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Regulatory mechanism of NFATc1 in RANKL-induced osteoclast activation

Insun Song, Jung Ha Kim, Kabsun Kim, Hye Mi Jin, Bang Ung Youn, Nacksung Kim *

National Research Laboratory for Regulation of Bone Metabolism and Disease, Medical Research Center for Gene Regulation, Brain Korea 21, Chonnam National University Medical School, 5 Hak-Dong, Dong-Ku, Gwangju 501-746, Republic of Korea

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ABSTRACT

NFATc1 is a master regulator of RANKL-induced osteoclast differentiation and herein we investigate the regulatory mechanism of NFATc1 in osteoclast activation. Inactivation of NFATc1 strongly attenuates RANKL-induced bone resorption and overexpression of a constitutively active form of NFATc1 in osteoclasts induces formation of actin rings and resorption pits on dentin slices. We demonstrate that NFATc1 binds directly to the promoter regions of its target genes and induces expression of various genes, including LTBP3, ClC7, cathepsin K, MMP9, and c-Src, which are key players in bone resorption. Thus, NFATc1 is essential for RANKL-induced osteoclast activation via up-regulation of osteoclast-activating genes.
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1. Introduction

The balance between osteoblasts and osteoclasts is essential for normal skeletal formation and maintenance as well as mineral homeostasis [1,2]. Osteoclasts are large multinucleated cells derived from monocyte/macrophage precursors which can resorb bone. Activation of these polykaryon cells is required for proper bone resorption mediated by pro-resorptive cytokines such as IL-1.

Receptor activator of NF-κB ligand (RANKL) plays a key role in osteoclast differentiation, fusion, and activation [3]. RANKL induces c-Fos expression during osteoclastogenesis and the binding of c-Fos to the NFATc1 promoter region leads to NFATc1 gene expression. NFATc1 auto-amplifies its own expression by binding to its own promoter region resulting in robust NFATc1 expression during RANKL-induced osteoclast differentiation [4].

NFATc1-deficient embryonic stem cells fail to differentiate into osteoclasts in response to RANKL [5] suggesting that NFATc1 acts as a key modulator of osteoclastogenesis. Recently, we have demonstrated that NFATc1 plays a pivotal role in the osteoclast fusion process via up-regulation of the dendritic cell-specific transmembrane protein (DC-STAMP) and the d2 isoform of vacuolar ATPase V_0 domain (Atp6v0d2) [6]. Recently, NFATc1 has been shown to activate Atp6v0d2 via co-activation with MEF2 and Mitf [7]. In addition to NFATc1's role in osteoclast differentiation and fusion, there is mounting evidence that NFATc1 also regulates osteoclast activation. It has been shown that activation of NFATc1 increases osteoclast formation and activation in vivo [8]. Although the regulatory mechanism of NFATc1 during osteoclast differentiation and fusion is well defined, little is known about mechanism by which NFATc1 regulates osteoclast activation.

Previous studies demonstrated that osteoclast activation is mediated by various genes, including c-Src [9], chloride channel 7 (ClC7) [10], cathepsin K [11], latent transforming growth factor beta binding protein 3 (LTBP3) [12], and matrix metallopeptidase 9 (MMP9) [13]. Besides Atp6i [14], TRAP, integrins, and carbonic anhydrase II are directly or indirectly involved in bone resorption [15,16].

Herein, we provide clear evidence that NFATc1 plays a key role in RANKL-induced osteoclast activation, and that NFATc1 directly regulates various genes associated with osteoclast activation such as: LTBP3, ClC7, cathepsin K, MMP9, and c-Src.

2. Materials and methods

2.1. Reagents

All cell culture media and supplements were obtained from HyClone (South Logan, UT). Soluble recombinant mouse RANKL was purified from insect cells as described previously [17]. Recombinant human M-CSF was a gift from D. Fremont (Washington University, St. Louis, MO). TRIZOL was obtained from Invitrogen (San Diego, CA). Cyclosporin A was purchased from Calbiochem (San Diego, CA).

