Int J Clin Exp Pathol 2017;10(6):6536-6543
www.ijcep.com /ISSN:1936-2625/IJCEP0051573

Original Article

MicroRNA-215 promotes proliferation and differentiation of osteoblasts by regulation of c-fos

Chia-Hsien Chen[1,3], Hsien-Tsung Lu[2,3], Yang-Hwei Tsuang[1,3], Yi-Jie Kuo[2,3]

[1]Department of Orthopedics, Shuang Ho Hospital, Taipei Medical University, New Taipei, Taiwan; [2]Department of Orthopedic Surgery, Taipei Medical University Hospital, Taipei, Taiwan; [3]Department of Orthopedic Surgery, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

Received February 23, 2017; Accepted April 25, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Background: Exploration of the molecular mechanisms governing osteoblast proliferation and differentiation is very important for improving the treatment of osteoporosis. MicroRNAs (miRNAs) have been shown to act as a regulator during osteoblastic differentiation. In this study, we examined the role of miR-215 in the proliferation and differentiation of MC3T3-E1 cells. Methods: The murine pre-osteoblast cell line MC3T3-E1 was used in the experiment. After transfected with miR-215 mimic, miR-215 inhibitor, or negative control, the expressions of miR-215, Runx2, Ocn, c-fos, MAPK, and JAK/STAT were assessed using qRT-PCR. Cell viability and migration were analyzed by Cell Counting Kit-8 assay and the level of expressions of Runx2, Ocn, c-fos, MAPK, and JAK/STAT were detected by western blotting. Results: MiR-215 expression was significantly upregulated during osteoblastic differentiation. Overexpression of miR-215 significantly promoted viability, migration, and differentiation of MC3T3-E1 cells, whereas silencing of miR-215 inhibited these processes. Furthermore, it was found that overexpression of miR-215 significantly upregulated the expression of c-fos, MAPK, and JAK/STAT proteins, while silencing of c-fos reversed these effects. These findings together indicate that miR-215 promotes proliferation and differentiation of osteoblasts by upregulating the expression of c-fos. Conclusion: Our findings imply that miR-215 promotes osteoblastic differentiation of MC3T3-E1 cells by regulating c-fos expression, and thus represent a novel and potential therapeutic target for treatment of osteoporosis.

Keywords: MicroRNA-215, osteoporosis, osteoblast differentiation, c-fos, cell proliferation

Introduction

Bone homeostasis is maintained by a balance between bone formation and bone resorption. During bone remodeling, osteoclasts are activated first which lead to bone resorption, followed by activation of osteoblasts leading to bone formation. Osteoporosis occurs when osteoclastic bone resorption exceeds osteoblastic bone formation [1]. Osteoporosis is a common skeletal disorder characterized by poor bone strength that predisposes patients to increased risk of fracture [2]. The progress of osteoporosis depends on the balance between osteoclasts and osteoblasts activities. Typical treatment strategy for osteoporosis focuses on inhibiting the excessive activation of osteoclasts [1]. However, mechanism of osteoblast differentiation is not much explored for treatment of osteoporosis. Therefore, understanding the mechanisms of osteoblast differentiation and maturation will assist in developing novel treatments options for osteoporosis.

MicroRNAs (miRNAs) are small, non-coding RNAs that enter the RNA interference pathway to regulate the expression of protein-encoding genes at the post-transcriptional level. miRNAs are involved in various cellular processes such as cell proliferation, migration, apoptosis, and differentiation [3-5]. miRNAs have been shown to be involved in osteoporosis in many studies, as miR-21, miR-23a, miR-24, miR-93, miR-100, miR-122a, miR-124a, miR-125b, and miR-148a were found to be significantly upregulated in the serum of patients with osteoporosis [6]. Additionally, miR-21 and miR-31 have been reported to play a critical role in osteoclast differentiation [1]. De-Ugarte et al showed that miR-320a and miR-483-5p were overexpressed in osteoporotic samples and expressed in primary osteoblasts [7].

MiR-215 is a p53-inducible miRNA that has the capability of increasing p21 level and arresting cell cycle [8-10]. Altered expression of miR-215 has been reported in several types of cancers [11-13]. However, the role of miR-215 in osteoporosis is not yet studied. Here, we assessed the expression of miR-215 during osteoblastic differentiation and the effect of forced expression of miR-215 on osteoblast proliferation and differentiation. In addition, we also analyzed the underlying molecular mechanisms by measuring the expressions of c-fos gene, mitogen-activated protein kinases (MAPK), Janus kinase/signal transducers, and activators of transcription (JAK/STAT) pathways.

