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Mir-98 reduces the expression of HMGA2 and promotes osteogenic differentiation of mesenchymal stem cells

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Abstract. – OBJECTIVE: To study effects of microRNA-98 (miR-98) on osteogenic differentiation of mesenchymal stem cells (MSCs).

PATIENTS AND METHODS: We predicted target gene of miR-98 with software test, and detected expression changes of miR-98, as well as its target gene HMGA2, in the process of osteogenic differentiation of mesenchymal stem cells. After transfection of miR-98 mimic and HMGA2 siRNA, we induced osteogenic differentiation and detected expression changes of osteogenic differentiation markers (RUNX2, ALP, OCN, and BSP).

RESULTS: MiR-98 combined directly with target gene HMGA2 and inhibited its expression. During the process of osteogenic differentiation, expression of miR-98 was up-regulated, while HMGA2 expression was down-regulated. In addition, the expression of osteogenesis maker genes increased in cells being transfected with miR-98 mimics and HMGA2 siRNA.

CONCLUSIONS: MiR-98 can promote osteogenic differentiation of mesenchymal stem cells by targeting gene HMGA2.

Key Words:
MiR-98, HMGA2, Osteogenic differentiation, Mesenchymal stem cells.

Introduction

The chronic inflammatory bone disease is a kind of disorder characterized by osteopenia, which mainly includes postmenopausal osteoporosis, rheumatoid arthritis, and periodontal disease. Impairment of bone formation is one of the major pathogenesis of these diseases. Autogenous and allogeneic bone grafts have been successfully applied in clinical treatment, but there are still some drawbacks, such as multiple surgeries, graft failure, immunological rejection, bone graft insufficiency, and infection[1]. Therefore, osteogenesis using MSCs as seeding cells is an efficient treatment[2].

Mesenchymal stem cells are a group of adult stem cells with both self-renewal ability and multidirectional differentiation potential[3]. They are derived from a variety of adult tissues, including bone marrow, adipose tissue, umbilical cord/umbilical cord blood and skin, etc.[4,5]. Under a given condition, mesenchymal stem cells are able to differentiate into osteoblast, lipoblast, chondroblast, and myoblast[6]. Mesenchymal stem cells have great abilities of self-replication and proliferation. In addition, mesenchymal stem cells have many advantages, such as widespread sources, easy access to separate, easy to *in vitro* culture and proliferate. Based on above features, mesenchymal stem cells have quickly become a hotspot in the field of cell regenerative medicine[7,8]. A variety of biological factors have an impact on proliferation, differentiation or apoptosis of mesenchymal stem cells, in which genes, signaling proteins and epigenetic modifications can act on the fate-decided process of MSCs[9-11].

MicroRNAs (miRNAs), as a kind of small non-coding RNA molecules, have been proven to be functional in negatively regulating expression of the target gene at transcriptional and posttranscriptional levels[12]. Evidence is accumulating that miRNAs are involved in regulation of many molecular biological processes, such as cell proliferation and differentiation, tissue and organ development, tumorigenesis[13,14]. Several miRNAs have been found to play an important role in balance regulation of osteogenic differentiation and adipogenic differentiation. For

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instance, overexpression of miR-455, miR-204, and miR-17-5p can inhibit osteogenic differentiation of mesenchymal stem cells and promote the differentiation of them into adipose cells at the same time[15-17].

The primary purpose of this study was to investigate the effects of microRNA-98 (miR-98) on osteogenic differentiation of mesenchymal stem cells (MSCs)

Patients and Methods

Culture of Human Bone Marrow-Derived Mesenchymal Stem Cells (hBMMSCs)

