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ORIGINAL ARTICLE

Alpha-mangostin Suppresses Receptor Activator Nuclear Factor-κB Ligand-induced Osteoclast Formation and Bone Resorption in RAW264.7 Cells by Inhibiting the Extracellular Signal-Regulated Kinase and c-Jun N-Terminal Kinase Signaling

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ABSTRACT

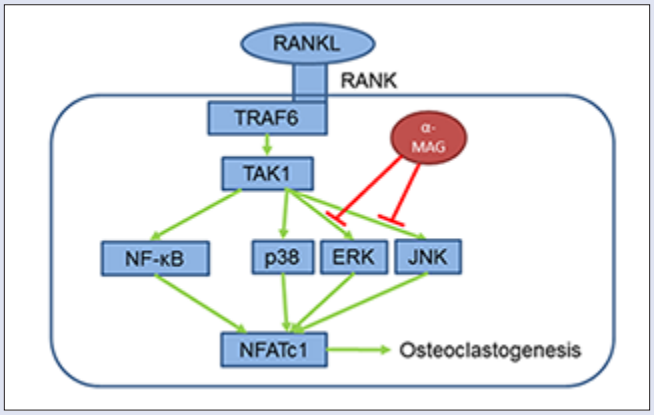
Background: Excessive osteoclast formation and over-activated function lead to a series of osteoclast-related diseases. Suppression of osteoclastogenesis is likely to be an effective means for the treatment of these diseases. **Objective:** In this study, we investigated the effects of alpha-mangostin (α-MAG), a natural compound derived from *Garcinia mangostana*, on osteoclast formation and function in RAW264.7 cells. **Materials and Methods:** Different concentrations of α-MAG were used to explore its effects on receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis and further, we investigated the mechanism by Western Blotting. **Results:** The study revealed that α-MAG attenuated RANKL-induced osteoclastogenesis. Moreover, the effects were confirmed to be caused by the suppression of the phosphorylation of the extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling and this inhibitory effect could be rescued by the administration of the JNK and p38 agonist anisomycin. **Conclusion:** The study results demonstrated that α-MAG could impair RANKL-induced osteoclastogenesis by inhibiting the ERK and JNK signaling pathways in RAW264.7 cells.

Key words: Alpha-mangostin, c-Jun N-terminal kinase, extracellular signal-regulated kinase, osteoclast

SUMMARY

- Alpha-mangostin (α-MAG) inhibited osteoclastogenesis and bone resorption.
- α-MAG exerted the inhibitory effects on osteoclastogenesis by suppressing the c-Jun N-terminal kinase and extracellular signal-regulated kinase signaling
- The inhibitory effects of α-MAG on osteoclast could be rescued by anisomycin.

Abbreviations used: α-MAG: α-mangostin, RANKL: Receptor activator of nuclear factor-κB ligand, ERK: Extracellular signal-regulated kinase, JNK: c-Jun N-terminal kinase, ANI: Anisomycin, M-CSF: Macrophage colony-stimulating factor, MAPK: Mitogen-activated protein kinase, IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, NFATc1: Nuclear factor of activated T cells c1, TRAP: Tartrate-resistant acid phosphatase, SEM: Scanning electron microscope, CTSK: Cathepsin K, CTR: Calcitonin receptor, DC-STAMP: Dendritic cell-specific transmembrane protein, TRAF6: Tumor necrosis factor receptor-associated factor 6, AP-1: Activator protein-1



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INTRODUCTION

Bone metabolism is a dynamic process of that maintains a balance between bone formation and bone resorption, which is controlled by the osteoblasts and osteoclasts, respectively Osteoclasts, derived from monocyte-macrophage precursors, are the only cells *in vivo* with the ability of bone resorption.[1] Bone resorption is induced by the stimulation of receptor activator nuclear factor-κB (NF-κB) ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), subsequently activating related pathway signaling for osteoclastogenesis, such as the mitogen-activated protein kinase (MAPK) family signalling cascade and NF-κB.[2,3] Abnormal osteoclasts formation and function induces the imbalance of bone homeostasis, resulting in a series of osteoclastic related diseases, such as osteoporosis, rheumatoid arthritis, and osteopetrosis.[4-6] Therefore, the treatment targeting at osteoclasts tend to be potential ways for these diseases by the regulation of bone homeostasis.

