ARTICLE
Journal of Cellular Biochemistry 116:969–976 (2015)

Journal of Cellular Biochemistry

MiR-540 as a Novel Adipogenic Inhibitor Impairs Adipogenesis Via Suppression of PPARγ

Lin Chen,[1,2] Yuanwei Chen,[1] Sheng Zhang,[2] Lanfeng Ye,[3] Junhui Cui,[1] Quan Sun,[1] Kaide Li,[3] Hanjiang Wu,[2] and Lei Liu[1,3]*

[1]*State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, P. R. China*

[2]*Department of Oral and Maxillofacial Surgery, The Second Xiangya Hospital, Central South University, Changsha 410011, P. R. China*

[3]*Department of Oral and Maxillofacial Surgery, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, P. R. China*

ABSTRACT

A better understanding of the molecular mechanisms in adipogenesis may provide new insights into adipose tissue-related diseases. Recently, microRNAs (miRNAs) have emerged as a class of epigenetic regulators of stem cell differentiation. In this study, we found that miR-540 was an essential negative regulator of adipogenic differentiation in adipose tissue-derived stromal cells (ADSCs). Lentivirus-mediated overexpression of miR-540 resulted in blockade of the expression of C/EBP-α and PPARγ, the two master transcription factors of adipogenesis, and deficient lipid accumulation, whereas inhibition of miR-540 promoted these processes. Target gen e reporter assays showed that miR-540 directly targeted the 3′-untranslated region (3′UTR) of PPARγ, resulting in a decrease of PPARγ protein expression. Collectively, these data suggest that miR-540 represents a new adipogenic inhibitor by, at least in part, targeting PPARγ. J. Cell. Biochem. 116: 969–976, 2015.

KEY WORDS: miR-540; ADIPOSE TISSUE-DERIVED STROMAL CELLS; ADIPOGENIC DIFFERENTIATION; PPAR-γ

Obesity is a metabolic syndrome that has gradually become a major public health concern, and presents a formidable therapeutic challenge as well as socioeconomic problems [Lean et al., 1998]. It is conservatively estimated that approximately half of the population is currently overweight, and obesity levels are expected to increase to 700 million people in the next several years [Lean et al., 1998; Cummings and Schwartz, 2003]. Moreover, the incidence of complications related to obesity, such as cardiovascular and cerebrovascular diseases, insulin resistance, type 2 diabetes mellitus, and obstructive sleep apnea hypopnea syndrome, continues to rise, and contributes significantly to global morbidity and mortality [Lean et al., 1998; Cummings and Schwartz, 2003; Salvador et al., 2004]. Thus, effective prevention and treatment of obesity is of paramount importance. However, current treatment modalities for obesity including lifestyle modification, pharmacologic agents, and bariatric surgery are usually complicated and carry inherent risks [Avenell et al., 2004]. Therefore, it is necessary to focus on improving our understanding of the molecular mechanisms of obesity to develop novel and valid therapeutic strategies.

It is widely accepted that abnormal adipose tissue formation is associated with obesity and its related metabolic diseases [Kopelman, 2000]. Within adipose tissue, adipocytes are derived from adipose tissue-derived stromal cells (ADSCs), also called adipose-derived stromal/stem cells (ASCs) [Baer and Geiger, 2012]. ADSCs have a propensity for adipogenic differentiation, and play a key role in the generation and metabolism of adipose tissue [Zuk et al., 2002; Gimble and Guilak, 2003]. Therefore, understanding the molecular events involved in adipogenesis may contribute to the development

The authors declare no competing financial interests.

Grant sponsor: National Natural Science Foundation of China; Grant numbers: 81070802, 81271096; Grant sponsor: Ministry of Finance of P. R. China; Grant number: 2011SCUD4B14; Grant sponsor: The National High Technology Research and Development Program of China; Grant number: 2011AA030107.

*Correspondence to: Lei Liu, State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, P. R. China. E-mail: drliulei@163.com

Manuscript Received: 14 October 2014; Manuscript Accepted: 16 December 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 5 January 2015

DOI 10.1002/jcb.25050 • © 2015 Wiley Periodicals, Inc.

of preventive measures and treatments for obesity-related metabolic diseases. However, the details of such regulatory mechanisms remain obscure.

MicroRNAs (miRNAs) are endogenous RNAs of approximately 21–25 nucleotides in length, and some of which play important regulatory roles in cells by post-transcriptionally regulating gene expression [Bartel, 2009]. Over the past decade, miRNAs have been found to play important regulatory roles in fat metabolism, metabolic integration, and appetite regulation [Teleman et al., 2006; Herrera et al., 2010; Parra et al., 2010]. Recently, several studies reported that miRNAs might be involved in adipogenesis. Esau et al. [2004] found that miR-143 was capable of promoting the adipogenic differentiation of 3T3-L1 preadipocytes possibly through targeting ERK5. Further, Yi et al. [2011] reported that miR-143 enhances adipogenesis partly by targeting pleiotrophin (PTN), which plays a negative role during adipogenesis via the PTN/PI3K/AKT signaling pathway. Ahn et al. [2013] reported that miR-146b played a role on promoting adipogenesis by suppressing the SIRT1-FOXO1 cascade, which is a critical signaling in the maintenance of metabolic homeostasis and promotes fat mobilization in white adipose tissue. Additionally, some other studies found that miRNAs were involved in brown adipose tissue formation [Sun et al., 2011; Mori et al., 2012]. In our previous study, we provided evidence that miR-143 played the role of regulating adipogenesis in part by promoting the transition from clonal expansion stage to terminal differentiation stage [Chen et al., 2014a], and miR-363 suppressed adipogenesis by blocking clonal expansion via directly targeting E2F3 [Chen et al., 2014b].

