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# MicroRNA-138 Suppresses Adipogenic Differentiation in Human Adipose Tissue-Derived Mesenchymal Stem Cells by Targeting Lipoprotein Lipase

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**Purpose:** Adipogenic differentiation of adipose tissue-derived mesenchymal stem cells (AMSCs) is critical to many disease-related disorders, such as obesity and diabetes. Studies have demonstrated that miRNA-138 (miR-138) is closely involved in adipogenesis. However, the mechanisms affected by miR-138 remain unclear. This work aimed to investigate interactions between miR-138 and lipoprotein lipase (LPL), a key lipogenic enzyme, in AMSCs.

**Materials and Methods:** Human AMSCs (hAMSCs) isolated from human abdomen tissue were subjected to adipogenic differentiation medium. Quantitative real-time polymerase chain reaction and Western blot assay were applied to measure the expressions of miR-138, LPL, and the two adipogenic transcription factors cytidine-cytidine-adenosine-adenosine-thymidine enhancer binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ). The relationship between miR-138 and LPL was predicted utilizing the miRTarBase database and validated by dual luciferase reporter assay.

**Results:** Showing increases in C/EBPα and PPARγ expression levels, hAMSCs were induced into adipogenic differentiation. During adipogenesis of hAMSCs, miR-138 expression was significantly downregulated. Overexpression of miR-138 by transfection inhibited hAMSCs adipogenic differentiation in vitro. Mechanically, LPL was a target of miR-138. LPL expression was upregulated during adipogenesis of hAMSCs, and this upregulation was reversed by miR-138 overexpression. Functionally, silencing of LPL by transfection exerted similar inhibition of the expressions of C/EBPα and PPARγ. Meanwhile, LPL ectopic expression was able to partly abolish the suppressive effect of miR-138 overexpression on adipogenic differentiation of hAMSCs.

**Conclusion:** Upregulation of miR-138 inhibits adipogenic differentiation of hAMSCs by directly downregulating LPL.

**Key Words:** miR-138, adipogenic differentiation, hAMSCs, lipoprotein lipase (LPL)

## INTRODUCTION

Attracting considerable attention in the fields of cell and gene therapy, mesenchymal stem cells (MSCs) are multipotent stem cells holding the capacity of differentiating into multiple lineages, such as adipocytes, osteoblasts, or chondrocytes,[1-3] which is determined by coordination of transcription factors and epigenomic regulators.[4] Adipose tissue-derived mesenchymal stem cells (AMSCs) provide an excellent cell model through which to investigate adipogenic differentiation of adult stem cells.[5] These MSCs undergo induction of adipocyte-specific genes for mitotic clonal expansion and terminal differentiation to exhibit the adipocyte phenotype. Adipogenic differentiation is a complex process, comprising differentiation from AMSCs into adipocyte precursor cells, then pre-adipocytes, and finally adipocytes.[6,7] During adipogenesis, several transcription factors participate in regulating adipogenic commitment, such as peroxisome proliferator-activated receptor-γ (PPARγ) and members of the cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding family of proteins (C/EBP).[8] Moreover, a number

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of recent studies have considered the pivotal role of microRNAs (miRNAs) in adipogenic differentiation of MSCs.[9]

miRNAs are endogenously expressed non-coding RNAs of 19–25 nucleotides in length. Molecularly, miRNAs can repress protein translation or induce mRNA degradation by direct base pairing with target sequences in a broad range of biological systems. Recent evidence has shown that miRNAs influence functions of stem cells, including differentiation, by negatively regulating functional gene expression at the post-transcriptional level.[9] In addition, miRNAs have been found to be involved in human adipocyte maturation.[10] For example, Skårn, et al.[11] identified 12 miRNAs that were differentially expressed during adipocytic differentiation of human bone marrow-derived stromal cells and functionally validated miRNA-155, miRNA-221, and miRNA-222 as negative regulators. Hu, et al.[12] identified 25 downregulated miRNAs and seven upregulated miRNAs after adipogenic differentiation of hAMSCs, and demonstrated that miR-27b impaired adipocyte differentiation of hAMSCs by targeting lipoprotein lipase (LPL).

Researchers have reported that miRNA-138 (miR-138) plays a crucial role in adipogenesis. Emerging evidence has demonstrated dysregulation of miR-138 in adipocytes.[13,14] In tendon stem cells from injured mice, silencing of lncRNA KCNQ1OT1 exerted anti-adipogenic function by directly upregulating miR-138.[15] Additionally, miR-138 was claimed to be downregulated in adipogenic differentiation of hAMSCs.[12,16] Although a potential role has been proposed for miR-138 in modulating adipogenic differentiation,[15,16] the precise mechanism underlying this remains unclear.

LPL, a key lipogenic enzyme, is most abundantly expressed in parenchymal cells, including adipose tissue.[17] The role of LPL in adipogenesis has been previously studied, as well as associations between miRNAs and LPL.[18,19] In this study, we aimed to investigate the effects of miR-138 and LPL on adipogenic differentiation of hAMSCs and to explore potential molecular mechanisms.

