European Review for Medical and Pharmacological Sciences 2019; 23: 7757-7765

LncRNA GAS5 overexpression alleviates the development of osteoporosis through promoting osteogenic differentiation of MSCs *via* targeting microRNA-498 to regulate RUNX2

J. FENG[1], J.-X. WANG[2], C.-H. LI[3]

[1]Health Management Centre, Weifang People's Hospital, Weifang, China
[2]Department of Orthopedics, Rizhao People's Hospital, Rizhao, China
[3]Laboratory Medicine, Ju County Hospital of Traditional Chinese Medicine, Rizhao, China

Abstract. – OBJECTIVE: The aim of this study was to elucidate whether long non-coding RNA (lncRNA) GAS5 could target microRNA-498 to regulate RUNX2, thus alleviating the development of osteoporosis.

PATIENTS AND METHODS: Human multipotential mesenchymal stem cells (hMSCs) were isolated from bone marrow of osteoporosis patients or healthy controls. Quantitative Real-time polymerase chain reaction (qRT-PCR) was performed to detect the expression levels of GAS5, microRNA-498 and RUNX2 in hMSCs of osteoporosis patients and controls, respectively. Meanwhile, the protein level of RUNX2 in hMSCs was detected by Western blot. Furthermore, alkaline phosphatase (ALP) activity assay and ALP staining were performed to evaluate the degree of osteogenic differentiation under the control of GAS5, microRNA-498 and RUNX2.

RESULTS: MicroRNA-498 was highly expressed in hMSCs derived from osteoporosis patients, whereas GAS5 and RUNX2 were lowly expressed. GAS5 overexpression significantly increased ALP activity and promoted osteogenic differentiation of hMSCs derived from osteoporosis patients. Meanwhile, GAS5 significantly promoted osteogenic differentiation by mediating microRNA-498 expression to up-regulate RUNX2. Co-overexpression of GAS5 and microRNA-498 could remarkably reverse the increase of RUNX2 expression. Besides, RUNX2 overexpression markedly elevated ALP activity.

CONCLUSIONS: LncRNA GAS5 is lowly expressed in patients with osteoporosis. Overexpression of GAS5 promotes osteogenic differentiation of hMSCs through regulating microRNA-498 to up-regulate RUNX2 expression, thus alleviating the development of osteoporosis.

Key Words:
Osteoporosis, LncRNA GAS5, MicroRNA-498, RUNX2.

Introduction

Osteoporosis refers to a systemic bone disease caused by the decrease in bone mass and degradation of bone microstructure. This may eventually result in increased bone fragility and reduced strength[1]. The main symptoms of osteoporosis include severe pain, multiple fractures, and difficulty breathing caused by thoracic cavity reduction[2]. Osteoporosis patients are prone to suffer from bone fracture, especially in the elderly population. Therefore, effective prevention and treatment of osteoporosis are of great significance. Human multi-potential mesenchymal stem cells (hMSCs) are progenitor cells with multiple differentiation potentials. Previous studies have demonstrated that hMSCs exert a crucial role in the maintenance and repair of various connective tissues, including bone and adipose tissues, cartilages and muscle tissues[3]. Numerous advantages of hMSCs have been found, such as easy isolation and cell culture, high acceptability and plasticity. Therefore, they have been widely applied in stem cell transplantation for various diseases. In this study, hMSCs isolated from osteoporosis patients and healthy controls were selected for a series of *in vitro* experiments. Long non-coding RNA (LncRNA) is a class of ncRNA transcripts transcribed from RNA polymerase 2 with about 200-100 000 nt in length. LncRNA barely has protein-coding function; however, it is able to interact with small interfering RNA (siRNA), miRNA and Piwi protein[4]. Current studies[5,6] have proved that lncRNAs exert epigenetic, transcriptional and post-transcriptional regulations on gene expressions. Meanwhile,

Corresponding Author: Caihua Li, BM; e-mail: licaihua12@163.com

they are involved in various regulatory processes, including X chromosome silencing, genomic imprinting, chromatin modification and nuclear transport. The major functions of lncRNAs can be summarized as follows: (1) interference with downstream gene transcription by inhibition of RNA polymerase 2 recruitment, (2) regulation of chromatin remodeling and histone modification, (3) interference with mRNA cleavage by formation of heterozygous duplexes with sense transcripts, (4) production of endogenous interfering RNA by binding and cleaving gene transcripts, (5) regulation of protein function and localization by binding to specific proteins, (6) formation of small RNA precursors and (7) inhibition of miRNA function as miRNA sponges[7]. In recent years, the functions of lncRNA in regulating MSCs and osteoblasts have been identified, greatly affecting bone metabolism[8]. LncRNA-GAS5, originally isolated from NIH 3T3 cells[9], encodes a lymphoma-associated chromosomal locus (1q25). Recent studies have confirmed that it exerts an important regulatory role in tumorigenesis. Currently, another study has found that GAS5 is associated with the occurrence and development of malignant tumors[10]. GAS5 participates in the development of bone diseases as well. Song et al[11] have shown that lncRNA GAS5 regulates the pathogenesis of osteoarthritis by inhibiting miR-21 activity. However, the exact role of lncRNA GAS5 in osteoporosis, as well as the possible underlying mechanism remains unclear. MicroRNAs are classified as non-coding, endogenous and single-stranded RNAs with 19-25 nucleotides in length. MicroRNAs are highly conserved, and can bind to target genes by incompletely pairing. It is estimated that microRNAs account for 1% of human genome, regulating 30% of human gene expressions[12]. Multiple microRNAs have been found to be involved in the development of osteoporosis, osteosarcoma and osteoarthritis[13-16]. Differentially expressed microRNAs have been verified in hMSCs, including miR-204, miR-30a, miR-17-5p, miR-106a, miR-22, miR-705, miR-3077-5p, miR-637 and etc. These microRNAs contribute to maintain the balance between adipogenic differentiation and osteogenic differentiation through regulating the expressions of RUNX2 and osterix[17-23]. Hence, it is of extremely importance to elucidate the potential roles of microRNAs in osteoporosis. In this study, we determined the expression levels of GAS5, microRNA-498, and RUNX2 in hMSCs derived from osteoporosis patients and healthy controls, respectively. Meanwhile, their functions in osteogenic differentiation were further elucidated. Our results might provide a theoretical basis for the prevention and treatment of osteoporosis.

