European Review for Medical and Pharmacological Sciences 2019; 23: 10195-10203

Long noncoding RNA MIRG induces osteoclastogenesis and bone resorption in osteoporosis through negative regulation of miR-1897

L. LING, H.-L. HU, K.-Y. LIU, Y.-I. RAM, J.-L. GAO, Y.-M. CAO

Orthopedic Department, the Second Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Abstract. – OBJECTIVE: To investigate the expression of long noncoding RNA (LncRNA) MIRG and its potential functions in regulating osteoclastogenesis and bone resorption function through modulating miR-1897 in bone marrow macrophages (BMMs).

MATERIALS AND METHODS: qRT-PCR was performed to detect the expressions of MIRG and its co-expression mRNA NFATc1 at different stages during osteoclastogenesis. The CCK-8 assay was performed to evaluate cell proliferation and differentiation. The correlation between miR-1897 and MIRG was detected by statistical analysis. Bioinformatics and luciferase assay were performed to explore the interaction and binding site of MIRG and miR-1897. We also cloned the mice NFATc1 3′-UTR into the luciferase reporter vector and constructed miR-1897 binding mutants to validate the inhibited regulation of miR-1897 to the expression of NFATc1.

RESULTS: Results showed that expressions of MIRG and NFATc1 were upregulated during osteoclastogenesis. qRT-PCR and CCK-8 assay showed that MIRG expression is associated with osteoclastogenesis and bone resorption. The bioinformatics prediction and luciferase assay suggested that by interacting with miR-1897, MIRG acts as a molecular sponge for the miR-1897 target NFATc1, to partly modulate the inhibitory effect of miR-1897 on NFATc1.

CONCLUSIONS: We found that lncRNA-MIRG was upregulated in osteoclasts, which could promote osteoclastogenesis and bone resorption function as a molecular sponge by modulating the inhibitory effect of miR-1897 on NFATc1.

Key Words:

Osteoporosis, Bone resorption, Long noncoding RNA MIRG, Osteoclastogenesis.

Introduction

Bone is a dynamic organ continuously undergoing remodeling[1]. Bone remodeling is maintained by the osteoblastic bone formation and osteoclastic bone resorption[2]. Osteoclasts, a type of bone-specific multinucleated cell, play a vital role in bone remodeling process[3,4]. Osteoclasts are bone resorbing cells differentiated from monocyte/macrophage lineage hematopoietic stem cells[5]. Receptor activator of nuclear factor κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF) are two important regulating factors in osteoclastogenesis[6,7]. Osteoclast differentiation and function dysregulation can cause various bone pathologies, including osteoporosis, Paget's disease, and pycnodysostosis[8-10]. Therefore, finding the ways and mechanisms to regulate osteoclasts is pivotal to find potential targets for the treatment of bone diseases.

Long noncoding RNAs (lncRNAs) represent a group of non-protein-coding RNA transcripts, which have more than 200 nucleotides. They have recently been reported to play pivotal roles in various biological functions such as gene expression, cell growth and differentiation[11-13]. The expression profile of lncRNAs is tissue-specific and differentially expressed across various stages during cell differentiation[14,15]. In particular, it has been demonstrated that lncRNAs can regulate bone development and remodeling[16,17]. However, its role in these processes still remains unknown. The regulating function of lncRNAs is not solitary but by the diverse and complex mechanism with the participation of DNA, microRNAs, mR-

Corresponding Authors: Yanming Cao, MD; e-mail: 13729813888@139.com
Long Ling, MD; e-mail: 525563925@qq.com

NAs, and proteins[18,19]. MicroRNAs are a class of noncoding small RNAs composed of approximately 22 nucleotides in length. Previous studies demonstrated that microRNAs are essential players in biological processes including bone development and remodeling[20]. MicroRNAs regulate over 30% of the genes in eukaryotic organisms and play essential roles in osteoclastogenesis[20-22]. In recent studies, a promising view has revealed that lncRNAs are supposed to be sponges, which can make microRNAs exhausted and preventing mRNA from degradation by microRNA[23-26].

LncRNA MIRG is located on chromosome 12F1, and its expression is upregulated in several tissues such as limb, liver and placenta[27]. Surprisingly, our previous study showed that MIRG was upregulated in osteoclasts[28]. However, the roles and functions of MIRG in osteoclast differentiation are currently poorly understood so far. In this study, we aimed to explore the functional roles of MIRG in osteoclastogenesis, as well as to disclose the molecular mechanisms. Firstly, we measured the MIRG levels in osteoclasts at different stages during osteoclastogenesis. Secondly, the proliferation ability and differentiation functions were measured after transfection with lentiviral MIRG. At last, we assessed the regulatory relationship between MIRG and miR-1897 and the miR-1897-mediated roles of MIRG. Our study uncovered a critical function of lncRNA MIRG in osteoclastogenesis and bone resorption function.

Materials and Methods

Cell Culture

Murine monocyte-macrophage RAW264.7 cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Rockville, MD, USA) supplied with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin-streptomycin (Gibco) and incubated at 37°C in an atmosphere of 5% CO_2.

