



Membrane-bound or soluble, mesenchymal or myeloid: which RANKL drives osteoclastogenesis?

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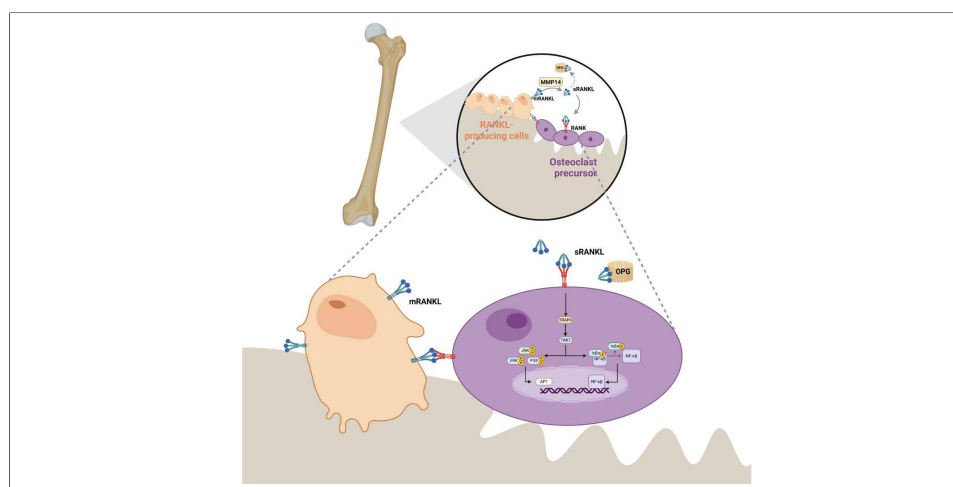
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Abstract

Receptor activator of nuclear factor- κ B ligand (RANKL) is indispensable for osteoclast differentiation, activation, and bone resorption. While the relative contributions of membrane-bound RANKL (mRANKL) and soluble RANKL were historically debated, evidence from the past decade increasingly establishes mRANKL as the primary driver of osteoclastogenesis. However, a systematic literature synthesis integrating these diverse cellular sources and structural modalities remains lacking. It has also not been systematically defined which cellular sources of RANKL - arising from bone marrow mesenchymal lineage cells or hematopoietic lineage cells - play dominant roles in driving osteoclast activation. Clarifying these distinct forms and sources of RANKL will refine the conceptual framework of osteoclast regulation and provide a mechanistic basis for next-generation antiresorptive therapies that selectively target specific RANKL modalities to achieve more durable skeletal protection.

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INTRODUCTION

Osteoclast precursors originate from monocytes derived from hematopoietic stem cells. Their activation involves the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT)-mechanistic target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) signaling pathways. This activation occurs under stimulation by the macrophage colony-stimulating factor (M-CSF)-colony-stimulating factor 1 receptor (CSF1R) axis. These pathways drive the differentiation of monocytes into macrophages. Subsequently, macrophages further differentiate into osteoclasts in response to receptor activator of nuclear factor- κ B ligand (RANKL) stimulation. During osteoclastogenesis, three major regulatory cascades operate: the M-CSF signaling pathway, the RANKL/receptor activator of nuclear factor- κ B (RANK) /osteoprotegerin (OPG) axis, and the nuclear factor- κ B (NF- κ B) signaling pathway. Among them, the RANKL/RANK/OPG axis represents the most crucial and classical pathway governing osteoclast differentiation^[1,2].

RANKL plays a central role in all stages of osteoclastogenesis. It regulates multiple key processes, including the fusion of osteoclast precursors into multinucleated cells, differentiation into mature osteoclasts, attachment to the bone surface, activation for bone resorption, and survival by preventing apoptosis. OPG, as the natural decoy receptor for RANKL, together with RANK and RANKL, forms the RANKL-RANK-OPG “tripartite regulatory system”, governing the rate of bone remodeling. An abnormal increase in the RANKL/OPG ratio within bone tissue is closely associated with pathological conditions such as osteoporosis, inflammatory bone destruction, and tumor-induced bone metastasis^[3].

The RANKL/OPG axis has been successfully translated into clinical practice for the treatment of osteoporosis. Denosumab, a widely used anti-osteoporotic monoclonal antibody, mimics the endogenous function of OPG by neutralizing RANKL, thereby suppressing RANKL-dependent osteoclast differentiation and activation. However, in the absence of appropriate sequential therapy, its therapeutic effects dissipate rapidly after discontinuation. This often results in a pronounced “bone metabolism rebound”, characterized by sharply increased bone resorption, accelerated loss of bone mineral density, and, in severe cases, multiple vertebral compression fractures^[3].

Therefore, elucidating the mechanisms through which the RANKL/OPG axis regulates osteoclast differentiation and function is essential for advancing its therapeutic translation. This review evaluates the spatiotemporal hierarchy of various cellular sources of tumor necrosis factor superfamily member 11 (*Tnfsf11*, mouse gene encoding RANKL) and clarifies the distinct, albeit secondary, role of soluble RANKL (sRANKL) in pathological bone resorption. We aim to provide a mechanistic framework for understanding why systemic *Tnfsf11* blockade leads to metabolic rebound by examining the localized feedback loops between osteocytes and osteoclasts.