Patients and Methods

Isolation and Culture of hMSCs

Bone marrow samples were first collected from osteoporosis patients (n=30) and healthy controls (n=30), followed by isolation of hMSCs. Informed consent was obtained from all participants prior to bone marrow collection. This study was approved by the Ethics Committee of Weifang People's Hospital. Bone marrow samples were placed on 10-mm plates, and cultured in 10 mL of low-glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 100 U/mL penicillin, 100 μg/mL streptomycin and 0.5 μg/mL of fungizone. All cells were maintained in an incubator with 5% CO_2 at 37°C. To induce osteogenic differentiation, osteogenic inducer was applied until 70-80% of cell density. Osteogenic differentiation was induced by DMEM (Gibco, Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS), 10 mmol/L sodium β-glycerophosphate, 50 μg/mL Vitamin C, 1% HEPES and 1% penicillin/streptomycin.

Cell Transfection

hMSCs were transfected with pcDNA-GAS5 (pcDNA as control), si-GAS5 (si-control as control), microRNA-498 inhibitor (NC as control) or microRNA-498 mimics (pre-NC as control) according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). 48 h after transfection, the expressions of relative genes in transfected cells were determined by quantitative Real-time polymerase chain reaction (qRT-PCR).

QRT-PCR

Total RNA in hMSCs was extracted in strict accordance with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA integrity was evaluated by agarose gel electrophoresis, and RNA concentration and purity were assessed by spectrophotometry. Subsequently, 1 μg of total RNA was reverse transcribed into cDNA using a Primescript RT kit (TaKaRa Bio Inc., Otsu, Shiga, Japan). QRT-PCR was performed according to the instructions of SYBR premix Ex TaqII kit

(TaKaRa, Otsu, Shiga, Japan) on an ABI Prism 7500 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Specific qRT-PCR conditions were as follows: pre-denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 20 s, and extension at 50°C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was utilized as an internal reference. Primer sequences used in this study were as follows: GAS5, F: 5'-CAAGGACTTCCTCCTCTTAC-3', R: 5'-CGGTAGCCATGTGTCTTAAGGA-3'; microRNA-498, F: 5'-GCTCTGTAAACATACTCGGCTG-3', R: 5'-AGTGTCGCCGAGTTGCTCG-3'; RUNX2, F: 5'-ACGGCAGCGGACAGCAGA-3', R: 5'-TGCGGATAGCAACACAGTTCT-3'. U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Total protein in cells was extracted using radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China). Extracted total proteins were separated by electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) at 300 mA for 100 min. After blocking with 5% skimmed milk for 2 hours, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were incubated with corresponding secondary antibodies at room temperature for 2 h. Immuno-reactive bands were exposed by enhanced chemiluminescence (ECL) method and analyzed by Image Software (NIH, Bethesda, MD, USA).

Alkaline Phosphatase (ALP) Activity Determination

After 7 days of osteogenic differentiation, hMSCs were fixed with 4% paraformaldehyde and 5% citric acid for 30 s. After washing with phosphate-buffered saline (PBS), the cells were incubated with 0.2% naphthol and 0.2% diazonium salt for 15 min. Finally, ALP activity was detected.

ALP Staining

After 14 days of osteogenic differentiation, hMSCs were induced for osteogenic differentiation. Subsequently, the cells were washed with PBS twice, fixed with 4% paraformaldehyde for 15 min and stained with 1% alizarin red staining for 5 min. Finally, calcified nodules were observed and captured using an inverted microscope.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Data were represented as mean ± SD (Standard Deviation). *t*-test was used to compare the differences between two groups. One-way analysis of variance (ANOVA) was performed to compare the differences among different groups, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Expressions of GAS5, microRNA-498 and RUNX2 in Osteoporosis

QRT-PCR was first conducted to detect the expression levels of lncRNA GAS5, microRNA-498 and RUNX2 in hMSCs isolated from osteoporosis patients and non-osteoporosis controls. Results showed that GAS5 was lowly expressed, whereas microRNA-498 was highly expressed in osteoporotic hMSCs relative to controls (Figure 1A, 1B). Meanwhile, both the mRNA and protein levels of RUNX2 in osteoporotic hMSCs were significantly lower than those of controls (Figure 1C, 1D). Therefore, GAS5 was significantly down-regulated in hMSCs isolated from osteoporosis patients. However, high expression of microRNA-498 was accompanied by decreased RUNX2 expression in osteoporosis.