Materials and methods

Cell culture and differentiation induction

MC3T3-E1 is an osteoblast precursor cell line derived from C57BL/6 mouse calvaria. MC3T3-E1 cells were plated in 100-mm dishes and incubated in α-Minimum Essential Medium (MEM), with 10% fetal bovine serum (FBS; Atlanta), 100 units/ml of penicillin, and 100 g/ml of streptomycin. At confluence (Day 0), these cells were treated with osteogenic differentiation media containing 10 mM β-glycerophosphate and 50 μg/ml of ascorbic acid. The differentiation media was refreshed every 48 hourly after the initial differentiation treatment.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the cells and tissues using Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II with the TaqMan MicroRNA Assay of miR-215 and U6 (Applied Biosystems, Foster City, CA, USA) were used for testing the expression levels of miR-215 in cells.

miRNAs transfection

MiR-215 mimic, si-miR-215, si-c-fos, and negative control (NC) were obtained from GenePharma Co. (Shanghai, China). Cell transfections were conducted using Lipofectamine 3000 reagent (Invitrogen) following the manufacturer's protocol.

Cell Counting Kit-8 (CCK-8) assay

Cells were seeded in 96-well plate with 5000 cells/well. Cell proliferation and migration were assessed by Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD) assay. Briefly, after stimulation, the CCK-8 solution was added to the culture medium, and the cultures were incubated for 1 hour at 37°C in humidified 95% air and 5% CO_2. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

Alkaline phosphatase activity

Osteoblasts were cultured for up to 21 days in osteogenic media containing 10 μM ascorbic acid and 50 μM β-glycerolphosphate. For alkaline phosphatase (ALP) staining, cells were fixed in 10% formalin and stained as previously detailed [14].

Western blot analysis

The protein used for western blotting was extracted using RIA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was purchased from Sigma. Primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1,000. Primary antibody was incubated with the membrane at 4°C overnight, followed by wash and incubation with secondary antibody marked by horseradish peroxidase for 1 hour at room temperature. After rinsing, the polyvinylidene difluoride membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented

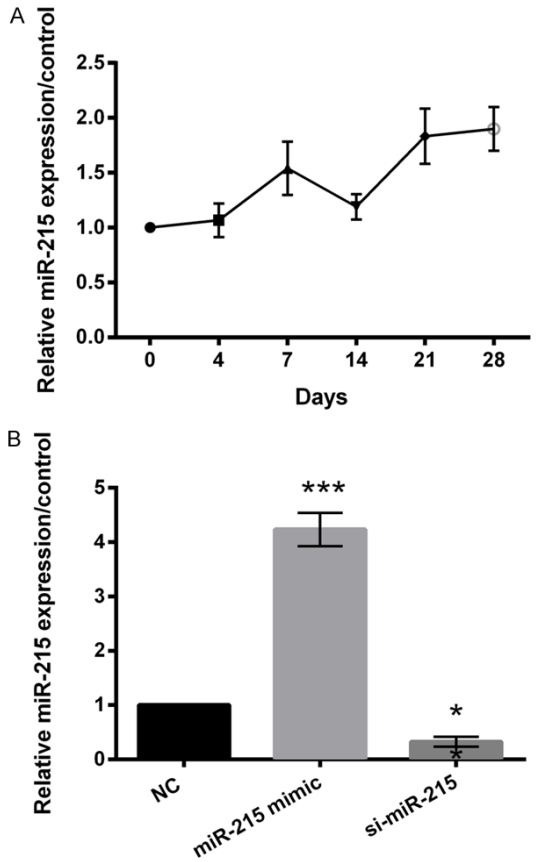

Figure 1. MiR-215 expression is increased during differentiation of osteoblasts. A. Total RNA was analyzed for expression of miR-215 by qRT-PCR at the indicated time points using MC3T3 cells. Absolute expression (y axis) was normalized to U6 small RNA. B. MC3T3 cells were transfected with miR-215 mimic, si-miR-215, or negative control (NC) to measure the transfection efficiency of miR-215 mimic and si-miR-215 in the cells using qRT-PCR. *P<0.05, ***P<0.001.

