Biochemical and Biophysical Research Communications 451 (2014) 329–333

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

PU.1 promotes miR-191 to inhibit adipogenesis in 3T3-L1 preadipocytes

Shengyue Ji [a,1], Weili Li [b,1], Lijuan Bao [a], Peng Han [a], Weiping Yang [a], Li Ma [a], Fanxu Meng [a], Binyun Cao [a,*]

[a] College of Animal Science and Technology, Northwest A&F University, 22 Xinong Road, Yangling 712100, Shaanxi, PR China
[b] Beijing Shuita Aged-vinegar Research Institute for Biosciences, Beijing 102200, PR China

ARTICLE INFO

Article history:
Received 19 July 2014
Available online 2 August 2014

Keywords:
PU.1
miR-191
C/EBPβ
3T3-L1 preadipocytes
Adipogenesis

ABSTRACT

PU.1, an Ets family transcription factor, was previously demonstrated expressed in 3T3-L1 preadipocytes and had an negative effect on adiopogenesis. However, the underlying mechanism remains elusive. Here, miR-191 was identified as an inhibitor of adipocyte differentiation through targeting the 3′ untranslated regions of C/EBPβ, the initial factor in the C/EBPα/β-PPARγ terminal pathway of adipogenic differentiation. MiR-191 suppressed the lipid accumulation by Oil Red O staining and downregulated the levels of adipogenic marker genes PPARγ ($P < 0.01$), aP2 ($P < 0.01$) and FAS ($P < 0.05$). Then, we found that PU.1 overexpression resulted in upregulation of miR-191 and adipogenic inhibition. Likewise, PU.1 overexpression rescued the miR-191 decrease and resisted the adipogenic promotion caused by miR-191 oligonucleotide inhibitor. Collectively, these results revealed that PU.1 promoted miR-191 to suppress adipogenesis 3T3-L1 preadipocyte and indicated a new mechanism of PU.1 inhibiting adipogenesis.

1. Introduction

PU.1 (PU box binding protein) is a vital transcription factor in biological processes, for it played important roles not only in the hematopoiesis and immune system development [1], but also in cell cycle exit [2,3] and epigenetic silencing [4]. However, recent studies demonstrated that PU.1 was expressed in 3T3-L1 preadipocytes, caused insulin resistance and inhibited adipogenic differentiation [5–7]. Overexpression of PU.1 downregulated key adipogenic genes C/EBPβ and PPARγ in the C/EBPα/β-PPARγ terminal pathway of adipogenic differentiation [5], however, the underlying mechanism that PU.1 suppressed the expression of C/EBPβ and PPARγ remains elusive.

MiR-191 plays crucial roles not only in normal development and differentiation processes, but also in a emultitude of diseases including cancer, neurodegenerative diseases and type 2 diabetes [8–11]. MiRNA microarray results revealed that the level of miR-191 in human mature adipocytes was 3.22 higher than that in preadipocytes [12]. Likewise, miRNA high-throughput sequencing showed that miR-191 was significantly rising during the differentiation of 3T3-L1 preadipocytes [13]. Therefore, miR-191 was likely to function in adipogenesis.

In this study, miR-191 was identified as an inhibitor of adipocyte differentiation through targeting the 3′ untranslated regions (UTRs) of C/EBPβ, the initial factor in the terminal pathway of adipogenic differentiation. MiR-191 suppressed the lipid accumulation by Oil Red O staining and downregulated the levels of adipogenic marker genes PPARγ, aP2 and FAS. Moreover, online browse ChIPBase searching displayed that miR-191 was a PU.1-mediated miRNA. PU.1 overexpression rescued the miR-191 decrease and the adipogenic promotion caused by miR-191 oligonucleotide inhibitor. In general, PU.1 promoted miR-191 to suppress adipogenesis 3T3-L1 preadipocyte and indicated a new mechanism of PU.1 inhibiting adipogenesis.

2. Materials and methods

2.1. Cell culture and differentiation

3T3-L1 cells were seeded in culture dishes at a density of 5×10^4 cells/cm^2 and cultured at 37 °C in humidified atmosphere with 5 % CO_2. Culture medium was changed every 2 days. Two days after cells reach to 100% confluence, which was defined as the day 0, the cells were induced to differentiate with cocktail way for 2 days. The cells were then maintained in DMEM with 10% FBS and 5 μg/ml insulin for another 4–6 days. During the differentiation process, media were changed every 2 days.