## MATERIALS AND METHODS

### Clinical specimens

Human adipose tissues were obtained from four patients who underwent elective abdominoplasty in the Yuhuangding Hospital of Yantai, and informed consent was signed by each individual. All protocols involving human subjects were approved by the Ethics Committee of the Yuhuangding Hospital of Yantai.

### Cell isolation and culture

The hAMSCs were isolated according to the methods described in previous studies.[20] After isolation, re-suspended cells were cultured in proliferation medium consisting of Dulbecco's Modified Eagle Medium (Gibco, Carlsbad, CA, USA), 10% fetal bovine serum (FBS; Gibco), and 100 U/mL of penicillin (Invitrogen, Carlsbad, CA, USA) and 100 μg/mL of streptomycin (Invitrogen). Non-adherent cells in the proliferation medium were removed, and the adherent cells were cultured with fresh proliferation medium after 24 h. At 10 days post primary culture, the cells were passaged. Cells cultured between passage 3 and 5 were used for subsequent experiments.

Human embryonic kidney 293T cells were acquired from American Type Culture Collection (ATCC; Manassas, VA, USA) and seeded into 24-well plates (Corning, NY, USA) in proliferation medium prior to transfection. All cells were cultured at 37°C in a humidified environment containing 5% $CO_2$ for the indicated times.

### Adipogenic induction of hAMSCs

For the induction of adipogenic differentiation, cells between passage 3 and 5 were incubated in adipogenic differentiation medium supplemented with proliferation medium, 50 nM insulin, 100 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 200 μM indomethacin (all purchased from Sigma-Aldrich, St. Louis, MO, USA). On days 0, 3, and 7 of adipogenic differentiation induction, the cells were collected for detection of total RNA and protein.

### Cell transfection

For overexpression, hsa-miR-138 mimics were obtained from GenePharma (Shanghai, China), as well as its negative control (miR-NC mimics). The coding domain sequence of LDL (NM_000237) was cloned into pcDNA 3.1 vector (Invitrogen). For knockdown, hsa-miR-138 inhibitors (anti-miR-138) and miR-NC inhibitors (anti-miR-NC) were acquired from GenePharma. hAMSCs were cultured in 6-well plates (Corning), and transfection experiments were performed using Lipofectamine 2000 (Invitrogen), following the standard instructions provided by the manufacturer. Transfected cells were cultured for another 48 h prior to further studies.

### Quantitative real-time polymerase chain reaction

Total RNA was isolated with TRIzol reagent (Thermo, Waltham, MA, USA), following the manufacturer's protocol. Total RNA was subjected to first-strand cDNA synthesis using a PrimeScript RT reagent kit (Takara, Shiga, Japan). Quantification of the miRNAs was performed with a miScript SYBR Green PCR Kit (Qiagen, Redwood City, CA, USA). The amplification of mRNAs was performed using SYBR-Green I Master Mix (Takara) on the ABI PRISM 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). To normalize the expression levels of miR-138 and mRNAs, U6 small nuclear RNA (U6, for miRNA) and glyceraldehyde-phosphate dehydrogenase (GAPDH, for mRNAs) were used as endogenous controls, according to the comparative threshold cycle value ($2^{-\Delta\Delta Ct}$) method. The reactions were performed in quadruplicate for each sample over at least three independent runs. QPCR primers are shown in Supplementary Table 1 (only online).


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### Western blot assay

Total protein was extracted using RIPA lysis buffer (Beyotime, Shanghai, China), and 20 μg of protein were loaded, referring to the standard procedures for Western blotting. β-actin on the same membrane was included as an internal standard to normalize protein levels. Primary antibodies for enhancer binding protein alpha (C/EBPα) (#40764, 1:1000), PPARγ (#209350, 1:1000), LPL (#93898, 1:500), and β-actin (#8227, 1:5000) were purchased from Abcam (Cambridge, United Kingdom). The protein bands were visualized using chemiluminescent ECL reagent (Millipore, Billerica, MA, USA).

### Dual-luciferase reporter assay

The wild type of LPL 3'-untranslated regions fragment (LPL WT 3'UTR) containing the potential target site of miR-138 at position 1481–1503 was mutated. Then, LPL WT 3'UTR and its mutant (LPL MUT 3'UTR) were cloned into pGL3 vector (Invitrogen), respectively. The LPL MUT 3'UTR was constructed by replacing the UGAUU with the complementary sequence ACUAA in the LPL sequence. 293T cells were transfected with LPL WT 3'UTR+miR-NC mimics, LPL WT 3'UTR+miR-138 mimics, LPL MUT 3'UTR+miR-NC mimics, LPL MUT 3'UTR+miR-138 mimics, LPL WT 3'UTR+anti-miR-NC, LPL WT 3'UTR+anti-miR-138, LPL MUT 3'UTR+anti-miR-NC, or LPL MUT 3'UTR+anti-miR-138. After 48-h incubation, cells were collected to measure luciferase activity using the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

### Statistical analyses

Data are presented as a mean±standard error of mean. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Inc., La Jolla, CA, USA). $p$ values were evaluated using a one-way analysis of variance (ANOVA). $p<0.05$ was considered to indicate statistical significance.

