

RESEARCH ARTICLE

F4/80 inhibits osteoclast differentiation via downregulation of nuclear factor of activated T cells, cytoplasmic 1

Ju-Hee Kang¹ · Jung-Sun Sim¹ · Ting Zheng¹ · Mijung Yim¹

Received: 15 August 2016 / Accepted: 12 February 2017 / Published online: 16 February 2017
© The Pharmaceutical Society of Korea 2017

Abstract Osteoclastogenesis is an essential process in bone metabolism, which can be induced by RANKL stimulation. The F4/80 glycoprotein is a member of the EGF-transmembrane 7 (TM7) family and has been established as a specific cell-surface marker for murine macrophages. This study aimed to identify the role of F4/80 in osteoclastogenesis. Using mouse bone marrow-derived macrophages (BMMs), we observed that the mRNA level of F4/80 was dramatically reduced as these cells differentiated into osteoclasts. Furthermore, osteoclastogenesis was decreased in F4/80^{high} BMMs compared to F4/80^{-/low} BMMs. The inhibitory effect of F4/80 was associated with decreased expression of nuclear factor of activated T cells, cytoplasmic 1 (NFATc1). Ectopic overexpression of a constitutively active form of NFATc1 rescued the anti-osteoclastogenic effect of F4/80 completely, suggesting that the anti-osteoclastogenic effect of F4/80 was mainly due to reduction in NFATc1 expression. As an underlying mechanism, we demonstrated that the presence of F4/80 abrogated the effect of RANKL on the phosphorylation of CREB and activated the expression of IFN- β , which are restored by cyclic AMP. Collectively, our results demonstrate that the

presence of F4/80 suppresses RANKL-induced osteoclastogenesis by impairing the expression of NFATc1 via CREB and IFN- β . Therefore, F4/80 may hold therapeutic potential for bone destructive diseases.

Keywords F4/80 · Osteoclast · RANKL · NFATc1 · Bone loss

Introduction

Bone remodeling is a highly regulated process that involves osteoblast-induced bone formation and osteoclast-mediated bone resorption. An imbalance between osteoclasts and osteoblasts leads to either osteopetrosis or excessive bone resorption (Rodan and Martin 2000; Kar-senty and Wagner 2002). Osteoclasts are generated from monocytes or macrophage populations (Tinkler et al. 1981; Quinn et al. 2002). The formation of osteoclasts takes place in several stages, including commitment, differentiation, multinucleation, and activation of immature osteoclasts; the process is regulated by both systemic hormones and cytokines in the bone microenvironment, which contains stromal cells, osteoblasts, and other local factors (Yavropoulou and Yovos 2008).

One of the key factors needed for osteoclast formation is the receptor activator of nuclear factor- κ B (RANK) ligand (RANKL) (Anderson et al. 1997), which is a major osteoclastogenic cytokine of the tumor necrosis factor (TNF) family. It has been reported that osteoblastic cells express RANKL on their surfaces and promote the differentiation of monocytes/macrophages into osteoclasts (Lacey et al. 1998; Teitelbaum 2000). In osteoclast precursor cells, the binding of RANKL to its receptor, RANK, induces the activation of multiple intracellular

Ju-Hee Kang and Jung-Sun Sim have contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12272-017-0900-7) contains supplementary material, which is available to authorized users.

✉ Mijung Yim
myim@sookmyung.ac.kr

¹ College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Republic of Korea

signaling pathways that are necessary for osteoclast differentiation and activation (Teitelbaum and Ross 2003; Asagiri and Takayanagi 2007). RANK recruits adaptor molecules such as TNF receptor-associated factor (TRAF) 6 to its cytoplasmic domain, resulting in the activation of NF- κ B and mitogen-activated protein kinases (MAPKs) (Lee and Kim 2003). Nuclear factor of activated T cells (NFATc1) is a downstream transcription factor in the RANKL/RANK signal pathway and plays a crucial role in osteoclastogenesis (Walsh et al. 2006; Kim and Kim 2014). As a key molecule of osteoclastogenesis, NFATc1 induces a series of osteoclast-specific genes, including cathepsin K, tartrate-resistant acid phosphatase (TRAP), calcitonin receptor, and osteoclast-associated receptor (Walsh et al. 2006; Kim and Kim 2014). c-Fos and cAMP response element binding protein (CREB) are also essential transcription factors for osteoclastogenesis and positively regulate osteoclastogenesis via NFATc1 activation (Lee and Kim 2003; Sato et al. 2006).

Although RANKL stimulates osteoclast differentiation, it also initiates a negative auto-regulatory effect on osteoclasts through the induction of interferon beta (IFN- β) expression (Takayanagi et al. 2002), which has an inhibitory effect on osteoclasts by interfering with RANKL-induced expression of c-Fos and NFATc1 (Takayanagi et al. 2002). Besides RANKL, macrophage colony-stimulating factor (M-CSF) is also a key factor in the differentiation and activation of osteoclasts. M-CSF induces the proliferation, survival, and RANK-upregulation of osteoclast precursor cells (Arai et al. 1999).

