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RANKL inhibits cell proliferation by regulating MALAT1 expression in a human osteoblastic cell line hFOB 1.19

W. Che[1], Y. Dong[2] and H-B. Quan[3]

[1] Department of Orthopedics Surgery, Zhongshan Hospital, Fudan University, Shanghai, 200032, PR China.
[2] Department of Neurology and Institute of Neurology, Shanghai Medical College, Fudan University, Shanghai, 200032, China.
[3] Department of Internal Medicine, Zhongshan Hospital, Fudan University, Shanghai, 200032, PR China

Corresponding author: Hong-Bo Quan, Department of Internal Medicine, Zhongshan Hospital, Fudan University, Shanghai, 200032, PR China. Tel: +86-013817394612, Email: QUANHONGBO2004@hotmail.com

Abstract

Receptor activator of NF-κB ligand (RANKL), a TNF-related protein, is a key factor regulating bone metabolism. It has been well known that RANKL-mediated signaling regulates the formation, activation and survival of osteoclast in normal bone modeling and remodeling, and also plays an important role in a variety of pathologic conditions. However, there is no direct evidence about the effect of RANKL on osteoblast. Herein, we investigated whether RANKL had effect on cell proliferation in a normal human fetal osteoblastic cell line hFOB 1.19. MTT assay showed that RANKL inhibited hFOB 1.19 cells growth in a dose-dependent and time-dependent manner. Importantly, we found that RANKL induced the expression of a lncRNA, *MALAT1,* for the first time. Knockdown of RANK by siRNA blocked the induction of *MALAT1* by RANKL. By infection with *MALAT1* siRNA, *MALAT1* knockdown reversed RANKL-induced cells growth inhibition and cell cycle arrest. In addition, *MALAT1* also regulated OPG expression in hFOB 1.19 cells. In conclusion, RANKL, binding to its receptor RANK, inhibited cell proliferation via *MALAT1* upregulation in osteoblast cells *in vitro*.

Key words: RANKL, osteoblast, proliferation, lnc *MALAT1,* OPG.

Introduction

Bone metabolic homeostasis mainly depends on the balance between osteoblastic bone formation and osteoclast bone resorption. Osteoblast lineage cells regulate the number and activity of osteoclasts through expression of receptor activator for nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) (1, 2). RANKL binds to its receptor, RANK, on the surface of osteoclasts, stimulating the formation and activation of osteoclasts and the subsequent induction of bone resorption (1, 3, 4). OPG acts as a soluble receptor antagonist which neutralizes RANKL, and thus blocks RANKL-RANK interaction. The severe osteoporosis and significant increasing osteoclasts in transgenic mice overexpressing RANKL (5) demonstrates the fundamental role of RANKL in bone metabolism. It also has been reported that inhibiting RANKL actions resulted in a remarkable reduced rate of bone resorption both clinically and in mouse models (6, 7).

RANKL is produced by osteoblastic lineage cells in membrane-bound form and expressed by activated T cells in soluble form. In addition, a truncated ectodomain form of RANKL is derived from the membrane-bound form after cleavage by the enzyme TACE. All three RANKL variants can bind to their specific receptor, RANK, which is located on osteoclastic and dendritic cells (8). Moreover, studies have demonstrated that soluble RANKL might have less potent activity in osteoclastogenesis induction than membrane-bound RANKL *in vitro* (3, 9). In rat models of inflammatory arthritis *in vivo*, increased soluble RANKL levels is associated with bone loss, indicating the important role of soluble RANKL in bone disease (10). In addition, mice injected with RANKL increased serum calcium levels in a short period of time (< 1 day), suggesting that the RANKL activates mature osteoclasts, which accelerates the resorption of bone (11). There have been lots of researches between RANKL and osteoclasts, however, to the precise role of RANKL (soluble or membrane-bound) in osteoblast has not been properly and fully elucidated to date.

Mammalian cells produces a large number of noncoding RNAs, including small and long noncoding RNAs (lncRNAs) (12-14). Except for the class of well-characterized small ncRNAs, such as microRNAs, lncRNAs are large intervening non-coding RNAs, and have recently emerged as important molecules in diverse cellular processes such as proliferation, cell-cycle progression, apoptosis, or cell growth (15). *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1) lncRNA, also referred to as *NEAT2* (nuclear-enriched abundant transcript 2), is highly abundant in many human cell types and highly conserved over its full length (8 kb) across mammalian species (16). *MALAT1* alternatively splices a subset of pre-mRNAs by modulating serine/arginine splicing factor activity in tissue-type- or cell-type-specific manner (17, 18). Previous study revealed that *MALAT1* depletion results in arrest at G1 phase and leads to activation of p53 and its target genes in human fibroblasts (19). In contrast, it also showed that transient overexpression of *MALAT1* promoted cellular proliferation and tumor formation in nude mice, while depletion of *MALAT1* decreased tumorigenicity in tumor cells (20, 21). However, the role of *MALAT1* or other lnc RNAs in bone metabolism is never been

reported before. Meanwhile, it remains to be elucidated whether the ubiquitously expressed *MALAT1* has one universal function or whether its mechanisms of action might be tissue-specifically different.

