

## Review

# TNF Super Family Members OPG, RANKL, RANK and Osteoporosis: a link

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## Abstract:

*Osteoclasts and osteoblasts dictate skeletal mass, structure, and strength via their respective roles in resorbing and forming bone. Bone remodeling is a spatially coordinated lifelong process whereby old bone is removed by osteoclasts and replaced by bone-forming osteoblasts. The refilling of resorption cavities is incomplete in many pathological states, which leads to a net loss of bone mass with each remodeling cycle (Hasan W et al, 2014). Postmenopausal osteoporosis and other conditions are associated with an increased rate of bone remodeling, which leads to accelerated bone loss and increased risk of fracture. Osteosynthesis of the bone matrix is achieved by osteoblasts and coordinated within this complex machinery of bone remodeling*

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*with resorption of extracellular bone matrix performed by osteoclasts. Bone resorption is dependent on a cytokine known as RANKL (receptor activator of nuclear factor  $\kappa$ B ligand), a TNF family member that is essential for osteoclast formation, activity, and survival in normal and pathological states of bone remodeling. (Kohli SS and Kohli VS, 2011; Kearns AE et al., 2008). The catabolic effects of RANKL are prevented by osteoprotegerin (OPG), a TNF receptor family member that binds RANKL and thereby prevents activation of its single cognate receptor called RANK. Osteoclast activity is likely to depend, at least in part, on the relative balance of RANKL and OPG. This review highlights the complex interplay of the RANKL–RANK/OPG axis and their management in bone health.*

**Key words:** RANKL, RANK, OPG, osteoclastogenesis, TNF- $\alpha$ , estrogen, T cells, NF- $\kappa$ B, osteoporosis.

Osteoporosis is a degenerative bone disease in elderly men and women characterized by low bone mineral density, increased fragility and deterioration of bone tissue leading to high fracture risk. In recent years, it has become a major health hazard afflicting more than 200 million people worldwide, and it is thought to be one of the highest incidence of diseases in aged people (Hamdy RC, 2002). This metabolic bone disease, over a life time results in fractures in 40% of aging women and 15% of aging men. (Riggs BL et al, 2002). Usually, osteoporosis is thought to be an age-adjusted symptom, and hypogonadism is the most well-established cause of osteoporosis (NIH Consensus Statement 2000)

Maintenance of bone mass is continuously controlled in adults by osteoblasts, which form new bone, and osteoclasts, which is the primary multinucleated bone-resorbing cell involved in bone remodeling (Park CK et al, 2007). Imbalance between bone formation and bone resorption is the key pathophysiological event in many metabolic bone disorders in adult humans, including osteoporosis, which results in bone loss (Fazzalari NL, 2008; Kearns AE et al, 2008). To resorb

bone effectively, osteoclasts attach themselves firmly to the bone surface using specialized actin-rich podosomes, which they use to form tightly sealed roughly circular extensions of their cytoplasm with the underlying bone matrix. Within these sealed zones called as resorption lacuna, they form ruffled membranes that increase the surface area of the cell membrane for secretion protons, proteases, superoxides, hydrochloric acid and the proteolytic enzyme cathepsin K onto the bone surface through ruffled borders (Shen CL et al., 2009; Yavropoulou MP & Yovos JG, 2008). They thereby simultaneously dissolve the mineral and degrade the matrix of bone, while protecting neighboring cells from harm by this sealing mechanism. (Boyce BF and Xing L., 2007). Other main characteristics of osteoclasts are: multinuclearity, formation of actin ring structure, a polar cell body during resorption, vitronectin receptor (integrin  $\alpha v \beta 3$ ) and contraction in response to calcitonin.(Susa M et al, 2004). Differentiation of osteoclasts is characterized by acquisition of mature phenotypic markers such as the calcitonin receptor (CTR), tartrate resistant acid phosphatase (TRACP), receptor activator of NF $\kappa$ B ligand (RANKL), and ability to resorb bone. Resorption involves synthesis of cysteine proteinases, such as Cathepsin K, and matrix metalloproteinases (MMPs) (Wittrant Y et al., 2008). The completion of resorption is associated with apoptosis of osteoclasts, followed by a reversal phase during which additional cells including preosteoblasts move to the bone surface. Osteoblasts orchestrate the formation of new bone matrix and regulate its mineralization. The return of the bone surface to its quiescent state involves the apoptosis of osteoblasts, their incorporation into the bone as osteocytes, or their transformation into bone surface-lining cells. Osteocytes, which mature to differentiate into active osteoblasts, also release diffusible chemical signals that regulate the resorptive potential in their local surroundings (You L et al., 2008, Kearns AE et al., 2008).