Variety of drugs aiming at inhibiting osteoclast formation and functions are widely used for the treatment of osteoclast-related diseases clinically,

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such as bisphosphonates and denosumab.[7,8] However, side effects are observed during the period of drug use. Thus, findings an effective compound with fewer adverse effects is still of great value. In addition, a number of natural compounds have exhibited the property to regulate the osteoclast formation or/and functions, such as icariin and lycorine.[9,10]

Alpha-mangostin (α-MAG) [Figure 1a], a natural xanthonoid as secondary metabolite derived from the pericarp, dried sap, and bark of *Garcinia mangostana*, has been widely used in Asian conventional medicine. It has been reported to exert multiple pharmaceutical properties, including anti-inflammatory, anti-tumor, cardioprotective, anti-diabetic, antifungal, antioxidant, antiobesity, and antibacterial effects.[11-16] Suvitha Sya *et al*. demonstrated that Mangostin inhibited lipopolysaccharide-induced inflammatory response by attenuating NF-κB signaling activation in RAW 264.7 macrophages *in vitro* and carrageenan-induced peritonitis *in vivo*.[17] Previous researches showed that α-MAG exerted its inhibitory effects on tumor cell through down-regulating MAPK and NF-κB signaling pathways.[13,18,19] However, to the best of our knowledge, the effects α-MAG on osteoclast formation and functions have not been reported so far.

Based on the crucial roles of MAPK and NF-κB signaling on osteoclastogenesis, we hypothesize that α-MAG might have an inhibitory effect on osteoclast formation and bone resorption. In this study, we utilized murine monocytic cell line RAW 264.7 cell to detect the effects of α-MAG on osteoclast formation and bone resorption, and further clarify the underlying mechanisms.

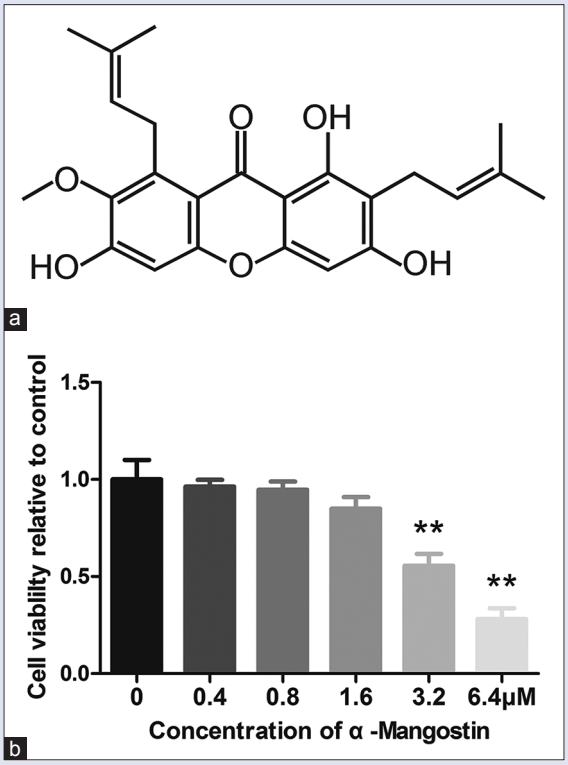

Figure 1: No cytotoxicity of alpha-mangostin was found to RAW264.7 cells at the concentration below 1.6 μM. (a) The chemical structure of alpha-mangostin. (b) The cytotoxicity of alpha-mangostin on RAW264.7 cells were determined by cell counting kit-8 assay at 96 h (**$P < 0.01$)

MATERIALS AND METHODS

Cells, media, and reagents

Mouse macrophage cell line RAW264.7 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The alpha modification of Eagle's medium (α-MEM), fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Gibco-BRL (Gaithersburg, MD, USA). Anisomycin (ANI) and α-MAG were purchased from Sigma Aldrich (St. Louis, MO), dissolved in dimethyl sulfoxide (DMSO) and stored in −20°C. The cell counting kit (CCK-8) was supplied by Dojindo Molecular Technology (Kumamoto, Japan). Recombinant bacteria-derived soluble RANKL was purchased from R and D system (Minneapolis, MN, USA). Bone slices were obtained from Immunodiagnostic Systems Limited (Boldon, UK). The Prime Script RT reagent kit and SYBR® *Premix Ex Taq*™ II were supplied by TaKaRa Biotechnology (Otsu, Shiga, Japan). Primary antibodies against phospho-extracellular signal-regulated kinase (ERK) (Thr202/Tyr204), phospho-c-Jun N-terminal kinase (JNK) (Thr183/Tyr185), phospho-p38 (Thr180/Tyr182), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, c-Fos and nuclear factor of activated T cells c1 (NFATc1) were obtained from Cell Signalling Technology (Cambridge, MA, USA), and β-actin was supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The diagnostic acid phosphatase kit for tartrate-resistant acid phosphatase (TRAP) staining and all other reagents were obtained from Sigma.

