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Curcumol suppresses RANKL-induced osteoclast formation by attenuating the JNK signaling pathway

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ABSTRACT

Osteoclasts, derived from hemopoietic progenitors of the monocyte/macrophage lineage, have a unique role in bone resorption, and are considered a potential therapeutic target in the treatment of such pathologic bone diseases as osteoporosis, rheumatoid arthritis, and periodontitis. In the present study, we demonstrate that curcumol, one of the major components of the essential oil of Rhizoma Curcumae, exhibits an inhibitory effect on receptor activator of nuclear factor kappaB ligand (RANKL)-induced osteoclast differentiation with both bone marrow-derived macrophages and RAW264.7 cells in a dose-dependent manner. In addition, RANKL-induced mRNA expression of osteoclast-specific genes, such as tartrate-resistant acid phosphatase, calcitonin receptor, and cathepsin K, is prominently reduced in the presence of curcumol. Furthermore, the molecular mechanism of action was investigated, and curcumol inhibited osteoclastogenesis by specifically impairing RANKL-induced c-Jun N-terminal kinase (JNK)/activator protein-1 (AP-1) signaling, which was further identified in rescue studies by means of anisomycin, a JNK signaling-specific activator. Taken together, these findings suggest that curcumol suppresses RANKL-induced osteoclast differentiation through the JNK/AP-1 signaling pathway, and may be useful as a therapeutic treatment for bone resorption-associated diseases.

1. Introduction

Osteoclast-induced bone resorption and osteoblast-mediated bone formation together determine the balance of bone mass. Disbalance in bone metabolism results in various bone-related diseases, such as osteoporosis, rheumatoid arthritis, and periodontitis [1,2]. Osteoclasts are derived from monocyte/macrophage lineage precursor cells, primarily sustained by macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappaB ligand (RANKL). More specifically, M-CSF guarantees cell multiplication and survival during osteoclastogenesis, while RANKL plays an essential role in osteoclast differentiation [3,4]. RANKL specifically binds to its receptor, RANK, and activates the nuclear factor kappaB (NF-κB) pathway and 3 major mitogen-activated protein kinases (MAPKs), including c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 [5]. These signaling pathways ultimately lead to induction and activation of transcription factors involved in the expression of genes that characterize osteoclasts. These transcription factors include nuclear factor of activated T cells c1 (NFATc1) and activator protein-1 (AP-1) [5–7]. Impairment of these osteoclastic-related cascades might lead to inhibition of osteoclast formation; for example, a RANKL-induced JNK pathway might activate AP-1 transcriptional activity, which plays a critical role in osteoclastogenesis, retarding JNK signaling and resulting in severe disorders in osteoclast differentiation and function [8].

Curcumol, a guaiane-type sesquiterpenoid hemiketal, is one of the major components of the essential oil of Rhizoma Curcumae. Recent pharmacologic studies have reported that curcumol has antiproliferation [9], antihepatic fibrosis [10], antitumor [11], antimicrobial [12], and anti-inflammatory [13] properties. However, to the best of our knowledge, there is very little evidence regarding the effect of curcumol on osteoclast function. In the

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present study, we tested the effect of curcumol on RANKL-induced osteoclastogenesis using both primary osteoclast precursors and RAW264.7 cell line. Curcumol significantly suppressed osteoclast differentiation induced by RANKL.

2. Materials and methods

2.1. Media and reagents

Curcumol and anisomycin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Alpha-MEM, fetal bovine serum (FBS), and penicillin were purchased from Gibco BRL (Gaithersburg, MD, USA). Soluble mouse recombinant M-CSF and RANKL were purchased from R&D Systems (USA). Tartrate-resistant acid phosphatase (TRAP) staining solution and P-nitrophenyl phosphate were purchased from Sigma–Aldrich. Primary antibodies targeting β-actin, IκBα, phospho-JNK, JNK, and c-Fos were purchased from Cell Signaling Technology (Danvers, MA, USA).

2.2. Cell viability assay

The antiproliferative effect of curcumol on bone marrow-derived macrophages (BMMs) and RAW264.7 cells was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). Briefly, cells were pretreated with indicated doses of curcumol for 48 h followed by addition of 10 μL CCK-8 solution to each well. After 4-h incubation, absorbance was measured at 450 nm using a microplate reader. The effect of curcumol on cell viability was expressed as percent cell viability, with vehicle-treated control cells set at 100%.

2.3. Osteoclastogenesis and TRAP activity assay

Bone marrow cells were obtained from the femur and tibia of 4- to 6-week-old C57/BL6 mice. Bone marrow cells were cultured in the presence of M-CSF (30 ng/mL) for 3–5 days to generate BMMs. To examine the effect of curcumol on osteoclast formation, BMMs were treated with indicated doses of curcumol in the presence of M-CSF (30 ng/mL) and RANKL (100 ng/mL) in 96-well culture plates (Corning, MA, USA). Seven days later, cells were fixed and stained with TRAP, and TRAP-positive cells with $\geqslant 3$ nuclei were counted as osteoclasts.

TRAP activity assay was carried out as previously described [14]. Briefly, multinucleated osteoclasts were fixed with 4% formalin for 30 min and 95% ethanol for 1 min followed by 100 mL citrate buffer (50 mM; pH, 4.6) containing 10 mM sodium tartrate. Then, 5 mM p-nitrophenyl phosphate was added to the dried cell-containing wells. After 1-h incubation, the enzyme reaction mixtures were transferred into new plates containing an equal volume of 0.1 N NaOH. Absorbance was measured at 410 nm, and TRAP activity was presented as a percentage of the control.

