FEBS Letters 585 (2011) 3303–3309

journal homepage: www.FEBSLetters.org

MiR-143 enhances adipogenic differentiation of 3T3-L1 cells through targeting the coding region of mouse pleiotrophin

Can Yi [a,b,1], Wei-dong Xie [b,1], Fu Li [a,b,1], Qing Lv [a,b], Jie He [a,b], Jiangbin Wu [a,b], Dayong Gu [c], Naihan Xu [b,*], Yaou Zhang [b,*]

[a] School of Life Sciences, Tsinghua University, Beijing 100084, PR China
[b] Division of Life Science, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, PR China
[c] Institute of Disease Control and Prevention, Shenzhen International Travel Health Care Center, Shenzhen Entry–Exit Inspection and Quarantine Bureau, Shenzhen 518045, China

ARTICLE INFO

Article history:
Received 29 June 2011
Revised 5 September 2011
Accepted 6 September 2011
Available online 19 September 2011

Edited by Tamas Dalmay

Keywords:
Mir-143
Pleiotrophin
3T3-L1 preadipocyte
Adipogenesis
Rare codon

ABSTRACT

Adipogenic differentiation of preadipocytes is a complex process regulated by various factors including miRNAs and cytokines. MiR-143 is a well known miRNA that enhances adipogenesis. Pleiotrophin (PTN), a heparin-binding growth factor, plays a negative role in adipogenesis. In this investigation, we demonstrate that PTN is a target gene of miR-143 during adipogenic differentiation in 3T3-L1 preadipocytes. MiR-143 down regulates PTN expression through interaction with a target site of miR-143 in the coding region of mouse PTN. The rare codons upstream of the target site regulate miR143-induced translational knockdown of PTN, which provides more insight into the mechanism of adipogenic differentiation.

1. Introduction

MiRNAs are recognized as important players of gene regulation through translational repression or mRNA degradation in a sequence-specific manner. MiRNAs regulate cell proliferation, apoptosis, tumorigenesis and many other physiological or pathological processes [1–12]. MiR-143 is a well known miRNA that is involved in adipogenesis. Christine Esau and coworkers identified miR-143 as one of the regulators of white adipocyte differentiation, which normally promotes adipogenic differentiation of preadipocytes through target gene ERK5 [13,14]. MiR-143 also impairs insulin-stimulated AKT activation and glucose homeostasis by down-regulating oxysterol-binding-protein-related protein (ORP) 8 in genetic and dietary mouse models of obesity [12].

On the contrary, pleiotrophin (PTN) inhibits 3T3-L1 preadipocyte differentiation [15]. PTN is an 18-kDa heparin-binding growth factor with pleiotrophic effects, including mitogenesis, angiogenesis, and differentiation [14,16–18]. Previously, we demonstrate that PTN plays a negative role during adipogenesis through PTN/PI3K/AKT/GSK-3β/β-catenin signaling pathway, which cross-talks with the Wnt/Fz/GSK-3β/β-catenin pathway to regulate adipogenesis [15]. Although it has been reported that miR-143 enhance adipogenesis of 3T3-L1 cells through targeting ERK5 [14], ERK5 should not be the only target gene of miR-143 because of the multi-target characteristics of miRNAs [9]. The expression of PTN during 3T3-L1 preadipocyte differentiation correlates negatively with that of miR-143, suggesting that miR-143 may regulate the expression of PTN. Here, we investigated if PTN is a direct target of miR-143 during adipogenic differentiation in 3T3-L1 preadipocytes. We show that miR-143 down regulates PTN expression through interaction with a target site of miR-143 in the coding region of mouse PTN. The rare codons upstream of the target site make the target site more accessible and affect miR143-induced translational knockdown of PTN. Furthermore, ectopic expression of PTN with miR-143 target site or rare codon mutations could enhance the inhibitory effect of PTN on adipogenesis. Taken together, this investigation provides more insight into the mechanism of preadipocyte differentiation.

2. Materials and methods

2.1. Cell culture and differentiation assays

Mouse 3T3-L1 preadipocytes (ATCC, USA) were cultured in DMEM supplemented with 10% calf serum (CS) and incubated in

Abbreviations: PTN, pleiotrophin; GSK-3b, glycogen synthase kinase 3b; PI3K, phosphoinositide 3-kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

* Co-corresponding authors. Fax: +86 0755 26036884 (Y. Zhang).
E-mail addresses: xu.naihan@sz.tsinghua.edu.cn (N. Xu), zhangyo@sz.tsinghua.edu.cn (Y. Zhang).

[1] These authors contributed equally to this work.

0014-5793/$36.00 © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2011.09.015

a humidified atmosphere of 5% CO_2 at 37 °C. For spontaneous differentiation, 3T3-L1 cells were cultured in DMEM with 10% FBS for 14–16 days, the medium was changed every 3 days until some cells differentiated into mature adipocytes [15].

