RANK signaling pathways as potent and specific therapeutic targets for bone erosion in rheumatoid arthritis

Xu Feng
University of Alabama at Birmingham, Department of Pathology, Birmingham, AL 35294, USA
Tel.: +1 205 975 0990;
Fax: +1 205 934 1775;
xfeng@path.uab.edu

Bone erosion is a major complication associated with rheumatoid arthritis (RA) and is a key contributing factor to the functional disability of RA patients. Tumor necrosis factor-α and interleukin-1 are well recognized as important factors causing bone erosion in RA. As such, current therapeutic regimens have relied primarily on the agents blocking the function of tumor necrosis factor-α and interleukin-1. The discovery of the receptor activator of NF-B (RANK) and its ligand (RANKL) has not only revealed an essential role for the RANKL/RANK system in arthritic bone erosion, but also indicated that the system can serve as effective therapeutic targets for arthritic bone erosion. In this review, the role of the RANKL/RANK system in bone erosion in RA, and the therapeutic agents, which are currently under development that target the system, will be discussed. Moreover, the rationale for exploring certain RANK signaling pathways as better therapeutic targets will also be discussed.

Keywords: bone erosion, interleukin-1, osteoclast, osteoprotegerin RANK, RANK ligand, rheumatoid arthritis, tumor necrosis factor-α

future medicine

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammatory synovitis and progressive destruction of cartilage and bone in joints [1]. The structural damage to cartilage and bone contributes significantly to the decline in the functionality and often has detrimental effect on quality of life in RA patients [2]. Previously, the cartilage and bone destruction were viewed as end-point results of the disease, but several studies have revealed that the cartilage and bone loss occurs at early stages of the disease [3,4]. Thus, an effective early intervention may be critical for preventing the progression of the structural deterioration in the cartilage and bone.

While cartilage degradation in RA results from action of matrix metalloproteinase produced by chondrocytes and pannus [5], bone destruction is primarily mediated by osteoclasts, the sole bone resorbing cells that have been convincingly identified to date [6]. Two inflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-1, which are involved in the pathogenesis of inflammation in RA, also play a role in arthritic bone erosion [2]. Both IL-1 and TNF-α are potent pro-osteoclastogenic factors and stimulate bone loss in RA by activating the formation and function of the osteoclasts [6]. An anti-IL-1 agent (anakinra, IL-1 receptor antagonist) and several TNF-α-blocking drugs (infliximab, etanercept and adalimumab) have been developed for RA [7,8]. However, these agents are only moderately effective in preventing arthritic bone destruction [7,8]. Moreover, it has increasingly become clear that these drugs may cause several adverse sideeffects in RA patients [7,8].

The discovery of the receptor activator of nuclear factor-κB ligand (RANKL)/RANK system in the late 1990s has not only significantly advanced our understanding of osteoclast biology, but also revealed that aberrant alterations in the system are implicated in bone loss associated with various pathological conditions, including RA [9]. More significantly, it has also become clear that RANKL is essential for IL-1 and TNF-α-mediated osteoclastogenesis, establishing the RANKL/RANK system as a potent therapeutic target for arthritic bone erosion [6]. Consequently, tremendous efforts have been devoted to developing therapeutic agents targeting the system for bone loss. The agents currently under development include osteprotegerin (OPG), RANK-Fc and anti-RANKL antibodies, which all function to block the RANKL–RANK interaction [10–12].

This article will start with an updated review of the cellular and molecular mechanisms underlying the pathogenesis of bone destruction in RA, with a special emphasis on the essential role of the RANKL/RANK system in the pathological process. Then, the therapeutic potentials and associated limitations of the therapeutic agents targeting the RANKL/RANK system, which are currently under development, will be discussed. Moreover, the author's perspectives on exploring certain RANK signaling pathways as more specific therapeutic targets will be provided to encourage further discussion, which may help harness the ultimate therapeutic potential of the RANK/RANK system for RA therapy in the future.

Cellular & molecular mechanisms of bone erosion in RA

Osteoclast as the principal cell mediating bone erosion

The definitive identification of cell type(s) involved in bone erosion in RA is critical for developing effective therapy for arthritic bone loss. Numerous early studies suggested that bone erosion in RA may result from combined actions of several distinct cell types, including osteoclasts, synovial fibroblasts and macrophages. While an electron-microscopic study initially implicated the macrophage as a major cell type causing arthritic bone erosion (primarily due to the predominant presence of macrophages at the synovial–bone junction [13]), numerous other studies indicated that osteoclasts are also abundantly localized in the areas of bone erosion in the inflamed joints [14,15], suggesting that the osteoclast is also involved in arthritic bone destruction. Moreover, synovial fibroblasts were also suggested to play a role in arthritic bone loss [16]. However, several recent studies using animal models demonstrated that the osteoclast is the principal cell mediating bone destruction in RA. For instance, OPG, a strong inhibitor of osteoclastogenesis, blocks bone erosion in both TNF-α and collagen-induced arthritis in animal models [17,18].

The most unambiguous evidence supporting the osteoclast as the principal cell type causing bone loss in RA came from the following two animal model studies using mice lacking osteoclasts [19,20]. In the first study, inflammatory arthritis was induced in the RANKL$^{-/-}$ mice, which completely lack osteoclasts using a serum transfer model [20]. The experimental animals exhibited minimal bone erosion while they developed significant inflammation [20], supporting the suggestion that the osteoclast plays a predominant role in inducing arthritic bone erosion. The second animal model study involved the cross-breeding of transgenic mice expressing human TNF (hTNFtg), which develop a severe and destructive arthritis, with osteopetrotic c-fos-deficient mice (c-fos$^{-/-}$), which completely lack osteoclasts [19]. The resulting mutant mice (c-fos$^{-/-}$hTNFtg) developed a TNF-dependent arthritis in the absence of osteoclasts and, importantly, these mice were completely protected against bone destruction, despite the presence of severe inflammatory changes, confirming that the osteoclast is the principal cell type causing arthritic bone erosion [19].