In this study, we investigated the role of another markedly down-regulated miRNA, miR-540, during adipogenic differentiation of ADSCs, and identified its direct molecular targets related to adipogenesis. Our data are the first to demonstrate that miR-540 impairs adipogenesis via binding to target sequences in the 3′-untranslated region (3′UTR) of PPARγ.

MATERIALS AND METHODS

CELL CULTURE AND DIFFERENTIATION

ADSCs were isolated from the inguinal fat tissue of 2-week-old female Sprague-Dawley rats following the International Guiding Principles for Animal Research (1985). After anesthesia by intramuscular injection of nembutal (25 mg/kg), fat tissue was obtained aseptically from the groin followed by removal of the soft tissue and small vessels. To remove the extracellular matrix, the adipose tissue was incubated at 37 °C for 40 min with 0.075% type I collagenase and then filtered through a 100-mm nylon mesh. The resulting cells were incubated in 0.16 M NH_4Cl at room temperature for 10 min to lyse any red blood cells and then centrifuged at 1,000 rpm for 5 min. The cells were incubated in α-MEM with 10% fetal bovine serum (Gibco) in a 5% CO_2 humidified atmosphere and analyzed for their multilineage differentiation potential by flow cytometry ($CD29^+$, $CD31^-$, and $CD45^-$), plastic adherence, and colony formation ability as described previously [Lin et al., 2006a,b]. At 80% confluence, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 5 μg/mL insulin (MDI) were added to induce adipogenic differentiation. The induction medium was replaced every 3 days until the ADSCs had differentiated into mature adipocytes. At day 7 of adipogenic induction, mature adipocytes were stained with oil red O.

LENTIVIRAL VECTOR CONSTRUCTION

The miR-540 (MIMAT0003174) sequence was obtained from the miRBase database. Briefly, a pGCsil-H1-CMV-GFP vector (Genechem) was linearized using AgeI and EcoRI (New England Biolabs). Fragments containing miR-540 and a miR-540 antagomir were introduced into the pGCsil-H1-CMV-GFP vector to create pGC-LV recombinant vectors. Then, competent cells were transformed with the pGC-LV recombinant vectors. After confirmation by PCR and sequencing, the pGC-LV recombinant vectors and lentiviral packaging vectors pHelper1.0 and pHelper2.0 (Genechem) were concurrently transduced into 293 T packaging cells using Lipofectamine 2000 (Invitrogen) to produce the virus. The virus titer was determined using the hole dilution method. The lentiviral vectors were then stored at −80 °C until use.

CELL TRANSDUCTION ASSAY

The lentiviral multiplicity of infection (MOI) of rat ADSCs was determined to be 50 in a preliminary experiment. Second passage ADSCs in 12-well plates were grown to the optimal confluence for infection (30–50%). ADSCs in the miR-540 group were infected with 5 μL/well miR-540 lentivirus (titer: 1×10^9 TU/mL; MOI: 50). ADSCs in the anti-miR-540 group were infected with 3.3 μL/well anti-miR-540 lentivirus (titer: 1.5×10^9 TU/mL; MOI: 50), and ADSCs in the vector alone group were infected with 2.5 μL/well lentiviral vector alone (titer: 2×10^9 TU/mL; MOI: 50). The untreated group was not infected with lentivirus. The culture medium was replaced at 12 h post-infection. Fluorescent protein expression in the ADSCs was observed using an inverted fluorescence microscope from day 0 to 7 post-infection to assess the infection efficiency. After 3 days, the levels of fluorescent protein expression reached their maximum. When the infected cells reached 80% confluence (3–4 days), the cells were subjected to adipogenic induction.

QUANTITATIVE REAL-TIME PCR

Quantification of mRNA was performed using MMLV reverse transcriptase (Epicentre) and SYBR green-based quantitative PCR (qPCR) with an ABI PRISM 7500 system (Applied Biosystems). qPCR was carried out by initial denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 60 s. The expression levels of each mRNA were normalized to the levels of *GAPDH* and the relative expression levels of miR-540 were normalized to the levels of *U6*. All the data were calculated using the ΔΔCt method. The following primer pairs were used for qPCR analyses: *C/EBPα*, 5′CGGGAACGCAACAACATC3′ (forward) and 5′TCCAGCGACCCTAAACCAT3′ (reverse); *PPARγ*, 5′CAGAGTCTGCTGATCTGCGAG3′ (forward) and 5′TGCAGGGGGGTGATATGTT3′ (reverse); *GAPDH*, 5′GGAAAGCTGTGGCGTGAT3′ (forward) and 5′AAGGTGGAAGAATGGGAGTT3′ (reverse).

