

RANKL-RANK/OPG MOLECULAR COMPLEX - CONTROL FACTORS IN BONE REMODELING

Doina Drugarin, Malina Drugarin, Serban Negru, Ramona Cioaca

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 phenotype, function and ontogenesis of osteoblasts and osteoclasts are essentially different. Osteoblasts arise from bone-marrow stromal cells, and osteoclasts arise from hematopoietic myeloid progenitor cells—monocyte/macrophage. Both types of cells have a common target - the bone structure. The imbalance between the effector activities of osteoblasts and osteoclasts has clinical implications associated with the decrease or increase of bone mass mineral density, osteoporosis or osteopetrosis respectively. Reports from 1977-1988 has offered new explanations that have changed the perception on bone metabolism and has opened new immunoclinical interpretations with therapeutic prospects. The balance of the trimolecular complex composed of Osteoprotegerin (OPG), RANKL (osteoprotegerin-ligand) and RANK, which are control factors through osteoclastogenesis modulation, governs homeostasis of bone remodeling. These molecules, OPG, RANKL and RANK, function as receptors and ligand and belong to the superfamily of tumor necrosis factor (TNF).

Key Words: bone remodeling, bone metabolism, trimolecular complex - OPG/RANKL-RANK

RANKL

RANKL is also known as receptor activator of NF κ B ligand or OPGL (osteoprotegerin ligand) or ODF (osteoclast differentiation factor) or TRANCE (TNF related activation-induced cytokine).

RANKL/OPGL/ODF/TRANCE was cloned and identified through different strategies by four independent groups of researchers: Yasuda¹, Lacey², Anderson³ and Wong⁴. RANKL is a member of the TNF family and is the only cytokine which has a role in the development and activation of osteoclasts.^{2,5,6} The *rankl* gene encodes a 316 AA (38 kDa) protein which is structurally a monomer and is functionally active as a homotrimer.

A trimeric RANKL can be expressed in two forms: as a *membrane* anchored molecule on the cell surface and as a *soluble* molecule released through enzymatic

cleavage by membrane metalloprotease - disintegrin TNF-alpha convertase (TACE).⁷ Both membrane bound and soluble forms of RANKL can function as potentially agonistic ligands which can interact with RANK and/or OPG receptors.

The cellular source of RANKL production are 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), parathyroid hormone-related protein (PTHrP), vitamin D₃, interleukin-1 (IL-1), IL-11, IL-17, TNF-alpha, PGE₂ and CD40L.^{8,9}

Biological effects of RANKL are focused on bone metabolism. *RANKL mediates osteoclastogenesis*. The differentiation of hematopoietic myeloid monocyte progenitors in mature osteoclasts is achieved under the associated control of RANKL and M-CSF (monocyte/macrophage colony stimulating factor).^{1,2} *Activation of mature osteoclasts* exclusively depends on RANKL. The activated osteoclasts are the main effector cells of bone resorption.

The RANKL effects were identified using *rankl* gene knockout animal models. The *rankl*^{-/-} mice display severe osteopetrosis and defects in tooth eruption.¹⁰

Department of Immunology,
Victor Babes University of Medicine and Pharmacy Timisoara

Correspondence to:
Doina Drugarin
Str. Soimos nr. 6, Timisoara
tel/fax 0256-192129; mobile 0723-149294; e-mail drugarin@yahoo.com

The osteoblasts obtained from the *rankl*^{-/-} mice cannot support osteoclastogenesis, which indicates an intrinsic defect in osteoblastic stroma.^{10,11} In the presence of a RANKL defect, osteoclastogenesis cannot be ensured because M-CSF alone cannot make the phenotypic and functional differentiation of myeloid progenitor cells in precursors of osteoclasts unless it is associated with RANKL.^{1,2,11} The administration of recombinant RANK (RANKr) restores osteoclastogenesis.

The osteoclastogenesis and bone resorption mediated by RANKL are achieved by interaction with the RANK receptor expressed on osteoclasts. 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. Thus RANKL modulates the osteoclastogenesis and the bone resorption achieved by osteoclasts.

RANK (receptor activator of NFκB), also known as TRANCE-R, is a heterotrimer expressed in a transmembrane way on the surface of osteoclast progenitor cells, mature osteoclasts, chondrocytes, dendritic cells, trophoblasts and epithelial cells of mammary glands. Structurally RANK is a TNF-R related heterotrimer protein.³

The interaction of the RANK receptor with its ligand RANKL represents the essential stage in initiating osteoclastogenesis and osteoclast activation.^{12,13} The encoding gene knockout mice model for *rank* (*rank*^{-/-}) appears as phenocopies of *rankl*^{-/-} mice and is characterized by osteoclastogenesis inhibition, absence of osteoclasts, associated with severe osteopetrosis.¹⁴ Under these circumstances 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. *Rank*^{-/-} mice display an intrinsic defect in osteoclast function, because the introduction of RANKr in the hematopoietic progenitor cells restores osteoclast ontogenesis.¹⁴

Similar results to the experiments on animal models have a correspondent in humans *in vivo* and *in vitro* (in cellular systems). Thus the interaction between RANKL, the free or membrane form expressed on osteoblasts and bone-marrow stromal cells or activated T cells, with the membrane RANK receptor expressed on osteoclasts is the key stage in triggering osteoclastogenesis.

