Molecular mechanism of bone destruction in rheumatoid arthritis

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There is accumulating evidence that osteoclasts, the primary cells responsible for bone resorption, are involved in bone and joint destruction in rheumatoid arthritis (RA). Preventing joint destruction is one of the most challenging issues in RA therapy. The recent elucidation of the various intracellular signaling pathways in osteoclasts has brought a tremendous understanding of the pathophysiology of inflammatory bone loss, and has heightened expectation of a novel intervention. We here highlight the molecular mechanism of bone and joint destruction in RA and the role of intracellular signaling pathways in osteoclastogenesis and mature osteoclast function. We also describe the recent trials on inhibitor drug and anticytokine therapies of arthritic joint disease-targeting osteoclasts.

Keywords: bone resorption, cytokines, osteoclastogenesis, receptor activator of nuclear factor-κB ligand, rheumatoid arthritis, survival

future medicine part of fsg

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by invasive synovial hyperplasia and associated with localized and generalized bone loss. Proliferation of the synovial cells leads to pannus tissue that invades the bare area between cartilage and bone, finally resulting in progressive bone and joint destruction in the affected joints. Radiographic studies have also shown that the bone erosion in RA begins at the early stage of the disease, and gradually exacerbates. Localized bone loss, including periarticular osteopenia and subchondrial bone erosions, constitutes an important feature in diagnosing and directing treatment in RA. Subchondral bone erosions reflect ongoing disease activity of inflammatory arthritis. Early intervention to prevent the natural progression of joint destruction can substantially improve functional status [1]. Some of the therapeutic agents, such as steroids, nonsteroidal anti-inflammatory drugs, and disease-modifying antirheumatic drugs, can reduce the joint inflammation. However, very few of them can effectively suppress bone destruction in RA. Since ameliorating joint destruction is one of the most important issues in the treatment of RA, more efficient therapies against it are needed. Recently, biological agents such as the anti-tumor necrosis factor (TNF)-α antibody, have been shown to ameliorate the progression of bone destruction in RA [2]. Although the bone-protective effect of these agents is limited and the prolonged usage of these medicines sometimes causes severe side effects, these biological agents possibly reverse early focal bone loss in some cases, increasing the interest in developing therapies specifically aimed at inhibiting the progression of erosions and joint destruction in the various inflammatory arthritides. There is accumulating evidence that osteoclasts, the primary cells responsible for bone resorption, are involved in bone and joint destruction in RA. In this article, we focus on the role of osteoclasts in the bone pathology of RA, and propose that they can be a potential target for RA treatment.

Physiological bone remodeling

Remodeling of bone, which begins early in fetal life, is a continuous process in the adult skeleton that permits the repair of microdamage while still regulating the mechanical strength and structure of bone. The bone remodeling cycle involves a series of highly regulated steps that depend on the interactions of two cell lineages, the mesenchymal bone-forming osteoblastic lineage and the hematopoietic bone-resorbing osteoclastic lineage [3]. Physiological bone remodeling is initiated by cells lining the bone surface, which are of osteoblastic lineage. When activated, the osteoblastic cells release several cytokines and chemokines that, in turn, recruit and induce osteoclast precursors. The interaction of osteoclast and osteoblast precursors leads to the differentiation, migration and fusion of the large multinucleated osteoclasts. These mature osteoclasts attach to the mineralized bone surface and initiate resorption through the secretion of hydrogen ions and lysosomal enzymes, particularly cathepsin K, which can degrade all the components of bone matrix, including collagen, at low pH. Osteoclastic resorption produces irregular scalloped cavities on the trabecular bone surface, termed

Howship lacunae, or cylindrical Haversian canals in cortical bone. Following the resorptive phase, orchestrated by the osteoclasts, the bone surface is repopulated by osteoblasts that deposit bone matrix, which eventually undergoes mineralization to form the new bone surface. In physiological remodeling, the amount of bone removed is matched exactly by the amount of bone laid down. An imbalance between these two processes, which favors increased osteoclastic activity, results in focal articular bone loss and generalized osteoporosis.

Evidence for a pivotal role of osteoclasts in inflammatory bone disease

Osteoclasts are multinucleated, terminally differentiated cells derived from the mononuclear cell precursors of the monocyte/macrophage lineage [4,5]. The pivotal role that osteoclasts perform by degrading mineralized matrix during normal and pathological bone turnover is well described in the scientific literature [6]. Animal studies reported that mice lacking osteoclasts are resistant to arthritis-induced bone erosion [7,8]. Bromley and Woolley observed a number of acid phosphatase-positive multinucleated cells in the erosive joint areas of RA patients [9]. Since then, many other studies have provided ample evidence to implicate the crucial role of osteoclasts in the pathogenesis of erosions in patients with RA [10–14]. Abundant multinucleated giant cells were also observed at the bone–pannus interfaces of arthritic joints in collagen-induced arthritic rats [15,16]. Multinucleated cells were positive for unique markers of osteoclasts, such as tartrate-resistant acid phosphatase (TRAP), cathepsin K and calcitonin receptors, satisfying the major criteria of mature osteoclasts [17]. In addition, synovial cells, which were isolated from RA synovium at the time of knee replacement surgeries, can support osteoclast differentiation from monocyte–macrophage lineage precursor cells in the presence of 1α,25-dihydroxyvitamin D3 and macrophage colony-stimulating factor (M-CSF) [18]. The important role of osteoclasts in bone resorption has been found to be true of other inflammatory arthritides as well. The demonstration that osteoclasts are largely responsible for focal bone erosions has increased efforts to understand the exact role played by a number of cytokines and inflammatory mediators that posseses the capacity to induce the recruitment, differentiation and activation of osteoclasts.