* Corresponding author. Fax: +82 62 223 4018.
E-mail addresses: nacksung@chonnam.ac.kr, nacksungkim@hotmail.com (N. Kim).

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2.2. Constructs

The 5 kb promoter regions of LTBP3, ClC7, cathepsin K, c-Src and MMP9 were prepared from DNA extracted from RAW264.7 cells by long-range PCR amplification with Expand Long Template PCR System (Roche Applied Sciences, Mannheim Germany) according to the manufacturer's instructions using following primers: LTBP3 sense, CTC GAG AAA TGA CAT AAC CAT GTA AAC ATT G; LTBP3 antisense, AAG CTT GCA GGG GAT CGA GTG TTG GTA GCA G; ClC7 sense, GGT ACC TTC AAA AGC AAG AAA GTG GCA CTG; ClC7 antisense, CTC GAG GAC CAA ACT CAA CAA GAA CAG; cathepsin K sense, GGT ACC AAT GAA ACT TAC TGT GAG ACT GAG; cathepsin K antisense, CTC GAG TCG GAT AGG AAG CGT GGC TAC TGT G; c-Src sense, GGT ACC ATT TGC TTT ACT TCG CAC CCA CTG G; c-Src antisense, CTC GAG CGG AGA GAC AGA CCG AGA GAA AGG; MMP9 sense, AGG CGA GTG AAG TGG TAC CCA CTG; MMP9 antisense, CTC GAG AAA GCC AGG AGA GCC AGG AGC AG. The PCR products were then cloned into the pGL2 basic luciferase vector (Promega, Madison, WI). The OSCAR reporter vector and expression vectors for NFATc1 were previously described [18].

2.3. Osteoclast formation

Murine osteoclasts were prepared from bone marrow cells as previously described [19]. In brief, bone marrow cells were cultured in α-minimal essential medium (α-MEM) containing 10% fetal bovine serum (FBS) with M-CSF (5 ng/ml) for 16 h. Non-adherent cells were harvested and cultured for 3 days in the presence of M-CSF (30 ng/ml). Floating cells were removed and adherent cells (bone marrow-derived macrophages; BMMs) were used as osteoclast precursors. To generate osteoclasts, BMMs (3×10^5 cells/well/96-well plate) were cultured with M-CSF (30 ng/ml) and RANKL (100 ng/ml) for 4 days. Cultured cells were fixed and stained for TRAP as previously described [20]. TRAP-positive multinuclear cells [TRAP(+) MNCs], containing more than three nuclei, were counted as osteoclasts.

2.4. Retroviral infection

To generate retroviral supernatant, retroviral vectors were transfected into the Plat E packaging cell line (3×10^5 cells/well/6-well plate) using FuGENE 6 (Roche Applied Sciences) [6,18]. Viral supernatant was collected from cultured media 24–48 h after transfection. BMMs (2×10^6 cells/well/6-well plate) were incubated with viral supernatant for 8 h in the presence of 10 μg/ml polybrene (Sigma–Aldrich, St., Louis, MO). After removing the viral supernatant, BMMs were used to generate osteoclasts.

2.5. Pit formation assay and actin ring staining

To generate mature osteoclasts, calvarial osteoblasts (1×10^6 cells) and bone marrow cells (1×10^7 cells) were co-cultured for 6 days with vitamin D_3 (10^{-8} M) and PGE_2 (10^{-6} M) in 10 cm plates. Mature osteoclasts isolated from cultured cells were plated on dentine slices and cultured for an additional 24 h with M-CSF (30 ng/ml) alone or with M-CSF/RANKL (100 ng/ml) in the absence or presence of cyclosporine A (CsA, 5 μg/ml) as indicated in Fig. 1. For the experiments shown in Fig. 2, BMMs were transduced with either a control vector or a constitutively active NFATc1 (Ca-NFATc1) retrovirus. Cells were plated on dentine slices and cultured for an additional 6 days in the presence of M-CSF. The slices were recovered, cleaned by ultrasonication in 0.5 M NH_4OH to remove adherent cells, and stained with accustain (Sigma–Aldrich) to visualize resorption pits. For actin ring staining, cultured cells were fixed and stained with Texas red-X phalloidin (Invitrogen).

