Defective Entry into Mitosis 1 (Dim1) Negatively Regulates Osteoclastogenesis by Inhibiting the Expression of Nuclear Factor of Activated T-cells, Cytoplasmic, Calcineurin-dependent 1 (NFATc1)

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Background: The potential role of Dim1 in RANKL-mediated induction of osteoclast differentiation is unknown. Results: Dim1 directly interacts with c-Fos to diminish the expression of NFATc1 target genes that are essential for RANKL-induced osteoclastogenesis. Conclusion: Dim1 functions as a negative regulator of RANKL-induced osteoclast differentiation. Significance: Understanding of the inhibitory role of Dim1 in RANKL-induced osteoclastogenesis might provide therapeutic benefits for treating various types of bone diseases.

Bone remodeling is a continuous process of osteoblastic bone formation and osteoclastic bone resorption to maintain normal bone mass. NFATc1 is the master regulator of osteoclastogenesis and transcriptionally activated by c-Fos and NF-κB in response to receptor activator of NF-κB ligand (RANKL) treatment. Defective entry into mitosis 1 (Dim1) is a nuclear protein that is implicated in pre-mRNA splicing and cell cycle progression, but the possible role of Dim1 in regulating other cellular processes remains unknown. Here, we demonstrate that Dim1 attenuates RANKL-induced osteoclastogenesis by targeting NFATc1 signaling pathway. Expression levels of Dim1 and NFATc1 are significantly increased during the formation of multinucleated osteoclasts. RNAi-mediated knockdown of Dim1 markedly enhances the expression of NFATc1 and its target genes, leading to the increase of RANKL-induced osteoclastogenesis in bone marrow-derived macrophages. Conversely, ectopic expression of Dim1 decreases RANKL-induced osteoclast differentiation by silencing NFATc1 and its target genes, further linking Dim1 to the dynamic regulation of osteoclastogenesis. Consistent with this notion, ChIP and interaction studies show that Dim1 directly associates with c-Fos and prevents c-Fos from binding to the NFATc1 promoter, resulting in targeted inactivation of the NFATc1 gene. Therefore, our studies reveal an unrecognized role for Dim1 as a master modulator of osteoclast differentiation, as well as the molecular mechanism underlying its repressive action toward osteoclastogenesis.

New bone formation and maintenance in vertebrate animals are carried out by the coordinated action of osteoblasts and osteoclasts. Osteoblasts are mesenchymal stem cells that form bone matrix, whereas osteoclasts are large multinucleated cells whose function is to resorb bone matrix (1–3). The excess formation or activity of osteoclasts in humans leads to many pathological bone diseases, including osteoporosis, rheumatoid arthritis, Paget’s disease, and tumor bone metastases (4–6). Osteoclast differentiation is triggered by the stimulation of receptor activator of NF-κB ligand (RANKL), also called TRANCE, TNSF11, OPGL, and ODF, which is expressed as a membrane-bound protein in osteoblasts and provides osteoclasc-specific differentiation signals (7–10). RANKL binds to its cognate receptor RANK on pre-osteoclast cell membrane and initiates multiple signal transduction pathways to turn on the expression of several transcription factors such as NF-κB, c-Fos, and NFATc1 in precursor cells (11). Activation of RANKL–RANK signaling leads to the recruitment of the adapter molecule TRAF6, followed by the activation and nuclear translocation of NF-κB (12, 13). When NF-κB is activated, it triggers c-Fos signals, which then induce the expression of NFATc1 (14). After the initial induction, NFATc1 autoamplifies its expression by binding to its own promoter, induces the expression of a number of genes, and activates differentiation of osteoclast, leading to bone resorption and remodeling (15–19).