We selected some patients who underwent iliac bone graft surgery and open fracture surgery After getting approval of Ethics Committee and patients' informed consent, we selected some patients whose disease required iliac crest bone graft and open fractures operation (excluding other systemic diseases and osteoporosis), and aspirated 5 mL of their bone marrow during operations. The bone narrow was mixed with the same volume of serum-free low-glucoseα-minimum essential medium (α-MEM), and centrifuged at 400 g for 10 min at room temperature. Then, after removing the adipose layer and resuspending the cells in the low-glucose α-MEM, the cells were centrifuged at 400 g for 30 min in the Percoll separation medium. Next, intermediate mononuclear cell layer was extracted, washed twice with phosphate buffered saline (PBS), and then, resuspended in the low-glucose α-MEM. Cells were seeded in 25 cm^2 culture flask after adjustment of cell density to 1×10^6/L, and were maintained in a 37°C incubator with 5% CO_2. The third generation cells in logarithmic growth period were used for the experiment.

Osteoinductive Differentiation In vitro

MSCs were seeded into 6-well plates after different treatments. When the cells reached 60%-70% confluence, osteogenic induction medium was added to induce osteogenic differentiation. The osteogenic induction medium was α-MEM with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Inc. Waltham, MA, USA), including 0.1 μmol/L dexamethasone, 50 μg/mL Vitamin C, and 10 mmol/L β-sodium glycerophosphate. Then, the full volume of the medium was changed every three days.

Prediction of miRNA Target Genes

The following three databases were used to predict target genes in this article: TargetScan (http://www.targetscan.org/), miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/php/search.php) and miRDB (http://www.mirdb.org/).

Transfection of miRNA and siRNA

Lyophilized powder of miRNA and siRNA were dissolved in diethyl pyrocarbonate (DEPC)-treated water as stock solution of 10 μmol/L and reserved. Cells of logarithmic growth phase were seeded in 6-well plates at a density of 1×10^5/cm^2. When the cell confluence reached 50%, the culture medium was abandoned, cells were washed three times with PBS and then dried. Subsequently, 4 μL Lipofectamine2000 (Thermo Fisher Scientific, Inc. Waltham, MA, USA) were added to 200 μL Opti-mem culture medium and mixed, then let stand for 5 minutes. Likewise, the corresponding volume of siRNA was added to 200 μL Opti-mem culture medium and mixed. Next, two tubes of mixed solutions were blended and let stand for 20 minutes at room temperature. The culture medium was renewed after 5 hours.

Dual-Luciferase Reporter Assay

A reporter plasmid containing wild-type or mutant sequences of HMGA2 3'UTR region was constructed. The 3'UTR sequence of HMGA2 in human's genome was amplified by polymerase chain reaction (PCR). Specific primers were used to add XbaI or Nde I restriction enzyme cutting sites at the ends of the sequence. The PCR products were purified and pGL3M vectors were ligated respectively into them after double enzyme digestion. Then, recombinant plasmids were transformed, identified and sequenced. The constructed plasmids were named pGL3-HMGA2-wild and pGL3-HMGA2-mut, respectively.

Cells were planted in 24-well plates one day before transfection. 500 μL culture medium, reporter plasmids, internal control plasmids and transfection reagent were put in Eppendorf tube and incubated for 5 minutes. Then, two 250 μL of mixed solutions were added to mimics and NC separately. Cells were taken out for detection of dual-luciferase reporter assay after being transfected for 48 hours. The result was based on average value of four replicate wells.

Alkaline Phosphatase Staining

The cultured cells were washed three times with PBS to remove culture medium. Next, the cells were fixed with 95% ethanol for 10 minutes and dried, Then, alkaline phosphatase reaction solutions were added to them to incubate for four hours

at 37°C. Thereafter, cells were treated with 1% cobaltous nitrate for two minutes and rinsed one to two minutes. Finally, cells were dried and photographed after one to two minutes of treatment by ammonium sulfide and five minutes of rinse.

