Biochemical and Biophysical Research Communications 460 (2015) 482–488

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MicroRNA-194 promotes osteoblast differentiation via downregulating STAT1

Jun Li [a], Xijing He [b, **], Wenzhi Wei [a], Xiaobo Zhou [c, *]

[a] Department of Emergency, Shannxi Province People's Hospital, Third Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710052, China
[b] Department of Orthopaedics, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China
[c] Department of Immunology and Pathogenic Biology, Medical School, Xi'an Jiaotong University, Xi'an 710061, China

ARTICLE INFO

Article history:
Received 9 March 2015
Available online 19 March 2015

Keywords:
Osteoblast differentiation
miR-194
STAT1
Runx2

ABSTRACT

Osteoblast differentiation is a vital process in maintaining bone homeostasis in which various transcriptional factors, signaling molecules, and microRNAs (miRNAs) are involved. Recently, signal transducer and activator of transcription 1 (STAT1) has been found to play an important role in regulating osteoblast differentiation. Here, we identified that STAT1 expression was regulated by miR-194. Using mouse bone mesenchymal stem cells (BMSCs), we found that miR-194 expression was significantly increased following osteoblast differentiation induction. Overexpression of miR-194 by lentivirus-mediated gene transfer markedly increased osteoblast differentiation, whereas inhibition of miR-194 significantly suppressed osteoblast differentiation of BMSCs. Using a dual-luciferase reporter assay, a direct interaction between miR-194 and the 3′-untranslated region (UTR) of STAT1 was confirmed. Additionally, miR-194 regulated mRNA and protein expression of STAT1 in BMSCs. Further analysis showed that miR-194 overexpression promoted the nuclear translocation of runt-related transcription factor 2 (Runx2), which is critical for osteoblast differentiation. In contrast, inhibition of miR-194 blocked the nuclear translocation of Runx2. Moreover, overexpression of STAT1 significantly blocked Runx2 nuclear translocation and osteoblast differentiation mediated by miR-194 overexpression. Taken together, our data suggest that miR-194 regulates osteoblast differentiation through modulating STAT1-mediated Runx2 nuclear translocation.

1. Introduction

The main cells that regulate bone homeostasis in the bone are osteoblasts and osteoclasts that cooperatively mediate bone-forming and bone-resorbing [1]. The imbalance between bone-forming and bone-resorbing results in bone loss leading to osteoporosis or bone fractures [2]. However, current therapies for the treatment of bone loss are still limited. Osteoblasts are the major cells that contribute to bone formation by secreting alkaline phosphatase (ALP) and bone matrix proteins that induce bone matrix mineralization [3,4]. In recent years, targeting osteoblast differentiation has been suggested to be a promising therapeutic method to treat bone damage and bone loss. Therefore, it is of great importance to find a novel molecular target that regulates osteoblast differentiation.

In recent years, microRNAs (miRNAs), a subset of small non-coding RNAs, have been classified as regulatory RNAs that regulate multiple gene expression by targeting the 3′-untranslated region (UTR) of messenger RNA (mRNA) [5,6]. Therefore, miRNAs have been anticipated to play an important role in cell development, including cell proliferation, apoptosis and differentiation, and thus, in the pathogenesis of various diseases [7,8]. Nowadays, the important role of miRNAs in bone formation and osteoblast differentiation has been highlighted [9–11]. The critical roles of several miRNAs in regulating osteoblast differentiation have been reported. For instance, miR-141 and -200a are reported to regulate osteoblast differentiation through the translational repression of distal-less homeobox 5, an important bone-generating transcription factor [12]. Mizuno et al. revealed that miR-210 is capable of promoting osteoblast differentiation via targeting and inhibiting activin-A receptor type 1B [13]. A group of miRNAs, including miR-23a, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-204,

* Corresponding author. Department of Immunology and Pathogenic Biology, Medical School, Xi'an Jiaotong University, No. 76 Yanta West Road, Xi'an 710061, China. Fax: +86 029 82657814.
** Corresponding author.
E-mail address: xiaobozhouxa@163.com (X. Zhou).

http://dx.doi.org/10.1016/j.bbrc.2015.03.059
0006-291X/

miR-205, miR-217 and miR-338, have been demonstrated to impede osteoblast differentiation through targeting runt-related transcription factor 2 (Runx2), which has been taken as the master transcription factor for osteoblast differentiation and bone formation [14]. These findings suggest that targeting critical miRNAs to develop methods for improving osteoblast differentiation is feasible. However, the precise mechanism of miRNAs in regulating osteoblast differentiation remains largely unknown.