The abbreviations used are: RANKL, receptor activator of NF-κB ligand; Dim1, defective entry into mitosis 1; BM, bone marrow; BMM, bone marrow-derived macrophage; TRAP, tartrate-resistant acid phosphatase; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium; qRT, quantitative reverse transcription.
Dim1 is a small protein with the molecular mass of 15 kDa and was initially identified in fission yeast as an essential protein for cell cycle regulation as well as for chromosome segregation during mitosis (20). Dim1 is extraordinarily well conserved throughout the eukaryotic kingdom and is present in numerous species including mammals, plants, and yeasts, with ~80% sequence identity throughout the entire length of 142 amino acids (21, 22). Dim1 has been reported to interact with components of pre-mRNA splicing machinery such as hnRNPF, hnRNP-H, and the RNA-binding protein Npw38/PQBP-1 (23). Under some circumstances, Dim1 has effects on cell cycle progression via its role in the control of pre-mRNA splicing of proteins responsible for accurate cell cycle progression. The three-dimensional structure of Dim1 has been determined by both nuclear magnetic resonance (21) and x-ray crystallography (24). The results indicate that Dim1 contains a common thioredoxin-like fold characterized by a four-stranded β-sheet comprising pairs of parallel and antiparallel strands flanked by three α-helices with a C-terminal extension. In addition to its function as a cell cycle regulator, Dim1 seems to participate in regulating other cellular reactions, such as host-virus interaction and viral replication, although the details remain largely unknown at present (25).

Here, we demonstrate that Dim1 functions as a negative regulator of RANKL-induced osteoclast differentiation. Specifically, Dim1 directly interacts with c-Fos and suppresses c-Fos transcriptional activity toward the NFATc1 gene, thereby diminishing the expression of NFATc1 target genes and inhibiting RANKL-induced osteoclastogenesis. Consistent with these results, Dim1-deficient osteoclast precursors exhibit increased expression of NFATc1 target genes and show up-regulation of RANKL-mediated induction of osteoclast differentiation. Our studies uncover a novel role for Dim1 in regulating RANKL-induced osteoclastogenesis and may provide the basis for novel therapeutic approaches to various types of bone diseases.

**EXPERIMENTAL PROCEDURES**

*Plasmids and Antibodies—* For mammalian expression of Dim1, FLAG-Dim1 cDNA was amplified by PCR and inserted into retroviral expression vector pMX. To generate GST-Dim1, Dim1 cDNA was ligated into pGEX4T-1 vector. Antibodies for c-Fos, Dim1, HA, NFATc1, and NF-κB p65 were from Santa Cruz Biotechnology; antibodies for actin and FLAG were from Sigma; antibody for Mitf was from Active Motif.

*Osteoclast Formation—* Bone marrow (BM) cells were collected by flushing femurs and tibias from 6- to 8-week-old C57BL/6 mice. Cells were cultured in α-minimum essential medium (α-MEM) supplemented with 10% FBS and M-CSF (5 ng/ml) for 16 h. Non-adherent cells were harvested and cultured with M-CSF (30 ng/ml) for 3 days. Floating cells were removed and adherent cells were used as bone marrow-derived macrophage (BMM) cells. To generate osteoclasts, BMMs were cultured in the presence of M-CSF (30 ng/ml) and RANKL (50 ng/ml). On day 3, the cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using the acid phosphatase, leucocyte kit (Sigma). TRAP-positive multinucleated cells containing three or more nuclei are counted as osteoclasts under a light microscope.

**Protein-Protein Interactions—* For* in vitro pulldown assays, whole cell lysates from 293T cells expressing Mitf, c-Fos, and NF-κB p65 were incubated with GST-Dim1 (2 μg) immobilized on glutathione-Sepharose beads in 750 μl of binding buffer (20 mM HEPES-KOH, pH 7.9, 0.5 mM EDTA, 200 mM NaCl, 1 mM dithiothreitol, 10% glycerol, and 0.1% Nonidet P-40) for 16 h at 4 °C. After washing beads three times with washing buffer (20 mM HEPES-KOH, pH 7.9, 0.5 mM EDTA, 250 mM NaCl, 1 mM dithiothreitol, 10% glycerol, and 0.1% Nonidet P-40), bound proteins were detected by immunoblotting. For *in vivo* interaction studies, RAW 264.7 cells were stably infected with retroviral vectors encoding FLAG-Dim1. Cell lysates were subjected to anti-FLAG immunoprecipitation, and the bound proteins were analyzed by immunoblotting.