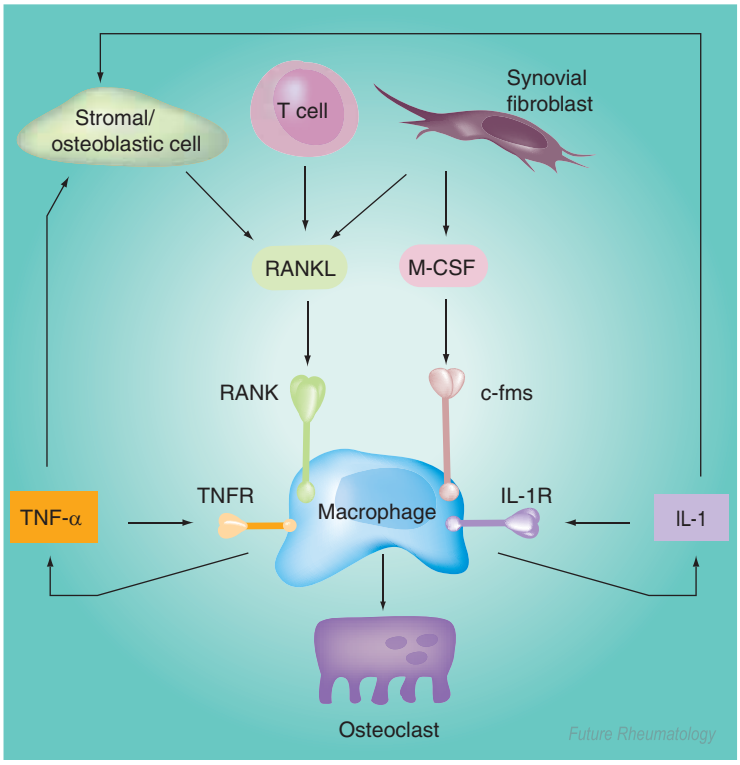
Inflammatory cytokines & bone erosion

Now that the osteoclast has been established as the principal cell mediating bone destruction in RA, the next critical issue is how osteoclast differentiation and function are regulated in the inflamed joints. Osteoclasts differentiate from cells of the monocyte/macrophage lineage upon stimulation of two essential factors: monocyte/macrophage-colony-stimulating factor (M-CSF) and RANKL (Figure 1) [21,22]. Thus, the osteoclast formation requires three basic components: the osteoclast precursors (namely macrophages), M-CSF and RANKL. Inflamed joints possess a pro-osteoclastogenic environment. Macrophages are abundantly present in inflamed joints (Figure 1) [1]. RANKL is produced by both activated T cells [23] and synovial fibroblasts [24]. The other essential osteoclastogenic factor M-CSF is mainly produced by synovial fibroblasts [25,26]. While M-CSF and RANKL serve as essential factors for osteoclastogenesis in arthritic joints, two proinflammatory cytokines IL-1 and TNF-α also play significant roles in osteoclast formation and function (Figure 1) [5]. IL-1 and TNF-α are believed to be produced primarily by macrophages [27], and they regulate osteoclastogenesis by two distinct mechanisms: directly and indirectly (Figure 1). First, macrophages express receptors for these two cytokines and these two cytokines directly bind to their receptors on macrophages to activate intracellular pathways involved in osteoclastogenesis [22,28]. Moreover, they also indirectly enhance osteoclast formation and function by stimulating the RANKL expression in stromal/osteoclastic cells (Figure 1) [29].

RANKL/RANK system as a potent therapeutic target for bone erosion in RA

RANKL, also known as OPGL, ODF and TRANCE, was identified independently by two bone biology groups [30,31] and two immunology groups [32,33] in the late 1990s. RANKL exerts its functions by binding and activating its receptor RANK expressed on target cells [32]. RANKL also has a decoy receptor, OPG, which inhibits RANKL function by competing with RANK for binding RANKL [34,35]. To date, the RANKL/RANK system has been shown to play pivotal roles in regulating various biological processes, such as immune function [32,36], mammary gland development [37] and bone homeostasis [31,38]. In the immune system, RANKL is expressed by T cells, and it binds to RANK on dendritic cells to regulate dendritic cell function and survival [32,33,39]. In mammary glands, both

Figure 1. Molecular mechanism of bone erosion in rheumatoid arthritis.



RANKL, primarily produced by activated T cells, synovial fibroblasts and stromal/osteoblastic cells in inflamed joints, plays an essential role in osteoclastogenesis in rheumatoid arthritis (RA). The other critical osteoclastogenic factor M-CSF is expressed mainly by synovial fibroblasts. Macrophages are major sources of TNF-α and IL-1, two key proinflammatory cytokines implicated in the pathogenesis of RA, which are also important osteoclastogenic factors. TNF-α and IL-1 promote osteoclastogenesis either directly, by targeting macrophages in an autocrine manner to activate pro-osteoclastogenic signaling pathways, or indirect, by stimulating stromal/osteoblastic cells to produce RANKL.
c-fms: Receptor for M-CSF; IL: Interleukin; IL-1R: IL-1 receptor; M-CSF: Monocyte/macrophage-colony-stimulating factor; RANK: Receptor activator of nuclear factor κB; RANKL: RANK ligand; TNF-α: Tumor necrosis factor-α; TNFR: TNF-α receptor.

RANKL and RANK are expressed on mammary gland epithelial cells, and they stimulate mammary gland epithelial cell proliferation in an autocrine fashion [37].

In normal bone remodeling, RANKL is expressed by osteoblasts/stromal cells [30,31] and binds to RANK on osteoclast precursors to regulate osteoclast formation [9,22,38]. Mice lacking the gene for either RANK or RANKL develop osteopetrosis due to complete failure to form osteoclasts, indicating that the RANKL/RANK system is essential for osteoclast differentiation [40–42]. Consistently, mice deficient in OPG develop early onset of osteoporosis due to elevated osteoclast differentiation [34], whereas transgenic mice overexpressing OPG exhibit osteopetrosis, resulting from a decrease in late stages of osteoclast differentiation [35]. Moreover, RANKL is also an important modulator of osteoclast function and survival [43–46].

While the studies with RANKL$^{-/-}$ and RANK$^{-/-}$ mice revealed that the RANKL/RANK system is absolutely required for osteoclast differentiation during normal bone remodeling [40–42], subsequent investigations further established that the system is also essential for osteoclastogenesis in pathological conditions such as RA. In particular, it has been established that IL-1- and TNF-α-mediated osteoclastogenesis requires RANKL. For example, administration of IL-1 to RANK$^{-/-}$ mice fails to promote osteoclast formation [42]. Consistently, IL-1 is unable to mediate osteoclast formation *in vitro* in the presence of M-CSF [47,48]. Similarly, TNF-α-mediated osteoclastogenesis also requires RANKL [49]. Given that the RANKL/RANK system is essential for osteoclast formation in RA, therapeutic targeting of the system has a great potential to give rise to unprecedented potency.

Current strategy for targeting the RANKL/RANK system

Shortly after the unraveling of the essential role for RANKL/RANK regulatory system in osteoclast formation, the promising potential of the system as a therapeutic target for bone loss associated with various pathological conditions was quickly appreciated. As a result, a significant amount of effort has been focused on developing agents capable of blocking RANKL–RANK interaction, which include OPG, soluble RANK-Fc and anti-RANKL antibodies [10–12]. Among these agents, OPG and a highly specific anti-RANKL antibody (denosumab, formerly known as AMG 162) have been advanced to be tested in clinical trials [11,50]. Denosumab may be considered as the more promising candidate since it has been the only one shown to be able to cause an increase in bone mineral density (BMD) in the clinical trial to date [11]. However, the increase in BMD is similar to or slightly higher than that of a bisphosphonate-based therapy [11], indicating that the new agent is not significantly more effective than the currently available antiresorptive drugs in treating generalized bone loss, such as postmenopausal osteoporosis. While the efficacy of the new agent in treating arthritic bone loss remains to be determined, there is a concern about the potential risk

associated with long-term use of this class of the agents, including denosumab, which act to block the RANKL–RANK interaction. As discussed above, the RANKL/RANK system not only plays a pivotal role in osteoclast formation and function [51], but is also involved in other biological processes, such as the immune system [36,39] and mammary gland development [37]. Therefore, use of the agents capable of blocking RANKL–RANK interaction to treat bone erosion will inevitably cause adverse side effects on the immune system in most RA patients and on mammary glands in juvenile RA (JRA) patients. In particular, the adverse effect of the agents on immune system may cause serious clinical complications in RA patients.