Cell culture and cell cytotoxicity assay

RAW264.7 cells were cultured in α-MEM with 10% FBS and 100 U/mL penicillin/streptomycin (complete culture media) in the condition of 37°C and 5% CO_2. Media was changed every 2–3 days.

The cytotoxicity of α-MAG on RAW264.7 cells was determined using CCK-8 assay according to the manufacturer's introductions. Cells were seeded in a 96-well plate at the density of 5×10^3/well and maintained with complete culture media for 12 h. Subsequently, complete culture media was replaced by fresh media comprising of different concentrations of α-MAG (0, 0.4 μM, 0.8 μM, 1.6 μM, 3.2 μM, and 6.4 μM) for 96 h. Then, 10 μL CCK-8 reagent was added to each well and cells were incubated in 37°C for another 2 h. The optical density (OD) was assessed at a wavelength of 450 nm (630 nm as reference) using an ELX800 absorbance microplate reader (Bio-tek, USA). The experiment was performed for 3 times and the cell viability was calculated by the ratio of experimental group OD/control group OD.

Osteoclast differentiation and tartrate-resistant acid phosphatase staining

RAW264.7 cells were seeded in a 96-well plate at a density of 2×10^3/well in the complete culture media with the stimulation of 50 ng/mL RANKL (differentiation culture media) for 4 days. Various concentrations of α-MAG (0, 0.4 μM, or 0.8 μM) with or without ANI (2.5 pg/mL) were added to each well in triplicate to explore its effects on osteoclastogenesis. Media was replaced every 2 days. Cells were then fixed with paraformaldehyde for 20 min and washed for twice with PBS. TRAP staining was performed using the diagnostic acid phosphatase kit. Cells with three or more nuclei were defined as osteoclasts, and the images were taken using a microscope. The number and the area of osteoclasts were measured using Image J software (National Institutes of Health, Bethesda, MD, USA).

Bone resorption assay

RAW264.7 cells were plated at a density of 2×10^3/well on the bovine bone slices in a 96-well plate in differentiation culture media with the treatment of different doses of α-MAG (0, 0.4 μM, or 0.8 μM). Seven days later, bone slices were fixed with paraformaldehyde and cells on slices were removed by mechanical agitation and sonication. Scanning electron microscope (FEI Quanta 250) was used to detect the resorption

pits on the surface of bone slices. The resorption area of bone slices were quantified by the measurement of five random fields on each slice using Image J software. Similar experiments were performed for 3 times.

RNA isolation and quantitative polymerase chain reaction assay

RAW264.7 cells were seeded in a 6-well plate at a density of 1×10^5/well and cultured in osteoclast differentiation media with different doses of α-MAG (0, 0.4 μM or 0.8 μM) for 4 days. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's introductions. Complementary DNA (cDNA) was synthesized using reverse transcriptase with 1 μg of total RNA from each sample. Real-time polymerase chain reaction (PCR) was performed on the ABI 7500 Sequencing Detection System (Applied Biosystems, Foster City, CA, USA) with the SYBR Premix Ex Tag kit following the introductions. The PCR cycling conditions were utilized as follows: 40 cycles of denaturation for 5 s under 95°C and amplification for 24 s under 60°C. Beta-actin was used here as the housekeeping gene and all the primer sequences were listed as follows: β-actin, forward 5'-ACCCAGAAGACTCTGGATGG-3' and reverse 5'-CACATTGGGTAGGAACAC-3'; Cathepsin K (CTSK), forward 5'-CTTCCAATACGTGCAGCAGA-3' and reverse 5'-TCTTCAGGGCTTTCTCGTTC-3' Calcitonin receptor (CTR), forward 5'-TGCAGACAACTCTTGGTTGG-3' and reverse 5'-TCGGTTTCTTCTCCTCTGGA-3'; TRAP, forward 5'-CTGGAGTGCACGATGCCAGCGACA-3' and reverse 5'-TCCGTGCTCGGCGATGGACCAGA-3'; c-Fos, forward 5'-CCAGTCAAGAGCATCAGCAA-3' and reverse 5'-AAGTAGTGCAGCCCGGAGTA-3'; NFATc1, forward 5'-CCGTTGCTTCCAGAAAATAACA-3' and reverse 5'-TGTGGGATGTGAACTCGGAA-30'; V-ATPase d2, forward 5'-AAGCCTTTGTTTGACGCTGT-3' and reverse 5'-TTCGATGCCTCTGTGAGATG-3'; V-ATPase a3, forward 5'-TGGCTACCGTTCCTATCCTG-3' and reverse 5'-CTTGTCCGTGTCCTCATCCT-3'; dendritic cell-specific transmembrane protein (DC-STAMP), forward 5'-AAAACCCTTGGGCTGTTCTT-3' and reverse 5'-AATCATGGACGACTCCTTGG-3'.

