FEBS Letters 587 (2013) 3032–3037

journal homepage: www.FEBSLetters.org

LMO4 modulates proliferation and differentiation of 3T3-L1 preadipocytes

Ning Wang *, Xichen Wang, Mingxin Shi, Hongyan Shi, Xiaohong Yan, Hui Li, Shouzhi Wang, Yuxiang Wang

College of Animal Science and Technology, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin, Heilongjiang 150030, China

ARTICLE INFO

Article history:
Received 10 June 2013
Revised 6 July 2013
Accepted 14 July 2013
Available online 24 July 2013

Edited by Laszlo Nagy

Keywords:
LMO4
3T3-L1 preadipocyte
Cell proliferation
Preadipocyte differentiation

ABSTRACT

Previous microarray analyses revealed that LMO4 is expressed in 3T3-L1 preadipocytes, however, its roles in adipogenesis are unknown. In the present study, using RT-PCR sequencing and quantitative real-time RT-PCR, we confirmed that *LMO4* gene is expressed in 3T3-L1 preadipocytes and its expression peaks at the early stage of 3T3-L1 preadipocyte differentiation. Further analyses showed that LMO4 knockdown decreased the proliferation of 3T3-L1 preadipocytes, and attenuated the differentiation of 3T3-L1 preadipocytes, as evidenced by reduced lipid accumulation and down-regulation of *PPARγ* gene expression. Collectively, our findings indicate that LMO4 is a novel modulator of adipogenesis.

1. Introduction

LIM-only protein 4 (LMO4) belongs to LIM only protein family of four mammalian nuclear LMO proteins characterized by two tandem LIM domains and no other functional domains [1]. LMO proteins function as a molecular adaptor for protein–protein interactions, and all LMO proteins interact with a transcriptional cofactor of LIM domains (Clim)/LIM domain binding protein (Ldb)/Nuclear LIM interactors (Nli), which has no known enzymatic or DNA binding activity. LMO/Clim complexes regulate gene expression via association with LIM homeodomain factors and some other DNA-binding proteins [1]. LMO proteins play critical roles in mammalian development and their deregulation is linked to oncogenesis [2].

LMO4 shares only 50% homology with the LIM domains of other LMO proteins. It regulates signaling activity and gene expression by interacting with multiple signaling molecules and transcription factors [3–7], and plays critical roles in neuron, skin, mammary gland and retina development. LMO4 is highly expressed in human breast tumors [8], prostate cancer [9], small cell lung carcinomas [10] and pancreas cancer [11], and implicated in the cause or progression of these cancers. LMO4 promotes cell proliferation of several breast cancer cell lines and normal mammary epithelial cells [12,13], and inhibits differentiation of mammary epithelial cells in vitro [8,13].

Adipogenesis involves cell proliferation and differentiation. Adipocyte differentiation is regulated by multiple transcription factors, coregulators and miRNAs [14–17]. Transcription factor peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) are recognized as master regulators of adipogenesis. PPARγ and C/EBPα act in concert to orchestrate the differentiation of preadipocytes into adipocytes. PPARγ is both necessary and sufficient for adipocyte differentiation [15]. Ectopic expression of PPARγ is sufficient to induce adipocyte differentiation in fibroblasts, and no factor has been reported to promote adipogenesis in the absence of PPARγ [15]. On the other hand, C/EBPα-deficient cells are capable of cell differentiation [15]. Recent studies have demonstrated that a number of new transcription factors, coregulators and miRNAs are involved in the regulation of adipogenesis [18,19].

The mouse 3T3-L1 preadipocyte cell line has been an ideal in vitro system for dissecting the underlying mechanism of adipogenesis [20]. The entire process of 3T3-L1 preadipocyte differentiation consists of several stages. First, proliferating preadipocytes become growth-arrested by contact inhibition. Second, after hormone induction, the growth-arrested 3T3-L1 preadipocytes reenter the cell cycle and undergo several rounds of cell division, a process known as mitotic clonalexpansion (MCE). Finally, after MCE, the cells become quiescent and express genes which lead to terminal

* Corresponding author.
E-mail address: wangning@neau.edu.cn (N. Wang).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.07.033

adipocyte differentiation [21]. Microarray data have shown that LMO4 is expressed in 3T3-L1 preadipocytes, and its expression peaks on day 1 of 3T3-L1 preadipocyte differentiation [22,23]. Moreover, a recent genome-wide profiling of histone H3 lysine 56 (H3K56) acetylation in human mesenchymal stem cell-derived adipocytes revealed that *LMO4* gene is highly acetylated at H3K56 [24]. Together, these data suggest the existence of a biological relevance of LMO4 in adipogenesis. However, the role and significance of LMO4 in adipogenesis has not been explored in vitro. In the present study, we first confirmed LMO4 expression in 3T3-L1 preadipocytes by RT-PCR and sequencing, and then investigated therole of LMO4 in the proliferation and differentiation of 3T3-L1 preadipocytes using siRNA approach. Our findings indicate LMO4 modulates proliferation and differentiation of 3T3-L1 preadipocytes.