MiRNA concentrations were detected by TaqMan qPCR. PCR was carried out in a final volume of 20 μL using an ABI PRISM 7900 system

(Applied Biosystems). Each reaction contained PCR primers, gene-specific stem-loop primers, and probes. PCR was performed by initial denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 60 s. The primers and probes were as follows: U6, 5′GCTTCGGCAGCACATATACTAAAAT3′ (forward) and 5′CG CTTCACGAATTTGCGTGTCAT3′ (reverse); miR-540, 5′GTCGTATC CAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCCCAG-G3′ (RT primer), 5′GGAGGTCAGAGGTCGATC3′ (gene-specific stem-loop primer), and 5′CAGTGCGTGTCGTGGAG3′ (probe).

WESTERN BLOT ANALYSIS

Cell lysates were prepared on ice and then protein concentrations were determined using a protein assay kit (Bio-Rad Laboratories). Equal amounts of protein were separated by 10% SDS-polyacrylamide electrophoresis and then transferred to cellulose membranes. The membranes were then incubated overnight with the following primary antibodies: anti-PPARγ (1:500, Sigma–Aldrich), anti-CEBPα (1:200, Sigma–Aldrich), or anti-β-actin (1:1000, Santa Cruz Biotechnology). Then, the membranes were immunoblotted with a horseradish peroxidase-conjugated secondary antibody (1:5000, Santa Cruz Biotechnology). Immunoreactivity was visualized using enhanced chemiluminescence (ECL kit, Santa Cruz Biotechnology) and analyzed by the Quantity One system (Bio-Rad Laboratories). The expression of β-actin was used for normalization and all the data were acquired from three independent experiments.

FLUORESCENT REPORTER ASSAY

The target prediction program 'miRanda' recommended by miRBase was used to predict adipogenesis-relating target genes of miR-540. PPAR-γ was predicted as a target gene with a high possibility score. The 3′UTR sequence of PPAR-γ mRNA was acquired from NCBI, and the sequence of miR-540 was obtained from miRBase. According to these data, we constructed enhanced green fluorescent protein (EGFP)/red fluorescent protein (RFP) reporter vectors. At 1 day before transduction, 293T cells were seeded in 24-well plates at 3×10^4 cells/well. The cells were cotransduced with miR-540 mimics or negative control oligonucleotides and then reporter vectors pcDNA3/EGFP-PPARγ 3′UTR or pcDNA3/EGFP-PPARγ 3′UTR mutant. The RFP expression vector pDsRed2-C1 was used for normalization. At 48 h post-transduction, EGFP and RFP expression were measured with an F-4500 fluorescence spectrophotometer (Hitachi).

STATISTICAL ANALYSIS

Quantitative data are shown as the means ± standard deviation (SD). For comparisons between two groups and multiple comparisons, one-way analysis of variance was applied using the SPSS software program for Windows. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

EXPRESSION OF MIR-540 DURING ADIPOGENESIS OF ADSCS AND IN ADSCS INFECTED WITH LENTIVIRAL VECTORS EXPRESSING MIR-540

The ADSCs were induced to undergo adipogenic differentiation using the standard MDI cocktail. RNA was purified from the cells at 0, 1, 3, 5, and 7 days following adipogenic induction, and then analyzed by qPCR. We found that the expression of miR-540 was decreased during adipogenic differentiation (Fig. 1A–B). To investigate whether miR-540 plays a role in molecular regulation of adipogenesis in ADSCs, lentiviral vectors expressing either miR-540 or a miR-540 antagomir were transduced into ADSCs. The efficiency of lentiviral transduction was determined by fluorescence microscopy (Fig. 1C). qPCR results showed that the expression of miR-540 peaked from day 3 and then maintained a high level until day 7 (Fig. 1D). Next, the transduced cells underwent adipogenic induction. qPCR results showed that the expression of miR-540 was maintained at a high level from day 0 to 7 post-induction (Fig. 1E).

MIR-540 INHIBITS ADIPOGENIC DIFFERENTIATION OF ADSCS

Seventy-two hours after infected with lentivirus, cells were harvested to detect the infectious efficiency. Additionally, cell proliferation and survival were also detected after infection (Figure S1). At 7 days post-adipogenic induction, we compared the degree of differentiation in uninfected cells (untreated group), cells infected with the lentiviral vector alone (vector alone group), and lentiviral vectors expressing miR-540 (miR-540 group) or the miR-540 antagomir (anti-miR-540 group). Lipid accumulation was determined on day 7 post-induction and the expression of two principal transcription factors in adipogenesis, C/EBP-α and PPARγ, as well as the glucose metabolism associated transporter GLUT4 were detected by qPCR and western blot analyses at the indicated time points. The results showed that cells in the miR-540 group produced significantly fewer lipid droplets, whereas cells in the anti-miR-540 group, which down-regulated the expression of miR-540, accumulated significantly more lipid droplets (Fig. 2A–B). qPCR results showed that the mRNA levels of *C/EBP*-α, *PPAR*γ, and *GLUT4* were significantly lower in the miR-540 group and significantly higher in the anti-miR-540 group (Fig. 2C–E). In western blot experiment, the relative protein levels (ratios between values of C/EBP-α or PPARγ and values of β-actin in their respective lane) analyzed by Quantity One system in each group were significantly different, which were similar to results of RT-qPCR (Fig. 2F–G).

