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TOPIC HIGHLIGHT

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Molecular mechanisms of triggering, amplifying and targeting RANK signaling in osteoclasts

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Abstract

Osteoclast differentiation depends on receptor activator of nuclear factor-κB (RANK) signaling, which can be divided into triggering, amplifying and targeting phases based on how active the master regulator nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) is. The triggering phase is characterized by immediate-early RANK signaling induced by RANK ligand (RANKL) stimulation mediated by three adaptor proteins, tumor necrosis factor receptor-associated factor 6, Grb-2-associated binder-2 and phospholipase C (PLC)γ2, leading to activation of IκB kinase, mitogen-activated protein kinases and the transcription factors nuclear factor (NF)-κB and activator protein-1 (AP-1). Mice lacking NF-κB p50/p52 or the AP-1 subunit c-Fos (encoded by *Fos*) exhibit severe osteopetrosis due to a differentiation block in the osteoclast lineage. The amplification phase occurs about 24 h later in a RANKL-induced osteoclastogenic culture when Ca^{2+} oscillation starts and the transcription factor NFATc1 is abundantly produced. In addition to Ca^{2+} oscillation-dependent nuclear translocation and transcriptional auto-induction of NFATc1, a Ca^{2+} oscillation-independent, osteoblast-dependent mechanism stabilizes NFATc1 protein in differentiating osteoclasts. Osteoclast precursors lacking PLCγ2, inositol-1,4,5-trisphosphate receptors, regulator of G-protein signaling 10, or NFATc1 show an impaired transition from the triggering to amplifying phases. The final targeting phase is mediated by activation of numerous NFATc1 target genes responsible for cell-cell fusion and regulation of bone-resorptive function. This review focuses on molecular mechanisms for each of the three phases of RANK signaling during osteoclast differentiation.

Key words: Receptor activator of nuclear factor-κB ligand; Tumor necrosis factor receptor-associated factor 6; c-Fos; Nuclear factor of activated T-cells cytoplasmic 1; Immunoreceptor tyrosine-based activation motif; Ca^{2+} oscillation

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INTRODUCTION

Osteoclasts are bone-resorbing cells derived from hematopoietic precursor cells[1-3]. Macrophage-colony stimulating factor (M-CSF) stimulation up-regulates expression of receptor activator of nuclear factor-κB (RANK, encoded by *Tnfrsf11a*) in the osteoclast precursor cell[4]. RANK, a type I transmembrane receptor with a C-terminal cytosolic tail, is responsible for osteoclast differentiation and

function. RANK signaling is induced by RANK ligand (RANKL, encoded by *Tnfsf11*), which is a type Ⅱ transmembrane protein (i.e., with a cytoplasmic N-terminus and an extracellular C-terminus). Mice with genetic deletion of *Tnfrsf11a* or *Tnfsf11* lack osteoclasts and exhibit severe osteopetrosis[5,6]. In humans, mutations in genes encoding RANK or RANKL are associated with osteoclast poor, autosomal recessive osteopetrosis[7,8]. RANK signaling is also modified by osteoprotegerin (encoded by *Tnfrsf11b*), a soluble decoy receptor of RANK that blocks RANKL binding to RANK[9,10].

RANK is a member of the tumor necrosis factor receptor (TNFR) superfamily consisting of 616 and 625 amino acid residues in human and mouse, respectively[11]. RANKL is produced by osteoblasts and osteocytes[12,13] and binds in a trimeric form to RANK, initiating signaling[14,15]. Like other TNFR superfamily members, RANK lacks intrinsic enzymatic activity and transduces intracellular signals by recruiting adaptor proteins including TNFR-associated factors (TRAFs), activating nuclear factor (NF)-κB and downstream mitogen activated protein kinase (MAPK) and Akt signaling[16-18]. RANK exhibits one of the longest cytoplasmic tails of any TNFR superfamily protein, and this domain is responsible for the osteoclast-specific signaling pathway[19,20].

Spatio-temporal control of signaling downstream of RANK[21] is divided into three phases in this review (Figure 1). In the triggering phase, NF-κB, activator protein-1 (AP-1), and MAPKs are rapidly activated within an hour of RANKL stimulation in a culture system[22]. Then, during the amplifying phase, nuclear factor of activated T-cells cytoplasmic 1 (NFATc1, encoded by *Nfatc1*) begins to accumulate approximately 24 h after RANKL stimulation as cytosolic Ca^{2+} levels begin to oscillate[23]. Finally, in the targeting phase, RANK signaling regulates multinucleation and bone resorptive function mainly through activation of NFATc1 target genes. Concerted action of RANK and its adaptor proteins as well as immunoreceptors and other co-stimulatory molecules drive these phases. Here we review literature relevant to the molecular mechanism of RANK signaling at each phase during osteoclast differentiation.