**Lentiviral-mediated RNA Interference—* For* shRNA-based knockdown, DNA oligonucleotides encoding shRNA specific for Dim1 mRNA (5'- CAAGCAAGAAATGGTTGACAT-3') were annealed and ligated into the lentiviral expression vector pLKO.1 (Addgene). Lentivirus particles were generated in 293T cells by co-transfecting plasmids encoding VSV-G, NL-BH, and the shRNA. For Dim1 knockdown, BMM cells were infected with these viruses and selected with puromycin (2 μg/ml) for 3 days. After selection, BMM cells were cultured for additional 3 days in the presence of M-CSF (30 ng/ml) and RANKL (50 ng/ml).

**Retroviral-mediated Gene Transfer—* To generate retroviral particles, pMX-FLAG-Dim1 was transfected into the packaging cell line Plat-E. Viral soup was collected from cultured media 2 days after transfection. BMM cells were infected with viral soup and selected with puromycin (2 μg/ml) for 3 days. After selection, cells were cultured with M-CSF (30 ng/ml) and RANKL (50 ng/ml) for 3 days.

**Cell Proliferation Assays—* Cell proliferation was assessed by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. In brief, BMM cells were seeded in 24-well tissue culture plates at a density of 2 × 10⁴ and treated with the MTT labeling reagent (0.5 mg/ml) at 37 °C for 1 h. The blue MTT formazan precipitate was dissolved with the MTT solvent (0.2 ml) and measured at a wavelength of 570 nm using a microplate reader (Bio-Rad).

**Reporter Gene Assays—* RAW 264.7 cells were plated in 12-well plates at 50% confluence and transfected with reporter plasmids and expression vectors for c-Fos, NF-κB p65, and/or Dim1 in the presence or absence of RANKL (30 ng/ml) for 24 h. Cells were lysed in Reporter Lysis buffer (Promega) and assayed for luciferase activity using Plate Chameleon (Hidex).

**Microarray and qRT-PCR—* BMM cells were treated with M-CSF and RANKL for 0 and 3 days. Total RNA was isolated and analyzed by gene expression microarray using the MouseRef-8 Expression BeadChip (version 2.0). Differential gene expression analysis was carried out using the ArrayPipe software. Genes that are up-regulated with RANKL treatment in BMM cells were functionally analyzed in the context of gene ontology and molecular networks by using Ingenuity Pathway Analysis (IPA) software. For quantitative reverse transcription (qRT)-PCR analysis, total RNA was isolated as for microarray and subjected to RT reactions (26). Assays were normalized to β-actin mRNA levels. The following primers were used for
RT-PCR to quantify target gene expression: β-actin (5'-GCAAGTGCTTCTAGGCGGAC-3' and 5'-AAGAAAGGTTGTAACACGCAGC-3'), c-fos (5'-CCAGTCAAGAGCATCCAA-3' and 5'-AAATGATGAGCCCAGGTA-3'), Ctsk (5'-ACGGAGGCATTGACTCTGAAGATG-3' and 5'-GGAAGCACCAACGAGAGGAGAAAT-3'), Dim1 (5'-CATCGCAGAAAAGGTTAAA-3' and 5'-GGCCCAGTTGATCTTGTTGT-3'), integrin-β3 (5'-GAATGAATGCGCAGCACAGAGC-3' and 5'-ACAGAGACTGGACCGAAACCAC-3'), Nfatc1 (5'-CTCGAAAGACAGCACTGGAGCAT-3' and 5'-CGGCTGCCTTCCGTCTCATAG-3'), OSCAR (5'-CTGCTGGTACGCTCCCAGA-3' and 5'-CCAAGGAGCCAACCTTCGAAA-3'), and GAPDH (5'-GGTCCTCAGTGAAGCCAAAG-3' and 5'-AATGTGTCCGCTGTGATTGAT-3').