Alizarin Red Staining

Cells were washed three times with PBS and fixed with 95% ethanol for 10 minutes, then washed three times with deionized water. Next, cells were incubated with 0.1% alizarin red staining for 30 minutes at 37°C so that positives cells were dyed orange. After that, cells were washed twice with distilled water and mounted with glycerol, examined and photographed under a microscope.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Quantitative Real-time RT-PCR was used to detect the gene expression in cells after osteogenic induction. After extracting total RNA by TRIzol (Thermo Fisher Scientific, Inc. Waltham, MA, USA), RNA samples were used to synthesize complementary Deoxyribose Nucleic Acid (cDNA) by PrimeScript TMRT reagent Kit (TaKaRa, Dalian, China) with gDNA Eraser according to the manufacturer's instructions. Then, cDNA templates were amplified using quantitative polymerase chain reaction (Q-PCR) according to the manufacturer's instructions of SYBR Green PCR Master Mix (TaKaRa, Dalian, China). Amplification protocol consisted of an initial denaturation step at 95°C (5 min) followed by 40 cycles of a thermal step protocol consisting of 95°C (5 s) and 60°C (20 s). Cycle threshold values (Ct value) of each sample and inner reference glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were obtained after the reaction. The primer sequences used in this experiment are shown in Table I.

Western Blot

After seven days osteogenic induction, cells transfected with miR-98 mimics and mimics control in each group were digested by trypsin (Gibco, Rockville, MD, USA) and collected in Eppendorf tube by centrifugation. Cell lysate was added to extract and prepare protein samples. Based on the molecular weight of the target protein, different concentrations of sodium dodecyl sulphate (SDS)-polyacrylamide gel were selected for protein electrophoresis. Protein molecules in the gel were transferred to polyvinylidene difluoride (PVDF) membrane under electric field (Millipore, Billerica, MA, USA). And the specific binding of antigen and antibody was used to specifically mark (primary antibody and secondary antibody) the target gene protein binding on the membrane. Then, the light signal was converted into visible bands on X-ray film.

Statistical Analysis

The experimental data were analyzed by statistical product and service solutions (SPSS) 17.0 (Chicago, IL, USA). All quantitative data were expressed as mean ± standard deviation. The *t*-test was used for the comparison of the two groups. The difference was statistically significant when $p<0.05$.

Results

Expression and Function of miR-98 in Osteogenic Differentiation

In the period of inducing mesenchymal stem cells differentiation, the expression of miR-98 increased gradually and reached the peak on the 14th day, then deceased slightly at 21st day, but it was still higher than baseline (Figure 1A). After transfection with mir-98 mimics, expression of mir-98 significantly increased (Figure 1B). High expression of mir-98 markedly promoted the expression of mRNA (Figure 1C) and protein (Figure 1D) of osteogenic differentiation genes RUX2, BSP and OCN. In addition, by alkaline phosphatase staining, we found that overexpression markedly promoted osteogenic differentiation (Figure 1E), and the results of alizarin red staining indicated a significant calcium deposition in high-expression miR-98 group. The above results suggested that miR-98 played an important role in osteogenic differentiation.

Table I. Primers used in this study.

Gene	Sense primer	Antisense primer
RNX2	ACTTCCTGTGCTCGGTGCT	GACGGTTATGGTCAAGGTGAA
OCN	CCTCACACTCCTCGCCCTAT	GTGGTCAGCCAAGCTGGTCAC
BSP	TGGATGAAAACGAACAAGGCA	AAACCCACCATTTGGAGACGT
GAPDH	TCCATGACAACTTTGGTATCG	TGTAGCCAAATTCGTTGTCA