## **RANK**

Receptor activator of nuclear factor kappa B (RANK), a member of the TNF receptor superfamily, is a type I transmembrane protein containing four cysteine-rich pseudorepeat domains in the extracellular region, a hallmark of the TNF-R family. The mouse and human RANK contain 625 and 616 aa residues, respectively, the latter having a signal peptide (28 aa), an N-terminal extracellular domain (184 aa), a transmembrane spanning domain (21 aa), and a large C-terminal cytoplasmic tail (383 aa). In the human genome, RANK is present on chromosome 18q22.1. The extracellular part of TNF-R-like receptors forms elongated structures by a scaffold of disulfide bridges, which fit into the inverted bell/ groove of the ligand trimer in a 3:3 complex (Locksley RM et al., 2001). This has recently been demonstrated to be true for the interaction between RANKL and RANK (Lam et al., 2001). Although RANK is ubiquitously expressed in human tissues, its cell surface expression is limited to CD4+ T cell line MP-1, foreskin fibroblasts, OC precursors, and certain Hodgkin lymphomas (Hsu et al., 1999; Flumara et al., 2001). RANK knockout mice lack peripheral lymph nodes and have defective T and B cell maturation (Li J et al., 2000). RANK is also expressed in several cancer cell lines where it seems to play a central role in tumor cell migration and invasion (Santini D et al, 2011).

## **RANKL**

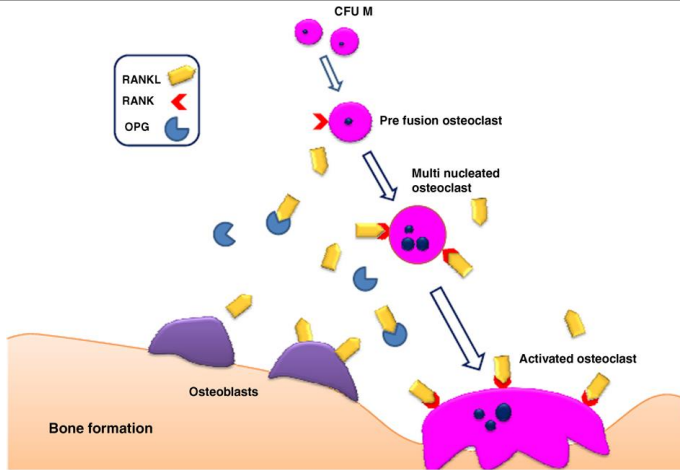
Human RANKL [also called TNF-related activation-induced cytokine (TRANCE)/ osteoprotegerin ligand (OPGL) /osteoclast differentiation factor (ODF)], a cytokine independently discovered by four different groups (Wong BR et al., 1997; Yasuda H et al., 1998; Lacey DL et al., 1998), is a member of the TNF ligand superfamily. It is a cytokine family that also includes TNF-  $\alpha$ , TNF-  $\alpha$  CD40 ligand, Fas ligand, CD30 ligand,

TWEAK, and TRAIL (Locksley RM et al., 2001). Like other members of the TNF-like family of cytokines, RANKL is a type II transmembrane protein, with a large extracellular receptor-binding domain, a membrane-anchoring domain, and a connecting stalk. The RANKL gene is present on human chromosome 13q14 and on mouse chromosome 14. The shape of the ligand is that of an inverted bell, which at the base interacts with the receptors in 3:3 symmetric complexes. The trimeric protein contains four unique surface loops that create the specificity in its interaction with the receptor RANK (Lerner UH, 2004). RANKL trimers exist either as membrane-anchored proteins or in a soluble cleaved form, which is derived from the membrane form as a result of either proteolytic cleavage or alternative splicing, both being functionally active (Nakashima T et al., 2000).

RANKL expression is stimulated in osteoblast/stromal cells by most of the factors that are known to stimulate osteoclast formation and activity. RANKL is a TNF family member that stimulates the fusion of pre-osteoclasts, (Lacey DL et al., 1998), the attachment of osteoclasts to bone (O'Brien EA et al., 2000), their subsequent activation (Burgess T et al., 1999), and their survival (Lacey DL et al., 2000, Kostenuik PJ, 2005). It is highly expressed in lymph nodes, thymus and lung, and at low levels in a variety of other tissues including spleen and bone marrow (Wada T et al., 2006). In inflamed joints RANKL is expressed by synovial cells and secreted by activated T cells, binds to RANK that is expressed on the dendritic cells, and such binding regulates the function and survival of these latter cells. (Schett G et al., 2005, Tat SK et al, 2009). It is also expressed by some malignant tumor cells that also express RANK, and thus it may play a role in inducing tumor cell proliferation by an autocrine mechanism or in a paracrine manner (Kim NS et al., 2006).

## **OPG**

Osteoprotegerin (OPG); also known as osteoclastogenesis inhibitory factor (OCIF) or TNF receptor-like molecule (TR 1), is a secreted protein that regulates bone mass by inhibiting Osteoclast differentiation and activation (Simonet WS et al., 1997; Yasuda H et al., 1998). OPG is synthesized in humans, rats, and mice as a 401-aa protein, which, after cleavage of 21-aa signal peptide, results in a 380-aa mature protein. In humans, the OPG gene is located on chromosome 8q23-24. The aa sequence of OPG displays several homologies to members of the TNF-R superfamily, including RANK. OPG contains four cysteine-rich domains in the N-terminal end, two homologous 'death domains' (DDH) in the C-terminus, a heparin-binding site, and a cysteine residue required for homodimerisation but, in contrast to other members of the TNF-R superfamily, lacks a transmembrane spanning domain and a cytoplasmic tail. Secreted OPG acts as a 'decoy receptor' due to its affinity to both membrane-bound and soluble RANKL and prevents the activation of RANK. OPG mRNA has been detected in bone, cartilage, aorta, skin, lung, heart, kidney, liver, brain, and in several other tissues. At the cellular level, OPG is expressed in OBs, stromal cells, endothelial cells, aortic smooth-muscle cells, fibroblasts, dendritic cells, and lymphoid cell lines (Lerner UH, 2004).