2.2. MiRNA and siRNA transfection

MiRNA mimics and PTN siRNA were synthesized by Shanghai GenePharma Co. (Shanghai, China). MiRNA were designed according to the miRBase sequence database (http://microrna.sanger.ac.uk). The sequence of miR-143 mimic was ugagaugaagcacuguagcuc; The target sequence of PTN-siRNA was 5′-GCGGAGTCAAAGAAGA AGA-3′. A small RNA with random sequence was used as negative control (NC). Mouse 3T3-L1 preadipocytes cultured on 24 well plates were transfected with siRNA or miRNA mimics at a concentration of 20 pmol per well using Lipofectaime 2000 (Invitrogen, USA) according to manufacturer's instructions. After 36 h of transfection, cells were collected for total RNA and protein isolation or continuously cultured for spontaneous differentiation.

2.3. Oil Red-O staining of cytoplasmic triglycerides and quantification

On day 14 (spontaneous differentiation), 3T3-L1 cell monolayers were fixed with 10% formalin for 20 min. The fixed samples were stained with 0.5% Oil Red-O/isopropyl alcohol solution for 1 h at room temperature. The stained cytoplasmic triglycerides were visualized and the images were captured by phase contrast microscope. To quantify the efficiency of differentiation, the stained cells were eluted with isopropanol and the OD values were detected by spectrophotometer at a wavelength of 510 nm [15].

2.4. RT-PCR assay of PTN expression

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. RT-PCR was carried out by TaKaRa's One Step RNA PCR kit (TaKaRa Dalian, China). PTN expression was detected using the primers shown in Table S1. The PCR product was 504 bp in length. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as control [15].

2.5. Western blotting

3T3-L1 cells were lysed as previously described [15]. Whole cell extracts were separated by 12% SDS–PAGE gel and transferred to nitrocellulose membrane. The membranes were probed with primary antibody against PTN (Santa Cruz. USA) or GAPDH (Kangchen Bio-tech, Shanghai, China), then incubated with corresponding secondary antibodies. The blots were detected with an ECL blotting analysis system (Kirkegaard & Perry Laboratories, Inc.).

2.6. Preparation of reporter vectors

Reporter vectors containing the coding fragment (nt 20-151) of PTN with the target site of miR-143, the full length PTN cds (PTN WT) and its corresponding mutated coding region, including mutants of the rare codons (PTN rare-m) and miR-143 binding site (PTN site-m), were amplified by PCR by using the primers shown in Table S1. The target sequence of PTN with the target site of miR-143 was cloned into the pRL-TK reporter vector (Promega, USA) and the full length PTN cds was cloned into pcDNA3.1 expression vector (Invitrogen, USA). Cos-7 cells cultured on 24-well plates were co-transfected with 300 ng PTN constructs and 20 pmol small RNAs by Lipofectamine 2000. Cell lysates were collected for RT-PCR and western blotting. Renilla luciferase activities were measured using a Luciferase Reporter Assay System (Promega, USA). Each experiment was repeated in triplication.

3. Results

3.1. MiR-143 enhances spontaneous differentiation of 3T3-L1cells

It has been reported previously that miR-143 enhance 3T3-L1 adipogenesis by targeting ERK5 [13,14]. Because of the multi-target characteristics of miRNAs, we predict that ERK5 is not the only target gene of miR-143 during adipogenesis. Earlier study by our laboratory suggests that PTN inhibit adipogenesis during spontaneous differentiation of 3T3-L1cells [15]. Thus it is interesting to know if PTN is a potential target of miR-143. Firstly, we examined if miR-143 could stimulate spontaneous differentiation of 3T3-L1cells. We transfected miR-143, PTN siRNA, or miR-143 inhibitor into 3T3-L1 cells, small RNA with random sequence (NC) and miR-29b were used as negative control. After 14 days, cells were fixed and stained with Oil Red-O. Similar to PTN siRNA, miR-143 stimulated spontaneous differentiation of 3T3-L1cells, while miR-143 inhibitor slightly suppressed adipogenesis compared with negative control (Fig. 1A). To quantify, the cells were destained with isopropanol and the OD values were determined by spectrophotometer. As shown in Fig. 1B, both PTN siRNA and miR-143 significantly enhanced spontaneous differentiation of 3T3-L1 cells.

3.2. MiR-143 suppresses PTN expression during adipogenesis in 3T3-L1 cells

The expression of PTN during 3T3-L1 preadipocyte differentiation correlates negatively with that of miR-143, indicating that miR-143 may regulate the expression of PTN. To examine this possibility, we transfected 3T3-L1 cells with miR-143, miR-143 inhibitor or PTN siRNA. Small interfering RNA with random sequence (NC) was used as a negative control. The effect of endogenous miR-143 was evaluated by miR-143 inhibitor. 3T3-L1 cells were collected at 48 h after transfection, total RNA and the protein were extracted for RT-PCR or western blotting. As shown in Fig. 2B and C, PTN siRNA suppressed the expression of both PTN mRNA and the protein. MiR-143 had no effect on PTN expression in mRNA level but significantly reduced the protein expression compared with negative control (NC). By contrast, miR-143 inhibitor slightly enhanced PTN expression in both mRNA and protein level.