2.2. Constructs and transfection

The pLentiHI-PU.1 shRNAs or scrambled shRNA (9 μg), combined with 6 μg Δ8.9 packaging plasmid and 9 μg VSV-G envelope

* Corresponding author. Fax: +86 29 87092164.
E-mail address: jiziqing2010@163.com (B. Cao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.07.130
0006-291X/

Table 1
Sequences of oligonucleotide primers used in strand-specific PCR and quantitative real-time PCR.

Gene name	Primer sequences (5′ → 3′)	Amplicon length (bp)
PPARγ	F: AGGACTACCAAAGTGCCATCAAA	142
	R: GAGGCTTTATCCCCACAGACAC	
FAS	F: AATCGGCAAATTCGACCTTTC	264
	R: ACCTGGATGACCACTTTGCCTAT	
aP2	F: GAGCACCATAACCTTAGATGGA	121
	R: AAATTCTGGTAGCCGTGACA	
GAPDH	F: AAGAGCACGCGAGGAGGA	139
	R: GTCTGGGATGGAAACTGGAAG	

protein plasmid were co-transfected into HEK293T packaging cells (2×10^5 cell per well) with the calcium phosphate method [14]. Forty-eight hours post transfection, the supernatant containing virus particles was collected and passed through a 0.45 μm filter to remove cellular debris. On preadipocytes reaching 70–80% confluence, the viral suspension of pLentiHI-PU.1 shRNAs or scrambled shRNA with 6 μg/ml polybrene were added for 6–8 h respectively and then were exchanged by the normal DMEM medium.

Overexpression constructs pcDNA-PU.1, pcDNA-C/EBPβ was presented by Department of Pediatrics, Baylor College of Medicine, and pcDNA-miR-191 was obtained from Laboratory of Animal Fat Deposition and Muscle Development, Northwest A&F University.

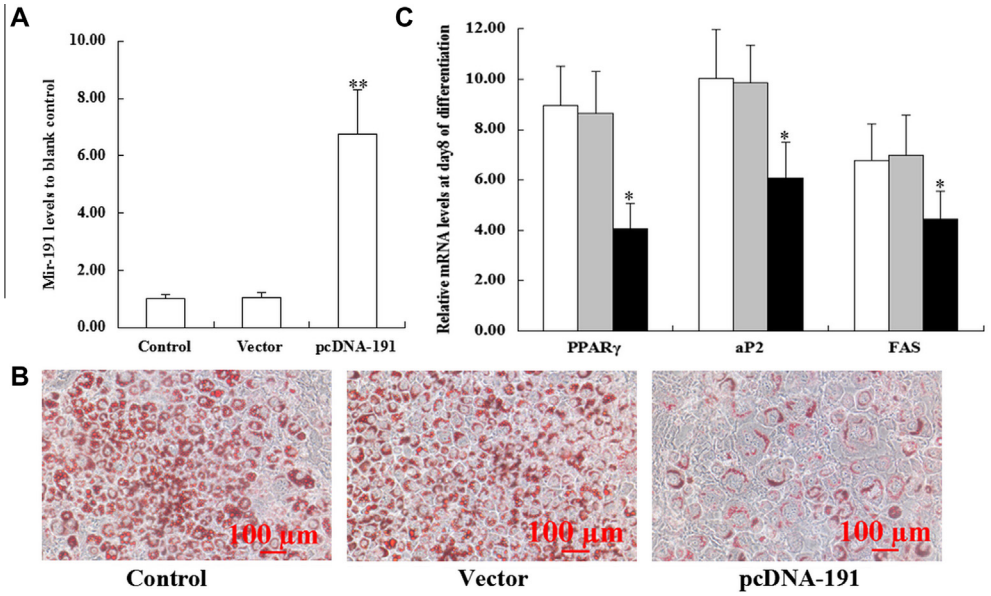

Fig. 1. Overexpression of miR-191 suppress adipogenic differentiation of 3T3-L1 preadipocytes. (A) Overexpression efficiency of pcDNA-miR-191 after transfected into HEK-293T cells 48 h. (B) Oil Red O staining of 3T3-L1 preadipocytes at day 8 of adipogenic differentiation treated with empty pcDNA3.1 vector or pcDNA-miR-191 or negative control. (C) The mRNA levels of the adipogenic marker genes PPAR, aP2 and FAS at day 8 of adipogenic differentiation after treated with empty pcDNA3.1 vector or pcDNA-miR-191.