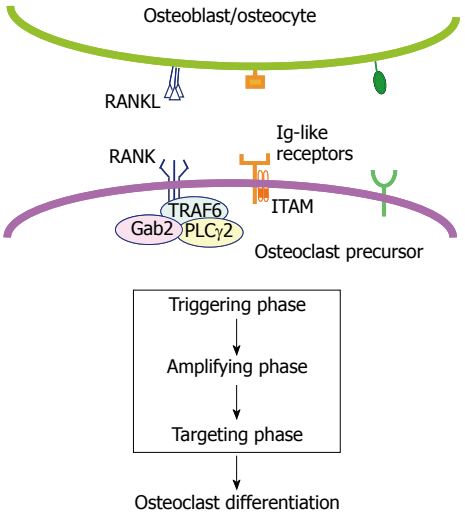

Figure 1 Three phases of receptor activator of nuclear factor-κB signaling during osteoclast differentiation. Osteoclast differentiation downstream of receptor activator of nuclear factor-κB (RANK) signaling is divided into triggering, amplifying and targeting phases, based on the nuclear factor of activated T-cells cytoplasmic 1 activation state. ITAM: Immunoreceptor tyrosine-based activation motif; TRAF6: Tumor necrosis factor receptor-associated factor 6; Gab2: Grb-2-associated binder-2; PLCγ2: Phospholipase C γ2; RANKL: RANK ligand; Ig: Immunoglobulin.

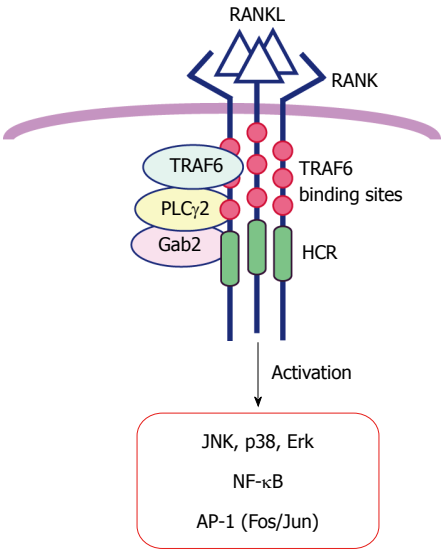

Figure 2 Triggering phase. Trimerization of receptor activator of nuclear factor-κB (RANK) by binding of RANK ligand (RANKL) immediately activates mitogen-activated protein kinases (MAPKs), nuclear factor (NF)-κB, and activator protein-1 (AP-1). An adaptor molecule complex including tumor necrosis factor receptor-associated factor 6 (TRAF6), Grb-2-associated binder-2 (Gab2) and phospholipase C (PLC)γ2 on TRAF6 binding sites of RANK is essential to induce the triggering phase. HCR: Highly conserved domain in RANK; JNK: c-Jun N-terminal kinase; Erk: Extracellular signal-regulated kinase.

TRIGGERING PHASE

Once homotrimeric RANKL forms complex with its receptor RANK[14,15], a cascade of downstream signaling is initiated. RANK recruits adaptor proteins to specific motifs in its C-terminal cytoplasmic tail, which contains three TRAF6 binding sites near the transmembrane domain, the a highly conserved domain in RANK (HCR) motif, and two binding sites for TRAF2 or TRAF5 near the C-terminus[20] (Figure 2). These motifs have been analyzed using various mutant RANK proteins[16,22]. Inoue and colleagues generated a CD40/RANK chimeric receptor carrying the N-terminal extracellular domain of human CD40 (*TNFRSF5*) and the cytoplasmic tail of mouse RANK (*Tnfrsf11a*), which can be specifically activated by anti-CD40 antibody and found that TRAF6 binding sites, but not the HCR, are essential for RANK signaling in the immediate-early phase[16,22]. At least three molecules, TRAF6, Grb-2-associated binder-2 (Gab2) and phospholipase C (PLC)γ2, function as adaptor molecules for RANK. TRAF6 is a really interesting new gene (RING) E3 ubiquitin ligase and Lys63-linked

