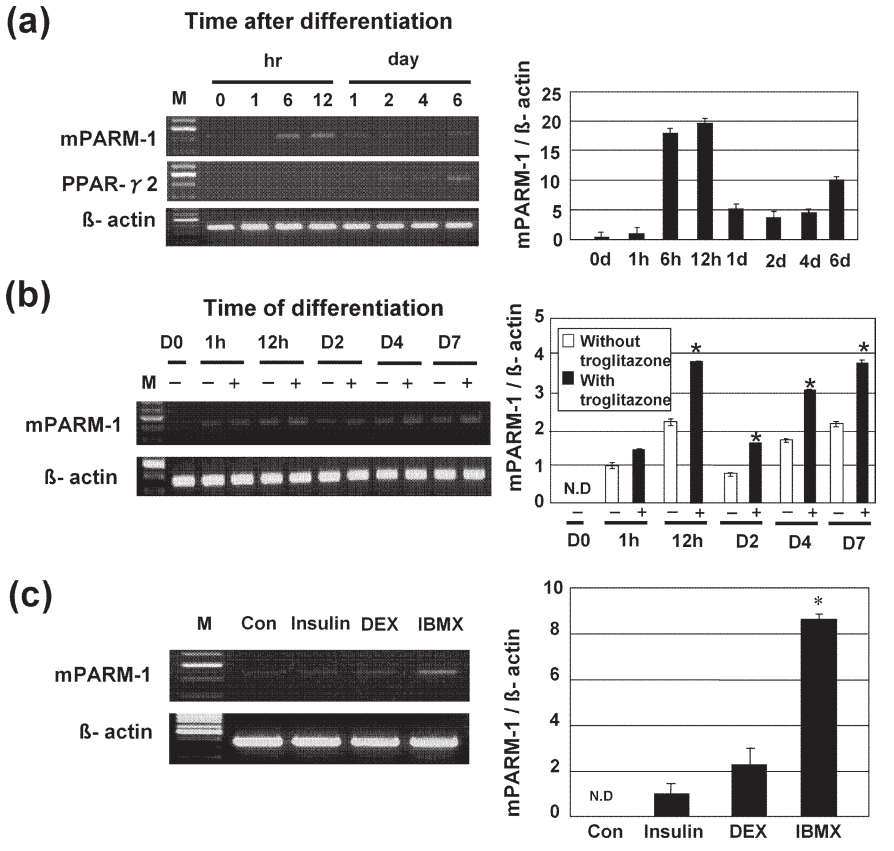

Fig. 4. The expression levels of mPARM-1 mRNA during differentiation of 3T3-L1 preadipocytes.
(a) Levels of mPARM-1 and PPAR-γ2 mRNA during the differentiation of 3T3-L1 cells. Total RNA was extracted from 0 h to day 6 after the differentiation medium was changed. Left panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1, PPAR-γ2, and β-actin on days of differentiation indicated at the top. Right panel: data normalized using β-actin mRNA, and expressed as a fold of the value obtained at day 0. All data are represented as the mean ± SEM of three independent experiments.
(b) Levels of mPARM-1 mRNA in 3T3-L1 adipocytes treated with (+) or without (-) troglitazone (5 μM) during differentiation. Left panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1 and β-actin on days of differentiation indicated at the top. Right panel: data normalized using β-actin mRNA, and expressed as a fold of the value obtained at 1 h with no treatment. All data are represented as the mean ± SEM of three independent experiments. N.D: None detected; *$P < 0.05$ vs. no treatment.
(c) Levels of mPARM-1 mRNA in 3T3-L1 cells treated with insulin, DEX, and IBMX. 3T3-L1 cells were proliferated to confluence, and were then treated with 1.7 μM insulin, 1 μM Dex, and 0.5 mM IBMX for 2 days (day 0). Total RNA was then extracted. Left panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1 and β-actin. Right panel: data normalized using β-actin mRNA, and expressed as a fold of the value obtained at day 0 with insulin treatment. All data are represented as the mean ± SEM of three independent experiments. *$P < 0.05$ vs. Control.

mice fed the high-fat diet (Fig. 3). The expressions of PPAR-γ2, leptin mRNA, and adipose tissue specific genes were also increased in the high-fat diet group as compared to the normal diet group.