MIR-540 IMPAIRS ADIPOGENIC DIFFERENTIATION OF ADSCS BY TARGETING PPARΓ

To identify the genes through which miR-540 exerts its effects on adipogenic differentiation, we first used bioinformatics to predict and rank putative target genes related to adipogenesis. PPARγ was predicted as a target gene of miR-540 with a high score by miRanda target prediction programs, and the alignment of miR-540 with the PPAR-γ 3′UTR is illustrated in Figure 3B. To confirm that PPAR-γ is a target gene of miR-540, we used an EGFP reporter assay to demonstrate that PPAR-γ expression is directly inhibited by miR-540 (Fig. 3A). In this assay, the 3′UTR sequence of PPAR-γ was cloned downstream of the EGFP coding sequence (pcDNA3/EGFP/PPAR-γ). We found that the EGFP expression was strongly reduced by overexpression of miR-540. However, when three mutations were introduced into the seed sequence of the PPAR-γ 3′UTR, which is complementary to miR-540, we found that the EGFP expression

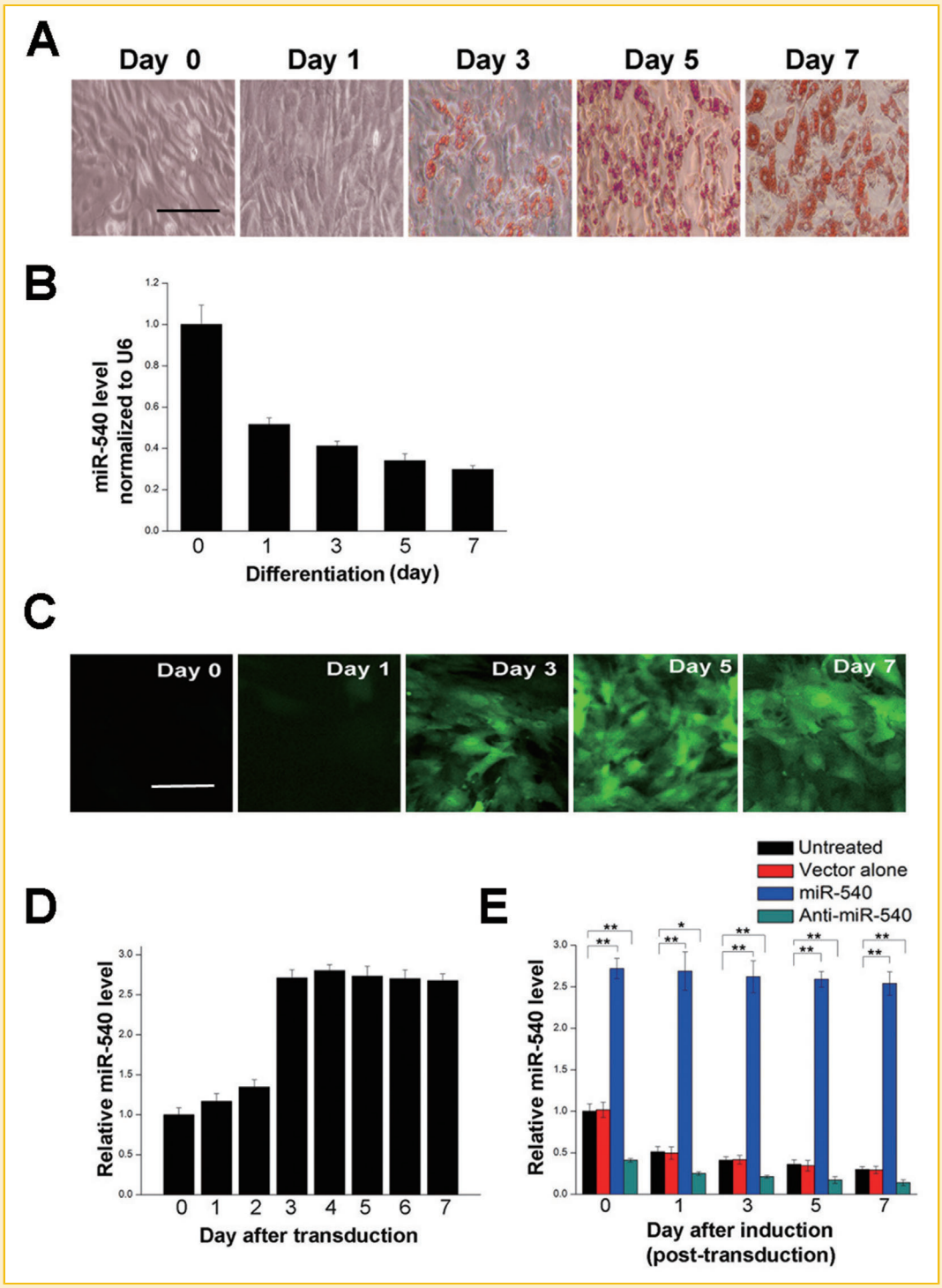

Fig. 1. Expression of miR-540 during adipogenesis of ADSCs and in ADSCs infected with lentiviral vectors expressing miR-540. (A) Oil red O staining demonstrated lipid accumulation during differentiation of ADSCs into adipocytes. Bar, 100 μm. (B) qPCR analysis of miR-540 expression at the indicated time points during ADSC differentiation. U6 was used for normalization. (C) EGFP expression in ADSCs transduced with lentiviral vectors. Bar, 100 μm. (D) Expression of miR-540 in cells infected with miR-540 expressing lentivirus as monitored by qPCR at the indicated time points after transduction. (E) After lentiviral infection, expression of miR-540 was analyzed by qPCR at the indicated time points post-induction. Data are the means ± SD from three qPCR assays. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus untreated cells or cells infected with vector alone.