2.4. Real-time polymerase chain reaction analysis

Total RNA was extracted from BMMs by using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). cDNA was synthesized from total RNA using reverse transcriptase (TaKaRa Biotechnology, Otsu, Japan), and amplified using polymerase chain reaction (PCR). Real-time PCR was performed using the SYBR Premix Ex Tag kit (TaKaRa Biotechnology) and the ABI 7500 Sequencing Detection System (Applied Biosystems, Foster City, CA, USA). The detector was programmed with the following PCR conditions: 40 cycles of 5-s denaturation at 95 °C and 34-s amplification at 60 °C. All reactions were run in triplicate and were normalized to the housekeeping gene β-actin. Primers for osteoclastogenic genes used in this study were as follows: mouse β-actin: forward, 5′-tttgatgtcacgcacgatttcc-3′ and reverse, 5′-tgtgatggtgggaatgggtcag-3′; mouse TRAP: forward, 5′-ctggagtgcacgatgccagcgaca-3′ and reverse, 5′-tccg tctcggcgatggaccaga-3′; mouse cathepsin K (CTSK): forward, 5′-cttcc aatacgtgcagcaga-3′ and reverse, 5′-acgcaccaatatcttgcacc-3′; mouse calcitonin receptor (CTR): forward, 5′-tttcaagaaccttagctg ccagag-3′ and reverse, 5′-tgtgatggtgggaatgggtcag-3′; mouse NFATc1: forward, 5′-ccgttgcttccagaaaataaca-3′ and reverse, 5′-tgtg ggatgtgaact cggaa-3′.

2.5. Western blot analysis

Cells were lysed with a buffer containing 20 mM Tris–HCl, 150 mM NaCl, 1% Triton X-100, protease-inhibitor cocktail (Sigma–Aldrich), and phosphatase-inhibitor cocktail (Sigma–Aldrich). The lysate was centrifuged at 12,000 rcf for 10 min, and the protein in the supernatant was collected. Protein concentrations were measured through BCA assay. Fifty micrograms of each protein lysate was resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using 8–10% gels, and then proteins were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% nonfat milk in TBST, and then immunostained with anti-phospho-JNK (1:1000), anti-phospho-ERK (1:1000), anti-phospho-p38 (1:1000), anti-JNK (1:1000), anti-ERK (1:1000), anti-p38 (1:1000), anti-IκBα (1:1000), anti-c-Fos (1:1000), and anti-β-actin (1:4000) followed by secondary horseradish peroxidese-conjugated antibody (1:5000). Antibody reactivity was detected by exposure in an Odyssey infrared imaging system (LI-COR).

2.6. Luciferase reporter gene activity assay

The effect of curcumol on RANKL-induced NF-κB activation was measured using RAW264.7 cells that had been stably transfected with an NF-κB luciferase reporter construct, as previously described [15,16]. Briefly, cells were seeded into 48-well plates and maintained in cell culture media for 24 h. Then, cells were pretreated with or without indicated concentrations of curcumol for 1 h followed by addition of RANKL (100 ng/mL) for 8 h. Luciferase activity was measured using the Promega Luciferase Assay System (Promega, Madison, WI, USA), and normalized to that of the vehicle control. Similarly, the effect of curcumol on RANKL-induced AP-1-dependent luciferase reporter assays was determined as described previously [17,18].

2.7. Osteoclastogenesis rescue assay

For rescue assay, BMMs were plated triply in 96-well plates at a density of 6×10^3 cells/well followed by stimulation of M-CSF (30 ng/mL), RANKL (100 ng/mL), and curcumol (160 μM), with or without indicated doses of anisomycin, a specific JNK activator [19,20], for 5 days. Cells were fixed and stained with TRAP, and TRAP-positive cells with $\geqslant 3$ nuclei were counted as osteoclasts. TRAP activity assay was carried out as previously described [14]. Absorbance was measured at 410 nm, and TRAP activity was presented as a percentage of the control.

2.8. Statistical analysis

All quantitative data are presented as mean ± SD ($n \geqslant 3$). Two-group comparisons were performed with Student's t tests, while multiple-group comparisons were performed by one-way analysis of variance followed by Tukey's post hoc test, using SPSS statistical package version 12 (SPSS Inc.). P values of <0.05 were considered significant.

3. Results

3.1. Effect of curcumol on cell viability

Both BMMs and RAW264.7 cells were treated with various concentrations of curcumol for 48 h, and cell viability was assessed with the CCK-8 assay kit. Curcumol had no cytotoxic effects on either cell at concentrations less than 160 μM, compared with the control treatment (Fig. 1B and C). In order to exclude the cytotoxic effects, in the following study, curcumol concentrations less than 160 μM were adopted for further analysis.

3.2. Effect of curcumol on osteoclast differentiation in RANKL-stimulated BMMs and RAW264.7 cells

To determine the effect of curcumol on osteoclast differentiation, we treated primary BMMs with various concentrations of curcumol in the presence of RANKL and M-CSF. Mature TRAP-positive multinucleated osteoclasts were seen in the control group. However, curcumol greatly retarded osteoclast differentiation in a dose-dependent manner (Fig. 1D and E). The inhibitory effect also was observed on RAW264.7 cells (Fig. 1D and E). Meanwhile, TRAP activity, a marker enzyme of osteoclasts, also was significantly reduced in the presence of curcumol (Figs. 1F). These data suggest that curcumol effectively suppresses both number of osteoclasts and TRAP activity, and that curcumol may be a potent inhibitor of osteoclastogenesis.