RANK signaling pathways as specific therapeutic targets for bone erosion in rheumatoid arthritis

Given the various deficiencies associated with the agents currently under development, such as the low efficacy and potential adverse side effects, it is wise to seek alternative approaches for more effective and specific targeting of the RANKL/RANK system for treating bone loss in RA. It is widely known that different cell types employ distinct sets of cellular signaling pathways to control cell proliferation, differentiation and function. Moreover, the same receptor may activate discrete signaling pathways in different cell types. Thus, it is possible that the RANKL/RANK system may activate certain unique signaling pathways to regulate osteoclast formation, function and/or survival, thus permitting cell type-specific targeting of the RANKL/RANK system for more effective treatment of bone erosion in RA.

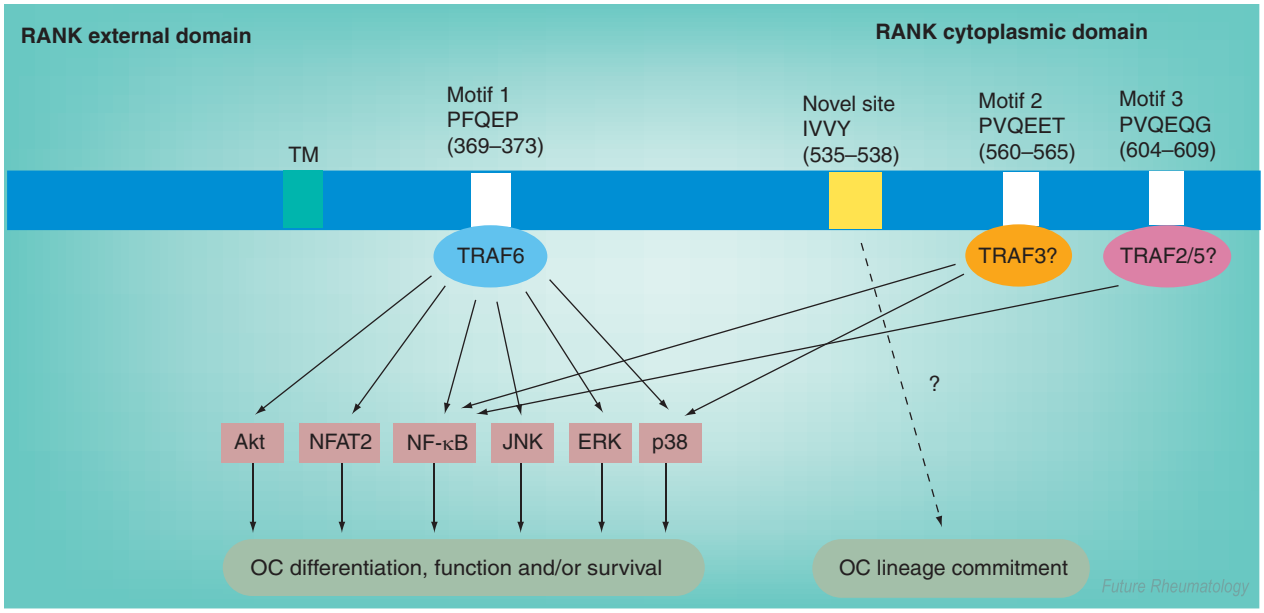
RANK signaling in osteoclasts

RANKL was cloned as a member of the TNF family [30,31], while RANK was identified as a member of the TNF receptor (TNFR) family [32]. Since members of this family primarily employ TNFR-associated factors (TRAF) to transmit nonapoptotic downstream signaling [52,53], most previous studies have been undertaken to identify and characterize the TRAF-dependent signaling pathways in osteoclasts. Despite the numerous studies demonstrating that, collectively, five TRAF proteins (TRAF 1, 2, 3, 5 and 6) interact with RANK in *in vitro* binding assays and/or in transformed cells in context of overexpression [51,54–58], subsequent functional studies indicated that RANK contains only three functional motifs: $PFQEP^{369–373}$, $PVQEET^{560–565}$ and $PVQEQG^{604–609}$, which are able to independently mediate osteoclast formation and function (Figure 2) [59,60]. More interestingly, $PVQEET^{559–564}$ and $PVQEQG^{604–609}$ are more potent than $PFQEP^{369–373}$ in mediating osteoclast formation [60].

Typically, the recruitment of a TRAF to its binding motif in a TNFR family member triggers the formation of a signaling complex containing multiple proteins that activate downstream signaling cascades [52,53]. It has consistently been established that Motif 1 recruits TRAF6 to activate downstream signaling pathway [61]. Upon binding to Motif 1, TRAF6 then recruits distinct downstream signaling molecules, such as c-Src, TAB2, TAK1 and TAB1, to form a signaling complex. This complex subsequently activates several signaling pathways including Akt, nuclear factior (NF)-κB, c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK) pathways (Figure 2) [46,61–68]. In addition, the same TRAF6-dependent signaling complex is also implicated in RANKL-induced activation of NFAT2 expression in osteoclast precursors [67] (Figure 2). Unlike Motif 1, the identities of TRAF proteins interacting with Motif 2 or 3 have not been unambiguously confirmed. Several *in vitro* interaction assays suggested that Motif 2 possibly recruits TRAF3, while Motif 3 is likely to utilize TRAF2 or TRAF5 to transmit downstream signals [51,58]. Nonetheless, it was demonstrated that Motif 2 initiate signaling pathways leading to the activation of NF-κB and p38 pathways, whereas Motif 3 mediates only the activation of the NF-κB pathway in osteoclast precursors [60]. The precise components of the signaling complex formed upon the recruitment of TRAF proteins at Motif 2 and 3 remain unknown. Collectively, these three functional RANK motifs activates six major signaling pathways (NF-κB, JNK, ERK, p38, NFAT2 and Akt), which eventually lead to the activation of various transcription factors that regulate the expression of genes important for osteoclast formation, function and/or survival (Figure 2) [9,22,69].

On the other hand, shortly after the unraveling of the essential role for the RANKL/RANK in osteoclastogenesis, it was proposed that RANK may activate unidentified and unique signaling pathways that are essential for osteoclastogenesis [70]. This idea was subsequently supported by the comparison of the osteoclastic generation potential of RANKL and IL-1. For example, it is well established that

Figure 2. Current understanding of RANK signaling in osteoclasts.