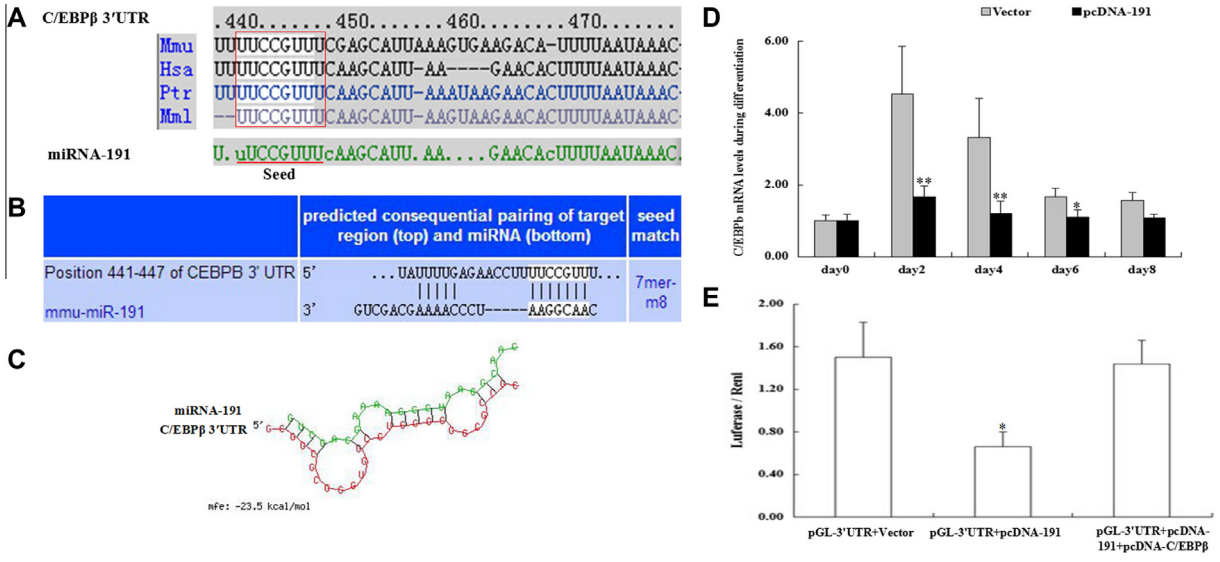

Fig. 2. MiR-191 target the 3′UTR of C/EBPβ mRNA. (A) Alignment of miR-191 seed and the 3′UTR of C/EBPβ mRNA among mouse and the mammals. Evaluation for the binding potential of miR-191 and C/EBPβ 3′UTR by Targetscan (B) and RNAhybrid (C). (D) C/EBPβ mRNA levels during 3T3-L1 preadipocyte differentiation after miR-191 overexpression. (E) Luciferase reporter gene assay for miR-191 and the 3′UTR of C/EBPβ mRNA sequence. Data expressed as mean ± S.E.M., compared with scrambled group (control), * indicated $P < 0.05$, ** indicated $P < 0.01$.

All the Overexpression constructs were transfected with X-tremeGENE HP DNA Transfection Reagent (Roche, USA) according to the manufacture's instruction.

2.3. Bioinformatics tools

MiRNA targets predicted by computational prediction programs were obtained from TargetScan (http://www.targetscan.org/vert50//), and RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid).

2.4. 3′UTR luciferase reporter assays

cDNA fragments corresponding to the 3′UTR containing the binding site of miR-191 of C/EBPβ mRNA with Xho I and Not I cutting sites (primer sequences were listed in Table 1). The construct were confirmed by sequencing. The fragments were cloned into psiCHECK™-2 Vectors (Promega, Madison, WI, USA) at the 3′-end of the Renilla gene. $20 \times 10^3 \geqslant$ HEK293T cells were seeded in 96-well plates, and 24 h later, transfections were performed using 1.0 μl X-tremeGENE HP DNA Transfection Reagent (Roche, USA), 100 ng of vector constructs and either 50 nM of miR-139-5p mimic or miR-NTC per well. Cells were harvested at 48 h after transfection. Luciferase activity was measured using the DualGlo Luciferase Assay System (Promega, USA). Renilla luciferase activity was measured and normalized to corresponding firefly luciferase activity.

