

# Role of RANKL–RANK/osteoprotegerin molecular complex in bone remodeling and its immunopathologic implications

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### ABSTRACT

Bone remodeling is a cyclic and continuous physiological process, which ensures the conservation and renewal of the bone matrix. Osteosynthesis of the bone matrix is achieved by osteoblasts and coordinated within this complex machinery of bone remodeling with resorption of extracellular bone matrix performed by osteoclasts. The mismatch between the activities of osteoblasts and osteoclasts has immunopathologic implications associated with either a decrease or increase of bone mass mineral density. The balance of the trimolecular control factor complex composed of osteoprotegerin (OPG), RANKL (osteoprotegerin ligand) and RANK maintains physiologic bone remodeling. This trimolecular complex functions as receptors and ligands and belongs to the superfamily of tumor necrosis factor (TNF). This mini review highlights the complex interplay of the RANKL–RANK/OPG axis and their immunopathologic implications in clinical medicine.

**Key words:** Bone metabolism, bone remodeling, RANK–RANKL/OPG

## INTRODUCTION

Bone as an organ has multiple functions in vertebrates, including protection of vital organs and hematopoietic marrow, structural support for muscles, and storage and release of vital ions, such as calcium, and of growth factors stored in the bone matrix.

## NORMAL BONE MODELING

All bones in the mammalian skeleton except calvarial bones are preformed in cartilage moulds from undifferentiated mesenchymal progenitors. Chondrocytes proliferate near

the ends of the cartilage moulds to drive their longitudinal growth, whereas those in the center undergo hypertrophic differentiation. The hypertrophic chondrocytes at the periphery of these centers are invaded by blood vessels and undergo apoptosis. Some of these hypertrophic cartilages survive as thin islands of cartilage in the centers of ossification in growing bones. Osteoblasts, which differentiate from these progenitors in a collar of connective tissue around the middle of the bones where vascular invasion takes place, follow the endothelial cells and lay down bone matrix on the surfaces of these islands of cartilage to form bone trabeculae.<sup>[1]</sup> Osteoclast precursors (OCPs), derived from progenitors in the spleen and liver, are attracted from blood in the invading blood vessels close to newly formed bone trabeculae. These OCPs fuse with one another to form multinucleated osteoclasts, which resorb most of the newly formed bone, leaving only a few trabeculae to comprise the spongy bone of the secondary spongiosa. Osteoblasts lay down new bone on parts of the surfaces of these surviving trabeculae where there has been osteoclastic resorption and much of this new bone

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is subsequently resorbed by osteoclasts in a remodeling process which ensures that the volume of bone in the medullary cavity is limited and does not fill the space.<sup>[2,3]</sup>

## NORMAL BONE REMODELING

Bone in the adult skeleton is renewed continuously in response to a variety of stimuli by the process of bone remodeling. This involves removal of trenches and tunnels of bone from the surfaces of trabecular and cortical bone, respectively, by osteoclasts.<sup>[4]</sup> Osteoblasts subsequently fill in these trenches by laying down new bone matrix in them. Bone formation matches resorption during normal bone remodeling.

The processes that drive bone remodeling are still not fully explained, but they include damage to parts of bones in response to normal wear and tear, changes in mechanical forces following alterations in body shape or weight or exercise, and local release of cytokines or growth factors due to alterations in levels of systemic hormones. It is a tightly regulated process in that formation follows resorption in a site-specific manner and there are more than 1 million of these microscopic foci of remodeling at any time in the adult skeleton.

Molecular biology in the last two decades has witnessed an exponential increase of knowledge in bone biology. The scientific study of the regulation and maintenance of bone as an organ by hitherto unknown systemic and local factors has opened interesting and intriguing vistas in learning about bone remodeling and bone metabolism. This has been possible due to several key discoveries; these include identification of sex steroid receptors in bone cells,<sup>[5,6]</sup> the recognition and categorization of parathyroid hormone related peptide (PTHrP) as being majorly responsible for humoral hypercalcemia in malignancy,<sup>[7,8]</sup> recognition of the master gene behind osteoblast differentiation, which is Core Binding Factor-1 (*Cbfa-1*);<sup>[9-11]</sup> and the significance of apoptosis in the regulation of osteoblasts and osteoclasts. However, there is one finding which overshadows all these accomplishments, which is the recognition and categorization and significance of the RANKL-RANK/OPG system in bone biology<sup>[12]</sup> [Figure 1]. This important discovery was first brought to light in 1997 in a paper by Simonet *et al.*<sup>[13]</sup>