The F4/80 glycoprotein is a member of the EGF-transmembrane 7 (TM7) family, which in turn forms a subgroup within the superfamily of G-protein-coupled receptors (GPCRs). The F4/80 molecule has been established as one of the most specific cell-surface markers for murine macrophages (Gordon et al. 2011). F4/80 knock out animal models showed that F4/80 is not required for macrophage development (Lin et al. 2005) or responses to infectious challenge with *Listeria* (Schaller et al. 2002). Nevertheless, the F4/80 was found to be necessary for the induction of efferent CD8+ regulatory T cells responsible for peripheral immune tolerance (Lin et al. 2005).

Since the role of F4/80 in osteoclast formation has not been investigated, we report the inhibitory function of F4/80 on osteoclastogenesis by using mouse bone marrow-derived macrophages (BMMs). Our findings suggest that F4/80 is a negative regulator of the early stages of osteoclast differentiation and F4/80 could be exploited for therapeutic potential to prevent the development of osteoclast-associated bone diseases.

Materials and methods

Reagents

Forskolin was purchased from Cayman Chemical Company (Ann Arbor, USA). Antibodies against phospho-CREB, c-Fos, and NFATc1 were purchased from Cell Signaling Technology (Beverly, USA). All other reagents were purchased from Sigma-Aldrich (St. Louis, USA).

Cells and culture system

All experiments were performed in accordance with institutional guidelines approved by the Sookmyung Women's University Animal Care and Use Committee. Bone marrow cells were isolated from the long bones of 4- to 6-week-old C57BL6 (Samtako, Inc. Osan, Korea) mice. Bone marrow cells were cultured in α -MEM/10% FBS, supplemented with M-CSF (5 ng/ml, R&D Systems Inc., Minneapolis, USA), for 12 h to separate adherent and non-adherent cells. The non-adherent cells were then harvested and cultured with M-CSF (30 ng/ml) for 4 days. The floating cells were removed and the attached cells were used as bone marrow-derived monocytes/macrophages (BMMs). All cells were cultured in α -MEM/10% FBS at 37 °C in 5% CO₂.

BMM cell sorting and osteoclast differentiation

F4/80^{-low} and F4/80^{high} BMM cells populations were isolated by fluorescence-activated cell-sorter (FACS) (FACS AriaTM, BD Bioscience, San Jose, USA). Briefly, BMMs were stained with FITC-labeled anti-F4/80 (eBioscience, San Diego, USA). After washing the cells three times with phosphate buffered saline (PBS) containing 2 mM EDTA, the cells were sorted into F4/80^{-low} and F4/80^{high} populations by F4/80 positive selection using FACScaliburTM flow cytometer (BD Biosciences). F4/80^{-low} or F4/80^{high} cells were differentiated into osteoclasts with 25 ng/ml M-CSF and 200 ng/ml RANKL (R&D Systems Inc.) for 4 days (Lee et al. 2016). Cytokines were replaced every 2 days.

Tartrate-resistant acid phosphatase (TRAP) staining

To confirm the generation of osteoclast cells, cells were fixed with 3.7% formalin for 15 min, fixed again with ethanol-acetone (50:50, v/v) for 1 min, and incubated with the TRAP-staining solution—0.05 M sodium acetate buffer (pH 5.0) containing naphthol AS-BI phosphoric acid sodium salt (Sigma-Aldrich) and fast red ITR salt (Sigma-Aldrich) in the presence of 10 mM sodium tartrate (Sigma-Aldrich). TRAP-positive cells with three or more nuclei

formed in the culture system were considered to be osteoclast-like multinuclear cells.

Retroviral gene transduction

Plat-E retroviral packaging cells were seeded in culture dishes 1 day before transfection. The following day, PMX-puro GFP (GFP-vector) or PMSCV-GFP CA-NFATc1 (CA-NFATc1) was transfected into Plat-E cells using Lipofectamine 2000 CD (Invitrogen by Life Technologies, Inc., Grand Island, NY, USA). After 2 days, culture supernatants of the retrovirus-producing cells were collected. F4/80^{high} BMMs were seeded with the culture supernatants of PMX-puro GFP or PMSCV-GFP CA-NFATc1 virus-producing Plat-E cells together with polybrene (10 mg/ml, Santa Cruz Biotechnology, Inc., Santa Cruz, USA) and M-CSF (30 ng/ml) overnight. Infected cells were then selected with puromycin (2 mg/ml) for 2 days and then further cultured in the presence of M-CSF (30 ng/ml) and RANKL (200 ng/ml) for 3 days.