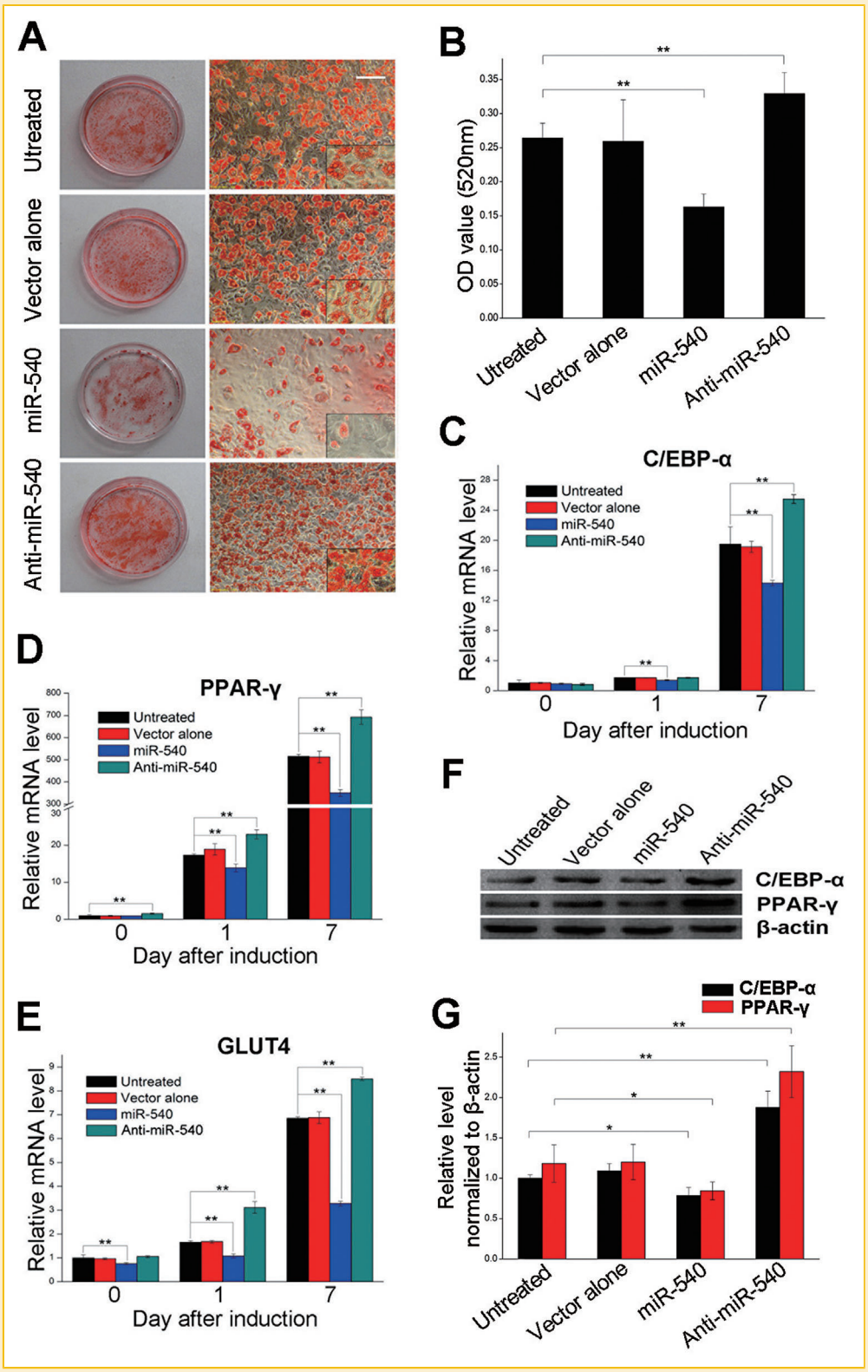

Fig. 2. MiR-540 inhibits adipogenic differentiation of ADSCs. (A) Lipid droplets were formed on day 7 after adipogenic induction as detected by oil red O staining. Lipid accumulation was significantly decreased in the miR-540 group. Bar, 100 μm. (B) The stained cultures were rinsed with 60% isopropanol and thoroughly washed with PBS. Quantification of oil red O staining was carried out by extracting the dye with 100% isopropanol, and the absorbance was measured at 520 nm. (C–E) Relative mRNA levels of *C/EBP-α*, *PPARγ*, and *GLUT4* at the indicated time points during differentiation. (F) Protein levels of C/EBP-α and PPARγ on day 7 post-induction as determined by western blot analysis. (G) Quantification of C/EBP-α and PPARγ levels normalized to β-actin. Data are the means ± SD from three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus untreated cells or cells infected with vector alone.