**Figure 1:** RANK/RANKL/OPG (receptor activator of nuclear factor kappa-B)/receptor activator of nuclear factor kappa-B ligand/osteoprotegerin) pathway of osteoclastogenesis (Dufresne A. et al., 2012).

OPG is a soluble decoy receptor that binds to RANKL and prevents RANKL from binding and activating receptor activator of nuclear factor-kB (RANK). This triad of proteins — OPG/RANKL/RANK — has been shown in genetic and pharmacology studies to have a critical role in the regulation of osteoclasts and bone resorption (Figure 1). Genetic variations in the genes coding for RANK, RANKL and OPG are thought to play roles in the susceptibility to osteoclastogenesis (Assmann G et al., 2010). Although other hormones and cytokines are able to influence osteoclasts in various ways, RANK/RANKL signaling is indispensable for the existence and activity of osteoclasts (Boyce BF and Xing L, 2007).

OPG has also been an invaluable tool for understanding the bone remodelling process, because OPG rapidly reduces osteoclast numbers while having no direct effect on osteoblasts. OPG inhibits the formation (Martin TJ and Sims NA, 2005, Karsenty G and Wagner EF, 2002), attachment to bone, activation (Ma YL et al., 2003) and survival of osteoclasts. OPG rapidly decreases osteoclast numbers and increases osteoclast apoptosis within hours after injection (Lacey DL et al., 1998).

OPG is not incorporated into bone matrix and its effects on bone resorption are therefore fully reversible. Osteoclast numbers recover within days of stopping treatment as recombinant OPG is gradually cleared from the blood (Simonet WS et al., 1997, Lacey DL et al., 1998). Serum OPG has been reported to increase in conditions such as aging (Chen G et al., 2006, Kapur RP et al., 2004) and postmenopausal osteoporosis (Hughes AE et al., 2000). In some observational studies, serum OPG levels showed negative correlation with bone resorption, positive correlation with BMD and reduced levels in patients with prevalent fractures. Thus, there are contradictory data, in general, that the upregulation of RANKL is associated with downregulation of OPG (Hofbauer LC and Schoppet M, 2004). At this point, it is not possible to reconcile the many contradictory observations reported with serum analyses of OPG and RANKL (Kostenuik PJ, 2005).

### **RANK/RANKL/OPG - Molecular triad**

RANKL, RANK, and OPG are three key molecules that regulate osteoclast recruitment and function. Macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor  $\kappa$ B (NF- $\kappa$ B) ligand (RANKL), a novel member of Tumour Necrosis Factor (TNF) family of cytokines, are produced as membrane-bound or secreted form in osteoblasts, are essential for osteoclast differentiation, function, and survival (Lacey DL et al, 2012; Kohli SS and Kohli VS, 2011; Shen CL et al, 2009; Ando K et al 2008; Matsuo K & Irie N, 2008).

Proinflammatory cytokines like Tumor necrosis factor- $\alpha$  (TNF), Interleukin-1 (IL-1&IL-6), RANKL are abundant in sites of inflammation and are known to promote osteoclast recruitment, differentiation, and activation, playing a pivotal role in induction of bone remodeling (Clowes JA et al, 2005; Gravallesse EM et al, 2000; Takayanagi H et al, 2000). These



cytokines, primarily, TNF- $\alpha$  and IL-1 $\beta$ , modulate this system primarily by stimulating M-CSF production, which further triggers the pool of preosteoclastic cells and by directly increasing RANKL expression, thus augmenting NF- $\kappa$ B; and inhibiting osteoblast survival. In addition, a number of other cytokines and hormones, such as TGF- $\beta$  (increased OPG production), PTH (increased RANKL/decreased OPG production), 1,25-dihydroxyvitamin D<sub>3</sub> (increased RANKL production), glucocorticoids (increased RANKL/decreased OPG production), and estrogen (increased OPG production) (Saika M et al., 2001) exert their effects on osteoclastogenesis by regulating osteoblastic/stromal cell production of OPG and RANKL (Khosla S, 2001). However, other hormones like calcitonin acts directly on osteoclastic cells, and estrogen has been shown to induce apoptosis of osteoclasts as well as inhibit osteoclast differentiation by interfering with RANK signaling (Hughes AE et al., 2000, Khosla S, 2001). Moreover, TGF- $\beta$  can also stimulate RANK expression on preosteoclastic cells, and thus enhance osteoclastic sensitivity to RANKL (Yan T et al., 2000).