Construction of Lentivirus and Cell Transfection

Lentiviral Lnc MIRG and lnc MIRG shRNA were synthesized and constructed by Shanghai GenePharma Co., Ltd., (Shanghai, China). For miR analysis, the miR-1897 mimic, miR-1897 inhibitor and the negative control were constructed by Shanghai GenePharma Co., Ltd., (Shanghai, China). To knock down NFATC1, si-NFATC1 plasma and negative control plasma were constructed by Shanghai GenePharma Co., Ltd., (Shanghai, China). For transfection, 1×10^4 cells were seeded in 6-well plates and cultured with RANKL (100 ng/mL) and M-CSF (100 ng/mL). Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA) and Opti-MEM® I reduced serum medium were used for transfection. For analysis of Lnc-MIRG, cells were transfected with Lnc-MIRG shRNA (referred as to sh) and negative control shRNA (referred as to nc), respectively. For analysis of miR-1897, cells were transfected with miR-1897 inhibitor, and control cells were transfected with an empty vector, respectively. The cells without transfection were used as the control (referred as to control). After cultures were incubated for 30 min, they were replaced with Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS. Then, at an indicated time point after transfection, cells were harvested for further study.

Osteoclast Differentiation

5×10^3 cells were seeded in 96-well plates uniformly. After one day, the medium was changed with DMEM containing M-CSF (100 ng/mL) and RANKL (100 ng/mL) according to the experiments. And the culture medium was refreshed every day. After induction for 3 days, tartrate-resistant acid phosphatase (TRAP) stain was performed using TRAP stain kit. Next, cells were harvested for real-time PCR.

RNA Extraction and qRT-PCR

After taking out the culture plates, the cells were washed with PBS. After treatment, total RNA of cells was extracted by using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions and samples were stored at room temperature for 30 min. The reverse transcription of cDNA was performed with a PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. And for qRT-PCR, PCR primers were synthesized by GenePharma (Shanghai Gene Pharma, Shanghai, China) and sequences were listed in Table I. SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) was used to detect the expression.

CCK8 Assay

The CCK-8 kit (Dojindo Molecular Technologies, Kumamoto, Japan) was used to measure the cells proliferation according to the manufacturer's instructions. In brief, 5×10^3 cells were seeded in 96-well

Table I. Primer sequences for qRT-PCR.

Genes	Forward	Reverse	Tm (°C)
NFATc1	5'-GACCCGGAGTTCGACTTCG-3'	5'-TGACACTAGGGGACACATAACTG-3'	61
MIRG	5'-AACCCAGACACAAGCATTCC-3'	5'-GAGACATTTTCCCGTTCACC-3'	60
miR-1897	5'-CAATGCAGCCCTAGAGAGACA-3'	5'-CCACACCGGAAGACATTTACA-3'	61
CTSK	5'-GCTCCTCTTAGGGGCCACT-3'	5'-ATTGGGGACCCTTAGGCCAT-3'	62
MMP-9	5'-AAGTCTCAAGGTTATAGGGACGG-3'	5'-CCATGCTTGTCTGGGTATAGTGT-3'	62
TRAP	5'-CACTCCCACCCTGAGATTTGT-3'	5'-CATCGTCTGCACGGTTCTG-3'	60
C-fos	5'-TTGAGCGATCATCCCGGTC-3'	5'-GCGTGAGTCCATACTGGCAAG-3'	61
DC-STAMP	5'-CGGCGGCCAATCTAAGGTC-3'	5'-CCCACCATGCCCTTGAACA-3'	61
GAPDH	5'-TGGATTTGGACGCATTGGTC-3'	5'-TTTGCACTGGTACGTGTTGAT-3'	62

plates uniformly. After treated with regulated medium, the medium was removed, and cells were washed with PBS solution 3 times. Then, CCK8 dilution was added to the 96-well plates and incubated at 37°C in an atmosphere of 5% CO_2 for 2 hours. After incubation, the plates were taken out, and cell proliferation was measured using a multi-detection microplate reader. And the absorbance (OD) value at 490 nm of each well was detected.

Luciferase Assay

After transfection for 48 h, the luciferase activities were measured by using the dual luciferase reporter assay system (Promega, Madison, WI, USA) according to the manufacturer's protocol. Renilla luciferase activities were normalized to the firefly luciferase activities and the data were expressed as the fold change relative to the corresponding control groups, which were defined as 1.0.

Statistical Analysis

Unless otherwise indicated, all data were processed by Statistical Product and Service Solutions (SPSS) 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Each assay was applied at least three independent experiments or replicates. All data were presented as mean ± SD. Student's *t*-test, one-way analysis of variance (ANOVA) and multiple comparison between the groups was performed by using SNK method, in which $*p < 0.05$, $**p < 0.01$ represented as the difference significance.

Results

MIRG and its Co-Expressed mRNA NFATc1 Was Highly Expressed in Osteoclasts

For osteoclast differentiation, BMMs isolated from mouse femur were cultured with RANKL and M-CSF to generated osteoclasts, following confirmed by RT-QPCR analysis of osteoclastogenesis specific genes (Figure 1A). By bioinformatics analysis of lncRNAs expression data of osteoclasts at different stages during osteoclastogenesis (0 h, 24 h and 72 h), we screened out MIRG and its co-expression gene NFATc1, both of which had high specific expression in osteoclasts (Figure 1B). We then performed RT-QPCR analysis for further validation; the results indicated that both the expressions of MIRG and NFATc1 were significantly upregulated in osteoclasts compared with BMMs ($p<0.05$) (Figure 1C).