3.3. Effect of curcumol on expression of osteoclastic marker gene expression

To further examine the role of curcumol in osteoclast differentiation, we detected its effect on osteoclast-specific gene expression during osteoclastogenesis. Osteoclast-specific genes,

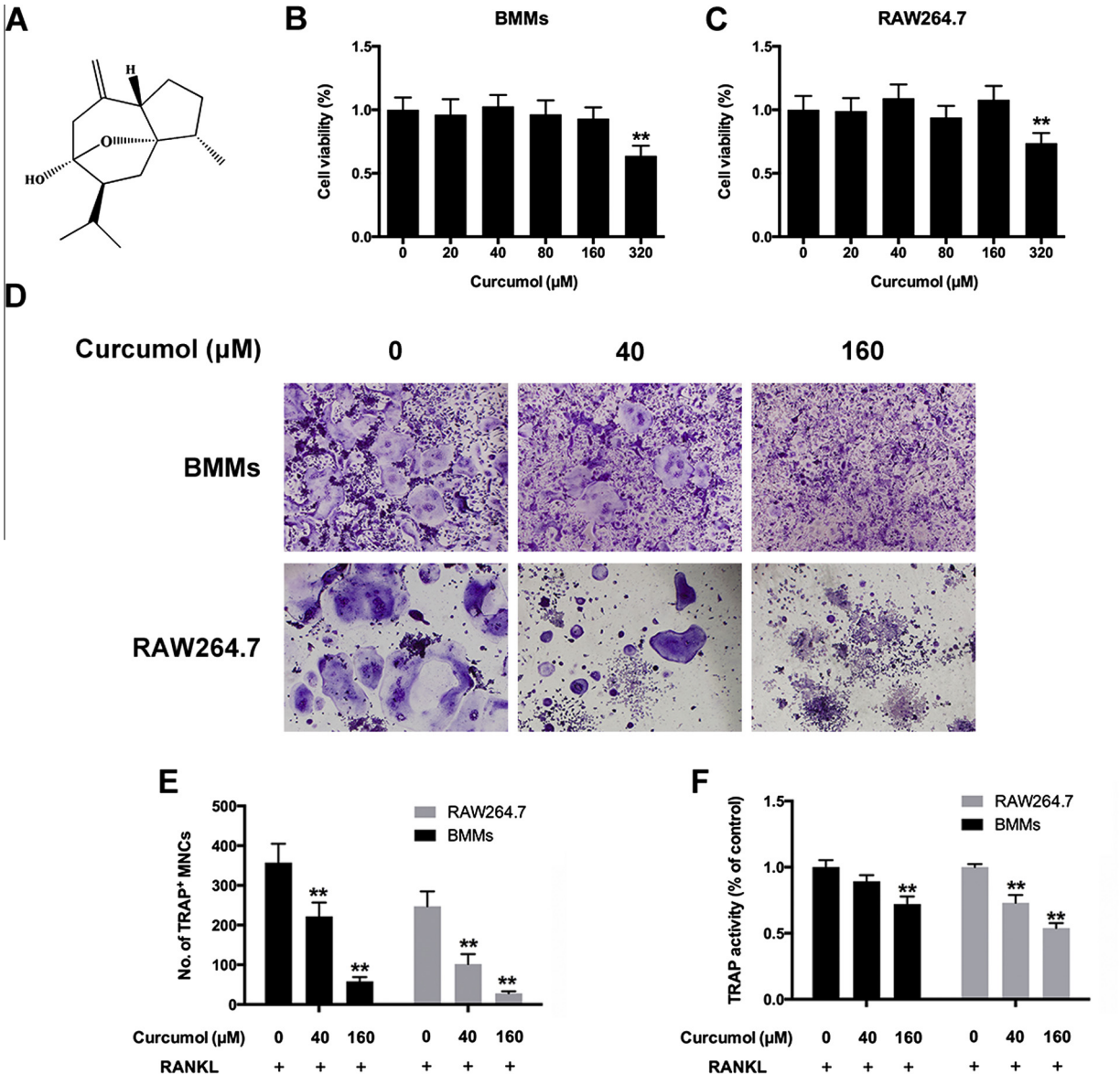

Fig. 1. (A) Structure of curcumol. (B) Viability of curcumol-treated bone marrow-derived macrophages (BMMs). (C) Viability of curcumol-treated RAW264.7 cell line. (D) BMMs were treated with various concentrations of curcumol followed by macrophage colony-stimulating factor (30 ng/mL) and RANKL (50 ng/mL) stimulation for 5 days. Then, cells were fixed with 4% PFA and subjected to tartrate-resistant acid phosphatase (TRAP) staining. RAW264.7 cells were treated with various concentrations of curcumol followed by RANKL (50 ng/mL) stimulation for 5 days. Then, cells were fixed with 4% PFA and subjected to TRAP staining. (E) TRAP-positive multinuclear cells were counted. (F) TRAP activity was quantitatively measured. All experiments were carried out at least 3 times, and significance was determined by Student–Newman–Keuls tests ($^{*}P < 0.05$, $^{**}P < 0.01$).

including TRAP, CTR, and CTSK, were significantly upregulated with RANKL stimulation. However, this trend was suppressed by addition of curcumol persistently (Fig. 2A). Moreover, curcumol suppressed osteoclastic marker expression in a dose-dependent manner (Fig. 2B). Taken together, these data further support the hypothesis that curcumol is a potent inhibitor of osteoclastogenesis.

3.4. Effect of curcumol on RANKL-induced JNK/AP-1 signaling

Previous researchers have found that MAPK (p38, ERK1/2, and JNK) signaling plays a critical role in osteoclast differentiation [8,21]. In order to demonstrate the underlying molecular mechanism of curcumol's inhibitory effect on osteoclast differentiation, RANKL-induced signaling pathways were investigated. JNK is a member of the MAPK family, and can be activated by a RANKL/RANK combination [22]. In this study, JNK phosphorylation was increased under RANKL stimulation, relative to a control group. However, JNK phosphorylation was greatly impaired after treatment with curcumol (Fig. 3A). Quantitative analysis confirmed these observations (Fig. 3C). Furthermore, levels of c-Fos, proteins downstream of JNK, also were reduced dramatically by curcumol treatment (Fig. 3B). These results suggest that curcumol inhibits JNK phosphorylation during osteoclast differentiation.