Recent studies suggest that at least in response to mechanical forces, osteocytes regulate the recruitment of osteoclasts to sites of bone resorption by inducing the expression of RANKL by osteoblastic cells in the local micro-environment.<sup>[14]</sup> They communicate with one another and with osteoblastic cells on the surface of

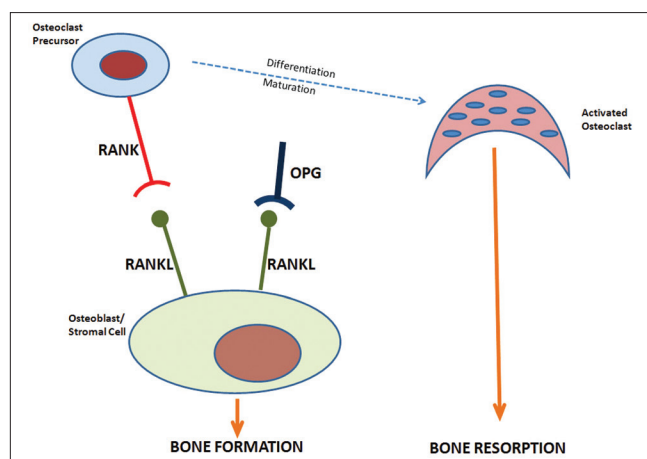


Figure 1: Interaction of the RANKL-RANK/OPG biomolecular complex

overlying bone and through them with osteoblastic cells in the bone marrow cavity and in this way are thought to regulate RANKL expression by these latter cells, although they do not appear to express RANKL themselves. Most of these factors induce bone resorption predominantly by an indirect mechanism that involves upregulation of the expression of macrophage colony stimulating factor (M-CSF) and RANKL by osteoblastic and other cells.

## RANKL-RANK

RANKL is the abbreviation for receptor activator of nuclear factor kappa beta (NFκB ligand). It is also commonly referred to as osteoprotegerin ligand (OPGL) or osteoclast differentiation factor (ODF) or TNF related activation-induced cytokine (TRANCE). Four independent researchers almost simultaneously identified the RANKL/OPGL/ODF/TRANCE system for which they cloned utilizing different strategies.<sup>[15-18]</sup> RANKL is identified to belong to the tumor necrosis factor (TNF) family and has been recognized to be the only cytokine to play an essential role in bone metabolism as it regulates the development, maintenance and activation of osteoclasts.<sup>[16,19,20]</sup> The *RANKL* gene encodes a 316AA protein which is structurally a monomer but in function exists as a homotrimer. RANKL is expressed in two forms: as a membrane adhered molecule on the cell surface and as a soluble molecule released by TNF-alpha convertase (TACE).<sup>[21]</sup>

The cellular reservoir of RANKL is formed by osteoblasts, bone marrow stromal cells, chondrocytes, activated T lymphocytes, TCD4+, TCD8+, and CD4 CD8 thymocytes. The calciotropic factors with a stimulating role on RANKL production are factors that stimulate bone resorption [i.e. parathyroid hormone (PTH), PTHrP, vitamin D3,

interleukin-1 (IL-1), IL-11, IL-17, TNF alpha, prostaglandin E2 (PGE2) and CD40L].<sup>[22,23]</sup>

The functions of RANKL are concentrated on bone biology, more specifically bone metabolism. RANKL plays a vital role in osteoclastogenesis. It is under the complex interplay of RANKL and M-CSF that monocyte progenitor from the hematopoietic myeloid reservoir differentiates into mature osteoclasts.<sup>[15,16]</sup> The osteoclasts are primarily responsible for bone resorption and RANKL influences their activation. For effective bone resorption, osteoclasts attach themselves to the bone surface via podosomes. By virtue of these podosomes, they form tight seals with the underlying bone matrix in roughly circular extensions of their cytoplasm and within these sealed zones they form ruffled border membranes. Ruffling of the cytoplasmic membrane increases the area of the cell surface for secretion of proteolytic enzymes, cathepsin K, and hydrochloric acid (HCl) onto the bone surface.<sup>[24]</sup> By this sealing and secretory mechanism, the bone matrix is simultaneously degraded and bone minerals are dissolved, while protecting neighboring cells from the harmful effects of HCl. RANKL and beta integrin-mediated signaling from bone matrix activate osteoclasts.<sup>[25-27]</sup> OCPs fuse with one another and become multinucleated under the influence of RANKL. RANKL also induces expression of tartrate-resistant acid phosphatase and cathepsin K through nuclear factor of activated T-cells,<sup>[28]</sup> thus establishing the link between RANKL and the activation of osteoclasts. Several RANKL gene knockout studies in animals support this theory.