## RESULTS

### MiR-138 expression downregulated during adipogenic differentiation of hAMSCs

hAMSCs were cultured in adipogenic differentiation medium for the indicated days, and total RNA and protein were isolated after induction for 0, 3, and 7 days. Adipogenic transcription factors C/EBPα and PPARγ were examined to reflect adipogenesis. As shown in Fig. 1A, quantitative real-time polymerase chain reaction (qRT-PCR) data showed that the mRNA levels of C/EBPα and PPARγ were elevated with the stages of adipogenic differentiation. Consistently, protein levels of C/EBPα and PPARγ were increased in a time-dependent manner (Fig. 1B). Therefore, we successfully induced hAMSCs to differentiate to adipocytes. At the same time, miR-138 expression was dramatically downregulated in hAMSCs during adipogenesis (Fig. 1C). The above findings suggested that miR-138 might play a potential role in adipogenic differentiation of hAMSCs.

### Overexpression of miR-138 inhibits adipogenic differentiation of hAMSCs

To verify the role of miR-138 in regulating adipogenic differentiation, we divided the transfected hAMSCs into two groups: miR-NC mimics and miR-138 mimics. The transfection efficiency was determined by qRT-PCR, and miR-138 levels were upregulated in miR-138 mimics-transfected hAMSCs for 2 d (Fig. 2A). Subsequently, the effect of miR-138 upregulation on adipogenic differentiation was examined. After induction, we observed a gradual, but significant, decrease in C/EBPα and PPARγ mRNA levels in the miR-138 group on days 3 and 7 (Fig. 2B and C). Moreover, Western blot assay suggested that C/EBPα and PPARγ protein levels in the miR-138 group were also reduced during adipogenic differentiation (Fig. 2D-F). Therefore, we deemed that overexpression of miR-138 might result in adipogenesis inhibition of hAMSCs.

### MiR-138 targets and downregulates LPL in hAMSCs

Combinatorial regulation of LPL by miRNA has been reported during mouse adipogenesis,[16] and LPL has been shown to be a gene target for miR-138 in human amniotic MSCs.[17] To explore

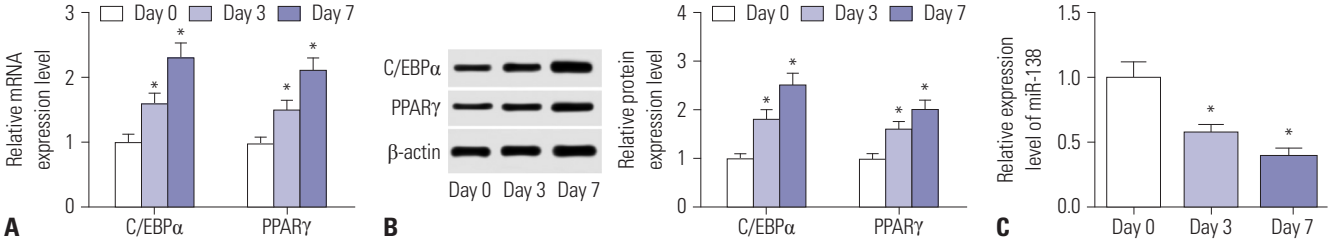


**Fig. 1.** Induction and identification of adipogenic differentiation in human adipose tissue-derived mesenchymal stem cells (hAMSCs). hAMSCs were cultured in adipogenic differentiation medium to induce adipogenic differentiation for the indicated days. (A) Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of enhancer binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) was conducted. (B) Western blot was used to measure the expression of C/EBPα and PPARγ, and the quantitation was determined with an image analyzer. (C) The expression of miR-138 was assessed by qRT-PCR. Data represent means±standard error of means. *n=3, $p<0.05$ compared with control hAMSCs on day 0.