MIRG Expression is Associated with Osteoclastogenesis and Bone Resorption Function

To explore the functions of MIRG in osteoclasts, lentiviral MIRG was constructed, and subsequently transfected into BMMs; next, MIRG expression was detected. RT-QPCR analysis showed that MIRG expression was remarkably downregulated after lentiviral MIRG transfection (Figure 2A). The CCK-8 assay was performed and showed that MIRG inhibition significantly suppressed the cell proliferation activity of BMMs after 1 d and 3 d (Figure 2B). MIRG-inhibited BMMs were stimulated with RANKL and M-CSF for 3 days for osteoclast differentiation, which showed a poor efficiency of osteoclastogenesis compared with control BMMs (Figure 2C), indicating that MIRG plays an important role in the regulation of osteoclastogenesis. RT-QPCR analysis was also performed to detect osteoclastogenesis related genes between MIRG-inhibited cells and control cells after osteoclast induction for 3 days. The results showed a significant down-regulation of osteoclast differentiation-associated

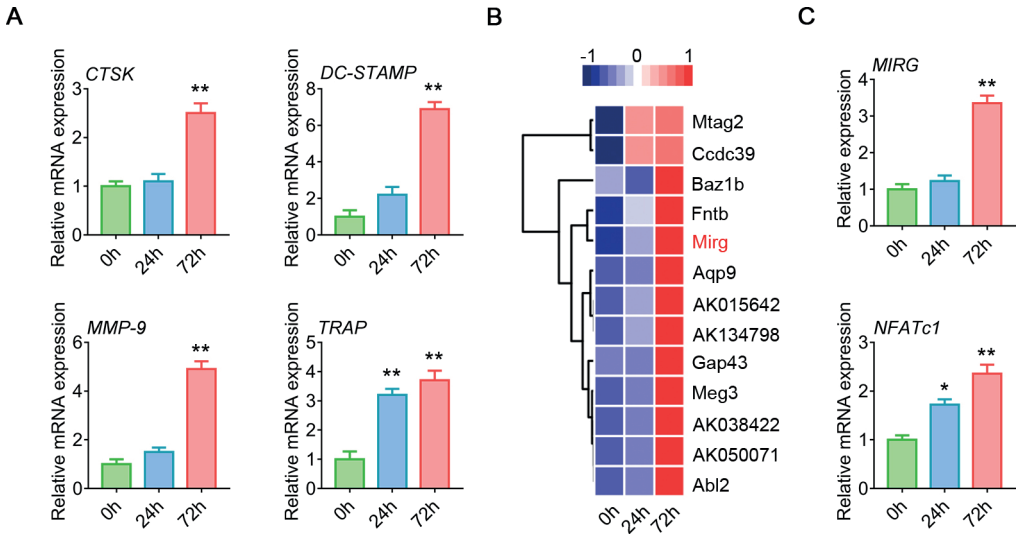

Figure 1. MIRG and its co-expressed mRNA NFATc1 were highly expressed in osteoclasts. **(A)** Relative mRNA expression levels of CTSK, DC-STAMP, MMP-9, TRAP of osteoclasts at different stages during osteoclastogenesis (0 h, 24 h and 72 h). (**B**) The cluster heat map of expression profiles of 13 lncRNAs at different stages during osteoclastogenesis. (**C**) Relative expression of MIRG and its co-expression gene NFATc1 at different stages during osteoclastogenesis (0 h, 24 h and 72 h) assessed by qRT-PCR. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

genes such as NFATc1, c-FOS and TRAP and bone resorption function-related genes including CTSK, MMP9 and DC-STAMP in osteoclasts after MIRG inhibition (Figure 2D, 2E). Together, these results suggested that MIRG expression is important for osteoclastogenesis and bone resorption function, and that inhibition of MIRG leads to a decreased of osteoclastogenesis and dysfunction of bone resorption.

MiR-1897 was Downregulated in Osteoclasts and Negatively Correlated with MIRG

In order to investigate whether MIRG was correlated miRNA, we used starBase 2.0 to predict the target miRNA of MIRG and found that miR-1897 is one of the target miRNAs of MIRG. Therefore, we used RT-QPCR analysis to detect the miR-1897 expressions of osteoclasts. Results showed that miR-1897 was downregulated in osteoclasts compared with BMMs (Figure 3A). We then used correlation analysis to explore the relationship between MIRG and miR-1897 further; the results showed that miR-1897 was significantly negatively correlated with MIRG, indicating that miR-1897 might be regulated by MIRG (Figure 3B). These results suggested that MiR-1897 was low expressed in osteoclasts and negatively correlated with MIRG.