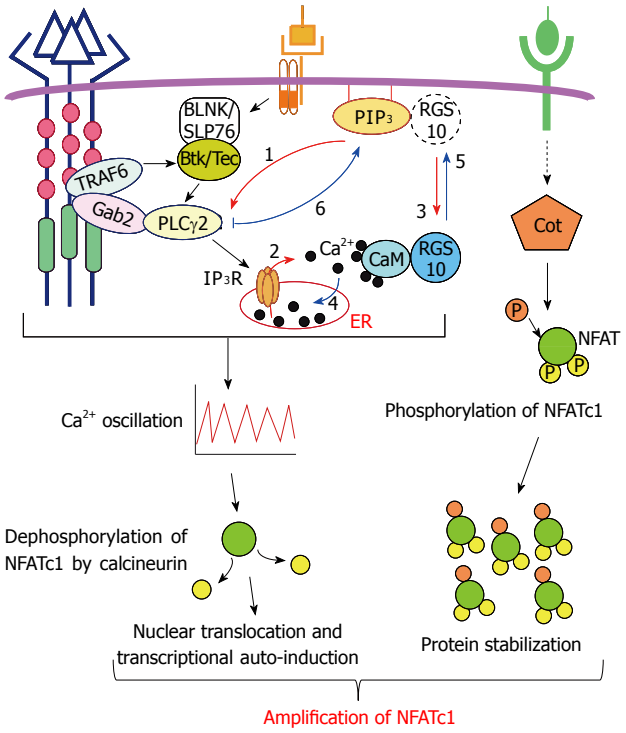

Figure 3 Amplifying phase. Both Ca^{2+} oscillation-dependent and -independent nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) amplification are induced. Highly conserved domain in receptor activator of nuclear factor-κB (RANK)-mediated RANK signaling and immunoreceptor tyrosine-based activation motif (ITAM) signaling lead to continuous phospholipase C (PLC)γ2 activation. Regulator of G-protein signaling 10 (RGS10) determines the Ca^{2+} oscillation pattern through control of PLCγ2 by competitive binding of Ca^{2+}/calmodulin and phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3). Sustained Ca^{2+} oscillation contributes to NFATc1 amplification mediated by transcriptional auto-induction. In the Ca^{2+} oscillation-independent pathway, Cot kinase enhances NFATc1 stabilization through direct phosphorylation and contributes to its accumulation. ER: Endoplasmic reticulum; BLNK: B cell linker protein; SLP76: Src homology 2 domain-containing leukocyte protein of 76 kD; TRAF6: Tumor necrosis factor receptor-associated factor 6; Gab2: Grb-2-associated binder-2; IP_3R: IP_3 receptor; CaM: Calmodulin; Btk: Bruton's tyrosine kinase.

auto-ubiquitination is necessary for the signal transduction to activate IκB kinase and NF-κB during osteoclast differentiation[24]. Mutational analysis of PLCγ2 revealed that catalytic activity of PLCγ2 is dispensable at the triggering phase but necessary for the amplifying phase of RANK signaling[25] (see below). These adaptor proteins activate diverse signaling molecules, phosphatidylinositol-3 kinase, Akt kinase, and MAPKs including c-Jun N-terminal kinase, p38, and extracellular-regulated kinase, leading to activation of the dimeric transcription factors NF-κB and AP-1[20,25,26]. Production of reactive oxygen species *via* a RANKL-TRAF6-Rac1-nicotinamide adenine dinucleotide phosphate oxidase-dependent pathway is also required for MAPK activation and osteoclastogenesis[27]. In osteoclast lineage cells, NF-κB and AP-1 are composed of two molecules among p65, RelB, p50 and p52 for NF-κB, and c-Fos (also Fra-1, Fra-2 or FosB) and c-Jun (also JunB or JunD) for AP-1. Double knockout mice lacking both p50 and p52 and single knockout mice lacking c-Fos lack osteoclasts and exhibit severe osteopetrosis[28-32]. Mice overexpressing dominant negative c-Jun also develop osteopetrosis[33]. These studies demonstrate the importance of NF-κB and AP-1 activation by RANK signaling in osteoclast differentiation.

TRANSITION TO THE AMPLIFYING PHASE

The transition from triggering to amplifying phase requires induction of *Nfatc1* transcription, which allows cooperation with signaling downstream of immune receptors. NFAT was first identified in nuclear extracts of activated T-cells as a transcription factor that binds to the interleukin-2 (IL-2) promoter[34]. NFAT regulates not only differentiation and activation of immune cells but also the development of tissues such as skeletal muscle, cardiac valve, and bone[35]. Since the promoter of the osteoclast-specific tartrate-resistant acid phosphatase (*TRAP*) gene carries an evolutionarily conserved AP-1/NFAT binding element similar to the cooperative AP-1/NFAT binding site in the IL-2 promoter, it was hypothesized that c-Fos/AP-1 is required for NFAT function in osteoclasts[36]. It was demonstrated that *Nfatc1* itself is a major c-Fos target gene during osteoclast differentiation[37-39]. In cells lacking c-Fos, NF-κB activity is unexpectedly elevated[40], supporting the idea that *Fos* and *Nfatc1* induction is downstream of NF-κB p50 and p52 activation in RANK signaling[41]. It is likely that NF-κB, c-Fos/AP-1 and NFATc2 mediate basal expression of *Nfatc1* in preparation for the amplification phase[42].

In concert with RANK signaling, immunoglobulin-like receptors such as osteoclast-associated receptor (OSCAR) and the triggering receptor expressed in myeloid cells (TREM)-2 transduce *Nfatc1* induction signals[43,44]. Both are associated with adaptor proteins containing the immunoreceptor tyrosine-based activation motif (ITAM), such as DNAX-activation protein 12 or the Fc receptor common γ subunit[45]. After ITAM tyrosine phosphorylation, a complex containing the tyrosine kinases Bruton's tyrosine kinase and Tec and the adaptor molecules B cell linker protein and Src homology 2 domain-containing leukocyte protein of 76 kD may facilitate cooperation between RANK and ITAM signaling (Figure 3)[46]. This combined signaling selectively leads to PLCγ phosphorylation, suggesting that integration of RANK and ITAM signaling is required for efficient activation of PLCγ during the amplifying phase. Furthermore, following elevation of intracellular Ca^{2+} levels, prior to the beginning of Ca^{2+} oscillation, *Nfatc1* transcription is enhanced by Ca^{2+}/calmodulin-dependent kinase Ⅳ, which phosphorylates the cAMP response element-binding protein, inducing *Fos* expression[47].

AMPLIFYING PHASE

During the amplifying phase starting approximately 24 h after RANKL stimulation in osteoclastogenic cultures,

intracellular Ca^{2+} levels oscillate, and activate the Ca^{2+}/calmodulin-dependent phosphatase calcineurin, which dephosphorylates NFATc1 and induces its nuclear translocation. On the HCR of the RANK C-terminal tail, PLCγ2 forms a complex with the TRAF6 and Gab2 adapter proteins in a stimulation-dependent manner[20]. An HCR deletion mutant of CD40/RANK chimeric receptor does not alter NF-κB and MAPK activation in the triggering phase but abolishes Ca^{2+} oscillation, indicating that HCR-mediated signaling is indispensable for continuous PLCγ2 activation.