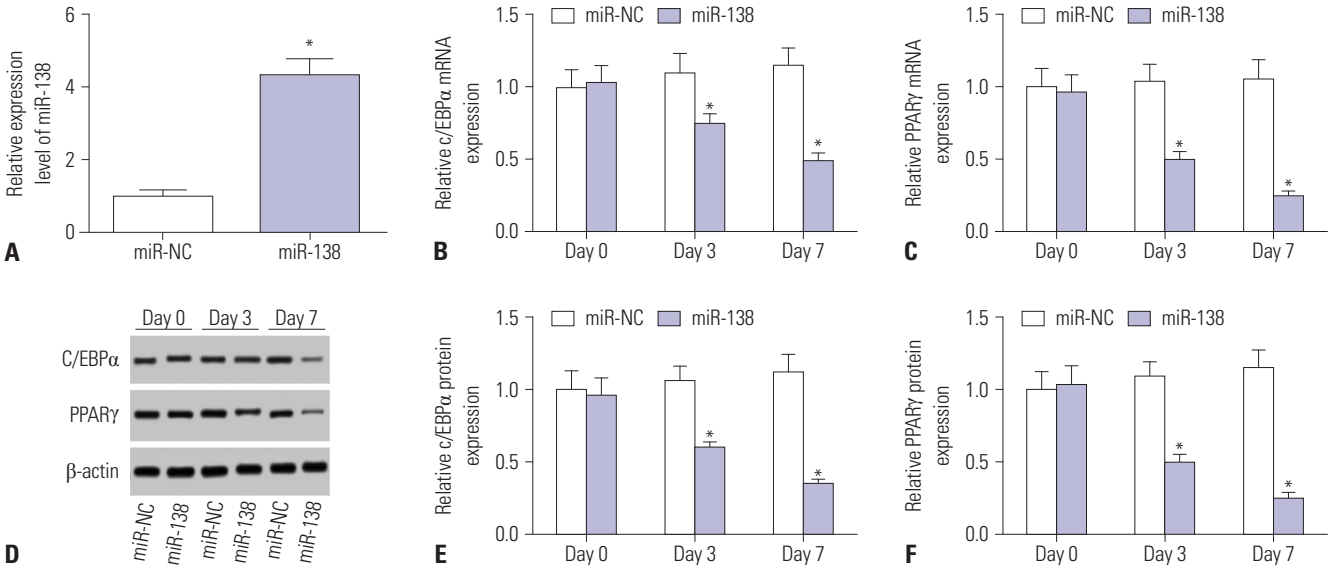


**Fig. 2.** Overexpression of miR-138 inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells (hAMSCs). Overexpression of miR-138 in hAMSCs was obtained by transfection of miR-138 mimic (miR-138) for 2 d. (A) Transfection efficiency was determined with quantitative real-time polymerase chain reaction. (B and C) Expression of enhancer binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) mRNA was monitored after adipogenic differentiation induction. (D–F) Expression of C/EBPα and PPARγ protein was monitored after adipogenic differentiation induction via Western blot assay. Data represent means±standard error of means. *n=3, $p<0.05$ compared with the miR-NC group at the indicated times.

the relationship between miR-138 and LPL in hAMSCs, the miRTarBase database was used to search for complementary binding sites between them. There were three potential binding sites (positions 1481–1503, 113–135, and 460–481) for miR-138 in the 3'UTR of LPL, and we focused on the position 1481–1503 (Fig. 3A). We constructed the mutant type of LPL 3' UTR by replacing the UGAUU with ACUAA. Dual-luciferase reporter assay showed that miR-138 mimics reduced the relative luciferase activity of LPL WT 3'UTR (Fig. 3B), whereas anti-miR-138 caused a distinct increase thereof (Fig. 3C). These results suggested a direct target relationship between LPL and miR-138. Furthermore, we detected a regulatory effect for miR-138 on LPL expression during adipogenesis. Western blot analysis demonstrated that LPL expression is gradually increased with the progression of adipogenic differentiation of hAMSCs, while this upregulation was inhibited by miR-138 ectopic expression upon transfection (Fig. 3D and E). These outcomes indicated that miR-138 inhibits hAMSCs adipogenic differentiation by targeting and downregulating LPL.

## Silencing of LPL suppresses adipogenic differentiation of hAMSCs

As a downstream target gene of miR-138, the biologic activity of LPL was also researched across the adipogenesis of hAMSCs. LPL expression was knocked down in hAMSCs by transfection. qRT-PCR revealed the transfection efficiency thereof, and LPL mRNA and protein levels were distinctly downregulated after transfection with si-LPL for 2 d (Fig. 4A and B). Subsequently, the effect of LPL silencing on adipogenic differentiation was examined. After induction, we observed that the mRNA levels of C/EBPα and PPARγ were reduced in the si-LPL group on days 3 and 7, compared to those in the si-NC group (Fig. 4C and D). Similarly, the protein levels of C/EBPα and PPARγ in the si-LPL group were also decreased, which was determined by Western blot (Fig. 4E-G). Hence, we deemed that downregulation of LPL has a suppressive impact on the adipogenesis of hAMSCs.

## MiR-138 upregulation inhibits hAMSCs adipogenic differentiation by downregulating LPL

To further clarify the mechanism of miR-138 in regulating adipogenic differentiation of hAMSCs, the cells were divided into four transfection groups: miR-NC mimics, miR-138 mimics, miR-138 mimics+pcDNA, and miR-138 mimics+pcDNA-LPL. The expression of adipogenic transcription factors was detected after adipogenic induction for 0, 3, and 7 d. As shown in Fig. 5A and B, introduction of LPL effectively restored the inhibition of C/EBPα and PPARγ mRNA levels mediated by miR-138 mimics. Furthermore, miR-138 mimic-triggered reductions of C/EBPα and PPARγ protein expression were notably overturned by upregulation of LPL (Fig. 5C-F). Additionally, miR-138 mimics-transfected cells exhibited lower levels of LPL after adipogenic differentiation for 7 d, and pcDNA-LPL co-transfection improved this depressing LPL expression (Supplementary Fig.1, only online). Moreover, LPL expression status on day 3 (data not shown) was similar to that on day 7. These findings indicated that LPL, a downstream target gene of miR-138, partly abolishes the suppressive role of miR-138 in adipogenic differentiation of hAMSCs.