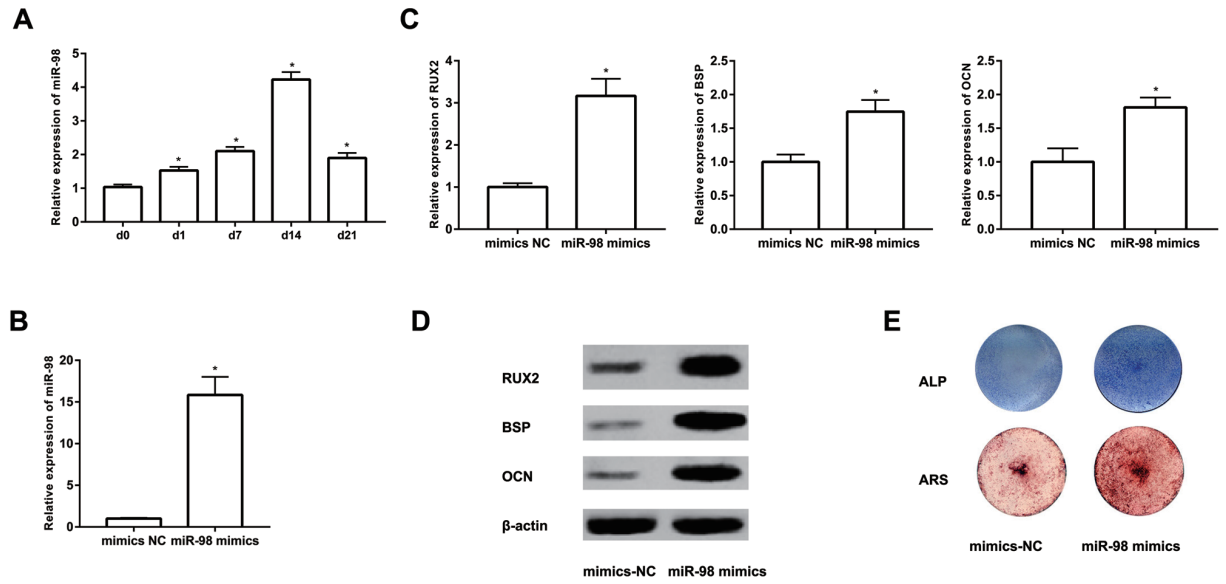

Figure 1. Expression and function of miR-98 in osteogenic differentiation. ***A,*** Expression of miR-98 increased in the period of inducing mesenchymal stem cells differentiation. ***B,*** Expression of mir-98 transfected with mir-98 mimics was significantly increased. ***C,*** High expression of mir-98 markedly promoted expression of mRNA of osteogenic differentiation genes RUX2, BSP and OCN. ***D,*** High expression of mir-98 markedly promoted the expression of protein of osteogenic differentiation genes RUX2, BSP and OCN. ***E,*** Alkaline phosphatase staining and alizarin red staining presented that high expression of miR-98 promote osteogenic differentiation.

Prediction and Verification of miR-98's Target Gene

We obtained 7 potential target genes (Figure 2A) through TargetScan, miRTarBase and miRDB, three online prediction websites. It has been previously reported[18] that HMGA2 is involved in stem cell differentiation, so we chose HMGA2 as the target gene for the study. High expression of mir-98 markedly promoted the expression of mRNA (Figure 2B) and protein (Figure 2C) of HMGA2. Further analysis suggested that the binding sites of mir-98 and HMGA2 were