Quantitative real-time PCR

Total RNA was extracted from cells by Easy-Blue (iNtRON Biotechnology, Inc., Seongnam, Korea). cDNA was synthesized from total RNA by using the Revert AidTM first strand cDNA synthesis kit (iNtRON, Biotechnology, Inc.) and was amplified with gene-specific primers using Applied Biosystems SYBR Green super-mix (Applied Biosystems Inc., Foster City, USA) in an Applied Biosystems 7500/7500 Fast Real-time PCR system (Applied Biosystems Inc.). Specific primer sequences for PCR were designed as follows: cathepsin K (CTK), 5'-cttccaatacgtgcagcaga-3' (forward), 5'-acgcaccaatatcttgacc-3' (reverse); Atp6v0d2, 5'-tcagatctctcaaggtgtgctg-3' (forward), 5'-gtgc caaatgagttcagagtgtg-3' (reverse); DC-STAMP, 5'-tggaa gttcactgaaactactgtg-3' (forward), 5'-ctcggttcccgcagcctctctc-3' (reverse); β 3-integrin, 5'-gatgacatcgagcaggtgaaagag-3' (forward), 5'-ccggatcatgaatggtgatgtag-3' (reverse); F4/80, 5'-gaatcttgccaagaagagac-3' (forward), 5'-gaattctcctgtatatcatcagc-3' (reverse); GAPDH, 5'-tgcaccaccaactgcttagc-3' (forward), 5'-ggcatggactgtggtcatgag-3' (reverse). The real-time PCR program was set as follows: 40 cycles, after an initial holding stage at 95 °C for 10 min, then the cycling stage at 95 °C for 15 s, and annealing at 53–57 °C for 1 min.

siRNA transfection

BMMs were plated on 48-well plates at a density of 3.5×10^4 cells/well with 30 ng/ml M-CSF. After 24 h, cells were transfected with 50 nM mouse F4/80 on-target plus smart pool siRNAs (Santa cruz, sc-42865) using Lipofectamine

2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The control contained 50 nM non-targeting siRNA (BIONEER). The transfection took place in 2.5 ml of serum free media for 6 h; the cells were then cultured for 4 days in complete media containing 30 ng/ml M-CSF and 100 ng/ml RANKL to form osteoclasts.

Immunoblot analysis

Total cell lysates were isolated, separated by SDS-PAGE, and transferred onto Immobilon-P membranes (Millipore, Bedford, USA). The membranes were blocked with 5% nonfat-milk in PBS-T (Phosphate Buffered Saline, 0.1% Tween 20), and then immunostained with specific antibodies. Immuno-reactivity was detected by using an advanced chemiluminescence detection kit (Amersham Biosciences, Buckinghamshire, UK).

Statistical analyses

Data are presented as the mean \pm SD from at least three independent experiments. Statistical analysis was performed by using the Student's *t* test followed by one-way analysis of variance. A *p* value <0.05 was considered statistically significant.

Results

The presence of F4/80 suppresses osteoclast formation via NFATc1

BMMs are osteoclast precursors and they differentiate into mature osteoclasts in the presence of M-CSF and RANKL (Quinn et al. 2002; Teitelbaum and Ross 2003; Asagiri and Takayanagi 2007). To investigate the role of F4/80 in osteoclastogenesis, we first investigated the mRNA expression level of F4/80 using mouse BMMs. Real-time PCR analysis revealed that RANKL stimulation decreases the expression level of F4/80 in BMMs (Fig. 1a). Next, we sorted mouse BMMs into F4/80^{high} and F4/80^{low} cells, and induced osteoclast differentiation by culturing them with M-CSF and RANKL. Figure 1b depicts the flow cytometry data of total BMMs. The mRNA level of F4/80 is in agreement with the isolation of F4/80^{high} and F4/80^{low} cells (Fig. 1c). As shown in Fig. 1d, osteoclastogenesis was severely impaired in the population with high F4/80 expression. In accordance with the mRNA expression levels of cathepsin K, levels of DC-stamp, ATP6v0d2, and β 3-integrin, which are markers of differentiated osteoclasts, were significantly decreased in F4/80^{high} cells treated with M-CSF and RANKL (Fig. 1e). To further confirm the role of F4/80 on osteoclastogenesis, we investigated