LncRNA GAS5 Overexpression Promoted Osteogenic Differentiation of hMSCs

To assess the effect of GAS5 on osteogenic differentiation, pcDNA or pcDNA-GAS5 was transfected into hMSCs isolated from osteoporosis patients. QRT-PCR verified that a remarkable increase was observed in GAS5 expression after transfection of pcDNA-GAS5 in hMSCs. GAS5 overexpression significantly down-regulated microRNA-498, whereas up-regulated RUNX2 level in hMSCs (Figure 2A). In addition, we further detected the influence of GAS5 on ALP activity. As shown in Figure 2B, hMSCs overexpressing GAS5 exhibited significantly higher level of ALP activity when compared with controls (Figure 2B). Similarly, ALP staining revealed ALP ex-

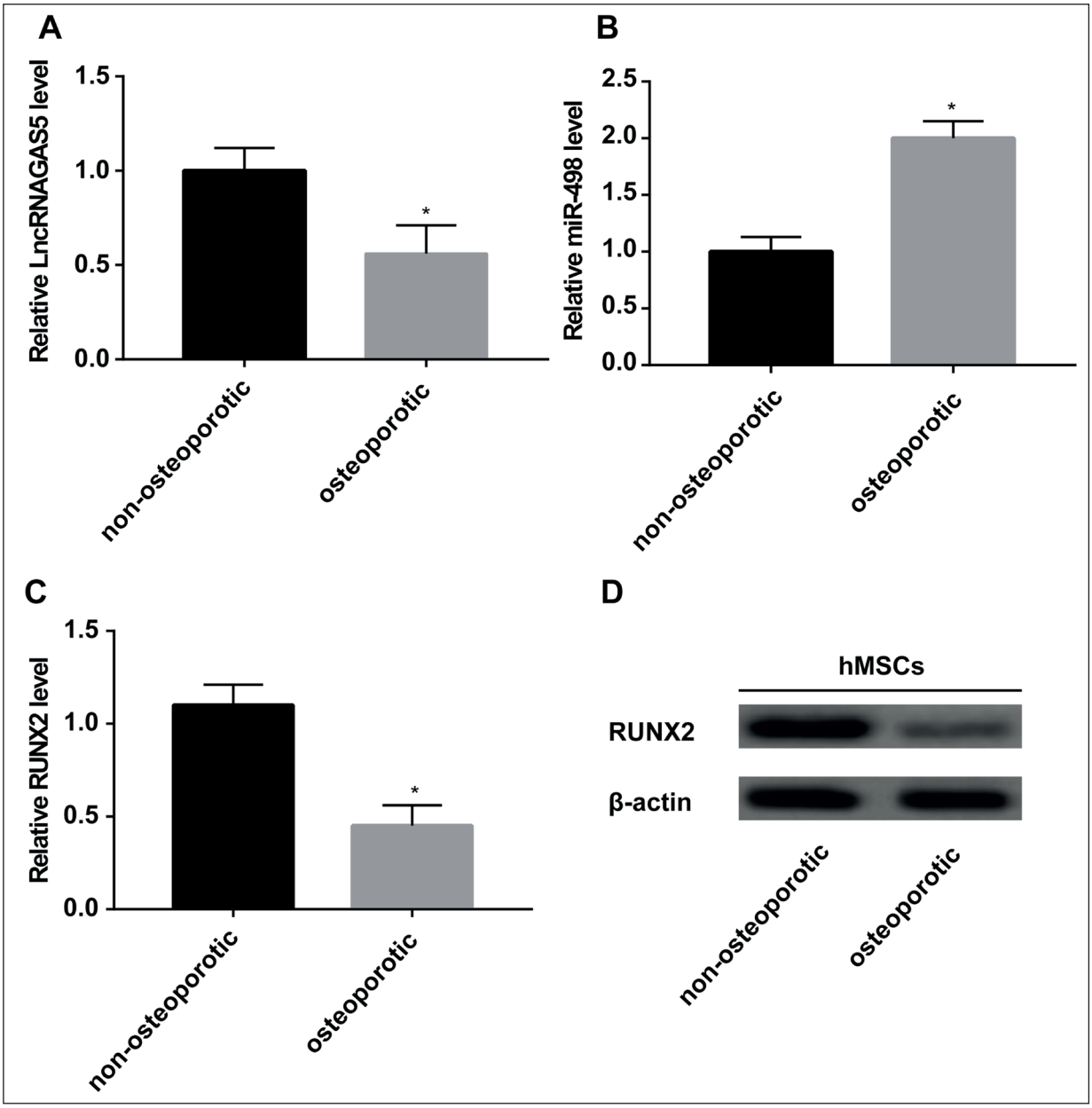

Figure 1. LncRNA GAS5 was lowly expressed in osteoporosis. **A,** LncRNA GAS5 was lowly expressed in osteoporosis patients when compared with healthy controls. **B,** MiR-498 was highly expressed in osteoporosis patients when compared with healthy controls. **C,** RUNX2 was lowly expressed in osteoporosis patients when compared with healthy controls. **D,** The protein level of RUNX2 in osteoporosis patients was significantly lower than healthy controls. *$p<0.05$.

pression in pcDNA-GAS5 group was remarkably elevated than that of control group (Figure 2C). These results indicated that GAS5 overexpression promoted osteogenic differentiation of hMSCs.