The signaling mechanism through RANK receptor after binding to RANKL consists in transducing activating signals into the osteoclasts through *adapter proteins*. The long intracytoplasmic segment (383 AA) of the RANK receptor contains two domains of combinative sites for the *associated factors of TNF receptors* called TRAF. The interaction between RANK and protein molecules TRAF 1, 2, 3, 5 and especially 6 has a functional role in the activation of the nuclear transcription factor NFκB followed by translocation in the nucleus and the activation of the JNK kinase.¹⁵⁻¹⁸ As a matter of fact RANK is considered a receptor activator of the NFκB factor, similar to the TNF-R signaling.

These intracellular signaling mechanisms trigger the differentiation, survival and activation of osteoclasts and bone resorption, which signify the RANK activation through the ligand or RANKL.

OSTEOPROTEGRIN

Structurally, osteoprotegerin (OPG="bone protector") or OCIF (osteoclasts inhibitor factor) is a posttranslationally glycosylated protein (55kDa) secreted as a homodimer.¹⁸⁻²⁰ OPG is a *soluble* receptor with homology to TNF-R²⁰, but made up only of extracellular area. The osteoprotegerin cellular sources are: osteoblasts, bone-marrow stromal cells and follicular dendritic cells.

Osteoprotegerin functions as a soluble decoy receptor, which binds to RANKL, through competition with the RANK receptor. Both receptors, OPG and RANK, show affinity for the same ligand, RANKL. Osteoprotegerin has *antiosteoclastogenic effect* through its

Table 1. Osteoclastogenesis control factors

	Cellular source	Type of molecule	Molecular form	Biologic effect
RANKL (OPGL, ODF)	osteoblasts stromal cells activated T cells	TNF family cytokine ligand for RANK	membrane soluble	activates osteoclasts stimulates osteoclastogenesis
RANK	osteoclast progenitors mature osteoclasts dendritic cells	TNF-R family receptor receptor for RANKL	membrane	RANKL-RANK stimulates osteoclastogenesis
OPG	Osteoblasts TNF-R family receptor receptor for RANKL	decoy receptor	soluble	RANKL-OPG inhibits osteoclastogenesis

role of antagonistic endogenous receptor which, after binding to RANKL, inhibits osteoclast maturation and activation via RANKL and blocks bone resorption.^{18,20} This bone protective action justifies the name of osteoprotegerin.

The OPG/RANKL interaction counterbalances the stimulating couple RANKL/RANK, so that RANKL – RANK and/or OPG complex has a pivotal role in bone remodeling through the action of osteoclasts.^{19,21} The transgenic mice model with hyperexpression of the OPG gene shows the increase of OPG systemic concentration associated with the decrease of RANKL concentration, which generates severe osteopetrosis as a consequence of the reduction of the amount of osteoclasts in contrast with the normal population of monocytes/macrophages.¹⁹ In exchange, in mice with OPG gene deletion (*opg*^{-/-}) severe osteoporosis is quickly installed, with the increase of spontaneous fractures and arterial calcification due to the compensating RANKL excess.²²

These experiments prove that the bone mass density is correlated with the OPG level, and the OPG/RANKL ratio may constitute a monitoring parameter of the osteoclast function and bone turnover. There is also a basis for the argumentation of the hypothesis on the OPG/RANKL effect in the regulation of vascular calcification.

IMMUNOPATHOGENIC IMPLICATIONS OF THE RANKL - RANK - OPG SYSTEM

Bone Remodeling

Bone remodeling is a cyclic process of bone matrix osteosynthesis/degradation rigorously controlled by the RANKL/RANK/OPG system together with other factors of bone metabolism. This trimolecular complex controls bone remodeling by means of osteoclasts bone resorption.

The most frequent imbalance of bone remodeling in which bone destruction prevails is generated by the stimulating effect of the RANKL/RANK interaction which, through osteoclast activation, contributes to the immunopathogeny of some osteopenic diseases. These osteopenic diseases include postmenopausal osteoporosis, bone metastases (associated to prostate or breast cancer), autoimmune diseases (insulin-dependent diabetes, SLE), chronic inflammatory diseases (rheumatoid arthritis, periodontitis), chronic viral infections (hepatitis C, HIV).