Identification of osteoclast differentiation factor: receptor activator of nuclear factor-κB ligand

Bone-resorbing osteoclasts originate from hemopoietic cells probably of the colony-forming-unit macrophage-derived monocyte–macrophage family. Takahashi and colleagues developed a mouse coculture system of hemopoietic cells and primary osteoblasts to investigate osteoclast formation *in vitro* [19–21]. In this coculture system, several systemic and local factors induced formation of TRAP-positive multinucleated cells, which satisfied most of the osteoclast criteria [22]. In this system, cell-to-cell contact between osteoblastic cells and osteoclast progenitors was essential for inducing osteoclastogenesis. From these findings, Suda's group proposed that osteoblastic cells induce osteoclast differentiation factor (ODF) as a membrane-associated factor in response to various osteotropic factors [22]. In 1997, receptor activator of nuclear factor (NF)-κB ligand (RANKL) and its receptor RANK were first described as regulators of interactions between dendritic cells and T cells [23]. ODF, which was cloned from a cDNA library of mouse stromal ST2 cells treated with bone-resorbing factors [24], was found to be identical to RANKL, TNF-related activation-induced cytokine and osteoprotegerin (OPG) ligand, which were independently identified by three other research groups [23–28]. RANKL induced osteoclast differentiation from mouse hemopoietic cells and human peripheral blood mononuclear cells in the presence of M-CSF [24,27]. RANK is the sole signaling receptor for RANKL in inducing osteoclastogenesis and activation of mature osteoclasts [28]. OPG, a decoy receptor for RANKL, lacks transmembrane and cytoplasmic domains and is released in a soluble form by a variety of cells including osteoblasts. OPG inhibits the differentiation and activity of osteoclasts to compete against RANK [28].

Synovial tissue in rheumatoid arthritis is a source of RANKL

Inflamed synovial fibroblasts produce a variety of other cytokines and hormones that can also affect the physiological bone remodeling by influencing osteoclastogenesis. These factors include interleukin (IL)-1α, -1β, -6, -11 and -17 and TNF-α, M-CSF and parathyroid hormone (PH)-related peptide [29,30]. These factors may play important roles not only in the immune response and development of inflammation but also in joint

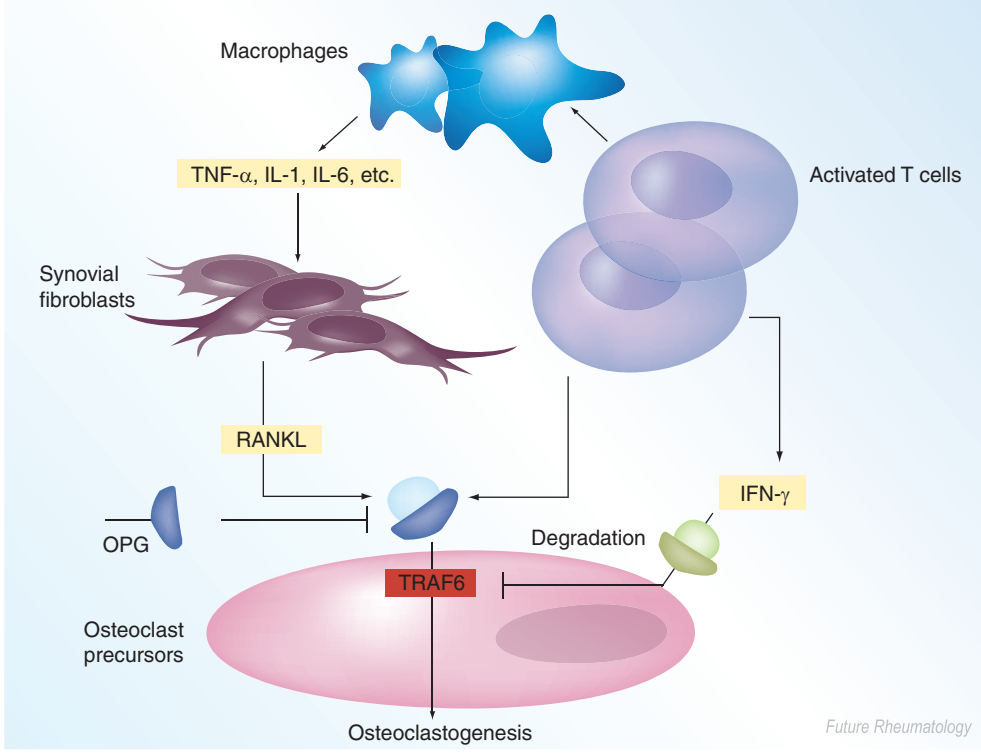
destruction in RA, by upregulating RANKL expression in synovial fibroblasts. In addition, RANKL and TNF-α act in synergy to enhance osteoclast differentiation [31,32]. Meanwhile, IL-1 acts primarily to directly activate osteoclastic bone resorption and prolong osteoclast suvival [28,33,34]. The role of these cytokines in inflammation and bone erosions provides further evidence of a link between immune system activation and bone resorption. Following extensive studies into the signaling mechanism of RANKL in bone remodeling, it was hypothesized that RANKL may play a major pathophysiological role in the bone and joint destruction observed in inflammatory arthritides such as RA. Studies by Takayanagi and colleagues provided initial insights into the role of RANKL in the pathogenesis of rheumatoid bone destruction [35]. Other studies have provided compelling evidence that activated T cells from the RA synovium and synovial fibroblasts express RANKL [29,30,36,37]. The primary role of RANKL in the immune system was recently demonstrated: mice with a disrupted RANKL gene lacked all lymph nodes and exhibited defects in T- and B-lymphocyte development [27]. However, it remains to be elucidated whether RANKL expressed on synoviocytes modulates immune responses, including T cells and dendritic cells, in RA. On the other hand, activated T cells stimulate macrophages to secrete proinflammatory cytokines such as TNF-α and IL-1, which strongly induce RANKL on synovial fibroblasts [18,35]. Although T cells themselves express RANKL, interferon (IFN)-γ production by activated T cells strongly suppresses osteoclastogenesis by rapid degradation of the RANK adapter protein, TNF receptor-associated factor (TRAF)6 [38]. The effect of T cells on osteoclastogenesis depends on the balance between RANKL and IFN-γ [38,39]. Since there appears to be a very low level of IFN-γ in synovial tissues, the imbalance may underlie the aberrant activation of osteoclast formation in inflammatory bone destruction observed in RA (**Figure 1**) [38,40].