Recently, the role of signal transducer and activator of transcription 1 (STAT1), which is an important signaling molecule in the interferon signaling pathway [15,16], has been widely studied in regulating osteoblast differentiation [17]. Although, it has been found that mice lacking STAT1 showed increased osteoclasts and bone resorption [18,19], the mice also have increased bone formation and bone mass in vivo [20]. Generally, it is suggested that STAT1 interacts with and impedes nuclear translocation in Runx2, thus leading to a blockage of osteoblast differentiation [20]. Runx2 is an important transcription factor for osteoblastogenesis, and mice lacking Runx2 completely abrogate bone formation [21–23]. Therefore, STAT1 has been considered an important component in maintaining skeletal homeostasis. Given that, one can speculate that targeting the gene regulation of STAT1 could provide a potential strategy for improving osteoblast differentiation. Interestingly, we found that STAT1 was a putative target gene of miR-194 using a bioinformatics analysis. Accordingly, we hypothesized that miR-194 might be involved in osteoblast differentiation through regulating STAT1 expression. Mesenchymal stem cells (MSCs) are pluripotent progenitors that are capable of undergoing osteoblast differentiation in certain situations [24]. In this study, we used mouse bone mesenchymal stem cells (BMSCs) to investigate the potential role and underlying mechanism of miR-194 in regulating osteoblast differentiation.

2. Materials and methods

2.1. Cell culture

Mouse bone mesenchymal stem cells (BMSCs) were provided by Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in an α-minimal essential medium (α-MEM; Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) plus 1% penicillin/streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C. To induce osteoblast differentiation, cells were cultured in an osteogenic differentiation medium (HyClone, Logan, UT, USA) containing 10% FBS, 50 μg/ml ascorbic acid, 5 mM sodium β-glycerophosphate and 2 mM L-glutamine, which was refreshed every two days [25].

2.2. Quantitative real-time PCR (qPCR)

Total RNA was extracted with the miRNeasy mini kit (Qiagen, Dusseldorf, Germany) according to the protocol provided by the manufacturer. For mRNA expression analysis, cDNA was synthesized using M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA). For miRNA expression analysis, a cDNA synthesis was performed using a miScript reverse transcription kit (Qiagen). The gene expression level was quantified using SYBR Green Master Mix (Life Technologies, Carlsbad, CA, USA). The relative expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for mRNA or RNU6 for miRNA.

2.3. Cell infection

Recombinant lentivirus vectors carrying miR-194 precursor (LV-miR-194) or miR-194 inhibitor (LV-anti-miR-194) were purchased from Genepharma (Shanghai, China). Lentivirus vectors were transduced into cells according to the supplier's instructions. Briefly, cells were cultured in a normal medium for 24 h. Then they were cultured in a medium containing Polybrene (Cruz Biotechnology, Santa Cruz, CA, USA). Thereafter, cells were infected with the lentivirus at the 0.5×10^5 plaque-forming unit (pfu) overnight. The medium was refreshed and incubated overnight without Polybrene. Then, stable clones expressing miR-194 or anti-miR-194 were selected with Puromycin dihydrochloride (Santa Cruz Biotechnology). For STAT1 overexpression, 0.5 μg of pcDNA3/STAT1 was transfected into cells using Lipofectamine™ 2000 (Life Technologies, Carlsbad, CA, USA).

2.4. Detection of osteoblast differentiation

Alkaline phosphatase (ALP) activity was measured using an ALP assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the standard protocol. Briefly, cells stably expressing miR-194 or miR-anti-194 were cultured in an osteogenic differentiation medium for three, seven and nine days. Cells were harvested, washed with a phosphate buffer solution (PBS), and lysed with a lysis buffer. After centrifugation (2500 g for 15 min at 4 °C), the supernatants were collected and incubated with Senso-Lyte p-nitrophenylphosphate. The absorbance at 405 nm was determined by an ELISA reader (Bio-tek, Winooski, VT, USA). Matrix mineralization was detected with Alizarin red S staining. Briefly, cells were fixed with 70% ethanol for 1 h, and then washed with distilled PBS. Then, cells were stained with Alizarin red S solution (40 mM) for 10 min. Excessive stain was removed using distilled PBS washes. Alizarin red S stained mineral deposits were extracted and dissolved in NaOH (0.1 N), and the absorbance at 540 nm was determined by an ELISA reader (Bio-tek).

2.5. Nuclear protein extraction

Nuclear protein was isolated using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Haimen, China) according to the manufacturer's instructions. Briefly, cells were harvested and washed with PBS, and then were centrifuged at 8000 g for 10 min. The cell sediments were collected and resuspended in 20 μl of PBS and mixed with 200 μl of Buffer A containing 1 mM Phenylmethylsulfonyl fluoride (PMSF). After being vibrated, cells were subjected to an ice bath for 10 min. Thereafter, 10 μl of Buffer B was added. The cells were again vibrated and subjected to an ice bath for one minute followed by centrifugation at 12,000 g for five minutes at 4 °C. The supernatants containing cytoplasmic protein were collected. The remaining sediments were collected and resuspended in 50 μl of a nuclear protein extraction agent and subjected to an ice bath for 30 min with a vortex at an interval of two minutes. After centrifugation (12,000 g for 10 min at 4 °C), the supernatants containing nuclear protein were collected and stored at −70 °C for further analysis.