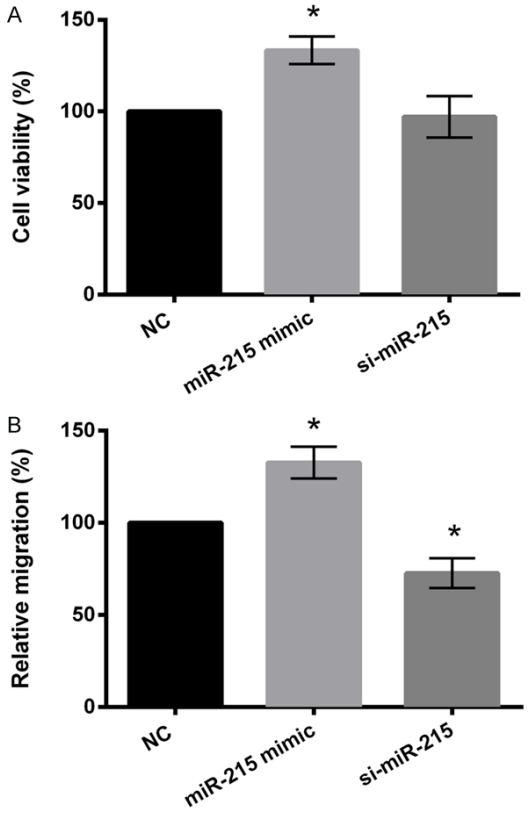

Figure 2. Overexpression of miR-215 promotes cell viability and migration. Cell Counting Kit-8 assay was used to measure MC3T3 cells viability (A) and migration (B). The cells were transfected with miR-215 mimic, si-miR-215, or negative control (NC). *P<0.05.

as the mean ± standard deviation. Statistical analyses were performed using SPSS 19.0 statistical software. The *P*-values were calculated using a one-way analysis of variance (ANOVA). *P*-value of <0.05 was considered to indicate a statistically significant result.

Results

MiR-215 expression is increased during differentiation of osteoblasts

Differentiation of MC3T3 pre-osteoblasts occurs in three stages of phenotypic maturation: Day 7 (post-confluency, early differentiation), Day 12 (mature osteoblasts), and Days 15-21 (mineralization). We measured total RNA for expression of miR-215 during this maturation program using qRT-PCR at indicated time points using MC3T3 cells. The results showed that relative expression of miR-215 continuously increased during this differentiation process, with a short decline during Days 7 to 14 (**Figure 1A**). Furthermore, we showed that transfection of the osteoblasts with miR-215 mimic significantly increased relative miR-15 expression as compared with negative control (P<0.05; **Figure 1B**). However, silencing the expression of miR-215 using si-miR-215 significantly decreased the expression of miR-215 in osteoblasts (P< 0.05; **Figure 1B**). These findings show that miR-215 is upregulated during differentiation of osteoblasts.

Overexpression of miR-215 promotes cell viability and migration

We then evaluated effect of miR-215 on viability and migration of osteoblasts. Cell viability