In the present study, we examined the role of RANKL in osteoclasts. We demonstrated that in a human fetal osteoblastic cell line hFOB 1.19, soluble RANKL treatment inhibited hFOB 1.19 cells growth in a does-dependent and time-dependent manner. Furthermore, RANKL induced the expression of *MALAT1* and knockdown of RANK blocked the induction of *MALAT1* by RANKL in hFOB 1.19 cells. *MALAT1* knockdown reversed RANKL-induced cells growth inhibition and cell cycle arrest Finally, we established that the proliferation inhibitory effect of RANKL is accomplished by its involvement in up-regulating the expression of *MALAT1,* accompanied by upregulation of OPG.

Materials and methods

Cell culture and reagents

The human fetal osteoblastic cell line hFOB 1.19 obtained from American Type Culture Collection (ATCC), was cultured in a 1:1 mixture of Ham's F12 Medium Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA) without phenol red, supplemented with 10% fetal bovine serum (FBS) (HyClone, Thermo, Fremont, USA). Growth medium was further supplemented with 50 μg/mL penicillin and 50 mg/L gentamicin (Sigma, St. Louis, MO, USA), and was adjusted to pH 7.2 under the 5% CO_2 atmosphere at 37°C. All hFOB cells cultures were maintained at at 37°C in humidified atmosphere of 5% CO_2.

Propidium iodide (PI) were obtained from Sigma (St. Louis, MO, USA). Primary monoclonal antibodies for OPG, RANK and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The HRP-conjugated anti-rabbit antibody was purchased from Abcam Inc. (Cambridge, MA, USA).. Soluble RANKL was obtained from Millipore (Billerica, MA).

RNA extraction and Quantitative real-time PCR (qRT-PCR)

Total RNAs of hFOB 1.19 cells were extracted respectively using the Trizol solution (Invitrogen, Carlsbad, CA) and reversely transcribed using the PrimeScript™ RT Reagent Kit (Perfect Real Time) (TaKaRa Biotechnology). RANK mRNA and *MALAT1* mRNA expressions were evaluated by real-time PCR using the SYBR Premix Ex TaqTM Perfect Real Time (TaKaRa, Shiga, Japan) on an ABIPRISM 7500 Real-Time System. GAPDH was applied as the input reference. The primers used are as follows:

RANK: Forward, 5'- CGTTGCAGCTCAACAAGGAC-3' Reverse, 5'- TCCGTGGAGGAAAAGGCATC -3'

MALAT1 : Forward, 5'- GGATCCTAGACCAGCATGCC -3' Reverse, 5'- AAAGGTTACCATAAGTAAGTTCCAGAAAA -3'

GAPDH: Forward, 5'- GTGGACATCCGCAAAGAC -3' Reverse, 5'- AAAGGGTGTAACGCAACTA -3'

Relative mRNA was calculated by using the formula $2^{-\Delta CT}$(CT; cycle threshold) which was described previously (22).

RNA Interference Targeting RANK and lnc MALAT1

The RANK siRNA and *MALAT1* siRNA sequences were designed by a commercial software (Applied Biosystems/Ambion, Austin, TX). For RANK, the siRNA sense sequence is 5'-GCGCAGACUUCACUCCAUAUU-3', and the anti-sense sequence is 5'-UAUGGAGUGAAGUCUGCGCUU-3'. The sequence of the *MALAT-1* siRNA corresponded to nt 5765 to 5783 of *MALAT-1* (5'-GCAGAGGCAUUUCAUCCUU-3'). The scamble control siRNA sense sequence is 5'-UUCUCCGAACGUGUCACGUdTdT-3', and the antisense sequence is 5'-ACGUGACACGUUCGGAGAAdTdT-3'. The siRNAs were synthesized and sequenced by Thermo Scientific (Waltham, MA).