2.6. Semiquantitative RT-PCR

RT-PCR was performed as previously described [18]. In brief, RT-PCR analysis was performed using cDNA from cultured samples. Total RNA was extracted from cultured cells using TRIZOL. First strand cDNA was transcribed from 1 μg of RNA using Superscript RT (Invitrogen) following the protocol provided by the supplier. Primer sequences of TRAP, OSCAR, NFATc1, HPRT, c-Src, cathepsin K, integrins, and ATP6i were previously described [6,18].

2.7. Luciferase assay

For transfection of reporter plasmids, HEK 293T cells were plated on 6-well plates at a density of 2×10^5 cells/well 1 day before transfection. A total of 500 ng of plasmid DNA was mixed with FuGENE 6 and transfected into the cells following the manufacturer's protocol [18]. The amount of transfected DNA was held constant to 500 ng by addition of empty vector DNA where necessary. After 48 h of transfection, the cells were washed twice with PBS buffer and then lysed in reporter lysis buffer. Luciferase activity was measured with a luciferase assay system (Promega) according to the manufacturer's instructions. Luciferase activity was measured in triplicate, averaged, and then normalized with β-galactosidase activity using

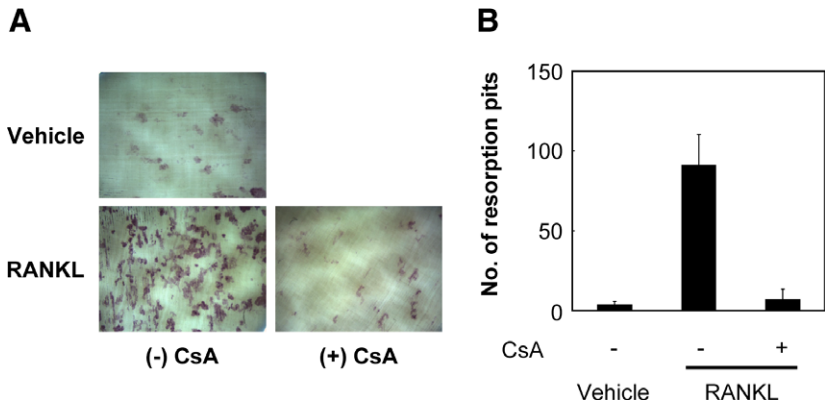

Fig. 1. The effect of NFATc1 inactivation in RANKL-induced bone resorption. Calvarial osteoblasts and bone marrow cells were co-cultured for 6 days with $1.25(OH)_2D_3$ (10^{-8} M) and PGE_2 (10^{-6} M). Mature osteoclasts were isolated from the cultured cells, plated on dentin slices, and cultured for an additional 24 h with M-CSF (30 ng/ml) alone or M-CSF/RANKL (100 ng/ml) in the absence or presence of cyclosporine A (CsA, 5 μg/ml). (A) The slices were stained with hematoxylin to visualize resorption pits. (B) Number of pits formed on dentin slices was counted. Results are representative of at least three independent sets of similar experiments. Data are expressed as means ± S.D. of quadruplicate cultures.