Binding of RANKL with its receptor, RANK, initiates signal transduction events by eliciting recruitment of the adaptor proteins, the TNF receptor-associated factor 2 (TRAF2), TRAF5, and TRAF6 (Park CK et al, 2007). These further activate downstream signalling pathways and several transcription factors. Since TNF- $\alpha$  is abundant in inflamed bone sites and plays a major role in progression of the disease, it implies that TNF closely regulates RANK/ RANKL-induced osteoclastogenesis. Stimulation of RANK results in strong NF- $\kappa$ B activation (Lacey DL et al., 2012) Various TNF-receptor (TNFR) associated factor (TRAF) proteins associate with the cytoplasmic domain of RANK and relay RANK stimulation to NF- $\kappa$ B. TRAFs are cytoplasmic adaptor proteins that bind to the intracellular domains of various receptors of the TNFR superfamily. The TNFR superfamily member RANK contains

three putative TRAF-binding domains and each TRAF-binding domain has a different affinity for TRAF2, 5 and 6 (Walsh MC and Choi Y,2003; Darnay BG et al. (1998). RANK activation by RANKL is followed by its interaction with TNF receptor-associated factors (TRAF) family members TRAF2, TRAF3, TRAF5, and TRAF6 (Wong BR et al.,1998; Darnay BG et al., 1998) which in turn recruits NF- $\kappa$ B inducing kinase leading to the activation of NF- $\kappa$ B (Darnay BG et al., 1999). Among TRAFs, TRAF6 is the most important adaptor for RANK–RANKL-induced osteoclastogenesis. The TRAF6 binding region in RANK was shown to be necessary for RANK-induced NF- $\kappa$ B activation and in vitro osteoclastogenesis (Darnay BG et al., 1998). In particular, genetic experiments have shown that TRAF6 is required for osteoclast formation and osteoclast activation. Importantly, TRAF6 deficient mice develop severe osteopetrosis. TRAF6 and NF- $\kappa$ B play an indispensable role in OC differentiation as demonstrated by the osteopetrotic phenotype of TRAF6 and NF- $\kappa$ B knockouts. (Wada T et al 2006).

NF- $\kappa$ B is a dimer consisting of the NF- $\kappa$ B / Rel family proteins which include p50, p52, p65, c-Rel, and Rel-B. Mice lacking both the NF- $\kappa$ B p50 and p52 proteins are osteopetrotic . The upstream subunits that mediate NF- $\kappa$ B activation, comprising the catalytic subunits I $\kappa$ B kinase a (IKK $\alpha$ ) and IKK $\beta$  and the non-catalytic subunit IKK $\gamma$  (also called NEMO), are also important for RANK–RANKL signaling and osteoclastogenesis (Wada.T et al., 2006) In the unstimulated cells, NF- $\kappa$ B dimer in cytosol is present as an inactive form complexed with an inhibitory protein I $\kappa$ B, two major forms of which are I $\kappa$ B- $\alpha$  and I $\kappa$ B-  $\beta$  (Thompson et al., 1995). When stimulated by stimulatory cytokine, TNF-  $\alpha$ , the NF- $\kappa$ B dimer dissociates from I $\kappa$ B and translocates to the nucleus, the process being called ‘activation of NF- $\kappa$ B’. The activated NF- $\kappa$ B dimer binds to the regulatory NF- $\kappa$ B elements in the target

genes and regulates their transcription (Hehlgans T & Pfeffer K, 2005; Yan-Hong Zhang et al, 2001).

In addition to the local regulation of osteoclasts, one surprising finding was that RANKL is upregulated on T cells upon activation. It has, therefore, been speculated that RANK–RANKL might be an important regulator of interactions between T and dendritic cells and might have a role in the regulation of the T-cell-dependent immune response (Wada T et al., 2006; Tat SK et al, 2009). However, dendritic cells, where RANK was initially identified, appear normal in their function to induce T cell proliferation. These data indicate that RANKL has a crucial role in lymph node organogenesis and that RANKL has a role in monocyte function and in mediating inflammatory response (Seshasayee, D. et al, 2004).

The central role of estrogen deficiency in the pathogenesis of osteoporosis in postmenopausal women has been clearly established. Postmenopausal osteoporosis and other conditions are associated with an increased rate of bone remodeling, which leads to accelerated bone loss and increased risk of fracture. Estrogen deficiency results in increased bone resorption over bone formation and net bone loss. Estrogen has been demonstrated to up-regulate gene expression and protein synthesis of OPG in human osteoblastic cells (Hofbauer LC et al., 1999; Michael H et al, 2005) *in vitro*. It is logical from animal and *in vitro* studies that the RANKL/OPG system is involved in the pathogenesis of postmenopausal osteoporosis (Kearns AE et al., 2008). There is general agreement that OPG levels increase after the menopause. (Michael H et al, 2005; Mezquita-Raya P et al., 2005). Estrogens attenuate osteoclastogenesis and stimulate osteoclast apoptosis, but the molecular mechanism and contribution of these effects to the overall antiosteoporotic efficacy of estrogens remain controversial (Millan MM et al., 2010). Unpredictably, genetic inactivation of RANKL and RANK revealed surprising novel functions. Mice lacking RANKL or its receptor RANK had