The hFOB 1.19 cells (2×10^5 cells per well) were plated in 6-well plates and cultured overnight. 200 pmol siRNA and 5 μL Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) were diluted in OPTI-MEM (Invitrogen, Carlsbad, CA) to a total volume of 250 μL. The diluted siRNA and Lipofectamine™2000 were mixed and incubated at room temperature for 20 minutes. The cells were firstly washed with serum-free DMEM medium, and then the diluted siRNA mix was added to the 6-well plates for 5 hours. After then, the siRNA and Lipofectamine™2000 mix was replaced with growth medium to further incubated for 48-72 hours.

Western blot

The hFOB 1.19 cells were extracted with lysis buffer (150 mMNaCl, 1% NP-40, 0.1% SDS, 2 μg/mL aprotinin, 1 mM PMSF) for 30 min at 4°C. Extracts were then centrifuged at 12,000 g for 20 min at 4°C and the supernatant was harvested. The total protein concentration was determined by 280nm OD on NanoDrop 2000 (Thermo Scientific, MA, USA). Protein lysates (50 μg/mL) were electrophoresed on 10% SDS-PAGE gels, transferred to PVDF membranes, and blotted with Primary monoclonal OPG, RANK or β-actin antibodiesin Tris-buffered saline containing 0.1% Tween-20 (TBST) and 5% non-fat dry milk overnight at 4°C. After the overnight incubation, membranes were followed by HRP-conjugated anti-rabbit secondary antibody and detection by chemiluminescence ECL.

MTT assay

The hFOB 1.19 cell proliferation was determined using a colorimetric assay with 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, hFOB cells or hFOB cells interfered with siRNAs were seeded onto 96-well plates (5,000 cells/well) for 24 hours and the medium was then replaced with 10% growth medium containing 0.2% DMSO or RANKL at specific concentrations for 24, 48, 72 or 96 hours. After treatment, 10 μL of MTT reagent (5 mg/mL in PBS) was added to the cells and incubated for 4 hours at 37°C. The medium was removed and 200 μL of isopropanol were added. The amount of formazan crystals formed correlates directly with the number of viable cells. The reaction product was quantified by measuring the absorbance at 595 nm using an ELISA plate reader. Experiments were repeated three times.