2.6. Western blot analysis

Equal amounts of proteins were loaded on a 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) apparatus, separated and transferred to a nitrocellulose membrane (Miltenyi Biotec, Auburn, CA, USA). The membranes were then blocked with 3.0% nonfat milk for one hour at 37 °C and incubated overnight with primary antibodies at 4 °C. After they were washed with a Tris buffered saline with Tween20 (TBST), secondary antibodies (1:2000; Bioss, Beijing, China) were added for one hour at room temperature. After they had been washed thrice with TBST, the signals were detected by an enhanced chemiluminescence

reagent. Quantitation analysis for Western blotting was performed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). The data were presented after normalization with the control group. The primary antibodies used in the experiment were as follows: anti-STAT1, anti-Runx2, anti-GAPDH and anti-Lamin B purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.7. Dual-luciferase reporter assay

The STAT1 3′-UTR cDNA fragment containing the putative binding site of miR-194 was amplified and subcloned into a pGL3 luciferase promoter vector (Promega, Madison, WI, USA). Human embryo kidney 293 (HEK293) cells were seeded into six-well plates at 1×10^5 cells per well proceeding LV-miR-194 or LV-anti-miR-194 infection for 24 h. About 0.5 μg pGL3-STAT1 3′-UTR vectors transfected into HEK293 cells using a Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After 48 h of incubation, cells were harvested and lysed in a reporter lysis buffer, and the relative luciferase activity was determined using a dual-luciferase reporter assay kit (Promega).

2.8. Data analysis

Data were expressed as mean ± standard deviation (SD). Statistical differences were analyzed with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) using one-way ANOVAs followed by Bonferroni post hoc tests. Differences were considered statistically significant if they had p-values less than 0.05.

3. Results

3.1. miR-194 expression is elevated during osteoblast differentiation

To explore whether miR-194 is involved in regulating osteoblast differentiation, we analyzed the expression of miR-194 in BMSCs post osteoblast differentiation by qPCR. The results showed that miR-194 expression was significantly increased post osteoblast differentiation (Fig. 1A), implying that miR-194 plays an important role in osteoblast differentiation. To further validate the critical role of miR-194 in regulating osteoblast differentiation, we generated BMSCs stably expressing miR-194 precursor or miR-194 inhibitor by lentivirus mediated gene transfer in which miR-194 expression was significantly increased or decreased, respectively (Fig. 1B). Furthermore, we demonstrated that miR-194 overexpression by LV-miR-194 markedly increased ALP activity (Fig. 1C) and matrix mineralization (Fig. 1D) in BMSCs during osteoblast differentiation. In contrast, inhibition of miR-194 by LV-anti-miR-194 significantly inhibited ALP activity and matrix mineralization (Fig. 1C and D).

3.2. STAT1 is a target gene of miR-194

Next, we sought to investigate the target gene of miR-194 that contributed to regulating osteoblast differentiation. Intriguingly, we found that STAT1, which was a critical gene for osteoblast differentiation, was a predicted target gene of miR-194 (Fig. 1E) by bioinformatics analysis. To validate the interaction between miR-194 and STAT1, we performed a dual-luciferase reporter assay. The results showed that overexpression of miR-194 significantly decreased the luciferase activity in pGL3-STAT1 3′-UTR transfected cells, whereas it had no effect on pGL3-mut STAT1 3′-UTR (Fig. 1F).

3.3. miR-194 regulates the expression of STAT1 in BMSCs

To evaluate whether miR-194 regulated STAT1 expression, we detected the mRNA and protein expression level of STAT1 in LV-miR-194 or LV-anti-mRNA infected cells. qPCR analysis showed that the mRNA level of STAT1 was significantly downregulated or increased by miR-194 overexpression or inhibition, respectively (Fig. 2A). Furthermore, Western blot analysis showed that miR-194 overexpression markedly decreased the protein level of STAT1, whereas miR-194 inhibition increased the protein expression of STAT1 (Fig. 2B).

3.4. miR-194 mediates Runx2 nuclear translocation

Previously, studies have reported that STAT1 regulates osteoblast differentiation associated with modulating Runx2 nuclear translocation [20], a master transcription factor of osteoblast differentiation [14]. Considering the regulatory effect of miR-194 on STAT1 expression, it was expected that miR-194 played an important role in regulating Runx2. Next, we aimed to determine whether miR-194 regulated Runx2 nuclear translocation. Western blot analysis showed that miR-194 overexpression significantly increased the nuclear translocation of Runx2, whereas Runx2 nuclear translocation was significantly inhibited by miR-194 inhibition (Fig. 2C and D).

3.5. Overexpression of STAT1 blocks the Runx2 nuclear translocation induced by miR-194 overexpression

To further validate miR-194 regulated Runx2 nuclear translocation through modulating STAT1, we detected the effect of STAT1 overexpression (Fig. 3A) on Runx2 nuclear translocation induced by miR-194 overexpression. The results showed that when the effect of miR-194 overexpression on Runx2 nuclear translocation was enhanced, it was apparently blocked by STAT1 overexpression (Fig. 3B). As expected, the osteoblast differentiation indicated by ALP activity (Fig. 4A) and matrix mineralization (Fig. 4B) upregulated by miR-194 overexpression was also notably impeded by STAT1 overexpression.