2. Materials and methods

2.1. Cell culture and differentiation

Mouse 3T3-L1 preadipocytes were purchased from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and were grown in Dulbecco's modified Eagle's medium (no pyruvate, high glucose formulation; Gibco-BRL) supplemented with 10% heat inactivated fetal bovine serum (FBS; Gibco) and incubated in a humidified atmosphere of 5% CO_2 at 37 °C, with media changes every other day. For 3T3-L1 differentiation, 3T3-L1 preadipocytes were grown to confluence, two days after reaching confluence, cells were induced to differentiate (designated as day 0) by adding standard differentiation cocktail (DMI) consisting of 1 μM dexamethasone (Dex) (Sigma–Aldrich), 0.5 mM 1-methyl-3-isobutylxanthin (IBMX) (Sigma–Aldrich) and 10 μg/ml insulin (Sigma–Aldrich) to the culture medium. After 2 days, the medium was replaced with DMEM containing 10% FBS and 10 μg/ml insulin, and the cells were incubated for another 2 days. Thereafter, until the cells were fully differentiated, the cells were maintained in DMEM with 10% FBS, with media changes every other day.

2.2. RNA isolation, cDNA synthesis and cloning

Total RNA was isolated from proliferating or differentiating 3T3-L1 preadipocytes using Trizol reagent (Invitrogen) following the manufacturer's instructions, and RNA quality was assessed by agarose gel electrophoresis. The cDNA synthesis was performed by using 1 μg of total RNA, oligo (dT) primer (Promega), and reverse transcriptase (Promega) according to the manufacturer's protocol. The cDNAs were subjected to partial cloning and subsequent quantitative real-time PCRs. PCR conditions for the partial cloning of *LMO4* cDNA were 95 °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 finally a cycle at 72 °C for 10 min. Ex Taq polymerase (TaKaRa, Dalian, China) was used for PCR amplification of *LMO4*. Primer sequences for cloning the partial cDNA of mouse *LMO4* were as follows: sense 5′-GACCGCTTTCTGCTCTATG-3′, antisense 5′-AGTAGTGGATTGCTCTGAAG-3′. The PCR products were separated by electrophoresis on a 1.5% agarose gel, gel purified using an AxyPrep™ DNA Gel Extraction Kit (AxyPrep Biosciences), and cloned into pMD18T-Vector (TaKaRa, Dalian, China) and sequenced (Invitrogen).

2.3. siRNA oligoduplexes and transfection

Small interfering RNA (siRNA) oligo for LMO4 and negative control were synthesized and annealed by GenePharma (Shanghai, China). The sequences of each oligoduplex were as follows: LMO4-specific siRNA (siLMO4), 5′-GAUCGGUUUCACUACAUCAdTdT-3′, and negative control siRNA (ncLMO4), 5′- GUCCAUUUCUCGGCGUUAAdTdT-3′. 3T3-L1 preadipocytes were transfected with siLMO4 or ncLMO4 using DharmaFECT3 (Dharmacon) according to the manufacturer's protocols. Transfection efficiency was assessed by real-time PCR analysis of *LMO4* expression levels in the transfected cells.

2.4. Quantitative real-time PCR

Real-time PCR was performed on a 7500 real-time PCR System (Applied Biosystem) using SYBR Premix Ex Taq (TaKaRa, Dalian, China). The PCR conditions were 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. The primer sequences usedfor quantitative real-time PCR were as follows: *LMO4* (sense 5′-GACCGCTTTCTGCTCTATG-3′, antisense 5′-AGTAGTGGATTGCTCTGAAG-3′), *cyclin D1* (sense 5′-GCGTACCCTGACACCAATC-3′, antisense 5′-CCTCCTCTTCGCACTTCTG-3′), *cyclin E* (sense 5′-GCCTCGGAAAATCAGACCA-3′, antisense 5′-CCATTAGCCAATCCAGAAGAAC-3′, *Cullin*-3 (sense 5′-CGGGATATTGGCCTACTCA-3′, antisense 5′-GACCATAAAAGGTGGCATTG-3′), *C/EBPβ* (sense 5′- TTTCGGGACTTGATGCAATC-3′, antisense 5′-CGCAGGAACATCTTTAAGGTGT-3′), *PPARγ* (sense 5′-CGGTTTCAGAAGTGCCTTC-3′, antisense 5′-CCGCCAACAGCTTCTCCTT-3′), *aP2* (sense 5′-TGAAGAGCATCATAACCCTA-3′, antisense 5′-TCATAACACATTCCACCACC-3′), *β-actin* (sense 5′-GCCACATCGCTCAGACAC-3′, antisense 5′-CATCACGCCACAGTTTCC-3′). All the primers were designed to span genomic introns to avoid the amplification of contaminated genomic DNA. Gene expression was normalized with *β-actin* expression level. Quantitative real-time PCR analyses were performed in triplicates and repeated at least three times.

2.5. Protein isolation and Western blot analysis

Cultured cells were washed twice with PBS, and lysed using RIPA Buffer (Beyotime Institute of Biotechnology, Jiangsu, China) supplemented with 1% Phenylmethanesulfonyl fluoride (PMSF; Beyotime Institute of Biotechnology). Lystates were fractionated on 12.5% SDS–PAGE gel and transferred onto Immun-Blot PVDF membranes (Millipore). The membranes were blocked with 5% nonfat dry milk in PBST (PBS with 0.05% Tween-20) for 1 h at room temperature, and immunoblotted with primary antibodies against cyclin D1 (1:1000 dilution; Beyotime Institute of Biotechnology, Jiangsu, China) or β-actin (1:1000 dilution; Beyotime Institute of Biotechnology, Jiangsu, China) at room temperature for 1 h. The blots were washed four times with PBST, incubated with goat anti-mouse IgG conjugated with horseradish peroxidase (1:5000) (ZSGB-BIO, Beijing, China) in PBST for 1 h at room temperature, and then washed four times with PBST for 20 min. The immunoreactive bands were visualized using an ECL Plus detection kit (HaiGene Biotechnology, Harbin, China) and exposed to X-ray film. Each Western blot was performed at least three times.