Western blotting

To determine the underlying signaling pathway which was inhibited by α-MAG, 1×10^6/well RAW264.7 cells were seeded in 6-well plates and pretreated with or without 0.8 μM α-MAG for 4 h. Subsequently, cells were stimulated by 50 ng/mL RANKL for 0, 5, or 15 min. To explore the effects of α-MAG on NFATc1 and c-Fos, 1×10^5/well RAW264.7 cells were plated in 6-well plates under the stimulation of 50 ng/mL RANKL for 0, 48 h and 72 h, being treatment with or without 0.8 μM α-MAG. Cells were lysed using radioimmunoprecipitation assay lysis buffer (Sigma Aldrich, St Louis, MO, USA) and whole cell lysates were centrifuged for 15 min at the speed of 12,000 × g. The supernatants were collected and protein concentrations were measured using a bicinchoninic acid kit (BCA, Thermo Fisher, MA, USA). A total of 30 g of total protein of each sample was added to each lane and separated on 10% gels, and then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). Specific primary antibodies were used on these membranes overnight at 4°C after 1 h nonspecific blocking with 5% skimmed milk in Tris-buffered saline-Tween (Invitrogen, San Diego, CA, USA). After being washed for twice, membranes were incubated with the appropriate secondary antibodies at 4°C for 2 h. Subsequently, specific bands were detected using a Bio-Rad XRS chemiluminescence detection system (Bio-Rad, Hercules, CA, USA) after adding electrochemical luminescence reagent (Millipore, Billerica, MA, USA) on the membranes. The protein bands were quantified using Image J software.

Statistical analysis

Data were shown as the mean ± standard deviation. Each experiment was repeated at least 3 times. Results were analyzed using the SPSS 16.0 software (SPSS Inc., USA). The one-way ANOVA test was used for the statistical analysis. Student's *t*-test was used to determine the significant difference between different groups. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Alpha-mangostin inhibits receptor activator of nuclear factor-κB ligand-induced osteoclastogenesis in RAW264.7 cells with no cytotoxic effects

To exclude the cytotoxic effect of α-MAG on RAW264.7 cells, CCK-8 assay was performed. As shown in Figure 1b, no cytotoxicity of α-MAG was detected at the concentration lower than 1.6 μM. However, α-MAG at doses higher than 3.2 μM demonstrated its cytotoxicity to RAW264.7 cells. To explore the effect of α-MAG on RANKL-induced osteoclastogenesis, RAW264.7 cells were cultured in the osteoclastic differentiation media with different doses of α-MAG (0, 0.4 μM or 0.8 μM). Figure 2a exhibited that α-MAG markedly suppressed the formation of osteoclasts in a dose-dependent manner in the comparison with the control group. The number of osteoclasts was 88.6/well in the group without α-MAG treatment while the number of osteoclasts decreased to 27.2/well in the presence of 0.8 μM α-MAG [Figure 2b]. The osteoclast area reduced significantly to 12.01% with the treatment 0.8 μM α-MAG compared to area in the control group (43.75%) [Figure 2c]. Together, these results indicate that α-MAG inhibits osteoclast formation in a dose-dependent manner.

Alpha-mangostin inhibits the ability of bone resorption *in vitro*

Since α-MAG had exerted the inhibitory effects on osteoclast differentiation, we further explored its effects on the osteoclastic bone resorption. As shown in Figure 3a, extensive bone resorption was detected on the bone surface of the control group. In contrast, the area of bone resorption reduced obviously under the treatment of α-MAG. Quantification of the bone resorption further confirmed the results [Figure 3b].

Alpha-mangostin attenuates osteoclastic specific gene expression

As the expression of a series of specific genes increase during the process of osteoclast differentiation, we therefore utilized quantitative PCR to access the effects of α-MAG on the mRNA expression of these osteoclast-related genes, including TRAP, CTSK, CTR, DC-STAMP V-ATPase a3, V-ATPase d2, c-fos, and NFATC1. The expression of the osteoclast-related genes was inhibited significantly by the treatment of α-MAG in a dose-independent manner after 4-day RANKL stimulation [Figure 4a-h].