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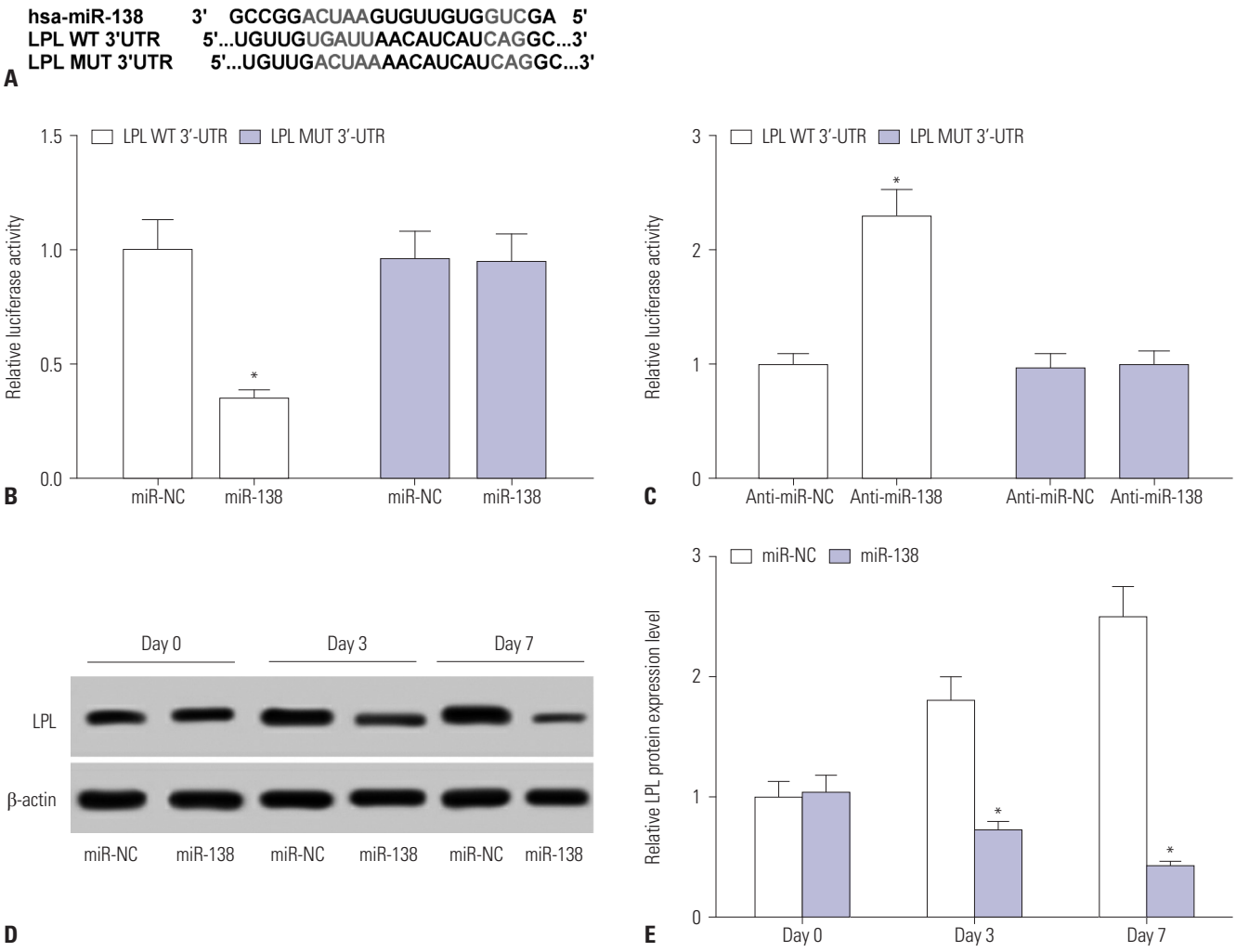


**Fig. 3.** miRNA-138 (miR-138) targets lipoprotein lipase (LPL) and negatively regulates LPL expression in human adipose tissue-derived mesenchymal stem cells (hAMSCs). (A) The binding sites between miR-138 and the 3'-UTR of LPL predicted by the miRTarBase database and the mutant are shown. (B and C) Dual-luciferase reporter assay was employed to detect the luciferase activity of wild type of LPL 3'UTR (LPL WT 3'UTR) and mutant of LPL 3'UTR (LPL MUT 3'UTR) after co-transfection with miR-138 or miR-NC. (D and E) Western blot was conducted to measure the effect of miR-138 on LPL protein expression during adipogenic differentiation of hAMSCs. Data represent means±standard error of means. *n=3, $p<0.05$ compared with the miR-NC group at the indicated times.