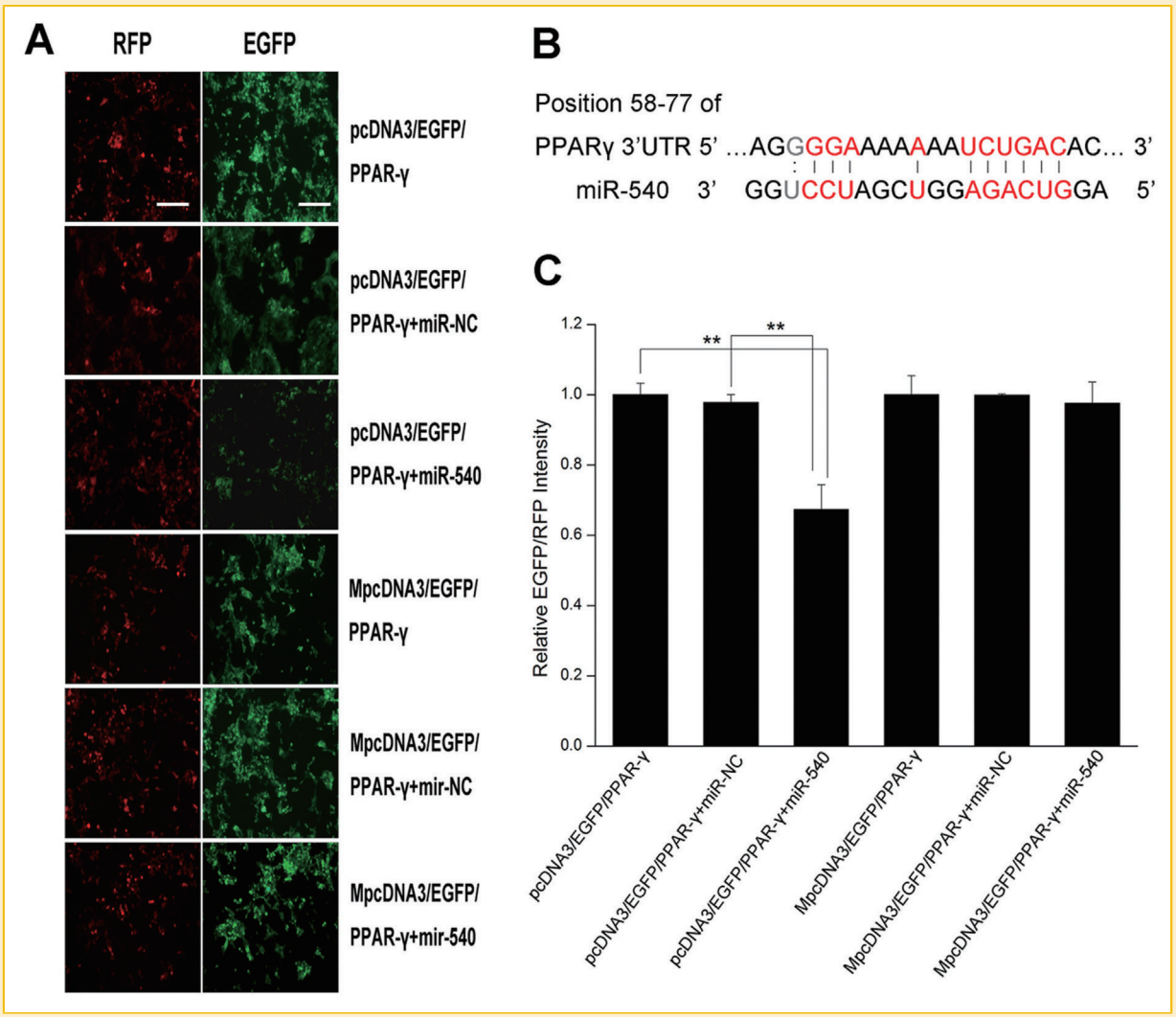

Fig. 3. MiR-540 impairs adipogenic differentiation of ADSCs by targeting PPARγ. (A) 293T cells were cotransfected with an EGFP reporter plasmid pcDNA3/EGFP/PPARγ or mutant plasmid MpcDNA3/EGFP/PPARγ and RFP reporter pDsRed-C1 plasmid either alone or in combination with a miR-540 mimic. At 48 h post-transfection, EGFP and RFP expression in each group were analyzed by fluorescence microscopy. Bar, 50 μm. (B) A sequence within PPARγ mRNA, which is complementary to miR-540, was identified by publicly available algorithms. (C) EGFP and RFP levels were measured using an F-4500 fluorescence spectrophotometer. The fluorescence value in the control group was set to 1 and histograms show normalized means ± SD of the fluorescence intensity from three independent experiments.

from the mutated vector (MpcDNA3/EGFP/PPAR-γ) was not influenced by miR-540 overexpression (Fig. 3C).

DISCUSSION

For decades, studies have shown that adipose tissue is not only a energy storage organ but also the largest endocrine organ. It plays an important role in the regulation of insulin sensitivity and energy metabolism [Rosen and Spiegelman, 2006]. Recently, research showed that excessive deposition of adipose tissue was closely related to obesity, type II diabetes, hyperlipemia, fatty liver, cardiovascular disease, and breast cancer [Lean et al., 1998; Kopelman, 2000; Cummings and Schwartz, 2003; Salvador et al., 2004]. Therefore, a better understanding of the molecular mechanisms of adipogenesis is crucial to develop novel therapeutic strategies for obesity-related diseases.