Alpha-mangostin impairs the extracellular signal-regulated kinase and c-Jun N-terminal kinase signaling pathway during osteoclastogenesis in RAW264.7 cells

As NFATc1 and c-Fos play a crucial role in RANKL-induced osteoclastogenesis[20,21] and α-MAG attenuated the mRNA expression of these two genes, we next investigated the effects of α-MAG on their

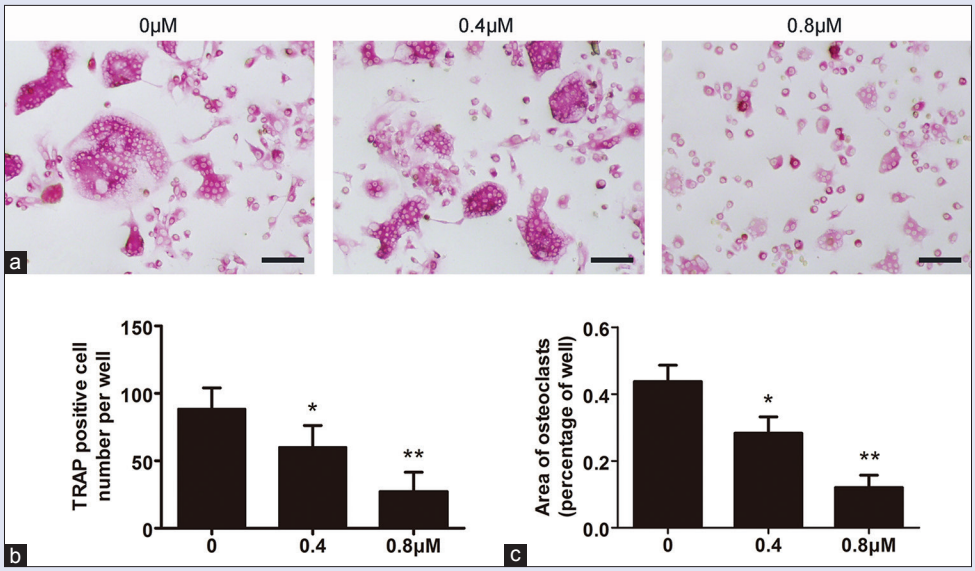

Figure 2: Alpha-mangostin inhibited receptor activator of nuclear factor-κB ligand -induced osteoclast formation *in vitro*. (a) RAW264.7 cells were cultured in osteoclast differentiation media with different doses of alpha-mangostin for 4 days. Cells were fixed and done with tartrate-resistant acid phosphatase staining. (b) The number and (c) the area of osteoclasts in each well were quantified. (*$P < 0.05$; **$P < 0.01$)

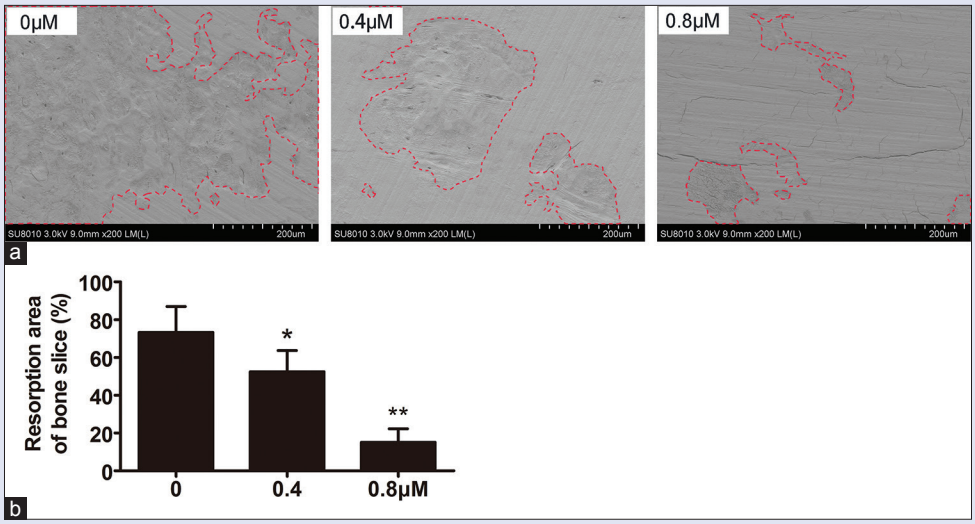

Figure 3: Alpha-mangostin suppressed bone resorption. (a) RAW264.7 cells were maintained on bone slices and cultured in osteoclast differentiation media with different doses of alpha-mangostin for 7 days. The surface of each bone slice is exhibited by scanning electron microscope images. (b) The area of bone resorption pit was measured using Image J. (*$P < 0.05$; **$P < 0.01$)

protein expression. As expected, the protein expression of NFATc1 and c-Fos increased significantly during the process of osteoclastogenesis. However, the protein levels were markedly diminished after the treatment of α-MAG [Figure 5a]. The Western blotting results were also confirmed by quantification [Figure 5b-c].