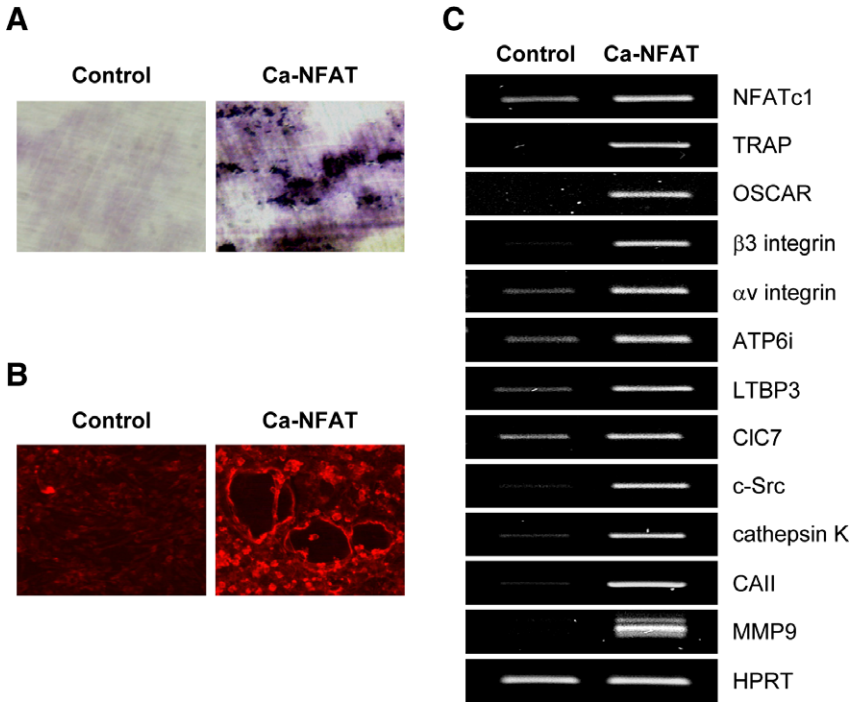

Fig. 2. The effect of NFATc1 overexpression on osteoclast-associated gene expression. BMMs were transduced with pMX-IRES-EGFP (control) or constitutively active NFATc1 (Ca-NFAT). (A) Transduced BMMs were cultured for 6 days with M-CSF alone on dentin slices. The slices were stained with hematoxylin to visualize resorption pits. (B) Transduced BMMs were cultured for 6 days with M-CSF alone. Cultured cells were fixed and stained with Texas red-X phalloidin. (C) Total RNA was collected from cultured cells and analyzed by RT-PCR to assess the expression levels of the indicated genes.

o-nitrophenyl-β-D-galactopyranoside (Sigma–Aldrich) as a substrate.

2.9. Chromatin immunoprecipitation (ChIP) assay

A ChIP assay was performed with a ChIP kit (Upstate Biotechnologies, Temecula, CA) as previously described [18]. The precipitated DNA was subjected to PCR amplification with primers specific for the LTBP3, ClC7, cathepsin K, c-Src and MMP9 promoter regions containing NFATc1-binding sites. The following primers were used for PCR: LTBP3 sense, AAT GGG CAA AAG TTA ACA GAC CT; LTBP3 antisense, AGG GAG AAG TGA GCA GTT GTT TT; ClC7 sense, TTG TTG GGA AAG TTT TTA TCT ATC G; ClC7 antisense, CTA GTC CTC CAA GAG ATG ATA TGG A; cathepsin K sense, AGT CTC CTA GAT CAA CAG CCT TTT T; cathepsin K antisense, TCT TCA GAA GCC CTG TAA TAA CTG T; c-Src sense, AAA GTT TAT GCT TTG AAG CTC TCT G; c-Src antisense, CTC CAT GCC TAA GAT TTG TCT AAT G; MMP9 sense, AAG CTT TCC TGA GTG GAG CAG; MMP9 antisense, GCT CTT TGA GGC AGG ATT TG.

3. Results

3.1. NFATc1 inactivation attenuates RANKL-induced bone resorption

To investigate whether NFATc1 plays a role in osteoclast activation, the effect of NFATc1 inactivation on bone resorption was examined. Mature osteoclasts isolated from co-culture of primary calvarial osteoblasts and bone marrow cells were plated on dentin slices and were cultured for an additional 24 h. Compared to control cultures, RANKL strongly induced pit formation (Fig. 1A and B). However, RANKL-induced bone resorption was strongly attenuated by cyclosporine A (CsA) which inhibits calcineurin activity [21], and subsequently inhibits NFATc1 activation. These data suggest that NFATc1 acts as an important regulator of osteoclast activation.