RANK contains three motifs (Motif 1, 2 and 3), which utilize TRAF proteins to activate six major signaling pathways (NF-κB, JNK, ERK, p38, NFAT2 and Akt) implicated in osteoclast differentiation, function and/or survival. In addition, RANK also possesses a newly identified and TRAF-independent cytoplasmic motif (Novel Site), which plays a crucial role in the osteoclast lineage commitment. The sequence and location of these motifs are shown.

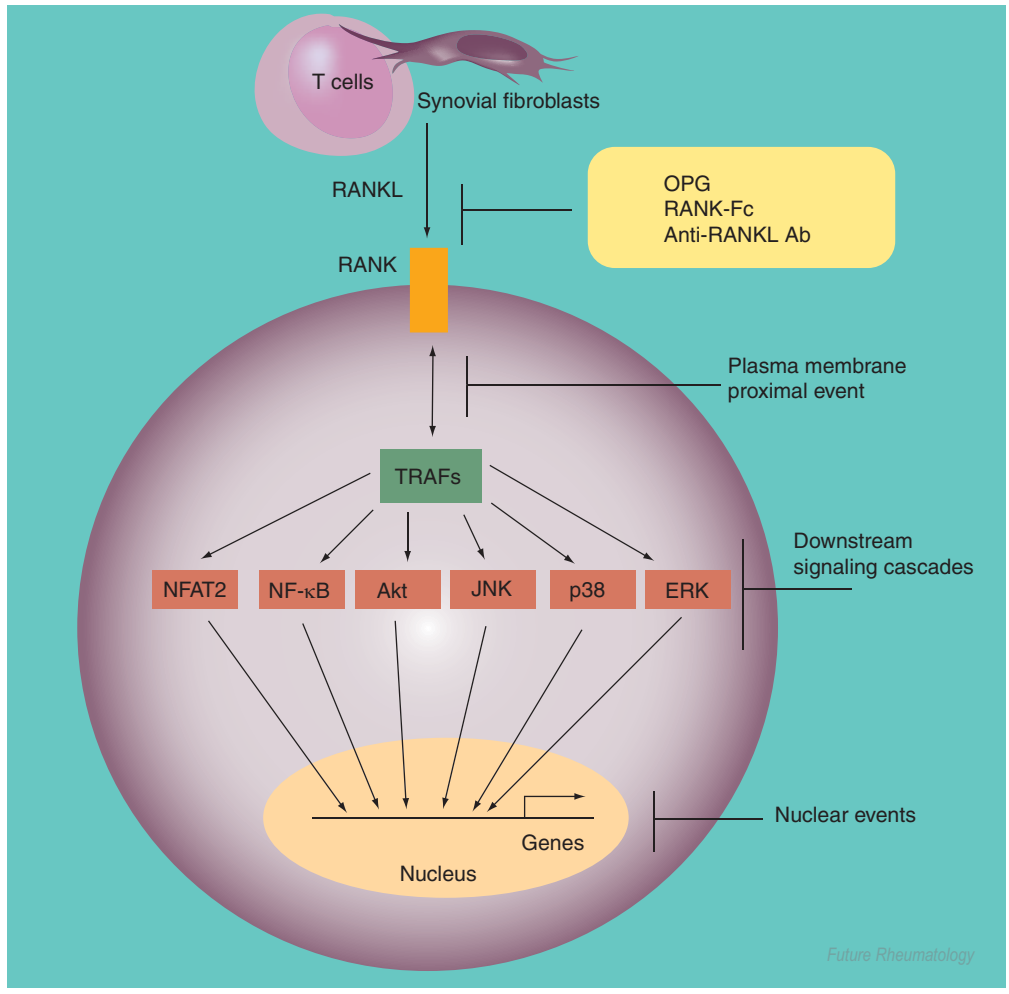
ERK: Extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; NFAT: Nuclear factor of activated T-cell; NF-κB: Nuclear factor κB; OC: Osteoclast; RANK: Receptor activator of NF-κB; TM: Transmembrane domain; TNF: Tumor-necrosis factor; TRAF: TNF receptor-associated factor.

TRAF6 acts as a key downstream signaling molecule for both RANK and IL-1R [71]. Moreover, TRAF6 is involved in osteoclastogenesis [72,73]. In particular, a single TRAF6-binding motif is able to promote osteoclastogenesis [60,61]. However, administration of IL-1 to RANK$^{-/-}$ mice failed to induce any osteoclastogenesis *in vivo*, thus indicating that RANK activates TRAF6-independent signaling pathways essential for osteoclastogenesis [42]. Consistent with this *in vivo* finding, *in vitro* studies also demonstrated that IL-1 failed to stimulate osteoclastogenesis [47,48]. Furthermore, RANK has a very long cytoplasmic domain (mouse RANK is 391 amino acids long and human RANK is 383 amino acids long), which shares no homology with any known members of the TNFR family, suggesting that it may activate downstream signals different from those arising from its cousins [32]. Consistently, a recent study identified a specific 4-amino acid RANK motif (IVVY$^{535–538}$), which plays an essential role in osteoclastogenesis by committing macrophages to the osteoclast lineage (Figure 2) [74]. Moreover, this RANK motif does not activate the known TRAF-dependent RANK signaling pathways, indicating that this motif employ a novel mechanism to regulate the osteoclast lineage commitment. Nonetheless, there is convincing evidence that this novel RANK motif is likely to exert its effect on osteoclast lineage commitment by binding an unidentified signaling molecule to transmit downstream signaling pathways required for the commitment [74].

RANK membrane-proximal signaling events as ideal therapeutic targets

The major RANK signaling events are summarized in Figure 3. Briefly, upon the binding by RANKL, RANK undergoes trimerization, which results in the recruitment of various TRAF proteins at the three functional TRAF-binding motifs in the RANK cytoplasmic domain. The recruited TRAF proteins then mediate the formation of distinct signaling complexes, which often contains multiple signaling molecules. The signaling complexes then activates six major signaling pathways (NF-κB, JNK, ERK, p38, NFAT2 and Akt), which eventually lead to the activation of various transcription factors that regulate the expression of genes important for osteoclast formation, function and/or survival (Figure 3) [9,22,69]. As such, there are three major possible approaches for targeting RANK signaling pathways (Figure 3):

Figure 3. Major strategies for targeting the RANKL/RANK regulatory system.