RANKL/RANK signaling in osteoclastogenesis

Most TNF receptor family members, including RANK, interact with the TRAF family of adaptor proteins [41]. Among the known TRAF family members, TRAF2, 5 and 6 can activate transcription factors, such as NF-κB and activator protein (AP)-1. RANK interacts with most of the TRAF family members; however, TRAF6 appeared to have a critical role in osteoclastogenesis mediated by RANK. TRAF6 can mediate signaling not only from RANK but also from other TNF receptor family members, such as IL-1R, Toll-like receptor and CD40 [41–43]. Despite the activation of overlapping TRAF6-dependent signaling cascades by these receptors, only RANK has been shown to induce osteoclast differentiation. TRAF6 activates the NF-κB, Akt and mitogen-activated protein kinase (MAPK) pathways, including extracellular-regulated kinase (ERK), JNK and p38 [44–47]. RANKL also activates the NF-κB and AP-1 via TRAF6 (**Figure 2**). Although the critical role of each MAPK or Akt has not been genetically shown, the essential role of TRAF6, NF-κB (p50/p52) and c-Fos in osteoclastogenesis was fully underscored by gene disruption studies [44,46,48,49]. Furthermore, stimulation by RANKL results in the induction of mRNA of IFN-β in osteoclast precursor cells and IFN-β strongly inhibits the osteoclast differentiation by interfering with the RANKL-induced expression of c-Fos, indicating that RANKL autoregulates its signaling [50].

Takyanagi and colleagues found that nuclear factor of activated T cells (NFAT)c1, a member of the NFAT family of transcription factor genes [51], is the most strongly induced transcription factor gene following RANKL stimulation. The transcription factors of the NFAT family, originally discovered in the context of T-cell activation [52], are also involved in the function and development of diverse cells in other biological systems, where they are under the control of the calcium-regulated phosphatase calcineurin [53]. RANKL also induces and activates NFATc1 through calcium signaling, and calcineurin inhibitors, such as FK506 and cyclosporin A, strongly inhibit osteoclastogenesis. The essential and sufficient role of the NFATc1 gene in osteoclast differentiation has been shown by the observation that NFATc1$^{-/-}$ embryonic stem cells cannot differentiate into osteoclasts and that NFATc1 overexpression induces osteoclast differentiation without RANKL stimulation (**Figure 2**) [54,55]. Recent studies by Ikeda and colleagues indicated that NFATc2 functions as an essential upstream regulator of NFATc1 [56]. In addition, *in vitro* osteoclastogenesis in NFATc1$^{-/-}$ cells was rescued by forced expression of NFATc2 [57]. However, NFATc2 deficiency did not have any effect on osteoclastogenesis and bone-resorbing activity [57]. Further studies are necessary in order to elucidate the precise regulatory mechanism of NFAT family members in osteoclastogenesis.

Figure 1. Involvement of RANKL/RANK pathways in osteoclast differentiation and bone destruction in RA.