ChIP—Mock-depleted or Dim1-depleted BMM cells, either treated or not treated with RANKL, were cross-linked with 1% formaldehyde for 10 min and processed for ChIP. All samples were run in triplicate, and results were averaged. Sequences of the primers used for quantitative real time PCR are as follows: AP-1 binding site (5'-CCGGGAGCCGCATGCAATCTGTTAGTAATT-3' and 5'-GCCGGTGCCCTGGAGAAAGCTACTTCCTCCT-3') and distal region (5'-TCTGAGAGGGAGTGCTGAT-3' and 5'-CTTGCTCGGTGGTATGAT-3').

Accession Number—The NCBI GEO accession number for microarray data reported in this paper is GSE57468.

RESULTS

RANKL-induced Osteoclastogenesis Coincides with High Expression of Dim1 and Nfatc1—Osteoclastogenesis is a complex process that reflects numerous changes in gene expression and cellular pathways. We used BMM cells to study the global transcription network during RANKL-induced osteoclastogenesis. Our initial TRAP staining confirmed that a significant number of multinucleated osteoclast-like cells were formed from BMM cells when cultured in the presence of soluble RANKL (Fig. 1 A). To screen for altered gene expression during osteoclastogenesis, RANKL-treated BMM cells were subjected to gene expression profiling using the MouseRef-8 Expression microarrays (version 2.0). With a fold-change cutoff of >2.0, the expression profiling showed that 1073 genes were activated and 1070 genes were repressed in RANKL-treated cells versus the day 0 control were selected (fold change > 2.0) and were sorted by fold changes. C, BMM cells were cultured for 0, 1, 2, and 3 days in the presence of M-CSF (30 ng/ml) and RANKL (50 ng/ml). Total RNA was isolated from cell lysates and analyzed by qRT-PCR. mRNA levels were normalized against an internal β-actin control for each time point, and values at the zero time point are set to 1. The results shown are mean values from three independent experiments for each time point. **, p < 0.01; ***, p < 0.001. D, whole cell lysates were prepared from M-CSF/RANKL-treated BMM cells as described in C and analyzed by immunoblot with Dim1 and Nfatc1 antibodies. β-Actin was probed as a loading control.
entially expressed in the RANKL-treated cells (supplemental Table S1).

Unexpectedly, our profiling data also indicated that Dim1 expression was up-regulated in RANKL-treated cells relative to its untreated controls (Table S1). Although Dim1 has been mainly characterized as an essential factor in the control of pre-RNA splicing in human cells, these results suggest that Dim1 may play a role in the regulation of osteoclastogenesis. To explore this possibility and validate the microarray data, we analyzed the expression of Dim1, NFATc1, and NFATc1 target genes, which showed the consistent response to RANKL treatment in the gene expression data, by qRT-PCR (Fig. 1C). The results correlated well with those obtained from the gene expression microarrays and demonstrated that RANKL-induced osteoclastogenesis is linked to transcriptional activation of these genes over a period of 3 days. In determining the protein levels of Dim1 and NFATc1 by immunoblot, we again found that RANKL-induced osteoclastogenesis is associated with the up-regulation of Dim1 and NFATc1 expression (Fig. 1D).