MIRG Could Directly Bind with miR-1897 in Osteoclasts

Previous studies demonstrated that lncRNAs might act as a competing sponge in regulating the biological functions of miRNAs. As discussed above, miR-1897 was negatively correlated with MIRG, so we hypothesized that MIRG regulated osteoclast differentiation and bone resorption through interaction with miR-1897. To further detect the relationship between miR-1897 and MIRG, MIRG-wt luciferase reporter vector and MIRG-mut 3'UTR luciferase reporter vector were synthesized (Figure 4A) and luciferase reporter assay was performed. Compared with the control, the luciferase activity of BMMs that co-transfected with miR-1897 mimic and MIRG-wt was significantly decreased ($p<0.05$), and it was reversely increased in MIRG-mut compared with MIRG-wt ($p<0.01$) (Figure 4B). These data demonstrated that miR-1897 could directly bind to MIRG. Besides, MIRG overexpression suppressed miR-1897 expression and MIRG inhibition reversely facilitated miR-1897 expression in BMMs (Figure 4C, 4D). Additionally, we also transfected miR-1897 mimic and miR-1897 inhibitor into BMMs. The results revealed that miR-1897 mimic inhibited MIRG expression and miR-1897 inhibitor increased MIRG expression (Figure 4E, 4F). These findings suggested that

miR-1897 directly bound to MIRG at the recognition sites.

MIRG Acts as a Molecular Sponge for miR-1897 and Controls the miR-1897 Target, NFATc1

NFATc1 is one of the most important transcription factors regulating osteoclastogenesis. To test whether miR-1897 targets the NFATc1, we performed RT-QPCR analysis for NFATc1 in the presence of miR-1897 mimics or inhibitor. We observed decreased NFATc1 expression when BMMs were treated with the miR-1897 mimics, indicating that miR-1897 downregulated NFATc1 expression (Figure 5A). To validate this mechanism, we cloned the mice NFATc1 3′-UTR into the luciferase reporter vector and constructed miR-1897 binding mutants in which the putative

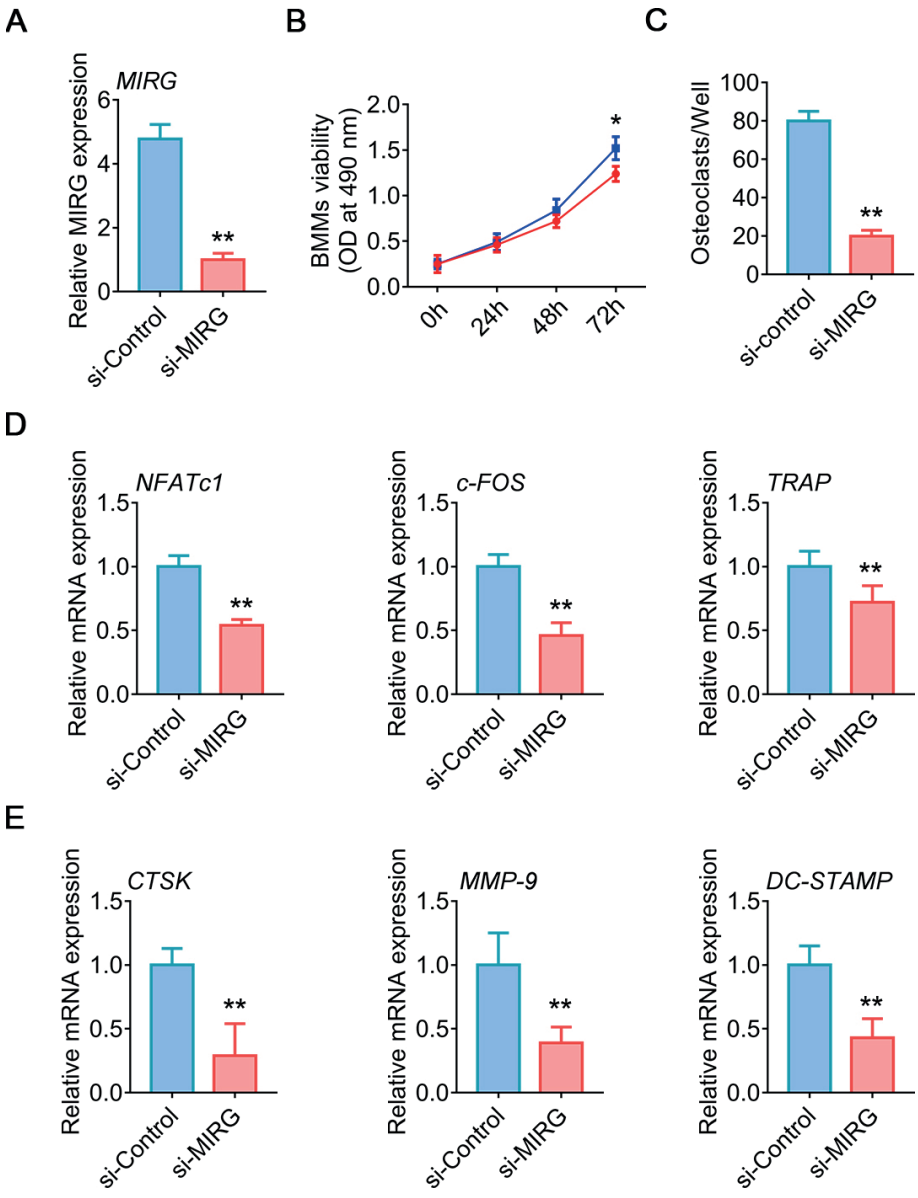

Figure 2. MIRG expression is associated with osteoclastogenesis and bone resorption function. (**A**) Relative mRNA expression levels of MIRG in osteoclasts transfected with si-control and si-MIRG. (**B**) Absorption at 490 nm of BMMs treated with MIRG detected by CCK-8 assay at 1 d, 2 d and 3 d. (**C**) Quantification of osteoclasts in each well. (**D**) Relative mRNA expression levels of NFATc-1, c-FOS and TRAP in osteoclasts transfected with si-control and si-MIRG detected by qRT-PCR. (**E**) Relative mRNA expression levels of CTSK, MMP-9 and DC-STAMP in osteoclasts transfected with si-control and si-MIRG detected by qRT-PCR. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