Furthermore, by using AP-1 luciferase reporter gene assays, we also examined whether curcumol suppresses AP-1 activity. Activation of AP-1 transcriptional activity sharply increased when exposed to RANKL. However, curcumol suppressed AP-1 luciferase activity in a dose-dependent manner (Fig. 3D), suggesting that curcumol can inhibit RANKL-induced activation of JNK/AP-1.

3.5. Effect of curcumol on RANKL-induced NFATc1 expression

NFATc1 is a well-known master regulator of osteoclastogenesis and osteoclast function [23]. The MAPK (JNK)/AP-1 pathway is a main activator that can regulate the NFATc1 promoter and NFATc1 expression [17,24]. To determine whether curcumol regulates expression of NFATc1 by inhibiting the JNK/AP-1 signaling pathway, we examined expression of NFATc1 at the mRNA level. NFATc1 was increased when cells were exposed to RANKL. However, curcumol abrogated the RANKL-induced increase in NFATc1 at the mRNA level in a dose-dependent manner (Fig. 3E), suggesting that curcumol can suppress RANKL-induced NFATc1

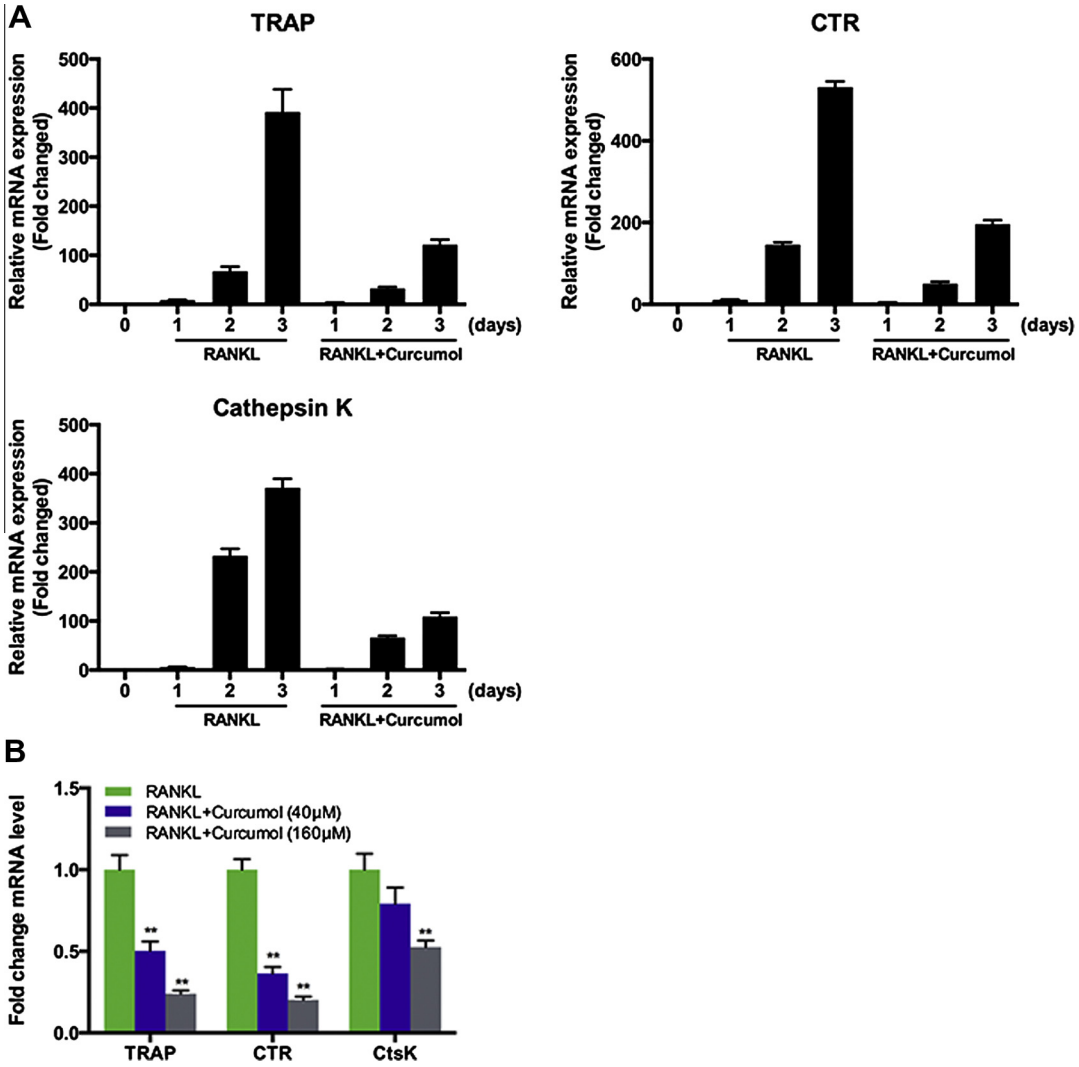

Fig. 2. (A) Bone marrow-derived macrophages (BMMs) were cultured with macrophage colony-stimulating factor (M-CSF) (30 ng/mL) and RANKL (50 ng/mL), with or without curcumol (160 μM), for 0, 1, 2, and 3 days. Osteoclast-specific gene expression (tartrate-resistant acid phosphatase [TRAP], calcitonin receptor [CTR], and cathepsin K [CTSK]) was analyzed by real-time polymerase chain reaction (PCR), and results were normalized to expression of β-actin. (B) BMMs were cultured with M-CSF (30 ng/mL) and RANKL (50 ng/mL), with indicated concentrations of curcumol, for 5 days. Osteoclast-specific gene expression (TRAP, CTR, and CTSK) was analyzed by real-time PCR, and results were normalized to expression of β-actin. All experiments were performed at least 3 times ($^{*}P < 0.05$, $^{**}P < 0.01$).