To elucidate the underlying mechanism how α-MAG inhibited osteoclast formation and function, we investigated the main signaling pathways of osteoclastogenesis by western blotting, including the MAPK (ERK, p38 and JNK) signaling pathway and the NF-κB signaling pathway. RAW264.7 cells were treated with RANKL for 0, 5 and 15 min after 4 h pretreatment of α-MAG or DMSO. As demonstrated in Figure 6a, the western blotting results revealed that the phosphorylation of ERK and JNK were almost completely inhibited by the administration of α-MAG while no suppressive effects were found on the phosphorylation of p38. In addition, little effects on inhibiting the degradation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha were observed in the α-MAG group compared to the control group, which clarified that α-MAG had no effects on the NF-κB signaling pathway. Therefore, these data illuminated that α-MAG suppressed osteoclastogenesis by impairing the ERK/JNK-c-Fos-NFATc1 signaling pathways without affecting the p38 and NF-κB signaling pathways.

Anisomycin partially rescues the inhibitory effects on osteoclastogenesis by alpha-mangostin

As the data shown above, α-MAG attenuated osteoclast formation through suppression of ERK and JNK signaling pathways. To further confirm this, we used ANI, an effective p38 and JNK agonist, to explore whether it could rescue the inhibitory effects of α-MAG on osteoclast

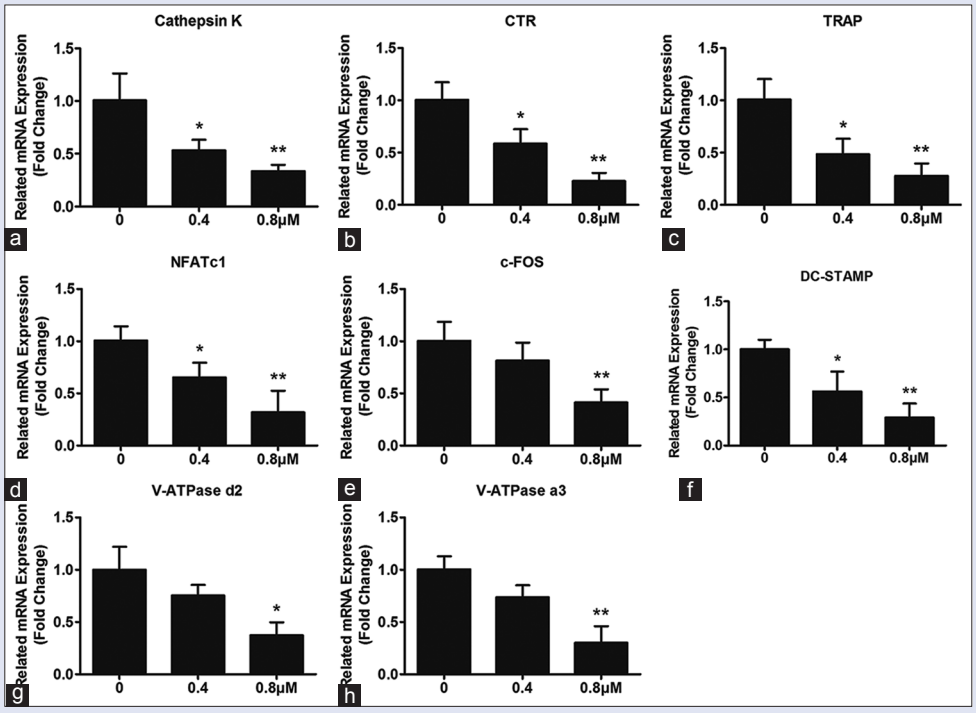

Figure 4: Alpha-mangostin attenuated the expression of osteoclastic specific genes. (a-h) The expression of tartrate-resistant acid phosphatase, cathepsin K, calcitonin receptor, dendritic cell-specific transmembrane protein V-ATPase a3, V-ATPase d2, c-fos, and nuclear factor of activated T cells c1 genes were detected by quantitative polymerase chain reaction after 4-day receptor activator of nuclear factor-κB ligand stimulation combined with different doses of alpha-mangostin. The mRNA expression level of each gene was relative to the expression of β-actin (*$P < 0.05$; **$P < 0.01$)