3.3. PTN is a target gene of miR-143 during adipogenesis in 3T3-L1 preadipocytes

To investigate if PTN is a direct target of miR-143, we used bioinformatics software including PITA (http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html) and Findtar (http://bio.sz.tsinghua.edu.cn/lab/findtar) to predict the target sites of miR-143 in mouse PTN. Mouse PTN has a very short 3′-UTR, there is no miR-143 target site has been found in this region. Interestingly, both PITA and Findtar predicted a putative miR-143 target site with high binding score in the coding region of PTN (Fig. 3A). Therefore, we generated two constructs to test if the putative target site of miR-143 has biological function.

Firstly, the coding fragment (nt 20-151) of PTN with the miR-143 binding site was inserted into the 3′-UTR of luciferase expression vector to generate a luciferase reporter construct. Then we co-transfected the reporter construct with miR-143, miR-143 inhibitor or NC into Cos-7 cells for luciferase activity assay. As shown in Fig. 3B, miR-143 reduced 29% of luciferase activity compared with NC, suggesting that the miR-143 binding site could be a functional site if it located in 3′-UTR region of PTN. Then, we cloned the coding region of PTN into pcDNA3.1vector to generate a wild-type PTN construct, then we transfected Cos-7 cells with both PTN construct and

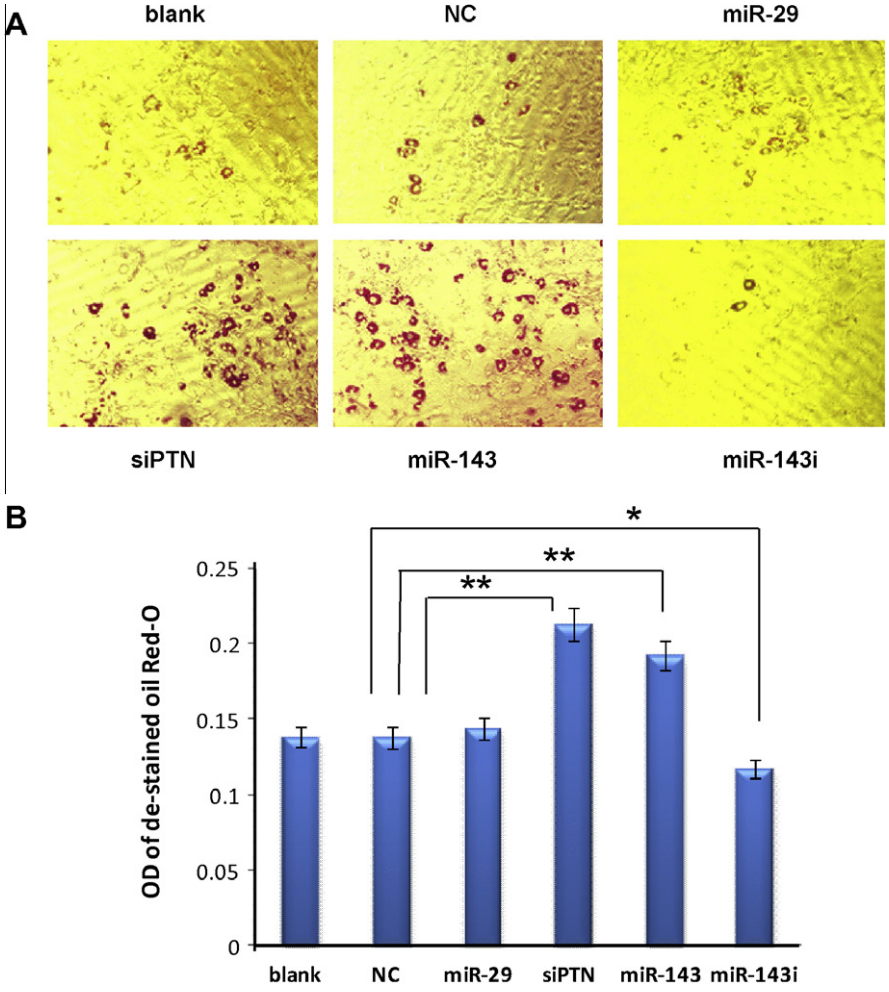

Fig. 1. MiR-143 enhances spontaneous differentiation of 3T3-L1 cells. (A) Oil Red-O staining of 3T3-L1 cells transfected with miRNAs or siRNA. (B) The Oil Red-O staining was eluted by isopropanol, the OD values were determined by spectrofluorometer. Blank: cells without transfection; NC: small RNA with random sequence; siPTN: PTN-siRNA; miR-143i: miR-143 inhibitor. $^{*}P < 0.05$; $^{**}P < 0.01$.