It was observed that RANKL animal models do not display osteoclastogenesis. Because of a RANKL defect, there is no osteoclastogenesis as M-CSF alone is not capable to aid in differentiation of myeloid progenitor cells in the absence of RANKL.<sup>[29,30]</sup> However, the administration of recombinant RANK (rRANK) in these animal models reinstates the process of osteoclastogenesis. At the same time, RANKL is a ligand for the soluble receptor OPG and this interaction blocks osteoclastogenesis via RANKL. Thus, RANKL has a dual antagonistic type action on osteoclastogenesis, depending on the type of receptor it interacts with: RANK or OPG, although both receptors belong to the same TNF receptor family. RANKL thus plays a key role in activation of osteoclasts, thereby influencing bone resorption.

The interaction between RANK and RANKL signals the initiation of both osteoclastogenesis and activation of osteoclasts.<sup>[31,32]</sup> RANK is the abbreviation of receptor activator of NFkB or commonly also known as TRANCE-R. Structurally, RANK is a heterotrimer. RANK is found to be expressed on the surface of osteoclast

progenitor cells, mature osteoclasts, chondrocytes, dendritic cells and trophoblasts.<sup>[33]</sup> Studies conducted on RANK-gene knockout animal models revealed that in these mice, osteoclastogenesis inhibition, absence of osteoclasts, associated with severe osteopetrosis was observed.<sup>[16]</sup>

Thus, it is theorized that the molecular mechanism consists in binding the RANKL ligand to the soluble decoy receptor OPG, in competition with RANK, followed by the inhibition of osteoclast development via RANKL.<sup>[33]</sup> RANK is considered to be a receptor activator of the NFkB factor, similar to the TNF-R signaling. This complex intracellular signaling mechanism which is responsible for differentiation, survival and activation of osteoclasts and bone resorption signifies the RANK activation through the ligand or RANKL.<sup>[34,35]</sup>

### Osteoprotegerin

Osteoprotegerin (OPG) is likened to a bone protector. It is also known as osteoclasts inhibitor factor (OCIF). OPG is secreted as a homodimer and is a post-translationally glycosylated protein.<sup>[36-38]</sup> OPG is a soluble receptor homologous to TNF-R.<sup>[38]</sup> The cellular reservoir of osteoblasts, are the bonemarrow stromal cells and follicular dendritic cells. OPG is a soluble decoy receptor, which in competition with RANK receptor binds to RANKL. Both OPG and RANK are receptors which show affinity to the same ligand RANKL.<sup>[36,38]</sup> OPG is an antagonistic endogenous receptor and upon binding with RANKL inhibits osteoclastogenesis, thus jamming the process of bone resorption.

The OPG-RANKL complex counterbalances the effect of the RANK-RANKL complex, thus playing the most important role in bone homeostasis.<sup>[37-39]</sup> This can be further substantiated by the fact that in transgenic mice in which the *OPG* gene was knocked out, severe osteoporosis quickly set in. Spontaneous fractures were observed in these animal models due to excess formation of RANKL-RANK complex.<sup>[40]</sup>

These experiments prove that formation of RANKL-OPG complex and RANKL-RANK complex is the paramount factor in osteoclast differentiation and activation, thus influencing cumulative bone turnover.

## SYSTEMIC IMMUNOPATHOGENIC IMPLICATIONS OF RANKL-RANK-OSTEOPROTEGERIN COMPLEX

Bone remodeling is a cyclic physiological process encompassing both periods of osteoblastogenesis and osteoclastogenesis with a quiescent period interposed

between them. Along with other systemic and local factors, the RANKL–RANK–OPG trimolecular complex rigorously regulates bone metabolism. An imbalance between these two complexes causes an altered rate of bone turnover, which gives rise to osteopenic diseases. In these cases, there is bone resorption and destruction in local or general osteolysis focal areas.