2.6. Cell proliferation assay

Cell proliferation was assessed by MTT assay. 3T3-L1 preadipocytes were seeded at a density of 2500 cells/well into 96-well plates in culture medium. After 24 h of culture, cells were transfected with siLMO4 or ncLMO4. At each designated time points after transfection, 20 μl MTT reagent (5 mg/ml; Invitrogen) was added to each well and incubated for 4 h at 37 °C in the dark. Following the incubation, culture medium was replaced with DMSO (150 μl/well), and plates were vibrated for 10 min. When MTT-formanzan crystals were solubilized, the absorbance was measured at a wavelength of 490 nm with an ELISA plate Reader.

2.7. Oil Red O staining

Culture medium was removed from the plate and cells were washed twice with PBS and fixed in 10% paraformaldehyde in PBS for 30 min at room temperature. Then cells were rinsed three times with PBS and distilled water, and stained with Oil Red O staining solution (0.5% Oil Red O in isopropanol, diluted 3:2 in water and filtered with a 0.22 μm filter), at room temperature for 40 min. After staining, the cells were washed three times with PBS and photographed. Alternatively, for a quantitative assay, Oil Red O dye was extracted with isopropanol and the absorbance was measured at 510 nm wavelength with a UV spectrophotometer (Ultrospec 1000, Biochrom Ltd., Cambridge, UK).

2.8. Statistical analysis

The results are presented as means ± standard deviations. Student's two tailed *t*-test was performed for comparison of differences between experimental and control groups. A P value of <0.05 (*) was considered significant; a P value of <0.01 (**), very significant.

3. Results

3.1. Expression of LMO4 in 3T3-L1 preadipocytes

Microarray gene expression analysis revealed that *LMO4* was expressed in 3T3-L1 preadipocytes [22,23]; however, this finding has not yet been validated by other techniques. Therefore, we first validated *LMO4* gene expression in 3T3-L1 preadipocyte samples using RT-PCR and sequencing. Based on *LMO4* mRNA sequence (NM_001161769.1), we designed a pair of specific primers for *LMO4* mRNA expression and performed RT-PCR using the total RNA extracted from three randomly chosen samples of 3T3-L1 preadipocytes. RT-PCR products yielded the expected amplified fragment of 248 bp in all the three samples (Fig. 1A). Sequence analysis showed that the amplified fragment shared 99.5% identity with *LMO4* mRNA sequence (NM_001161769.1) (Fig. 1B). Taken together, our data confirm that LMO4 is expressed in 3T3-L1 preadipocytes. There was one single nucleotide difference between the amplified LMO4 RT-PCR fragment and *LMO4* mRNA sequence. The nucleotide difference was located in the coding region of *LMO4* gene and caused an amino acid change of serine to proline at residue 152. The amino acid change, which was located at the extreme C-terminus of LMO4, did not reside in any known functional domains and sites of LMO4, including LIM domains, Lbd1 binding site, and Zn binding site.

3.2. Impact of LMO4 knockdown on proliferation of 3T3-L1 preadipocytes

Adipogenesis involves in cell proliferation and differentiation. In order to investigate the functional roles of LMO4 in 3T3-L1 adipogenesis, we first used small interfering RNA (siRNA) to assess the role of LMO4 in the proliferation of 3T3-L1 preadipocytes. Before we performed the experiment, we functionally tested transfection efficacy of siRNA-mediated knockdown of LMO4. Quantitative real-time RT-PCR analysis showed that transfection with LMO4-specific siRNA (siLMO4) significantly reduced *LMO4* mRNA expression in 3T3-L1 preadipocytes at 48 h after transfection, on average, by around 80% compared with the control siRNA (ncLMO4), ($P < 0.01$; Fig. 2A), confirming the utility of this approach. We then addressed whether LMO4 regulates proliferation of 3T3-L1 preadipocytes by siRNA-mediated suppression of LMO4. The impact of LMO4 silencing on 3T3-L1 preadipocyte proliferation was assessed using the MTT assay. As shown in Fig. 2B, siLMO4 significantly reduced cell proliferation in proliferating 3T3-L1 preadipocytes on days 2, 3, and 4 post siRNA transfection, compared to ncLMO4 ($P < 0.05$). LMO4 promotes cell proliferation of several breast cancer cell lines and normal mammary epithelial cells [12,13]. Recently, it has been shown that LMO4 is a general cell cycle regulator, and it is required for sustained expression of key cell cycle regulators: cyclin D1 and cullin-3 [25]. To understand the underlying mechanism by which LMO4 regulates 3T3-L1 preadipocyte proliferation, we evaluated the impact of LMO4 knockdown on the expression of *cyclin D1*, *cyclin E* and *cullin*-3 in 3T3-L1 preadipocytes using quantitative real-time RT-PCR. The results showed that LMO4 knockdown significantly reduced the mRNA expression of *cyclin D1* compared to ncLMO4 ($P < 0.05$; Fig. 2C). We also observed a trend for decreasing expression of *cyclin E* and *cullin-3* in siLMO4-transfected cells compared to ncLMO4-transfected cells, but the differences did not reach statistical significance ($P > 0.05$; Fig. 2C). Consistently, Western blot analysis also confirmed that LMO4 Knockdown reduced cyclin D1 expression (Fig. 2D). Our findings suggest that LMO4 may promote proliferation of 3T3-L1 preadipocytes via upregulation of cyclin D. Overall, these results demonstrate that LMO4 knockdown inhibits proliferation of 3T3-L1 preadipocytes and suggest its involvement in the regulation of 3T3-L1 preadipocyte proliferation.