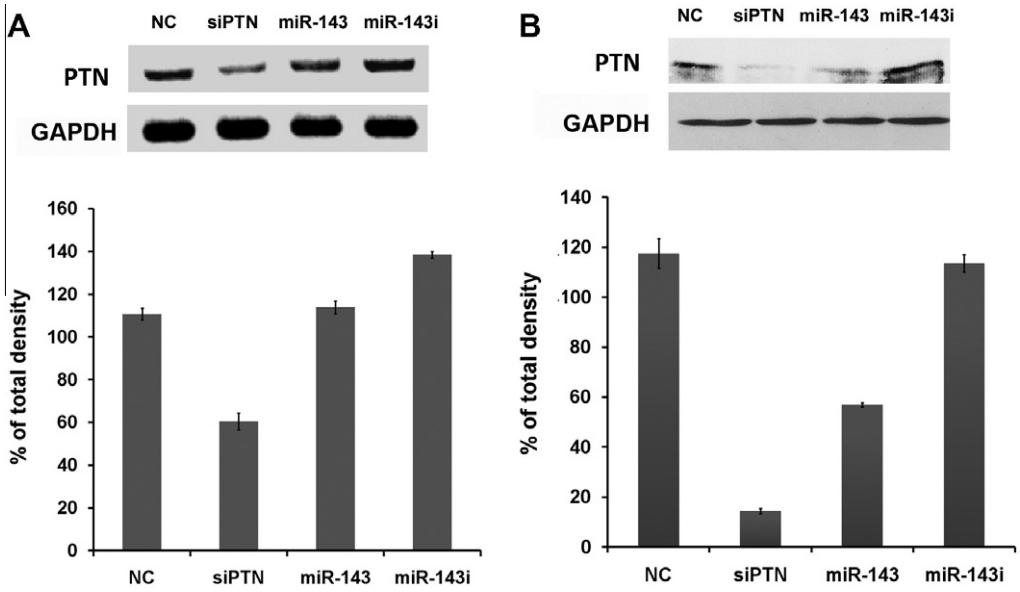

Fig. 2. MiR-143 represses PTN expression. 3T3-L1 cells were transfected with miRNAs or PTN siRNA, the samples were collected at 48 h after transfection. RT-PCR (A) and Western blotting (B) were carried out to detect PTN expression. GAPDH was used as an internal control. Blank: cells without transfection; NC: small RNA with random sequence; siPTN: PTN- siRNA; miR-143i: miR-143 inhibitor.