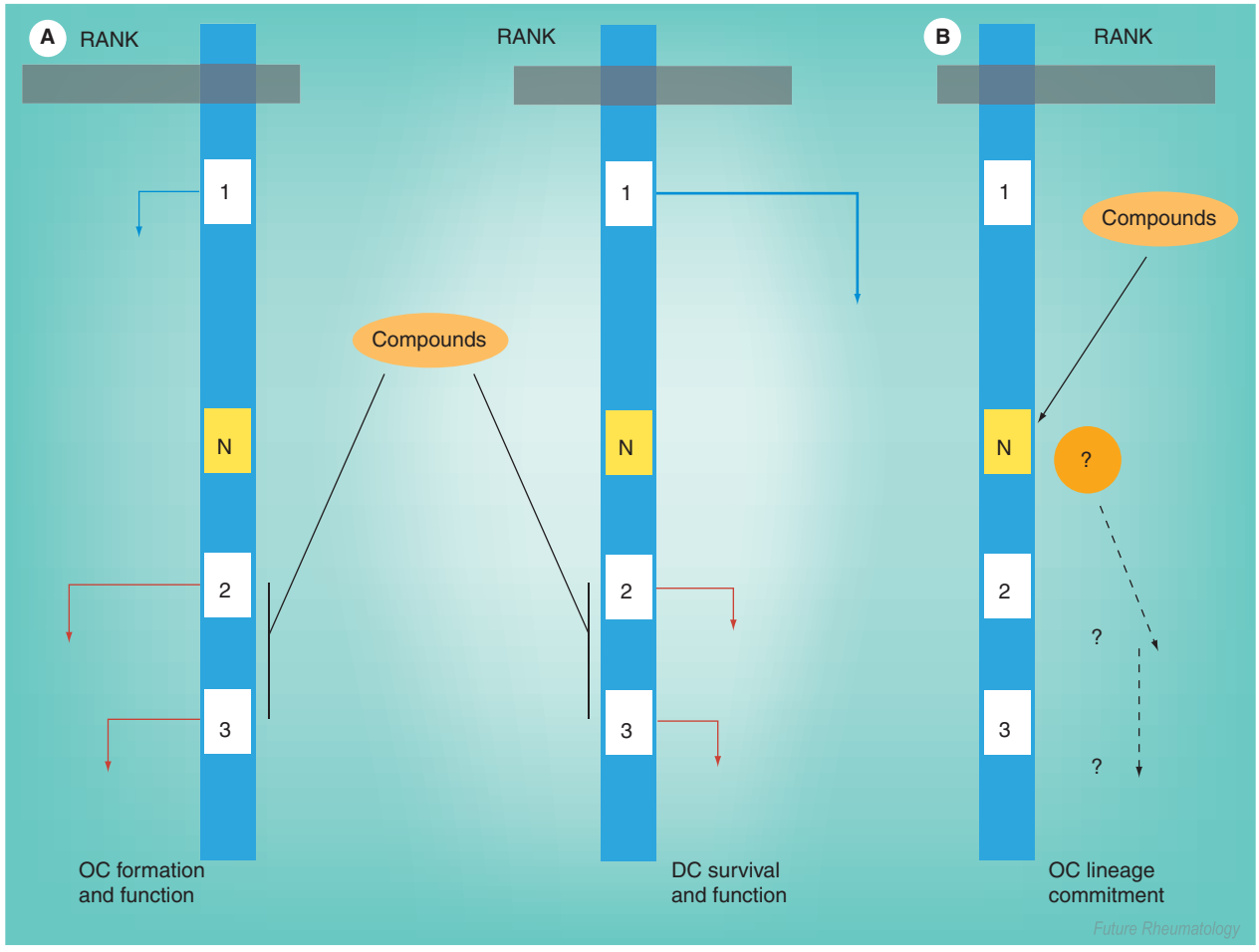
The therapeutic agents under current development include OPG, RANK-Fc and anti-RANKL Ab, which all function as blockers of the RANKL–RANK interaction. There are three major approaches for targeting RANK signaling pathways: plasma membrane-proximal events; downstream signaling cascades; and nuclear events.

Ab: Antibody; ERK: Extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; NF-κB: Nuclear factor-κB; NFAT: Nuclear factor of activated T-cell; OPG: Osteoprotegerin; RANK: Receptor activator of NF-κB; RANK-Fc: Fusion protein containing extracellular domain of RANK linked to the Fc portion of human IgG; RANKL: RANK ligand; TNF: Tumor necrosis factor; TRAF: TNF-receptor-associated factor.

- Targeting the plasma membrane-proximal events; specifically, the interaction between RANK and TRAF proteins;
- Targeting downstream signaling cascades; specifically, disrupting the formation of signaling complexes and subsequent signaling steps;
- Targeting nuclear events; specifically, inhibiting activation of transcription factors and/or binding of transcription factors to promoters for genes involved in osteoclast formation, function and/or survival.

Among the three strategies, the plasma membrane-proximal events may represent the best choice. First, the RANK cytoplasmic motifs are most accessible to prospective compounds. For instance, to target the downstream signaling cascades or the nuclear events, a compound must not only cross the plasma membrane, but also penetrate potential barriers, such as cytoskeleton networks and/or other cellular organelles, to reach its target (Figure 3). In contrast, a compound only needs to cross the plasma membrane to interfere with the plasma membrane-proximal events (Figure 3). Second,

Figure 4. RANK motifs serve as specific therapeutic targets for bone erosion in rheumatoid arthritis.



(A) Motif 2 and 3 primarily play significant roles in osteoclastogenesis (red arrows), while Motif 1 is predominantly implicated in dendritic cell function and survival (blue arrows). Blockage of signaling from both Motif 2 and 3 by prospective compounds results in cell type-specific inhibition of receptor activator of nuclear factor-kB ligand (RANKL) function in osteoclasts. **(B)** The newly identified and TNF receptor-assocaited factor-independent RANK motif mediates the osteoclast lineage commitment. Investigation of the role of the motif in other biological systems and identification of the signaling pathway(s) activated by the motif may address whether this motif and its downstream signaling pathway(s) can also serve as specific therapeutic targets for arthritic bone loss.
?: Signaling molecule(s)/pathway(s) that remain to be identified.
1: Motif 1; 2: Motif 2; 3: Motif 3; DC: Dendritic cell; N: Newly identified and TNF receptor-associated proteins-independent RANK motif; OC: Osteoclast; RANK: Receptor activator of nuclear factor-κB.

since the known RANK-activated signaling pathways (e.g., NF-κB, JNK, ERK, p38, NFAT2 and Akt) are also activated by a variety of other cytokines, including other members of the TNFR family, in a variety of cell types, targeting downstream signaling cascades may not give rise to desired cell type specificity. Similarly, the specificity concern also applies to the transcription factors since these transcription factors are also involved in the regulation of the expression of genes implicated in different biological processes in distinct cell types (Figure 3).

Motif 2 & 3 can serve as effective & specific targets for bone loss in RA

Motif 2 and 3 in the RANK cytoplasmic domain are very effective in mediating osteoclast formation and function, and mutation of both motifs, results in a significant impairment in osteoclast formation and function, thus indicating that these two motifs can serve as potent therapeutic targets (Figure 4A) [60]. Moreover, these two motifs also represent specific therapeutic targets for treating bone loss. It has been demonstrated that Motif 1 exerts a marginal effect on osteoclast formation and function (Figure 4A) [60].