### Postmenopausal osteoporosis

The *in vitro* expression of the OPG as evidenced in studies in the human stromal cell cultures is induced by estrogens.<sup>[41,42]</sup> Estrogens stimulate OPG secretion from osteoblasts and inhibit RANKL production. This effect attributes to the anti-resorbing property of estrogen. Thus, the production of estrogen is proportional to OPG secretion. Hence, in postmenopausal females as the production of estrogen is reduced, subsequently the production of OPG is hindered and postmenopausal osteoporosis sets in. As part of the counterbalancing phenomenon, the production of RANKL is increased which leads to the formation of RANKL–RANK complex as the competitive inhibition by OPG is stalled. This leads to greater bone resorption and decrease in bone mineral density. This forms a viable argument for the immunopathogenesis of postmenopausal osteoporosis. In addition, in individuals with osteoporosis, there is an associated increase in vascular calcification.<sup>[43,44]</sup>

Studies performed on ovariectomized female mice models lends credibility to this theory.<sup>[37]</sup> In these animal models, upon injection of recombinant OPG, there was reduction in bone destruction and the process of osteoporosis was mired.

Thus, it is hypothesized that recombinant OPG will be a viable future treatment possibility for postmenopausal osteoporosis and, to some extent, in osteoporosis. The administration of recombinant OPG in the form of a therapeutic agent will help prevent osteoclast activation and consequently bone destruction.

### Autoimmune and chronic inflammatory diseases

The RANKL/RANK/OPG system is an important molecular biologic link between the immune system and bone metabolism. The functioning of the OPG/RANKL system is similar to that of the interleukin–cytokine system. Research in cell cultures and animal models shows that activated T cells express membrane-soluble RANKL. This RANKL production leads to osteoclast formation and activation and hence stimulates osteolysis. In cases where there is chronic systemic activation of T cells, the process of osteoclast formation and activation is triggered due to the expression of RANKL complexes. In normal conditions,

this is followed by stage of bone formation/remodeling; in pathologic settings, this phenomenon of bone resorption gets intensified. Therapeutic administration of recombinant OPG is known to thwart this event of osteolysis due to the effect of the activated T cells osteoclasts.<sup>[45-48]</sup>

### Rheumatoid arthritis

Rheumatoid arthritis is described as an autoimmune chronic inflammatory disease which presents with inflammation of the synovium and the adjacent articular synovial tissue, associated with erosion and destruction of articular cartilage and bone, followed by articular dysfunction and disability. It is known to be an autoimmune disease with cellular mediated response. The main contributors of this orchestration of events are dominated by activated monocytes/macrophages, synovial fibroblasts, polymorphonuclear cells, mastocytes and activated TCD4+ lymphocytes. The activated synovial T cells express RANKL and initiate osteoclastogenesis via RANKL.<sup>[46-49]</sup> While activated non-T cells (monocytes/macrophages, fibroblasts) from the inflammatory synovium produce proinflammatory cytokines: IL-1, IL-6, IL-11, TNF-alpha, which indirectly mediate bone destruction, probably by stimulating the RANKL expression in osteoblasts and chondrocytes, but do not stimulate the OPG expression in osteoblasts. This bone destruction is mediated by the RANKL complex, but the development of bone response is independent of the alteration of the bone metabolism.

Therapeutic administration of the recombinant OPG would also have a beneficial effect by hindering the resorbing effects of the RANKL-mediated complex without influencing the evolution of the inflammatory process.<sup>[49-51]</sup>

### Dialysis related amyloid osteopathy

In individuals suffering from long-term amyloidosis, a common condition called dialysis related amyloid osteopathy (DRAO) is observed which primarily exhibits osteoarticular focal lytic lesion. The most affected sites are the epiphyses of the long bones and the spinal bones; these sites present with erosive ariculitis. This course of development of disease is termed as destructive spondyloarthropathy. The affected sites exhibit deposition of amyloid material both in the cavities of long bone cysts and in intervertebral discs. These conditions exhibit an increase in osteoclastogenesis without reactive bone formation. The lesions displayed in DRAO are believed to be related to the paracrine/autocrine mechanism because of the absence of any changes in the systemic bone metabolism.<sup>[52]</sup>