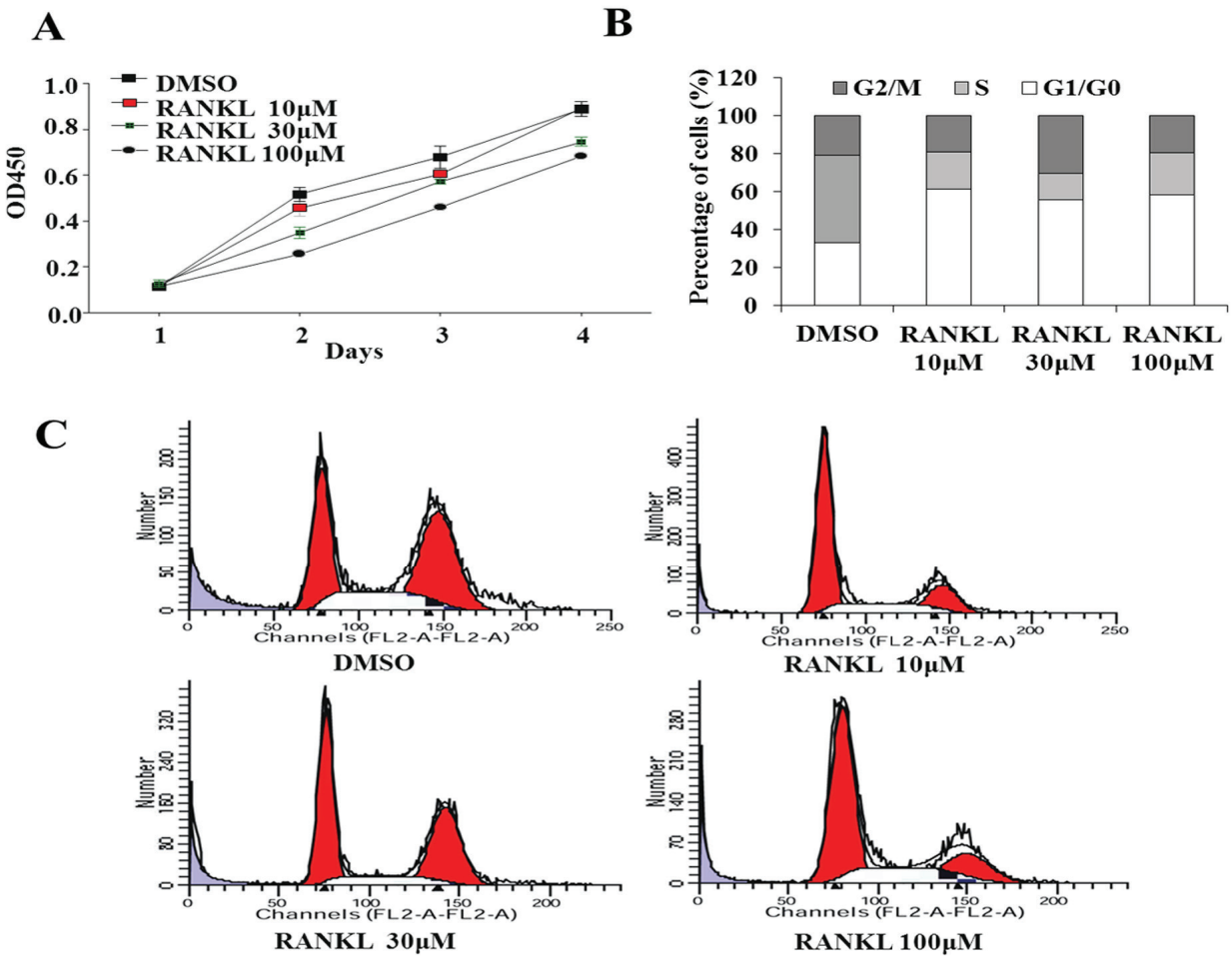

Figure 1. RANKL inhibited hFOB 1.19 cells growth. (A) The cells were treated with RANKL at various concentrations or DMSO for 1, 2, 3, or 4 days. After treatment, cell proliferation was estimated using MTT assay. **(B, C)** The cells were treated with RANKL at various concentrations or DMSO for 2 days, cell cycle distribution was estimated using flow cytometry as described in Material and methods.

Cell cycle analysis

After treatment with 100 nM, hFOB cells or hFOB cells interfered with siRNAs were harvested and washed twice with ice-cold PBS, and fixed in 70% ethanol at 4˚C overnight. The fixed cells were washed twice with cold PBS and then incubated for 30 min with RNase at 37°C. PI (50 mg/mL) (Sigma-Aldrich) was added directly to the cell suspension. The suspension was filtered through a 50-mm nylon mesh, and total of 10000 events per sample were analyzed by a flow cytometer (FACS Cali-bur, BD Biosciences).

Statistical analysis

Data were analyzed using GraphPad Prism software version 6.00 for Windows (GraphPad Prism Software, San Diego, CA, USA). Average values were expressed as mean ± SD. Statistical significance between different groups was determined by repeated-measures ANOVA test. A p value<0.05 was accepted as statistically significant.

Results

RANKL inhibited hFOB 1.19 cells growth

To investigate the role of RANKL at various concentrations on the proliferation of human osteoblasts, MTT assay was performed on the hFOB cells. As a conditionally immortalized human fetal osteoblastic cell line, hFOB 1.19 was established as a homogeneous, rapidly proliferating model system (23). As shown in Fig.1A, we monitored cell numbers at each time point (1, 2, 3, and 4 days) in cell viability assay, RANKL does-dependently inhibited the growth of hFOB 1.19 cells. At 100 μM concentration, RANKL significantly inhibited hFOB 1.19 cells proliferation.

To investigate whether the inhibitory effect of RANKL on osteoblast proliferation is, in part, related to cell cycle distribution, cell cycle analysis with PI staining was performed using flow cytometry. After treatment for 24 hr, RANKL (10-100 μM) significantly increased the percentages of cells in G0/G1 phase, compared with un-treatment hFOB 1.19 cells (Fig.1B). The results implicated that the inhibitory effect of RANKL on hFOB 1.19 cells proliferation was mediated by G1/S cell cycle arrest.

RANKL induced the expression of MALAT1 in hFOB 1.19 cells

Previous studies have demonstrated that the lncRNA *MALAT1* was involved in cellular proliferation and played a crucial determinant of cell cycle progression (19, 20, 24). In order to determine whether RANKL induces *MALAT1* expression in hFOB 1.19 cells, we firstly incubated the hFOB 1.19 cells with varying doses of RANKL (10, 30, and 100 μM) and then real-time PCR assays were performed. We found that RANKL dramatically induced the expression of *MALAT1* in a does-dependent manner, compared with DMSO-treatment