In contrast, Motif 1 plays a predominant role in dendritic cell maturation and function, since Motif 1 transmits intracellular signals by recruiting TRAF6, which plays a key role in immune responses by regulating dendritic cell maturation and development [75]. Thus, blockage of Motif 2- and Motif 3-initiated signaling pathways should have minimal effect on immune response (Figure 4), which suggests that Motif 2 and 3 can serve as specific therapeutic targets for bone loss in RA.

Novel motif may also serve as effective & specific targets for bone loss in RA

In addition to RANK cytoplasmic Motif 2 and 3, the recently identified novel RANK motif may also represent a promising therapeutic target for bone loss in RA (Figure 4B). Given that this novel RANK motif plays an essential role in the osteoclast lineage commitment, targeting of the novel RANK motif may result in great efficacy. However, it is still not clear whether this novel motif is involved in other biological processes, such as immune function. Thus, more studies are needed to establish specificity of this novel site as a drug target. Moreover, although the essential role of the novel motif in osteoclastogenesis has been established, the precise downstream signaling pathway activated by the novel motif has not yet been elucidated. The next critical step is to identify and characterize the signaling molecule that interacts with the novel motif to transmit the downstream signaling pathway(s). The identification of the signaling molecule may also facilitate the development of the assay system to identify the compounds that block the interaction.

Conclusion & future perspective

The discovery of the RANKL/RANK system and subsequently its pivotal role in osteoclast differentiation and function almost a decade ago has not only significantly advanced our understanding of osteoclast biology, but also generated enormous momentum to develop therapeutic agents targeting the system for preventing and treating bone loss in various pathological conditions including RA. Several therapeutics under current developments, such as OPG, RANK-Fc and anti-RANKL antibody, all act to block the RANKL–RANK interaction. To date, none of these agents have demonstrated significant improvement in efficacy compared with those on market. Moreover, given that the RANKL/RANK system is not only involved in osteoclast biology, but also regulates the immune response and mammary gland development, any agent that can efficiently block the RANKL–RANK interaction would inevitably have an impact on all these biological processes, raising concerns about potential serious side effects associated with this type of agent. Thus, to harness the therapeutic potential of the RANKL/RANK system for RA therapy more effectively, it is wise to shift our focus towards exploring the RANK signaling pathways for potent and specific therapeutic targeting.

To date, the signaling pathways activated by the RANKL/RANK system in osteoclasts have been partially elucidated. In particular, recent *in vitro* studies have identified several RANK cytoplasmic motifs, including two TRAF-binding motifs and one novel TRAF-independent motif, which may serve as potent and specific therapeutic targets. In order to better validate the potential of these motifs as therapeutic targets, these *in vitro* findings must be further investigated and confirmed *in vivo*. Moreover, future studies should also be actively pursued to elucidate the molecular mechanism by which the novel TRAF-independent motif regulates the osteoclast lineage commitment. A better understanding of the novel RANK motif-mediated osteoclast lineage commitment may elucidate additional, if not better, therapeutic targets/approaches.

Finally, it is worthwhile to point out that the National Institutes of Health has developed initiatives aimed at facilitating drug discovery by supporting the development of highly efficient assay systems for drug screening [101]. Thus, future efforts should also be directed at designing highly innovative and efficient assay systems for identifying compounds capable of blocking the signaling from these RANK cytoplasmic motifs.

Executive summary

Bone erosion as a severe complication associated with rheumatoid arthritis

- Bone erosion is a major complication associated with rheumatoid arthritis (RA) and is a key contributing factor to the functional disability of RA patients.
- An effective intervention of bone erosion is crucial for RA therapy.

Osteoclasts are the principal cells causing bone destruction in RA

- The osteoclast has been established as the principal cell type mediating bone destruction in RA.

Executive summary

RANKL–RANK plays an essential role in osteoclastogenesis

- Receptor activator of nuclear factor κB ligand (RANKL)/RANK plays an essential role in osteoclastogenesis in normal bone remodelling, since mice lacking the gene for either RANK or RANKL develop osteopetrosis due to complete failure to form osteoclasts.
- RANKL/RANK is also essential for osteoclastogenesis in various pathological conditions, including RA, since tumor necrosis factor (TNF)-α- and interleukin (IL)-1-mediated osteoclast differentiation depends on the action of the RANKL/RANK system.

Current strategy for targeting the RANKL/RANK system

- The current strategy for targeting the RANKL/RANK system primary involves the development of agents capable of blocking the RANKL–RANK interaction, including osteoprotegrin (OPG), RANK-Fc and anti-RANKL antibodies.
- These agents have not demonstrated significant improvement in efficacy compared with the drugs on market. Moreover, these agents have potential to cause serous side effects.

RANK signaling pathway as specific agents

- Given that different cell types employ distinct sets of cellular signaling pathways to control cell proliferation, differentiation and function, and that the same receptor may activate discrete signaling pathways in different cell types, it is possible that the RANKL/RANK system may activate certain unique signaling pathways to regulate osteoclast formation, function and/or survival, thus permitting cell type-specific targeting of the RANKL/RANK system for more effective treatment of bone erosion in RA.

Three major approaches to inhibit RANK signaling pathways

- Target the plasma membrane-proximal events, specifically, the interaction between RANK and TNF receptor-associated (TRAF) proteins.
- Target downstream signaling cascades (i.e., disrupting the formation of signaling complexes and subsequent signaling steps).
- Target nuclear events, specifically, inhibiting activation of transcription factors and/or binding of transcription factors to promoters for genes involved in osteoclast formation, function and/or survival.

Several RANK cytoplasmic motifs may serve as potent and specific therapeutic targets

- Two TRAF-binding motifs exist: Motif 2 and 3. These two motifs primarily play roles in osteoclastogenesis, but exert minimal effect on immune response. Thus, blockage of signaling from both Motif 2 and 3 results in cell type-specific inhibition of RANKL function in osteoclasts.
- The newly identified and TRAF-independent RANK motif mediates the osteoclast lineage commitment. Investigation of the role of the motif in other biological systems and identification of the signaling pathway(s) activated by the motif will address whether this motif and its downstream signaling pathway(s) can also serve as specific therapeutic targets for arthritic bone loss.

Conclusion

- We have not fully harnessed the therapeutic potential of the RANKL/RANK system for RA therapy.
- Future studies should be shifted towards exploring the RANK signaling pathways for potent and specific therapeutic targeting.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Feldmann M, Brennan FM, Maini RN: Role of cytokines in rheumatoid arthritis. *Ann. Rev. Immunol.* 14, 397–440 (1996).
2. Goldring SR: Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. *Rheumatology* 42(Suppl. 2), ii11–ii16 (2003).

• Reviews the cellular and molecular mechanisms underlying bone and cartilage destruction in rheumatoid arthritis (RA).