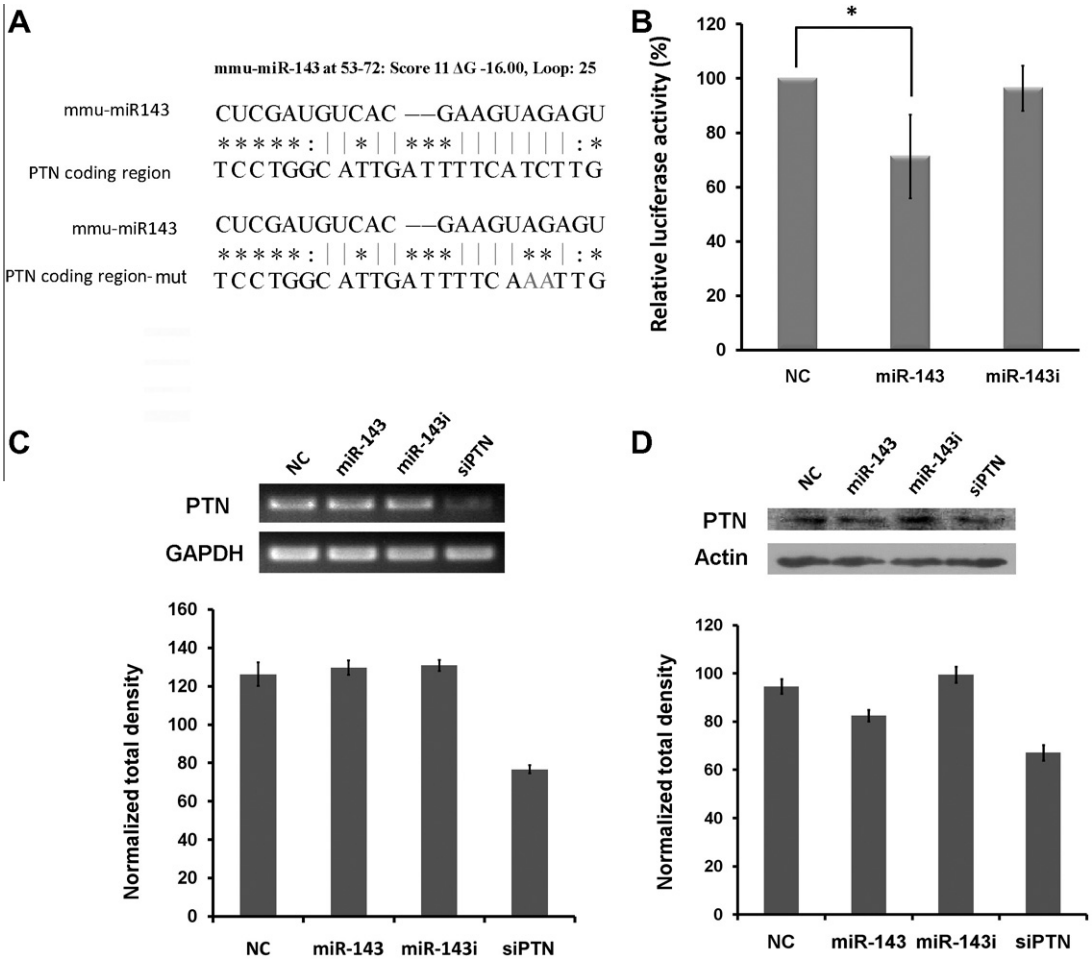

Fig. 3. PTN is a target of miR-143. (A) Diagram of PTN coding region containing the binding site of miR-143. (B) Luciferase activity assay. Cos-7 cells were co-transfected with miRNAs and PTN-pRL-TK, a luciferase report vector carrying a coding region fragment of PTN with the miR-143 binding site inserting in 3′-UTR. The cells were collected for Luciferase activity assay. Cos-7 cells were co-transfected with miRNAs and PTN-pcDNA3.1, a PTN expressive construct. The cells were collected for RT-PCR (C) and western blotting (D) to detect the expression of PTN. NC: small RNA with random sequence; siPTN: PTN-siRNA; miR-143i: miR-143 inhibitor ($n = 4$; $^*p < 0.05$).

miR-143 or miR-143 inhibitor, PTN siRNA or NC were used as control. The PTN mRNA and protein expression were examined by RT-PCR (Fig. 3C) or western blotting (Fig. 3D). MiR-143 significantly suppressed PTN protein level without affecting mRNA expression, while miR-143 inhibitor slightly enhanced the expression of both. To further investigate the interaction between miR-143 and the target site of miR-143 in the coding region of PTN, we generated a PTN mutant with 2 bp mutations in seed region of miR-143 binding site (PTN site-m) (Fig. 3A). The mutation eliminated the repressive effect of miR-143 on PTN protein expression (Figs. 4B and C). These results indicate that miR-143 may directly target the coding region of PTN.

3.4. The rare codons upstream of the miR-143 target site affect miR-143-induced translational knockdown of PTN

We analyzed the sequence of PTN mRNA and found that there is a cluster of rare codons upstream of the target site of miR-143 in the coding region of PTN, including UCG (Ser, 4–6), CAA (Gln, 13–15), CAA (Gln 25–27) and CGT (Arg 28–30) (Fig. 4A). To examine if the rare codons upstream of the target site affect miR-143-induced translational knockdown of PTN, we mutated these rare codons to common codons (Table S2). PTN with rare codon mutations was cloned into pcDNA3.1 vector to generate a mutated PTN expression construct (PTN rare-m). Cos-7 cells were co-transfected with miR-143 and PTN expression constructs with or without rare codon mutations. Similar to PTN miR-143 site mutation, the rare codon mutation also eliminated the repressive effect of miR-143 on PTN protein expression (Fig. 4B and C).

Next, we investigated if the mutations (miR-143 binding site mutation or rare codon mutation) affect the ability of PTN to suppress preadipocyte differentiation. 3T3-L1 cells were transfected with either wild-type or PTN mutants, the empty vector (pcDNA3.1) was used as a positive control. After 14 days, cells were fixed and stained with Oil Red-O. As shown in Fig. 5A, both the wild-type and the PTN mutants (site-m and rare-m) significantly inhibited spontaneous differentiation in 3T3-L1 cells preadipocytes. However, the inhibition of adipogenesis seems more significant in the presence of PTN mutants compared with wild-type PTN (Fig. 5B). These results further demonstrate that a cluster of rare codons upstream of miR-143 target site is required for miR-143-induced translational knockdown of PTN when the binding site is located in the coding region.