4. Discussion

In the present study, our findings have provided evidence that miR-194 is an important regulator of osteoblast differentiation through modulating STAT1, an inhibitor of osteoblasts. Our data demonstrated that miR-194 was increased during osteoblast differentiation and overexpression of miR-194 significantly increased osteoblast differentiation of BMSCs, whereas inhibition of miR-194 significantly blocked osteoblast differentiation. Using a dual-luciferase reporter assay, we validated a direct interaction between miR-194 and the 3′-UTR of STAT1. In BMSCs, the mRNA and protein expression of STAT1 was also regulated by miR-194, thus disrupting the interaction of STAT1 with Runx2 and promoting Runx2 nuclear translocation. Our data delineated a critical role and mechanism of miR-194 in osteoblast differentiation in which miR-194 regulates STAT1 expression.

The role of miR-194 in various cellular processes in various cell types has been widely investigated. MiR-194 has been earlier reported to be involved in intestinal epithelial cell differentiation, which is controlled by hepatocyte nuclear factor-1alpha [26,27]. The relevant role of miR-194 has been implicated in various cancers, including colon cancer, gastric cancer and liver cancer [28–31]. It has reported that miR-194 inhibits metastasis of non-small cell lung cancer via targeting bone morphogenetic protein 1 and cyclin-dependent kinase inhibitor 1B [32]. More recently,

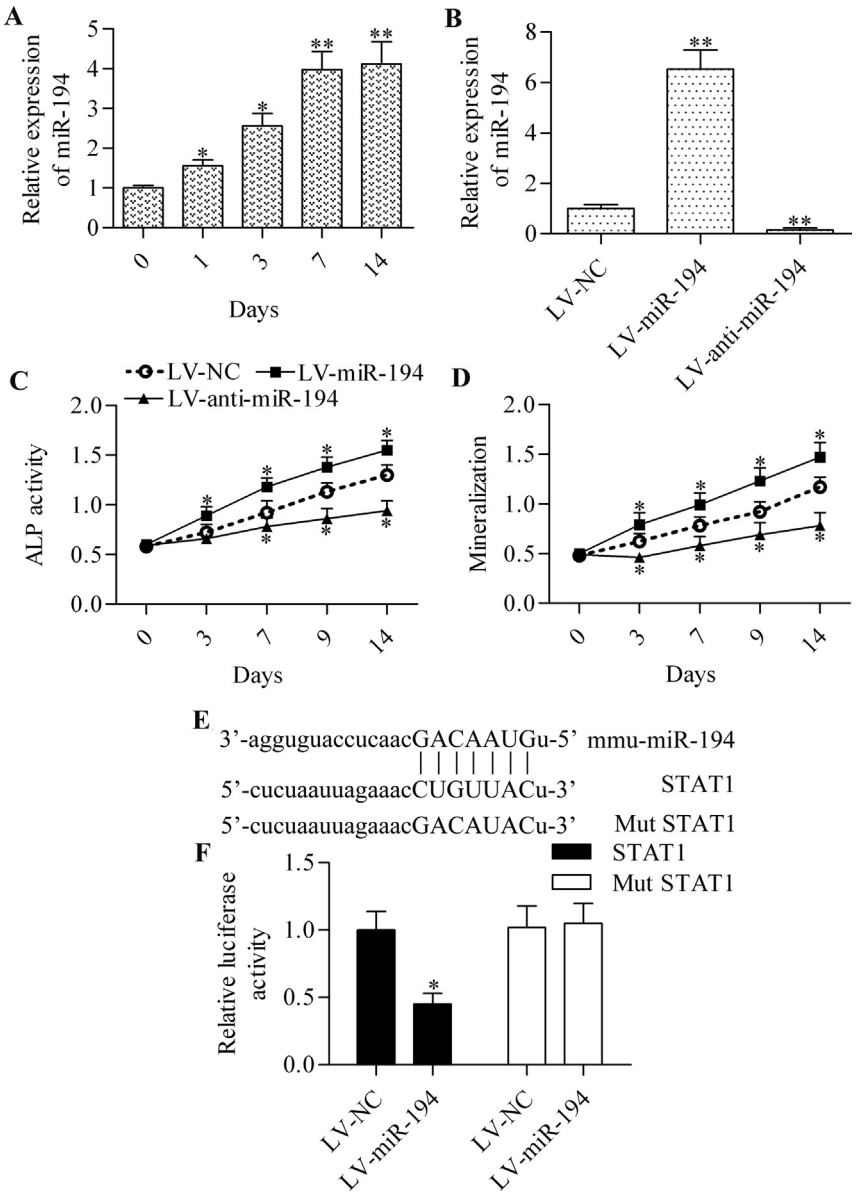

Fig. 1. The role of miR-194 in osteoblast differentiation. (A) Analysis of miR-194 expression during osteoblast differentiation of BMSCs by qPCR. BMSCs were cultured in an osteogenic differentiation medium, and miR-194 expression was detected at days 0, 1, 3, 7 and 14 post-osteogenic differentiation induction. N = 3, *p < 0.05, **p < 0.01 vs. day 0. (B) Detection of miR-194 expression in LV-miR-194 or LV-anti-miR-194 infected cells by qPCR. LV-NC encoding nonspecific miRNAs was taken as a control. N = 3, **p < 0.01 vs. LV-NC. (C) ALP activity was detected in LV-miR-194 or LV-anti-miR-194 infected cells post osteoblast differentiation using an ALP activity kit, and the absorbance was read at 405 nm. N = 6, *p < 0.05 vs. LV-NC. (D) Mineralization was detected by Alizarin red S staining, and the absorbance was quantified at 540 nm. N = 6, *p < 0.05 vs. LV-NC. (E) Schematic diagram of miR-194 target site in the 3′-UTR of STAT1 mRNA. (F) Dual-luciferase reporter assay was performed to detect the interaction between miR-194 and the 3′-UTR of STAT1. LV-miR-194 was co-transfected with pGl3 reporter vectors into HEK293 cells for 48 h. N = 6, *p < 0.01 vs. LV-NC.