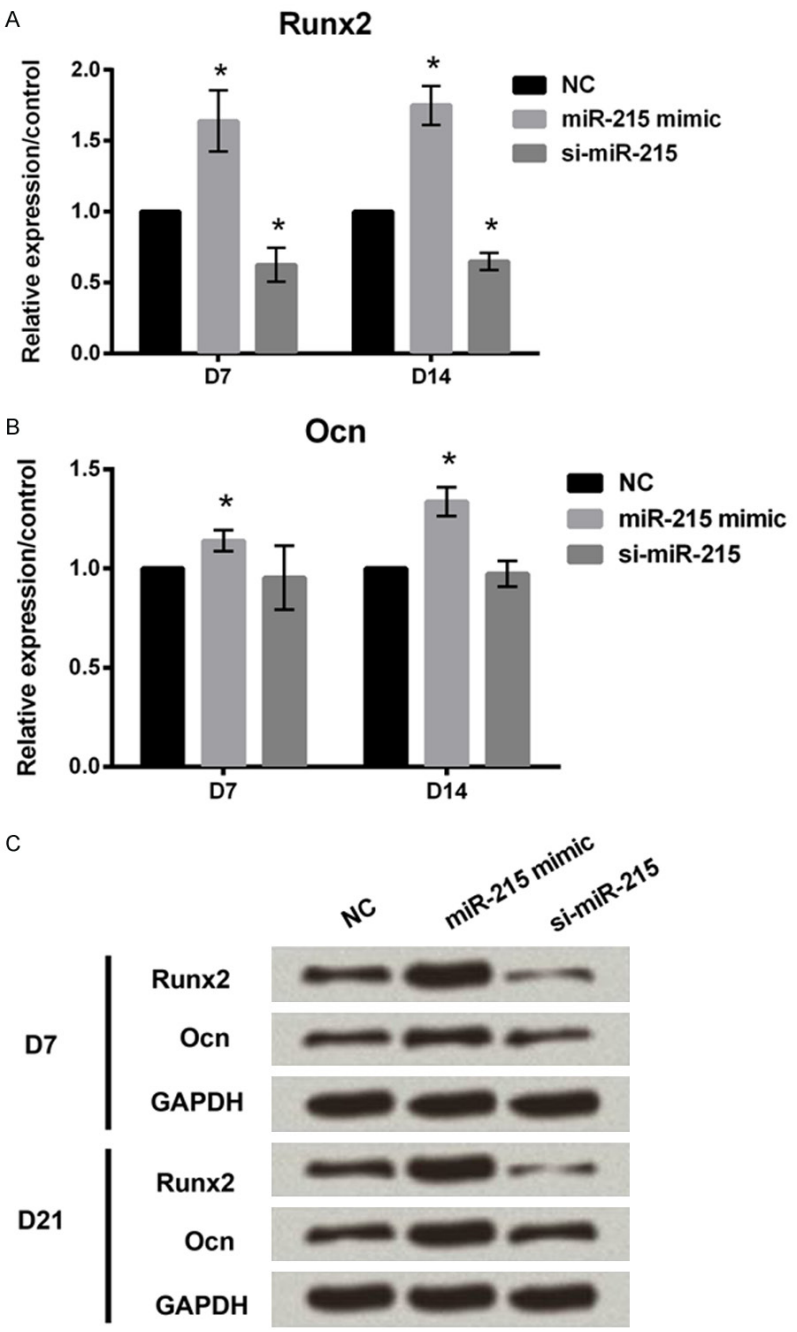

Figure 3. Overexpression of miR-215 promotes differentiation of osteoblasts. MC3T3 cells were transfected with miR-215 mimic, si-miR-215, or negative control (NC). Total RNA was analyzed by qRT-PCR for mRNA expression profile of bone marker genes Runx2 (A) and Ocn (B) at Day 7 and Day 14. The Runx2 transcription factor and Ocn are represented as markers of early and late stages of osteoblast lineage cells. (C) Western blot analysis at Day 7 and Day 21. GAPDH: glyceraldehyde 3-phosphate dehydrogenase. *P<0.05.

and migration were measured by CCK-8 assay. Introduction of miR-215 into osteoblasts significantly increased cell viability and migration (both P<0.05; **Figure 2A** and **2B**). In contrast, silencing the expression of miR-215 using si-miR-215 resulted in significant decrease in cell migration (P<0.05, **Figure 2B**); the decrease in cell viability was not statistically significant (**Figure 2A**).

Overexpression of miR-215 promotes differentiation of osteoblasts

To evaluate effect of miR-215 on osteoblast differentiation, we measured the expression of osteogenic differentiation markers, Runx2 and osteocalcin (Ocn), in osteoblasts transfected with negative control, miR-215-mimic, or si-miR-215. The qRT-PCR results (**Figure 3A** and **3B**) showed overexpression of miR-215 significantly increased expression of Runx2 and Ocn at Days 7 and 14 (P<0.05), while knockdown of miR-215 significantly decreased the expression of Runx2 (P<0.05) but had no significant effects on Ocn expression. The western blot analysis showed the similar results at Day 7 and Day 21 (**Figure 3C**). Thus, miR-215 overexpression promotes osteoblast differentiation and knockdown of miR-215 inhibits differentiation.

MiR-215 upregulates expression of c-fos

C-fos gene is a proto-oncogene which is involved in various important cellular processes, such as cell proliferation, differentiation, and survival. We measured the expression of c-fos in osteoblasts transfected with miR-215 mimic, si-miR-215, or negative control. The qRT-PCR results (**Figure 4A**) showed that overexpression of miR-215 significantly increased the relative expression of c-fos (P<0.05) while knockdown

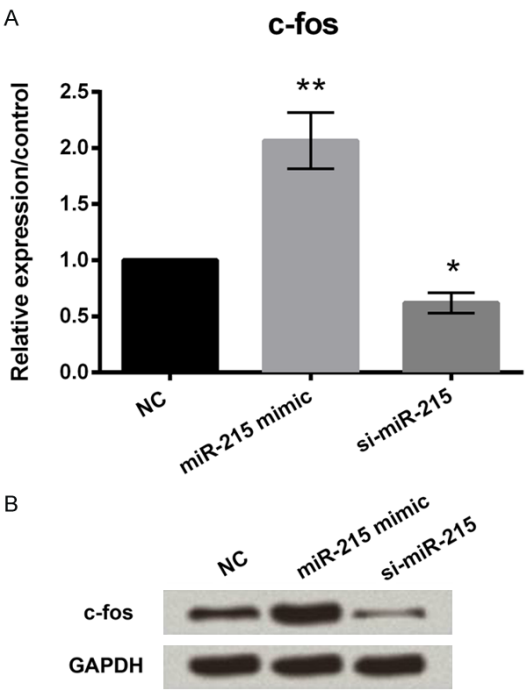

Figure 4. MiR-215 upregulates expression of c-fos. A. MC3T3 cells were transfected with miR-215 mimic, si-miR-215, or negative control (NC). Total RNA was analyzed by qRT-PCR for mRNA expression profile of c-fos. B. Western blot analysis was used to measure the expression of c-fos in the transfected cells. GAPDH: glyceraldehyde 3-phosphate dehydrogenase. *P<0.05, **P<0.01.