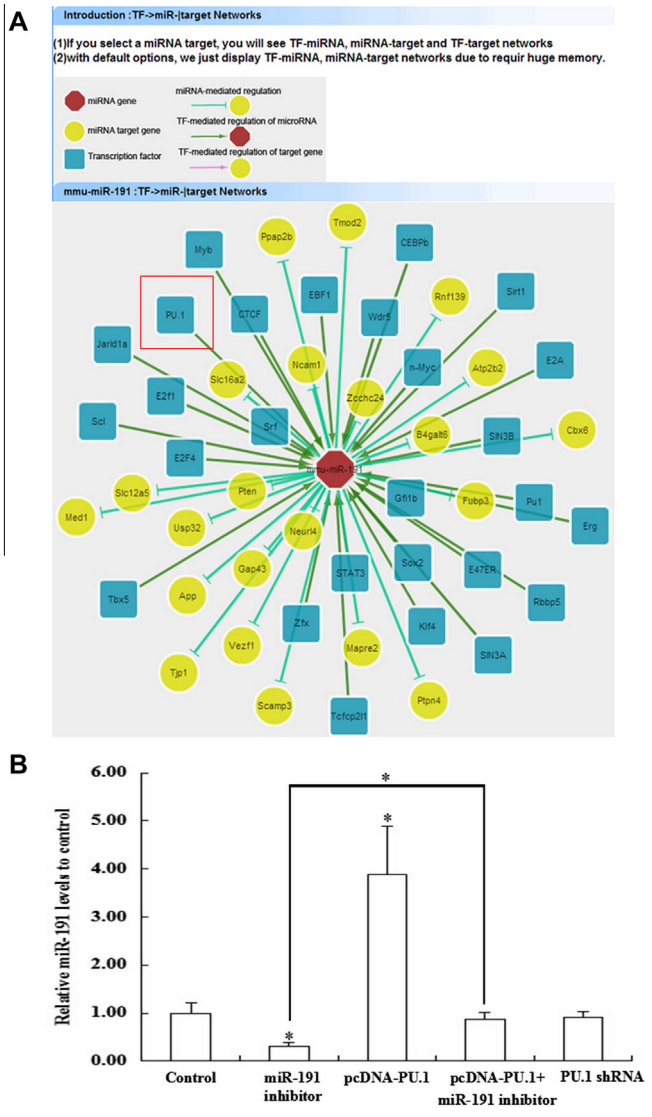

Fig. 3. PU.1 promoted the expression of miRNA. (A) MiR-191 is a PU.1-regulated miRNA in ChIPbase browse. (B) The alternation of mature miR-191 levels after treated with miR-191 inhibitor or/and pcDNA-PU.1 or PU.1 shRNA.

2.5. Oil Red O staining

Cultures were washed twice with PBS at day 8, fixed with 4% formalin in PBS, stained with 0.5% Oil Red O for 30 min. Cells were visualized under a microscope at a magnification of ×200.

2.6. Quantitative real-time PCR

Three micrograms of total RNA from each category as well as control was reverse transcribed to obtain cDNA using MMLV reverse transcriptase (Invitrogen, USA) and oligo (dT) 18 primer following the manufacturer's protocol. Real-time qPCR reactions were carried out in a final volume of 25 μl, using SYBR Premix Ex Taq (TaKaRa, Japan), 0.4 mM of each primer, and 200 ng of cDNA template. Each individual sample was run in triplicate wells. PCR amplification cycles were performed using iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad, USA) and SYBR Premix Ex Taq II kit (TaKaRa, Japan). The reactions were initially denatured at 95 °C for 3 min followed by 40 cycles of 95 °C for 15 s, 63 °C for 30 s and 72 °C for 20 s. The melting curve analysis was performed after amplification to verify the accuracy of each amplicon. The density of SYBR green I and determine the threshold cycle (Ct) value were analyzed by iQ™5 Optical System Software 2.1. The change of transcript abundance of all tested genes was calculated using $2^{-\Delta\Delta Ct}$ method. All mRNA amounts were normalized to GAPDH control.