LncRNA GAS5 Regulated RUNX2 Expression Through microRNA-498

The binding sites of GAS5 to microRNA-498, as well as microRNA-498 to RUNX2 were predicted using TargetScan7.1. It was found that microRNA-498 could bind to the 3'UTR of GAS5 and RUNX2 (Figure 3A). Furthermore, pcDNA-GAS5 or microRNA-498 mimics was transfected into hMSCs isolated from osteoporosis patients. Subsequent experiments revealed that overexpression of GAS5 significantly up-regulated RUNX2 expression at both mRNA and protein levels. However, co-overexpression of

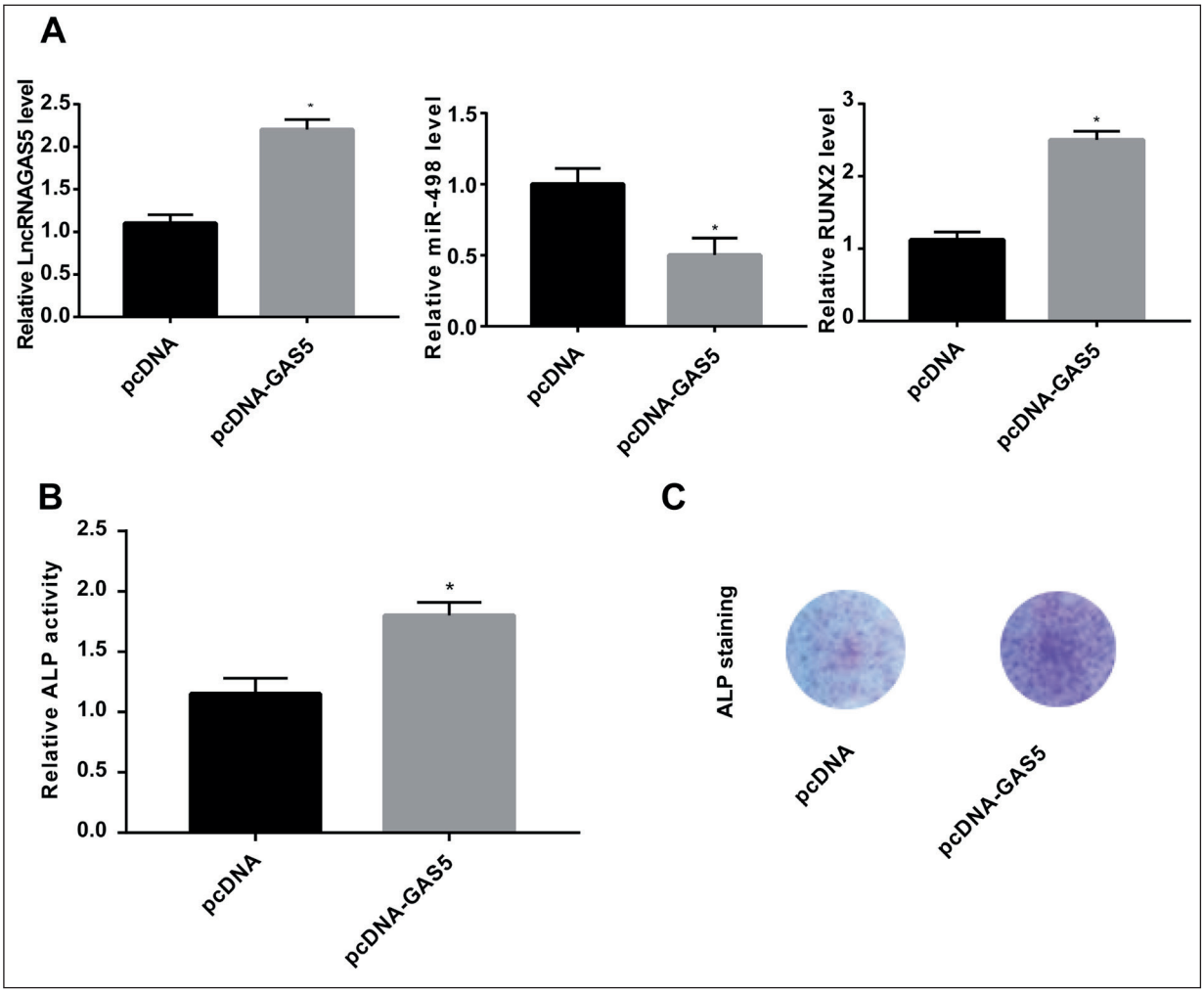

Figure 2. LncRNA GAS5 overexpression promoted osteogenic differentiation of hMSCs. **A,** Expression levels of GAS5, miR-498 and RUNX2 in hMSCs transfected with pcDNA or pcDNA-GAS5. **B,** Relative activity of ALP in hMSCs transfected with pcDNA or pcDNA-GAS5. **C,** ALP staining in hMSCs transfected with pcDNA or pcDNA-GAS5. *p<0.05.

microRNA-498 could reverse their expression levels (Figure 3B). We co-transfected si-GAS5 and microRNA-498 inhibitor into hMSCs as well. In contrast, the results demonstrated that GAS5 knockdown significantly inhibited the mRNA and protein expressions of RUNX2, which could be reversed by microRNA-498 knockdown (Figure 3C). These results indicated that GAS5 regulated the expression of RUNX2 through microRNA-498.