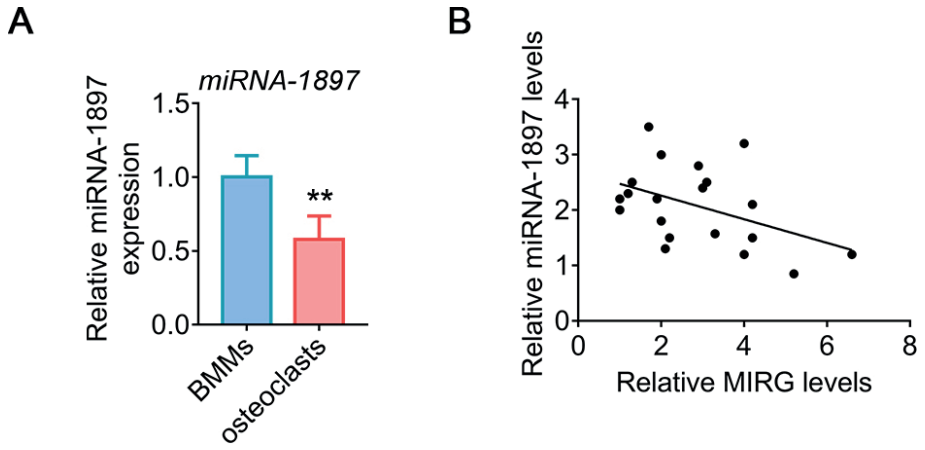

Figure 3. miR-1897 was downregulated in osteoclasts and negatively correlated with MIRG. (**A**) Relative miR-1897 expression in BMMs and osteoclasts detected by qRT-PCR. (**B**) Correlation analysis was performed to evaluate the relationship between miR-1897 and MIRG. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

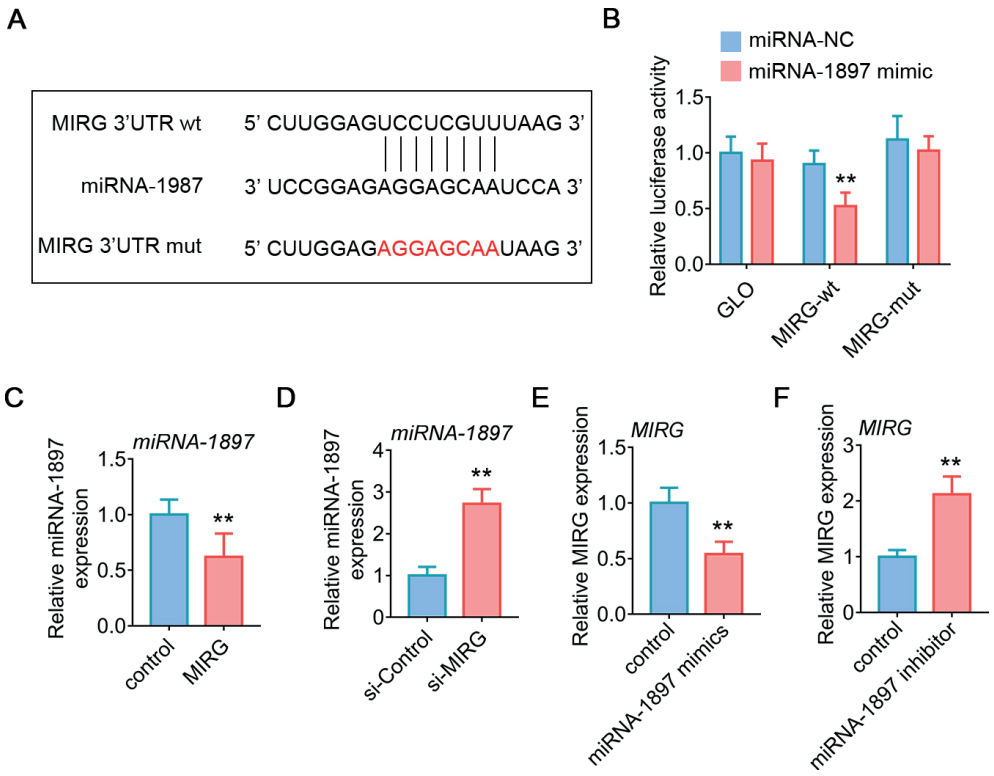

Figure 4. MIRG could directly bind with miR-1897 in osteoclasts**.** (**A**) Schematic illustration of the predicted miR-1897 binding sites and mutant sites in MIRG. (**B**) Relative luciferase activity of BMMs. (**C-D**) qRT-PCR analysis of miR-1897 expression level in BMMs transfected with lentiviral MIRG and si- MIRG. (**E-F**) Relative MIRG expression was detected in BMMs after miR-1897 mimic and miR-1897 inhibitor by RT-PCR. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

miR-1897 binding sites CGAGU in the NFTAc1 3′-UTR were mutated into GCUCA (Figure 5B). As expected, double luciferase report results showed that miR-1897 mimics significantly downregulated the NFATc1 expression whereas point mutations in the NFATc1 3′-UTR abrogated the suppressed effect of miR-1897 (Figure 5C). Next, we validated whether MIRG can regulate