impaired lobuloalveolar mammary structures during pregnancy, resulting in death of newborns. RANKL expression in mammary gland epithelial cells is induced in pregnancy in response to sex and/or pregnancy hormones and stimulates proliferation of mammary epithelial cells constitutively expressing RANK (Fata JE et al, 2000). Thus, RANKL and RANK, the master regulators of skeletal calcium release, are also essential for the sex and/or pregnancy hormone induced formation of the lactating mammary gland. The link between RANKL and RANK and sex and/or pregnancy hormones provides a rationale for hormonal regulation of bone metabolism and gender bias in osteoporosis.

OPG is a secreted TNFR-related protein acts as a decoy receptor by blocking RANKL binding to its cellular receptor RANK. The binding of OPG to RANKL inhibits the binding between RANKL and RANK; this, in turn, prevents osteoclast precursors from differentiating and fusing to form mature osteoclasts. In normal mice, recombinant OPG blocks the pro-resorptive effects of numerous hormones and cytokines, including IL-1 and TNF- $\alpha$ . OPG is also highly effective at preventing systemic bone loss in osteopenic mice that over express TNF- $\alpha$ . The ratio of RANKL: OPG may be the ultimate determinant of bone resorption (Hasan W et al., 2014; Lacey DL et al., 2012) 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 (Kohli SS and Kohli VS, 2011). In many situations, bone resorption is stimulated by both increased RANKL and decreased OPG, which can amplify pro-resorptive signals. Parathyroid hormone (PTH) is perhaps the best described mediator of this reciprocal regulation (Kostenuik PJ, 2005) .The OPG–RANKL complex counterbalances the effect of the RANK–RANKL complex, thus playing the most important role in bone homeostasis.

## **Summary and future directions**

Discovery of the RANKL/RANK/OPG system represents the most important advance in osteoclast biology in the past decade. This system helps maintain skeletal homeostasis and is disrupted in most diseases that affect the skeleton by altered levels of RANKL, OPG, or both, which are expressed by osteoblasts and stromal cells or other cells. Osteoclasts not only respond to signals from osteoblasts and other cell types involved in immune responses, but they also regulate osteoblast functions. In addition, RANKL/RANK signaling and OPG play important roles in the development of other tissues (at least in mice) and appear to be involved in the growth of some malignant tumors.

The RANKL-RANK system brought us a rapid progress in the understanding of the regulatory mechanism of osteoclast differentiation. The knowledge obtained from osteopetrotic mouse models is now being reconstituted in the context of the RANKL-stimulated signaling network. However, osteoclast differentiation is regulated not only by RANKL and OPG, instead is affected by various other hormones and cytokines. For example, RANKL induces its own inhibitor IFN- $\beta$  and autoregulates RANKL signaling. Also, in healthy subjects, serum OPG correlated negatively with bone resorption markers and positively with bone formation markers. These relationships suggest that high OPG levels are associated with a favorable balance of bone formation over bone resorption.

The identification of NF $\kappa$ B as the master transcription factor for osteoclastogenesis led us to realize the importance of RANK/RANKL signaling, crucial for osteoclast differentiation. Elucidation of the roles of RANKL/ RANK and OPG in these various types of cells, their signalling pathways in normal and disease states is likely to lead to the development of novel strategies and new drugs designed to specifically influence the production of these cytokines or responses to them. Thus the

costimulatory signal-activated by RANK/RANKL/OPG axis will open a new era of osteoimmunology, providing an unprecedented therapeutic strategy for skeletal and immune disorders.

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## REFERENCES

- Ando, K., Mori, K., Rédini, F., Heymann, D.2008. "RANKL/RANK/OPG: key therapeutic target in bone oncology." *Curr Drug Discov Technol* 5(3): 263-8.
- Assman, G., Koeing, J., Pfreundschuh, M., Epplen, J.T., Kekow, J., Roemer, K., Wieczorek, S.2010. "Genetic variations in genes encoding RANK, RANKL, and OPG in rheumatoid arthritis: a case-control study." *The Journal of Rheumatology* 37(5): 900-4.
- Boyce, B.F., and Xing, L.2007. "Biology of RANK, RANKL, and Osteoprotegerin." *Arthritis Research & Therapy* 9:S1.
- Burgess, T.L., Qian, Y.X., Kaufman, S., Ring, B.D., Van, G., Capparelli, C., Kelley, M., Hsu, H., Boyle, W.J., Dunstan, C.R., Hu, S., Lacey, D.L.1999. "The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts." *J Cell Biol* 145: 527-538.
- Chen, G., Sircar, K., Aprikian, A., Potti, A., Goltzman, D., Rabbani, S.A.2006. "Expression of RANKL/RANK/OPG in primary and metastatic human prostate cancer as markers of disease stage and functional regulation." *Cancer* 107: 289-298.