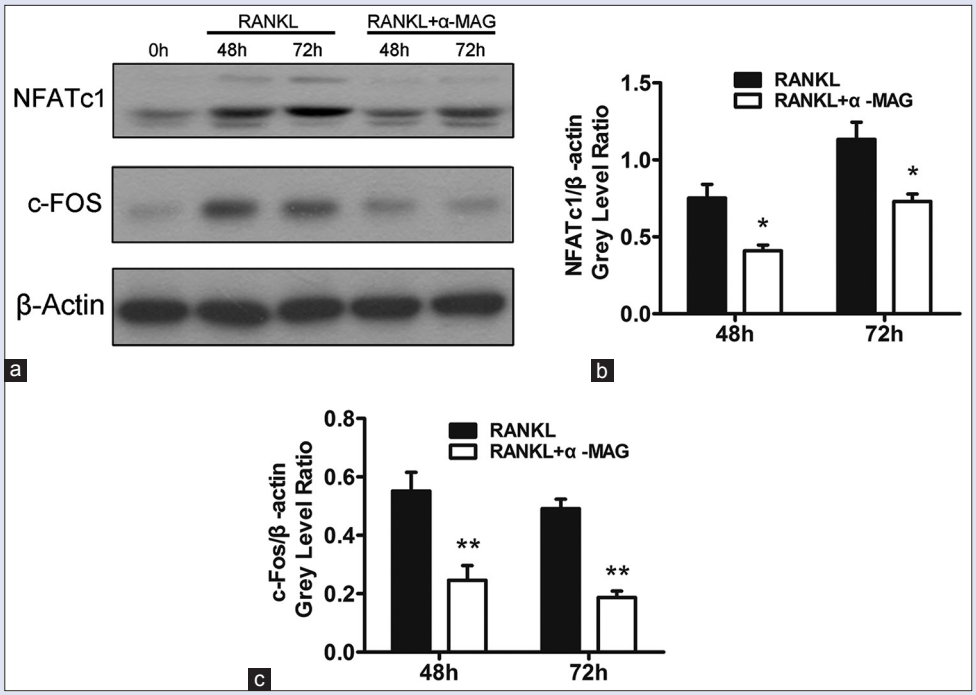

Figure 5: Alpha-mangostin diminished the protein expression of nuclear factor of activated T cells c1 and c-Fos in a time-independent manner. (a) RAW264.7 cells were cultured in osteoclast differentiation media with or without 0.8μM alpha-mangostin for 0, 48 h and 72 h. Western blotting was used to explore the protein expression of nuclear factor of activated T cells c1 and c-Fos. (b-c) The gray levels of nuclear factor of activated T cells c1 and c-Fos were quantified by being normalized to β-actin. (*$P < 0.05$; **$P < 0.01$)

formation. As shown in Figure 6b, the suppression of osteoclastogenesis by the treatment of α-MAG was partially rescued by the administration of ANI. More and larger osteoclasts were found in the ANI+α-MAG group in comparison with the α-MAG group. Moreover, Figure 6c revealed

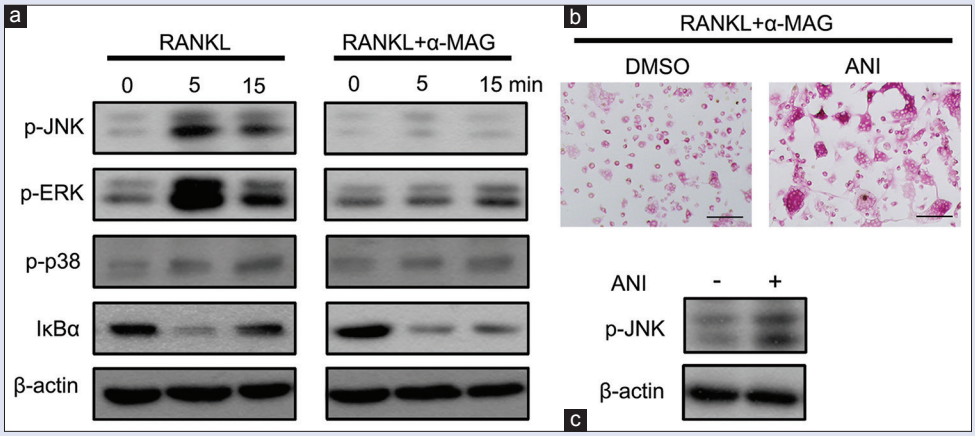

Figure 6: Alpha-mangostin impaired osteoclastogenesis by inhibiting the extracellular signal-regulated kinase and c-Jun N-terminal kinase signaling pathways and the inhibitory effects were rescued by anisomycin. (a) Cells were pre-treated with or without 0.8 μM alpha-mangostin for 4 h, followed by administrating receptor activator of nuclear factor-κB ligand for 0, 5 or 15 min. The phosphorylated levels of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and total nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha were assessed. (b) Cells were treated with/without anisomycin in the presence of alpha-mangostin for 4 days, then stained with tartrate-resistant acid phosphatase solution. (c) Cells were pre-treated with alpha-mangostin for 4 h and then stimulated by receptor activator of nuclear factor-κB ligand with Anisomycin/dimethyl sulfoxide for 5 min. The phosphorylated expression of c-Jun N-terminal kinase was evaluated

that the phosphorylation of JNK was higher in the ANI+α-MAG group than the α-MAG group.