The DRAO lesions are said to occur through three intricate pathways. Inflammatory cells infiltrating the



amyloid deposits secrete a series of proinflammatory cytokines such as IL-1, IL-11, IL-17, and TNF- $\alpha$ , which promote expression of RANKL on osteoblasts. The RANKL binds with RANK on osteoblast precursor cells and stimulates maturation of these precursors. RANKL also binds to mature osteoclasts to inhibit apoptosis of these cells and activation of their osteoclastic activity.<sup>[52]</sup> The cells of the inflammatory infiltrate, namely CD4+ helper T cells, can express RANKL upon their activation and sustain the aforementioned schema of stimulating osteoclastogenesis.<sup>[53]</sup> In addition, the inflammatory cells release cytokines, which in turn may act directly on osteoclasts (TNF- $\alpha$  helps in maturation of osteoblast precursors, while IL-1 $\beta$  promotes the inhibition of mature osteoclasts).<sup>[53,54]</sup>

Still it is important to fully understand the roles of these molecular-biologic pathways in DRAO lesions to develop a suitable therapy for it. Recombinant OPG therapy has been suggested, as this molecule holds the promise of blocking osteoclastogenic activity by acting as a decoy receptor which would help in preservation of bones in dialyzed patient from osteolytic lesions in the presence of local amyloid deposits.

## FUTURE PHARMACOTHERAPEUTIC AVENUES UTILIZING THE RANKL/RANK-OSTEOPROTEGERIN PATHWAY

Preclinical *in vivo* studies utilizing inhibitors of the RANKL/RANK signaling pathway have established the importance of this trimolecular system in non-human models of sex-steroid deficiency- and glucocorticoid-induced osteoporosis, rheumatoid arthritis, multiple myeloma, and metastatic bone disease.<sup>[55-58]</sup> Phase 1 clinical trials of two forms of OPG (Fc-OPG and OPG-Fc) were conducted wherein single injections of these OPG constructs were administered into normal volunteers which resulted in prolonged dose-dependent reductions in the biochemical markers of bone resorption.<sup>[59,60]</sup> However, neither of these proteins were developed for further clinical trials, probably because of concerns about the possibility of immune responses to them and consequent unwanted adverse effects on the immune system. They were replaced by Denosumab, a fully human monoclonal antibody developed by injecting mice with human RANKL, which binds to and inactivates RANKL, similar to the action of OPG. Denosumab is designed to target RANK ligand, a protein that acts as the primary signal to promote bone removal. Compared to OPG-Fc, Denosumab has a significantly longer circulating half-life and a more prolonged effect to reduce serum levels of markers of bone resorption and

formation.<sup>[61]</sup> There are several phase 2 and 3 clinical trials investigating the efficacy of Denosumab in patients with a variety of bone disorders, including postmenopausal osteoporosis, rheumatoid arthritis, multiple myeloma and metastatic bone disease.<sup>[62,63]</sup> To date, treatment of patients in these studies has resulted in significant inhibition of bone resorption without any obvious significant adverse effects.

Future vistas of clinical trials utilizing the RANKL/RANK-OPG axis may help in slowing the progression of alveolar bone destruction in both aggressive and chronic types of periodontitis,<sup>[64]</sup> and also may prove to be a valuable adjunct in orthodontic therapy by aiding in increasing the rate of tooth movement.<sup>[65,66]</sup>

## SUMMARY

The discovery of the RANKL/RANK/OPG system has been one of the most important advances in bone biology in the last decade. This signaling system is essential for skeletal homeostasis, and disruption of it leads to inhibition of bone resorption *in vitro* and in animal models of most bone diseases characterized by increased resorption. RANKL/RANK signaling plays important roles in tissues other than bone. Elucidation of the specific roles of RANKL/RANK in these various types of cells will likely link bone remodeling in normal and disease states with regulation of the function of other organ systems in health and disease. The discovery of RANKL, RANK and OPG has led to the development of specific inhibitors of RANKL, some of which, such as OPG and a monoclonal antibody to RANKL, have been tested in humans in clinical trials with successful inhibition of bone resorption. Thus, it will be important to determine if long-term inhibition of RANKL has any unwanted adverse effects in these tissues and on immune responses in particular.

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