NFATc1 expression via competition for miR-1897 binding. The results showed that MIRG could significantly increase NFATc1 expression; however, mutagenesis of the miR-1897 recognition element in MIRG abrogated the function effectively (Figure 5D). Conversely, inhibition of miR-1897 overcame the suppression of NFATc1 by MIRG knockdown (Figure 5E). Taken together, these data indicate that by interacting with miR-1897, MIRG acts as a molecular sponge for the miR-1897 target NFATc1, to partly modulate the inhibitory effect of miR-1897 on NFATc1.

Discussion

Osteoclastogenesis is a highly regulated process, which is important for bone health[29,30]. The expression of genes of osteoclasts undergoes great changes during osteoclastogenesis, resulting in differences in cells shape and function[29,31-33]. Therefore, the intervention and regulation of several osteoclast-associated genes during osteoclastogenesis is of great significance for regulating osteoclastogenesis[34-36].

As a "bridge" between DNA and protein, the complicated regulatory role of RNA has long been neglected. In eukaryotic cells, protein-coding RNA (mRNA) only accounts for about 2% of the genome, the rest large number of transcripts were categorized to non-protein coding RNAs (ncRNAs). Unlike ribosomal RNA (rRNA) and transfer RNA (tRNA), which have been well acknowledged, other noncoding RNAs (ncRNAs) were thought to be transcriptional "noises" once upon a time. However, more and more researches have revealed that ncRNAs play a pivotal role in the cellular process[37,38]. In the past decade, miRNAs (20–24 nt) were most intensively studied among all the ncRNAs. These small miRNAs bind to the complementary site on the 3′ untranslated region (UTR) of targeting mRNAs called miRNA binding

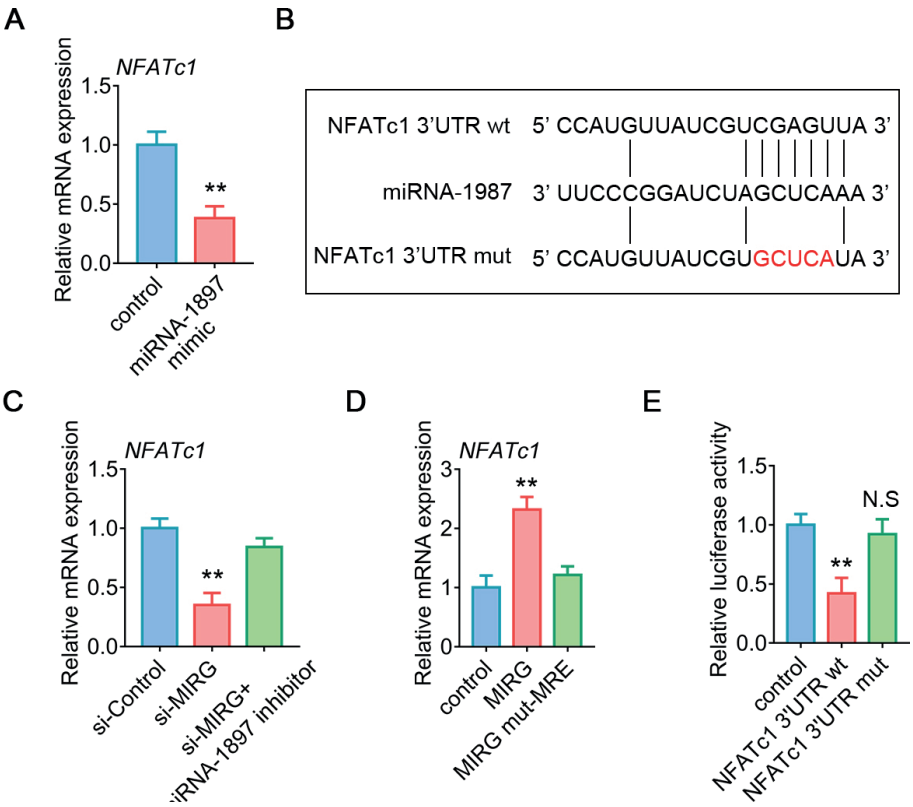

Figure 5. MIRG acts as a molecular sponge for miR-1897 and controls the miR-1897 target, NFATc1. (**A**) qRT-PCR analysis of NFATc1 mRNA expression level in BMMs treated with the miR-1897 mimics. (**B**) Schematic illustration of the predicted NFATc1 binding sites and mutant sites in miR-1897. (**C**) mRNA expression levels of NFATc1 in BMMs transfected with si-MIRG, si-MIRG and miR-1897 inhibitor by qRT- PCR. (**D**) mRNA expression levels of NFATc1 in BMMs transfected with MIRG and MIRG mut-MRE. (**E**) Relative luciferase activity of BMMs. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

elements (MREs) and block protein translation or modulate mRNA stability on a post-transcriptional level[39]. However, different from miRNAs, the role of lncRNAs is barely studied. LncRNAs have transcripts longer than 200 nucleotides. Due to its length, lncRNAs can fold into secondary or higher orders of structure, thus making it more flexible in targeting proteins or gene sites[40]. Moreover, because of the differential splicing and alternative transcription initiation sites, lncRNAs is becoming more and more complex[41].