2.7. Western blotting

Twenty five micrograms of protein of each sample were separated by 12% SDS–PAGE and electro-transferred to PVDF membrane (Millipore) for immunoblot analysis. The following primary antibodies were used: anti-PU.1 (Cell Signaling, #2258, 1:300), anti-PPARγ (Abcam, ab1948, 1:300), anti-C/EBPβ (Santa Cruz, sc-176940, 1:200), anti-aP2 (Santa Cruz, sc-18661, 1:500) and anti-GAPDH (Santa Cruz, sc-166574, 1:800) which was used to be the loading control. After incubation with the appropriate HRP-conjugate secondary antibody, proteins were detected using a ChemiDoc XRS imaging system and analysis software Quantity One (Bio-Rad, USA).

2.8. Statistical analysis

All statistical analyses were performed using SPSS 19.0 statistical software (SPSS, Inc.). Data were presented as means ± S.E.M. Comparisons were made by one-way ANOVA. Significance was set at $P < 0.05$.

3. Results

3.1. Overexpression of miR-191 suppressed adipogenic differentiation of 3T3-L1 preadipocytes

MiRNA microarray and sequencing suggested that miR-191 was likely to function in adipogenesis. Thus a pcDNA-miR-191 overexpression vector was constructed and detected about 6-fold overexpression efficiency (Fig. 1A). 3T3-L1 preadipocytes were treated with empty vector or pcDNA-miR-191 overexpression vector (pcDNA-191) and induced to differentiate at day 2 of confluent. At day 8 of differentiation, Oil O Red staining revealed that the

differentiation was suppressed by pcDNA-191 treatment compared to negative control group and empty vector group (Fig. 1B). Adipogenic marker genes PPARγ, aP2 and FAS were also significant downregulated (Fig. 1C). Thus miR-191 was identified as an inhibitor of adipogenic differentiation.

3.2. miR-191 targeted the 3′UTR of C/EBPβ mRNA

Then several miRNA target predict tools were applied to seeked potential targets for miR-191. We found that the 3′UTR of C/EBPβ mRNA sequences were highly conserved among mouse and the mammals, in which 8 consecutive bases completely matched with the seed of miR-191 (Fig. 2A). Targetscan and RNAhybrid results showed a very high target potential between mature miR-191 and C/EBPβ 3′UTR sequences (Fig. 2A and B). During the differentiation, miRNA overexpression significantly downregulated the mRNA levels of C/EBPβ supporting the above forcast results by the bioinformatics tools (Fig. 2D). Moreover, luciferase reporter gene assay revealed that miR-191 notebaly weakened the fluorescence of the reporter construct attached the C/EBPβ 3′UTR sequence, while pcDNA-C/EBPβ rescued the fluorescence intensity (Fig. 2E).

3.3. PU.1 promoted miR-191 expression to suppress the C/EBPβ level inhibiting adipogenesis

In the online database ChIPbase, which decodes transcription factor (TF) binding maps and transcripitional regulation, transcription factor PU.1 was predicted to potentially mediate regulation of miRNA-191 (Fig. 3A). A dramatic elevation of miR-191 level was detected after pcDNA-PU.1 transfected 48 h. Even more, pcDNA-PU.1 remedied the miR-191 descend by miR-191 inhibitor (Fig. 3B).

Next, 3T3-L1 preadipocytes were treated with miR-191 inhibitor or pcDNA-PU.1+miR-191 inhibitor and then induced to differentiate. At day 8 of differentiation, Oil O Red staining revealed that miR-191 inhibitor distinctly promoted the differentiation, likewise, pcDNA-PU.1 blocked the promotive effect caused by miR-191 inhibitor (Fig. 4A). As we expected, C/EBPβ mRNA and protein levels raised after miR-191 inhibitor treated and pcDNA-PU.1 reduced the C/EBPβ level nearly to control (Fig. 4B–D). Consequently, the levels of the downstream adipogenic marker genes PPARγ, aP2 and FAS followed the alteration of C/EBPβ (Fig. 4B–D). These results indicated PU.1 promoted miR-191 expression to suppress the C/EBPβ level hence inhibiting adipogenesis.

4. Discussion

MiR-191 plays versatile roles in numerous physiological and pathological processes. MiRNA microarray and sequencing suggested that miR-191 functioned in adipogenesis, so first, we overexpressed miR-191 in 3T3-L1 preadipocytes induced to adipogenic differentiation. Oil O Red staining and Adipogenic marker genes detection revealed that miR-191 played an negative role in adipogenesis.