ADSCs are somatic stem cells in adipose tissue, which have an extensive proliferative potential and the ability to differentiate into adipogenic, osteogenic, chondrogenic, and myogenic lineages, and the natural differentiation trend of ADSCs is adipogenesis [Zuk et al., 2002; Gimble and Guilak, 2003; Rodriguez et al., 2005]. Within adipose tissue, adipocytes are derived from ADSCs via a specialized process of cell lineage differentiation, including commitment, growth arrest, clonal expansion, and terminal differentiation. Although the signaling cascades associated with this process have been actively investigated [Lean et al., 1998; Kahn and Flier, 2000; Otto and Lane, 2005] and the transcription factors involved in adipogenesis have been identified [Gregoire et al., 1998; Feve, 2005; Farmer, 2006], what regulates these transcription factors is poorly

understood. In this study, we identified a non-coding microRNA miR-540 as a novel regulator of adipogenesis from ADSCs. We found that miR-540 was down-regulated after adipogenic induction by the standard MDI cocktail. Furthermore, ectopic overexpression of miR-540 impaired adipogenic differentiation of ADSCs as measured by lipid accumulation and marker gene expression. Notably, PPARγ, a transcription factor that is highly expressed in adipose tissue, was predicted and validated as a direct target gene of miR-540.

PPARγ is a member of the nuclear hormone receptors that regulate gene expression by direct binding of steroid and thyroid hormones, vitamins, lipid metabolites, and xenobiotics [Chawla et al., 2001]. It is expressed at the highest level in adipose tissue and adipocyte cell lines [Ntambi and Kim, 2000; Lehrke and Lazar, 2005; Farmer, 2006]. The role of PPARγ as a critical transcription factor of adipogenesis has been supported by overwhelming evidence from both in vivo and in vitro studies. PPARγ$^{-/-}$ mouse embryonic fibroblasts demonstrate that ectopic PPARγ is capable of inducing adipogenesis [Mueller et al., 2002]. Furthermore, adipose-selective knockout of PPARγ in the mouse gives rise to insulin-insensitive animals with reduced fat [Zhang et al., 2004]. The expression of PPARγ is induced early during adipocyte differentiation. Once expressed, it activates adipocyte-specific genes resulting in the adipocyte phenotype and ensuring maintenance of terminal differentiation [Ntambi and Kim, 2000; Lehrke and Lazar, 2005].

In this study, several lines of evidence indicate that *PPARγ* mRNA is a direct target of miR-540. First, the 3′UTR of PPARγ contains a sequence that is complementary to the miR-540 seed region. Second, overexpression of miR-540 suppresses PPARγ protein expression, whereas inhibition of miR-540 enhances PPARγ expression. Third, miR-540 overexpression in 293 T cells results in reduced expression of a fluorescent reporter containing the PPARγ 3′UTR. Finally, specific mutations in the miR-540 target site within the PPARγ 3′UTR of the reporter eliminate regulation by miR-540. Therefore, these results strongly indicate that PPARγ is a direct target gene of miR-540.

In conclusion, we provide evidence that miR-540 is down-regulated during adipogenic differentiation of ADSCs, overexpression of miR-540 impairs differentiation, and inhibition of miR-540 promotes this process. Furthermore, we predicted and validated that miR-540 plays a role in adipogenic differentiation, at least in part, through directly targeting PPARγ. Our findings suggest that miR-540 plays an important role in adipose tissue formation, and may be implicated in metabolic diseases that show alterations of the amount and/or function of adipose tissue. Therefore, this finding may improve our understanding of the molecular mechanisms of obesity and be helpful to develop novel and valid therapeutic strategies.

ACKNOWLEDGMENTS

We appreciate Prof. Xiaoyu Li in State Key Laboratory of Oral Diseases in Sichuan University for her excellent assistance, critical comments, and stimulating discussions. We acknowledge National Natural Science Foundation of China, Contract grant number: 81070802, 81271096; Ministry of Finance of P.R.China, Contract grant number: 2011SCUD4B14; The National High Technology Research and Development Program of China, Contract grant number: 2011AA030107 for financial support.

REFERENCES

Ahn J, Lee H, Jung CH, Jeon TI, Ha TY. 2013. MicroRNA-146b promotes adipogenesis by suppressing the SIRT1-FOXO1 cascade. EMBO Mol Med 5:1602–1612.

Avenell A, Broom J, Brown TJ, Poobalan A, Aucott L, Stearns SC, Smith WCS, Jung RT, Campbell MK, Grant AM. 2004. Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. Health Technol Assess 8:1–182.

Baer PC, Geiger H. 2012. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. Stem Cells Int 2012:812693.DOI: 10.1155/2012/812693

Bartel DP. 2009. MicroRNAs: Target recognition and regulatory functions. Cell 136:215–233.

Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. 2001. Nuclear receptors and lipid physiology: Opening the X-files. Science 294:1866–1870.

Chen L, Cui J, Hou J, Long J, Li C, Liu L. 2014b. A novel negative regulator of adipogenesis: MicroRNA-363. Stem Cells 32:510–520.