4. Discussion

Earlier studies demonstrate that the up-regulation of microRNA-143 is associated with obesity in adipose tissue of mice fed high-fat diet [13]. MiR-143 enhances white adipocyte differentiation by targeting ERK5, a mitogen-activated protein kinase [14]. However, it is highly possible that ERK5 is not the only target gene

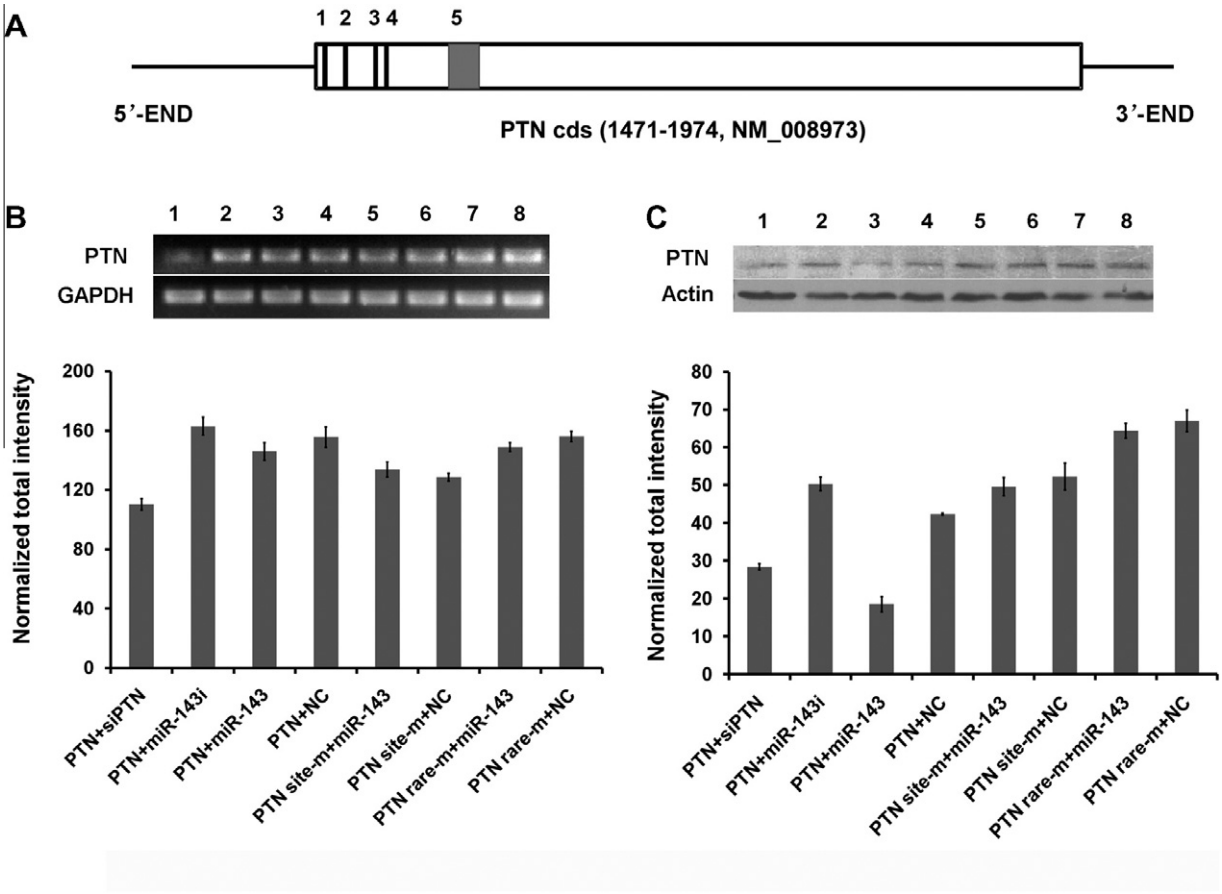

Fig. 4. The effect of rare codons upstream of the miR-143 target site on PTN expression. (A) Diagram of PTN coding region containing the binding site of miR-143 and the rare codons upstream of the target site. 1–4: the rare codons upstream of the miR-143 target site. 5: the miR-143 target site in PTN coding region. Both miRNAs and PTN were co-transfected into Cos-7 cells, the cells were collected for RT-PCR (B) and western blotting (C). NC: small RNA with random sequence; siPTN: PTN-siRNA; miR-143i: miR-143 inhibitor; PTN site-m: PTN with miR-143 binding site mutation of; PTN rare-m: PTN with rare codon mutation.