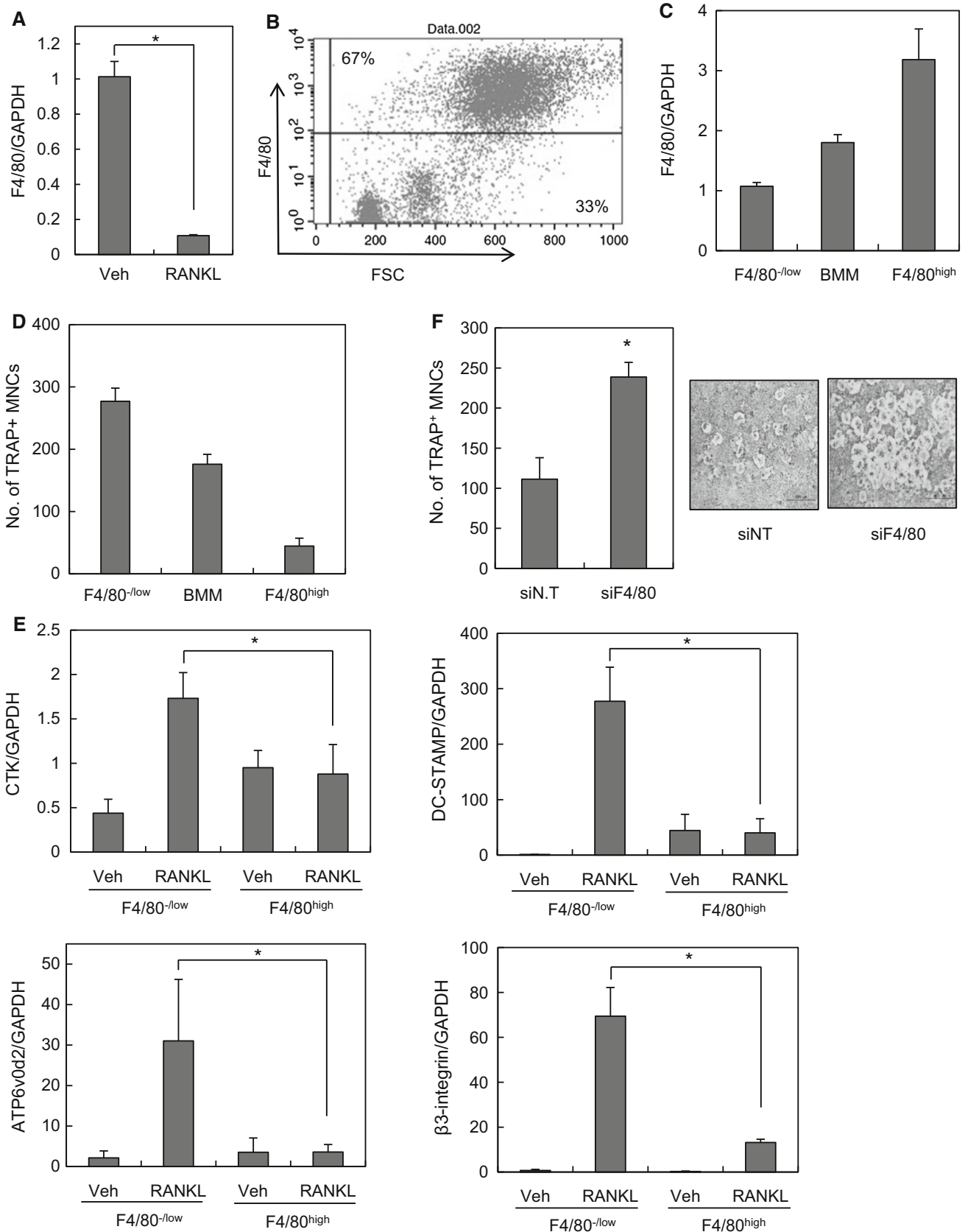


Fig. 1 The presence of F4/80 negatively regulates RANKL-induced osteoclast formation. **a** BMMs were cultured with RANKL (100 ng/ml) and M-CSF (30 ng/ml) for 4 days. The mRNA expression level of F4/80 was determined by real-time PCR. **b** BMMs were stained with FITC-labeled anti-F4/80 and analyzed by FACS. **c** F4/80^{-low} or F4/80^{high} BMMs were sorted and the mRNA expression level of F4/80 was determined by real-time PCR. **d** F4/80^{-low} or F4/80^{high} BMMs were sorted and differentiated into osteoclasts in the presence of RANKL (100 ng/ml) and M-CSF (30 ng/ml) for 4 days. TRAP⁺ multinucleated cells (MNCs) were counted. **e** The mRNA expression level of osteoclastogenic marker genes was determined by real-time PCR. **f** The siRNA-transfected BMMs were cultured with RANKL (100 ng/ml) for 4 days, and then TRAP⁺ osteoclasts were counted. Data are expressed as the mean \pm SD from at least three independent experiments. *veh* vehicle. **p* < 0.05. Scale bar 200 μ m

whether down-regulation of F4/80 by siRNA would modulate the osteoclast formation. Compared with the control siRNA, the knock-down of F4/80 resulted in a significant increase in the formation of TRAP⁺ MNCs by RANKL (Fig. 1f). This result indicates that F4/80 is involved in suppressing osteoclastogenesis by acting directly on osteoclast precursors.

It is well known that RANKL stimulation increases the expression of NFATc1 in osteoclast precursors. The NFATc1 pathway plays a critical and fundamental role in osteoclast development and the lack of NFATc1 arrests osteoclastogenesis (Lee and Kim 2003; Asagiri and Takayanagi 2007). To clarify the involvement of the inhibitory effect of F4/80 on osteoclast formation, the expression level of NFATc1 was examined at 24 h after RANKL treatment. Significant increase in NFATc1 protein levels was detected in F4/80^{-low} cells treated with RANKL. This NFATc1 upregulation decreased in F4/80^{high} cells (Fig. 2a). We next investigated whether the anti-osteoclastogenic effect of F4/80 was rescued by overexpression of NFATc1. The constitutively active form of NFATc1 (CA-NFATc1) genes were overexpressed in F4/80^{high} BMMs (Fig. 2b) and these NFATc1-transduced BMMs were cultured with M-CSF and RANKL. As shown in Fig. 2c, the suppression of osteoclastogenesis in F4/80^{high} cells was efficiently overcome by the forced expression of CA-NFATc1, suggesting that the anti-osteoclastogenic effect of F4/80 is mainly due to reduction in NFATc1 expression.