3. McGonagle D, Conaghan PG, O'Connor P *et al.*: The relationship between synovitis and bone changes in early untreated rheumatoid arthritis: a controlled magnetic resonance imaging study. *Arthr. Rheum.* 42, 1706–1711 (1999).
4. McQueen FM, Stewart N, Crabbe J *et al.*: Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals a high prevalence of erosions at four months after symptom onset. *Ann. Rheum. Dis.* 57, 350–356 (1998).
5. Dayer JM: The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. *Rheumatology* 42(Suppl. 2), ii3–ii10 (2003).
6. Teitelbaum SL: Osteoclasts; culprits in inflammatory osteolysis. *Arthr. Res. Ther.* 8, 1–8 (2006).

• Excellent and recent review on basic osteoclast biology and the pathological role of osteoclasts in inflammatory osteolysis.

7. Furst DE: Anakinra: review of recombinant human interleukin-1 receptor antagonist in the treatment of rheumatoid arthritis. *Clin. Ther.* 26, 1960–1975 (2004).
8. Hochberg MC, Lebwohl MG, Plevy SE *et al.*: The benefit/risk profile of TNF-blocking agents: findings of a consensus panel. *Sem. Arthritis Rheum.* 34, 819–836 (2005).
9. Boyle WJ, Simonet WS, Lacey DL: Osteoclast differentiation and activation. *Nature* 423, 337–342 (2003).

•• Comprehensive review on the molecular mechanism controlling osteoclast differentiation and function, with a special focus on the role of the receptor activator of nucelar factor κB ligand (RANKL)/RANK system in the biological process.

10. Wittrant Y, Theoleyre S, Chipoy C *et al.*: RANKL/RANK/OPG: new therapeutic targets in bone tumours and associated osteolysis. *Biochim. Biophys. Acta* 1704, 49–57 (2004).

11. McClung MR, Lewiecki EM, Cohen SB *et al.*: For the Bone Loss Study Group: Denosumab in postmenopausal women with low bone mineral density. *N. Engl. J. Med.* 354, 821–831 (2006).
• Reports the clinical trial designed to determine the effect of an anti-RANKL antibody, Denosumab, on bone mineral density (BMD) in postmenopausal women.
12. McClung MR: Inhibition of RANKL as a treatment for osteoporosis: preclinical and early clinical studies. *Curr. Osteo. Reports* 4, 28–33 (2006).
13. Ishikawa H, Ohno O, Hirohata K: An electron microscopic study of the synovial-bone junction in rheumatoid arthritis. *Rheumatol. Int.* 4, 1–8 (1984).
14. Bromley M, Woolley DE: Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. *Arthritis Rheum.* 27, 968–975 (1984).
15. Gravallese EM, Harada Y, Wang JT *et al.*: Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. *Am. J. Pathol.* 152, 943–951 (1998).
16. Pap T, Meinecke I, Muller-Ladner U *et al.*: Are fibroblasts involved in joint destruction? *Ann. Rheum. Dis.* 64(Suppl. 4), iv52–iv54 (2005).
17. Romas E, Sims NA, Hards DK *et al.*: Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. *Am. J. Pathol.* 161, 1419–1427 (2002).
18. Redlich K, Hayer S, Maier A *et al.*: Tumor necrosis factor α-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. *Arthritis Rheum.* 46, 785–792 (2002).
19. Redlich K, Hayer S, Ricci R *et al.*: Osteoclasts are essential for TNF-α-mediated joint destruction. *J. Clin. Invest.* 110, 1419–1427 (2002).
•• Seminal work establishing that osteoclasts are essential for bone erosion in RA.
20. Pettit AR, Ji H, von SD *et al.*: TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. *Am. J. Pathol.* 159, 1689–1699 (2001).
•• Another critical work establishing that osteoclasts play an essential role in arthritic bone destruction.
21. Teitelbaum SL, Ross FP: Genetic regulation of osteoclast development and function. *Nat. Rev. Genetics* 4, 638–649 (2003).
22. Feng X: Regulatory roles and molecular signaling of TNF family members in osteoclasts. *Gene* 350, 1–13 (2005).
•• Provides a comprehensive review of regulatory roles and molecular signaling of tumor necrosis factor (TNF) family members with a focus on the current understanding of RANK signaling osteoclasts.
23. Kong YY, Feige U, Sarosi I *et al.*: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* 402, 304–309 (1999).
24. Wu Y, Liu J, Feng X *et al.*: Synovial fibroblasts promote osteoclast formation by RANKL in a novel model of spontaneous erosive arthritis. *Arthritis Rheum.* 52, 3257–3268 (2005).
25. Seitz M, Loetscher P, Fey MF *et al.*: Constitutive mRNA and protein production of macrophage colony-stimulating factor but not of other cytokines by synovial fibroblasts from rheumatoid arthritis and osteoarthritis patients. *Br. J. Rheum.* 33, 613–619 (1994).
26. Hamilton JA, Filonzi EL, Ianches G: Regulation of macrophage colony-stimulating factor (M-CSF) production in cultured human synovial fibroblasts. *Growth Factors* 9, 157–165 (1993).
27. Arend WP, Dayer JM: Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum.* 33, 305–315 (1990).
28. Kwan TS, Padrines M, Theoleyre S *et al.*: IL-6, RANKL, TNF-α/IL-1: interrelations in bone resorption pathophysiology. *Cytokine Growth Factor Rev.* 15, 49–60 (2004).
29. Romas E, Gillespie MT, Martin TJ: Involvement of receptor activator of NF-κB ligand and tumor necrosis factor-α in bone destruction in rheumatoid arthritis. *Bone* 30, 340–346 (2002).
30. Lacey DL, Timms E, Tan HL *et al.*: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93, 165–176 (1998).
31. Yasuda H, Shima N, Nakagawa N *et al.*: Osteoclast differentiation factor is a ligand for osteoprotegerin/ osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl Acad. Sci. USA* 95, 3597–3602 (1998).
32. Anderson DM, Maraskovsky E, Billingsley WL *et al.*: A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 390, 175–179 (1997).
33. Wong BR, Rho J, Arron J *et al.*: TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. *J. Biol. Chem.* 272, 25190–25194 (1997).
34. Bucay N, Sarosi I, Dunstan CR *et al.*: Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev.* 12, 1260–1268 (1998).
35. Simonet WS, Lacey DL, Dunstan CR *et al.*: Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 89, 309–319 (1997).
36. Wong BR, Josien R, Choi Y: TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. *J. Leukocyte Biol.* 65, 715–724 (1999).
37. Fata JE, Kong YY, Li J *et al.*: The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. *Cell* 103, 41–50 (2000).
38. Teitelbaum SL: Bone resorption by osteoclasts. *Science* 289, 1504–1508 (2000).
39. Wong BR, Josien R, Lee SY *et al.*: TRANCE (tumor necrosis factor TNF-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J. Exp. Med.* 186, 2075–2080 (1997).
40. Kong YY, Yoshida H, Sarosi I *et al.*: OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 397, 315–323 (1999).
41. Dougall WC, Glaccum M, Charrier K *et al.*: RANK is essential for osteoclast and lymph node development. *Genes Dev.* 13, 2412–2424 (1999).
42. Li J, Sarosi I, Yan X-Q *et al.*: RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. *Proc. Natl Acad. Sci. USA* 97, 1566–1571 (2000).
• Demonstrated that administration of interleukin (IL)-1 to RANK knockout mice was unable to induce osteoclast formation *in vivo*, thus establishing that IL-1-mediated osteoclastogenesis depends on the RANKL/RANK system.
43. Burgess TL, Qian Y, Kaufman S *et al.*: The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. *J. Cell. Biol.* 145, 527–538 (1999).