3.2. Exogenous expression of NFATc1 induces formation of actin rings and resoprtion pits

It has been shown that overexpression of constitutively active NFATc1 in BMMs induces osteoclast differentiation and fusion even in the absence of RANKL [5]. Therefore, we examined whether

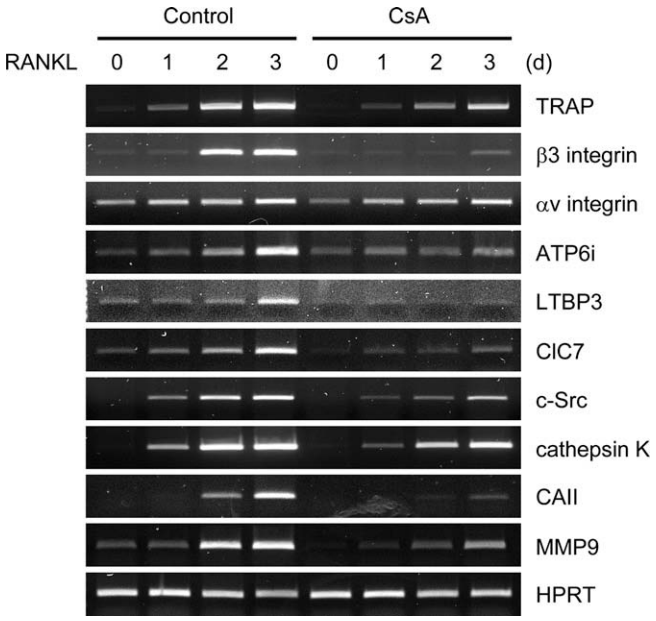

Fig. 3. The effect of NFATc1 inactivation on RANKL-induced up-regulation of genes involved in osteoclast activation. BMMs were cultured with M-CSF and RANKL in the absence or presence of CsA for the indicated times. Total RNA was collected from each time point. RT-PCR was performed to assess the expression of the indicated genes.

osteoclasts cells over-expressing NFATc1 could resorb bone. Compared to control cells, exogeneous expression of NFATc1 in osteoclasts strongly induced pit formation (Fig. 2A). In addition, NFATc1 over-expressing osteoclasts caused the formation of actin rings which is important for bone resorption. These data indicate that NFATc1 alone is sufficient to induce bone resorption.

Next, we examined the expression patterns of various genes by RT-PCR and found that NFATc1 overexpression of induced expression of various osteoclast-associated genes such as tartrate-resistant acid phosphatase (TRAP) and osteoclast-associated receptor (OSCAR); as well as known osteoclast-activating genes such as ATP6i, LTBP3, ClC7, c-Src, cathepsin K, carbonic anhydrase II, and MMP9 (Fig. 2C). Together, these data imply that NFATc1 is an important mediator of osteoclast activation through up-regulation of various osteoclast-activating genes.

3.3. Inactivation of NFATc1 attenuates RANKL-induced up-regulation of osteoclast-activating genes

Given that RANKL induces osteoclast activation, we examined whether RANKL could induce the expression of genes known to be associated with osteoclast activation and function. As shown in Fig. 3, RANKL strongly induced the expression of various osteoclast-activating genes, but this expression was abrogated by treatment with CsA (Fig. 3). These data suggest that NFATc1 plays a pivotal role in RANKL-induced up-regulation of key genes associated with osteoclast activation.

3.4. NFATc1 directly induces the expression of key genes associated with osteoclast activation

To investigate whether NFATc1 directly causes the expression of genes associated with osteoclast activation, we used a reporter assay involving transient transfections into HEK 293T cells with a reporter constructs containing the 5 kb promoter regions of LTBP3, ClC7, c-Src, cathepsin K, and MMP9. NFATc1 increased luciferase activity of these genes as well as OSCAR in a dose-dependent manner (Fig. 4A).