RANKL/OPG IN OSTEOCLAST ACTIVATION AND BONE RESORPTION

RANKL's role in osteoclast activation

RANKL is a type II transmembrane protein encoded by a gene on human chromosome 13q14. It belongs to the tumor necrosis factor ligand family. The murine RANKL protein comprises 316 amino acids and shares approximately 85% sequence identity with its human ortholog, which contains 317 amino acids^[4]. RANKL exists in both membrane-bound (40-45 kDa) and soluble (~31 kDa) forms, with the soluble form generated

by proteolytic cleavage of the full-length protein near residues 140/145. RANKL is most abundantly expressed in bone tissue, but it is also detectable in several lymphoid organs, including lymph nodes, thymus, spleen, fetal liver, and Peyer's patches^[5].

RANKL plays a central role in bone homeostasis by regulating osteoclast differentiation and bone resorption. Its principal function is mediated by the binding of trimeric RANKL to homotrimeric RANK. This interaction induces receptor clustering and recruits tumor necrosis factor receptor associated factor 6 (TRAF6), thereby activating multiple downstream signaling cascades. These include the inhibitor of I κ B kinase (IKK)/NF- κ B, c-Jun N-terminal kinase (JNK)/activator protein-1 (AP-1), cellular myelocytomatosis oncogene (c-Myc), and calcineurin pathways, which collectively orchestrate osteoclast differentiation. In addition, the non-receptor tyrosine kinase Src and mitogen-activated protein kinase kinase 6-p38 mitogen-activated protein kinase (p38-MAPK)-melanocyte-inducing transcription factor pathways regulate osteoclast activation, whereas Src in conjunction with ERK promotes osteoclast survival^[6-8]. Beyond the canonical kinase cascades, regulation of Ca²⁺ signaling in this pathway relies on co-stimulatory input mediated by immunoreceptor tyrosine-based activation motif (ITAM)^[9,10].

OPG's role in osteoclast activation

The OPG gene (*Tnfrsf11b*, *tumor necrosis factor receptor superfamily member 11B*) is located on human chromosome 8q23-24 and encodes a secreted, non-membrane-bound member of the tumor necrosis factor receptor (TNFR) superfamily. OPG is synthesized as a precursor of approximately 401 amino acids (aa) that includes a 21-aa signal peptide; removal of this signal peptide yields a mature polypeptide of ~380 aa. The N-terminal region of OPG contains four TNFR-like cysteine-rich domains (CRD1-CRD4). OPG is secreted predominantly as a disulfide-linked homodimer (~110 kDa), with a ~55 kDa monomer also detectable, and it is a glycoprotein that migrates at ~40 kDa after deglycosylation^[11].

OPG acts as a soluble decoy receptor for RANKL. It directly binds the ligand, preventing its interaction with RANK, thereby suppressing osteoclastogenesis and bone resorption at their source^[10]. Studies have found that the N-terminal CRDs mediate ligand recognition; truncation near Cysteine 185 abolishes activity, indicating that this segment is critical for RANKL neutralization^[11]. Both mesenchymal and hematopoietic lineages have been established as important sources of OPG. This creates a local "sponge effect" for RANKL within the bone marrow, constraining its diffusion and reducing the likelihood of RANK engagement^[6]. It is well established that multiple hormones and cytokines coordinately regulate expression of RANKL and OPG, thereby altering the RANKL/OPG ratio. An increase in this ratio augments osteoclast number and activity, whereas a decrease has the opposite effect, establishing the RANKL/OPG ratio as a functional rheostat of bone resorption^[6,12].

mRANKL VS. sRANKL: WHICH FORM PLAYS THE DOMINANT ROLE IN OSTEOCLAST ACTIVATION

mRANKL's role in osteoclast activation

To determine whether membrane-bound RANKL (mRANKL) or sRANKL predominantly regulates osteoclast differentiation, genetic approaches are essential, given their potential spatiotemporal and signaling differences. Xiong *et al.* used the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 to delete amino acids isoleucine 133-serine 149 of the *Tnfsf11* and substitute Arginine for the former tryptophan 150 residue, thereby generating a sheddase-resistant (SR) variant of the *Tnfsf11*^[13]. This mouse strain was designated *Tnfsf11*^{SR}. In *Tnfsf11*^{SR/SR} mice, plasma sRANKL is undetectable, leaving only mRANKL. At 5 weeks of age, bone mass, tooth eruption, and the development of lymphoid organs and the mammary gland are essentially normal, indicating that mRANKL can execute most developmental functions of RANKL during the bone-modeling stage.