Dim1 Exerts Repressive Effects on NFATc1 and Its Target Genes during RANKL-induced Osteoclastogenesis—One of the key observations made in our gene expression profiling is that high-level expression of Dim1 coincides with transcriptional activation of NFATc1 and its target genes, which are critical for RANKL-induced osteoclastogenesis. To assess whether Dim1 directly influences osteoclastogenesis, we first depleted Dim1 in BMM cells and checked its effects on osteoclastic cell growth and the NFATc1 transcription pathway. Immunoblotting confirmed that infection of BMM cells with lentivirus expressing Dim1 shRNA efficiently silenced Dim1 expression (Fig. 2A). Because osteoclastogenesis enhances the expression of Dim1, we expected that Dim1 knockdown would negatively affect NFATc1 transactivation and thus RANKL-induced osteoclastogenesis. Contrary to these expectations, we found that osteoclast development was significantly accelerated in Dim1-depleted BMM cells in the presence of RANKL (Fig. 2B). Moreover, the finding that the proliferation of osteoclast precursors was not affected by Dim1 knockdown suggests that Dim1 inhibits the differentiation, not the proliferation, of pre-osteoclasts (Fig. 2C).
We next determined whether Dim1 would participate in regulating the expression of NFATc1 and its target genes in the context of RANKL-induced osteoclast differentiation by qRT-PCR. Consistent with the results of osteoclastogenesis assays in Fig. 2B, knockdown of Dim1 resulted in higher expression of NFATc1 over 3 days of RANKL treatment (Fig. 2D, NFATc1). Further analyses revealed a similar increase in the expression of the four NFATc1 target genes, which were identified from our gene expression profiling, in Dim1-depleted BMM cells during osteoclast differentiation (Fig. 2D). In consonance with our observations from knockdown experiments, ectopic expression of Dim1 suppressed RANKL-induced osteoclast formation in BMM cells by ~20%, but did not affect the proliferation of BMM cells (Fig. 3, A–C). Expression of NFATc1 was also repressed by Dim1 expression, and the observed repression was paralleled by inactivation of the four NFATc1 target genes (Fig. 3D). These observations are consistent with the hypothesis that Dim1-mediated suppression of RANKL-induced osteoclastogenesis involves transcriptional alterations of NFATc1 and its target genes.

**Dim1 Down-regulates NFATc1 Expression by Antagonizing the Recruitment and Function of c-Fos**—RANKL-induced transcriptional activation of the Nfatc1 gene during osteoclastogenesis is dependent on both c-Fos and NF-κB pathways (26–30). Previous studies identified several factors negatively regulating RANKL-induced osteoclastogenesis by interfering with the DNA-binding activities of c-Fos and NF-κB, thereby reducing the expression of NFATc1 (31, 32). Considering the transrepression activities of Dim1 toward NFATc1 expression, we were interested in investigating whether the observed function of Dim1 in attenuating osteoclastogenesis is the consequence of inhibiting the expression of these upstream signaling components. Therefore, we examined the relative levels of c-Fos and NF-κB p65 subunit in control and Dim1-depleted osteoclastogenic cells by qRT-PCR. Knocking down Dim1 led to a distinct increase in NFATc1 transcription during osteoclast formation (Fig. 4A, NFATc1), but transcription of c-Fos and NF-κB p65 was only weakly affected by Dim1 knockdown (c-fos and p65). Similarly, we did not see any obvious antagonistic regulation of c-fos and NF-κB p65 genes by Dim1 expression during RANKL-
induced osteoclastogenesis (Fig. 4B). Consistent with these results, reporter gene assays showed that Dim1 selectively suppresses NFATC1 transcription without affecting the transcription of c-Fos and NF-κB p65 (Fig. 4C). This observation strongly suggests that it is the function of c-Fos/NF-κB, not their expression, which is disrupted by Dim1 for NFATC1 suppression. To check this possibility, we first sought to determine whether the impact of Dim1 on NFATC1 expression is due to its direct interaction with c-Fos and/or NF-κB. GST-fused Dim1 was immobilized to glutathione-Sepharose beads, and incubated with cell lysates of 293T cells expressing Mitf, c-Fos, or NF-κB p65. The same binding assays were performed with GST alone showing no interaction binding to Dim1. The observed interaction between Dim1 and c-Fos appears to be specific as GST alone showed no interaction with c-Fos. To further validate these in vitro binding results, extracts from RAW 264.7 cells infected with virus expressing FLAG-Dim1 were co-immunoprecipitated with endogenous c-Fos, but failed to show any coprecipitation of Mitf and NF-κB p65 (Fig. 5B).