Both HCR-dependent RANK signaling and ITAM signaling lead to long-term induction of PLCγ2 catalytic activity. PLCγ2 increases intracellular Ca^{2+} levels by producing inositol-1,4,5-trisphosphate (IP_3). Since Ca^{2+} oscillation during osteoclast differentiation is abolished in IP_3 receptor (IP_3R) knockout cells, Ca^{2+} release from endoplasmic reticulum (ER) *via* IP_3Rs is required to generate Ca^{2+} oscillation[48]. The PLCγ family consists of PLCγ1, which is widely distributed, and PLCγ2, which is primarily limited to hematopoietic cells[49]. PLCγ2 null mice exhibit an osteopetrotic phenotype[25], indicating that PLCγ2, independent of PLCγ1, is required for osteoclastogenesis.

Intracellular Ca^{2+} levels (approximately 100 nmol/L) are 20 000-fold lower than outside the cell (approximately 2 mmol/L)[50]. Ca^{2+} oscillation in osteoclasts is tightly controlled by the regulator of G-protein signaling 10 (RGS10)[51]. RGS10 is competitively bound by phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3) and Ca^{2+}/calmodulin, and intracellular Ca^{2+} concentration shifts the balance between RGS10-PIP_3 and RGS10-Ca^{2+}/calmodulin complexes[51] (Figure 3). PIP_3 is required for membrane localization and subsequent activation of PLCγ2. As the first peak formation of Ca^{2+} oscillation, PLCγ2 activation induces transient release of Ca^{2+} from the ER, elevating intracellular Ca^{2+} concentration (Figure 3, arrows 1 and 2). RGS10 forms a complex with the Ca^{2+}/calmodulin complex and increases levels of free PIP_3, further activating PLCγ2 until the intracellular Ca^{2+} level reaches its peak (Figure 3, arrow 1 and 3). Empty ER Ca^{2+} stores reload through sooth endoplasmic reticular Ca^{2+} ATPase, decreasing intracellular Ca^{2+}, increasing RGS10-PIP_3, and reducing PLCγ2 activity (Figure 3, arrows 4-6). A repeat of these processes may generate Ca^{2+} oscillation through oscillatory regulation of PLCγ2 activation[51]. RGS10 knockout mice exhibit severe osteopetrosis caused by a defect in osteoclasts *in vivo*, indicating that Ca^{2+} oscillation is a crucial mechanism underlying NFATc1 activation and amplification during osteoclast differentiation[51].

NFATc1 is also activated by an osteoblast-induced Ca^{2+} oscillation-independent pathway. When osteoclast precursors are co-cultured with osteoblasts, osteoblasts increase NFATc1 levels in osteoclast precursors, and promote osteoclast differentiation even in the presence of the calcineurin inhibitor FK506. Furthermore, wild-type osteoblasts induce differentiation of osteoclast precursors derived from IP_3R type 2 and type 3 double knockout mice without detectable RANKL-induced Ca^{2+} oscillation[48]. Indeed, Cot (cancer osaka thyroid) serine/threonine kinase, also known as tumor progression locus 2, is activated by cell-cell interaction of osteoclasts with osteoblasts and promotes Ca^{2+} oscillation/calcineurin-independent osteoclastogenesis[52]. Furthermore, Cot increases NFATc1 protein levels through phosphorylation-dependent protein stabilization thereby amplifying NFATc1 activity in the absence of Ca^{2+} oscillation. Cot likely phosphorylates residues that differ from those targeted by calcineurin-mediated dephosphorylation required for nuclear translocation. At present, the identity of osteoblast-derived molecules that activate Cot in osteoclasts is unknown, but Cot-mediated NFATc1 stabilization clearly contributes to osteoclastogenesis *in vivo*. Collectively, NFATc1 amplification is achieved by both upregulated expression and enhanced stability.

TARGETING PHASE

NFATc1 induction and amplification regulate mRNA levels of target genes driving osteoclast differentiation, fusion and function. While forced NFATc1 expression directs osteoclast differentiation, NFATc1-deficient embryonic stem cells fail to differentiate into osteoclasts following RANKL-stimulation[23,42].

In osteoclast differentiation, the immunoglobulin-like receptor OSCAR, but not TREM-2, is an NFATc1 target gene[53,54]. During differentiation, positive regulators of NFATc1 are enhanced while negative regulators are suppressed. The transcriptional repressor B-lymphocyte-induced maturation protein-1 (Blimp1) is induced by RANKL-stimulation and down-regulates three negative regulators: the v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B; interferon regulatory factor-8; and B cell lymphoma 6. All of these proteins repress *Nfatc1* transcription[55-58] (Figure 4). Evidence showing that Blimp1 is a direct NFATc1 target[55] suggests that NFATc1 maintains expression of itself *via* NFATc1/Blimp1 signaling.