There is bare of research about ncRNAs in the skeletal system. Pioneering studies were performed on the expression profile of lncRNAs during chondrogenic differentiation and osteogenic differentiation. Furthermore, research was done on the relationship between lncRNAs and bone diseases such as osteoarthritis, osteoporosis and bone tumors. More and more studies have revealed that lncRNAs is tissue-specific and differentially expressed across diverse stages during cell differentiation[41,42]. Moreover, recent research from Zhu et al[43] have found out that lncRNAs play a vital role in regulating bone development and remodeling processes.

Conclusions

In this study, we first verified the high expression of MIRG in osteoclasts and found its important role in osteoclastogenesis, a bone resorption function. Through target prediction, we found miR-1897 as a target miRNA of MIRG and validate the combination relationship of MIRG and miR-1897 using luciferase reporter assay. Next, we observed that miR-1897 can bind with MIRG co-expression gene NFATc1 and downregulate the expression of NFATc1. MIRG could significantly upregulate NFATc1 gene expression; however, mutagenesis of the miR-1897 recognition element in MIRG abrogated the function effectively. Therefore, we proved that MIRG served as ceRNA of miR-1897 to upregulate NFATc1 expression.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Grabowski P. Physiology of bone. Endocr Dev 2009; 16: 32-48.
2) Xiao W, Li S, Pacios S, Wang Y, Graves DT. Bone remodeling under pathological conditions. Front Oral Biol 2016; 18: 17-27.
3) Kylmaoja E, Nakamura M, Tuukkanen J. Osteoclasts and remodeling based bone formation. Curr Stem Cell Res Ther 2016; 11: 626-633.
4) Hadjidakis DJ, Androulakis II. Bone remodeling. Ann N Y Acad Sci 2006; 1092: 385-396.
5) Jin Z, Li X, Wan Y. Minireview: nuclear receptor regulation of osteoclast and bone remodeling. Mol Endocrinol 2015; 29: 172-186.
6) Sakaguchi Y, Nishikawa K, Seno S, Matsuda H, Takayanagi H, Ishii M. Roles of enhancer RNAs in RANKL-induced osteoclast differentiation identified by genome-wide cap-analysis of gene expression using CRISPR/Cas9. Sci Rep 2018; 8: 7504.
7) Abe T, Sumi K, Kunimatsu R, Oki N, Tsuka Y, Nakajima K, Ando K, Tanimoto K. The effect of mesenchymal stem cells on osteoclast precursor cell differentiation. J Oral Sci 2019; 61: 30-35.
8) Obaid R, Wani SE, Azfer A, Hurd T, Jones R, Cohen P, Ralston SH, Albagha OME. Optineurin negatively regulates osteoclast differentiation by modulating NF-kappaB and interferon signaling: implications for Paget's disease. Cell Rep 2015; 13: 1096-1102.
9) Sun Q, Zhang B, Zhu W, Wei W, Ma J, Tay FR. A potential therapeutic target for regulating osteoporosis via suppression of osteoclast differentiation. J Dent 2019; 82: 91-97.
10) Sánchez Lázaro JA, Linares Álvarez L. [Pycnodysostosis: a rare disease with frequent fractures]. Semergen 2014; 40: e47-50.
11) Zhang Y, Tao Y, Liao Q, Long noncoding RNA: a crosslink in biological regulatory network. Brief Bioinform 2018; 19: 930-945.
12) Hu G, Niu F, Humburg BA, Liao K, Bendi S, Callen S, Fox HS, Buch S. Molecular mechanisms of long noncoding RNAs and their role in disease pathogenesis. Oncotarget 2018; 9: 18648-18663.
13) Rafiee A, Riazi-Rad F, Havaskary M, Nuri F. Long noncoding RNAs: regulation, function and cancer. Biotechnol Genet Eng Rev 2018; 34: 153-180.
14) Wu QY, Li X, Miao ZN, Ye JX, Wang B, Zhang F, Xu RS, Jiang DL, Zhao MD, Yuan FL. Long non-coding RNAs: a new regulatory code for osteoporosis. Front Endocrinol (Lausanne) 2018; 9: 587.
15) Grote P, Wittler L, Hendrix D, Koch F, Währisch S, Beisaw A, Macura K, Bläss G, Kellis M, Werber M, Herrmann BG. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell 2013; 24: 206-214.
16) Quan H, Liang M, Li N, Dou C, Liu C, Bai Y, Luo W, Li J, Kang F, Cao Z, Yang X, Jiang H, Dong S. LncRNA-AK131850 sponges MiR-93-5p in newborn and mature osteoclasts to enhance the secretion of vascular endothelial growth factor a promoting vasculogenesis of endothelial progenitor cells. Cell Physiol Biochem 2018; 46: 401-417.