Further studies in 8-month-old *Tnfrsf11^{SR/SR}* mice showed that osteoclast numbers were reduced and trabecular bone mass increased, while cortical thickness remained unchanged. These results indicate that mRANKL is the principal driver of adult osteoclastogenesis/remodeling, whereas sRANKL primarily acts as an amplifier^[13]. Accordingly, we speculate that the influence of sRANKL on skeletal development may increase with age. In addition, after undergoing ovariectomy (OVX), *Tnfrsf11^{SR/SR}* mice exhibit bone loss and increased bone remodeling comparable to wild-type controls, indicating that OVX-induced bone loss is independent of sRANKL and can be fully driven by mRANKL alone^[13].

sRANKL's role in osteoclast activation

sRANKL is generated primarily through proteolytic cleavage of the extracellular domain of mRANKL, and this ectodomain-shedding event is mediated by matrix metalloproteinase 14, a membrane-type matrix metalloproteinase^[14]. Compared with mRANKL, sRANKL is dispensable during development but contributes to physiological trabecular bone remodeling in adulthood. Mizuno *et al.* generated two transgenic mouse lines overexpressing sRANKL and characterized their phenotypes^[15]. Mice with global embryonic sRANKL overexpression died during late gestation, whereas mice in which sRANKL expression was restricted to the liver postnatally survived to adulthood. In the latter, trabecular bone began to decline by 6 weeks and was nearly absent by 3-4 months, while cortical bone remained largely unchanged^[15]. Compared with OPG-deficient mice, bone loss in the sRANKL-overexpressing line progressed more slowly and was not accompanied by increased osteoblast numbers, suggesting that the impact of sRANKL on bone development is strongly age-dependent. Asano *et al.* generated a selectively sRANKL-deficient mouse model (*Tnfrsf11^{Δs/Δs}*) through targeted genetic deletion of its cleavage sites^[16]. Their study demonstrated that sRANKL is physiologically dispensable for skeletal homeostasis and, importantly, does not drive the bone loss induced by estrogen deficiency^[16].

Bone marrow mesenchymal lineage cell (BMSC)- VS. Hematopoietic stem cell (HSC)-DERIVED RANKL: WHICH SOURCE DRIVES OSTEOCLAST ACTIVATION

BMSC-derived RANKL

Prx1⁺ stem cells

Paired related homeobox-1 (Prx1)-positive cells mark early progenitors of the embryonic mesenchymal stem-cell lineage. They are localized primarily within the mesenchymal condensations of the limbs and parts of the craniofacial skeleton, but are largely absent from the axial skeleton and visceral organs^[17].

Conditional deletion of RANKL in Prx1⁺ cells produces profound osteopetrosis in the appendicular long bones. At 5 weeks of age, trabecular bone volume fraction increases by approximately 4.5-fold, osteoclasts are nearly abolished, and hypertrophic cartilage persists at the growth plate, consistent with defective removal of calcified cartilage. In contrast, the spine is only minimally affected. These findings provide direct evidence that mesenchyme-derived RANKL is indispensable for osteoclastogenesis in developing long bones^[17,18].

Because Prx1-cyclization recombination (Cre) is active in both chondrocyte and osteoblast lineages, the recombination encompasses both compartments. Further lineage-specific analyses are therefore required to delineate the respective contributions of these cell types [Figure 1].

Osteoblast

In the early stages of research, the hypothesis was proposed that osteoblasts and their precursors are the principal sources of RANKL in bone that facilitate osteoclast formation^[19-21]. Osteoblast lineage and bone-marrow stromal cells constitute the most robust and consistent reservoir of RANKL; In combination with co-secreted M-CSF, they can drive the differentiation of monocytes into osteoclasts *in vitro*^[22,23]. RANKL

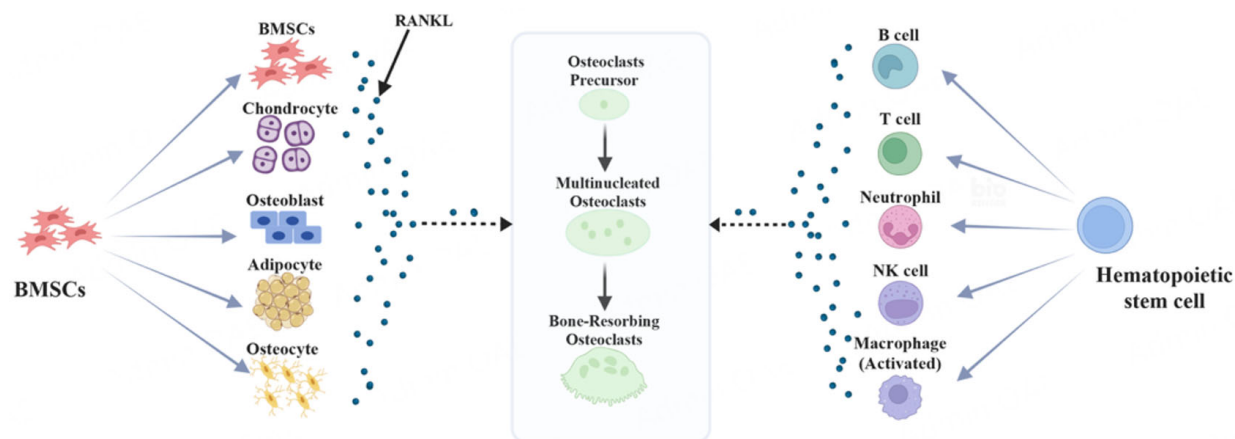


Figure 1. Schematic model illustrating how RANKL derived from BMSCs and HSCs regulates osteoclast precursor differentiation and activation. BMSCs: Bone marrow mesenchymal lineage cells; RANKL: receptor activator of nuclear factor-κB ligand. Created in BioRender. Sun, H. (2026) <https://BioRender.com/6ic15f4>.