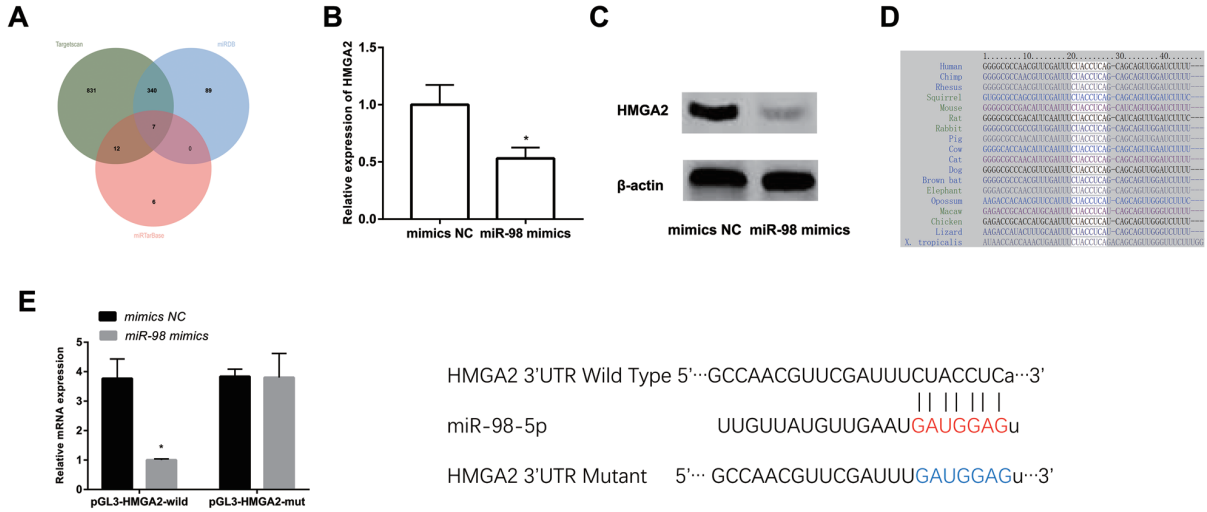

Figure 2. Prediction of miR-98's target gene. ***A,*** Target gene intersection in different prediction websites. ***B,*** miR-98 inhabited expression of HMGA2 mRNA. ***C,*** miR-98 inhabited expression of HMGA2 protein. ***D,*** Conservative analysis of miR-98 and HMGA2 in within vertebrates. ***E,*** miR-98 can be only combined with the HMGA2 3 ' UTR after being transfected with wild type plasmid and mutant sequences plasmid respectively, **p*<0.05.

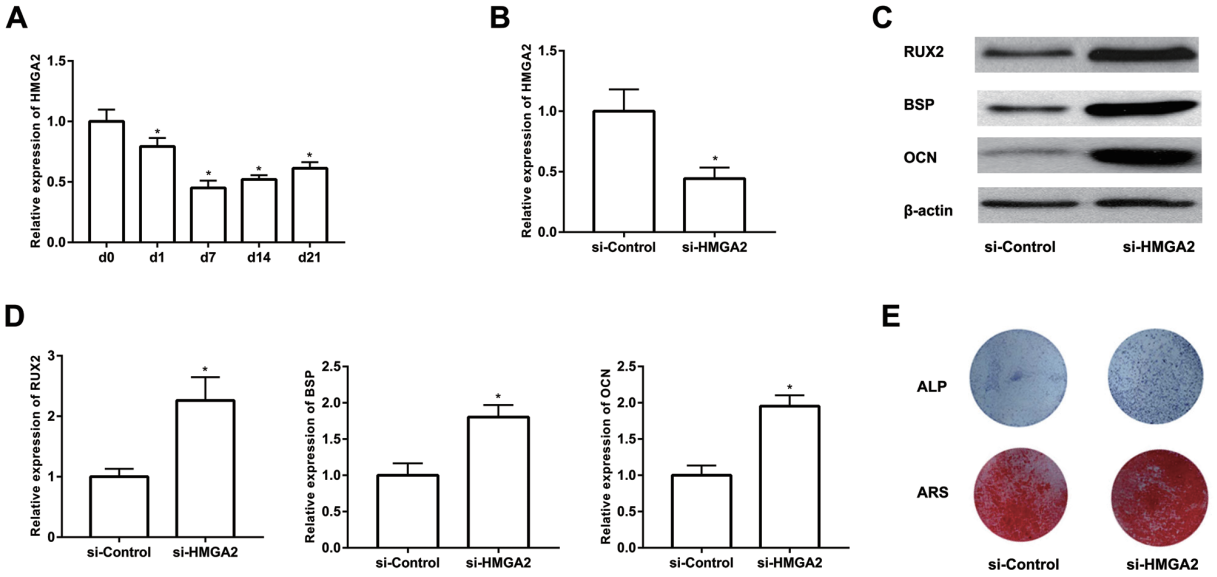

Figure 3. Expression and function of HMGA2 in osteogenic differentiation. ***A,*** Expression and function of HMGA2 in osteogenic differentiation. ***B,*** Expression of HMGA2 transfected with HMGA2 siRNA mimics was significantly decreased. ***C,*** Low expression of HMGA markedly promoted the expression of protein of osteogenic differentiation genes RUX2, BSP and OCN. D: Low expression of HMGA markedly promoted the expression of mRNA of osteogenic differentiation genes RUX2, BSP and OCN. E: Alkaline phosphatase staining and alizarin red staining presented that low expression of HMGA2 promote osteogenic differentiation, *$p<0.05$.