various target genes of miR-194, including RING box protein 1 [33], mitogen activated protein kinase 4 kinase [34], AKT2 [35], and an insulin-like growth factor 1 receptor [36] have been reported. Moreover, Xu et al. have revealed that miR-194 mediates chondrogenic differentiation by regulating the expression of SRY-related high mobility group-Box gene 5 [37]. In the present study, we found that miR-194 was involved in regulating osteoblast differentiation through targeting and regulating STAT1 expression. Our finding is consistent with the reports of Jeong et al., which demonstrate that miR-194 is increased during osteoblast differentiation or decreased in adipocyte differentiation, and overexpression of miR-194 promotes osteoblast differentiation [38]. However, they found that miR-194 reciprocally regulated osteogenesis and adipogenesis through regulating chicken ovalbumin upstream promoter-transcription factor II, which was an important regulator of the MSCs fate by suppressing Runx2 activity [39]. Here, our data indicated that STAT1 was a target gene of miR-194 through which miR-194 could also regulate Runx2 activity and osteoblast differentiation in BMSCs. Nonetheless, all these findings support the notion that miR-194 is an important regulator of osteoblast differentiation.

STAT1 is an important signaling molecule in interferon signaling pathways [15,16]. Previous studies have suggested that interferon systems, including interferon-α, β and γ, are involved in regulating interferon systems via inhibiting osteoclastogenesis, in which STAT1 is essential for interferon-mediated signal termination [18,40]. It has been reported that mice that lacked STAT1 showed increased osteoclast numbers and bone resorption in vivo [18,19]. Meanwhile, another study has demonstrated that the mice lacking

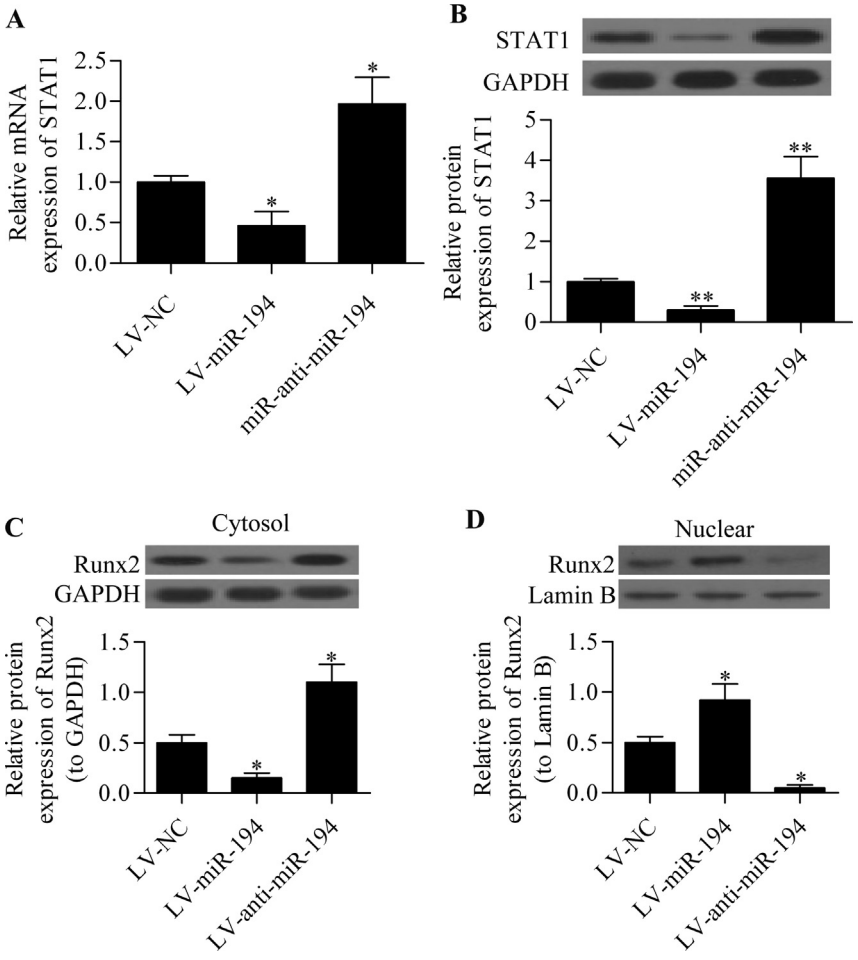

Fig. 2. Effect of miR-194 on STAT1 and Runx2 expression. The mRNA (A) and (B) expression levels were detected by qPCR and Western blot analysis, respectively, in BMSCs infected with LV-miR-194 or LV-anti-miR-194. Relative protein expression was measured using Image-Pro Plus 6.0 software, and was normalized to GAPDH. N = 3, *p < 0.05, *p < 0.01 vs. LV-NC. (C) Western blot analysis of Runx2 protein expression in the cytoplasm. GAPDH was used as a control for cytosol extracts. (D) Western blot analysis of Runx2 protein expression in the cell nucleus. Lamin B was used as a control for nuclear extracts. Relative protein expression was measured using Image-Pro Plus 6.0 software. N = 3, *p < 0.05 vs. LV-NC.