When adipocytes differentiate, lipid droplet was gradually generated, and then the cell filled with fatty droplets until the last large fat drop formed [15]. The process of differentiation of preadipocytes is the adipogenesis of fat cells, and its essence is the timing expression of a series of marker genes C/EBPα/β, PPARγ and FAS, etc. and regulation of their networks [16,17]. MiRNAs regulate adipocyte differentiation through targeting adipogenesis-related pathways or adipogenic key factors. For instance, miR-143 suppressed adipogenesis through targeting extracellular signal-regulated kinase 5 [12]. Here, we applied several miRNA target predict tools to seeked potential targets for miR-191 and C/EBPβ was a most potential one. Then luciferase reporter gene assay results supported the predict and miR-191 overexpression downregulating C/EBPβ level during differentiation provided an additional proof. However, it could not be evacuated that there are other adipogenesis-related target genes by miR-191. Thus C/EBPβ pathway might not be the only way by which miR-191 regulates adipogenesis.

It is a dynamic and complex process for adipocyte differentiation, including clonal expansion, cell cycle exit and terminal differentiation into mature adipocytes [18,19]. In previous studys, PU.1

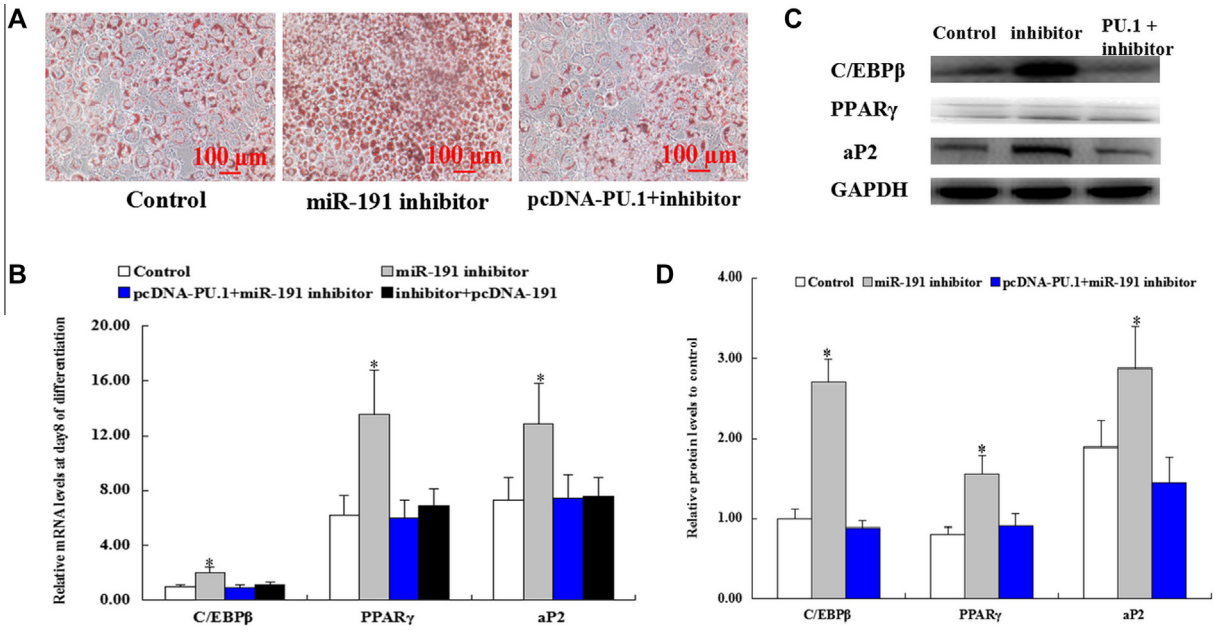

Fig. 4. PU.1 resists the adipogenic promotion by miR-191 inhibitor. (A) Oil Red O staining of 3T3-L1 preadipocytes at day 8 of differentiation treated with miR-191 inhibitor or miR-191 inhibitor+pcDNA-PU.1. (B) The mRNA of the adipogenic marker genes PPAR, aP2 and C/EBPβ at day 8 of differentiation. (C) and (D) Western blotting assay for protein levels of the adipogenic marker genes PPAR, aP2 and C/EBPβ at day 8 of differentiation.

was demonstrated to inhibit adipogenesis [5,7]. The inhibition of adipocyte differentiation by PU.1 was likely achieved through repression of the transcriptional activity of C/EBPα/β and PPARγ, but the underlying mechanism was not completely clear. Some clues hinted PU.1 protein interacted with C/EBPβ [17]. But in current study, we proved PU.1 exerts its function through regulating the expression of miR-191, an adipogenesis inhibitor, which target C/EBPβ, the initial factor in the C/EBPα/β-PPARγ terminal pathway of adipogenic differentiation. It is a completely new mechanism of PU.1 inhibiting adipogenesis.