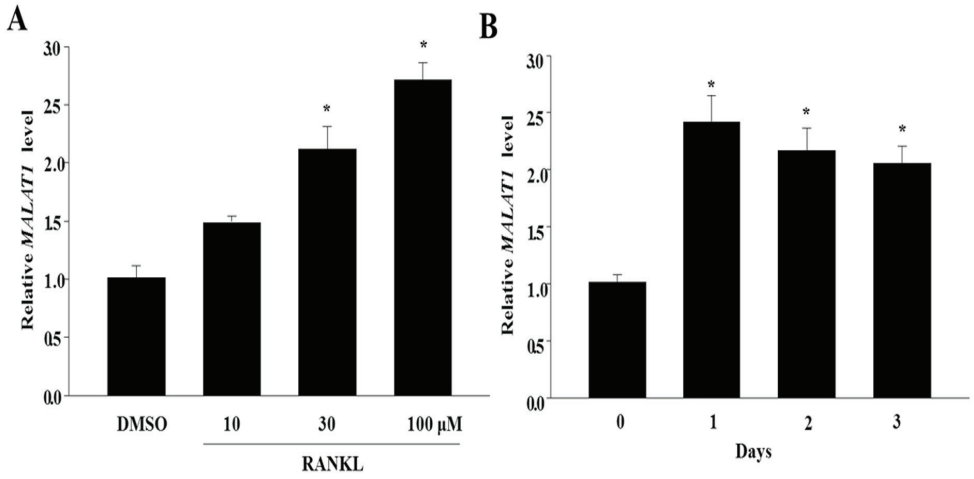

Figure 2. RANKL induced the expression of *MALAT1* in hFOB 1.19 cells. (A) The cells were treated with RANKL at various concentrations or DMSO for 2 days. After treatment, the expression of *MALAT1* was determined by real-time PCR. **(B)** The cells were treated with RANKL at the concentration of 100 nM or DMSO for 1, 2, or 3 days. After treatment, the expression of *MALAT1* was determined by real-time PCR.

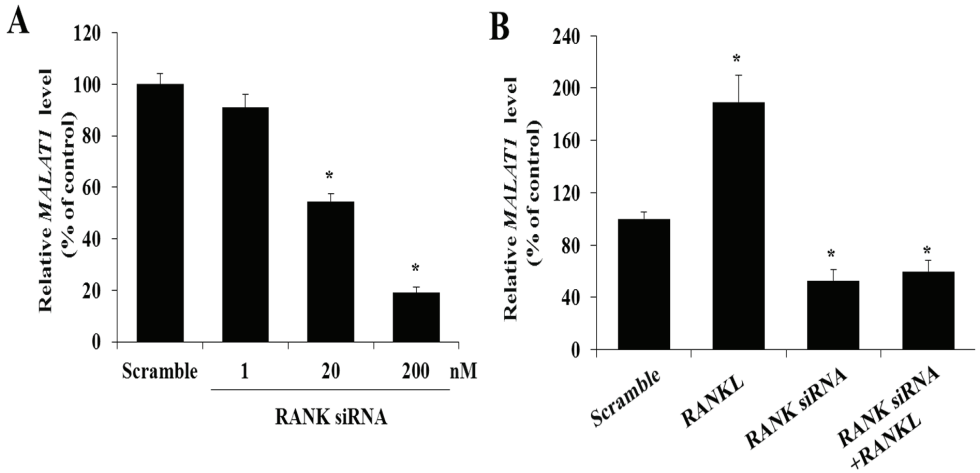

Figure 3. Knockdown of RANK blocked the induction of *MALAT1* by RANKL in hFOB 1.19 cells.(A) *MALAT1* level was measured after RANK siRNA treatment. RANK siRNA treatment decreased *MALAT1* level at 20 and 200 nM in comparison to scramble control siRNA. **(B)** *MALAT1* levels by RANK siRNA or scramble control siRNA interference with or without RANKL in culture of hFOB 1.19 cells cells. * indicate statistical difference of $P<0.05$.

control cells. At the maximum dose of RANKL (100 μM) used in this study, about 270% induction ($P<0.05$) was observed (Fig.2A). Similarly, at lower doses (30 μM), RANKL also resulted in a significant induction of *MALAT1* (Fig.2A).

In addition, we further extended the does-response studies to include time-course experiments. We analyzed *MALAT1* levels in hFOB 1.19 cells treated with 100 μM RANKL for desired times (1-3 days) by real-time PCR. As shown in Figure.2B, RANKL also significantly increased *MALAT1* mRNA level in a time-dependent manners. Collectively, these results suggested that RANKL induced the expression of *MALAT1* in osteoblastic cells.

Knockdown of RANK blocked the induction of MALAT1 by RANKL in hFOB 1.19 cells

To further conform the mechanisms through which RANKL mediated *MALAT1* upreuglation, the role of RANK was investigated in hFOB 1.19 cells. To identify the optimal concentration of RANK siRNA used to knockdown RANK expression**,** hFOB 1.19 cells were transfected with scramble siRNA or RANK siRNA varying concentrations of RANK siRNAs ranging from 1 to 200 nM for 24 hr. As shown in Figure.3A, RANK siRNA probes does-dependently reduced the mRNA levels of RANK gene in hFOB 1.19 cells. At the maximum dose of 200 nM, RANK siRNA probes significantly inhibited RANK mRNA levels more than 80% compared to the control scramble siRNA.