The presence of F4/80 suppresses osteoclastogenesis via CREB and IFN- β , which is rescued by cyclic adenosine monophosphate (cAMP)

CREB is an essential transcription factor for osteoclastogenesis and positively regulates osteoclastogenesis via NFATc1 activation (Sato et al. 2006). To define the molecular mechanism of the inhibitory effects of F4/80 on osteoclastogenesis, we examined the effects of F4/80 on CREB activation induced by RANKL. As shown in Fig. 3a,

phosphorylation of CREB was observed after RANKL treatment in F4/80^{-low} cells, which was diminished by the high expression of F4/80 in BMMs. Since cAMP is a well-known mediator of CREB activation (Wen et al. 2010), we evaluated whether the elevation of cAMP restores the RANKL-induced CREB activation in F4/80^{high} BMMs. We found that the treatment with forskolin, a cAMP elevating agent, significantly increased the RANKL-induced phosphorylation of CREB in F4/80^{high} BMMs (Fig. 3b). These data suggested that the presence of F4/80 inhibits RANKL-induced CREB activation, which is rescued by cAMP.

Since the presence of F4/80 was shown to decrease the pro-osteoclastogenic signals from RANKL, we next investigated whether F4/80 modulates the anti-osteoclastogenic mechanism of RANKL. IFN- β is a negative regulator of osteoclast differentiation (Takayanagi et al. 2002). Thus we analyzed whether the presence of F4/80 modulates the expression level of IFN- β by RANKL. Real-time PCR demonstrated that RANKL-induced mRNA expression level of IFN- β was significantly increased due to the high expression of F4/80 (Fig. 3c). Moreover, forskolin treatment in F4/80^{high} BMMs decreased the mRNA expression of IFN- β by RANKL (Fig. 3d).

Since forskolin was shown to modulate the effect of F4/80 on RANKL signaling, we finally evaluated the effect of forskolin on NFATc1 expression and osteoclast formation in F4/80^{high} BMMs. We found that forskolin increased RANKL-induced NFATc1 expression and osteoclast formation in F4/80^{high} BMMs (Fig. 4).

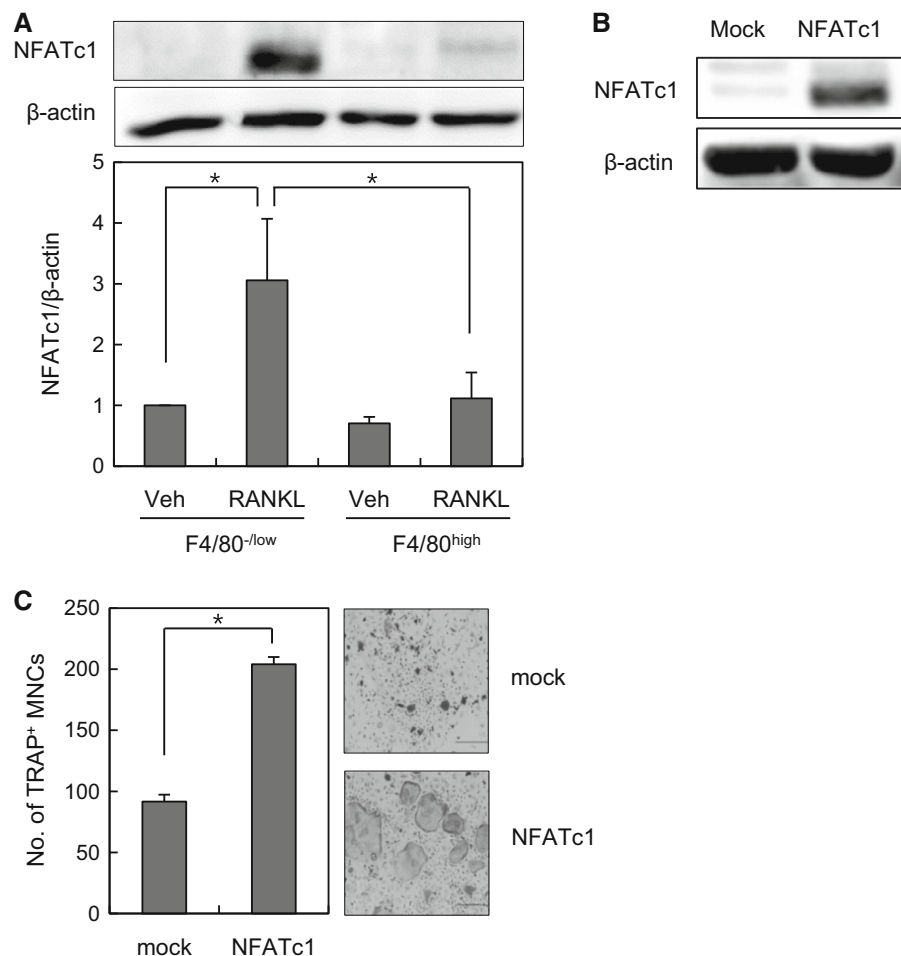
Taken together, these data suggested that the presence of F4/80 suppresses RANKL-induced osteoclastogenesis by impairing the activation of CREB and increasing IFN- β expression, which is restored by cAMP.