highly conserved invertebrates (Figure 2D). By analysis of dual-luciferase reporter assay, miR-98 could be directly combined with 3'UTR region of HMGA2 (Figure 2E). These above results indicated that miR-98 can be directly combined with HMGA2 to inhibit its expression.

Expression and Function of HMGA2 in Osteogenic Differentiation

During the *in vitro* induction of mesenchymal stem cells differentiation, expression of HMGA2 kept a moderate downward tendency and decreased to the lowest at 7th day. Thereafter, the expression increased slightly but still was lower than baseline (Figure 3A). After transfection with HMGA2 siRNA, expression of HMGA2 significantly declined (Figure 3B). Low expression of mir-98 significantly promoted the expression of the protein (Figure 3C) and mRNA (Figure 3D) of osteogenic differentiation genes RUX2, BSP, and OCN. By alkaline phosphatase staining, it could be seen that reduction of HMGA2 expression promoted osteogenic differentiation evidently (Figure 3E), and the results of alizarin Red S staining showed a significant calcium deposition in the low-expression HMGA2 group. The above results suggested that HMGA2 played an important role in osteogenic differentiation. In summary, we demonstrated that miR-98 was involved in stem cell differentiation by regulating expression of HGMA2 through comprehensive analysis.

Discussion

In 1999, Pittenger et al[3] isolated mesenchymal stem cells from the iliac bone marrow. He found that the cell populations were single phenotypic and could be induced to multiple differentiation. Furthermore, cloned cells from the cell populations were also found to have similar characteristics. Therefore, those cells are defined as bone marrow mesenchymal stem cells. MSCs are considered to be the advantageous resource for cell and gene therapy[19], with their treatment effectiveness having been reported[20,21] extensively in the field of hematopoiesis recovery, osteogenesis, *osteogenesis imperfecta*, acute myocardial infarction, and joint diseases. Therefore, it is extremely meaningful to study the differentiation of MSCs.

MiRNAs have been found to affect the activity of osteoblasts and osteoclasts. Wei et al[22] found that miR-34 inhibits the final differentiation of osteoblasts by inhibiting expression of SATB2 and inhibits the proliferation of mature osteoblasts by inhibiting expression of CyclinD1,

CDK4, and CDK6. Obvious skeletal defect was generated in transgenic mice with abnormal expression of miR-34c. Expression changes of miRNA in age-related osteoporosis had been revealed by high-throughput screening; as a result, it was found that expression of eight miRNAs increased and 30 miRNAs deceased in osteoporosis model of mice through overlapping detection of mesenchymal stem cells and bone tissue[23].

One of the important manifestations of the differentiation of BMSCs into osteoblasts is the secretion and synthesis of genes and proteins related to osteoblasts. Alkaline phosphatase is a marker enzyme produced in the early stage of osteogenic differentiation, which has the function of hydrolyzing phosphate ester in the process of osteogenesis, and is conducive to osteogenesis. The expression of alkaline phosphatase is time-dependent and its activity can be used as an indicator of osteogenic differentiation[24]. Osteoblast specific transcription factor is one of the important factors affecting the bone development. This transcription factor participates in the process of osteoblast differentiation and bone development of MSCs by regulating the expression of various osteogenesis-specific factors; it is another indicator to judge the ability of osteogenic differentiation[25]. Osterix (OSX), a kind of transcription factor with zinc-finger structure, has been found to be specifically expressed in osteoblasts and plays an extremely important role in bone formation and osteogenic differentiation. The mutation or deletion of OSX can cause delay and stagnation of bone formation[26]. Therefore, in this experiment, we examined the expression levels of above-mentioned markers to verify whether BMSCs differentiated into osteoblasts.