NFATc1 target genes encode proteins crucial for osteoclast cell-cell fusion such as a dendritic cell-specific transmembrane protein (DC-STAMP), vacuolar proton pump subunit Atp6v0d2 and the c-Src substrate Tks5 (tyrosine kinase substrate with five SH3 domains)[59-62]. Tks5 appears to be required not only for fusion but for circumferential podosome (actin ring or sealing ring) formation. Following Tks5 knockdown in osteoclasts, multinucleation is abolished although mononuclear osteoclasts still express and amplify NFATc1 in the presence of M-CSF and RANKL[62]. Furthermore, defects of c-Src knockout osteoclasts can be partially rescued by expression of a form of Tks5 carrying glutamate substitutions that mimic constitutive phosphorylation at c-Src phosphorylation target tyrosines[62]. The c-Src-Tks5 axis illustrates an additional signaling pathway induced by

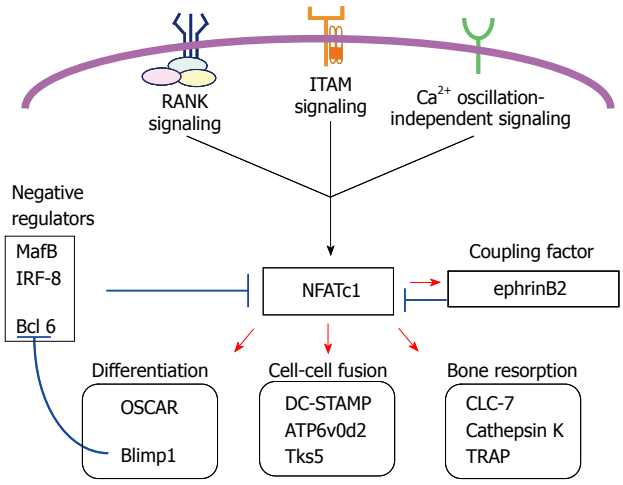

Figure 4 Targeting phase. Negative regulation of nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) is indicated by blue lines and activation of its targets, shown in boxes, is indicated by red arrows. RANK: Receptor activator of nuclear factor-κB; ITAM: Immunoreceptor tyrosine-based activation motif; OSCAR: Osteoclast-associated receptor; Blimp1: B-lymphocyte-induced maturation protein-1; DC-STAMP: Dendritic cell-specific transmembrane protein; TRAP: Tartrate-resistant acid phosphatase; IRF-8: Interferon regulatory factor-8; MafB: V-maf musculoaponeurotic fibrosarcoma oncogene family, protein B; Bcl6: B cell lymphoma 6; CLC: Chloride channel.

RANK signaling beyond NFATc1. In conjunction with ITAM-bearing proteins, c-Src also phosphorylates the tyrosine kinase Syk when integrin $\alpha_v\beta_3$ is activated by adhesion to bone matrix, in particular, vitronectin[63,64]. In these c-Src-Syk signaling components, integrin β_3 and c-Src are NFATc1 target gene products[65,66], suggesting that NFATc1 target genes include those critical for osteoclast-adhesion.

To resorb bone, osteoclasts secrete acid hydrogen chrolide and various hydrolases. Several NFATc1 target genes encode proteins required for acidification and proteolysis, such as the CLC-7 chloride channel (*Clc7*)[66], a late endosomal/lysosomal chloride channel localizing in ruffled borders, cathepsin K[67], which degrades collagens, and TRAP[23,39], which dephosphorylates the bone matrix phosphoproteins osteopontin and bone sialoprotein. Mice lacking *Clc7* or the V0-ATPase subunit a3 show severe osteopetrosis reminiscent of osteoclast-rich osteopetrosis in humans[68,69]. These mice show TRAP-positive osteoclasts with apparently normal NFATc1 amplification. Expression of the calcitonin receptor depends on NFATc1[23,39,53,70], and calcitonin receptor signaling inhibits both osteoclast formation and function independently of transcriptional regulation by RANK signaling[71].

Finally, NFATc1 induces transcription of ephrinB2[72]. Eph receptors and ephrin ligands are increasingly recognized as important in bone biology[73]. Reverse signaling into ephrinB2-expressing osteoclast lineage cells suppresses osteoclast differentiation by downregulating c-Fos and NFATc1, while forward signaling into receptor EphB4-expressing osteoblast lineage cells enhances osteoblastic differentiation and bone formation. Therefore, ephrinB2 is considered as a coupling factor inducible by RANK signaling[73].

In conclusion, RANK signaling appears to be a straightforward transcriptional cascade of "NF-κB/c-Fos induces NFATc1 induces target genes". Numerous signaling molecules including receptors, adaptors, kinases and lipases reinforce this cascade. Oscillation of intracellular Ca^{2+} levels drives the cascade, but a Ca^{2+} oscillation-independent mechanism also contributes to amplification of NFATc1 activity. RANK signaling stimulates the cell-cell fusion machinery (specifically, DC-STAMP and Tks5) and activates proteins located on or secreted from the osteoclast ruffled border (CLC-7 and cathepsin K, respectively). Numerous questions remain unanswered about RANK signaling, such as whether and how RANK signaling is connected to microRNA control[74-77] or to long noncoding RNAs (such as competing endogenous RNAs, or ceRNAs)[78]. Components of the RANK signaling pathway will continue to provide not only topics for investigation but novel therapeutic targets to prevent osteoporosis and other bone loss diseases.