is predominantly membrane-bound and requires direct contact with osteoclast precursors; mechanical microdamage, parathyroid hormone, and 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] have been shown to upregulate RANKL expression in osteoblast-lineage cells, thereby initiating osteoclastogenic programs.

Several *in vivo* studies have conditionally ablated matrix-producing osteoblasts to assess their role in bone resorption. Even when osteoblasts were completely depleted in mice and bone formation ceased, RANKL messenger RNA (mRNA) levels in bone remained unchanged over time, under both basal conditions and parathyroid hormone stimulation^[24,25]. Osteoblast-lineage-specific ablation of RANKL - using osterix (Osx, also known as Sp7, encoded by the mouse gene Sp7) 1-Cre or osteocalcin (Ocn)-Cre - produces osteopetrosis^[26-28]. At 5 weeks of age, mice exhibit an approximately four-fold increase in trabecular bone volume together with a marked reduction in osteoclasts within bone tissue. By contrast, the impact on adult bone remodeling is minimal: osteoclast numbers are not consistently reduced, osteopetrosis is not invariably induced, and osteoclast abundance during the remodeling phase remains unchanged. These findings may indicate that osteoclasts engaged in bone modeling are more sensitive to RANKL, or, alternatively, that osteoblast-derived RANKL is not an essential source during the remodeling phase^[17] [Figure 1].

Chondrocyte

Chondrocytes - particularly those at the hypertrophic stage - express RANKL, and bone morphogenetic protein 2 stimulation robustly increases RANKL mRNA/protein, raising the RANKL/OPG ratio. *In vitro*, co-culture of chondrocytes with splenic osteoclast precursors from wild-type mice is sufficient to induce spontaneous osteoclast formation^[29]. Moreover, 1,25(OH)₂D₃-VDR signaling induces RANKL in chondrocytes and promotes osteoclastogenesis both *in vitro* and *in vivo*. Conversely, loss of VDR in chondrocytes delays vascular invasion and osteoclast entry into the growth plate, indirectly indicating that the developmental “chondrocyte-to-osteoclast” sequence depends on a chondrocyte-derived RANKL pathway^[30]. Consistent with this, selective deletion of *Rankl* in hypertrophic chondrocytes (collagen type X alpha 1 chain/Collagen X-Cre) leads to retention of mineralized cartilage, mirroring the growth-plate phenotype observed in *Osx1/Ocn* models^[17,31]. Together, these findings demonstrate that RANKL from hypertrophic chondrocytes is a principal driver of mineralized cartilage clearance during endochondral development [Figure 1].

Osteocyte

Osteocytes modulate RANKL expression in neighboring cells in response to mechanical loading and injury cues. By sensing changes in load, they secrete RANKL to regulate osteoclastogenesis and cancellous bone remodeling, positioning osteocytes as a central source along the “mechanics-to-osteoclast” axis^[17].

In vivo, osteocyte-specific deletion of RANKL using the dentin matrix protein 1 (Dmp1)-Cre driver shows no discernible difference in bone mass or osteoclast number at 5 weeks of age compared with littermate controls. Unlike *Osx1*-Cre or *Ocn*-Cre models^[32-34], Dmp1-Cre-mediated deletion does not impair removal of calcified cartilage at the growth plate during the modeling phase. By approximately 6 months of age, however, Dmp1-Cre; *Tnfrsf11^{fl/fl}* mice exhibit a significant increase in bone mineral density. Specifically, the femoral trabecular bone volume fraction is roughly doubled, while the osteoclast surface is reduced by approximately 75%^[17,18], effects comparable in magnitude to systemic RANKL blockade^[35]. Moreover, in the hindlimb-unloading model, the knockout mice are markedly protected from bone loss, trabecular architectural deterioration, and cortical thinning; the unloading-induced rise in cortical RANKL observed in controls is absent, and the increase in osteoclast number is blunted^[17]. Together, these findings identify osteocyte-derived RANKL as a pivotal driver of bone remodeling and of disuse-induced bone loss [Figure 1].