LncRNA GAS5 Influenced Osteogenic Differentiation of hMSCs Through microRNA-498 to Upregulate RUNX2

To elucidate the regulatory mechanism of GAS5 on osteogenic differentiation, we co-transfected pcDNA-GAS5, microRNA-498 mimics and pcDNA-Runx2 into hMSCs. ALP activity assay showed that overexpression of GAS5 significantly increased ALP activity. However, co-overexpression of microRNA-498 obviously decreased ALP activity. Eventually, this was elevated with RUNX2 overexpression (Figure 4A). In contrast, knockdown of GAS5 decreased ALP activity, which was subsequently increased by microRNA-498 inhibitor. RUNX2 overexpression remarkably enhanced ALP activity in hMSCs co-transfected with si-GAS5 and microRNA-498 inhibitor (Figure 4B). The above results suggested that GAS5 up-regulated the expression of RUNX2 through microRNA-498, promoting osteogenic differentiation.

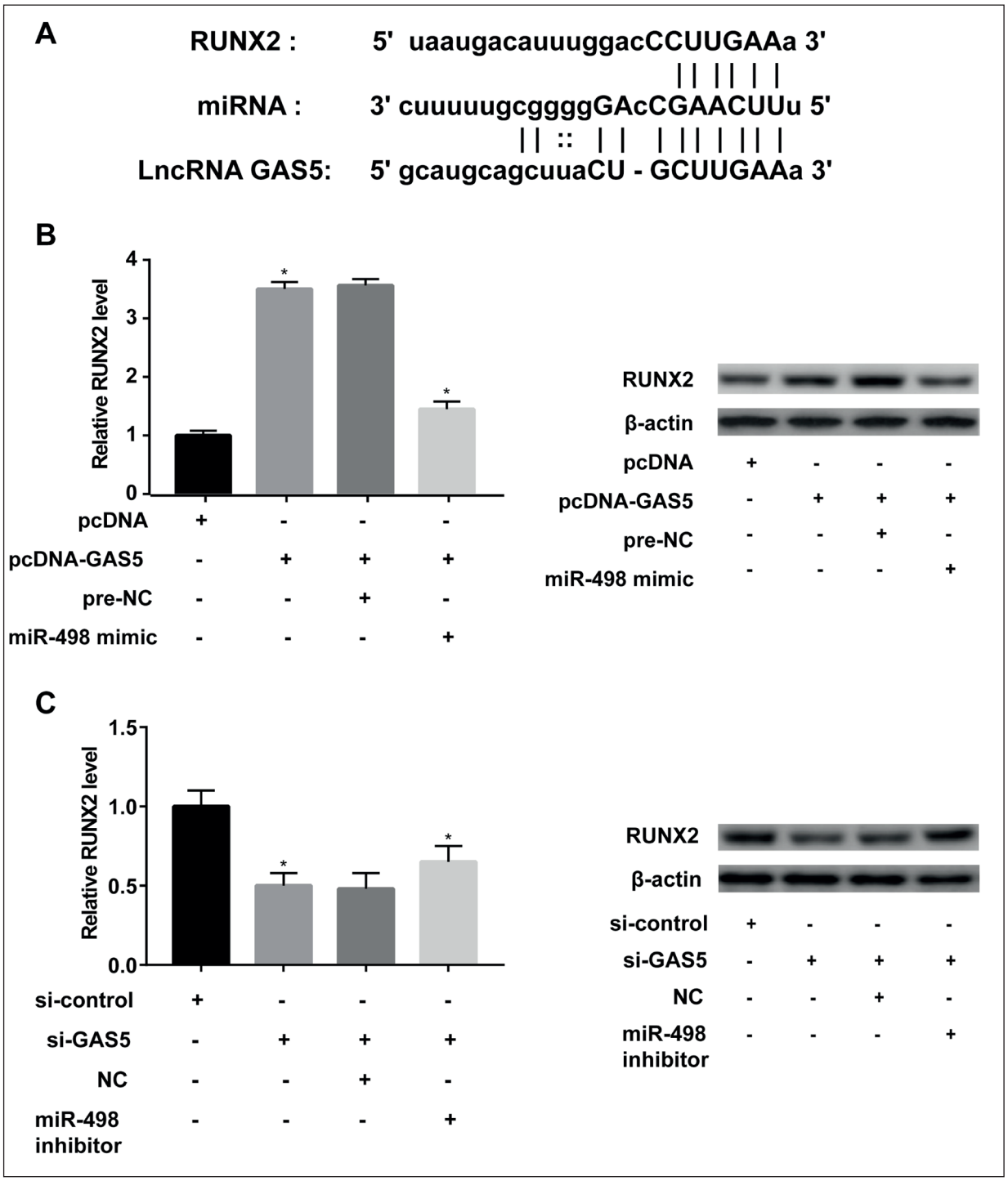