miR-98 was first cloned from HeLa cells and was expressed in many tissues. The miR-98 family is highly conserved and is closely related to a variety of physiological processes by regulating target genes. The dysregulated expression of miR-98 is involved in the occurrence, development, invasion, and metastasis of various tumors, such as lung cancer, breast cancer, and colon cancer[27,28]. Although miR-98 is also expressed in a variety of normal tissues, its function under physiological conditions is still not clear. We found that miR-98 gradually increased during the induction of mesenchymal stem cell differentiation. Highly expressed miR-98 could significantly promote the expression of osteogenic differentiation-related genes RUX2, BSP, and OCN, and promoted calcium deposition. The above results suggested that miR-98 played an important role in osteogenic differentiation.

To study the mechanism of miR-98 in regulating osteogenic differentiation of MSCs, we used bioinformatics prediction methods to find the potential target genes of miR-98. It has been reported[29] that HMGA2 was involved in the differentiation of stem cells. HMGA2 could promote the self-renewal of breast cancer stem cells, block their differentiation, and play a key role in maintaining their stemness. Therefore, we chose HMGA2 as a target gene for research. We showed that miR-98 can directly bind to HMGA2 and inhibit its expression. HMGA2 expression gradually decreased during the induction of mesenchymal stem cell differentiation *in vitro*. Decreasing HMGA2 significantly promoted osteogenic differentiation.

Conclusions

We showed that miR-98 is one of the key miRNAs that regulate the osteogenic differentiation of MSCs, and can promote the osteogenic differentiation of MSCs by negatively regulating the expression of HMGA2. This work is beneficial for us to profoundly understand the mechanism of osteogenic differentiation of MSCs and provide an important mean for the clinical treatment of osteoporosis caused by the decreased osteogenic capacity of MSCs.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg Am 2002; 84-A: 716-720.

2) Noda M, Ishijima M, Takamoto M, Tsuji K, Nifuji A. [Bone regeneration and Mesenchymal stem cells]. Clin Calcium 2002; 12: 187-192.

3) Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-147.

4) Dong CH, Deng YS, Yang XJ, Liu J, Liu R, Hou FY, Li SS, Zhen P. The interplay of transcriptional and post-transcriptional regulation of migration of mesenchymal stem cells during early stages of bone fracture healing. Eur Rev Med Pharmacol Sci 2017; 21: 5542-5547.

5) Miao Z, Jin J, Chen L, Zhu J, Huang W, Zhao J, Qian H, Zhang X. Isolation of mesenchymal stem cells from human placenta: Comparison with human bone marrow mesenchymal stem cells. Cell Biol Int 2006; 30: 681-687.

6) Oreffo RO, Bord S, Triffitt JT. Skeletal progenitor cells and ageing human populations. Clin Sci (Lond) 1998; 94: 549-555.

7) In TAP, Scherjon SA, Kleijburg-van DKC, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 2004; 22: 1338-1345.

8) Semenov OV, Koestenbauer S, Riegel M, Zech N, Zimmermann R, Zisch AH, Malek A. Multipotent mesenchymal stem cells from human placenta: Critical parameters for isolation and maintenance of stemness after isolation. Am J Obstet Gynecol 2010; 202: 191-193.

9) Bais MV, Shabin ZM, Young M, Einhorn TA, Kotton DN, Gerstnefeld LC. Role of Nanog in the maintenance of marrow stromal stem cells during post-natal bone regeneration. Biochem Biophys Res Commun 2012; 417: 211-216.

10) Kang SW, La WG, Kang JM, Park JH, Kim BS. Bone morphogenetic protein-2 enhances bone regeneration mediated by transplantation of osteogenically undifferentiated bone marrow-derived mesenchymal stem cells. Biotechnol Lett 2008; 30: 1163-1168.