3.3. LMO4 expression pattern during adipocyte differentiation of 3T3-L1 preadipocytes

Microarray analysis showed that *LMO4* expression peaked on day 1 of 3T3-L1 preadipocyte differentiation [22,23]. To confirm the microarray results, we analyzed *LMO4* expression pattern dur-

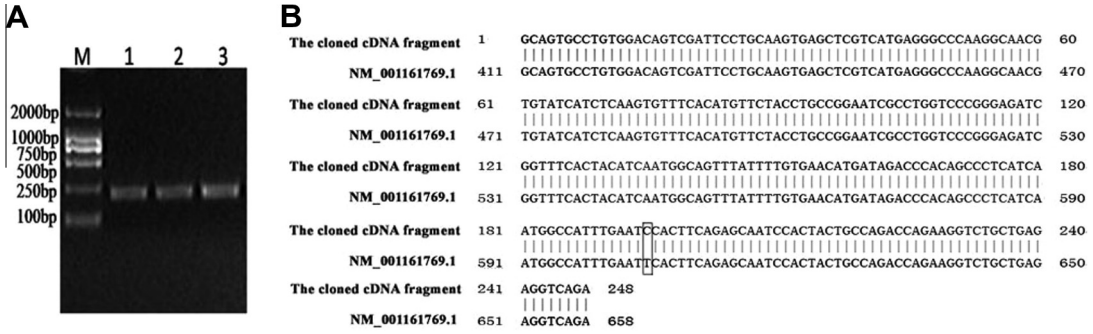

Fig. 1. Expression of *LMO4* in 3T3-L1 preadipocytes. (A) Reverse transcription-PCR analysis of *LMO4* mRNA expression in three randomly chosen samples of 3T3-L1 preadipocytes. M: DL2000 marker; lanes 1–3: *LMO4* RT-PCR products from three randomly chosen samples of 3T3-L1 preadipocytes. (B) Sequence alignment of the amplified *LMO4* RT-PCR product and mouse *LMO4* mRNA sequence (NM_001161769). Conserved nucleotides are indicated as vertical lines, and nucleotide differences are indicated as open boxes.