Noncoding RNAs accomplish a remarkable variety of biological functions. They regulate gene expression at the levels of transcription, RNA processing, and translation [20]. Recent years, the roles of noncoding RNAs, like miRNAs or long non-coding RNAs (lncRNAs), and their relevant transcription factors are increasingly focused. As a newly discovered adipogenic regulatory factors, PU.1 was closely related several non-coding RNAs. MiR-155, processed from B-cell integration cluster (BIC) gene and highly expressed in both activated B- and T-cells and in monocytes/macrophages [21], plays a critical role in various physiological and pathological processes but targeting PU.1 gene [22,23]. In addition, in most recent years, evidence showed that PU.1 was post-transcriptionally regulated by a lncRNA which was transcribed from the antisense strand of PU.1 gene locus and resulted in the disconsequent expression between PU.1 mRNA and protein [7,24]. Here, we found PU.1 overexpression rescued the miR-191 decrease and resisted the adipogenic promotion caused by miR-191 oligonucleotide inhibitor. This indicated miR-191 was promoted by PU.1. However, PU.1 knockdown did not lead to the miR-191 level descend. Thus further research would be focused on the interaction between PU.1 protein and the miR-191 promotor.

In conclusion, we reported a new adipogenic inhibitor miR-191 and its regulator transcription factor PU.1 functioned negatively in adipogenic differentiation. These data indicated a new mechanism of PU.1 inhibiting adipogenesis and provided an insight for the adipogenesis regulatory networks.

Acknowledgment

This work was supported by the National Science and Technology R&D Program of China (Grant No. 2011BAD28B05-3) and the Provincial Agriculture Special Fund Project of China (Grant No. 2011NYTT03).