Activated T cells stimulate macrophages to secrete proinflammatory cytokines, such as IL-1 and TNF-α, which strongly induce RANKL on synovial fibroblasts. In addition, activated T cells themselves express RANKL. RANKL binds to its specific receptor RANK, which is expressed in monocyte–macrophage lineage osteoclast precursor cells. The interaction between RANKL and RANK is blocked by OPG, a physiological inhibitor of RANKL. T-cell production of IFN-γ suppresses RANKL-induced osteoclastogenesis by TRAF6 degradation. IFN: Interferon; IL: Interleukin; OPG: Osteoprotegerin; RANK: Receptor activator of nuclear factor-κB; RANKL: Receptor activator of nuclear factor-κB ligand; TNF: Tumor necrosis factor; TRAF: TNF-receptor activated factor.

Intracellular signaling pathways regulating osteoclast survival

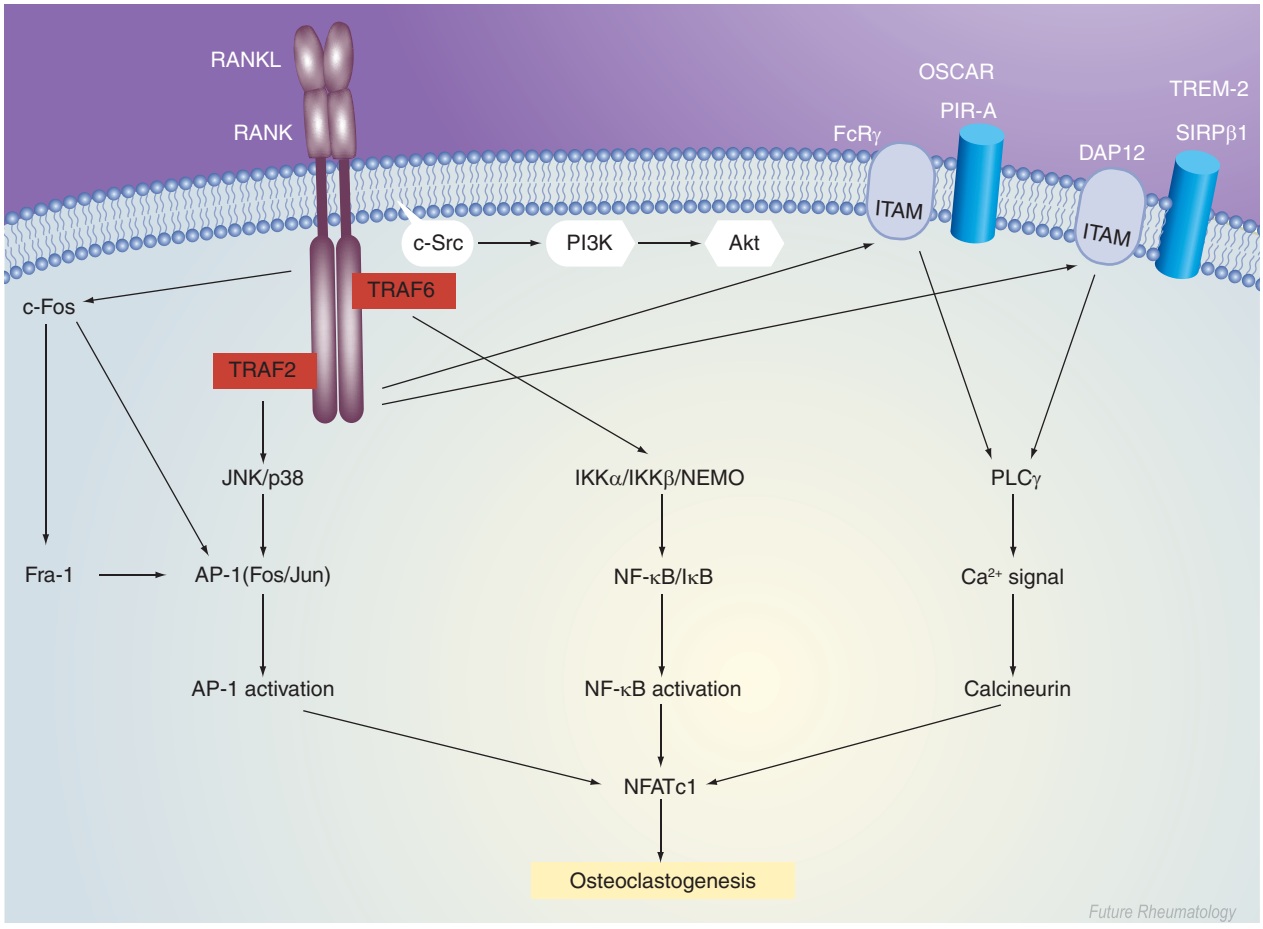
The success of bisphosphonates as the drug therapy of osteoporosis, together with the finding that bisphosphonates act directly on osteoclasts to induce their apoptosis, has attracted a great deal of attention to the molecular mechanism of osteoclast apoptosis, and therapeutics targeting osteoclast survival will provide a novel treatment for abnormal bone resorption, including RA. The lifespan of osteoclasts is relatively short both *in vitro* and *in vivo* and, once differentiated, they rapidly die in the absence of supporting cells such as osteoblasts or bone marrow stromal cells, or growth factors, such as IL-1, RANKL and M-CSF [58]. Antiresorptive drugs such as estrogen, raloxifene, and bisphosphonates are known to reduce the lifespan of osteoclasts [59]. Previously, we have reported that the activation of ERK markedly promoted the survival of osteoclasts [34]. Conversely, inhibiting ERK activation by overexpressing a dominant-negative *ras* gene mutant rapidly induced apoptotic cell death [34]. These findings, combined with the fact that antiapoptotic factors such as RANKL, ILH1 and M-CSF also induce ERK activation in osteoclasts, suggest that the Ras–ERK pathway plays an essential role in osteoclast survival. Recently, we demonstrated a novel and unique regulation of apoptosis by ubiquitylation-dependent degradation of Bim in osteoclasts [60]. In the presence of M-CSF, Bim is constitutively ubiquitylated and degraded, and cytokine deprivation induced rapid upregulation of Bim due to the reduced level of its ubiquitylation [60]. In addition, Bim-deficient osteoclasts exhibited prolonged survival both *in vitro* and *in vivo*. Concordant with our observation, Sugatani and Hruska reported that silencing the *bim* gene by small interfering