Bone marrow adipose lineage cells

Unlike peripheral adipose tissue, bone marrow adipose lineage cells (MALPs) arise from bone marrow mesenchymal lineage cells (BMSCs)^[36,37]. Prior studies have shown that obesity and aging expand this adipose lineage within the marrow, thereby impairing both hematopoietic and skeletal regeneration^[38]. Using adiponectin promoter-driven Cre (Adipoq-Cre) to selectively target MALPs, osteolineage-specific deletion of RANKL in Adipoq⁺ cells produced a modest increase in body weight relative to littermate controls. At 4, 8, and 16 weeks of age, these mice consistently showed increased trabecular bone in the femur and vertebrae, while cortical bone remained largely unaffected. Compared with *Tnfrsf11^{fl/fl}* controls, Adipoq-Cre; *Tnfrsf11^{fl/fl}* mice exhibited a marked reduction in osteoclast numbers within the marrow adjacent to trabeculae at 8 weeks, accompanied by diminished coupled bone formation; by contrast, osteoclasts beneath the growth plate and along the periosteal surface were unchanged^[39,40]. These findings indicate that RANKL produced by MALPs acts primarily during the remodeling phase of trabecular bone turnover. Moreover, adipose-lineage RANKL deletion conferred significant protection against OVX- and rosiglitazone-induced osteoclastogenesis and bone loss, but did not mitigate disuse osteoporosis in the hindlimb-unloading model^[40].

MALPs are cells that express adipogenic markers (e.g., Adipoq) yet lack lipid droplets and show minimal proliferative activity. Along the adipogenic trajectory, they occupy an intermediate position between mesenchymal progenitors and lipid-laden mature adipocytes^[41]. To eliminate developmental confounders and define the role of MALP-derived RANKL in adult bone, Lu *et al.* generated inducible conditional knockout mice (Adipoq-CreER; *Tnfrsf11^{fl/fl}*, iCKO), enabling tamoxifen-triggered deletion of RANKL specifically in MALPs^[42]. Inducible ablation of RANKL in adulthood rapidly increased trabecular bone mass in long bones and vertebrae and provided both prophylactic and therapeutic benefits in the OVX model of postmenopausal bone loss, while leaving cortical bone largely unchanged. Conversely, drill-hole injury repair was delayed, consistent with diminished coupling of bone formation to resorption^[42] [Figure 1].

HSC-derived RANKL

B cell

Historically, the high expression of *Tnfrsf11b* in B lymphocytes at physiological steady state suggested they were a principal source of this decoy receptor. However, recent functional studies using conditional gene deletion have redefined this hierarchy^[43]. Cawley *et al.* demonstrated that OPG derived from CD19⁺ B cells is

physiologically dispensable for bone homeostasis, as its deletion failed to alter bone mass or cortical thickness^[44]. Instead, mature osteoblasts emerged as the essential local source of OPG, directly suppressing RANKL activity to terminate the resorption phase of the bone remodeling cycle^[44]. In contrast, B cells contribute little to the RANKL pool under homeostasis. Under pathological conditions such as estrogen deficiency, they transition into RANKL-expressing amplifiers, thereby exacerbating trabecular bone loss. Under estrogen deficiency and chronic low-grade inflammation, however, the B-cell compartment is remodeled: expression of RANKL and factors such as granulocyte colony-stimulating factor increases, the RANKL/OPG balance shifts downward, and B cells switch from “brakes” to drivers of osteoclastogenesis, thereby amplifying trabecular bone resorption^[45,46].

In vivo, B cell-specific deletion of *Tnfsf11* leaves baseline bone mass and turnover essentially unchanged in the absence of estrogen deficiency or inflammatory stimuli, indicating that B cell-derived RANKL is dispensable for steady-state remodeling. Following OVX, by contrast, B cell-specific *Tnfsf11* deficiency confers robust protection against trabecular bone loss, with marked reductions in osteoclast number and osteoclast surface; this protective effect is largely confined to cancellous bone, with minimal impact on cortical bone^[47].

The contribution of B cells to RANKL production is environment dependent; in estrogen-deficient or chronically inflamed marrow, they transition from OPG-dominant inhibitors to RANKL-expressing amplifiers of osteoclastogenesis. Consistent with this, B cell-specific loss of RANKL selectively mitigates OVX-induced trabecular bone loss while exerting little influence on physiologic homeostasis or cortical bone^[46] [Figure 1].

T cell

In early postmenopausal women, the per-cell surface abundance of RANKL on bone-marrow T cells is markedly elevated and correlates positively with biochemical indices of bone resorption, indicating that under estrogen deficiency T cells can amplify osteoclastogenesis. In inflammatory arthritis models, activated T cells express RANKL and drive periarticular bone loss and erosion, underscoring T cells as one of the important sources of marrow RANKL activity^[48].