- Clowes, J.A., Riggs, B.L., Khosla, S.2005. "The role of the immune system in the pathophysiology of osteoporosis." *Immunol Rev* 208: 207–227.
- Darnay, B.G., Haridas, V., Ni, J., Moore, P.A., Aggarwal, B.B.1998. "Characterization of the intracellular domain of receptor activator of NF-kappaB (RANK) - Interaction with tumor necrosis factor receptor-associated factors and activation of NFkappa b and c-Jun N-terminal kinase." *J. Biol. Chem.* 273: 20551–20555.
- Darnay, B.G., Ni, J., Moore, P.A., Aggarwal, B.B.1999. "Activation of NF-kappaB by RANK requires tumor necrosis factor receptor-associated factor (TRAF) 6 and NF-kappaB-inducing kinase. Identification of a novel TRAF6 interaction motif." *J. Biol. Chem.* 274: 7724-7731.
- Dufresne, A., Derbel, O., Cassier, P., Vaz, G., Decouvelaere, A.V., Blay, J.Y.2012. "Giant-cell tumor of bone, anti-RANKL therapy. *Bonekey Rep.* 5(1):149
- Fata, J.E., Kong, Y.Y., Li, J., Sasaki, T., Irie-Sasaki, J., Moorehead, R.A., Elliott, R., Scully, S., Voura, E.B., Lacey, D.L., Boyle, W.J., Khokha, R., Penninger, J.M. 2000. "The osteoclast differentiation factor osteoprotegerin ligand is essential for mammary gland development." *Cell* 103: 41–50.
- Fazzalari, N.L.2008. "Bone remodeling: a review of the bone microenvironment perspective for fragility fracture (osteoporosis) of the hip". *SeminCell Dev Biol* 19(5): 467-72.
- Flumara, P., Snell, V., Li, Y., Mukhopadhyay, A., Younes, M., Gillenwater, A.M., Cabanillas, F., Aggarwal, B.B., Younes, A.2001. "Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines." *Blood* 98: 2784-2790.
- Gravallese, E. M., Manning, C., Tsay, A., Naito, A., Pan, C., Amento, E., and Goldring, S. R.2000. "Synovial tissue in

- rheumatoid arthritis is a source of osteoclast differentiation factor.” *Arth. Rheum.* 43: 250–258.
- Hamdy, R.C. 2002. “Osteoporosis, the deafening silent epidemic.” *Southern Med. J.* 95: 567–568.
- Hasan,W., Mahdi, A.A. and Islam, N.2014. “The Role of Molecular Triad OPG/RANKL/RANK Axis in Osteoporosis-A Mini-Review.” *World Journal of Biology and Medical Sciences* 1(1): 38-44.
- Hehlgans, T., Pfeffer, K.2005. “The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games.” *Immunology* 115:1–20.
- Hofbauer, L.C., Gori, F., Riggs, B.L., Lacey, D.L., Dunstan, C.R., Spelsberg, T.C., Khosla, S.1999.“Stimulation of osteoprotegerin ligand and inhibition of Osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis.” *Endocrinology* 140: 4382–4389.
- Hofbauer, L.C., Khosla, S., Dunstan, C.R., Lacey, D.L., Spelsberg, T.C., Riggs, B.L. 1999.“Estrogen stimulates gene expression and protein production of Osteoprotegerin in human osteoblastic cells.” *Endocrinology* 140: 4367–4370.
- Hofbauer, L.C., Schoppet, M.2004. “Clinical implications of the osteoprotegerin/ RANKL/RANK system for bone and vascular diseases” .*JAMA* 292: 490-495.
- Hsu, H., Lacey, D.L., Dunstan, C.R., Solovyev Colmbero, A., Timms, E., Tan, H.L., Elliot, G.1999. “Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin Ligand”. *Proc. Natl. Acad. Sci. (USA)* 96: 3540-3545.
- Hughes, A.E., Ralston, S.H., Marken, J., Bell, C., MacPherson, H., Wallace, R.G., van Hul, W., Whyte, M.P., Nakatsuka,



- K., Hovy, L., Anderson, D.M.2000. "Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis." *Nat Genet* 24: 45-48.
- Kapur, R.P., Yao, Z., Iida, M.H., Clarke, C.M., Doggett, B., Xing, L., Boyce, B.F.2004. "Malignant autosomal recessive osteopetrosis caused by spontaneous mutation of murine Rank." *J Bone Miner Res* 19:1689-1697.
- Karsenty, G., Wagner, E.F.2002. "Reaching a genetic and molecular understanding of skeletal development." *Dev Cell* 2: 389- 406.
- Kearns, A.E., Khosla, S., Kostenuik, P.J.2008. "Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulation of bone remodeling in health and disease." *Endocr Rev* 29: 155–92.
- Khosla, S.,2001. "Minireview: The OPG/RANKL/RANK System." *Endocrinology* 142: 5050-5055.
- Kim, N.S., Kim, H.J., Koo, B.K., Kwon, M.C., Kim, Y.W., Cho, Y., Yokota, Y., Penninger, J.M., Kong, Y.Y.2006. "Receptor activator of NF-kB ligand regulates the proliferation of mammary epithelial cells via Id2." *Mol Cell Biol* 26:1002-1013.
- Kohli,S.S., Kohli, V.S.2011. "Role of RANKL-RANK/osteoprotegerin molecular complex in bone remodeling and its immunopathologic implications." *Indian Journal of Endocrinology and Metabolism* 15(3): 175–181.
- Kong, Y.Y., Feige,U., Sarosi,I., Bolon, B., Tafuri, A., Morony,S., Capparelli, C., Li,J., Elliott,R., McCabe,S., Wong, T., Campagnuolo, G., Moran,E., Bogoch,E.R., Van , G., Nguyen L.T., Ohashi , P.S., Lacey, D.L., Fish,E., Boyle, W.J., Penninger, J.M.1999. "Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand." *Nature* 402: 304–309.