## DISCUSSION

In this study, we determined that miR-138 is expressed at lower levels during adipogenic differentiation of hAMSCs and that its overexpression could inhibit adipogenic differentiation of hAMSCs. Of note, we discovered that LPL was negatively regulated by miR-138 via targeted binding. The differential expression of key adipogenic transcription factors (C/EBPα and PPARγ) demonstrated that overexpression of miR-138 or silencing of LPL could suppress adipogenic commitment. On the contrary, however, overexpression of LPL could partly reverse the inhibitory effect of miR-138 on adipogenic commitment.

Previously, dysregulation of miR-138 was declared to determine MSC functions. For example, Hu, et al.[12] reported that miR-138, together with 24 other miRNAs, were expressed at a significantly lower levels after adipogenic differentiation of hAMSCs for 21 d. Consistently, NCode miRNA microarrays demonstrated that miR-138 was significantly downregulated after differentiation for 7 or 14 d following the protocols for adipocytes, compared with that in undifferentiated hMSCs.[13] Indeed, an inhibitory action for miR-138 towards adipogenic differentiation of hAMSCs was reported as early as 2011. In that investigation, miR-138 overexpression reduced lipid droplet accumulation and decreased the expression of key adipogenic transcription factors (C/EBPα and PPARγ) and adipogenic marker genes (FABP4 and LPL) in hAMSCs ex-vivo.[16] Moreover, miR-138, C/EBPα, PPARγ, and leptin appeared to play a vital part in the horizontal transfer of adipogenic transcripts from medullary adipocytes to osteoblasts through extracellular vesicles.[16] Interestingly, dysregulation of miR-138 has also been observed in stem cells from injury tissue. Yu, et al.[15] proposed that the expression of miR-138 is attenuated in tendon stem cells from injured mice and that miR-138 may mediate the anti-adipogenic differentiation function of lncRNA KCNQ1OT1 knockdown by targeting PPARγ. More-

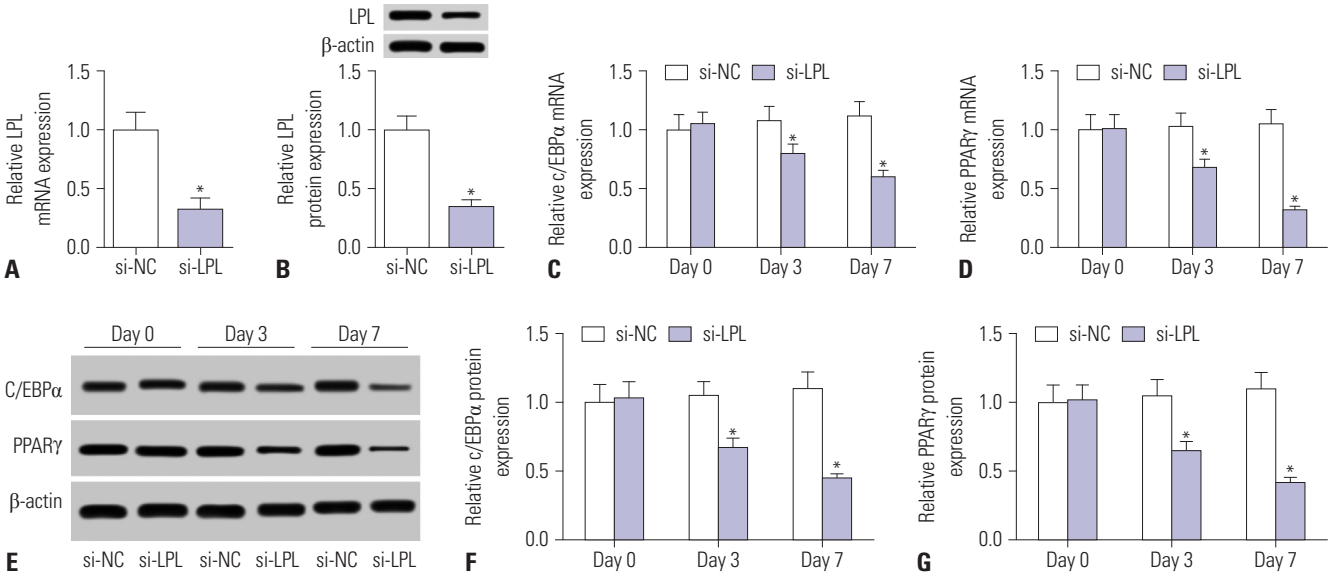


**Fig. 4.** Silencing of lipoprotein lipase (LPL) suppresses adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells (hAMSCs). Silencing of LPL in hAMSCs was obtained by transfection with si-LPL for 2 d. (A and B) Expression of LPL was determined through quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot assay. (C and D) The mRNA expression of enhancer binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) was examined after adipogenic differentiation induction via qRT-PCR. (E–G) Expression of C/EBPα and PPARγ protein was analyzed after adipogenic differentiation induction utilizing Western blot assay. Data represent means±standard error of means. *n=3, $p<0.05$ compared with the si-LPL group at the indicated times.