STAT1 have increased bone formation and bone mass in vivo [20], implying that STAT1 also participated in osteoblast differentiation. They found that lack of STAT1 resulted in an enhanced activation of Runx2 and osteoblast differentiation by disrupting the interaction between STAT1 and Runx2, which leads to an accumulation of Runx2 nuclear translocation [20]. In contrast, overexpression of STAT1 significantly impeded Runx2 nuclear translocation [20]. Consistent with the findings, our data demonstrated that inhibition of STAT1 by miR-194 significantly promoted osteoblast differentiation by upregulation of Runx2 nuclear translocation. Besides,

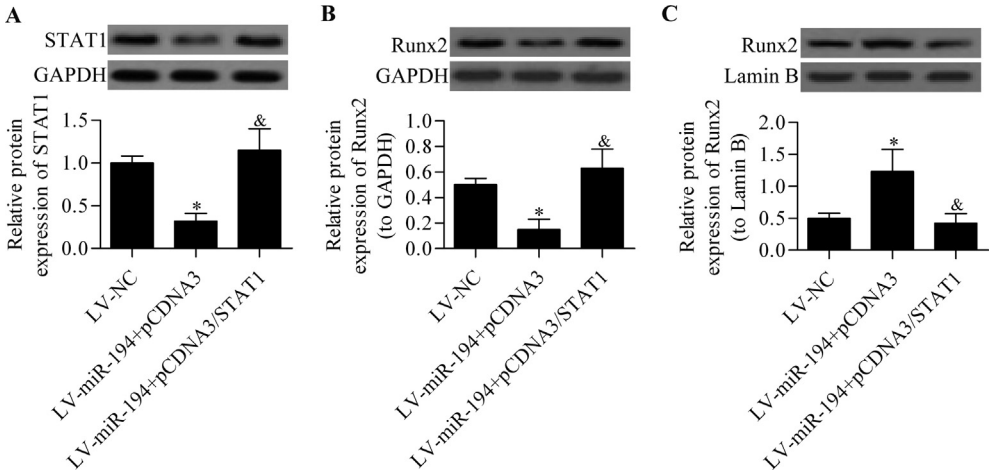

Fig. 3. Effect of STAT1 overexpression on Runx2 nuclear translocation. (A) Detection of STAT1 protein expression in different groups by Western blot analysis. Cells were co-transfected with LV-miR-194 and pcDNA3/STAT1 harboring no 3′-UTR. (B) Western blot analysis of Runx2 protein expression in the cell nucleus. Relative protein expression was measured using Image-Pro Plus 6.0 software. N = 3, *p < 0.01 vs. LV-NC; p < 0.05 vs. LV-miR-194 + pcDNA3.

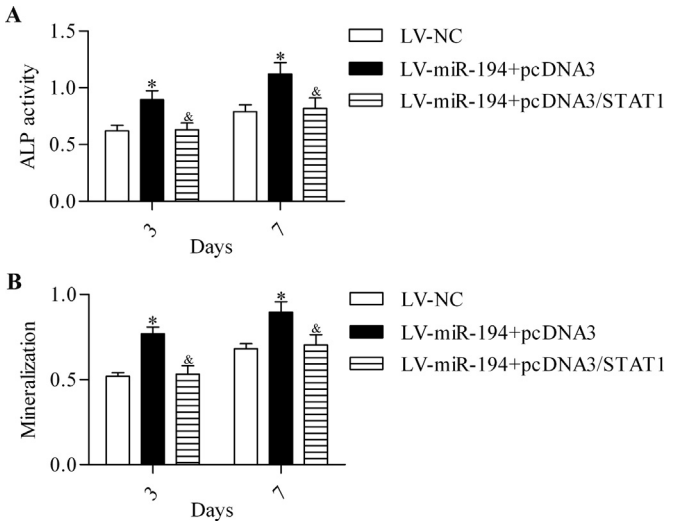

Fig. 4. Effect of STAT1 overexpression on osteoblast differentiation. Detection of ALP activity (A) and matrix mineralization (B) in LV-miR-194 and pcDNA3/STAT1 co-transfected cells. N = 6, *p < 0.01 vs. LV-NC; p < 0.05 vs. LV-miR-194 + pcDNA3.

overexpression of STAT1 could apparently abolish the positive effect of miR-194 overexpression on osteoblast differentiation and blocked Runx2 nuclear translocation. Overall, these data provided a better understanding of miR-194 in regulating osteoblast differentiation.