Furthermore, we examined whether RANKL induced the expression of *MALAT1* through RANK in hFOB 1.19 cells, we knocked down RANK by siRNA and subsequently measured *MALAT1* levels by real-time PCR assays. As shown in Figure.3B, RANKL alone significantly increased the expression of *MALAT1*, however knockdown of RANK by siRNA potently blocked the ability of RANKL to induce the expression of *MALAT1* in hFOB 1.19 cells.In addition, knockdown of RANK by siRNA with RANKL added in culture also resulted in a significant decrease of *MALAT1* in hFOB 1.19 cells, compared with scramble cells. . All these results indicated that RANKL induced the expression of *MALAT1* depending on RANK in osteoblastic cells.

MALAT1 knockdown reversed RANKL-induced cells growth inhibition and cell cycle arrest in hFOB 1.19 cells

To explore the effects of *MALAT1* knockdown on cells growth, cells were transfected with *MALAT1*

siRNA or treated with RANKL, and subsequently assessed for MTT assay. As shown in Figure.4A, *MALAT1* knockdown by siRNA alone significantly augmented hFOB 1.19 cells proliferation, as compared with DMSO-treated cells. In contrast, RANKL treatment significantly inhibited hFOB 1.19 cells proliferation, while *MALAT1* knockdown by siRNA potently reversed the inhibitory effect of RANKL.

To further investigate the effect of *MALAT1* knockdown on osteoblastic cell growth in more detail, we analyzed the effects of *MALAT1* knockdown on the cell cycle distribution of hFOB 1.19 cells. Cells were transfected with *MALAT1* siRNA or treated with RANKL, and then cell cycle distribution was monitored by flow cytometry analysis. As seen in Figure.4B, in comparison with DMSO-treated control cells, *MALAT1* knockdown by siRNA alone induced an accumulation of cells in the G2/M phase fractions. RANKL treatment alone significantly increased the fraction of cells in G0/G1 phase and decreased the fraction of cells in G2/M phase of the cell cycle while *MALAT1* knockdown before RANKL treatment significantly reduced the proportion in the G0/G1 phase and restored proportion in the G2/M phase of the cell cycle. Together, our findings demonstrated that RANK inhibited osteoblastic cells growth through upregulation of *MALAT1*.

MALAT1 regulated OPG expression in hFOB 1.19 cells

As a decoy receptor for RANKL, OPG plays crucial role in bone metabolism as well as immune system and arterial calcification (8). To investigate whether *MALAT1* regulated OPG expression in hFOB 1.19 cells, cells were transfected with *MALAT1* siRNA or together treated with RANKL, and the protein levels of OPG were assessed using western blot. As revealed in Figure.5, *MALAT1* knockdown significantly downregulated the expression of OPG in protein levels, but had no effect on RANK expression. Taken together, our data indicated that *MALAT1* regulated OPG expression in osteoblastic cells.