RNA prolonged the survival of osteoclasts [61]. These findings suggest that ERK signaling is a major pathway for downregulation of Bim by M-CSF in osteoclast survival (**Figure 3**). Further studies are required to elucidate the mechanism of action and the regulation of Bim in osteoclasts, and the role of Bim in skeletal disorders.

Intracellular signaling pathway regulating bone-resorbing activity of mature osteoclasts

Members of the TNF receptor family and the IL-1R are associated either directly or indirectly with TRAFs that recruit and activate downstream signaling transducers [62]. TRAF6 is involved in signaling from RANK and IL-1R, which can activate the bone-resorbing activity of mature osteoclasts [34,47,63–65]. Studies on TRAF6 knock-out mice demonstrated the essential role of TRAF6 on the activation of osteoclastic bone resorption [44,47]. Not only does TRAF6 activate NF-κB by signaling via NF-κB inducing kinase (NIK) and IκB kinase (IKK), but it also activates ERK in a Ras-independent manner [66]. Although the ERK pathway plays a critical role in osteoclast survival, neither inhibition nor activation of ERK affected the bone-resorbing activity of osteoclasts. Inhibition of the NF-κB pathway by dominant negative IKK expression suppressed the pit-forming activity of osteoclasts, and NF-κB activation

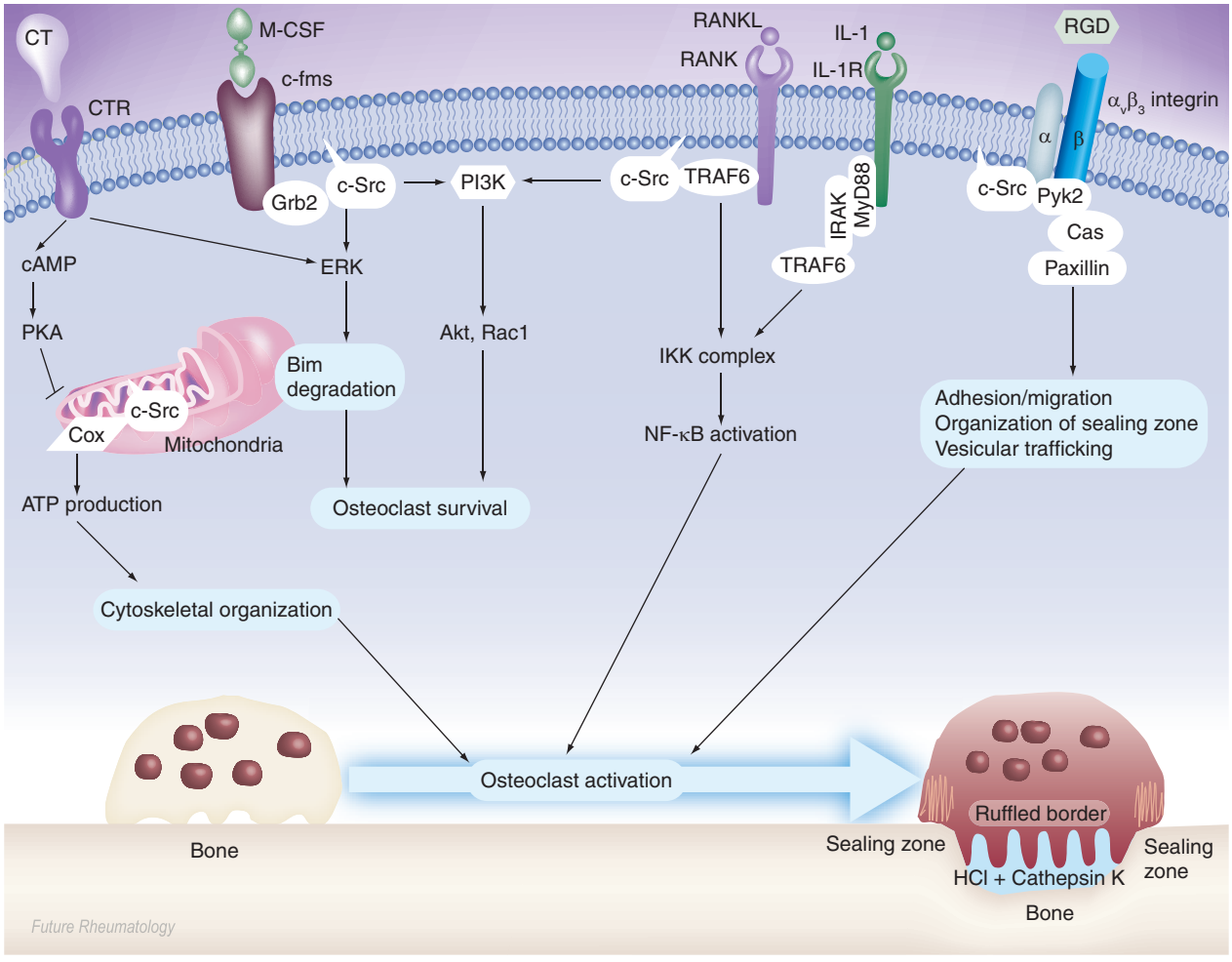
Figure 2. RANKL signaling in osteoclastogenesis.