Chen L, Hou J, Ye L, Chen Y, Cui J, Tian W, Li C, Liu L. 2014a. MicroRNA-143 regulates adipogenesis by modulating the MAP2K5–ERK5 signaling. Sci Rep 4:3819.

Cummings DE, Schwartz MW. 2003. Genetics and pathophysiology of human obesity. Annu Rev Med 54:453–471.

Esau C, Kang X, Peralta E, Hanson E, Marcusson EG, Ravichandran LV, Sun Y, Koo S, Perera RJ, Jain R, Dean NM, Freier SM, Bennett CF, Lollo B, Griffey R. 2004. MicroRNA-143 regulates adipocyte differentiation. J Biol Chem 279:52361–52365.

Farmer SR. 2006. Transcriptional control of adipocyte formation. Cell Metab 4:263–273.

Feve B. 2005. Adipogenesis: Cellular and molecular aspects. Best Pract Res Clin Endocrinol 19:483–499.

Gimble JM, Guilak F. 2003. Differentiation potential of adipose derived adult stem (ADAS) cells. Curr Top Dev Biol 58:137–160.

Gregoire FM, Smas CM, Sul HS. 1998. Understanding adipocyte differentiation. Physiol Rev 78:783–809.

Herrera BM, Lockstone HE, Taylor JM, Ria M, Barrett A, Collins S, Kaisaki P, Argoud K, Fernandez C, Travers ME, Grew JP, Randall JC, Gloyn AL, Gauguier D, McCarthy MI, Lindgren CM. 2010. Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes. Diabetologia 53:1099–1109.

Kahn BB, Flier JS. 2000. Obesity and insulin resistance. J Clin Invest 106: 473–481.

Kopelman P. 2000. Obesity as a medical problem. Nature 404:635–643.

Lean M, Han T, Seidell J. 1998. Impairment of health and quality of life in people with large waist circumference. Lancet 351:853–856.

Lehrke M, Lazar MA. 2005. The many faces of PPARγ. Cell 123:993–999.

Lin Y, Chen X, Yan Z, Liu L, Tang W, Zheng X, Li Z, Qiao J, Li S, Tian W. 2006a. Multilineage differentiation of adipose-derived stromal cells from GFP transgenic mice. Mol Cell Biochem 285:69–78.

Lin Y, Liu L, Li Z, Qiao J, Wu L, Tang W, Zheng X, Chen X, Yan Z, Tian W. 2006b. Pluripotency potential of human adipose-derived stem cells marked with exogenous green fluorescent protein. Mol Cell Biochem 291:1–10.

Mori M, Nakagami H, Rodriguez-Araujo G, Nimura K, Kaneda Y. 2012. Essential role for miR-196a in brown adipogenesis of white fat progenitor cells. PLoS Biol 10:e1001314.DOI: 10.1371/journal.pbio.1001314

Mueller E, Drori S, Aiyer A, Yie J, Sarraf P, Chen H, Hauser S, Rosen ED, Ge K, Roeder RG, Spiegelman BM. 2002. Genetic analysis of adipogenesis through peroxisome proliferator-activated receptor gamma isoforms. J Biol Chem 277:41925–41930.

Ntambi JM, Kim YC. 2000. Adipocyte differentiation and gene expression. J Nutr 130:3122–3126.

Otto TC, Lane MD. 2005. Adipose development: From stem cell to adipocyte. Crit Rev Biochem Mol 40:229–242.

Parra P, Serra F, Palou A. 2010. Expression of adipose microRNAs is sensitive to dietary conjugated linoleic acid treatment in mice. PLoS ONE 5:e13005.

Rodriguez AM, Elabd C, Amri EZ, Ailhaud G, Dani C. 2005. The human adipose tissue is a source of multipotent stem cells. Biochimie 87:125–128.

Rosen ED, Spiegelman BM. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444:847–853.

Salvador J, Iriarte J, Silva C, Gómez Ambrosi, Díez J, Caballero A, Frühbeck G. 2004. The obstructive sleep apnoea syndrome in obesity: A conspirator in the shadow. Rev Med Univ Navarra 48:55–62.

Sun L, Xie H, Mori MA, Alexander R, Yuan B, Hattangadi SM, Liu Q, Kahn CR, Lodish HF. 2011. Mir193b-365 is essential for brown fat differentiation. Nat Cell Biol 13:958–965.

Teleman AA, Maitra S, Cohen SM. 2006. Drosophila lacking microRNA miR-278 are defective in energy homeostasis. Genes Dev 20:417–422.

Yi C, Xie WD, Lia F, Lv Q, He J, Wu JB, Gu DY, Xu NH, Zhang YO. 2011. MiR-143 enhances adipogenic differentiation of 3T3-L1 cells through targeting the coding region of mouse pleiotrophin. FEBS Lett 585: 3303–3309.

Zhang J, Fu M, Cui T, Xiong C, Xu K, Zhong W, Xiao Y, Floyd D, Liang J, Li E, Song Q, Chen YE. 2004. Selective disruption of PPAR γ 2 impairs the development of adipose tissue and insulin sensitivity. Proc Natl Acad Sci USA 101:10703–10708.

Zuk PA, Zhu M, Ashjian P, De Ugarte A, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. 2002. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.