of miR-215 significantly decreased the expression of c-fos (P<0.05). The western blot analysis showed the similar results (**Figure 4B**). This result indicates that miR-215 positively regulates expression of c-fos.

MiR-215 regulates downstream signaling pathway

Lastly, we explored the possible roles of the MAPK and JAK/STAT pathways in proliferation and differentiation of osteoclasts. The qRT-PCR results (**Figure 5A**) showed that overexpression of miR-215 increased the relative expressions of p/t-p38MAPK, p/t-JAK1, p/t-STAT1, and p/t-STAT2, while knockdown of miR-215 expression reversed these results. Interestingly, the effects of miR-215 overexpression were also reversed by silencing of c-fos (**Figure 5A**). The western blot analysis showed the similar results (**Figure 5B**). These findings indicate that miR-215 upregulates the MAPK and JAK/STAT pathways.

Discussion

In the present study, we demonstrated the positive role of miR-215 during *in vitro* osteoblast differentiation. First, we provided evidence that miR-215 expression was increased during osteoblast differentiation. Further analysis demonstrated that overexpression of miR-215 promoted MC3T3-E1 cell proliferation, migration and differentiation into osteoblasts, while miR-215 silencing inhibited cell proliferation, migration and differentiation. The mechanism assays confirmed that miR-215 regulated osteoblast proliferation and differentiation by activating the MAPK and JAK/STAT signaling pathways via upregualtion of c-fos gene. These findings suggest the potential role of miR-215 in regulating the process of bone regeneration.

Recently, many miRNAs have emerged as important regulators of posttranscriptional gene expressions [15]. miRNAs play critical roles in osteogenesis [16]. Overexpression of several miRNAs during osteoblast differentiation has been reported in previous studies. In an *in vitro* study using human bone marrow stromal cells, miR-15b was found to be highly expressed in differentiated osteoblasts [17]. In a study using human mesenchymal stem cells, miR-21 expression was found to be elevated during osteoblast differentiation [18]. In another study using human mesenchymal stem cells, the expression of miR-27 was increased during osteoblast differentiation [19]. Consistent with these findings, we demonstrated that the expression of miR-215 is significantly increased during the maturation process of osteoblasts.

Reports show that miR-215 acts as a positive or negative regulator in several types of cancer. Mostly, miR-215 overexpression inhibits cell proliferation and migration in cancer. It has been demonstrated that the expression of miR-215 is downregulated in pancreatic and non-small lung cancer cells, and overexpression of miR-215 inhibits cell proliferation, migration and invasion by targeting ZEB2 [12, 20]. In contrast to this report, miR-215 was found to be upregulated in gastric cancer cells and it promoted cell proliferation, migration and invasion by directly targeting Runx1 [21]. Consistent with this report, we showed that miR-215 is upregulated in osteoblasts and it promotes cell proliferation and migration. Silencing of miR-