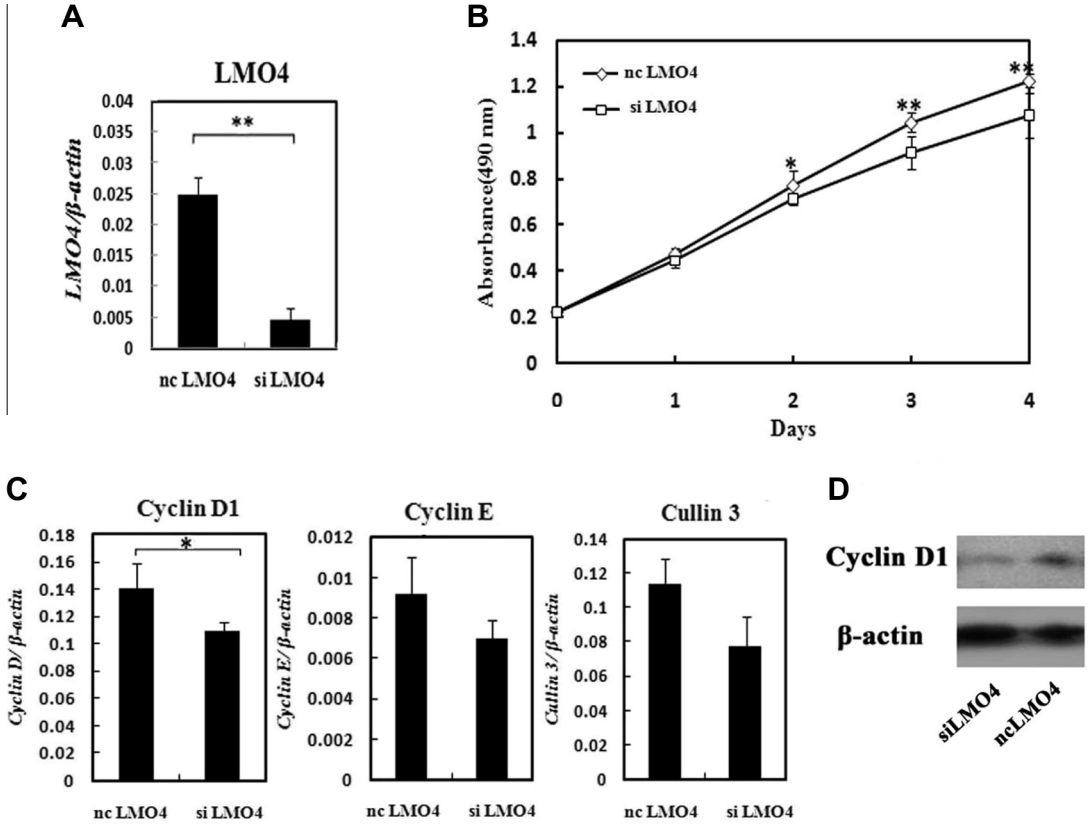

Fig. 2. LMO4 knockdown reduces 3T3-L1 preadipocyte proliferation. (A) Quantitative real-time RT-PCR analysis for *LMO4* mRNA knockdown efficiency in 3T3-L1 preadipocytes. After transfection with LMO4-specific siRNA (siLMO4) or negative control siRNA (ncLMO4), 3T3-L1 preadipocytes were cultured for 48 h. Total RNA was extracted, and gene expression was analyzed by quantitative real-time RT-PCR, and expression levels were normalized to *β-actin*, and the results were expressed as means ± standard deviations for three independent wells. (B) The Impact of LMO4 knockdown on 3T3-L1 preadipocyte proliferation. 3T3-L1 preadipocytes were transfected with siLMO4 or ncLMO4, respectively, and MTT assay was used to evaluate the proliferation of 3T3-L1 preadipocytes at the designated time points. Results were presented as means ± standard deviations for five independent wells. (C) Impact of LMO4 knockdown on the expression of *cyclin D1*, *cyclin E*, and *cullin-3* in 3T3-L1 preadipocytes. 3T3-L1 preadipocytes were transfected with siLMO4 or ncLMO4, and gene expression was analyzed by quantitative real-time RT-PCR. Expression levels were normalized to *β-actin*, and the results were expressed as means ± standard deviations for three independent wells. (D) Western blotting analysis of the impact of LMO4 knockdown on cyclin D1 protein expression in 3T3-L1 preadipocytes. β-Actin was used as a protein loading control. Asterisk (*) indicates $P < 0.05$; double asterisks (**) indicates $P < 0.01$ in Student's two tailed *t*-test.

ing the course of 3T3-L1 preadipocyte differentiation using quantitative real-time RT-PCR. As expected, the early differentiation marker gene, *C/EBPβ*, was highly expressed at the early stage of adipocyte differentiation, and the late differentiation marker genes, *PPARγ* and *aP2*, were highly expressed at the late stage of differentiation (Fig. 3), evidencing for successful adipogenic differentiation. As shown in Fig. 3, quantitative real-time RT-PCR analysis revealed that *LMO4* expression peaked on day 1 of 3T3-L1 differentiation (eightfold and fourfold increases compared to day 0 and day 2, respectively), which confirmed the previous microarray result [22,23].

3.4. Impact of LMO4 knockdown on 3T3-L1 preadipocyte differentiation

LMO4 expression fluctuates during 3T3-L1 preadipocyte differentiation, suggesting LMO4 may play a role in preadipocyte differentiation. To explore the role of LMO4 in 3T3-L1 preadipocyte differentiation, we explored the impact of *LMO4* knockdown on 3T3-L1 preadipocyte differentiation using oil red O staining for lipid droplets. As shown in Fig. 4A and B, Oil red O staining and its quantification clearly showed that LMO4 knockdown attenuated 3T3-L1 preadipocyte differentiation from day 4 onward, as demonstrated by the presence of fewer lipid accumulating cells. Consistent with oil red O staining results, at day 8 of preadipocyte differentiation, *PPARγ* mRNA expression was reduced in siLMO4-transfected cells compared with ncLMO4-tranfected cells (Fig. 4C) ($P < 0.05$). Taken together, our data indicate that LMO4 knockdown partially inhibits 3T3-L1 preadipocyte differentiation, thus suggesting that LMO4 is a positive regulator of 3T3-L1 preadipocyte differentiation.

4. Discussion

In the present study, we confirmed the previous microarray reports that the LMO4 is expressed in 3T3-L1 preadipocytes and demonstrated that LMO4 knockdown decreases the proliferation of 3T3-L1 preadipocytes, and attenuates 3T3-L1 preadipocyte differentiation. To our knowledge, this is first report that LMO4 modulates proliferation and differentiation of 3T3-L1 preadipocytes.

Previous studies have shown that LMO4 regulates cell proliferation in mammary gland development and several breast cancer cell lines [12,13]. Mammary gland-specific knockout LMO4 results in reduced lobuloalveolar development with a concomitant decrease in cell proliferation [26], and targeted overexpression of LMO4 in mouse mammary glands induceshyperplasia and tumors [13]. Moreover, the expression of the Engrailed-LMO4 fusion protein inhibits cell growth of human normal mammary epithelial cells [12]. Silencing of LMO4 in several breast cancer cell lines and HEK-293T leads to markedly reduced proliferation, accompa-