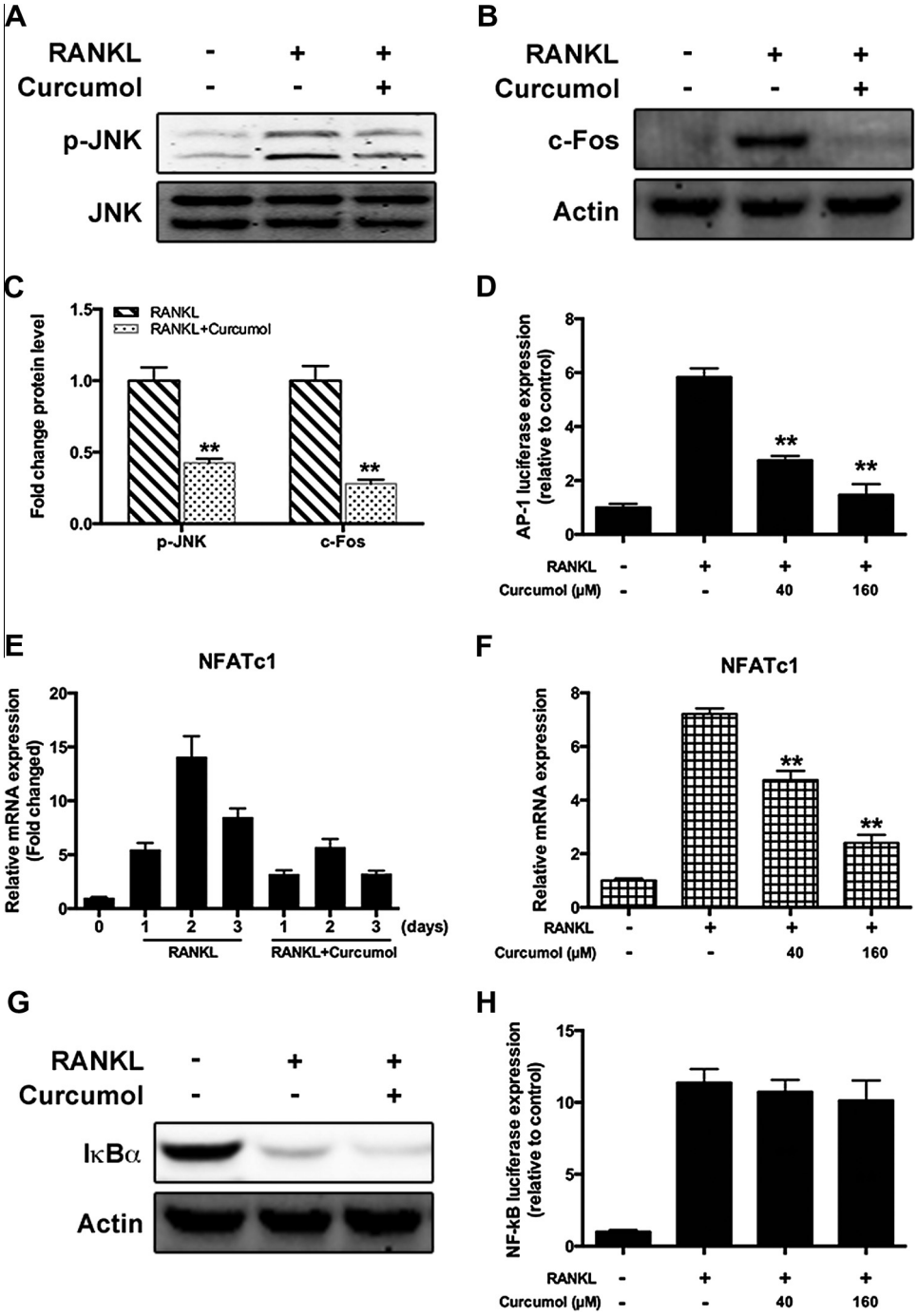

Fig. 3. (A) Bone marrow-derived macrophages (BMMs) were seeded at 5×10^5 cells/well in 6-well plates and pretreated with or without curcumol (160 μM) for 4 h prior to RANKL stimulation (50 ng/mL) for 10 min. Cells were lysed for Western blotting with specific antibodies against phospho-JNK and JNK. (B) BMMs were seeded at 5×10^5 cells/well in 6-well plates and pretreated with or without curcumol (160 μM) for 4 h prior to RANKL stimulation (50 ng/mL) for 24 h. Cells were lysed for Western blotting with specific antibodies against c-Fos and actin. (C) Fold changes in phospho-JNK and c-Fos protein levels were normalized to expression of actin. (D) Stably transfected RAW264.7 cells with an AP-1 luciferase reporter construct were seeded in 48-well plates and maintained in cell culture media for 24 h. Then, cells were pretreated with or without indicated concentrations of curcumol for 1 h followed by addition of RANKL (50 ng/mL) for 24 h. AP-1 luciferase activity was measured. (E) BMMs were cultured with macrophage colony-stimulating factor (M-CSF) (30 ng/mL) and RANKL (50 ng/mL), with or without curcumol (160 μM), for 0, 1, 2, and 3 days. Nuclear factor of activated T cells c1 (NFATc1) expression was analyzed by real-time polymerase chain reaction (PCR), and results were normalized to expression of β-actin. (F) BMMs were cultured with M-CSF (30 ng/mL) and RANKL (50 ng/mL), with indicated concentrations of curcumol, for 3 days. NFATc1 expression was analyzed by real-time PCR, and results were normalized to expression of β-actin. (G) BMMs were seeded at 5×10^5 cells/well in 6-well plates and pretreated with or without curcumol (160 μM) for 4 h prior to RANKL stimulation (50 ng/mL) for 10 min. Cells were lysed for Western blotting with specific antibodies against IκBα and actin. (H) Stably transfected RAW264.7 cells with a nuclear factor kappaB (NF-κB) luciferase reporter construct were seeded in 48-well plates and maintained in cell culture media for 24 h. Then, cells were pretreated with or without indicated concentrations of curcumol for 1 h followed by addition of RANKL (50 ng/mL) for 8 h. NF-κB luciferase activity was measured. All experiments were performed at least 3 times ($^*P < 0.05$, $^{**}P < 0.01$).

expression. To further confirm that curcumol inhibits NFATc1 expression, we examined various time points, and demonstrated that curcumol suppressed NFATc1 expression throughout the experimental period (Fig. 3F). Taken together, our results indicate that curcumol suppresses RANKL-induced NFATc1 expression in osteoclastogenesis.