References

[1] D.G. Tenen, R. Hromas, J.D. Licht, D.E. Zhang, Transcription factors, normal myeloid development, and leukemia, Blood 90 (1997) 489–519.
[2] H.Y. Kueh, A. Champhekhar, S.L. Nutt, M.B. Elowitz, E.V. Rothenberg, Positive feedback between PU.1 and the cell cycle controls myeloid differentiation, Science 341 (2013) 670–673.
[3] R. Ziliotto, M.R. Gruca, S. Podder, G. Noel, C.K. Ogle, D.A. Hess, R.P. DeKoter, PU.1 promotes cell cycle exit in the murine myeloid lineage associated with downregulation of E2F1, Exp. Hematol. 42 (2014) 204–217.
[4] M. Ridinger-Saison, E. Evanno, I. Gallais, P. Rimmele, D. Selimoglu-Buet, E. Sapharikas, Moreau-F. Gachelin, C. Guillouf, Epigenetic silencing of Bim transcription by Spi-1/PU.1 promotes apoptosis resistance in leukaemia, Cell Death Differ. 20 (2013) 1268–1278.
[5] F. Wang, Q. Tong, Transcription factor PU.1 is expressed in white adipose and inhibits adipocyte differentiation, Am. J. Physiol.-Cell Physiol. 295 (2008) C213–C220.
[6] L.G. Lin, W.J. Pang, K.Y. Chen, F. Wang, J. Gengler, Y.X. Sun, Q. Tong, Adipocyte expression of PU.1 transcription factor causes insulin resistance through upregulation of inflammatory cytokine gene expression and ROS production, Am. J. Physiol. 302 (2012) E1550–E1559.
[7] W.J. Pang, L.G. Lin, Y. Xiong, N. Wei, Y. Wang, Q.W. Shen, G.S. Yang, Knockdown of PU.1 AS lncRNA inhibits adipogenesis through enhancing PU.1 mRNA translation, J. Cell. Biochem. 114 (2013) 2500–2512.
[8] R. Kulshreshtha, N. Nagpal, MiR-191: an emerging player in disease biology, Front. Genet 5 (2014) 99.
[9] R. Saba, C.D. Goodman, R. Huzarewich, C. Robertson, S.A. Booth, A miRNA signature of prion induced neurodegeneration, PLoS One 3 (2008) e3652.
[10] G.X. Xu, Y. Zhang, H. Jia, J. Li, X.M. Liu, J.F. Engelhardt, Y.J. Wang, Cloning and identification of microRNAs in bovine alveolar macrophages, Mol. Cell. Biochem. 332 (2009) 9–16.
[11] A. Zampetaki, S. Kiechl, I. Drozdov, P. Willeit, U. Mayr, M. Prokopi, A. Mayr, S. Weger, F. Oberhollenzer, E. Bonora, A. Shah, J. Willeit, M. Mayr, Plasma MicroRNA profiling reveals loss of endothelial MiR-126 and other MicroRNAs in type 2 diabetes, Circ. Res. 107 (2010) 810–817.
[12] C. Esau, X.L. Kang, E. Peralta, E. Hanson, E.G. Marcusson, L.V. Ravichandran, Y.Q. Sun, S. Koo, R.J. Perera, R. Jain, N.M. Dean, S.M. Freier, C.F. Bennett, B. Lollo, R. Griffey, MicroRNA-143 regulates adipocyte differentiation, J. Biol. Chem. 279 (2004) 52361–52365.
[13] T.W. Sun, M.G. Fu, A.L. Bookout, S.A. Kliewer, D.J. Mangelsdorf, MicroRNA let-7 regulates 3T3-L1 adipogenesis, Mol. Endocrinol. 23 (2009) 925–931.
[14] M. Jordan, A. Schallhorn, F.M. Wurm, Transfecting mammalian cells: optimization of critical parameters affecting calcium-phosphate precipitate formation, Nucl. Acids Res. 24 (1996) 596–601.
[15] M.S. Desruisseaux, M.E. Nagajyothi, H.B. Trujillo, P.E. Tanowitz, Scherer, adipocyte, adipose tissue, and infectious disease, Infect. Immun. 75 (2007) 1066–1078.
[16] S. Gesta, Y.H. Tseng, C.R. Kahn, Developmental origin of fat: tracking obesity to its source, Cell 131 (2007) 242–256.
[17] W.J. Pang, N. Wei, Y. Wang, Y. Xiong, F.F. Chen, W.J. Wu, C.Z. Zhao, S.D. Sun, G.S. Yang, Obese and lean porcine difference of FoxO1 and its regulation through C/EBPβ and PI3K/GSK3β signaling pathway, J. Anim. Sci. 92 (2014) 1968–1979.
[18] B. Feve, Adipogenesis: cellular and molecular aspects, Best Pract Res. Clin. Endocrinol. Metab. 19 (4) (2005) 483–499.
[19] C. Gummersbach, K. Hemmrich, K.D. Kroncke, C.V. Suschek, K. Fehsel, N. Pallua, New aspects of adipogenesis: radicals and oxidative stress, Differentiation 77 (2009) 115–120.
[20] T.R. Cech, J.A. Steitz, The noncoding RNA revolution-trashing old rules to forge new ones, Cell 157 (2014) 77–94.
[21] T.S. Elton, H. Selemon, S.M. Elton, N.L. Parinandi, Regulation of the MIR155 host gene in physiological and pathological processes, Gene 532 (2013) 1–12.
[22] R.C. Thompson, M. Herscovitch, I. Zhao, T.J. Ford, T.D. Gilmore, NF-κB down-regulates expression of the B-lymphoma Marker CD10 through a miR-155/PU.1 pathway, J. Biol. Chem. 286 (2011) 1675–1682.
[23] R. Thompson, I. Vardinogiannis, T. Gilmore, Identification of an NF-κB p50/p65-responsive site in the human MIR155HG promoter, BMC Mol. Biol. 14 (2013) 14–24.
[24] A.K. Ebralidze, F.C. Guibal, U. Steidl, P. Zhang, S. Lee, B. Bartholdy, M.A. Jorda, V. Petkova, F. Rosenbauer, G. Huang, T. Dayaram, J. Klupp, K.B. O'Brien, B. Will, M. Hoogenkamp, K.B. Borden, C. Bonifer, D.G. Tenen, PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element, Genes Dev. 22 (2008) 2085–2092.