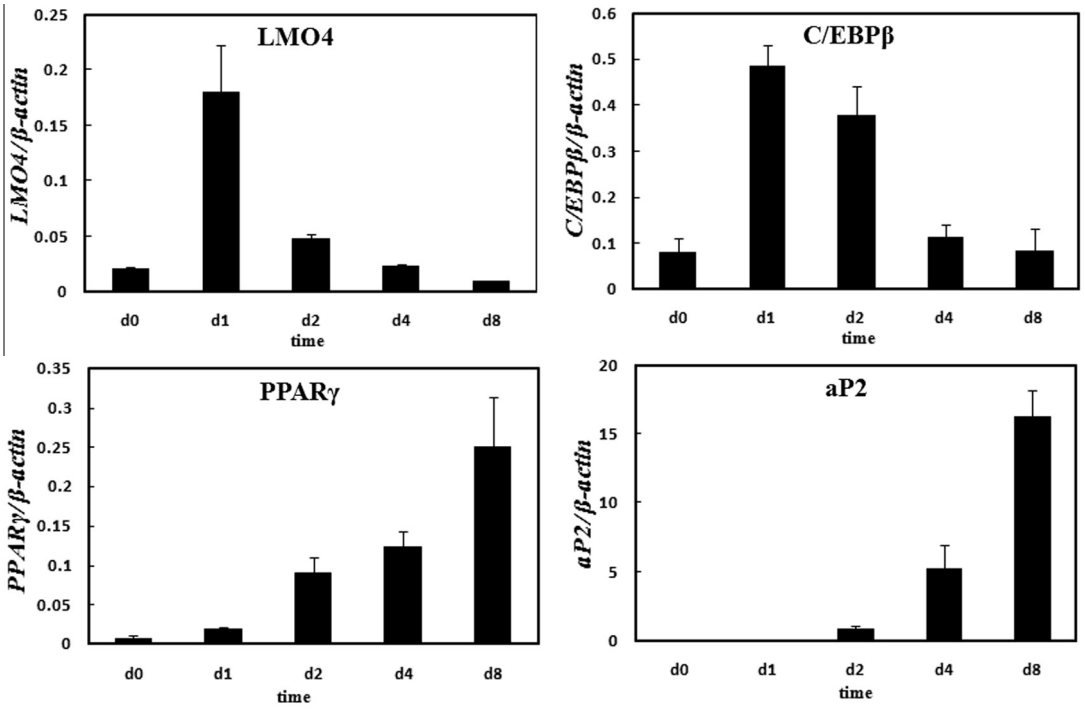

Fig. 3. The mRNA expression of *LMO4* and adipocyte differentiation marker genes (*C/EBPβ* and *PPARγ*) during 3T3-L1 preadipocyte differentiation. Gene expression was analyzed by quantitative real-time RT-PCR. Expression levels were normalized to *β-actin*, and the results were expressed as mean ± standard deviations for three independent wells.

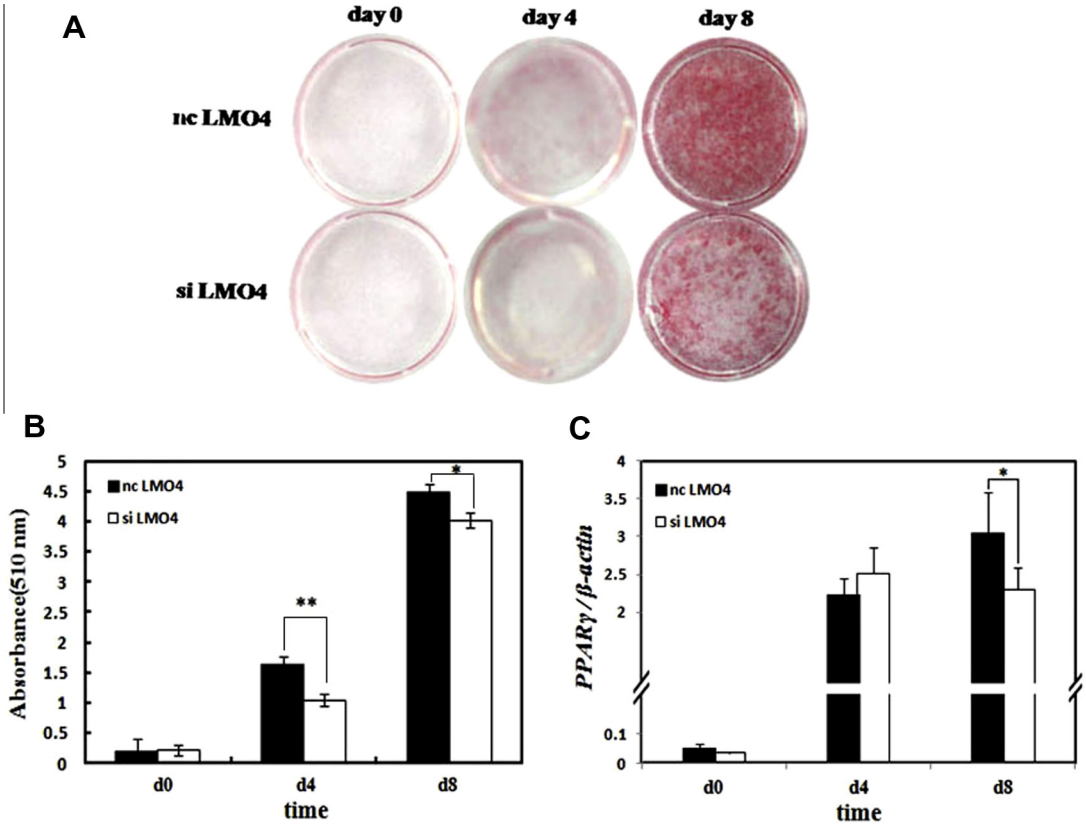

Fig. 4. Impact of LMO4 knockdown on 3T3-L1 preadipocyte differentiation. (A) Oil Red O staining of 3T3-L1 preadipocytes transfected with siLMO4 or ncLMO4 at the designated time points after differentiation. (B) Quantification of lipid accumulation in the differentiating 3T3-L1 preadipcytes on days 0, 4, and 8 of differentiation. (C) Impact of LMO4 knockdown on the expression of adipocyte differentiation marker genes. 3T3-L1 preadipocytes transfected with siLMO4 or ncLMO4, were induced to differentiate with DMI, total RNA was extracted at the designated time points after differentiation. Gene expression was analyzed by quantitative real-time RT-PCR. Expression levels were normalized to *β-actin* and the results were expressed as means ± standard deviations for three independent wells. Asterisk (*) indicates $P < 0.05$; double asterisks (**) indicates $P < 0.01$ in Student's two tailed *t*-test.