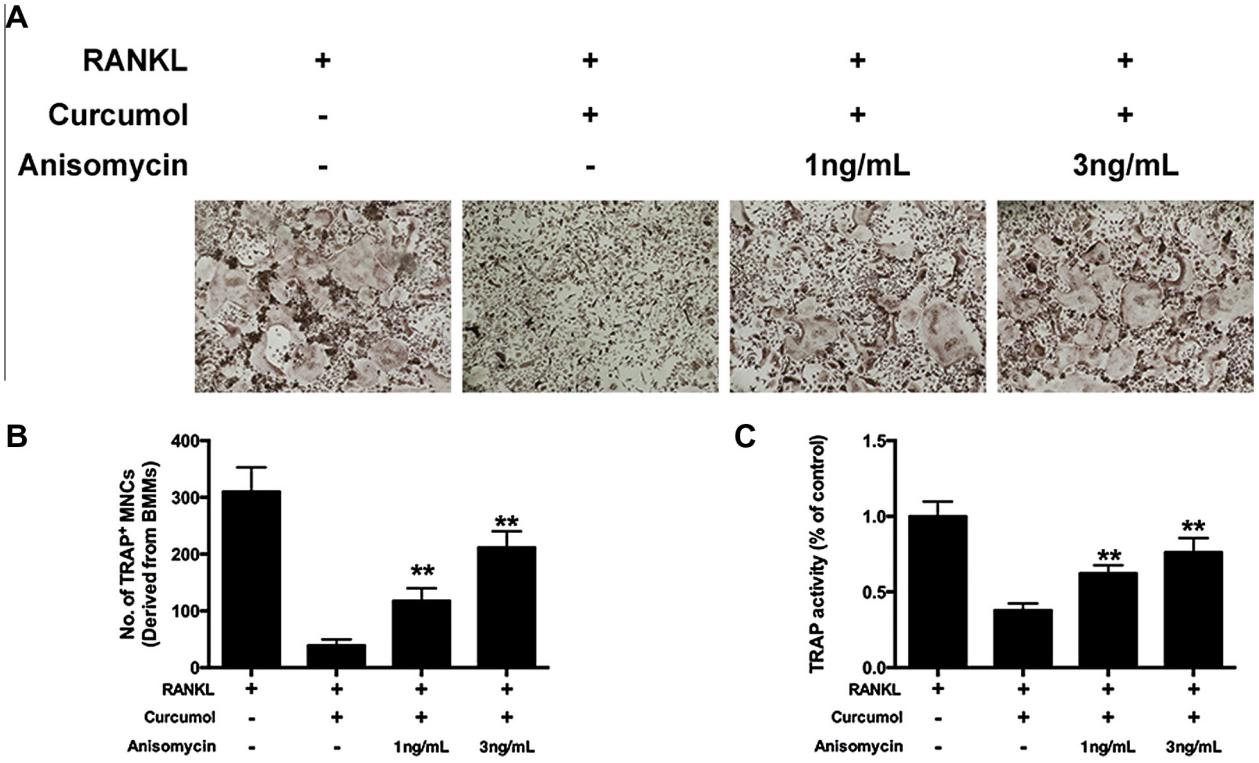

Fig. 4. (A) Bone marrow-derived macrophages (BMMs) were stimulated with macrophage colony-stimulating factor (30 ng/mL), receptor activator of nuclear factor kappaB ligand (50 ng/mL), and curcumol (160 μM), with or without indicated concentrations of anisomycin. Then, cells were subjected to tartrate-resistant acid phosphatase (TRAP) staining. (B) TRAP-positive multinuclear cells were counted. (C) TRAP activity was quantitatively measured. All experiments were carried out at least 3 times, and significance was determined by Student–Newman–Keuls tests ($^{*}P < 0.05$, $^{**}P < 0.01$).

3.6. Curcumol did not affect NF-κB, p38, or ERK activation

We next used Western blotting and luciferase assays to investigate additional signaling pathways (NF-κB, ERK, and p38) that are closely associated with osteoclast differentiation. RAW264.7 cells were treated with RANKL in the presence or absence of curcumol. Then, NF-κB, ERK, and p38 signaling pathways were examined using specific antibodies. As seen in Fig. 3G, curcumol had no effect on NF-κB; this was further confirmed by luciferase reporter assay (Fig. 3H). In addition, curcumol had no effect on ERK or p38 signaling pathways (data not shown).

3.7. Anisomycin rescued curcumol-treated impaired osteoclastogenesis

As described above, osteoclast formation was potently inhibited by curcumol, which may be due to reduced JNK phosphorylation. To confirm these observations, following treatment with curcumol, BMM cells were treated with anisomycin, which has been reported to strongly activate JNK cascades. As expected, osteoclast formation was inhibited in cells treated with curcumol only. However, in cells also treated with anisomycin, impaired osteoclastogenesis was rescued and mature osteoclasts were observed (Fig. 4).

4. Discussion

Excessive bone resorption plays a critical role in pathologic bone diseases [25]. Thus, suppressing osteoclast formation should be a potential treatment for osteoclast-related disorders. In our present research, we examined the effect of curcumol on osteoclast differentiation from both BMMs and RAW264.7 cells. Osteoclasts are generated under the activation of RANKL [26,27]. Our research shows that curcumol can retard RANKL-induced osteoclast formation from both precursor cells and cell line without cytotoxicity.