The expression levels of mPARM-1 mRNA during adipocyte differentiation of 3T3-L1 preadipocytes

To examine the expression pattern of mPARM-1 during the process of adipocyte differentiation, we examined mPARM-1 mRNA levels in 3T3-LI cells during differentiation (Fig. 4a). Our results demonstrated that mPARM-1 levels began to rise during the 0 day of treatment with a differentiation mixture, showed a spike in expression at 12 hr, and returned to a lower level by 24 h, followed by high levels in mature adipocytes. Notably, the induction of mPARM-1 expression at 12 hr preceded the induction of PPAR-γ2 expression at 24 h, suggesting that mPARM-1 functions upstream of PPAR-γ2.

To investigate how the expression of PPAR-γ2 is related to the expression of mPARM-1 mRNA, we treated 3T3-L1 cells with troglitazone during adipocyte differentiation. Troglitazone treatments stimulated PPAR-γ2 mRNA expression during differentiation for 7 days, during which time levels of mPARM-1 mRNA became significantly up-regulated compared with the no treatment group (Fig. 4b).

To examine the effects of adipogenic stimulation reagents on mPARM-1 expression, we treated confluent preadipocytes with insulin, DEX, or IBMX for 2 days. As shown in Fig. 4c, the mPARM-1 gene was significantly

induced by IBMX, but not by insulin or DEX.

The expression levels of mPARM-1 mRNA in 3T3-L1 adipocytes treated with TNF-α

TNF-α is known to induce lipolysis and apoptosis in adipocytes. To investigate the relationship between mPARM-1 and TNF-α, we treated the differentiated 3T3-L1 adipocytes with TNF-α (10 ng/ml) for 24 h. TNF-α significantly decreased the expression levels of mPARM-1, PPAR-γ2, leptin, and adipogenin mRNAs (Fig. 5).

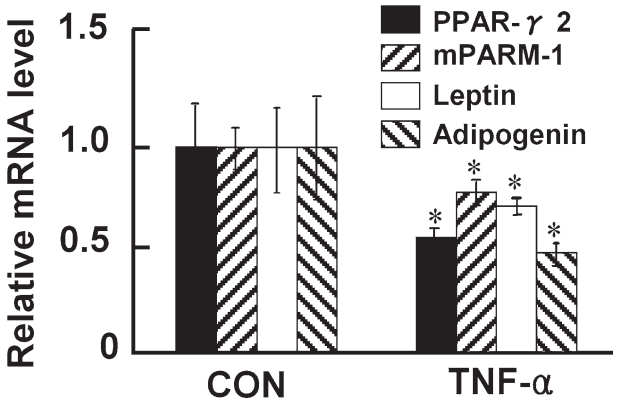

Fig. 5. Effects of TNF-α on the expression of mPARM-1 mRNA in differentiated 3T3-L1 adipocytes. After 10 days of differentiation, the 3T3-L1 adipocytes were treated with TNF-α (10ng/ml) for 24 h. Total RNA was then extracted. Data were normalized using β-actin mRNA and expressed as fold of the value obtained with control. $^{*}P < 0.05$ vs. control.

Inhibitory effects of mPARM-1 siRNA during adipocyte differentiation

In 3T3-L1 cells, mPARM-1 siRNA silences its target mRNA specifically and effectively. The number of oil red O staining cells was dramatically less in mPARM-1 siRNA-transfected cells (Fig. 6a). To determine if the reduction of mPARM-1 involves an alteration in the expression of genes encoding a key adipogenic transcription factor, we analyzed the expressions of PPAR-γ2 and aP2 during the differentiation of mPARM-siRNA transfected cells. PPAR-γ2 and aP2 mRNA levels declined in these cells compared to controls (Fig. 6b).

Discussion

Our results demonstrate that mPARM-1 may be involved or required in the development of adipose tissues and in the adipocyte differentiation process. By expressing a variety of proteins involved in lipid metabolism as well as several other molecules, adipose tissue plays a key role in energy regulation. Up-regulation of mPARM-1 mRNA occurred, in three different white fats, in mice fed a high-fat diet compared to those fed a control diet. Furthermore, mPARM-1 mRNA levels were low in S-V cell samples, but were high in adipocyte samples.