In these diseases the bone resorption and destruction are manifested in local or general osteolysis focal areas, frequently associated with disability, which entails high economic and social costs.

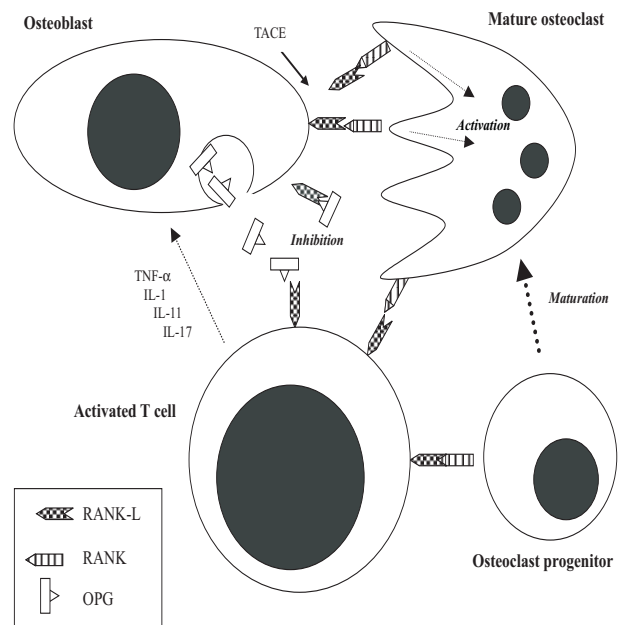


Figure 1. Interactions between different types of cells through the RANKL-RANK/OPG molecular complex

Postmenopausal Osteoporosis

Estrogens induce the *in vitro* expression of the OPG gene (cultures of human stromal cells).^{23,24} Estrogens stimulate the OPG secretion from osteoblasts and inhibit the RANKL production; this effect probably has an important role in the estrogen bone antiresorbing activity. The decrease of the ovarian function, associated with the decrease of the estrogen secretion, leads to the decrease of the OPG production in the source cells of the osteoblast lineage and may be an argument in the postmenopausal osteoporosis immunopathogeny in women. By counterbalance RANKL is released, escaping the competitive inhibition of the OPG decoy receptor, interacting with the receptor activator RANK. Thus the hyperexpression of RANKL and action of the RANKL/RANK couple stimulates osteoclastogenesis and bone resorption, and lowers the bone mass mineral density; consequently osteoporosis is installed. *In vivo* experiments have proved that the administration of recombinant OPG (OPGr) to ovariectomized female mice blocks bone destruction and installation of osteoporosis associated to the decrease of the ovarian function.¹⁹

In persons with osteoporosis, an increase of vascular calcification²⁴ has also been observed. Women with osteoporosis display an increase of vascular accidents and heart attacks through an incompletely deciphered mechanism in which the RANKL/OPG^{25,26} seems to be implicated. It is considered that OPGr or the functional modulation of RANKL/RANK can be regarded as a prospect for prevention of postmenopausal osteoporosis and osteoporosis in general.

The endogenous osteoprotegerin has been

reproduced by a recombinant method and has been experimentally used on animals, with prospects of being introduced in human therapy in order to reduce bone resorption in severe osteoporosis. OPGr mimics the effects of endogenous OPG, may contribute to the diminishing/amelioration of bone mass reduction and could represent a similar therapy to insulin used for diabetic patients. The constant administration of OPGr as a therapeutic agent prevents osteoclast activation and consequently bones destruction.

Autoimmune and Chronic Inflammatory Diseases

The RANKL/OPG/RANK system is essential for bone remodeling, but at the same time it represents the molecular link between the immune system and bone metabolism. The RANKL/OPG system is similar to the IL-1/IL-1ra cytokine system, which has a role in the modulation of the inflammatory response.

Recent research (animal models, cell cultures, and clinical observations) imposes a new paradigm regarding the role of T cells in bone pathogeny. Antigen activated T cells (through TCR: CD3, HLA class II, co-receptor CD4, co-stimulating molecules CD28 and CD40) express membrane and soluble RANKL. RANKL released by the activated T cells stimulates mature osteoclast ontogenesis and activation, with stimulating consequences on bone destruction and turnover.

Under the circumstances of chronic systemic activation of T cells *in vivo* osteoclastogenesis is triggered, via RANKL, followed by bone resorption, a stage of bone remodeling, but exacerbated in pathology. Therapeutic administration of recombinant decoy receptor OPGr blocks the effects of activated T cells on osteoclasts.^{29, 30}

Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune chronic inflammatory disease presenting inflammation of the synovium and the adjacent articular synovial tissue associated with erosion and destruction of articular cartilage and bone, followed by articular disfunction and disability.