Osteoclasts are derived from monocyte/macrophage lineages [28]. Mature osteoclasts are characterized by morphologic conversion into large multinucleated cells, as well as expression of specific phenotypic markers, including TRAP, CTSK, and CTR [29–32]. Our present study revealed that curcumol inhibits RANKL-induced osteoclast maker gene expression in a dose-dependent manner. Many studies have revealed that MAPKs (JNK, ERK, and p38) can be activated by RANKL stimulation, and are associated with osteoclastogenesis [5,8]. Specifically, dominant-negative JNK prevents RANKL-induced osteoclastogenesis [17], while p38 is important in the early stages of osteoclast generation because it regulates the microphthalmia-associated transcription factor [33]. Meanwhile, inhibition of ERK has been shown to decrease osteoclast formation [34]. To be more specific, previous research has demonstrated the importance of JNK signaling in osteoclastogenesis, and RANKL is the main activator in stimulating JNK [35]. Activated JNK subsequently phosphorylates downstream factors, including c-Fos and c-Jun. These 2 factors combine to form heterodimers of AP-1, which is an essential translation factor during osteoclast formation [36,37]. In our study, JNK phosphorylation and c-Fos expression were dramatically inhibited. AP-1 transcriptional activity was significantly suppressed in the presence of curcumol. Additionally, to further verify that curcumol's inhibitory effect on osteoclastogenesis was due to JNK suppression, anisomycin, an activator of JNK, was used to treat BMM cells following treatment with curcumol. Just as we hypothesized, impaired osteoclastogenesis was rescued. In addition, since NF-κB, ERK, and p38 are also osteoclastogenesis-related signaling pathways, we further detected the effect of curcumol on these related signaling pathways. Our results indicate that NF-κB, ERK, and

p38 pathways are not involved in the antiosteoclastogenic effect of curcumol.

In summary, the present study demonstrated that curcumol inhibits osteoclastogenesis from primary macrophages and cell line *in vitro*. Curcumol also reduces RANKL-induced expression of osteoclastic marker genes. In addition, curcumol attenuated attenuates RANKL-induced JNK/AP-1 activation. Although additional experiments are needed to confirm the efficacy of curcumol in treating disease conditions *in vivo*, our results indicate that curcumol has potential as a therapy for disorders associated with bone loss.

Conflict of interest

The authors have no conflict of interest to declare.