Figure 3. LncRNA GAS5 regulated RUNX2 expression through miR-498. **A,** The binding sites of GAS5 to miR-498, and miR-498 to RUNX2. **B,** The mRNA and protein levels of RUNX2 in hMSCs transfected with pcDNA-GAS5 and miR-498 mimics. **C,** The mRNA and protein levels of RUNX2 in hMSCs transfected with si-GAS5 and miR-498 inhibitor. *$p<0.05$.

Discussion

Osteoporosis is a severe disease characterized by systemic bone loss and bone microstructure deterioration. This may eventually result in decreased bone strength, increased fragility, and elevated fracture risk[24]. In recent years, researches have gradually focused on the ge-

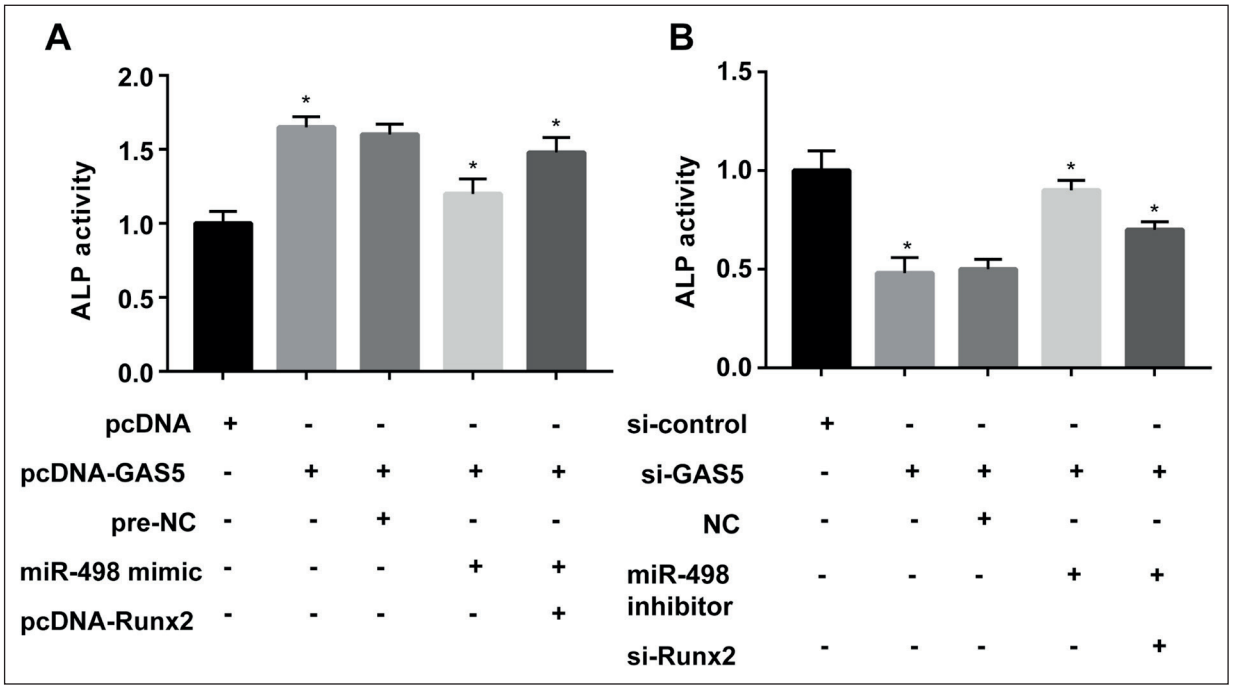

Figure 4. LncRNA GAS5 influenced osteogenic differentiation of hMSCs through miR-498 to upregulate RUNX2. **A,** ALP activity in hMSCs co-transfected pcDNA-GAS5, miR-498 mimics and pcDNA-Runx2. **B,** ALP activity in hMSCs co-transfected si-GAS5, miR-498 inhibitor and si-RUNX2. *$p<0.05$.