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REFERENCES

1 **Teitelbaum SL**. Bone resorption by osteoclasts. *Science* 2000; **289**: 1504-1508
2 **Karsenty G**, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* 2002; **2**: 389-406
3 **Asagiri M**, Takayanagi H. The molecular understanding of osteoclast differentiation. *Bone* 2007; **40**: 251-264
4 **Arai F**, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM, Suda T. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. *J Exp Med* 1999; **190**: 1741-1754
5 **Dougall WC**, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. *Genes Dev* 1999; **13**: 2412-2424
6 **Kong YY**, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* 1999; **402**: 304-309
7 **Sobacchi C**, Frattini A, Guerrini MM, Abinun M, Pangrazio A, Susani L, Bredius R, Mancini G, Cant A, Bishop N, Grabowski P, Del Fattore A, Messina C, Errigo G, Coxon FP, Scott DI, Teti A, Rogers MJ, Vezzoni P, Villa A, Helfrich MH. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet* 2007; **39**: 960-962
8 **Guerrini MM**, Sobacchi C, Cassani B, Abinun M, Kilic SS, Pangrazio A, Moratto D, Mazzolari E, Clayton-Smith J, Orchard P, Coxon FP, Helfrich MH, Crockett JC, Mellis D, Vellodi A, Tezcan I, Notarangelo LD, Rogers MJ, Vezzoni

P, Villa A, Frattini A. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am J Hum Genet* 2008; **83**: 64-76

9 **Simonet WS**, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309-319

10 **Yasuda H**, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 1998; **95**: 3597-3602

11 **Anderson DM**, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 1997; **390**: 175-179

12 **Nakashima T**, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF, Penninger JM, Takayanagi H. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 2011; **17**: 1231-1234

13 **Xiong J**, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011; **17**: 1235-1241

14 **Douni E**, Rinotas V, Makrinou E, Zwerina J, Penninger JM, Eliopoulos E, Schett G, Kollias G. A RANKL G278R mutation causing osteopetrosis identifies a functional amino acid essential for trimer assembly in RANKL and TNF. *Hum Mol Genet* 2012; **21**: 784-798

15 **Liu C**, Walter TS, Huang P, Zhang S, Zhu X, Wu Y, Wedderburn LR, Tang P, Owens RJ, Stuart DI, Ren J, Gao B. Structural and functional insights of RANKL-RANK interaction and signaling. *J Immunol* 2010; **184**: 6910-6919

16 **Wong BR**, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi Y. The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. *J Biol Chem* 1998; **273**: 28355-28359

17 **Galibert L**, Tometsko ME, Anderson DM, Cosman D, Dougall WC. The involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF-kappaB, a member of the TNFR superfamily. *J Biol Chem* 1998; **273**: 34120-34127

18 **Wong BR**, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. *Mol Cell* 1999; **4**: 1041-1049

19 **Gohda J**, Akiyama T, Koga T, Takayanagi H, Tanaka S, Inoue J. RANK-mediated amplification of TRAF6 signaling leads to NFATc1 induction during osteoclastogenesis. *EMBO J* 2005; **24**: 790-799

20 **Taguchi Y**, Gohda J, Koga T, Takayanagi H, Inoue J. A unique domain in RANK is required for Gab2 and PLC-gamma2 binding to establish osteoclastogenic signals. *Genes Cells* 2009; **14**: 1331-1345

21 **Takayanagi H**. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 2007; **7**: 292-304

22 **Kobayashi N**, Kadono Y, Naito A, Matsumoto K, Yamamoto T, Tanaka S, Inoue J. Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. *EMBO J* 2001; **20**: 1271-1280

23 **Takayanagi H**, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell* 2002; **3**: 889-901

24 **Lamothe B**, Besse A, Campos AD, Webster WK, Wu H, Darnay BG. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. *J Biol Chem* 2007; **282**: 4102-4112

25 **Mao D**, Epple H, Uthgenannt B, Novack DV, Faccio R. PLC-gamma2 regulates osteoclastogenesis via its interaction with ITAM proteins and GAB2. *J Clin Invest* 2006; **116**: 2869-2879

26 **Wada T**, Nakashima T, Oliveira-dos-Santos AJ, Gasser J, Hara H, Schett G, Penninger JM. The molecular scaffold Gab2 is a crucial component of RANK signaling and osteoclastogenesis. *Nat Med* 2005; **11**: 394-399

27 **Lee NK**, Choi YG, Baik JY, Han SY, Jeong DW, Bae YS, Kim N, Lee SY. A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* 2005; **106**: 852-859

28 **Wang ZQ**, Ovitt C, Grigoriadis AE, Möhle-Steinlein U, Rüther U, Wagner EF. Bone and haematopoietic defects in mice lacking c-fos. *Nature* 1992; **360**: 741-745

29 **Grigoriadis AE**, Wang ZQ, Cecchini MG, Hofstetter W, Felix R, Fleisch HA, Wagner EF. c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. *Science* 1994; **266**: 443-448

30 **Franzoso G**, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U. Requirement for NF-kappaB in osteoclast and B-cell development. *Genes Dev* 1997; **11**: 3482-3496

31 **Iotsova V**, Caamaño J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. *Nat Med* 1997; **3**: 1285-1289

32 **Matsuo K**, Owens JM, Tonko M, Elliott C, Chambers TJ, Wagner EF. Fosl1 is a transcriptional target of c-Fos during osteoclast differentiation. *Nat Genet* 2000; **24**: 184-187