RANKL signaling events are reconstituted in the context of NFATc1 induction and activation. RANKL induces the *NFATc1* gene via TRAF6 and c-Fos signaling pathways, both of which are essential for osteoclastogenesis. The phosphorylation of ITAM stimulated by immunoreceptors and RANKL–RANK interaction results in the activation of PLCγ and calcium signaling, which is critical for NFATc1 induction.

AP: Activator protein; DAP: DNAX-activating protein; FcRγ: Fc receptor common γ-chain; ITAM: Immunoreceptor tyrosine-based activation motif; NF: Nuclear factor; NFAT: Nuclear factor of activated T cell; OSCAR: Osteoclast-associated receptor; PIR: Paired immunoglobulin receptor; PLC: Phospholipase C; RANK: Receptor activator of nuclear factor-κB; RANKL: Receptor activator of nuclear factor-κB ligand; TRAF: TNF-receptor associated factors; TREM: Triggering receptor expressed in myeloid cells; SIRP: Signal-regulatory protein.

Figure 3. Intracellular signaling pathway in mature osteoclasts.



A variety of antiapoptotic factors, including RANKL, IL-1, M-CSF and calcitonin, activate the ERK pathway, resulting in Bim degradation. M-CSF and RANKL also activate PI3K/Akt pathway in c-Src dependent manner. These two pathways are important for osteoclast survival. For the bone-resorbing activity, there are three major signaling pathways in mature osteoclasts. These include: mitochondrial ATP production that is inhibited by calcitonin stimulation; RANKL- or IL-1-induced NF-κB activation through IKK complex; and integrin-dependent actin reorganization, leading to adhesion, migration and vesicular trafficking.
Cox: Cytochrome C oxidase; CT: Calcitonin; CTR: Calcitonin receptor; ERK: Extracellular-regulated kinase; HCL: Hydrochloric acid; IKK: IκB kinase; IL: Interleukin; IRAK: IL-1 receptor-associated kinase; M-CSF: Macrophage colony-stimulating factor; MyD: Myeloid differentiation factor; NF: Nuclear factor; PI3K: Phosphoinositide-3 kinase; PKA: Protein kinase A; Pyk: Proline-rich tyrosine kinase; RANK: Receptor activator of nuclear factor-κB; RANKL: Receptor activator of nuclear factor-κB ligand; RGD: Arg-Gly-Asp; TRAF: TNF receptor-associated factor.

by constitutively active IKK expression upregulated it without affecting their survival [34]. Taken together, it is likely that RANK and IL-1 can promote survival and activity of osteoclasts simultaneously via ERK and NF-κB activation, both of which are mediated by TRAF6. These results suggest that the NFκB pathway is involved in TRAF6-mediated activation for osteoclastic bone resorption.

Calcitonin, which is used therapeutically for diseases characterized by elevated bone resorption (i.e., osteoporosis, Paget's disease and humoneral hypercalcemia of malignancy), is known to inhibit osteoclastic bone resorption through its receptor, which is abundantly expressed on the plasma membrane of mature osteoclasts. Calcitonin inhibits osteoclastic bone resorption via cyclic (c)AMP-dependent protein kinase (PKA) by disrupting actin organization [67,68] and promoting osteoclast survival *in vitro* via ERK activation [69,70]. The stimulation of PKA activity in osteoclasts by calcitonin disrupts the actin ring [68]. In addition, we have reported that cytochrome C oxidase (Cox) activity, which plays a critical role in

ATP generation by mitochondrial oxidative phosphorylation, is required for maintaining osteoclast morphology and for normal bone resorption [70]. Furthermore, Yang and colleagues demonstrated that cAMP can negatively regulate Cox activity as a consequence of preventing the interaction between the PKA RIα regulatory subunit and Cox subunit Vb (CoxVb) [71]. We also found that calcitonin causes Cox activity to decrease and that reducing Cox activity prevents the formation of the actin ring and decreased bone resorption [70]. These findings raise the possibility that the inhibitory effect of calcitonin on bone resorption may be partly due to the downregulation of Cox activity in osteoclasts.