It is considered to be an autoimmune disease with cellular mediated response and production of cytokines with type TH1 profile. Although so far there have not been identified any particular (auto)antigens with pathogenic role, the major implication of T CD4⁺ lymphocytes is unanimously accepted.

The cellular inflammatory infiltrate of the articular synovium is dominated by activated monocytes/macrophages, synovial fibroblasts, polymorphonuclear cells, mastocytes and activated TCD4⁺ lymphocytes,

as main contributors to the orchestration of the immune response. The activated synovial T cells isolated from the synovial membrane and inflammatory synovial pannus directly express RANKL and initiate osteoclastogenesis – via RANKL – and articular bone destruction.^{27, 28, 30}

On the other hand 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 does not stimulate the OPG expression in osteoblasts. Thus the bone destruction is mediated by RANKL, but the inflammatory response develops independent of the alteration of bone metabolism or bone destruction respectively.

The therapeutic administration of OPGr would have a benefic effect inhibiting the RANKL resorbing effects and thus preventing the destruction of the articular bone and cartilage without influencing the evolution of the inflammatory process. All these mechanisms justify the use of OPGr as a successful therapeutic target in rheumatoid arthritis.³²

Periodontitis

Periodontitis is the main cause of tooth loss in human oral pathology and is implied as a risk factor for several systemic diseases such as: heart failure, vascular stroke and bacterial pneumonia.³⁶ The mechanism by which periodontitis and antibacterial immune response lead to the destruction of the alveolar bone and tooth loss is incompletely explained.

Periodontitis, with its various forms, has a heterogeneous etiology, but certainly the chronic infection with specific oral bacteria triggers the inflammatory immune response in which the population of non-T cells (macrophages, polymorphonuclear cells and fibroblasts) and activated antigen T CD4⁺ cells from the infiltration of the periodontium represent the main cells which mediate the destruction of alveolar bone.

The molecular mechanism of the alveolar bone destruction is mediated by RANKL produced by CD4⁺ cells continuously activated through local bacterial antigens. RANKL expression in activated T cells stimulates osteoclastogenesis, osteoclast activation and directly mediates bone destruction. The release of proinflammatory cytokines (IL-1, TNF-alpha) from non-T cells, involved in the periodontium affection, acts directly as well as indirectly by stimulating the RANKL expression in osteoblasts,³⁵ thus contributing to the stimulation of the local bone resorption.

The *in vivo* inhibition of the RANKL function

through the soluble decoy receptor OPGr (OPG-Fc fusion protein) has a therapeutic utility through the decrease of the alveolar bone destruction accompanied by the tooth mobility and loss, which generate functional and aesthetic oral deficits. At present the OPGr therapy might represent a palliative modality for secondary osteoporosis in rheumatoid arthritis, multiple myeloma, renal insufficiency, periodontitis and other inflammatory and autoimmune diseases.

Dialysis-related amyloid osteopathy

Dialysis-related amyloid osteopathy (DRAO) is a phenomenon often found in patients with long-term amyloidosis, consisting in osteoarticular focal lytic lesions. The epiphyses of long bones, which present erosive ariculitis, and bone cysts and the spinal bones, which fuse together while the intervertebral discs disappear, are affected the most. The process is known as destructive spondyloarthropathy.

Pathological findings showed depositions of amyloid at the affected sites, both in cavities of long bones cysts and in intervertebral discs. The lesions are demonstrated to be due to an activation of osteoclastogenesis without reactive bone formation. Because of the absence of any changes in the systemic bone metabolism, the locally restricted lesions in DRAO are thought to be related to a paracrine/autocrine mechanism. Three possibly intricate pathways were suggested to be responsible of the lesions in these patients:

Inflammatory cells infiltrating the amyloid deposits secrete a series of proinflammatory cytokines such as IL-1, IL-11, IL-17, TNF- α which promote expression of RANKL on osteoblasts. RANKL binds RANK on osteoclast precursor cells and stimulate maturation of these precursors. RANKL also binds to mature osteoclasts to inhibit apoptosis of these cells and activation of their osteoclastic activity.³⁷

Some cells from the inflammatory infiltrate, namely CD4+ helper T cells, could express RANKL following their activation and could sustain the above mentioned mechanism of stimulating the osteoclastogenesis.³⁸

Several cytokines secreted by inflammatory cells could directly act on osteoclasts. TNF- α promotes maturation of osteoclast precursors and IL-1 β stimulates the mature osteoclasts and inhibits their apoptosis.^{39, 40}

It is important to further clarify the roles of these pathways in DRAO lesions, in order to apply a suitable therapy for them. In this respect, a possible role for recombinant OPG therapy is suggested; this molecule should block the activation of osteoclastogenesis via RANK acting as a decoy receptor and should protect the bones of dialyzed patients from osteolytic lesions associated with amyloid deposits.

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