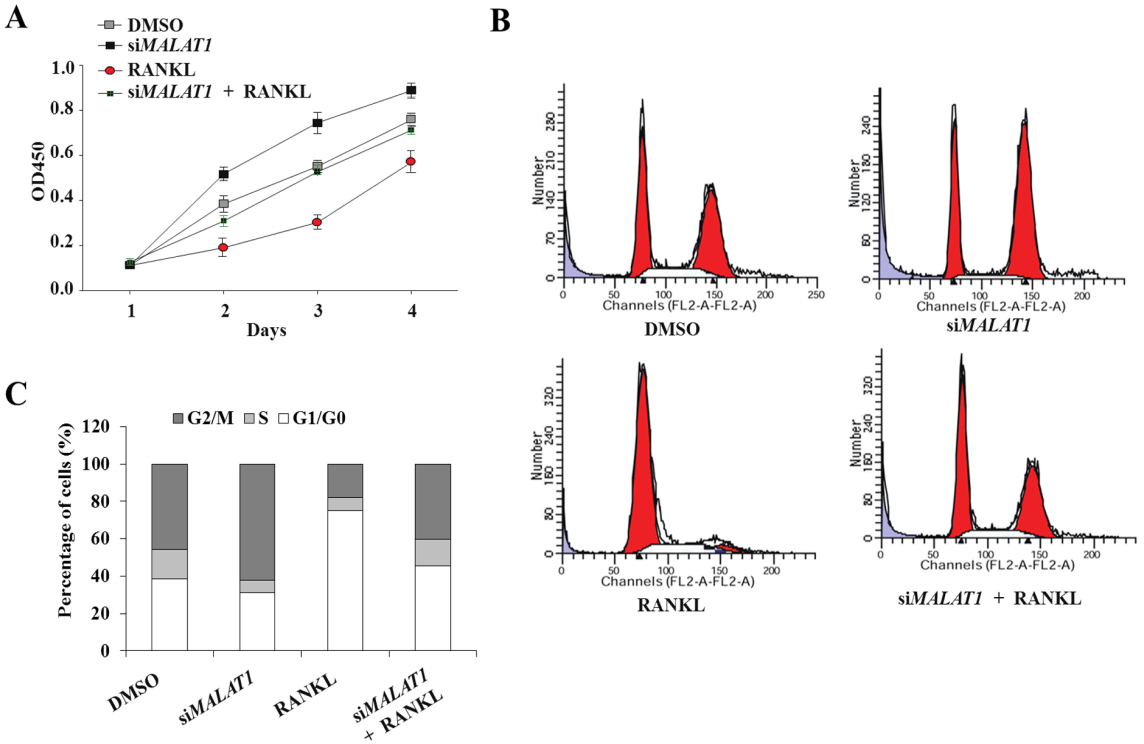

Figure 4. ***MALAT1*** **knockdown reversed RANKL-induced cells growth inhibition and cell cycle arrest in hFOB 1.19 cells. (A)** hFOB 1.19 cells were transfected by *MALAT1* siRNA or scramble control siRNA as control. After siRNA transfection for 48 hours, hFOB 1.19 cells were treated with RNKL (100nM) or DMSO for 1, 2, 3, or 4 days, and cell proliferation was estimated using MTT assay. **(B, C)** hFOB 1.19 cells were transfected by *MALAT1* siRNA or scramble control siRNA as control. After siRNA transfection for 48 hours, hFOB 1.19 cells were treated with RNKL (100 nM) or DMSO for 2 days, cell cycle distribution was estimated using flow cytometry.

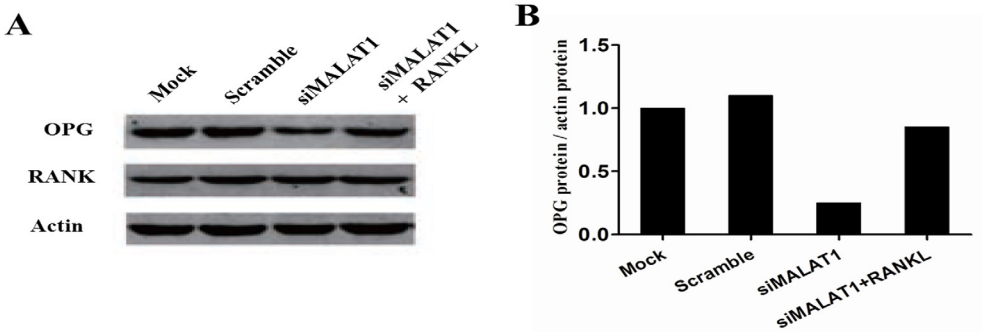

Figure 5. ***MALAT1*** **regulated OPG expression in hFOB 1.19 cells.** (A) hFOB 1.19 cells were transfected by *MALAT1* siRNA or scramble control siRNA as control. After siRNA transfection for 48 hours, hFOB 1.19 cells were further treated with RNKL (100nM) or DMSO for 2 days, OPG and RANK protein from above cells was detected by Western blotting. (B) OPG/β-actin were calculated for each sample.