Integrin $\alpha_v\beta_3$ has distinct functional properties that are mediated through interactions with a variety of extracellular matrix (ECM) proteins in addition to vitronectin [72]. Studies of macrophages and osteoclasts have shown that blocking $\alpha_v\beta_3$ inhibits adhesion, migration and, for osteoclasts, bone resorption [73–75]. Osteoclasts attach to the bone surface and form a tight sealing zone (or clear zone), enclosing the resorption lacunae, which was frequently compared with a large lysosome. Following the insertion of secretory vesicles, a highly convoluted membrane named the ruffled border is formed facing the bone surface [5]. $\alpha_v\beta_3$ and the actin cytoskeleton are enriched in the sealing zone of the resorbing osteoclasts. The sealing zone is believed to mediate attachment to the bone matrix and the formation of acidic resorption lacunae that are required for bone resorption [76]. Disrupting $\alpha_v\beta_3$ signaling molecules, such as gelsolin, impairs podosome formation, cell movements and bone resorption [77]. Osteoclast contact with ECM induces $\alpha_v\beta_3$ integrin clustering and initiates intracellular signals that lead to proline-rich tyrosine kinase (Pyk) phosphorylation at Tyr 402, creating the binding site for the Src homology (SH)2 domain of c-Src. Recruitment and activation of c-Src kinase can lead to enchanced phosphorylation of Pyk2 at other sites, which potentially serves as a binding site for other downstream signaling/adaptor molecules, such as Grb-2 and Cas, or other cytoskeletal molecules, such as paxillin [73,74]. The combined action of Pyk2, c-Src, Cas and paxillin modulates the integrin-dependent recruitment of cytoskeletal molecules that are important for adhesion, migration, survival and vesicular trafficking of mature osteoclast on bone [75]. As osteoclast-mediated bone resorption and macrophage-dependent inflammation are such central pathogenic features of RA, these data provide strong support for the concept that therapeutic inhibition of $\alpha_v\beta_3$ or c-Src kinase activity is sensible [16,78–81].

Emerging therapeutic strategies

As stated earlier, an important aim of treating RA is to limit the progression of bone and joint destruction. This may be done by suppressing bone resorption and/or increasing bone formation. Anticytokine therapy, in the form of anti-TNF-α and anti-IL-1 blockade, has provided evidence for its ability to retard bone resorption in randomized placebo controlled clinical trials [82–85]. In addition, Hasegawa and colleagues reported that bisphosphonate treatment, which is the gold-standard antiresorptive therapy for osteoporosis, was effective at inhibiting bone resorption and destruction and decreasing serum IL-6 concentration in patients with RA, raising a hope that bisphosphonates may be beneficial in RA [86]. However, the exact mechanism of how anti-TNF-α, anti-IL-1 and bisphosphonate therapies do so is not fully understood. Furthermore, there are still a number of patients with inflammatory arthritis who do not respond to these agents. This has led to research into finding alternative therapeutic strategies to control bone resorption in RA.

Since cytokines and hormonal factors implicated in bone resorption may act via a common final pathway, RANKL/RANK/OPG signaling, OPG and human monoclonal anti-RANKL antibody, AMG-162 (denosumab) [87], might be new therapies in several diseases characterized by excessive bone resorption. The studies to assess efficacy, safety and tolerability of these therapies are currently underway at multiple centers across the USA.

Small molecules and peptidomimetics to modulate the biologic targets, including mediators of inflammation and osteoclastogensis, may provide the new therapies. Aoki and colleagues reported that a TNF receptor loop peptide, which mimics a TNF receptor ligand contact site and inhibits the signaling pathways induced by TNF and RANKL, prevented the increased osteoclastogonesis and bone loss induced in mice by ovariectomy or low dietary calcium [88]. In addition, Jimi and colleagues reported that a cell-permeable peptide inhibitor of the IKK complex demonstrated efficacy in a murine collagen-induced arthritis (CIA) model with suppression of inflammation due to a decrease in proinflammatory cytokine production [89,90]. Calcinurin

inhibitor FK506 (tacrolimus), which primarily affects T-cell function by inhibiting NFAT activity and has been approved not only for prophylaxis of liver and kidney allograft rejection but also for RA treatment, was reported to strongly inhibit osteoclastogenesis and suppress inflammation and damage to bone and cartilage in rat CIA [54,91,92]. Efficacy of tacrolimus in preventing bone erosion of RA patients is now being assessed in clinical trials. Furthermore, it is reported that histone deacetylase inhibitor suppresses osteoclastogenesis and bone destruction by inducing the expression of IFN-β [93] and by suppressing NF-κB activation [94], suggesting that several inhibitors of histone deacetylases, including simple compounds such as butyrate, cyclic tetrapeptides, benzamides and hydroxamic acids, are being considered as potential therapeutic agents to treat RA as well as cancer.