Discussion

Osteoporosis is a disorder caused by increased bone resorption and reduced bone formation. As osteoporosis progresses, bone becomes fragile and bone fractures may readily occur even due to a small impact (Rodan and Martin 2000). The regulation of bone mass depends on the dynamic balance between bone formation and bone resorption, which are driven by osteoblast activation and osteoclast activation, respectively (Rodan and Martin 2000). RANKL is a key positive regulator of osteoclast differentiation and excessive RANKL signaling causes enhanced osteoclast formation and bone resorption (Lee and Kim 2003). As such, downregulation of RANKL-induced signals may be a valuable approach in the treatment of pathological bone loss, including osteoporosis.

In this report, we show for the first time that the F4/80 molecule is a negative regulator of RANKL-induced

Fig. 2 The presence of F4/80 suppresses RANKL-induced NFATc1 expression. **a** F4/80^{-/-} or F4/80^{high} BMMs were treated with or without 200 ng/ml of RANKL for 24 h. Cell lysates were then subjected to western blot analysis. **b** F4/80^{high} BMMs were infected through the retrovirus packaging system. Western blotting was performed for NFATc1 expression levels. **c** Infected BMMs were cultured with RANKL (100 ng/ml) and M-CSF (30 ng/ml) for 4 days. The recovery rate was defined as the percentage of the osteoclast formation in the presence of F4/80. The osteoclast formation in the presence of mock was given as 100%. Data are expressed as mean \pm SD from at least three independent experiments. * $p < 0.05$



osteoclast differentiation. We demonstrated that F4/80 modulates CREB and IFN- β signaling pathways via cAMP, which leads to the decrease of NFATc1-mediated osteoclast formation. These data suggest that NFATc1 is a target of the F4/80 inhibitory effect on osteoclast development. Although c-Fos and NF- κ B have been well known as regulators of NFATc1, they unlikely mediate the inhibitory function of F4/80 on osteoclastogenesis (data not shown). Given the lack of a known ligand for F4/80, it remains difficult to further define a molecular mechanism mediating the differentiation of osteoclasts. The elucidation of the functional F4/80 ligand may allow us to determine the mechanism by which F4/80 molecule triggers differentiation and activation of osteoclasts. Identifying the molecular mechanisms used by the F4/80 molecule to suppress the differentiation of osteoclasts will contribute to our understanding of the processes involved in physiological and pathological bone loss.

Many therapeutic agents for the treatment of osteoporosis have been developed and marketed. Bisphosphonate (FosamaxTM) and its derivatives are well-known

agents for patients with osteoporosis (Papapoulos 2000). However, these compounds have severe side-effects owing to their very low bioavailability (Iseri et al. 2005). Soluble estrogen receptor modulator (RaloxifeneTM) for estrogen replacement increases bone density by 3–4%, but carries the risk of breast cancer (Vogel et al. 2006). Parathyroid hormone is a novel drug for the activation of osteoblasts to balance bone remodeling. However, this drug enhances the risk of developing osteosarcoma (Cosman 2008). Recently, the humanized monoclonal antibody to RANKL (Denosumab; ProliaTM) has become available. The expected side-effect of this agent is immune deficiency due to RANKL scavenging (Josse et al. 2013). In our study, F4/80 suppresses osteoclast formation and holds promise as a novel therapeutic agent for osteoporosis.

In summary, we demonstrated that F4/80 functions as an inhibitor of RANKL-mediated osteoclastogenesis via CREB and IFN- β signaling pathways. Therefore, drug-targeting approaches for F4/80 may hold therapeutic potential for treating post-menopausal osteoporosis.