In vivo, T cell-specific *Tnfsf11* deletion leaves skeletal homeostasis essentially intact. Up to 7 months of age, whole-body and site-specific BMD are comparable to controls, and bone-marrow T/B-cell proportions are unaltered, implying that T cell-derived RANKL is not required for basal remodeling or modeling^[47]. Moreover, T cell-specific *Tnfsf11* deficiency does not mitigate OVX-induced bone loss. Declines in spinal, femoral, and whole-body BMD are similar to controls, and L4 trabecular bone volume/total volume (BV/TV) and trabecular thickness (Tb.Th), as well as femoral cortical thickness, show no improvement. This contrasts with B cell-specific *Tnfsf11* deletion, which protects trabecular bone after OVX, pointing to a division of labor under estrogen deficiency. Consistent with earlier observations that mice lacking T cells are relatively resistant to OVX-induced bone loss, T cells clearly participate in postmenopausal bone catabolism; however, their principal contribution likely operates through cytokine networks (e.g., tumor necrosis factor (TNF)- α , interleukin (IL)-17) and indirect regulation of other cells (such as B cells and osteoblasts) rather than through T cell-intrinsic RANKL^[49-52] [Figure 1].

Macrophage

In inflammatory bone-loss settings such as rheumatoid arthritis and periodontitis, macrophages can participate in - or themselves express - RANKL, thereby promoting focal osteoclastogenesis^[53-56]. Macrophages broadly polarize into pro-inflammatory M1 and anti-inflammatory, pro-regenerative M2

phenotypes^[57]. Multiple studies indicate that M1 macrophages inhibit osteogenic differentiation, whereas M2 macrophages more effectively support osteogenesis^[58]. Co-culture of M1 macrophages with mesenchymal stem cells downregulates OPG, implying an indirect facilitation of osteoclast formation. Notably, M2 macrophages differentiate into mature osteoclasts in the presence of RANKL only under estrogen-deficient conditions. Collectively, the effects of macrophages on bone cells are polarization-dependent and modulated by estrogen [Figure 1].

NK cell

In rheumatoid arthritis (RA) synovial fluid and synovium, natural killer (NK) cells express RANKL together with M-CSF and are positioned in close proximity to CD14⁺ monocytes; *in vitro*, NK-monocyte co-cultures drive osteoclast differentiation in a RANKL- and M-CSF-dependent manner. In the murine collagen-induced arthritis model, depletion of NK cells nearly abolishes bone erosion^[59], indicating that RANKL derived from NK cells can fuel inflammation-associated osteoclastic bone destruction. By contrast, during steady-state bone remodeling and in most systemic models, the dominant sources of RANKL remain osteocytes/osteoblastic lineage cells and MALPs [Figure 1].

Neutrophil

In RA, neutrophils isolated from synovial fluid and synovium have been shown to express RANKL at both the mRNA and protein levels and, *in vitro*, can directly drive monocytes to differentiate into osteoclasts^[46,60]. Mechanistically, neutrophils secrete oncostatin M (OSM), which robustly induces RANKL expression in osteogenic-lineage cells^[61]; moreover, neutrophil extracellular traps potentiate RANKL-induced osteoclastogenesis and thereby exacerbate bone erosion^[62].

In vivo, authoritative genetic evidence defining the skeletal phenotype of a neutrophil-specific *Tnfsf11* knockout is still lacking. Nevertheless, neutrophil depletion/inhibition studies - such as anti-lymphocyte antigen 6 complex locus G antibody treatment - in periodontitis and other inflammatory models consistently reduce RANKL⁺ cells, osteoclast numbers, and bone erosion^[63], providing proof-of-concept and a strong rationale for future neutrophil-specific *Tnfsf11* loss-of-function studies [Figure 1].

Spatiotemporal hierarchy and synergistic regulation of RANKL sources

The regulation of osteoclastogenesis relies on a sophisticated spatiotemporal hierarchy of RANKL sources that shifts across different skeletal life stages. During the bone modeling phase, RANKL derived from hypertrophic chondrocytes and early osteoblast-lineage cells serves as the primary driver^[17,29]. Specifically, chondrocyte-derived RANKL is essential for the clearance of mineralized cartilage during endochondral ossification, while osteoblasts provide the requisite signals for initial bone modeling in the appendicular skeleton.

As the skeleton transitions into the adult remodeling phase, the dominance of RANKL production shifts toward matrix-embedded osteocytes and MALPs. Osteocytes, acting as mechanosensors, provide the pivotal RANKL signals required for steady-state remodeling and disuse-induced bone loss. Concurrently^[18], MALPs emerge as a major regulator of trabecular bone turnover, particularly in the context of aging and metabolic changes^[39,40].

This mesenchymal-derived RANKL framework does not operate in isolation but synergizes with local immune cells to fine-tune bone resorption^[43]. Under physiological steady state, hematopoietic cells such as B cells primarily exert an anti-resorptive influence by maintaining a high OPG/RANKL ratio. However, in pathological contexts such as estrogen deficiency or chronic inflammation, the bone marrow

microenvironment facilitates a “role reversal”: B cells and activated T cells transition into potent RANKL-expressing amplifiers. The synergy between mesenchymal “commanders” (osteocytes/MALPs) and hematopoietic “amplifiers” (B/T cells) creates a hyper-resorptive milieu. This provides a mechanistic explanation for the rapid bone loss observed in postmenopausal osteoporosis and inflammatory bone diseases^[46,47] [Figure 1].