nied by a decrease in the expression of cyclin D1, cyclin E and cullin-3 [25]. Further investigation of the molecular mechanism of proliferative effect of LMO4 in the these cells revealed that LMO4 is a cell cycle regulator, which is required for proliferation and necessary for sustained expression of several key cell cycle regulators including cyclin D1 [25]. Consistent with these previous studies, we observed that LMO4 knockdown decreased proliferation of 3T3-L1 preadipocytes, accompanied bydown-regulation of cyclin D1, cyclin E and cullin-3. Our finding provides further evidence that LMO4 is a general cell cycle regulator.

Overexpression of LMO4 or its binding partner Ldb1 inhibits the differentiation of mammary epithelial cells [8]. In present study, we demonstrated that LMO4 knockdown led to partial inhibition of 3T3-L1 preadipocyte differentiation. We did not address the molecular mechanisms by which LMO4 regulates 3T3-L1 preadipocyte differentiation. There are at least two possible explanations for the partial inhibition 3T3-L1 preadipocyte differentiation by LMO4 knockdown. First, PPARγ is the master regulator of adipocyte differentiation, and a previous study has shown that LMO4 interacts in a ligand-dependent manner with PPARγ and promotes PPARγ-dependent gene activation in neurons [3]. Thus, it is possible that LMO4 knockdownmay interfere with the functional activity of PPARγ during adipocyte differentiation, resulting in the partial inhibition of 3T3-L1 adipocyte differentiation. Alternatively, MCE is a key event for initiating differentiation of 3T3-L1 preadipocytes into adipocytes [21]. LMO4 is a general cell cycle regulator [25], and its expression peak coincided with the onset of MCE of 3T3-L1 preadipocyte differentiation. LMO4 knockdown may interfere with MCE, leading to attenuation of 3T3-L1 preadipocyte differentiation.

Recently, LMO4 has been proved to be a novel modulator of leptin signaling in the hypothalamus and to regulate fat metabolism [27]. Together with our findings, these data suggest that LMO4 regulates energy homeostasis and fat metabolism at different levels and that LMO4 may be a potential targetfor future therapy for obesity. Future studies will focus on the molecular mechanisms by which LMO4 regulates the adipogenesis of 3T3-L1 preadipocytes.

In conclusion, although further studies are required to elucidate the molecular mechanisms underlying LMO4-mediated preadipocyte differentiation, our findings clearly identify LMO4 as a novel modulator of adipogenesis in 3T3-L1 preadipocytes.

Acknowledgments

This study was supported by a grant of the Key Programs for returned overseas Chinese scholars from the educational Commission of Heilongjiang Province of China (Grant No. 1153h04).