In an effort to directly assess c-Fos-targeted repressive action of Dim1, we next tested whether Dim1 can inhibit transcriptional activation of transiently transfected reporter plasmids by c-Fos. The expression of the reporter gene bearing c-Fos binding sites was highly activated by transient expression of c-Fos. Because co-expression of Dim1 generated a substantial inactivation of c-Fos-mediated reporter gene transcription (Fig. 5C), our findings suggest that the repressive function of Dim1 is highly dependent on c-Fos. To gain further insight into the mechanisms by which Dim1 modulates the expression of Nfatc1 gene, ChIP assays were performed in control and Dim1-depleted cells using c-Fos antibody. In control cells, a distinct localization of c-Fos at the proximal promoter of Nfatc1 gene was detected in response to RANKL stimulation (Fig. 5D). However, a clear increase in c-Fos occupancy was observed at the promoter region upon knocking down Dim1 by shRNA.
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Taken together, these findings support the view that Dim1 suppresses \textit{Nfatc1} gene transcription by affecting the recruitment of c-Fos to NFATc1 promoter, rather than affecting the expression of c-Fos, during osteoclastogenesis.

DISCUSSION

In the present study, we focused on potential roles of Dim1 in regulating the initial stage of the differentiation process in which quiescent osteoclast precursors are induced to become osteoclasts by RANKL stimulation. Our study indicates that Dim1 plays a critical role in controlling the expression of NFATc1, a pivotal component for osteoclast development, in osteoclast precursors by interfering with the transactivation function of c-Fos, which is an essential transcription factor for NFATc1 activation (26). Whereas Dim1 has no apparent effect on c-Fos expression, it seems to antagonize \textit{Nfatc1} gene transactivation through negatively regulating c-Fos localization at NFATc1 locus. This idea is supported by the fact that Dim1 knockdown caused an increase in c-Fos binding to the NFATc1 promoter. The strong correlation between the cellular levels of Dim1 and the efficiency of \textit{Nfatc1} gene silencing we have observed in Figs. 2 and 3 also fits well with the repression of NFATc1 target genes. This striking finding suggests that direct disruption of transcription integrity via the interaction between Dim1 and c-Fos is specific and significant in the regulation of bone remodeling. This is the first study indicating that Dim1 is a key molecule in determining the differentiation rate of osteoclasts as an upstream effector of RANKL signaling. Our study failed to show the binding of Dim1 to p65 subunit of NF-κB, suggesting that Dim1 is mainly targeting c-Fos to affect osteoclast differentiation. Our results, however, do not rule out the possibility that other signaling transduction pathways may also offer a molecular basis of Dim1-triggered repression of osteoclast differentiation. Thus, to better understand how much the Dim1-mediated regulatory mechanism contributes to osteoclast formation, it will be important to identify other osteoclast-specific genes whose transcription is particularly sensitive to Dim1 expression in future investigations.

Keeping the balance between bone formation and resorption is tightly regulated by various hormones and cytokines in local microenvironments. Aberrant regulation of osteoclast formation and function has been implicated in bone loss with concomitant suppression of bone growth and repair, which can weaken the skeleton and increase the risk of fracture. The controlled manipulation of osteoclastogenesis can be used to arrest such conditions, protect against accelerated bone loss, and preserve bone mass. The association of Dim1 with bone disorders has not been reported so far. Therefore, our present study raises the possibility that Dim1 could be a novel therapeutic tool for osteoclastogenic disorders because of its preferential expression on osteoclast cells as well as its significant inhibitory role in differentiation into mature osteoclasts. In light of this view, Dim1-mediated inactivation of NFATc1 expression described in this study will be very useful for the purpose of gaining further insight on the process to osteoclast formation and treating patients suffering from bone loss or skeletal complications of disease. In addition, osteoclast pathway is known to be regulated by a combination of gene specific and general transcription factors, which collectively maintain normal osteoclastic resorption within an acceptable level. In this respect, investigating how these multiple osteoclastic transcription factors functionally interact with Dim1 will increase our understanding of anti-osteoclastogenic function of Dim1.

In conclusion, we investigated the possible effects of Dim1 on osteoclastogenesis using defined experimental systems. Unexpectedly, our initial characterization of Dim1 demonstrated that Dim1 plays a critical role in controlling RANKL-induced osteoclast differentiation. We extended these findings by showing that c-Fos is the target for the observed function of Dim1 as an inhibitor of RANKL-induced osteoclastogenesis. Thus, our findings argue that the distinct interaction of Dim1 with c-Fos has a critical function in controlling RANKL-induced osteoclast differentiation. It remains a challenge to understand how Dim1 cooperates with other signaling factors that have been reported to be involved in osteoclast differentiation. Identification of additional factors as well as targets for Dim1 will definitely be required for deeper understanding of the molecular basis of disease associated with increased bone resorption.