netic level of bone disease rather than bone morphology. Meanwhile, non-coding RNAs in bone metabolism have become well concerned. LncRNAs relative to bone diseases have been discovered by high throughput sequencing or microarrays. Researchers have found that certain lncRNAs are capable of influencing bone metabolism through regulating osteogenesis, adipogenesis and osteoclast differentiation *via* epigenetic modification and miRNA adsorption[25,26]. For example, lncRNA p21 exerts a great impact on BMMSC[27]. Knockdown of lncRNA p21 promotes BMMSC to secret vascular endothelial growth factors, basic fibroblast growth factors, insulin-like growth factors and β-catenin by regulating Wnt/β pathway. This may finally accelerate BMMSC differentiation into osteoblasts. Wei et al[28] have shown that lncRNA HOTAIR regulates the expressions of miR-17-5p and its target gene Smad 7 through BMPs/TGF-β pathway. In this study, GAS5 was lowly expressed in osteoporosis patients. GAS5 overexpression could significantly promote osteogenic differentiation of hMSCs. Furthermore, we investigated the role of GAS5 in regulating osteogenic differentiation. MicroRNAs are involved in various functions of hMSCs, including self-renewal, multi-directional differentiation and functional exercise[29]. MiR-214 has been found significantly up-regulated in osteoblasts of aging people, suppressing bone formation by inhibiting TCF4[30]. The expression level of miR-188 is significantly elevated in aging mice and hMSCs, leading to differentiation defects in hMSCs[31]. As an anti-oncogene, microRNA-498 regulates multiple tumor-suppressor genes involved in tumor growth, invasion and metastasis regulation, thereby mediating the development of various malignancies[32-34]. However, the specific role of microRNA-498 in osteoporosis remained unclear, which was specifically elucidated in this paper. Competing endogenous RNA (CeRNA) hypothesis is a gene regulation pattern proposed recently. LncRNA controls the expression levels of other RNAs (including coding RNAs) by competitively binding to specific miRNAs. LncRNAs act as miRNA sponges or precursors to regulate osteogenic differentiation. Liang et al[35] have observed that H19 inhibits the expressions of miR-141 and miR-22 by interacting with RNA-induced silencing complex (RISC) Ago2, thus activating osteogenic differentiation. Song et al[36] have screened out three lncRNAs TCONS_00046478, TCONS_00027225, and

TCONS_00007697 by high throughput sequencing, Weighted Gene Co-expression Network Analysis (WGCAN) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Meanwhile, these lncRNAs are considered as miRNAs precursors (miR-689, miR-544 and miR-640) involving in bone differentiation. Our study found that microRNA-498 was highly expressed in hMSCs isolated from osteoporosis patients, showing a negative regulatory effect on osteogenic differentiation. Furthermore, we also evaluated the potential binding sites between microRNA-498 and GAS5, suggesting their crucial roles in regulating osteogenic differentiation. RUNX2 has been identified as a target for multiple miRNAs, such as miR-30b/c, miR-133a, miR-204, miR-217, miR-34, miR-23a, etc.[37]. In this study, we found that RUNX2 was the target of microRNA-498, whose expression was regulated by microRNA-498. This further demonstrated the important role of RUNX2 in osteogenic differentiation. GAS5 regulated RUNX2 expression *via* targeting microRNA-498, thus influencing osteogenic differentiation of hMSCs. Furthermore, overexpression of GAS5 significantly up-regulated RUNX2 expression by microRNA-498 to promote osteogenic differentiation of hMSCs, thereby alleviating the progression of osteoporosis. This study might provide a new perspective on the prevention and treatment of osteoporosis.

Conclusions

Overexpression of GAS5 promotes osteogenic differentiation of hMSCs through regulating microRNA-498 to up-regulate RUNX2 expression, thus alleviating the development of osteoporosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Diab DL, Watts NB. Postmenopausal osteoporosis. Curr Opin Endocrinol Diabetes Obes 2013; 20: 501-509.
2) Paolucci T, Saraceni VM, Piccinini G. Management of chronic pain in osteoporosis: challenges and solutions. J Pain Res 2016; 9: 177-186.
3) Abarrategi A, Marinas-Pardo L, Mirones I, Rincon E, Garcia-Castro J. Mesenchymal niches of bone marrow in cancer. Clin Transl Oncol 2011; 13: 611-616.
4) Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
5) Yoon JH, Abdelmohsen K, Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. J Mol Biol 2013; 425: 3723-3730.
6) Zhang L, Wang DL, Yu P. LncRNA H19 regulates the expression of its target gene HOXA10 in endometrial carcinoma through competing with miR-612. Eur Rev Med Pharmacol Sci 2018; 22: 4820-4827.
7) Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 2009; 23: 1494-1504.
8) Zhu L, Xu PC. Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. Biochem Biophys Res Commun 2013; 432: 612-617.
9) Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. Cell 1988; 54: 787-793.
10) Lucafo M, De Iudicibus S, Di Silvestre A, Pelin M, Candussio L, Martelossi S, Tommasini A, Piscianz E, Ventura A, Decorti G. Long noncoding RNA GAS5: a novel marker involved in glucocorticoid response. Curr Mol Med 2015; 15: 94-99.
11) Song J, Ahn C, Chun CH, Jin EJ. A long non-coding RNA, GAS5, plays a critical role in the regulation of miR-21 during osteoarthritis. J Orthop Res 2014; 32: 1628-1635.
12) Hu R, Li H, Liu W, Yang L, Tan YF, Luo XH. Targeting miRNAs in osteoblast differentiation and bone formation. Expert Opin Ther Targets 2010; 14: 1109-1120.
13) Vrtacnik P, Marc J, Ostanek B. Epigenetic mechanisms in bone. Clin Chem Lab Med 2014; 52: 589-608.
14) Barter MJ, Young DA. Epigenetic mechanisms and non-coding RNAs in osteoarthritis. Curr Rheumatol Rep 2013; 15: 353.
15) Delgado-Calle J, Garmilla P, Riancho JA. Do epigenetic marks govern bone mass and homeostasis? Curr Genomics 2012; 13: 252-263.
16) Gamez B, Rodriguez-Carballo E, Ventura F. MicroRNAs and post-transcriptional regulation of skeletal development. J Mol Endocrinol 2014; 52: R179-R197.
17) Li Z, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, Lian JB, Stein GS. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A 2008; 105: 13906-13911.
18) Berendsen AD, Olsen BR. Osteoblast-adipocyte lineage plasticity in tissue development, maintenance and pathology. Cell Mol Life Sci 2014; 71: 493-497.