33 **Ikeda F**, Nishimura R, Matsubara T, Tanaka S, Inoue J, Reddy SV, Hata K, Yamashita K, Hiraga T, Watanabe T, Kukita T, Yoshioka K, Rao A, Yoneda T. Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation. *J Clin Invest* 2004; **114**: 475-484

34 **Shaw JP**, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR. Identification of a putative regulator of early T cell activation genes. *Science* 1988; **241**: 202-205

35 **Hogan PG**, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* 2003; **17**: 2205-2232

36 **Matsuo K,** Tonko M, Wagner EF. Why do c-Fos deficient mice lack osteoclasts? *J Bone Miner Res* 1996; **11** Suppl 1: M377

37 **Ishida N**, Hayashi K, Hoshijima M, Ogawa T, Koga S, Miyatake Y, Kumegawa M, Kimura T, Takeya T. Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator. *J Biol Chem* 2002; **277**: 41147-41156

38 **Takayanagi H**, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, Yokochi T, Oda H, Nakamura K, Ida N, Wagner EF, Taniguchi T. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. *Nature* 2002; **416**: 744-749

39 **Matsuo K**, Galson DL, Zhao C, Peng L, Laplace C, Wang KZ, Bachler MA, Amano H, Aburatani H, Ishikawa H, Wagner EF. Nuclear factor of activated T-cells (NFAT) rescues osteoclastogenesis in precursors lacking c-Fos. *J Biol Chem* 2004; **279**: 26475-26480

40 **Ray N**, Kuwahara M, Takada Y, Maruyama K, Kawaguchi T, Tsubone H, Ishikawa H, Matsuo K. c-Fos suppresses