DISCUSSION

Osteoclasts exert the ability of bone resorption and play a key role in bone homeostasis.[22] Overactivated and increased number of osteoclasts results in excess of bone resorption and subsequently leads to a series of osteoclast-related diseases. Suppressing osteoclast formation and/or its function is likely to be a potential way for the treatment of these diseases. In this study, we have showed for the first time that α-MAG exerts inhibitory effects on RANKL-induced osteoclast formation, bone resorption, and osteoclastic-related gene expression in RAW264.7 cells with no cytotoxicity. Moreover, we further confirmed that α-MAG demonstrated its suppressive effects by inhibiting RANKL-induced activation of ERK/JNK-c-Fos-NFATc1 signaling pathway.

Alpha-MAG has been used in Asian conventional medicine for a long time for the treatment of a variety of diseases. Previous studies have showed that the suppression of osteoclast differentiation and function might prevent cancer bone metastasis, such as bisphosphonates.[23,24] Therefore, we propose that the application of α-MAG on treating cancer might also be due to its effects on preventing cancer bone metastasis. Here, we exhibited the inhibitory effects of α-MAG on osteoclast formation and osteoclastic bone resorption for the first time, as well as downregulating the expression of osteoclast-specific genes, making α-MAG a potential drug for treating osteoclast-related diseases.

The RANKL-RANK signaling pathway plays a key role in the regulation of osteoclast differentiation and osteoclastic bone resorption.[2] The trimerization of RANK formed when RANKL binding to its receptor RANK, leading to the recruitment of tumour necrosis factor receptor-associated factor 6 (TRAF6). Subsequently, the downstream signaling cascades were activated, including the p38, ERK, JNK, and NF-κB pathways, followed by mediating the downstream molecules to initiate the differentiation of osteoclasts.[25] The study results indicated that α-MAG affected osteoclastogenesis by inhibiting the phosphorylation of ERK and JNK, without influencing the p38 signaling and the NF-κB signaling. As reported, phosphorylated JNK induces osteoclast formation by regulating an important transcription factor activator protein-1 (AP-1).[26] The phosphorylation of ERK has also showed its effects on activating the downstream molecules AP-1 (Fos/Fra and Jun) and NFAT proteins for osteoclastogenesis.[27,28] The inhibition of the phosphorylation of ERK results in the reduction of osteoclast formation and functions.[21] To further confirm that α-MAG modulated the inhibition of osteoclast formation by suppressing the JNK and ERK signaling, we used ANI, an agonist of JNK and P38, to treat the RAW276.7 cells with α-MAG before the stimulation of RANKL. As expected, the administration of ANI partially rescued the inhibition of osteoclastogenesis by α-MAG, showing increased number and area of osteoclasts, which gave stronger evidence that the α-MAG attenuated RANKL-induced osteoclastogenesis through the suppression of JNK signaling.

NFATc1 is the central transcription regulator for the osteoclast formation and function, which is induced by the accumulation of those early RANKL-induced signaling events. The induction and effects of NFATc1 are promoted by autoamplification and the induction by the NF-κB and MAPK signaling pathways.[29,30] Previous studies have exerted that the deletion of NFATc1 in mice led to osteopetrosis due to the lack of osteoclast formation.[20,31] Besides, c-Fos is also very important in the NFATc1 signaling. Deficiency of c-Fos led to severe osteopetrosis as well due to short of NFATc1 expression. In the present study, we showed that α-MAG significantly reduced the expression of NFATc1 and c-Fos at both mRNA and protein levels and thus inhibited the expression of many osteoclast-specific genes, such as TRAP, CTR, CTSK DC-STAMP, V-ATPase a3 and V-ATPase d2, resulting in blocking osteoclast formation and the ability of bone resorption.

CONCLUSION

The study results revealed that α-MAG attenuated osteoclast differentiation and the activity of bone resorption in RAW264.7. Moreover, we further confirmed that the inhibitory effects were mediated by the suppression of the ERK and JNK signaling pathways. Hence, the study results suggest that α-MAG may be a potential drug for osteoclast-related diseases, but it should be further confirmed *in vivo*.

Acknowledgment

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Nil.

Conflicts of interest

There are no conflicts of interest.

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