References

[1] T. Alliston, R. Derynck, Medicine: interfering with bone remodelling, Nature 416 (2002) 686–687.
[2] G. Karsenty, E.F. Wagner, Reaching a genetic and molecular understanding of skeletal development, Dev. Cell 2 (2002) 389–406.
[3] T. Suda, N. Takahashi, T.J. Martin, Modulation of osteoclast differentiation, Endocr. Rev. 13 (1992) 66–80.
[4] T. Chambers, Regulation of the differentiation and function of osteoclasts, J. Pathol. 192 (2000) 4–13.
[5] Z.H. Lee, H.-H. Kim, Signal transduction by receptor activator of nuclear factor kappa B in osteoclasts, Biochem. Biophys. Res. Commun. 305 (2003) 211–214.
[6] D.M. Anderson, E. Maraskovsky, W.L. Billingsley, W.C. Dougall, M.E. Tometsko, E.R. Roux, M.C. Teepe, R.F. DuBose, D. Cosman, L. Galibert, A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function, Nature 390 (1997) 175–179.
[7] H. Takayanagi, S. Kim, T. Koga, H. Nishina, M. Isshiki, H. Yoshida, A. Saiura, M. Isobe, T. Yokochi, J.-I. Inoue, Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts, Dev. Cell 3 (2002) 889–901.
[8] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342.
[9] H. Wang, Y. Fang, Y. Wang, Z. Wang, Q. Zou, Y. Shi, J. Chen, D. Peng, Inhibitory effect of curcumol on Jak2-STAT signal pathway molecules of fibroblast-like synoviocytes in patients with rheumatoid arthritis, Evid. Based Complement. Altern. Med 2012 (2012).
[10] Y. Jiang, Z.-S. Li, F.-S. Jiang, X. Deng, C.-S. Yao, G. Nie, Effects of different ingredients of zedoary on gene expression of HSC-T6 cells, World J. Gastroenterol. 11 (2005) 6780.
[11] L. Xu, K. Bian, Z. Liu, J. Zhou, G. Wang, The inhibitory effect of the curcumol on women cancer cells and synthesis of RNA, Tumor 25 (2005) 570–572.
[12] Y. Lou, H. Zhang, H. He, K. Peng, N. Kang, X. Wei, X. Li, L. Chen, X. Yao, F. Qiu, Isolation and identification of phase 1 metabolites of curcumol in rats, Drug Metab. Dispos. 38 (2010) 2014–2022.
[13] X. Chen, C. Zong, Y. Gao, R. Cai, L. Fang, J. Lu, F. Liu, Y. Qi, Curcumol exhibits anti-inflammatory properties by interfering with the JNK-mediated AP-1 pathway in lipopolysaccharide-activated RAW264. 7 cells, Eur. J. Pharmacol. (2013).
[14] S.N. Kim, M.H. Kim, Y.K. Min, S.H. Kim, Licochalcone A inhibits the formation and bone resorptive activity of osteoclasts, Cell Biol. Int. 32 (2008) 1064–1072.
[15] C. Wang, J.H. Steer, D.A. Joyce, K.H.M. Yip, M.H. Zheng, J. XU, 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits osteoclastogenesis by suppressing RANKL-induced NF-κB activation, J. Bone Miner. Res. 18 (2003) 2159–2168.
[16] Z. Zhai, H. Li, G. Liu, X. Qu, B. Tian, W. Yan, Z. Lin, T. Tang, A. Qin, K. Dai, Andrographolide suppresses RANKL-induced osteoclastogenesis in vitro and prevents inflammatory bone loss in vivo, Br. J. Pharmacol. (2013).
[17] F. Ikeda, R. Nishimura, T. Matsubara, S. Tanaka, J.-I. Inoue, S.V. Reddy, K. Hata, K. Yamashita, T. Hiraga, T. Watanabe, Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation, J. Clin. Invest. 114 (2004) 475–484.
[18] T.J. Weber, L.M. Markillie, Regulation of activator protein-1 by 8-iso-prostaglandin E2 in a thromboxane A2 receptor-dependent and-independent manner, Mol. Pharmacol. 63 (2003) 1075–1081.
[19] E. Cano, C.A. Hazzalin, L.C. Mahadevan, Anisomycin-activated protein kinases p45 and p55 but not mitogen-activated protein kinases ERK-1 and-2 are implicated in the induction of c-fos and c-jun, Mol. Cell. Biol. 14 (1994) 7352–7362.
[20] C.A. Hazzalin, R. Le Panse, E. Cano, L.C. Mahadevan, Anisomycin selectively desensitizes signalling components involved in stress kinase activation and fos andjun induction, Mol. Cell. Biol. 18 (1998) 1844–1854.
[21] D.A. Stevenson, E. Schwarz, J.C. Carey, D.H. Viskochil, H. Hanson, S. Bauer, H.Y. Cindy Weng, T. Greene, K. Reinker, J. Swensen, Bone resorption in syndromes of the Ras/MAPK pathway, Clin. Genet. 80 (2011) 566–573.
[22] C. Davies, C. Tournier, Exploring the function of the JNK (c-Jun N-terminal kinase) signalling pathway in physiological and pathological processes to design novel therapeutic strategies, Biochem. Soc. Trans. 40 (2012) 85–89.
[23] Q. Zhao, X. Wang, Y. Liu, A. He, R. Jia, NFATc1: functions in osteoclasts, Int. J. Biochem. Cell Biol. 42 (2010) 576–579.
[24] M. Asagiri, H. Takayanagi, The molecular understanding of osteoclast differentiation, Bone 40 (2007) 251–264.
[25] S.R. Cummings, L.J. Melton, Epidemiology and outcomes of osteoporotic fractures, Lancet 359 (2002) 1761–1767.
[26] T. Suda, N. Takahashi, N. Udagawa, E. Jimi, M.T. Gillespie, T.J. Martin, Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families, Endocr. Rev. 20 (1999) 345–357.
[27] S.L. Teitelbaum, RANKing c-Jun in osteoclast development, J. Clin. Invest. 114 (2004) 463–465.
[28] S.G.-K. Mohamed, E. Sugiyama, K. Shinoda, H. Taki, H. Hounoki, H.O. Abdel-Aziz, M. Maruyama, M. Kobayashi, H. Ogawa, T. Miyahara, Interleukin-10 inhibits RANKL-mediated expression of NFATc1 in part via suppression of c-Fos and c-Jun in RAW264. 7 cells and mouse bone marrow cells, Bone 41 (2007) 592–602.
[29] O. Anusaksathien, C. Laplace, X. Li, Y. Ren, L. Peng, S.R. Goldring, D.L. Galson, Tissue-specific and ubiquitous promoters direct the expression of alternatively spliced transcripts from the calcitonin receptor gene, J. Biol. Chem. 276 (2001) 22663–22674.
[30] G. Motyckova, K. Weilbaecher, M. Horstmann, D. Rieman, D. Fisher, D. Fisher, Linking osteopetrosis and pycnodysostosis: regulation of cathepsin K expression by the microphthalmia transcription factor family, Proc. Natl. Acad. Sci. 98 (2001) 5798–5803.
[31] S. Reddy, J. Hundley, J. Windle, O. Alcantara, R. Linn, R. Leach, D. Boldt, G. Roodman, Characterization of the mouse tartrate-resistant acid phosphatase (trap) gene promoter, J. Bone Miner. Res. 10 (1995) 601–606.
[32] H.J. Choi, Y.R. Park, M. Nepal, B.-Y. Choi, N.-P. Cho, S.H. Choi, S.R. Heo, H.S. Kim, M.-S. Yang, Y. Soh, Inhibition of osteoclastogenic differentiation by Ikarisoside A in RAW 264.7 cells via JNK and NF-κB signaling pathways, Eur. J. Pharmacol. 636 (2010) 28–35.
[33] M. Matsumoto, T. Sudo, T. Saito, H. Osada, M. Tsujimoto, Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-κB ligand (RANKL), J. Biol. Chem. 275 (2000) 31155–31161.
[34] E. Ang, Q. Liu, M. Qi, H.G. Liu, X. Yang, H. Chen, M.H. Zheng, J. Xu, Mangiferin attenuates osteoclastogenesis, bone resorption, and RANKL-induced activation of NF-κB and ERK, J. Cell. Biochem. 112 (2011) 89–97.
[35] J.-P. David, K. Sabapathy, O. Hoffmann, M.H. Idarraga, E.F. Wagner, JNK1 modulates osteoclastogenesis through both c-Jun phosphorylation-dependent and-independent mechanisms, J. Cell Sci. 115 (2002) 4317–4325.
[36] A.E. Grigoriadis, Z.-Q. Wang, M.G. Cecchini, W. Hofstetter, R. Felix, H.A. Fleisch, E.F. Wagner, C-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling, Science 266 (1994) 443–448.
[37] N. Kobayashi, Y. Kadono, A. Naito, K. Matsumoto, T. Yamamoto, S. Tanaka, J.i. Inoue, Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis, EMBO J. 20 (2001) 1271–1280.