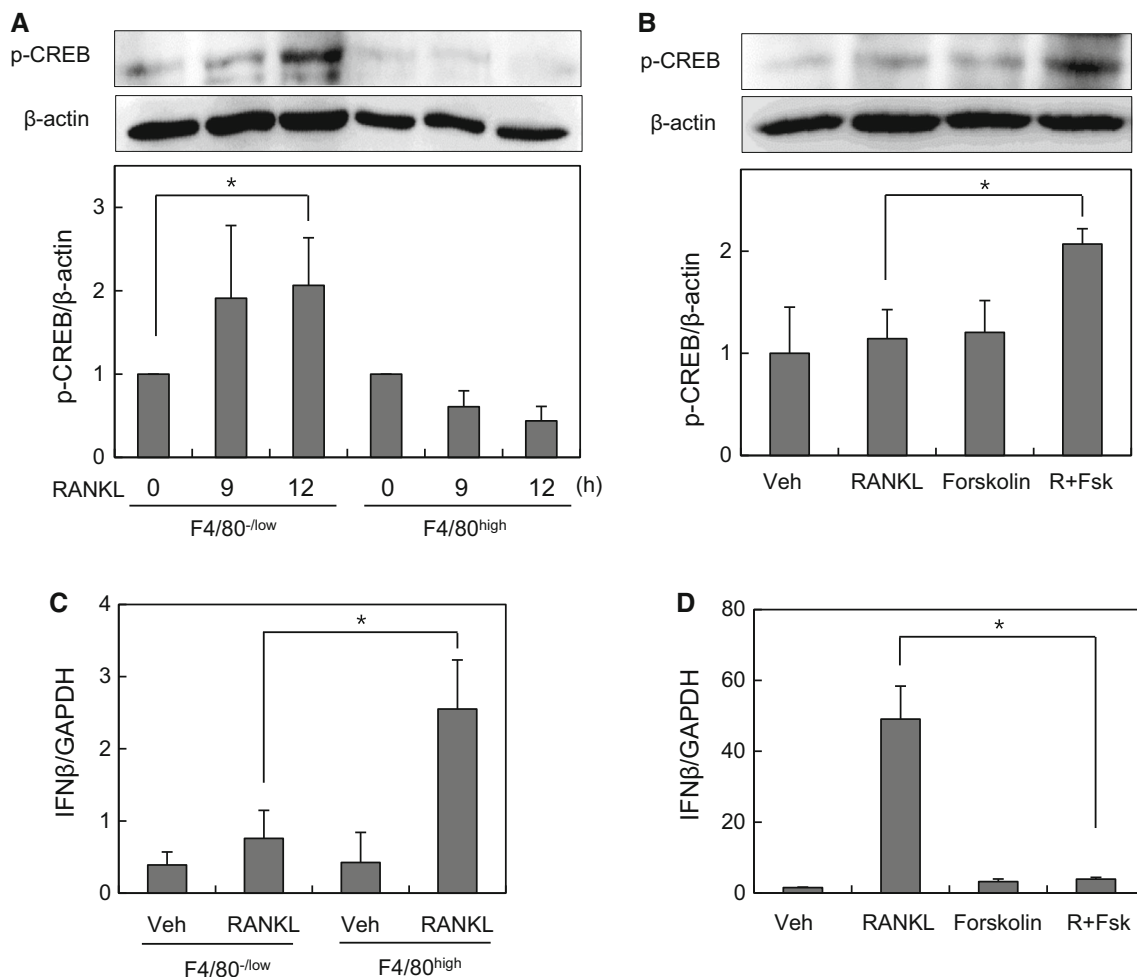


Fig. 3 The presence of F4/80 abrogates the effect of RANKL on CREB and IFN- β . **a, c** F4/80^{-low} or F4/80^{high} BMMs were treated with or without 200 ng/ml of RANKL. **b, d** F4/80^{high} BMMs were treated with 200 ng/ml of RANKL in the presence or absence of forskolin (0.1 μ M). Cells were then analyzed by western blotting (**a, b**) or real-time PCR (**c, d**). Data are expressed as mean \pm SD from at least three independent experiments. *veh* vehicle. * $p < 0.05$

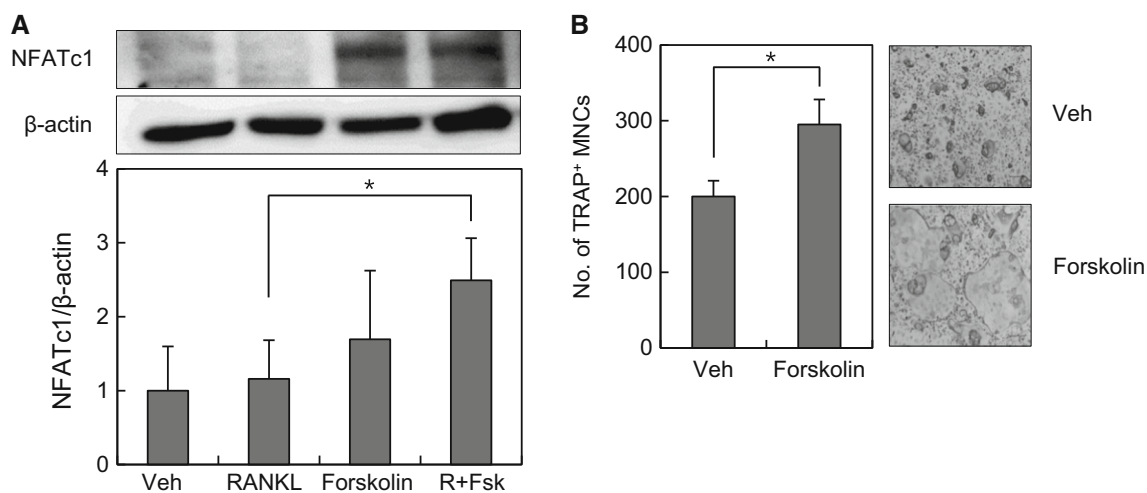


Fig. 4 The presence of F4/80 suppresses osteoclast formation via cAMP. **a** F4/80^{high} BMMs were treated with or without 200 ng/ml of RANKL for 24 h. Cell lysates were then subjected to western blotting analysis. **b** F4/80^{high} BMMs were treated with 200 ng/ml of RANKL in the presence or absence of forskolin (0.1 μ M) for 4 days. TRAP⁺ multinucleated cells (MNCs) were counted. Data are expressed as the mean \pm SD from at least three independent experiments. *veh* vehicle. * $p < 0.05$