11) Levy O, Ruvinov E, Reem T, Granot Y, Cohen S. Highly efficient osteogenic differentiation of human mesenchymal stem cells by eradication of STAT3 signaling. Int J Biochem Cell Biol 2010; 42: 1823-1830.

12) Ge DW, Wang WW, Chen HT, Yang L, Cao XJ. Functions of microRNAs in osteoporosis. Eur Rev Med Pharmacol Sci 2017; 21: 4784-4789.

13) Fu T, Seok S, Choi S, Huang Z, Suino-Powell K, Xu HE, Kemper B, Kemper JK. MicroRNA 34a inhibits beige and brown fat formation in obesity in part by suppressing adipocyte fibroblast growth factor 21 signaling and SIRT1 function. Mol Cell Biol 2014; 34: 4130-4142.

14) Huang J, Zhao L, Xing L, Chen D. MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. Stem Cells 2010; 28: 357-364.

15) Li H, Li T, Wang S, Wei J, Fan J, Li J, Han Q, Liao L, Shao C, Zhao RC. MiR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 2013; 10: 313-324.

16) Luo W, Wu H, Ye Y, Li Z, Hao S, Kong L, Zheng X, Lin S, Nie Q, Zhang X. The transient expression of miR-203 and its inhibiting effects on skeletal muscle cell proliferation and differentiation. Cell Death Dis 2014; 5: e1347.

17) Zhang H, Guan M, Townsend KL, Huang TL, An D, Yan X, Xue R, Schulz TJ, Winnay J, Mori M, Hirshman MF, Kristiansen K, Tsang JS, White AP, Cypess AM, Goodyear LJ, Tseng YH. MicroRNA-455 regulates brown adipogenesis via a novel HIF1an-AMPK-PGC1alpha signaling network. EMBO Rep 2015; 16: 1378-1393.

18) Zhang JF, Fu WM, He ML, Xie WD, Lv Q, Wan G, Li G, Wang H, Lu G, Hu X, Jiang S, Li JN, Lin MC, Zhang YO, Kung HF. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biol 2011; 8: 829-838.

19) Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: Characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med 2004; 8: 301-316.

20) Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci U S A 2002; 99: 8932-8937.

21) Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 2000; 18: 307-316.

22) Wei J, Shi Y, Zheng L, Zhou B, Inose H, Wang J, Guo XE, Grosschedl R, Karsenty G. MiR-34s inhibit osteoblast proliferation and differentiation in the mouse by targeting SATB2. J Cell Biol 2012; 197: 509-521.

23) He X, Zhang W, Liao L, Fu X, Yu Q, Jin Y. Identification and characterization of microRNAs by high through-put sequencing in mesenchymal stem cells and bone tissue from mice of age-related osteoporosis. PLoS One 2013; 8: e71895.

24) Aubin JE, Liu F, Malaval L, Gupta AK. Osteoblast and chondroblast differentiation. Bone 1995; 17(2 Suppl): 77S-83S.

25) Orfanidou T, Iliopoulos D, Malizos KN, Tsezou A. Involvement of SOX-9 and FGF-23 in RUNX-2 regulation in osteoarthritic chondrocytes. J Cell Mol Med 2009; 13: 3186-3194.

26) Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 108: 17-29.

27) Wang K, Dong L, Fang Q, Xia H, Hou X. Low serum miR-98 as an unfavorable prognostic biomarker in patients with non-small cell lung cancer. Cancer Biomark 2017; 20: 283-288.

28) Cai C, Huo Q, Wang X, Chen B, Yang Q. SNHG16 contributes to breast cancer cell migration by competitively binding miR-98 with E2F5. Biochem Biophys Res Commun 2017; 485: 272-278.

29) Wu J, Zhang S, Shan J, Hu Z, Liu X, Chen L, Ren X, Yao L, Sheng H, Li L, Ann D, Yen Y, Wang J, Wang X. Elevated HMGA2 expression is associated with cancer aggressiveness and predicts poor outcome in breast cancer. Cancer Lett 2016; 376: 284-292.