To determine whether NFATc1 binds directly to the promoter regions of these genes endogenously in osteoclasts, we performed a chromatin immunoprecipitation (ChIP) assay. After immunoprecipitation with anti-NFATc1 or control antibody from BMMs or osteoclasts, PCR was performed with primers specific for the promoter regions of genes containing NFATc1-binding sites. A single

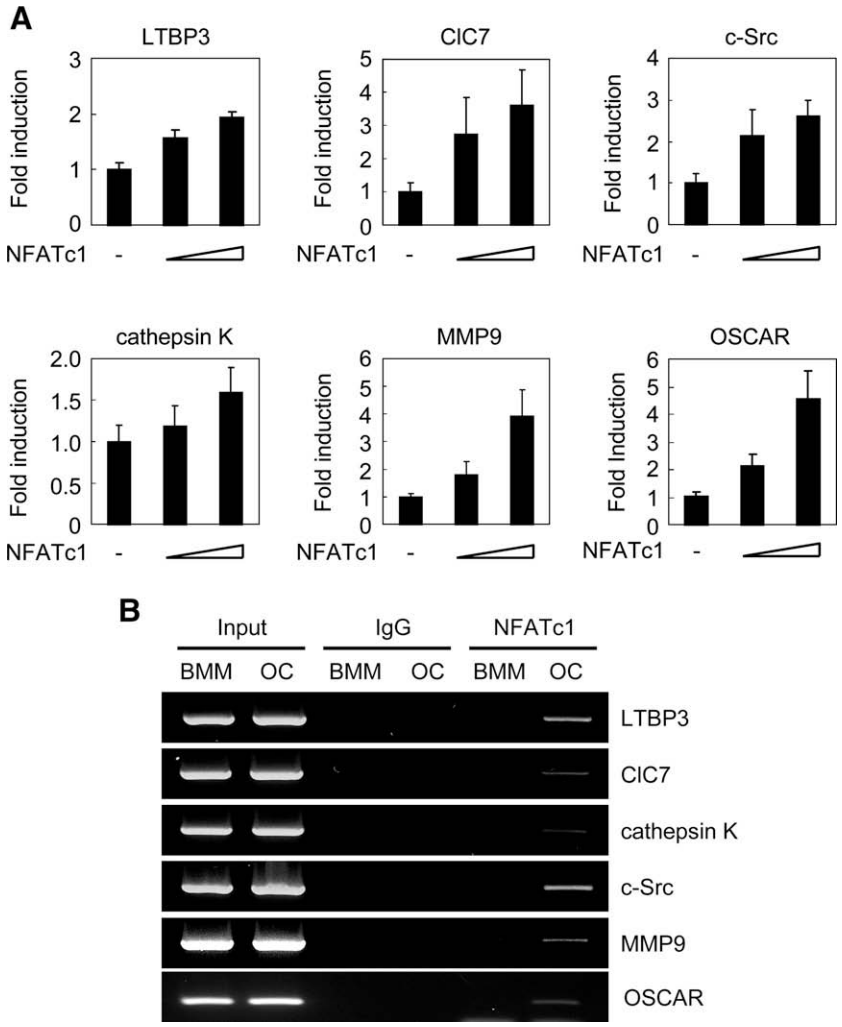

Fig. 4. NFATc1 directly binds to the promoter region of genes associated with osteoclast activation. (A) HEK 293T cells were co-transfected with various luciferase reporter vectors and increasing amounts of NFATc1 (300 and 600 ng). Results are representative of at least three independent sets of similar experiments. Data represent the means and S.D. of triplicate samples. (B) ChIP assay. BMMs were cultured with M-CSF alone (BMM) or M-CSF/RANKL (osteoclasts, OC) for 3 days before cross-linking. Samples were immune-precipitated with control IgG or anti-NFATc1 antibodies and subjected to PCR amplification with primers specific for NFATc1-binding sites in the promoter regions of the indicated genes.

PCR product was obtained from osteoclast DNA after immunoprecipitation with anti-NFATc1 antibody (Fig. 4B). These data indicate that NFATc1 can directly regulate genes associated with osteoclast activation.

4. Discussion

Based on data from osteopetrotic mice, many genes appear to be involved in bone degradation mediated by osteoclasts. Fully differentiated osteoclasts recognize and physically attach to bone during the degradation process. Mice lacking β3 integrin have an osteosclerotic phenotype due to abnormal cytoskeleton organization in osteoclasts [22]. Consistent with previous results [23], we show that NFATc1 induces the expression of β3 integrin and c-Src. Osteoclasts deficient for c-Src exhibit reduced motility and abnormal organization of the ruffle border and lack the cytoskeletal elements necessary for bone resorption. Our current data suggest that NFATc1 is a key regulator of osteoclast migration and adhesion to bone surface through induction of β3 integrin and c-Src.