- Kostenuik, Paul J.2005. "Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength." *Current Opinion in Pharmacology* 5: 618–625.
- Lacey, D.L., Boyle, W.J., Simonet, S.S., Kostenuik,P.J., Dougall, W.C., Sullivan, J.K., San Martin, J., Dansey,R.,2012. "Bench to bedside: elucidation of the OPG–RANK–RANKL pathway and the development of denosumab." *Nature Reviews Drug Discovery* 11: 401-419.
- Lacey, D.L., Tan, H.L., Lu, J., Kaufman, S., Van, G., Qiu, W., Rattan, A., Scully, S., Fletcher, F., Juan, T., Kelley, M.,Burgess, T.L., Boyle,W.J., Polverino, A.J.2000. "Osteoprotegerin ligand modulates murine osteoclast survival in vitro and in vivo." *Am J Path* 157: 435-448.
- Lacey, D.L., Timms, E., Tan, H.L., Kelley, M.J., Dunstan, C., Burgess, T., Elliot, R., Colombero, A., Elliot, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C.,Eli, A., Qian, Y.X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J., Boyle, W.J.1998. "Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation." *Cell* 93: 165-176.
- Lam, J., Nelso, C.A., Ross, F.P., Teitelbaum, S.L., Fremont, D.H.2001. "Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity" *J. Clin. Invest.* 108: 971-979.
- Lerner, U.H.2004. "New molecules in the tumor necrosis factor ligand and receptor superfamilies with importance for physiological and pathological bone resorption." *Crit. Rev. Oral. Biol. Med.* 15: 62-81.
- Li ,J., Sarosa, I., Yan, X.Q., Morony, S., Caparelli, C., Tan, H.L., McCabe, S., Elliot, R., Scully, S., Van, G., Kaufman, H., Juan, S.C., Sun,Y., Tarpley,J., Martini,L., Christensen,K., McCabe,J., Kostenuik,P., Hsu,H., Fletcher, F., Dunstan,C.R., Lacey,D.L., Boyle,W.J.2000.

- “RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism.” *Proc. Natl. Acad. Sci. (USA)* 97: 1566-1571.
- Locksley, R.M., Killeen, N., Lenardo, M.J. 2001. “The TNF and TNF receptor superfamilies: integrating mammalian biology.” *Cell* 104: 487-501.
- Ma, Y.L., Bryant, H.U., Zeng, Q., Schmidt, A., Hoover, J., Cole, H.W., Yao, W., Jee, W.S., Sato, M. 2003. “New bone formation with teriparatide [human parathyroid hormone-(1-34)] is not retarded by longterm pretreatment with alendronate, estrogen, or raloxifene in ovariectomized rats.” *Endocrinology* 144:2008-2015.
- Martin, T.J., Sims, N.A. 2005. “Osteoclast-derived activity in the coupling of bone formation to resorption.” *Trends Mol Med* 11:76- 81.
- Matsuo, K., Irie, N. 2008. “Osteoclast-osteoblast communication.” *Arch Biochem Biophys* 473(2): 201-9.
- Mezquita-Raya, P., De la Higuera, M., Fernandez Garcia, D., Alonso, G., Ruiz-Requena, M.E., de Dios Luna, J., Escobar-Jimenez, F., Munoz-Torres, M. 2005. “The contribution of serum osteoprotegerin to bone mass and vertebral fractures in postmenopausal women.” *Osteoporos Int* 16:1368–1374.
- Michael, H., Harkonen, P.L., Vaananen, H.K., Hentunen, T.A. 2005. “Estrogen and testosterone use different cellular pathways to inhibit osteoclastogenesis and bone resorption.” *J Bone Miner Res* 20:2224–2232.
- Millan, M.M., Almeida, M., Ambrogini, E., Han, L., Zhao, H., Weinstein, R.S., Jilka, R.L., O’ Brien, C.A., Manolagas, S.C. 2010. “The Estrogen Receptor- $\alpha$  in Osteoclasts Mediates the Protective Effects of Estrogens on Cancellous But Not Cortical Bone.” *Molecular Endocrinology* 24: 323–334.