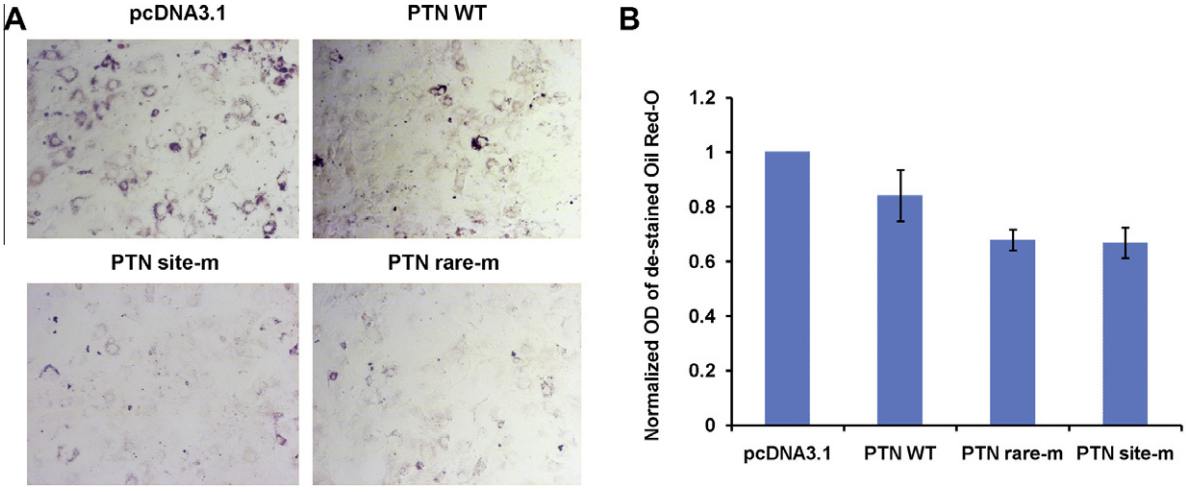

Fig. 5. PTN mutants slightly enhances the inhibitory effect of PTN on spontaneous differentiation of 3T3-L1 cells. (A) Oil Red-O staining of 3T3-L1 cells transfected with wild-type PTN or PTN mutants (site-m or rare-m), pcDNA3.1 was used as a positive control. (B) The stained cells were eluted with isopropanol, the OD value of the elution was determined by spectrophotometer at 510 nm.

of miR-143 during 3T3-L1 cell differentiation, because multi-targets are the most obvious characteristics of miRNAs comparing with other regulatory factors. Computational prediction and biologic data from investigations of genome-wide scale show that one miRNA may target tens to hundreds of genes [19,20]. By co-regulating functionally-related multi-genes miRNA can provoke obvious changes in function [9].

Previously, we demonstrated that PTN plays a negative role on adipogenesis in 3T3-L1 cells preadipocytes [15]. PTN expression is maintained at a nearly undetectable level before confluence. The

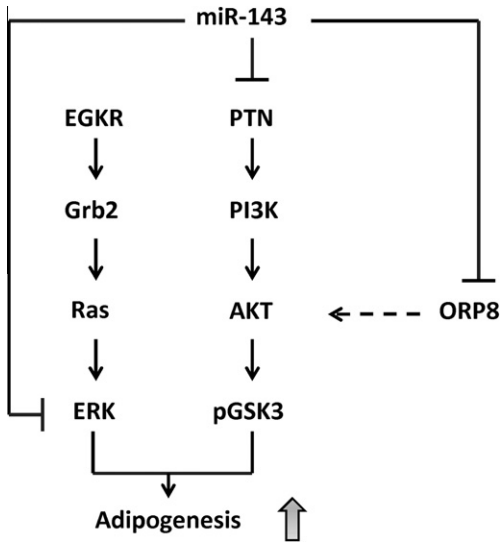

Fig. 6. Diagram of signaling pathways involved in MiR-143 mediated adipogenesis. Through regulation of PTN/AKT, ORP8/AKT and ERK signaling pathway, miR-143 enhances adipogenesis.

expression is significantly increased after confluence, but quickly deceases when differentiation is induced [15]. By contrast, the expression of miR-143 begins to increase when differentiation is induced [13]. Thus, it is highly possible that miR-143 may enhance adipogenesis by repressing PTN expression. The results from this investigation confirm that miR-143 interacts directly with the binding site in PTN coding region and represses PTN expression.

Usually, miRNAs repress gene expression by interacting with target sites located in 3′-UTRs of mRNAs, which leads to translational repression or mRNA degradation [21,22]. However under some circumstances, miRNAs can exercise their control on mRNAs by targeting the coding region or the promoter region [23–25]. It has been reported by Gu et al. that the accessibility of a miRNA target site in coding region is affected by the codons upstream of the target sites [26]. They generated a luciferase expression plasmid containing miR-30 target sites and the targets remain in the 3′-UTR of an mRNA but become embedded within the coding sequence with an additional single-base insertion, abolishing the stop codon and extending the ORF through the miR-30 sites. The luciferase activity of the construct containing the tandem miR-30 target sites in the extended ORF could not be repressed by miR-30. However, miRNA-induced translational knockdown was restored by the addition of rare but not optimal codons upstream of the extended ORF [26]. In this study, we found a cluster of rare codons upstream of the target site of miR-143 in the coding region of PTN. To test the importance of rare codons upstream of miRNA target sites in coding region of a native mRNA molecule for the regulation of miRNA-mediated translation suppression, we mutated these rare codons and found that the mutation of the rare codons eliminated the repressive effect of miR-143 on PTN expression. Interestingly, recent study suggests that miR-130 also acts through a coding region site of PPAR-γ to affect adipogenic differentiation [27]. PPAR-γ mRNA contains a high ratio (over 8.5%) of rare codons, a cluster of rare codons located in the region of 197-164nt upstream of miR-130 target sites are important for miRNA-induced translational knockdown when the binding sites of miRNAs are located in the coding region (Table S3).