After attaching to bone, osteoclasts extrude protons and chloride ions through proton and chloride transporters onto the bone surface and dissolve the inorganic components of the bone matrix. Carbonic anhydrase II, ClC7 chloride channel, and Atp6i, the osteoclast-specific proton pump a3 subunit, are important for the process of proton secretion. Mice deficient for these genes develop severe osteopetrosis because their osteoclasts cannot secrete acid and thus cannot dissolve bone [10,14,24,25]. Thus, our data indicate that NFATc1 also regulates osteoclast acidification.

Once mineralized compartments are dissolved by acidification, osteoclasts degrade the organic matrix in bone by the secretion of proteolytic enzymes such as cathepsin K and matrix metalloproteinases (MMPs). The osteopetrotic cathepsin K-deficient mice generate ruffled membranes and mobilize bone mineral, but fail to degrade the collagen fibers [26]. In addition, gelatinases such as MMP9 have been shown to be involved in cytokine-induced degradation of bone matrix [27]. LTBP3, an extracellular matrix glycoprotein, is essential for activation of TGF-β and LTBP3 null mice exhibit abnormally high bone mass due to defective bone resorption [12]. Taken together, these data indicate that LTBP3 regulates the level of TGF-β in bone and cartilage, which leads to compromised osteoclast function. Thus, our observation suggests that NFATc1 modulates degradation of organic matrix through up-regulation of cathepsin K, MMP9, and LTBP3.

Various transcription factors, including Mitf, PU.1 and NFATc1, are involved in RANKL-induced osteoclastogenesis. Among these transcription factors, NFATc1 appears to play a role in regulating downstream gene expression in mature osteoclasts; whereas Mitf and PU.1 are responsible for initiating induction of target genes during RANKL-induced osteoclastogenesis [28]. Consistent with this, our data clearly demonstrate that NFATc1 is important for activation of mature osteoclasts through direct regulation of various genes involved in osteoclast activation.

In this study, we provide the first line of evidence that NFATc1 plays a pivotal role in osteoclast activation via up-regulation of various genes responsible for osteoclast adhesion, migration, acidification, degradation of inorganic and organic bone matrix. Therefore, our data suggest that NFATc1 is a master modulator for osteoclast activation as well as osteoclast fusion and maturation.