19) Kawai M, de Paula FJ, Rosen CJ. New insights into osteoporosis: the bone-fat connection. J Intern Med 2012; 272: 317-329.

20) Liao L, Yang X, Su X, Hu C, Zhu X, Yang N, Chen X, Shi S, Shi S, Jin Y. Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. Cell Death Dis 2013; 4: e600.

21) Huang S, Wang S, Bian C, Yang Z, Zhou H, Zeng Y, Li H, Han Q, Zhao RC. Upregulation of miR-22 promotes osteogenic differentiation and inhibits its adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells by repressing HDAC6 protein expression. Stem Cells Dev 2012; 21: 2531-2540.

22) 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.

23) Gamez B, Rodriguez-Carballo E, Bartrons R, Rosa JL, Ventura F. MicroRNA-322 (miR-322) and its target protein Tob2 modulate Osterix (Osx) mRNA stability. J Biol Chem 2013; 288: 14264-14275.

24) Ahlborg HG, Rosengren BE, Jarvinen TL, Rogmark C, Nilsson JA, Sernbo I, Karlsson MK. Prevalence of osteoporosis and incidence of hip fracture in women--secular trends over 30 years. BMC Musculoskelet Disord 2010; 11: 48.

25) Chen J, Cui X, Shi C, Chen L, Yang L, Pang L, Zhang J, Guo X, Wang J, Ji C. Differential lncRNA expression profiles in brown and white adipose tissues. Mol Genet Genomics 2015; 290: 699-707.

26) Sun L, Goff LA, Trapnell C, Alexander R, Lo KA, Hacisuleyman E, Sauvageau M, Tazon-Vega B, Kelley DR, Hendrickson DG, Yuan B, Kellis M, Lodish HF, Rinn JL. Long noncoding RNAs regulate adipogenesis. Proc Natl Acad Sci U S A 2013; 110: 3387-3392.

27) Xia W, Zhuang L, Deng X, Hou M. Long noncoding RNAp21 modulates cellular senescence via the Wnt/betacatenin signaling pathway in mesenchymal stem cells. Mol Med Rep 2017; 16: 7039-7047.

28) Wei B, Wei W, Zhao B, Guo X, Liu S. Long non-coding RNA HOTAIR inhibits miR-17-5p to regulate osteogenic differentiation and proliferation in non-traumatic osteonecrosis of femoral head. PLoS One 2017; 12: e169097.

29) Dirks PB. MicroRNAs and parallel stem cell lives. Cell 2009; 138: 423-424.

30) Wang X, Guo B, Li Q, Peng J, Yang Z, Wang A, Li D, Hou Z, Lv K, Kan G, Cao H, Wu H, Song J, Pan X, Sun Q, Ling S, Li Y, Zhu M, Zhang P, Peng S, Xie X, Tang T, Hong A, Bian Z, Bai Y, Lu A, Li Y, He F, Zhang G, Li Y. miR-214 targets ATF4 to inhibit bone formation. Nat Med 2013; 19: 93-100.

31) Li CJ, Cheng P, Liang MK, Chen YS, Lu Q, Wang JY, Xia ZY, Zhou HD, Cao X, Xie H, Liao EY, Luo XH. MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation. J Clin Invest 2015; 125: 1509-1522.

32) Matamala N, Vargas MT, Gonzalez-Campora R, Arias JI, Menendez P, Andres-Leon E, Yanowsky K, Llaneza-Folgueras A, Minambres R, Martinez-Delgado B, Benitez J. MicroRNA deregulation in triple negative breast cancer reveals a role of miR-498 in regulating BRCA1 expression. Oncotarget 2016; 7: 20068-20079.

33) Kogo R, How C, Chaudary N, Bruce J, Shi W, Hill RP, Zahedi P, Yip KW, Liu FF. The microRNA-218~survivin axis regulates migration, invasion, and lymph node metastasis in cervical cancer. Oncotarget 2015; 6: 1090-1100.

34) Gopalan V, Smith RA, Lam AK. Downregulation of microRNA-498 in colorectal cancers and its cellular effects. Exp Cell Res 2015; 330: 423-428.

35) Liang WC, Fu WM, Wang YB, Sun YX, Xu LL, Wong CW, Chan KM, Li G, Waye MM, Zhang JF. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep 2016; 6: 20121.

36) Song WQ, Gu WQ, Qian YB, Ma X, Mao YJ, Liu WJ. Identification of long non-coding RNA involved in osteogenic differentiation from mesenchymal stem cells using RNA-Seq data. Genet Mol Res 2015; 14: 18268-18279.

37) Zhang Y, Xie RL, Croce CM, Stein JL, Lian JB, van Wijnen AJ, Stein GS. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proc Natl Acad Sci U S A 2011; 108: 9863-9868.