Interestingly, it has been demonstrated that STAT1-deficient mice showed increased fracture callus remodeling and membranous ossification in a mouse model of bone fractures [41]. Furthermore, administration of fludarabine, which is a potent STAT1 inhibitor, significantly promoted bone formation in a heterotopic ossification model [41]. It has been demonstrated that the acceleration of ubiquitination and degradation of STAT1 protein by double-stranded RNA-dependent protein kinase in a STAT-interacting LIM protein-dependent manner increased Runx2 activity and osteoblast differentiation [42]. More recently, Fujie et al. showed that B cell lymphoma 6, a transcriptional repressor, inhibits STAT1 expression by binding to the promoter region in osteoblasts that stimulate Runx2 nuclear translocation and osteoblast differentiation [43]. These findings indicate that targeting and inhibiting STAT1 is an effective way to improve osteoblast differentiation.

Inhibiting STAT1 using miRNAs has been demonstrated by several studies. For instance, miR-146a inhibits anti-hepatitis B virus immune response via downregulating STAT1 in hepatocytes [44]. In chronic hepatitis B patients, miR-146a is found to be highly upregulated, which inhibits T cell immune function by targeting and inhibiting STAT1 [45]. MiR-145 inhibits colon cancer by directly suppressing gene expression of STAT1 [46]. Therefore, these reports hint that inhibiting STAT1 expression with miRNAs is a feasible method. Taken together, our data suggest that suppressing STAT1 expression with miR-194 accelerates osteoblast differentiation, thus implying that miR-194 could be used as a potential molecular target for improving bone homeostasis.

Conflict of interest

None.

Acknowledgments

This work was supported by Shannxi Province Basic Research Grants (2014JM4197).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.059.

References

[1] T.D. Rachner, S. Khosla, L.C. Hofbauer, Osteoporosis: now and the future, Lancet 377 (2011) 1276–1287.
[2] G.A. Rodan, T.J. Martin, Therapeutic approaches to bone diseases, Science 289 (2000) 1508–1514.
[3] E. Canalis, A.N. Economides, E. Gazzerro, Bone morphogenetic proteins, their antagonists, and the skeleton, Endocr. Rev. 24 (2003) 218–235.
[4] R.T. Franceschi, The developmental control of osteoblast-specific gene expression: role of specific transcription factors and the extracellular matrix environment, Crit. Rev. Oral Biol. Med. 10 (1999) 40–57.
[5] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[6] J. Winter, S. Jung, S. Keller, et al., Many roads to maturity: microRNA biogenesis pathways and their regulation, Nat. Cell. Biol. 11 (2009) 228–234.
[7] J.T. Mendell, E.N. Olson, MicroRNAs in stress signaling and human disease, Cell 148 (2012) 1172–1187.
[8] K. Ranganathan, V. Sivasankar, MicroRNAs – biology and clinical applications, J. Oral Maxillofac. Pathol. 18 (2014) 229–234.
[9] Y. Arfat, W.Z. Xiao, M. Ahmad, et al., Role of microRNAs in osteoblasts differentiation and bone disorders, Curr. Med. Chem. (2014).
[10] G. Papaioannou, F. Mirzamohammadi, T. Kobayashi, MicroRNAs involved in bone formation, Cell. Mol. Life Sci. 71 (2014) 4747–4761.
[11] C. Pi, Y.P. Li, X. Zhou, et al., The expression and function of microRNAs in bone homeostasis, Front. Biosci. (Landmark Ed.) 20 (2015) 119–138.
[12] T. Itoh, Y. Nozawa, Y. Akao, MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5, J. Biol. Chem. 284 (2009) 19272–19279.
[13] Y. Mizuno, Y. Tokuzawa, Y. Ninomiya, et al., miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b, FEBS Lett. 583 (2009) 2263–2268.
[14] D. Chen, M. Zhao, G.R. Mundy, Bone morphogenetic proteins, Growth Factors 22 (2004) 233–241.
[15] I. Lodige, A. Marg, B. Wiesner, et al., Nuclear export determines the cytokine sensitivity of STAT transcription factors, J. Biol. Chem. 280 (2005) 43087–43099.
[16] K. Mowen, M. David, Regulation of STAT1 nuclear export by Jak1, Mol. Cell. Biol. 20 (2000) 7273–7281.
[17] H. Takayanagi, S. Kim, T. Koga, et al., Stat1-mediated cytoplasmic attenuation in osteoimmunology, J. Cell. Biochem. 94 (2005) 232–240.
[18] H. Takayanagi, K. Ogasawara, S. Hida, et al., T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma, Nature 408 (2000) 600–605.
[19] H. Takayanagi, S. Kim, K. Matsuo, et al., RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta, Nature 416 (2002) 744–749.
[20] S. Kim, T. Koga, M. Isobe, et al., Stat1 functions as a cytoplasmic attenuator of Runx2 in the transcriptional program of osteoblast differentiation, Genes. Dev. 17 (2003) 1979–1991.
[21] T. Komori, H. Yagi, S. Nomura, et al., Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts, Cell 89 (1997) 755–764.
[22] P. Ducy, R. Zhang, V. Geoffroy, et al., Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation, Cell 89 (1997) 747–754.
[23] K. Nakashima, X. Zhou, G. Kunkel, et al., The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation, Cell 108 (2002) 17–29.
[24] D.J. Prockop, Marrow stromal cells as stem cells for nonhematopoietic tissues, Science 276 (1997) 71–74.
[25] M.Q. Hassan, Y. Maeda, H. Taipaleenmaki, et al., miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells, J. Biol. Chem. 287 (2012) 42084–42092.
[26] K. Hino, T. Fukao, M. Watanabe, Regulatory interaction of HNF1-alpha to microRNA-194 gene during intestinal epithelial cell differentiation, Nucleic Acids Symp. Ser. (Oxf) (2007) 415–416.
[27] K. Hino, K. Tsuchiya, T. Fukao, et al., Inducible expression of microRNA-194 is regulated by HNF-1alpha during intestinal epithelial cell differentiation, RNA 14 (2008) 1433–1442.
[28] P. Sundaram, S. Hultine, L.M. Smith, et al., p53-responsive miR-194 inhibits thrombospondin-1 and promotes angiogenesis in colon cancers, Cancer Res. 71 (2011) 7490–7501.
[29] Y. Song, F. Zhao, Z. Wang, et al., Inverse association between miR-194 expression and tumor invasion in gastric cancer, Ann. Surg. Oncol. 19 (Suppl. 3) (2012) S509–S517.
[30] Z. Meng, X. Fu, X. Chen, et al., miR-194 is a marker of hepatic epithelial cells and suppresses metastasis of liver cancer cells in mice, Hepatology 52 (2010) 2148–2157.