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References

[1] Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M.T. and Martin, T.J. (1999) Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr. Rev. 20, 345–357.
[2] Walsh, M.C., Kim, N., Kadono, Y., Rho, J., Lee, S.Y., Lorenzo, J. and Choi, Y. (2006) Osteoimmunology: interplay between the immune system and bone metabolism. Annu. Rev. Immunol. 24, 33–63.
[3] Boyle, W.J., Simonet, W.S. and Lacey, D.L. (2003) Osteoclast differentiation and activation. Nature 423, 337–342.
[4] Asagiri, M. et al. (2005) Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. J. Exp. Med. 202, 1261–1269.
[5] Takayanagi, H. et al. (2002) Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev. Cell 3, 889–901.
[6] Kim, K., Lee, S.H., Ha Kim, J., Choi, Y. and Kim, N. (2008) NFATc1 induces osteoclast fusion via up-regulation of Atp6v0d2 and the dendritic cell-specific transmembrane protein (DC-STAMP). Mol. Endocrinol. 22, 176–185.
[7] Feng, H. et al. (2009) Myocyte enhancer factor 2 and microphthalmia-associated transcription factor cooperate with NFATc1 to transactivate the V-ATPase d2 promoter during RANKL-induced osteoclastogenesis. J. Biol. Chem. 284, 14667–14676.
[8] Ikeda, F., Nishimura, R., Matsubara, T., Hata, K., Reddy, S.V. and Yoneda, T. (2006) Activation of NFAT signal in vivo leads to osteopenia associated with increased osteoclastogenesis and bone-resorbing activity. J. Immunol. 177, 2384–2390.
[9] Soriano, P., Montgomery, C., Geske, R. and Bradley, A. (1991) Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 64, 693–702.
[10] Kornak, U. et al. (2001) Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 104, 205–215.
[11] Gowen, M. et al. (1999) Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. J. Bone Miner. Res. 14, 1654–1663.
[12] Dabovic, B., Levasseur, R., Zambuto, L., Chen, Y., Karsenty, G. and Rifkin, D.B. (2005) Osteopetrosis-like phenotype in latent TGF-beta binding protein 3 deficient mice. Bone 37, 25–31.
[13] Ishibashi, O., Niwa, S., Kadoyama, K. and Inui, T. (2006) MMP-9 antisense oligodeoxynucleotide exerts an inhibitory effect on osteoclastic bone resorption by suppressing cell migration. Life Sci. 79, 1657–1660.
[14] Li, Y.P., Chen, W., Liang, Y., Li, E. and Stashenko, P. (1999) Atp6i-deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nat. Genet. 23, 447–451.
[15] Sly, W.S., Hewett-Emmett, D., Whyte, M.P., Yu, Y.S. and Tashian, R.E. (1983) Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc. Natl. Acad. Sci. USA 80, 2752–2756.
[16] Teitelbaum, S.L. (2000) Bone resorption by osteoclasts. Science 289, 1504–1508.
[17] Kim, N., Takami, M., Rho, J., Josien, R. and Choi, Y. (2002) A novel member of the leukocyte receptor complex regulates osteoclast differentiation. J. Exp. Med. 195, 201–209.
[18] Kim, K. et al. (2005) Nuclear factor of activated T cells c1 induces osteoclast-associated receptor gene expression during tumor necrosis factor-related activation-induced cytokine-mediated osteoclastogenesis. J. Biol. Chem. 280, 35209–35216.
[19] Kim, K., Kim, J.H., Lee, J., Jin, H.M., Kook, H., Kim, K.K., Lee, S.Y. and Kim, N. (2007) MafB negatively regulates RANKL-mediated osteoclast differentiation. Blood 109, 3253–3259.
[20] Suda, T., Jimi, E., Nakamura, I. and Takahashi, N. (1997) Role of 1 alpha, 25-dihydroxyvitamin D3 in osteoclast differentiation and function. Meth. Enzymol. 282, 223–235.
[21] Liu, J., Farmer Jr., J.D., Lane, W.S., Friedman, J., Weissman, I. and Schreiber, S.L. (1991) Calcineurin is a common target of cyclophilin–cyclosporin A and FKBP-FK506 complexes. Cell 66, 807–815.
[22] McHugh, K.P. et al. (2000) Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. J. Clin. Invest. 105, 433–440.
[23] Crotti, T.N., Flannery, M., Walsh, N.C., Fleming, J.D., Goldring, S.R. and McHugh, K.P. (2006) NFATc1 regulation of the human beta3 integrin promoter in osteoclast differentiation. Gene 372, 92–102.
[24] Rousselle, A.V. and Heymann, D. (2002) Osteoclastic acidification pathways during bone resorption. Bone 30, 533–540.
[25] Sly, W.S. and Hu, P.Y. (1995) Human carbonic anhydrases and carbonic anhydrase deficiencies. Annu. Rev. Biochem. 64, 375–401.

[26] Nishi, Y., Atley, L., Eyre, D.E., Edelson, J.G., Superti-Furga, A., Yasuda, T., Desnick, R.J. and Gelb, B.D. (1999) Determination of bone markers in pycnodysostosis: effects of cathepsin K deficiency on bone matrix degradation. J. Bone Miner. Res. 14, 1902–1908.
[27] Kusano, K., Miyaura, C., Inada, M., Tamura, T., Ito, A., Nagase, H., Kamoi, K. and Suda, T. (1998) Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. Endocrinology 139, 1338–1345.
[28] Sharma, S.M., Bronisz, A., Hu, R., Patel, K., Mansky, K.C., Sif, S. and Ostrowski, M.C. (2007) MITF and PU.1 recruit p38 MAPK and NFATc1 to target genes during osteoclast differentiation. J. Biol. Chem. 282, 15921–15929.