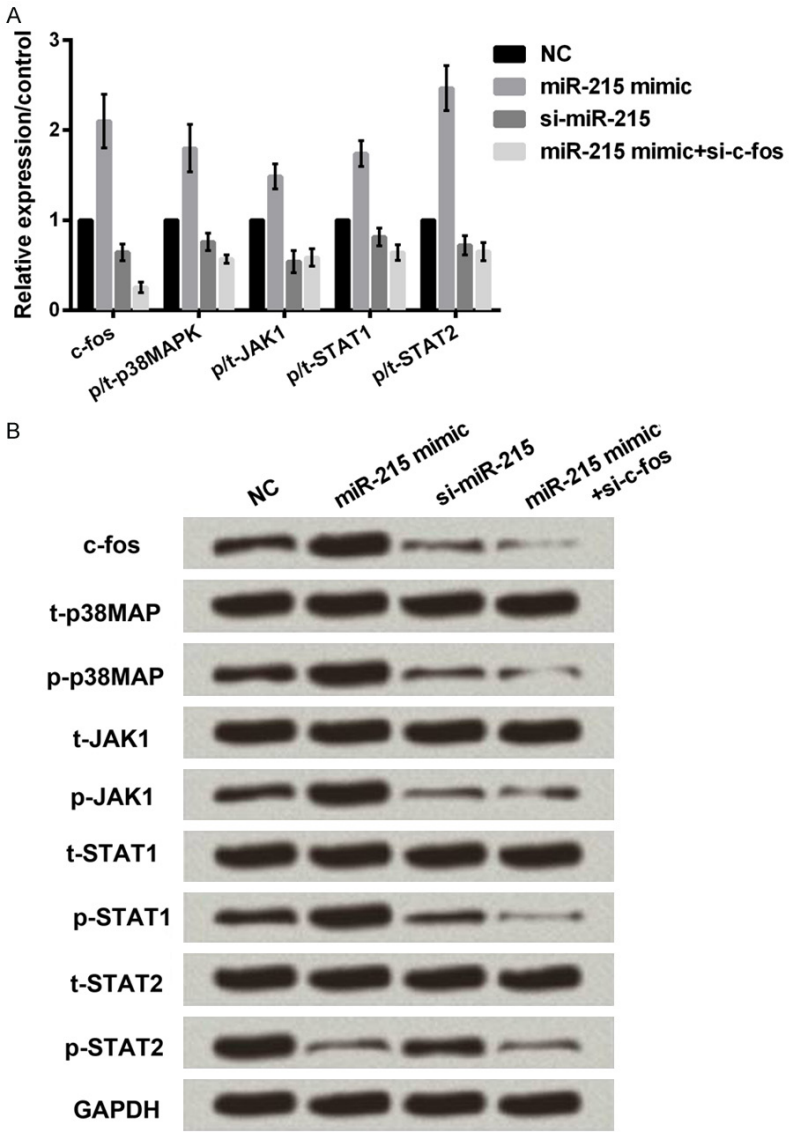

Figure 5. MiR-215 regulates downstream signaling pathway. A. MC3T3 cells were transfected with miR-215 mimic, si-miR-215, negative control (NC), or miR-215 + si-c-fos. Total RNA was analyzed by qRT-PCR for mRNA expression profile of c-fos, p/t-p38MAPK, p/t-JAK1, p/t-STAT1, and p/t-STAT2. B. Western blot analysis was used to measure the expression of these proteins in the transfected cells. GAPDH: glyceraldehyde 3-phosphate dehydrogenase; JAK: Janus kinase; MAPK: mitogen-activated protein kinases; STAT: signal transducers and activators of transcription.

215 expression inhibited proliferation and migration of osteoblasts.

Osteoblast differentiation is tightly controlled by several regulators including miRNAs [22, 23]. Recent studies suggest that miRNAs may act as positive or negative regulators in osteoblast differentiation. MiR-355 acts as a negative regulator and inhibits proliferation and migration of human mesenchymal stem cells by targeting Runx2 [24] and to name a few, other negative regulators include miR-204, miR-211, and miR-155 [25]. In contrast, there are several miRNAs which act as positive regulators of osteoblast differentiation. MiR-218 promotes osteoblast differentiation by inhibiting ERB1 and sclerostin. MiR-2861 promotes osteoblast differentiation by repressing histone deacetylase 5 expressions [26]. We demonstrated that miR-215 is a positive regulator which promotes differentiation of osteoblasts by increasing the expression of differentiation markers, Runx2 and Ocn. Runx2 is a bone-related transcription factor which is essential for osteoblast differentiation [27]. Overexpression of Runx2 in nonosseous mesenchymal cells increases expression of osteoblast phenotypic genes [28]. Runx2 can directly upregulate the expression of osteoblast marker genes, such as Ocn. Ocn is a late bone marker during osteogenic differentiation and mineralization [29].

The transcription factor activator protein 1 (AP-1) is composed of heterodimers of the c-fos and c-Jun family members [30]. AP-1 is a regulator of major biological functions, such as cell proliferation, migration, differentiation, and apoptosis [31]. Several reports suggest that c-fos plays a crucial role in differentiation of osteoblasts. In an *in vitro* study, the expression of c-fos was increased during osteoblast differentiation [32]. Kano et al., reported that c-fos gene is involved in the regulation of osteoblast proliferation and osteoclast differentiation [33]. In our study, forced expression of miR-215 significantly increased the expression of c-fos,

indicating that miR-215 promotes cell proliferation, migration and differentiation via upregulation of c-fos gene.