REGULATORY ROLE OF EXTRA-SKELETAL-DERIVED SRANKL IN OSTEOCLAST FUNCTION

As a secreted protein, sRANKL is also produced by various extra-skeletal organs and tissues, enters the circulation, and subsequently reaches bone, where it contributes to the regulation of osteoclast-mediated bone resorption.

Activated human T cells can produce a secreted splice variant of tumor necrosis factor ligand superfamily member 11 (*TNFSF11*, human gene encoding RANKL) that yields sRANKL, which by itself is sufficient to support osteoclastogenesis. In parallel, T cells can release sRANKL via proteolytic ectodomain shedding of membrane RANKL^[64]. In periodontitis and related inflammatory settings, activated B/lymphoid cells generate sRANKL through TNF- α converting enzyme/A disintegrin and metalloproteinase domain 17-mediated shedding, thereby amplifying osteoclast formation^[65]. Rheumatoid arthritis synovial fibroblasts overexpress RANKL and secrete osteoclastogenic mediators; their conditioned media can drive peripheral blood monocytes to differentiate into osteoclasts, positioning the synovial lesion as a major source of sRANKL^[66].

Human microvascular endothelial cells upregulate RANKL in response to inflammatory cytokines such as IL-1 and TNF- α , thereby promoting osteoclast formation^[67]. Concurrently, they increase the expression of intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and CD44, which markedly enhances the adhesion and transendothelial migration of circulating CD14⁺ osteoclast precursors, indirectly amplifying osteoclastogenesis and bone resorption at sites of inflammation^[68].

Multiple hepatic cell types - including hepatocytes, Kupffer cells, liver sinusoidal endothelial cells, and hepatic stellate cells - express RANKL and its decoy receptor OPG^[69]. Under pathological conditions such as chronic liver disease, trauma, or systemic inflammation, hepatic RANKL expression is typically induced in parallel with inflammatory activation. Histologically, increased densities of RANKL-positive cells are observed within portal tracts, along fibrous septa, and around bile ducts. Upregulated RANKL can be shed or otherwise released into the circulation, thereby contributing to the systemic pool of sRANKL and amplifying osteoclastogenic signaling^[69-71]. Consistent with this, several studies have reported elevated serum sRANKL in non-cirrhotic stages of chronic liver disease^[70]. Nevertheless, because bone remodeling is governed predominantly by local paracrine interactions, circulating RANKL/OPG concentrations only partially capture the effective signaling milieu within bone.

Tumor-associated cells also constitute important sources of sRANKL that promote local-and in some cases systemic-bone resorption. In multiple myeloma, malignant plasma cells themselves produce sRANKL, correlating with elevated circulating sRANKL and generalized bone loss^[72,73]. In addition, myeloma cells stimulate bone marrow stromal/osteoblastic cells to increase RANKL, creating a malignant bone-tumor feed-forward loop. Breast and prostate cancer cells increase the RANKL/OPG ratio in the bone microenvironment directly or indirectly (e.g., via parathyroid hormone-related protein, IL-6), thereby amplifying osteoclastogenesis, driving osteolysis, and facilitating skeletal metastasis^[16,74-76].

RANKL REVERSE SIGNALING: FROM OSTEOCLAST TO OSTEOBLAST AND BACK TO OSTEOCLAST

Beyond the functions described above, RANKL acts as a bidirectional signaling molecule. The canonical “forward” pathway is the RANKL-RANK interaction that promotes osteoclastogenesis. By contrast, “reverse” signaling is initiated within RANKL-expressing cells when multiple RANKL trimers are cross-linked into higher-order clusters. The cytoplasmic tail of RANKL contains proline-rich motifs that engage Src family kinases, thereby activating downstream pathways. In bone, vesicular RANK derived from osteoclasts can serve as a ligand to engage RANKL on osteoblasts and trigger RANKL reverse signaling. This signaling drives runt-related transcription factor 2 nuclear translocation and early osteoblast differentiation, thereby supporting the coupling between bone resorption and formation^[77]. RANKL is also subject to intrinsic negative feedback. In osteoclast precursors, RANKL induces expression of the deubiquitinase ubiquitin C-terminal hydrolase L1 (UCHL1), which directly interacts with CD13 to deubiquitinate and stabilize the CD13 protein, thereby promoting the release of soluble CD13 (sCD13). Acting in an autocrine manner, sCD13 attenuates RANKL-driven MAPK signaling (JNK/ERK/p38) with minimal impact on NF-κB, ultimately suppressing osteoclast differentiation. This mechanism provides a rationale for how, in the early stages of osteoarthritis, restricting osteoclastogenesis within the subchondral bone compartment can help protect the joint^[78].