REFERENCES

1. Karsenty, G., and Wagner, E. F. (2002) Reaching a genetic and molecular understanding of skeletal development. Dev. Cell 2, 389–406
2. Zaidi, M. (2007) Skeletal remodeling in health and disease. Nat. Med. 13, 791–801
3. Zelzer, E., and Olsen, B. R. (2003) The genetic basis for skeletal diseases. Nature 423, 343–348
4. Novack, D. V., and Teitelbaum, S. L. (2008) The osteoclast: friend or foe? Annu. Rev. Pathol. 3, 457–484
5. Rodan, G. A., and Martin, T. J. (2004) Therapeutic approaches to bone diseases. Science 289, 1508–1514
6. Tanaka, S., Nakamura, K., Takahasi, N., and Suda, T. (2005) Role of RANKL in physiological and pathological bone resorption and therapeutics targeting the RANKL-RANK signaling system. Immunol. Rev. 208, 30–49
7. Baron, R. (2004) Arming the osteoclast. Nat. Med. 10, 458–460
8. Nakashima, T., Hayashi, M., Fukunaga, T., Kurata, K., Oh-Hora, M., Feng, J. Q., Bonewald, L. F., Kodama, T., Wutz, A., Wagner, E. F., Penninger, J. M., and Takayanagi, H. (2011) Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat. Med. 17, 1231–1234
9. Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombo, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparella, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J., and Boyle, W. J. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93, 165–176
10. Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N., and Suda, T. (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc. Natl. Acad. Sci. U.S.A. 95, 3597–3602
11. Teitelbaum, S. L. (2000) Bone resorption by osteoclasts. Science 289, 1504–1508
12. Asagiri, M., and Takayanagi, H. (2007) The molecular understanding of osteoclast differentiation. Bone 40, 251–264
13. Gohta, J., Akiyama, T., Koga, T., Takayanagi, H., Tanaka, S., and Inoue, J. (2005) RANK-mediated amplification of TRAF6 signaling leads to NFATc1 induction during osteoclastogenesis. EMBO J. 24, 790–799
14. Yamashita, T., Yao, Z., Li, F., Zhang, Q., Badell, I. R., Schwarz, E. M., Takeshita, S., Wagner, E. F., Noda, M., Matsuo, K., Xing, L., and Boyce, B. F. (2007) NF-κB p50 and p52 regulate receptor activator of NF-κB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor
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differentiation by activating c-Fos and NFATc1. J. Biol. Chem. 282, 18245–18253

15. Ishida, N., Hayashi, K., Hoshijima, M., Ogawa, T., Koga, S., Miyatake, Y., Kumezawa, M., Kimura, T., and Takeya, T. (2002) Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator. J. Biol. Chem. 277, 41147–41156

16. Yagi, M., Miyamoto, T., Sawatani, Y., Iwamoto, K., Hosogane, N., Fujita, N., Morita, K., Ninomiya, K., Suzuki, T., Miyamoto, K., Oike, Y., Takeya, M., Toyama, Y., and Suda, T. (2005) DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. J. Exp. Med. 202, 345–351

17. Kim, Y., Sato, K., Asagiri, M., Morita, I., Soma, K., and Takayanagi, H. (2005) Contribution of nuclear factor of activated T cells c1 to the transcriptional control of immunoreceptor osteoclast-associated receptor but not triggering receptor expressed by myeloid cells-2 during osteoclastogenesis. J. Biol. Chem. 280, 32905–32913

18. Asagiri, M., Sato, K., Usami, T., Ochi, S., Nishina, H., Yoshida, H., Morita, I., Wagner, E. F., Mak, T. W., Serfling, E., and Takayanagi, H. (2005) Autoamplification of NFATC1 expression determines its essential role in bone homeostasis. J. Exp. Med. 202, 1261–1269