17) Puppo M, Taipaleenmäki H, Hesse E, Clézardin P. Non-coding RNAs in bone remodelling and bone metastasis: mechanisms of action and translational relevance. Br J Pharmacol 2019. doi: 10.1111/bph.14836. [Epub ahead of print]

18) Morriss GR, Cooper TA. Protein sequestration as a normal function of long noncoding RNAs and a pathogenic mechanism of RNAs containing nucleotide repeat expansions. Hum Genet 2017; 136: 1247-1263.

19) Jiang W, Zhan H, Jiao Y, Li S, Gao W. A novel lncRNA-miRNA-mRNA network analysis identified the hub lncRNA RP11-159F24.1 in the pathogenesis of papillary thyroid cancer. Cancer Med 2018; 7: 6290-6298.

20) Liu C, Cao Z, Bai Y, Dou C, Gong X, Liang M, Dong R, Quan H, Li J, Dai J, Kang F, Zhao C, Dong S. LncRNA AK077216 promotes RANKL-induced osteoclastogenesis and bone resorption via NFATc1 by inhibition of NIP45. J Cell Physiol 2019; 234: 1606-1617.

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

22) Yuan Y, Zhang L, Tong X, Zhang M, Zhao Y, Guo J, Lei L, Chen X, Tickner J, Xu J, Zou J. Mechanical stress regulates bone metabolism through microRNAs. J Cell Physiol 2017; 232: 1239-1245.

23) Zhang ZK, Li J, Guan D, Liang C, Zhuo Z, Liu J, Lu A, Zhang G, Zhang BT. A newly identified lncRNA MAR1 acts as a miR-487b sponge to promote skeletal muscle differentiation and regeneration. J Cachexia Sarcopenia Muscle 2018; 9: 613-626.

24) Han DX, Sun XL, Wang CJ, Yu ZW, Zheng Y, Huang YJ, Wang WH, Jiang H, Gao Y, Yuan B, Zhang JB. Differentially expressed lncRNA-m433s1 regulates FSH secretion by functioning as a miRNA sponge in male rat anterior pituitary cells. Biol Reprod 2019; 101: 416-425.

25) Liu H, Sun HL. LncRNA TCF7 triggered endoplasmic reticulum stress through a sponge action with miR-200c in patients with diabetic nephropathy. Eur Rev Med Pharmacol Sci 2019; 23: 5912-5922.

26) Liang L, Xu J, Wang M, Xu G, Zhang N, Wang G, Zhao Y. Correction: LncRNA HCP5A promotes follicular thyroid carcinoma progression via miRNAs sponge. Cell Death Dis 2019; 10: 639.

27) Ekita Y, Wagatsuma H, Irie M, Kobayashi S, Kohda T, Matsuda J, Yokoyama M, Ogura A, Schuster-Gossler K, Gossler A, Ishino F, Kaneko-Ishino T. Aberrant regulation of imprinted gene expression in Gtl-2lacZ mice. Cytogenet Genome Res 2006; 113: 223-229.

28) Dou C, Cao Z, Yang B, Ding N, Hou T, Luo F, Kang F, Li J, Yang X, Jiang H, Xiang J, Quan H, Xu J, Dong S. Changing expression profiles of lncRNAs, mRNAs, circRNAs and miRNAs during osteoclastogenesis. Sci Rep 2016; 6: 21499.

29) Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423: 337-342.

30) Charles JF, Aliprantis AO. Osteoclasts: more than 'bone eaters'. Trends Mol Med 2014; 20: 449-459.

31) Cappariello A, Maurizi A, Veeriah V, Teti A. The great beauty of the osteoclast. Arch Biochem Biophys 2014; 558: 70-78.

32) Boyce BF. Advances in the regulation of osteoclasts and osteoclast functions. J Dent Res 2013; 92: 860-867.

33) Tanaka S. RANKL-independent osteoclastogenesis: a long-standing controversy. J Bone Miner Res 2017; 32: 431-433.

34) Collison J. Bone: DJ-1 orchestrates osteoclastogenesis. Nat Rev Rheumatol 2018; 14: 2-3.

35) Miyamoto T. Regulators of osteoclast differentiation and cell-cell fusion. Keio J Med 2011; 60: 101-105.

36) Yavropoulou MP, Yovos JG. Osteoclastogenesis-current knowledge and future perspectives. J Musculoskelet Neuronal Interact 2008; 8: 204-216.

37) Costa FF. Non-coding RNAs: meet thy masters. Bioessays 2010; 32: 599-608.

38) Knowling S, Morris KV. Non-coding RNA and antisense RNA. Nature's trash or treasure? Biochimie 2011; 93: 1922-1927.

39) Ghildiyal M, Zamore PD. Small silencing RNAs: an expanding universe. Nat Rev Genet 2009; 10: 94-108.

40) Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012; 482: 339-46.

41) Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.

42) Chen L, Zhang S. Long noncoding RNAs in cell differentiation and pluripotency. Cell Tissue Res 2016; 366: 509-521.

43) Zhu J, Wang Y, Yu W, Xia K, Huang Y, Wang J, Liu B, Tao H, Liang C, Li F. Long noncoding RNA: function and mechanism on differentiation of mesenchymal stem cells and embryonic stem cells. Curr Stem Cell Res Ther 2019; 14: 259-267.