To further understand the underlying mechanism, we examined the MAPK and JAK/STAT pathways. The MAPK pathway is a downstream signaling pathway which regulates miRNAs: several miRNAs are upregulated (e.g., miR-155) while few are downregulated (e.g., miR-99a) by the MAPK [34]. The MAPK pathway can also activate c-fos gene [30]. Activation of the JAK stimulates cell proliferation, differentiation, migration and apoptosis [35]. It has been reported that JAK2 is essential for the activation of c-fos promoters [36]. This establishes that the MAPK and JAK/STAT pathways activate c-fos gene, and thus, altered expression of c-fos gene may be regulated by altered expression of these downstream signaling pathways. Consistent with this mechanism, we demonstrated that overexpression of miR-215 increased the expressions of p38MAPK, JAK1, STAT1, and STAT2 proteins. Furthermore, we found that silencing the expression of c-fos or miR-215 reversed these effects. These findings indicate that miR-215 can activate the MAPK and JAK/STAT pathways.

In conclusion, miR-215 was identified as a novel regulator in osteoblast proliferation and differentiation through upregulating c-fos expression in vitro. Therefore, this study may provide new insights into the possibility of miR-215 being a potential therapeutic target for the treatment of osteoporosis.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yi-Jie Kuo, Department of Orthopedic Surgery, Taipei Medical University Hospital, 252, Wu Hsing Street, Taipei 11031, Taiwan. E-mail: benkuo5@tmu.edu.tw

References

[1] Tang P, Xiong Q, Ge W and Zhang L. The role of microRNAs in osteoclasts and osteoporosis. RNA Biol 2014; 11: 1355-1363.

[2] Leali PT, Muresu F, Melis A, Ruggiu A, Zachos A and Doria C. Skeletal fragility definition. Clin Cases Miner Bone Metab 2011; 8: 11-13.

[3] Slezak-Prochazka I, Durmus S, Kroesen BJ and van den Berg A. MicroRNAs, macrocontrol: regulation of miRNA processing. RNA 2010; 16: 1087-1095.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Bhalala OG, Srikanth M and Kessler JA. The emerging roles of microRNAs in CNS injuries. Nat Rev Neurol 2013; 9: 328-339.

[6] Seeliger C, Karpinski K, Haug AT, Vester H, Schmitt A, Bauer JS and van Griensven M. Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures. J Bone Miner Res 2014; 29: 1718-1728.

[7] De-Ugarte L, Yoskovitz G, Balcells S, Guerri-Fernandez R, Martinez-Diaz S, Mellibovsky L, Urreizti R, Nogues X, Grinberg D, Garcia-Giralt N and Diez-Perez A. MiRNA profiling of whole trabecular bone: identification of osteoporosis-related changes in MiRNAs in human hip bones. BMC Med Genomics 2015; 8: 75.

[8] Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T, Orntoft TF, Andersen CL and Dobbelstein M. p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. Cancer Res 2008; 68: 10094-10104.

[9] Georges SA, Biery MC, Kim SY, Schelter JM, Guo J, Chang AN, Jackson AL, Carleton MO, Linsley PS, Cleary MA and Chau BN. Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. Cancer Res 2008; 68: 10105-10112.

[10] Pichiorri F, Suh SS, Rocci A, De Luca L, Taccioli C, Santhanam R, Zhou W, Benson DM Jr, Hofmainster C, Alder H, Garofalo M, Di Leva G, Volinia S, Lin HJ, Perrotti D, Kuehl M, Aqeilan RI, Palumbo A and Croce CM. Downregulation of p53-inducible microRNAs 192, 194, and 215 impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. Cancer Cell 2016; 30: 349-351.

[11] Wei Y, Sun J and Li X. MicroRNA-215 enhances invasion and migration by targeting retinoblastoma tumor suppressor gene 1 in high-grade glioma. Biotechnol Lett 2017; 39: 197-205.

[12] Li QW, Zhou T, Wang F, Jiang M, Liu CB, Zhang KR, Zhou Q, Tian Z and Hu KW. MicroRNA-215 functions as a tumor suppressor and directly targets ZEB2 in human pancreatic cancer. Genet Mol Res 2015; 14: 16133-16145.

[13] Chen Z, Han S, Huang W, Wu J, Liu Y, Cai S, He Y, Wu S and Song W. MicroRNA-215 suppresses cell proliferation, migration and invasion of colon cancer by repressing Yin-Yang 1. Biochem Biophys Res Commun 2016; 479: 482-488.

[14] Stanford CM, Jacobson PA, Eanes ED, Lembke LA and Midura RJ. Rapidly forming apatitic mineral in an osteoblastic cell line (UMR 106-01 BSP). J Biol Chem 1995; 270: 9420-9428.