Discussion

The multiple functions of bone in vertebrates includes protection of vital organs and hematopoietic marrow, structural support for muscles, and storage and release of vital ions, such as calcium. Normal bone homeostasis is mainly dependent on balancing the number and activity of bone forming osteoblasts and bone resorbing osteoclasts (25). RANKL is a homotrimeric protein, which is typically membrane-bound on osteoblastic and activated T cells or is secreted by activated T cells (25). RANKL is well-known to stimulate the formation and activation of osteoclasts via binding to its specific receptor, RANK. Previous study demonstrated that RANKL-deficient mice exhibit severe osteopetrosis and tooth eruption failure as a result of a complete absence of osteoclasts (26). In addition to the regulation of normal bone remodeling, it has also been reported that RANKL played vital roles in regulating the proliferation of mammary epithelial cells and cancer-induced bone disease (27, 28). However, the exact effect of RANKL on osteoblastic cells remains unclear to date. In this study, we investigated the effects of RANKL on a human fetal osteoblastic cell line hFOB 1.19 *in vitro*. We treated hFOB 1.19 cells with a variety concentration of soluble RANKL, the cell growth was inhibited in a does-dependent manner (Fig.1A). To further investigate the changes of cell cycle distribution, cell cycle analysis with PI staining was performed using flow cytometry and the results showed that RANKL (10-100 μM) significantly increased the percentages of cells in G0/G1 phase, while simultaneously reducing the proportion in the G2/M phase(Fig.1B and Fig.1C). These results suggested that RANKL has an inhibitory effect on hFOB 1.19 cells growth. Recently, studies have shown that mammalian cells produces a large number of lncRNAs, which plays an important role in diverse cellular processes such as proliferation, cell-cycle progression, apoptosis, or cell growth (12-15). Moreover, lncRNAs exhibit temporal and spatial expression patterns or their expression is tissue-specific, cell-specific, or restricted to particular cell cycle stages (29-31). The roles of lnc RNAs in bone metabolism, however, are still unknown since now. *MALAT1* (also known as NEAT2, HCN, PRO2853 and NCRNA00 047), located at chromosome 11q13.1, is highly conserved over its full length (8 kb) across mammalian species (16). Previous studies showed that *MALAT1* is overexpressed in metastasizing non-small-cell lung cancer (32), endometrial stromal sarcoma (33) and hepatocellular carcinoma, as well as widely expressed in different normal human tissues (34). In our study , we found that *MALAT1* was potently upregulated by RNAKL in hFOB 1.19 cells for the first time. Using real-time PCR assays, RNAKL treatment induces the expression of *MALAT1* in a does-dependent and time-dependent manner in hFOB 1.19 cells (Fig. 2). Furthermore, using RANK siRNA interference, we observed that knockdown of RANK blocked the induction of *MALAT1* by RANKL in hFOB 1.19 cells (Fig. 3), indicating that RNAKL induced the expression of *MALAT1* through its receptor RNAK. Recent study suggested that MALAT1 regulates the expression of cell cycle genes and is required for G1/S and mitotic progression in normal human diploid fibroblasts. Depletion of MALAT1 leads to activation of p53, and downregulation of B-MYB (Mybl2), an oncogenic transcription factor involved in G2/M progression, due to altered binding of splicing factors on B-MYB pre-mRNA and aberrant alternative splicing (19). In additon, a previous study showed that MALAT1 regulated the E2F1 transcription factor activity, which is a crucial determinant of cell cycle progression and tumorigenesis (24). Our results indicated that *MALAT1* knockdown reversed RANKL-induced cells growth inhibition and cell cycle arrest in hFOB 1.19 cells (Fig. 4). Cells transfected with *MALAT1* siRNA had significant growth activity when treated with RANKL, as compared with scramble siRNA transfected and RANKL-treated hFOB 1.19 cells (Fig. 4A). Furthermore, RANKL treatment alone significantly increased the fraction of cells in G0/G1 phase and decreased the fraction of cells in G2/M phase of the cell cycle while *MALAT1* knockdown before RANKL treatment significantly reduced the proportion in the G0/G1 phase and restored proportion in the G2/M phase of the cell cycle (Fig.4B and Fig.4C). All the results indicated that *MALAT1* played a growth inhibitory role and caused cell cycle arrest in osteoblast cells. The differences of *MALAT1* function between osteoblast cells and other types of cells, such as tumor cells, may result from its alternative splicing of pre-mRNAs of different cell cycle related proteins by modulating serine/arginine splicing factor activity in tissue-type- or cell-type-specific manner. On the other hand, we speculated that the differences may also be due to different degree of cell differentiation.

The RANKL/RANK/OPG system for the regulation of bone resorption explains the mechanism of how bone modeling and remodeling are regulated. The biological effects of OPG are opposite of the RANKL-mediated effects, by preventing RANKL interaction and subsequent stimulation with its receptor, RANK (3). Several studies have demonstrated that mice with excessive or defective expression of RANKL, RANK, or OPG displayed both extremes of skeletal phenotypes, ie, osteoporosis (OPG knockout) and osteopetrosis (OPG transgenic, RANKL knockout, RANK knockout) (2, 8, 26). In this study, we observed that *MALAT1* knockdown by siRNA significantly downregulated the expression of OPG in protein levels in hFOB 1.19 cells, indicating that *MALAT1* regulated OPG expression in osteoblastic cells. In conclusion, *MALAT1* involved in bone metabolism and osteoblast biology by modulating cytokines such as OPG.

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