Acknowledgments This work was supported by Grants from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI14C24470000) and from the National Research Foundation of Korea (NRF), funded by Ministry of Science, ICT and Future Planning (MSIP) (NRF-2014M1A3A3A02034917).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L (1997) A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 390:175–179
- Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM, Suda T (1999) Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappa B (RANK) receptors. *J Exp Med* 190:1741–1754
- Asagiri M, Takayanagi H (2007) The molecular understanding of osteoclast differentiation. *Bone* 40:251–264
- Cosman F (2008) Parathyroid hormone treatment for osteoporosis. *Curr Opin Endocrinol Diabetes Obes* 15:495–501
- Gordon S, Hamann J, Lin HH, Stacey M (2011) F4/80 and the related adhesion-GPCRs. *Eur J Immunol* 41:2472–2476
- Işeri SO, Sener G, Yüksel M, Contuk G, Cetinel S, Gedik N, Yegen BC (2005) Ghrelin against alendronate-induced gastric damage in rats. *J Endocrinol* 187:399–406
- Josse R, Khan A, Ngui D, Shapiro M (2013) Denosumab, a new pharmacotherapy option for postmenopausal osteoporosis. *Curr Med Res Opin* 29:205–216
- Karsenty G, Wagner EF (2002) Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* 2:389–406
- Kim JH, Kim N (2014) Regulation of NFATc1 in osteoclast differentiation. *J Bone Metab* 21:233–241
- Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli E, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93:165–176
- Lee ZH, Kim HH (2003) Signal transduction by receptor activator of nuclear factor kappa B in osteoclasts. *Biochem Biophys Res Commun* 305:211–214
- Lee JY, Kim KJ, Kim J, Choi SU, Kim SH, Ryu SY (2016) Anti-osteoclastogenic effects of isoquinoline alkaloids from the rhizome extract of *Sinomenium acutum*. *Arch Pharm Res* 39:713–720
- Lin HH, Faunce DE, Stacey M, Terajewicz A, Nakamura T, Zhang-Hoover J, Kerley M, Mucenski ML, Gordon S, Stein-Streilein J (2005) The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. *J Exp Med* 201:1615–1625
- Papapoulos SE (2000) Bisphosphonates in the treatment of osteoporosis. *Ann Med Interne (Paris)* 151:504–510
- Quinn JM, Whitty GA, Byrne RJ, Gillespie MT, Hamilton JA (2002) The generation of highly enriched osteoclast-lineage cell populations. *Bone* 30:164–170
- Rodan GA, Martin TJ (2000) Therapeutic approaches to bone diseases. *Science* 289:1508–1514
- Sato K, Suematsu A, Nakashima T, Takemoto-Kimura S, Aoki K, Morishita Y, Asahara H, Ohya K, Yamaguchi A, Takai T, Kodama T, Chatila TA, Bito H, Takayanagi H (2006) Regulation of osteoclast differentiation and function by the CaMK-CREB pathway. *Nat Med* 12:1410–1416
- Schaller E, Macfarlane AJ, Rupec RA, Gordon S, McKnight AJ, Pfeffer K (2002) Inactivation of the F4/80 glycoprotein in the mouse germ line. *Mol Cell Biol* 22:8035–8043
- Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, Yokochi T, Oda H, Nakamura K, Ida N, Wagner EF, Taniguchi T (2002) RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. *Nature* 416:744–749
- Teitelbaum SL (2000) Bone resorption by osteoclasts. *Science* 289:1504–1508
- Teitelbaum SL, Ross FP (2003) Genetic regulation of osteoclast development and function. *Nat Rev* 4:638–649
- Tinkler SM, Linder JE, Williams DM, Johnson MW (1981) Formation of osteoclasts from blood monocytes during 1 alpha-OH Vit D-stimulated bone resorption in mice. *J Anat* 133:389–396
- Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, Bevers TB, Fehrenbacher L, Pajon ER Jr, Wade JL 3rd, Robidoux A, Margolese RG, James J, Lippman SM, Runowicz CD, Ganz PA, Reis SE, McCaskill-Stevens W, Ford LG, Jordan VC, Wolmark N, National Surgical Adjuvant Breast and Bowel Project (NSABP) (2006) Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. *JAMA* 295:2727–2741
- Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, Choi Y (2006) Osteoimmunology: interplay between the immune system and bone metabolism. *Annu Rev Immunol* 24:33–63
- Wen AY, Sakamoto KM, Miller LS (2010) The role of the transcription factor CREB in immune function. *J Immunol* 185:6413–6419
- Yavropoulou MP, Yovos JG (2008) Osteoclastogenesis—current knowledge and future perspectives. *J Musculoskelet Neuronal Interact* 8:204–216