over, Nardelli, et al.[21] indicated that miR-138 together with miR-222-3p is overexpressed in hAMSCs from obese-pregnant women. Here, we recorded the downregulation of miR-138 during adipogenic differentiation of hAMSCs over 3 and 7 days and showed that its overexpression by transfection could slow down adipogenesis by decreasing expression of key adipogenic transcription factors C/EBPα and PPARγ.

Studies have revealed that various miRNAs affect adipogenesis by targeting several adipogenic transcription factors and key signaling molecules in vivo and in vitro.[5,9] Among them, PPARγ is an important regulator of adipogenic differentiation and is targeted by several miRNAs, such as miR-27a/b, miR-130, and miR-138.[15,20,22,23] The adipogenic commitment of tendon stem cells was suppressed by lncRNA KCNQ1OT1 knockdown via downregulating miR-138-targeted PPARγ, accompanying a loss of the adipogenic marker gene adiponectin.[15] The adenovirus early region 1-A-like inhibitor of differentiation (EID-1), a nuclear receptor co-regulator, was predicted to be another gene targeted by miR-138.[16] EID-1 was previously reported to be involved in adipogenesis by promoting small heterodimer partner, an endogenous enhancer of PPARγ, and TGFβ signaling pathways.[16] In this work, we identified LPL as a novel downstream target of miR-138 in adipogenesis of hAMSCs. Overexpression of miR-138 downregulated expression of C/EBPα and PPARγ directly in accordance with the downregulation of LPL, thereby resulting in the impairment of adipogenic commitment of hAMSCs in tissue from individuals undergoing elective abdominoplasty.

LPL is a common downstream target gene for several miRNAs during adipogenesis. Functionally, LPL mediates the hydrolysis of triglyceride-rich lipoproteins, which is important for the successful adipogenesis and maintaining adipose tissue.[24,25] The role of LPL in adipogenesis has been previously studied. For example, expression of LPL mRNA was observed in the early stages of adipogenesis and reached stable levels in mature adipocytes, which indicated LPL as a critical factor in adipogenic differentiation.[19] Moreover, LPL has been shown to be negatively regulated at the mRNA and functional level by miRNAs in a combinatorial fashion in maturing adipocytes. For instance, expression of miR-27a and miR-29a was inversely correlated with LPL and PPARγ mRNA levels by combinatorial targeting during 3T3-L1 adipocyte differentiation.[18] Another targetable regulation relationship between miR-27b and LPL was also demonstrated in regulating adipogenesis.[12] In that research, abnormal expression of miR-27b and LPL played a significant role in regulating adipogenic differentiation of hAMSCs. Here, we observed an upregulation of LPL in miR-NC-transfected hAMSCs during adipogenic differentiation, and miR-138 overexpression could restrain LPL expression during adipogenesis through targeted binding. Functionally, LPL silencing displayed a suppressive effect similar to miR-138 upregulation on C/EBPα and PPARγ expression, while its upregulation attenuated the suppressive role of miR-138. These results might offer an explanation to the dysregulation of miR-138/LPL axis in conditions like obesity and diabetes.

One of the limitations in this study is that the markers of adipogenic differentiation were only adipogenic transcription factors (C/EBPα and PPARγ). More adipogenic markers, such as FABP4 and leptin, need to be examined to better reflect adipogenesis.[16,26] Secondly, the classical morphological change of dif-


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ferentiated cells remains to be exhibited with oil red O staining, which could reveal the accumulation of lipid droplets.[12] Nonetheless, we discovered that adipogenic commitment was inhibited by miR-138 upregulation to a certain extent, as evidenced by decreased expression of C/EBPα and PPARγ, which are key adipogenic transcription factors.[8,18] Additionally, the molecular mechanism underlying miR-138 was confirmed through targeting LPL.

In conclusion, we identified a regulatory relationship between miR-138 and LPL in modulating hAMSC adipogenic differentiation. Our data describe a novel miR-138/LPL axis in adipogenesis and might suggest novel targets for cell and gene therapy in metabolic and cardiovascular diseases, such as obesity and diabetes.