44. Fuller K, Wong B, Fox S, Choi Y, Chambers TJ: TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. *J. Exp. Med.* 188, 997–1001 (1998).
45. Lum L, Wong BR, Josien R *et al.*: Evidence for a role of a tumor necrosis factor-α (TNF-α)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *J. Biol. Chem.* 274, 13613–13618 (1999).
46. Wong BR, Besser D, Kim N *et al.*: TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. Molecular Cell 4, 1041–1049 (1999).
47. Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A: Tumor necrosis factor-α induces differentiation of and bone resorption by osteoclasts. *J. Biol. Chem.* 275, 4858–4864 (2000).
48. Kobayashi K, Takahashi N, Jimi E *et al.*: Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J. Exp. Med.* 191, 275–285 (2000).
49. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL: TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J. Clin. Invest.* 106, 1481–1488 (2000).
• Describes the important study demonstrating that TNF-α-mediated osteoclastogenesis is dependent on the RANKL/RANK system.
50. Bekker PJ, Holloway D, Nakanishi A *et al.*: The effect of a single dose of osteoprotegerin in postmenopausal women. *J. Bone Miner. Res.* 16, 348–360 (2001).
51. Hsu H, Lacey DL, Dunstan CR *et al.*: Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proc. Natl Acad. Sci. USA* 96, 3540–3545 (1999).
52. Locksley RM, Killeen N, Lenardo MJ: The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104, 487–501 (2001).
53. Bodmer JL, Schneider P, Tschopp J: The molecular architecture of the TNF superfamily. *Trend. Biochem. Sci.* 27, 19–26 (2002).
54. Darnay BG, Haridas V, Ni J *et al.*: Characterization of the intracellular domain of receptor activator of NF-κB (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF-κb and c-Jun N-terminal kinase. *J. Biol. Chem.* 273, 20551–20555 (1998).
55. Wong BR, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi YW: The TRAF family of signal transducers mediates NF-κB activation by the TRANCE receptor. *J. Biol. Chem.* 273, 28355–28359 (1998).
56. Kim HH, Lee DE, Shin JN *et al.*: Receptor activator of NF-κB recruits multiple TRAF family adaptors and activates c-Jun N-terminal kinase. *FEBS Lett.* 443, 297–302 (1999).
57. Darnay BG, Ni J, Moore PA *et al.*: Activation of NF-κB by RANK requires tumor necrosis factor receptor-associated factor (TRAF) 6 and NF-κB-inducing kinase. Identification of a novel TRAF6 interaction motif. *J. Biol. Chem.* 274, 7724–7731 (1999).
58. Galibert L, Tometsko ME, Anderson DM *et al.*: The involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF-κB, a member of the TNFR superfamily. *J. Biol. Chem.* 273, 34120–34127 (1998).
59. Armstrong AP, Tometsko ME, Glaccum M *et al.*: A RANK/TRAF6-dependent signal transduction pathway is essential for osteoclast cytoskeletal organization and resorptive function. *J. Biol. Chem.* 277, 44347–44356 (2002).
60. Liu W, Xu D, Yang H *et al.*: Functional identification of three RANK cytoplasmic motifs mediating osteoclast differentiation and function. *J. Biol. Chem.* 279, 54759–54769 (2004).
• Describes the functional identification of the three TNF-receptor associated factor (TRAF)-binding motifs and provides evidence that these motifs regulate osteoclast formation and function.
61. Ye H, Arron JR, Lamothe B *et al.*: Distinct molecular mechanism for initiating TRAF6 signalling. *Nature* 418, 443–447 (2002).
62. Mizukami J, Takaesu G, Akatsuka H *et al.*: Receptor activator of NF-κB ligand (RANKL) activates TAK1 mitogen-activated protein kinase kinase kinase through a signaling complex containing RANK, TAB2, and TRAF6. *Mol. Cell Biol.* 22, 992–1000 (2002).
63. Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao Z, Matsumoto K: The kinase TAK1 can activate the NIK-I κB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature* 398, 252–256 (1999).
64. Shirakabe K, Yamaguchi K, Shibuya H *et al.*: TAK1 mediates the ceramide signaling to stress-activated protein kinase/c-Jun N-terminal kinase. *J. Biol. Chem.* 272, 8141–8144 (1997).
65. Lee SW, Han SI, Kim HH, Lee ZH: TAK1-dependent activation of AP-1 and c-Jun N-terminal kinase by receptor activator of NF-κB. *J. Biochem. Mol. Biol.* 35, 371–376 (2002).
66. Ge B, Gram H, Di Padova F *et al.*: MAPK-independent activation of p38α mediated by TAB1-dependent autophosphorylation of p38α. *Science* 295, 1291–1294 (2002).
67. Takayanagi H, Kim S, Koga T *et al.*: Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Develop. Cell* 3, 889–901 (2002).
68. Ishida N, Hayashi K, Hoshijima M *et al.*: Large scale gene expression analysis of osteoclastogenesis *in vitro* and elucidation of NFAT2 as a key regulator. *J. Biol. Chem.* 277, 41147–41156 (2002).
69. Takayanagi H, Kim S, Matsuo K *et al.*: RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-β. *Nature* 416, 744–749 (2002).
70. Yeh WC, Hakem R, Woo M, Mak TW: Gene targeting in the analysis of mammalian apoptosis and TNF receptor superfamily signaling. *Immunolog. Rev.* 169, 283–302 (1999).
71. Wu H, Arron JR: TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. *BioEssays* 25, 1096–1105 (2003).
72. Lomaga MA, Yeh WC, Sarosi I *et al.*: TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* 13, 1015–1024 (1999).
73. Naito A, Azuma S, Tanaka S *et al.*: Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. *Genes Cells* 4, 353–362 (1999).
74. Xu D, Wang S, Liu W, Liu J, Feng X: A novel RANK cytoplasmic motif plays an essential role in osteoclastogenesis by committing macrophages to the osteoclast lineage. *J. Biol. Chem.* 281, 4678–4690 (2005).
75. Kobayashi T, Walsh PT, Walsh MC *et al.*: TRAF6 is a critical factor for dendritic cell maturation and development. *Immunity* 19, 353–363 (2003).

Website

101. NIH roadmap for clinical health
nihroadmap.nih.gov

Affiliation

• *Xu Feng*
University of Alabama at Birmingham, Department of Pathology, Birmingham, AL 35294, USA
Tel.: +1 205 975 0990;
Fax: +1 205 934 1775;
xfeng@path.uab.edu