Executive summary

Pivotal role of osteoclasts in rheumatoid arthritis

- Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by invasive synovial hyperplasia associated with localized and generalized bone loss.
- Osteoclasts, the primary cells responsible for bone resorption, are involved in bone and joint destruction in RA.
- Animal studies reported that mice lacking osteoclasts are resistant to arthritis-induced bone erosion.

Synovial tissue in RA is a source of receptor activator of nuclear factor-κB.

- Receptor activator of nuclear factor-κB ligand (RANKL) is an osteoclast differentiation factor produced in response to various osteotropic factors.
- Activated T cells stimulate macrophages to secrete proinflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, which strongly induce RANKL on synovial fibroblasts.

RANKL/RANK signaling in osteoclastogenesis

- RANK interacts with TNF-receptor associated factor (TRAF)6, which activates the nuclear factor (NF)-κB, Akt and mitogen-activated protein kinases.
- The targeted disruption of RANKL, RANK, TRAF6, NF-κB (p50/p52) or c-Fos results in osteopetrosis due to the defective osteoclast formation.
- RANKL induces and activates nuclear factor of activated T cells c1, the master transcription gene for osteoclastogenesis.

Osteoclast survival

- The lifespan of osteoclasts is relatively short, both *in vitro* and *in vivo*.
- Antiresorptive drugs, such as estrogen, raloxifene and bisphosphonates, are known to reduce the lifespan of osteoclasts.
- The extracellular-regulated kinase pathway, which leads to Bim degradation, plays an essential role in osteoclast survival.
- Bim-deficient osteoclasts exhibited prolonged survival both *in vitro* and *in vivo*.

Bone-resorbing activity of mature osteoclasts

- RANKL and IL-1 activate osteoclastic bone resorption via TRAF6.
- The NF-κB pathway plays an important role in TRAF6-mediated activation for osteoclastic bone resorption.
- Calcitonin inhibits osteoclastic bone resorption via cyclic AMP-dependent protein kinase A.
- Cytochrome c oxidase activity, which plays a critical role for ATP generation by mitochondrial oxidative phosphorylation, is required for osteoclastic bone resorption.
- Studies of macrophages and osteoclasts have shown that blocking $\alpha_v\beta_3$ inhibits adhesion, migration, and, for osteoclasts, bone resorption.
- The combined action of proline-rich tyrosine kinase 2, c-Src, Cas and paxillin modulates integrin-dependent recruitment of cytoskeletal molecules that are important for adhesion, migration and vesicular trafficking of mature osteoclasts on bone.

Emerging therapeutic strategies

- Osteoprotegerin and anti-RANKL antibody can be good therapies in several diseases characterized by excessive bone resorption.
- Small molecules and peptidomimetics, such as the TNF receptor loop peptide and the cell-permeable peptide inhibitor of the IκB kinase complex, may provide the new therapies to modulate osteoclastic bone resorption.

Conclusion

- The combined approach of reducing inflammatory response, blocking osteoclast differentiation and activation and stimulating osteoblast activity, may achieve optimal control of RA.
- Further elucidation of various intracellular signaling pathways in osteoclasts will be important to direct new therapeutic intervensions.

Future perspective

Recent studies in animal models of inflammatory arthritis have provided considerable evidence that osteoclasts are important mediators of focal bone erosion and have provided insight into the pathways leading to osteoclast differentiation and activation in inflamed joints. These findings support the hypothesis that therapies directly targeting osteoclastogenesis or activation of mature osteoclasts may be useful in protecting against bone destruction in RA. On the other hand, there have been no studies in patients with RA that have explored treatment modalities specifically designed to increase bone formation rates, partly because of the absence of agents that can directly increase osteoblastic activity. However, recent animal studies revealed that the addition of PTH to anti-TNF-α and/or OPG therapies increased trabecular bone mass and osteoblast numbers [95,96]. These data suggest that inflammatory bone loss is reversible and that bone repair requires therapeutic intervention, which shifts the balance of osteoclasts to osteoblasts in favor of the latter. The combined approach of reducing inflammatory response, blocking osteoclast differentiation and activation and stimulating osteoblast activity may achieve optimal control of disease. The development of small molecules and peptidomimetics as therapeutic agents may provide alternatives to current therapies and offer the potential to treat other metabolic bone diseases.

Conclusion

Patients with RA face complications of the bony skeleton that result in joint destruction. The ultimate goal of the treatment of RA is to prevent bone and joint destruction and preserve the daily activity of the patients. Recent studies have demonstrated that osteoclasts are involved in the pathogenesis of bone and joint destruction and can be potent therapeutic targets for this disease, and that therapies which inhibit osteoclast formation or function can at least ameliorate the progression of these bone changes. Discovery of the RANKL/RANK system and other intracellular signaling pathways brought us a rapid increase in the understanding of the regulatory mechanism of osteoclast differentiation and function. Further elucidation of each of these mechanisms will be important to direct new therapeutic intervensions in this area.

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