DISCUSSION

The relative contributions of mRANKL and sRANKL to osteoclastogenesis have been historically debated. However, recent pivotal genetic studies have increasingly established mRANKL as the primary driver of both skeletal development and adult bone remodeling^[13]. Our synthesis confirms that mice selectively lacking sRANKL (*Tnfsf11*^{Δs/Δs}) exhibit normal bone density and skeletal microstructure, demonstrating that sRANKL is physiologically dispensable for basal homeostasis^[16]. In contrast, mRANKL serves as the indispensable ligand for physiological bone turnover and the accelerated resorption observed in estrogen-deficiency models. While sRANKL appears redundant for normal skeletal health, its role is markedly prominent in pathological settings. It acts as a chemotactic agent that facilitates the migration of RANK-expressing tumor cells to bone, thereby promoting skeletal metastasis without directly influencing osteoclast activation^[16].

The clinical translation of the RANKL-RANK-OPG axis, exemplified by Denosumab, has encountered significant challenges, most notably the “rebound bone turnover” following treatment cessation^[3]. Mechanistically, the withdrawal of RANKL inhibition leads to a localized surge in *Tnfsf11* levels, triggering the rapid fusion of accumulated osteoclast precursors - termed osteomorphs - into active, bone-resorbing cells^[2]. This metabolic burst, compounded by an insufficient compensatory OPG response, significantly elevates the risk of multiple vertebral compression fractures. To mitigate these risks, next-generation antiresorptive strategies must move toward precision targeting^[3].

FUTURE PERSPECTIVES

Despite substantial progress, several conceptual and translational gaps remain. A key priority is to delineate the spatiotemporal dynamics of RANKL production across mesenchymal and hematopoietic compartments, particularly in the contexts of aging, metabolic dysregulation, malignancy, and chronic inflammation. Advances in single-cell multi-omics and spatial transcriptomics are expected to uncover previously unrecognized microanatomical niches that fine-tune RANKL accessibility during skeletal remodeling.

Another promising direction is to define the functional significance of RANKL reverse signaling within osteoblast-lineage cells, which may represent an underexplored mechanism governing the coupling of bone

resorption and formation. Denosumab, a potent full-spectrum RANKL-neutralizing antibody, is frequently associated with a pronounced “rebound bone turnover” upon discontinuation, resulting in a rapid loss of bone mineral density^[3]. Recent mechanistic insights reveal that this phenomenon is driven by a localized surge in RANKL levels following treatment withdrawal, which triggers the swift fusion of long-term suppressed osteoclast precursors (osteomorphs) into active bone-resorbing cells^[2]. Furthermore, emerging evidence underscores the regulatory role of osteocyte-derived OPG; an insufficient OPG response fails to antagonize the excessive RANKL signaling, thereby exacerbating this metabolic dysregulation. Clinically, these abrupt fluctuations in bone turnover significantly elevate the risk of multiple vertebral compression fractures. To mitigate these skeletal events, current management strategies advocate for the immediate sequential administration of anti-resorptive agents, such as bisphosphonates, to dampen the transient burst in osteoclast activity^[3]. Additionally, the identification of the RANKL-UCHL1-sCD13 negative feedback loop provides a promising molecular target. Targeting this loop could help stabilize subchondral bone and prevent systemic bone loss^[78]. Looking forward, the development of precision therapies is essential; as proposed by Asano *et al.*, selective inhibitors targeting sRANKL represent a superior anti-metastatic strategy^[16]. By inhibiting sRANKL-mediated tumor cell migration while preserving the physiologically indispensable mRANKL, such agents are hypothesized to carry fewer systemic adverse effects and minimize the risks associated with total RANKL blockade^[16]. From a therapeutic standpoint, next-generation antiresorptive strategies should prioritize precision targeting - such as selectively blocking RANKL derived from specific cellular sources (e.g., MALPs or osteocytes) or preferentially inhibiting the soluble form - to minimize adverse effects and prevent systemic rebound in bone turnover.

CONCLUSION

A comprehensive body of genetic, cellular, and lineage-tracing evidence demonstrates that the RANKL/RANK/OPG axis serves as the central regulatory system governing osteoclast differentiation, activation, and skeletal turnover^[1,2]. This review integrates decades of research delineating the distinct contributions of mRANKL and sRANKL, as well as the diverse cellular sources of RANKL within the bone marrow and across extra-skeletal organs and tissues. Collectively, current evidence establishes that mRANKL is indispensable for developmental osteoclastogenesis and remains the predominant driver of physiological bone remodeling in adulthood, whereas sRANKL functions mainly as an age-dependent amplifier of trabecular bone resorption^[13-15].

Advances in conditional knockout models have further refined the hierarchy of RANKL sources, identifying osteocytes and MALPs as key regulators of adult skeletal remodeling^[17,39,40]. In addition, extra-skeletal tissues-including lymphoid organs, the liver, vascular endothelium, and various tumors-contribute to circulating sRANKL and may thereby influence systemic osteoclastogenic activity. Together, these findings redefine current concepts of RANKL-mediated osteoclast regulation and provide an updated mechanistic framework for understanding both physiological and pathological bone resorption.

DECLARATION

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Authors' contributions

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Conducted the literature search, performed data extraction from the included studies, and assisted in the synthesis of recent genetic evidence: Wang W (Wenzhao Wang)

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Conflicts of interest

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Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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