References

[1] Bach, I. (2000) The LIM domain: regulation by association. Mech. Dev. 91, 5–17.
[2] Rabbitts, T.H., Bucher, K., Chung, G., Grutz, G., Warren, A. and Yamada, Y. (1999) The effect of chromosomal translocations in acute leukemias: the LMO2 paradigm in transcription and development. Cancer Res. 59, 1794s–1798s.
[3] Schock, S.C., Xu, J., Duquette, P.M., Qin, Z., Lewandowski, A.J., Rai, P.S., Thompson, C.S., Seifert, E.L., Harper, M.E. and Chen, H.H. (2008) Rescue of neurons from ischemic injury by peroxisome proliferator-activated receptor-gamma requires a novel essential cofactor LMO4. J. Neurosci. 28, 12433–12444.
[4] Chen, H.H., Schock, S.C., Xu, J., Safarpour, F., Thompson, C.S. and Stewart, A.F. (2007) Extracellular ATP-dependent upregulation of the transcription cofactor LMO4 promotes neuron survival from hypoxia. Exp. Cell Res. 313, 3106–3116.
[5] Lu, Z., Lam, K.S., Wang, N., Xu, X., Cortes, M. and Andersen, B. (2006) LMO4 can interact with Smad proteins and modulate transforming growth factor-beta signaling in epithelial cells. Oncogene 25, 2920–2930.
[6] Yu, Z., Lin, K.K., Bhandari, A., Spencer, J.A., Xu, X., Wang, N., Lu, Z., Gill, G.N., Roop, D.R., Wertz, P. and Andersen, B. (2006) The Grainyhead-like epithelial transactivator Get-1/Grhl3 regulates epidermal terminal differentiation and interacts functionally with LMO4. Dev. Biol. 299, 122–136.
[7] Novotny-Diermayr, V., Lin, B., Gu, L. and Cao, X. (2005) Modulation of the interleukin-6 receptor subunit glycoprotein 130 complex and its signaling by LMO4 interaction. J. Biol. Chem. 280, 12747–12757.
[8] Visvader, J.E., Venter, D., Hahm, K., Santamaria, M., Sum, E.Y., O'Reilly, L., White, D., Williams, R., Armes, J. and Lindeman, G.J. (2001) The LIM domain gene LMO4 inhibits differentiation of mammary epithelial cells in vitro and is overexpressed in breast cancer. Proc. Natl. Acad. Sci. USA 98, 14452–14457.
[9] Mousses, S., Bubendorf, L., Wagner, U., Hostetter, G., Kononen, J., Cornelison, R., Goldberger, N., Elkahloun, A.G., Willi, N., Koivisto, P., Ferhle, W., Raffeld, M., Sauter, G. and Kallioniemi, O.P. (2002) Clinical validation of candidate genes associated with prostate cancer progression in the CWR22 model system using tissue microarrays. Cancer Res. 62, 1256–1260.
[10] Taniwaki, M., Daigo, Y., Ishikawa, N., Takano, A., Tsunoda, T., Yasui, W., Inai, K., Kohno, N. and Nakamura, Y. (2006) Gene expression profiles of small-cell lung cancers: molecular signatures of lung cancer. Int. J. Oncol. 29, 567–575.
[11] Yu, J., Ohuchida, K., Nakata, K., Mizumoto, K., Cui, L., Fujita, H., Yamaguchi, H., Egami, T., Kitada, H. and Tanaka, M. (2008) LIM only 4 is overexpressed in late stage pancreas cancer. Mol. Cancer 7, 93.
[12] Tian, Y., Wang, N. and Lu, Z. (2010) Repression of Lim only protein 4-activated transcription inhibits proliferation and induces apoptosis of normal mammary epithelial cells and breast cancer cells. Clin. Exp. Metastasis 27, 455–463.
[13] Sum, E.Y., Segara, D., Duscio, B., Bath, M.L., Field, A.S., Sutherland, R.L., Lindeman, G.J. and Visvader, J.E. (2005) Overexpression of LMO4 induces mammary hyperplasia, promotes cell invasion, and is a predictor of poor outcome in breast cancer. Proc. Natl. Acad. Sci. USA 102, 7659–7664.
[14] Wang, Q., Li, Y.C., Wang, J., Kong, J., Qi, Y., Quigg, R.J. and Li, X. (2008) MiR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. Proc. Natl. Acad. Sci. USA 105, 2889–2894.
[15] Farmer, S.R. (2006) Transcriptional control of adipocyte formation. Cell Metab. 4, 263–273.
[16] Rosen, E.D. and MacDougald, O.A. (2006) Adipocyte differentiation from the inside out. Nat. Rev. Mol. Cell Biol. 7, 885–896.
[17] Esau, C., Kang, X., Peralta, E., Hanson, E., Marcusson, E.G., Ravichandran, L.V., Sun, Y., Koo, S., Perera, R.J., Jain, R., Dean, N.M., Freier, S.M., Bennett, C.F., Lollo, B. and Griffey, R. (2004) MicroRNA-143 regulates adipocyte differentiation. J. Biol. Chem. 279, 52361–52365.
[18] Sacco, J. and Adeli, K. (2012) MicroRNAs: emerging roles in lipid and lipoprotein metabolism. Curr. Opin. Lipidol. 23, 220–225.
[19] Lefterova, M.I. and Lazar, M.A. (2009) New developments in adipogenesis. Trends Endocrinol. Metab. 20, 107–114.
[20] MacDougald, O.A. and Lane, M.D. (1995) Transcriptional regulation of gene expression during adipocyte differentiation. Annu. Rev. Biochem. 64, 345–373.
[21] Tang, Q.Q., Otto, T.C. and Lane, M.D. (2003) Mitotic clonal expansion: a synchronous process required for adipogenesis. Pro.c Natl. Acad. Sci. USA 100, 44–49.
[22] Burton, G.R. and McGehee, R.J. (2004) Identification of candidate genes involved in the regulation of adipocyte differentiation using microarray-based gene expression profiling. Nutrition 20, 109–114.
[23] Burton, G.R., Nagarajan, R., Peterson, C.A. and McGehee, R.J. (2004) Microarray analysis of differentiation-specific gene expression during 3T3-L1 adipogenesis. Gene 329, 167–185.
[24] Lo, K.A., Bauchmann, M.K., Baumann, A.P., Donahue, C.J., Thiede, M.A., Hayes, L.S., des Etagesand Fraenkel, E., S.A. and Fraenkel, E. (2011) Genome-wide profiling of H3K56 acetylation and transcription factor binding sites in human adipocytes. PLoS One 6, e19778.
[25] Montanez-Wiscovich, M.E., Seachrist, D.D., Landis, M.D., Visvader, J., Andersen, B. and Keri, R.A. (2009) LMO4 is an essential mediator of ErbB2/HER2/Neu-induced breast cancer cell cycle progression. Oncogene 28, 3608–3618.
[26] Wang, N., Lin, K.K., Lu, Z., Lam, K.S., Newton, R., Xu, X., Yu, Z., Gill, G.N. and Andersen, B. (2007) The LIM-only factor LMO4 regulates expression of the BMP7 gene through an HDAC2-dependent mechanism, and controls cell proliferation and apoptosis of mammary epithelial cells. Oncogene 26, 6431–6441.
[27] Zhou, X., Gomez-Smith, M., Qin, Z., Duquette, P.M., Cardenas-Blanco, A., Rai, P.S., Harper, M.E., Tsai, E.C., Anisman, H. and Chen, H.H. (2012) Ablation of LMO4 in glutamatergic neurons impairs leptin control of fat metabolism. Cell Mol. Life Sci. 69, 819–828.