[15] Khraiwesh B, Zhu JK and Zhu J. Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 2012; 1819: 137-148.
[16] van Wijnen AJ, van de Peppel J, van Leeuwen JP, Lian JB, Stein GS, Westendorf JJ, Oursler MJ, Im HJ, Taipaleenmaki H, Hesse E, Riester S and Kakar S. MicroRNA functions in osteogenesis and dysfunctions in osteoporosis. Curr Osteoporos Rep 2013; 11: 72-82.
[17] Vimalraj S, Partridge NC and Selvamurugan N. A positive role of microRNA-15b on regulation of osteoblast differentiation. J Cell Physiol 2014; 229: 1236-1244.
[18] Mei Y, Bian C, Li J, Du Z, Zhou H, Yang Z and Zhao RC. miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation. J Cell Biochem 2013; 114: 1374-1384.
[19] Wang T and Xu Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. Biochem Biophys Res Commun 2010; 402: 186-189.
[20] Hou Y, Zhen J, Xu X, Zhen K, Zhu B, Pan R and Zhao C. miR-215 functions as a tumor suppressor and directly targets ZEB2 in human non-small cell lung cancer. Oncol Lett 2015; 10: 1985-1992.
[21] Li N, Zhang QY, Zou JL, Li ZW, Tian TT, Dong B, Liu XJ, Ge S, Zhu Y, Gao J and Shen L. miR-215 promotes malignant progression of gastric cancer by targeting RUNX1. Oncotarget 2016; 7: 4817-4828.
[22] Lian JB, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T and Zhang Y. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol 2012; 8: 212-227.
[23] Bessa PC, Casal M and Reis RL. Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). J Tissue Eng Regen Med 2008; 2: 1-13.
[24] Tome M, Lopez-Romero P, Albo C, Sepulveda JC, Fernandez-Gutierrez B, Dopazo A, Bernad A and Gonzalez MA. miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells. Cell Death Differ 2011; 18: 985-995.
[25] Rahman MS, Akhtar N, Jamil HM, Banik RS and Asaduzzaman SM. TGF-beta/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res 2015; 3: 15005.
[26] Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, van Wijnen AJ, Stein JL, Stein GS and Lian JB. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J Biol Chem 2009; 284: 15676-15684.
[27] Franceschi RT, Xiao G, Jiang D, Gopalakrishnan R, Yang S and Reith E. Multiple signaling pathways converge on the Cbfa1/Runx2 transcription factor to regulate osteoblast differentiation. Connect Tissue Res 2003; 44 Suppl 1: 109-116.
[28] Shui C, Spelsberg TC, Riggs BL and Khosla S. Changes in Runx2/Cbfa1 expression and activity during osteoblastic differentiation of human bone marrow stromal cells. J Bone Miner Res 2003; 18: 213-221.
[29] Paredes R, Arriagada G, Cruzat F, Olate J, Van Wijnen A, Lian J, Stein G, Stein J and Montecino M. The Runx2 transcription factor plays a key role in the 1alpha,25-dihydroxy Vitamin D3-dependent upregulation of the rat osteocalcin (OC) gene expression in osteoblastic cells. J Steroid Biochem Mol Biol 2004; 89-90: 269-271.
[30] Whitmarsh AJ and Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. J Mol Med (Berl) 1996; 74: 589-607.
[31] Lee HJ, Palkovits M and Young WS 3rd. miR-7b, a microRNA up-regulated in the hypothalamus after chronic hyperosmolar stimulation, inhibits Fos translation. Proc Natl Acad Sci U S A 2006; 103: 15669-15674.
[32] Closs EI, Murray AB, Schmidt J, Schon A, Erfle V and Strauss PG. c-fos expression precedes osteogenic differentiation of cartilage cells in vitro. J Cell Biol 1990; 111: 1313-1323.
[33] Kano J, Sugimoto T, Kanatani M, Kaji H, Yamaguchi T, Fukase M and Chihara K. Involvement ofc-fos gene in the regulation of osteoblast proliferation and osteoclast differentiation by parathyroid hormone and parathyroid hormone-related protein. Journal of Bone and Mineral Metabolism 1994; 12: S39-S43.
[34] Zhou R, O'Hara SP and Chen XM. MicroRNA regulation of innate immune responses in epithelial cells. Cell Mol Immunol 2011; 8: 371-379.
[35] Rawlings JS, Rosler KM and Harrison DA. The JAK/STAT signaling pathway. J Cell Sci 2004; 117: 1281-1283.
[36] Watanabe S, Itoh T and Arai K. JAK2 is essential for activation of c-fos and c-myc promoters and cell proliferation through the human granulocyte-macrophage colony-stimulating factor receptor in BA/F3 cells. J Biol Chem 1996; 271: 12681-12686.