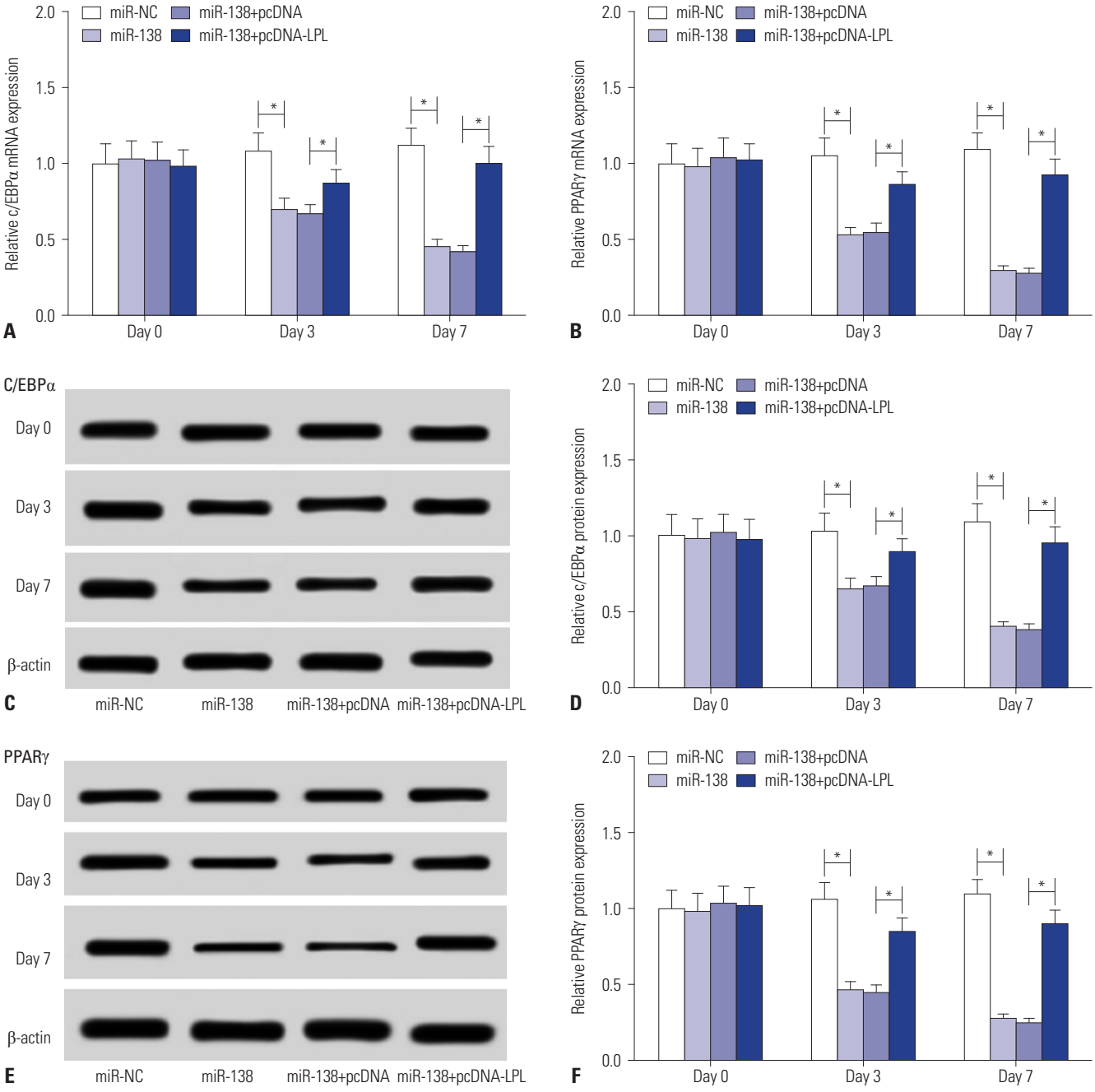


**Fig. 5.** Overexpression of lipoprotein lipase (LPL) partly reverses the inhibitory effect of miRNA-138 (miR-138) on adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells (hAMSCs). Four transfection groups were used to study adipogenic differentiation: miR-NC mimic, miR-138 mimic, miR-138 mimic+pcDNA, and miR-138 mimic+pcDNA-LPL. (A and B) Expression of enhancer binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) mRNA was evaluated by quantitative real-time polymerase chain reaction. (C–F) Protein expression of C/EBPα and PPARγ was tested with Western blot assay. Data represent means±standard error of means. *n=3, $p<0.05$ compared with the si-LPL group or miR-138 mimic+pcDNA group at the indicated times.

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## AUTHOR CONTRIBUTIONS

Conceptualization: Lixin Lin. Data curation: Lixin Lin and Peng Wang. Formal analysis: Yuting Wang and Junjun Sun. Funding acquisition: Xueming Wang. Investigation: Yong Huang and Xueming Wang. Methodology: Yuting Wang and Yong Huang. Project administration: Yuting Wang. Resources: Lixin Lin and Yong Huang. Software: Peng Wang. Supervision: Yuting Wang and Lixin Lin. Validation: Yuting Wang and Lixin Lin. Visualization: Yong Huang and Junjun Sun. Writing—original draft: Yuting Wang and Lixin Lin. Writing—review & editing: Lixin Lin and Yong Huang.

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