[31] H.S. Jung, Y.R. Seo, Y.M. Yang, et al., Galpha12gep oncogene inhibits FOXO1 in hepatocellular carcinoma as a consequence of miR-135b and miR-194 dysregulation, Cell. Signal 26 (2014) 1456–1465.
[32] X. Wu, T. Liu, O. Fang, et al., miR-194 suppresses metastasis of non-small cell lung cancer through regulating expression of BMP1 and p27(kip1), Oncogene 33 (2014) 1506–1514.
[33] X. Chen, Y. Wang, W. Zang, et al., miR-194 targets RBX1 gene to modulate proliferation and migration of gastric cancer cells, Tumour Biol. (2014).
[34] B. Wang, Z.L. Shen, Z.D. Gao, et al., MiR-194, commonly repressed in colorectal cancer, suppresses tumor growth by regulating the MAP4K4/c-Jun/MDM2 signaling pathway, Cell. Cycle (2015).
[35] H.J. Zhao, L.L. Ren, Z.H. Wang, et al., MiR-194 deregulation contributes to colorectal carcinogenesis via targeting AKT2 pathway, Theranostics 4 (2014) 1193–1208.
[36] Y. Yu, J. Chai, H. Zhang, et al., miR-194 promotes burn-induced hyperglycemia via attenuating IGF-IR expression, Shock 42 (2014) 578–584.
[37] J. Xu, Y. Kang, W.M. Liao, et al., MiR-194 regulates chondrogenic differentiation of human adipose-derived stem cells by targeting Sox5, PLoS One 7 (2012) e31861.
[38] B.C. Jeong, I.H. Kang, Y.C. Hwang, et al., MicroRNA-194 reciprocally stimulates osteogenesis and inhibits adipogenesis via regulating COUP-TFII expression, Cell. Death Dis. 5 (2014) e1532.
[39] K.N. Lee, W.G. Jang, E.J. Kim, et al., Orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) protein negatively regulates bone morphogenetic protein 2-induced osteoblast differentiation through suppressing runt-related gene 2 (Runx2) activity, J. Biol. Chem. 287 (2012) 18888–18899.
[40] H. Takayanagi, H. Iizuka, T. Juji, et al., Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis, Arthritis Rheum. 43 (2000) 259–269.
[41] K. Tajima, H. Takaishi, J. Takito, et al., Inhibition of STAT1 accelerates bone fracture healing, J. Orthop. Res. 28 (2010) 937–941.
[42] K. Yoshida, H. Okamura, B.R. Amorim, et al., PKR-mediated degradation of STAT1 regulates osteoblast differentiation, Exp. Cell. Res. 315 (2009) 2105–2114.
[43] A. Fujie, A. Funayama, Y. Miyauchi, et al., Bcl6 promotes osteoblastogenesis through Stat1 inhibition, Biochem. Biophys. Res. Commun. (2015).
[44] Z.H. Hou, Q.J. Han, C. Zhang, et al., miR146a impairs the IFN-induced anti-HBV immune response by downregulating STAT1 in hepatocytes, Liver Int. 34 (2014) 58–68.
[45] S. Wang, X. Zhang, Y. Ju, et al., MicroRNA-146a feedback suppresses T cell immune function by targeting Stat1 in patients with chronic hepatitis B, J. Immunol. 191 (2013) 293–301.
[46] L.H. Gregersen, A.B. Jacobsen, L.B. Frankel, et al., MicroRNA-145 targets YES and STAT1 in colon cancer cells, PLoS One 5 (2010) e8836.