The interesting point to consider is that regulation of mPARM-1 in the transcription network controls adipocyte differentiation. This study showed that mPARM-1 mRNA expression was temporally localized in the initial stages of adipogenesis, and preceded PPAR-γ2 induction. Pfam search and analysis demonstrated that mPARM-1 had a reti-

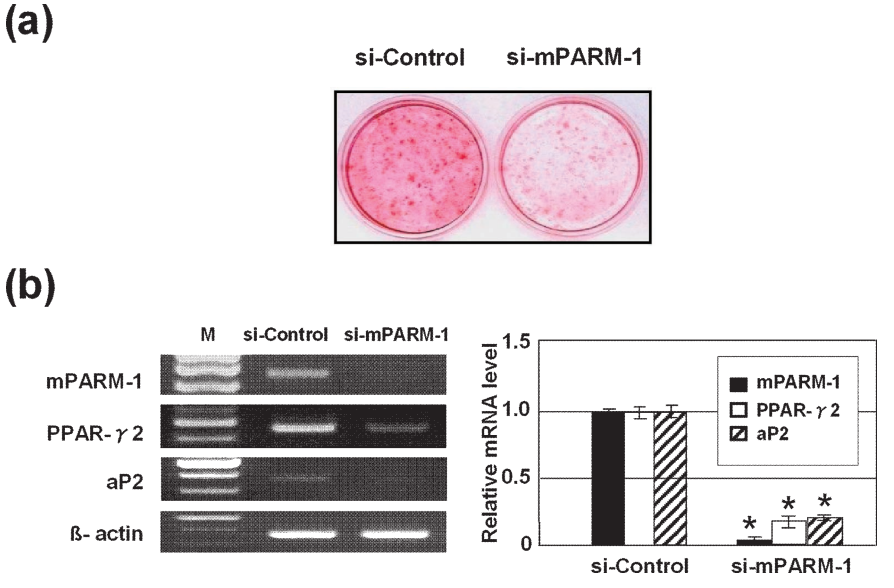

Fig. 6. Adipocyte differentiation in mPARM-1 siRNA-transfected 3T3-L1 cells. (a) 3T3-L1 cells cultured and transfected with 50 nM scrambled siRNA (control) and mPARM-1 siRNA (si-mPARM-1) prior to induction of cell differentiation. After 7 days, the cells were fixed and stained with Oil-Red-O to detect oil droplets. (b) The levels of mPARM-1, PPAR-γ2, and aP2 mRNA in 3T3-L1 cells transfected with mPARM-1 and scrambled siRNAs during differentiation for 7 days. Left panel: representative ethidium bromide-stained agarose gel showing amplified mPARM-1, PPAR-γ2, aP2, and β-actin. Right panel: data normalized using β-actin mRNA and expressed as a fold of the value obtained for controls. All data are represented as the mean ± SEM of three independent experiments. $^{*}P < 0.05$ vs. control.

noblastoma (RB)-like domain and a transmembrane domain. In this regard, the protein most closely related to mPARM-1 is RB. Recently, other roles in adipogenesis have been attributed to RB: attenuating the transcriptional activity of PPAR, avoiding adipocyte hypertrophy (Fajas 2003), and determining white versus brown adipocyte differentiation (Hansen et al. 2004). Of note, the gene for an RB-like protein, mPARM-1, is transiently expressed during major cell cycle-accompanied adipocyte differentiation.

It is well known that insulin, DEX, and IBMX are essential in the process of the 3T3-L1 cell adipocyte differentiation. IBMX, a potent cyclic nucleotide phosphodiesterase inhibitor, increases cyclic AMP in tissue, thereby activating cyclic nucleotide-regulated protein kinases (Elks et al. 1983). The fact that mPARM-1 is controlled by IBMX indicates that the activation process of cAMP may be related to the expression of mPARM-1.

TNF-α is known to inhibit the differentiation of preadipocytes to adipocytes (Xing et al. 1997). The addition of TNF-α suppressed the expression of PPAR-γ2, leptin, and adipogenin mRNAs, as well as the expression of mPARM-1 mRNA. This is further proof that mPARM-1 is among the genes regulated during the adipocyte regulation process.

In our functional analysis of mPARM-1 using the siRNA method, we found that when mPARM-1 expression was knocked down during adipogenesis, oil droplet accumulation decreased, and the level of PPAR-γ2 and aP2 mRNA declined during adipocyte differentiation. These results suggest that mPARM-1 plays an essential role in the transcriptional cascade during adipogenesis.

In conclusion, our results provide evidence that mPARM-1 mRNA is expressed in adipose tissues and during adipocyte differentiation as well. Furthermore, the level of mPARM-1 mRNA is up-regulated along with other adipogenic genes during adipose tissue development. Thus, we propose that mPARM-1 expression may be required for adipocyte function.

Acknowledgments

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