19. Chen, S., and Pan, M. (2013) NFAT signaling and bone homeostasis. Hematol. Thromb. Dis. 10.4172/2329-8790.1000102

20. Berry, L. D., Feoktistova, A., Wright, M. D., and Gould, K. L. (1999) The Schizosaccharomyces pombe dim1(+ ) gene interacts with the anaphase-promoting complex or cyclosome (APC/C) component lid1(+ ) and is required for APC/C function. Mol. Cell. Biol. 19, 2535–2546

21. Reuter, K., Nottrott, S., Fabrizio, P., Lührmann, R., and Ficner, R. (1999) Identification, characterization and crystal structure analysis of the human spliceosomal U5 snRNP-specific 15 kD protein. J. Mol. Biol. 284, 515–525

22. Zhang, Y. Z., Gould, K. L., Dunbrack, R. L., Cheng, H., Roder, H., and Golemis, E. A. (1999) The evolutionarily conserved Dim1 protein defines a novel branch of the thioredoxin fold superfamily. Physiol. Genomics 1, 109–118

23. Zhang, Y., Lindblom, T., Chang, A., Sudol, M., Shuder, A. E., and Golemis, E. A. (2000) Evidence that dim1 associates with proteins involved in pre-mRNA splicing, and delineation of residues essential for dim1 inter-actions with hnRNP F and Npw38/PQBP-1. Gene. 257, 33–43

24. Simeoni, F., Arvai, A., Bello, P., Gondev, C., Hopfner, K. P., Neyroz, P., Heitz, F., Tainer, J., and Divita, G. (2005) Biochemical characterization and crystal structure of a Dim1 family-associated protein: Dim2. Biochemistry 44, 11997–12008

25. Mehrabadi, M., Hussain, M., and Agari, S. (2013) Cloning and characterization of a Dim1-like mitosis gene of Spodoptera frugiperda cells (S9) induced by Autographa californica multiple nucleopolyhedrovirus. J. Invertebr. Pathol. 113, 152–159

26. Takayanagi, H., Kim, S., Koga, T., Nishina, H., Ishihiki, M., Yoshida, H., Saiura, A., Isobe, M., Yokochi, T., Inoue, J., Wagner, E. F., Mak, T. W., Kodama, T., and Taniguchi, T. (2002) Induction and activation of the transcription factor NFATC1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev. Cell 3, 889–901

27. Nishikawa, K., Nakashima, T., Hayashi, M., Fukunaga, T., Kato, S., Kodama, T., Takahashi, S., Calame, K., and Takayanagi, H. (2010) Blimp1-mediated repression of negative regulators is required for osteoclast differentiation. Proc. Natl. Acad. Sci. USA. 107, 3117–3122

28. Zhao, B., Takami, M., Yamada, A., Wang, X., Koga, T., Hu, X., Tamura, T., Ozato, K., Choi, Y., Ivashkiv, L. B., Takayanagi, H., and Kamijo, R. (2009) Interferon regulatory factor-8 regulates bone metabolism by suppressing osteoclastogenesis. Nat. Med. 15, 1066–1071

29. Matsuoka, S., Galson, D. L., Zhao, C., Peng, L., Laplace, C., Wang, K. Z., Bachler, M. A., Amano, H., Aburatani, H., Ishikawa, H., and Wagner, E. F. (2004) Nuclear factor of activated T-cells (NFAT) rescues osteoclastogenesis in precursors lacking c-Fos. J. Biol. Chem. 279, 26475–26480

30. Vaira, S., Alhawagri, M., Anwisye, I., Kitaura, H., Faccio, R., and Novack, D. V. (2008) RelA/p65 promotes osteoclast differentiation by blocking a RANKL-induced apoptotic JNK pathway in mice. J. Clin. Invest. 118, 2088–2097

31. 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

32. Zhao, B., and Ivashkiv, L. B. (2011) Negative regulation of osteoclastogenesis and bone resorption by cytokines and transcriptional repressors. Arthritis Res. Ther. 13, 234