systemic inflammatory response to endotoxin. *Int Immunol* 2006; **18**: 671-677
41 **Yamashita T**, Yao Z, Li F, Zhang Q, Badell IR, Schwarz EM, Takeshita S, Wagner EF, Noda M, Matsuo K, Xing L, Boyce BF. NF-kappaB p50 and p52 regulate receptor activator of NF-kappaB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. *J Biol Chem* 2007; **282**: 18245-18253
42 **Asagiri M**, Sato K, Usami T, Ochi S, Nishina H, Yoshida H, Morita I, Wagner EF, Mak TW, Serfling E, Takayanagi H. Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. *J Exp Med* 2005; **202**: 1261-1269
43 **Kim N**, Takami M, Rho J, Josien R, Choi Y. A novel member of the leukocyte receptor complex regulates osteoclast differentiation. *J Exp Med* 2002; **195**: 201-209
44 **Cella M**, Buonsanti C, Strader C, Kondo T, Salmaggi A, Colonna M. Impaired differentiation of osteoclasts in TREM-2-deficient individuals. *J Exp Med* 2003; **198**: 645-651
45 **Koga T**, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 2004; **428**: 758-763
46 **Shinohara M**, Koga T, Okamoto K, Sakaguchi S, Arai K, Yasuda H, Takai T, Kodama T, Morio T, Geha RS, Kitamura D, Kurosaki T, Ellmeier W, Takayanagi H. Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* 2008; **132**: 794-806
47 **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. Regulation of osteoclast differentiation and function by the CaMK-CREB pathway. *Nat Med* 2006; **12**: 1410-1416
48 **Kuroda Y**, Hisatsune C, Nakamura T, Matsuo K, Mikoshiba K. Osteoblasts induce Ca2+ oscillation-independent NFATc1 activation during osteoclastogenesis. *Proc Natl Acad Sci USA* 2008; **105**: 8643-8648
49 **Wilde JI**, Watson SP. Regulation of phospholipase C gamma isoforms in haematopoietic cells: why one, not the other? *Cell Signal* 2001; **13**: 691-701
50 **Clapham DE**. Calcium signaling. *Cell* 2007; **131**: 1047-1058
51 **Yang S**, Li YP. RGS10-null mutation impairs osteoclast differentiation resulting from the loss of [Ca2+]i oscillation regulation. *Genes Dev* 2007; **21**: 1803-1816
52 **Kuroda Y**, Hisatsune C, Mizutani A, Ogawa N, Matsuo K, Mikoshiba K. Cot kinase promotes Ca2+ oscillation/calcineurin-independent osteoclastogenesis by stabilizing NFATc1 protein. *Mol Cell Biol* 2012; **32**: 2954-2963
53 **Kim Y**, Sato K, Asagiri M, Morita I, Soma K, Takayanagi H. 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* 2005; **280**: 32905-32913
54 **Kim K**, Kim JH, Lee J, Jin HM, Lee SH, Fisher DE, Kook H, Kim KK, Choi Y, Kim N. 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* 2005; **280**: 35209-35216
55 **Nishikawa K**, Nakashima T, Hayashi M, Fukunaga T, Kato S, Kodama T, Takahashi S, Calame K, Takayanagi H. Blimp1-mediated repression of negative regulators is required for osteoclast differentiation. *Proc Natl Acad Sci USA* 2010; **107**: 3117-3122
56 **Kim K**, Kim JH, Lee J, Jin HM, Kook H, Kim KK, Lee SY, Kim N. MafB negatively regulates RANKL-mediated osteoclast differentiation. *Blood* 2007; **109**: 3253-3259
57 **Zhao B**, Takami M, Yamada A, Wang X, Koga T, Hu X, Tamura T, Ozato K, Choi Y, Ivashkiv LB, Takayanagi H, Kamijo R. Interferon regulatory factor-8 regulates bone metabolism by suppressing osteoclastogenesis. *Nat Med* 2009; **15**: 1066-1071
58 **Miyauchi Y**, Ninomiya K, Miyamoto H, Sakamoto A, Iwasaki R, Hoshi H, Miyamoto K, Hao W, Yoshida S, Morioka H, Chiba K, Kato S, Tokuhisa T, Saitou M, Toyama Y, Suda T, Miyamoto T. The Blimp1-Bcl6 axis is critical to regulate osteoclast differentiation and bone homeostasis. *J Exp Med* 2010; **207**: 751-762
59 **Yagi M**, Ninomiya K, Fujita N, Suzuki T, Iwasaki R, Morita K, Hosogane N, Matsuo K, Toyama Y, Suda T, Miyamoto T. Induction of DC-STAMP by alternative activation and downstream signaling mechanisms. *J Bone Miner Res* 2007; **22**: 992-1001
60 **Lee SH**, Rho J, Jeong D, Sul JY, Kim T, Kim N, Kang JS, Miyamoto T, Suda T, Lee SK, Pignolo RJ, Koczon-Jaremko B, Lorenzo J, Choi Y. v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. *Nat Med* 2006; **12**: 1403-1409
61 **Kim K**, Lee SH, Ha Kim J, Choi Y, Kim N. NFATc1 induces osteoclast fusion via up-regulation of Atp6v0d2 and the dendritic cell-specific transmembrane protein (DC-STAMP). *Mol Endocrinol* 2008; **22**: 176-185
62 **Oikawa T**, Oyama M, Kozuka-Hata H, Uehara S, Udagawa N, Saya H, Matsuo K. Tks5-dependent formation of circumferential podosomes/invadopodia mediates cell-cell fusion. *J Cell Biol* 2012; **197**: 553-568
63 **Zou W**, Kitaura H, Reeve J, Long F, Tybulewicz VL, Shattil SJ, Ginsberg MH, Ross FP, Teitelbaum SL. Syk, c-Src, the alphavbeta3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. *J Cell Biol* 2007; **176**: 877-888
64 **Chambers TJ**, Fuller K. How are osteoclasts induced to resorb bone? *Ann N Y Acad Sci* 2011; **1240**: 1-6
65 **Crotti TN**, Flannery M, Walsh NC, Fleming JD, Goldring SR, McHugh KP. NFATc1 regulation of the human beta3 integrin promoter in osteoclast differentiation. *Gene* 2006; **372**: 92-102
66 **Song I**, Kim JH, Kim K, Jin HM, Youn BU, Kim N. Regulatory mechanism of NFATc1 in RANKL-induced osteoclast activation. *FEBS Lett* 2009; **583**: 2435-2440
67 **Matsumoto M**, Kogawa M, Wada S, Takayanagi H, Tsujimoto M, Katayama S, Hisatake K, Nogi Y. Essential role of p38 mitogen-activated protein kinase in cathepsin K gene expression during osteoclastogenesis through association of NFATc1 and PU.1. *J Biol Chem* 2004; **279**: 45969-45979
68 **Frattini A**, Orchard PJ, Sobacchi C, Giliani S, Abinun M, Mattsson JP, Keeling DJ, Andersson AK, Wallbrandt P, Zecca L, Notarangelo LD, Vezzoni P, Villa A. Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. *Nat Genet* 2000; **25**: 343-346
69 **Scimeca JC**, Franchi A, Trojani C, Parrinello H, Grosgeorge J, Robert C, Jaillon O, Poirier C, Gaudray P, Carle GF. The gene encoding the mouse homologue of the human osteoclast-specific 116-kDa V-ATPase subunit bears a deletion in osteosclerotic (oc/oc) mutants. *Bone* 2000; **26**: 207-213
70 **Anusaksathien O**, Laplace C, Li X, Ren Y, Peng L, Goldring SR, Galson DL. Tissue-specific and ubiquitous promoters direct the expression of alternatively spliced transcripts from the calcitonin receptor gene. *J Biol Chem* 2001; **276**: 22663-22674
71 **Granholm S**, Lundberg P, Lerner UH. Calcitonin inhibits osteoclast formation in mouse haematopoetic cells independently of transcriptional regulation by receptor activator of NF-{kappa}B and c-Fms. *J Endocrinol* 2007; **195**: 415-427
72 **Zhao C**, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishi-

waki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* 2006; **4**: 111-121

73 **Matsuo K**, Otaki N. Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. *Cell Adh Migr* 2012; **6**: 148-156

74 **Lian JB**, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, Zhang Y. MicroRNA control of bone formation and homeostasis. *Nat Rev Endocrinol* 2012; **8**: 212-227

75 **Sugatani T**, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. *J Biol Chem* 2009; **284**: 4667-4678

76 **Mizoguchi F**, Izu Y, Hayata T, Hemmi H, Nakashima K, Nakamura T, Kato S, Miyasaka N, Ezura Y, Noda M. Osteoclast-specific Dicer gene deficiency suppresses osteoclastic bone resorption. *J Cell Biochem* 2010; **109**: 866-875

77 **Sugatani T**, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. *Blood* 2011; **117**: 3648-3657

78 **Cesana M**, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 2011; **147**: 358-369

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