In animals, most of the identified target sites for endogenous miRNAs are located in the 3′-UTR of target mRNAs [28], maybe because active translation of mRNAs impedes miRNA-programmed RISC association with target mRNAs in coding region and precludes miRNA-induced knockdown [26]. The clusters of rare codons upstream of miRNA target sites may cause ribosome pausing and let miRNA target sites become more accessible to miRNA-programmed RISC. Taken together, these findings demonstrate that miR-143 enhances adipogenic differentiation of preadipocytes through targeting different genes in different pathways (Fig. 6).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

This project is supported by National Natural Science Foundation of China (No. 30871428).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.09.015.

References

[1] Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297.
[2] He, L. and Hannon, G.J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531.
[3] Ambros, V. (2004) The functions of animal microRNAs. Nature 431, 350–355.
[4] Garzon, R., Calin, G.A. and Croce, C.M. (2009) MicroRNAs in cancer. Annu. Rev. Med. 60, 167–179.
[5] Johnson, C.D. et al. (2007) The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res. 67, 7713–7722.
[6] Makunin, I.V., Pheasant, M., Simons, C. and Mattick, J.S. (2007) Orthologous microRNA genes are located in cancer-associated genomic regions in human and mouse. PLoS ONE 2, e1133.
[7] Hua, Z. et al. (2006) MiRNA-directed regulation of VEGF, other angiogenic factors under hypoxia. PLoS ONE 1, e116.
[8] Kahai, S. et al. (2009) MicroRNA miR-378 regulates nephronectin expression modulating osteoblast differentiation by targeting GalNT-7. PLoS ONE 4, e7535.
[9] He, J. et al. (2010) miRNA-mediated functional changes through co-regulating function related genes. PLoS ONE 5, e13558.
[10] Akao, Y., Nakagawa, Y. and Naoe, T. (2007) MicroRNA-143 and -145 in colon cancer. DNA Cell Biol. 26, 311–320.
[11] Akao, Y., Nakagawa, Y. and Naoe, T. (2006) MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. Oncol. Rep. 16, 845–850.
[12] Jordan, S.D. et al. (2011) Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat. Cell Biol. 13, 434–446.
[13] Takanabe, R. et al. (2008) Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. Biochem. Biophys. Res. Commun. 376, 728–732.
[14] Esau, C. et al. (2004) MicroRNA-143 regulates adipocyte differentiation. J. Biol. Chem. 279, 52361–52365.
[15] Gu, D., Yu, B., Zhao, C., Ye, W., Lv, Q., Hua, Z., Ma, J. and Zhang, Y. (2007) The effect of pleiotrophin signaling on adipogenesis. FEBS Lett. 581, 382–388.
[16] Deuel, T.F., Zhang, N., Yeh, H.J., Silos-Santiago, I. and Wang, Z.Y. (2002) Pleiotrophin: a cytokine with diverse functions and a novel signaling pathway. Arch. Biochem. Biophys. 397, 162–171.
[17] Himburg, H.A. et al. (2010) Pleiotrophin regulates the expansion and regeneration of hematopoietic stem cells. Nat. Med. 16, 475–482.
[18] Kadomatsu, K. and Muramatsu, T. (2004) Midkine and pleiotrophin in neural development and cancer. Cancer Lett. 204, 127–143.
[19] Lim, L.P. et al. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433, 769–773.
[20] Grimson, A., Farh, K.K., Johnston, W.K., Garrett-Engele, P., Lim, L.P. and Bartel, D.P. (2007) MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol. Cell 27, 91–105.
[21] Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233.
[22] Nilsen, T.W. (2007) Mechanisms of microRNA-mediated gene regulation in animal cells. Trends Genet. 23, 243–249.
[23] Tay, Y., Zhang, J., Thomson, A.M., Lim, B. and Rigoutsos, I. (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455, 1124–1128.
[24] Place, R.F., Li, L.C., Pookot, D., Noonan, E.J. and Dahiya, R. (2008) MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc. Natl. Acad. Sci. USA 105, 1608–1613.

[25] Kim, D.H., Saetrom, P., Snove Jr, O. and Rossi, J.J. (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc. Natl. Acad. Sci. USA 105, 16230–16235.
[26] Gu, S., Jin, L., Zhang, F., Sarnow, P. and Kay, M.A. (2009) Biological basis for restriction of microRNA targets to the 3′ untranslated region in mammalian mRNAs. Nat. Struct. Mol. Biol. 16, 144–150.
[27] Lee, E.K. et al. (2011) MiR-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor gamma expression. Mol. Cell. Biol. 31, 626–638.
[28] Lai, E.C. (2002) Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. Nat. Genet. 30, 363–364.