- Morony, S., Capparelli, C., Lee, R., Shimamoto, G., Boone, T., Lacey, D.L., Dunstan, C.R.1999. "A chimeric form of osteoprotegerin inhibits hypercalcemia and bone resorption induced by IL-1b, TNF-a, PTH, PTHrP, and 1, 25(OH)2D3." *J Bone Miner Res* 14:1478-1485.
- Nakashima, T., Kobayashi, Y., Yamasaki, S., Kawakani, A., Eguchi, K., Sasaki, H., Sakai,H.2000. "Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappa B ligand: modulation of the expression by osteotropic factors and cytokines." *Biochem. Biophys. Res. Commun.* 275: 768-775.
- NIH Consensus Statement.2000. "Osteoporosis prevention, diagnosis, and therapy." *NIH Consensus Statement Online* 17, 1–36.
- O'Brien, E.A., Williams, J., Marshall, M.J.2000. "Osteoprotegerin Ligand regulates osteoclast adherence to the bone surface in mouse calvaria." *Biochem. Biophys. Res. Commun.* 274:281-290.
- Park,C.K., Hyung, J. K., Kim, H.J., Lee,T.H., Bang,M., Kim, M.C., Lee,Y., Chung,D.K., Baek,N., Kim,J., Lee,Z.H., Kim,H.H.2007. "Inhibitory effects of *Stewartia koreana* on osteoclast differentiation and bone resorption." *International Immunopharmacology* 7:1507–1516.
- Riggs, B.L., Khosla, S., Melton, L.J.2002. "Sex steroids and the construction and conservation of the adult skeleton." *EndocrRev* 23:279–302.
- Saika, M., Inoue, D., Kido, S., Matsumoto, T. 2001."17β-Estradiol stimulates expression of osteoprotegerin by a mouse stromal cell line, ST-2, via estrogen receptor." *Endocrinology* 142: 2205–2212.
- Santini, D., Perrone, G., Roato,I., Godio,L., Pantano, F., Grasso, D., Russo,A., Vincenzi, B., Fratto, M.E., Sabbatini, R., Pepa, C.D.,Porta, C., Del Conte, A., Schiavon, G., Berruti, A., Tomasino, R.M., Papotti, M.,

- Papapietro, N., Muda, A.O., Denaro, V., Tonini, G. 2011. "Expression pattern of receptor activator of NF- $\kappa$ B (rank) in a series of primary solid tumors and related bone metastases." *J. Cell. Physiol.* 226: 780–784.
- Schett, G., Hayer, S., Zwerina, J., Redlich, K., Smolen, J.S. 2005. "Mechanisms of disease: the link between RANKL and arthritic bone disease." *Nat Clin Pract Rheumatol* 1:47-54.
- Seshasayee, D., Wang, H., Lee, W.P., Gribling, P., Ross, J., Bruggen, V.N., Carano, R., and Grewal, I.S. 2004. "A novel in vivo role for Osteoprotegerin ligand in activation of monocyte effector function and inflammatory response." *J. Biol. Chem.* 279, 30202–30209.
- Simonet, W.S., Lacey, D.L., Dunstan, C.R., Kelley, M., Chang, M.S., Luthy, R., Nguyen, H.Q., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colombero, A., Tan, H.L., Trail, G., Sullivan, J., Davy, E., Bucay, N., Renshaw Gegg, L., Hughes, T.M., Hill, D., Pattinson, W., Capbell, P., Sander, S., Van, G., Tarpley, J., Derby, P., Lee, R., Boyle, W.J. 1997. "Osteoprotegerin: a novel secreted protein involved in the regulation of bone density." *Cell* 89:309-319.
- Takayanagi, H., Iizuka, H., Juji, T., Nakagawa, T., Yamamoto, A., Miyazaki, T., Koshihara, Y., Oda, H., Nakamura, K., and Tanaka, S. 2000. "Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis." *Arth. Rheum.* 43, 259–269.
- Tat, S.K., Amiable, N., Pelletier, J.P., Boileau, C., Lajeunesse, D., Duval, N., Martel Pelletier, J. 2009. "Modulation of OPG, RANK and RANKL by human chondrocytes and their implication during osteoarthritis." *Rheumatology (Oxford)* 48(12):1482-90.

- Thompson, J.E., Phillips, R.J., Erdjument, B.H., Tempst, P., Ghosh, S. 1995 "i kappa B-beta regulates the persistent response in a biphasic activation of NF-kappa." *B. Cell* 80: 573-582.
- Wada, T., Nakashima, T., Hiroshi, N., and Penninger, J.M. 2006. "RANKL-RANK signaling in osteoclastogenesis and bone disease." *TRENDS in Molecular Medicine* 12: 1471-1491.
- Walsh, M.C. and Choi, Y. 2003. "Biology of the TRANCE axis." *Cytokine Growth Factor Rev.* 14, 251-263.
- Wittrant, Y., Gorin, Y., Woodruff, K., Horn, D., Abboud H.E., Mohan, S., Abboud-Werner, S.L. 2008. "High D (+) glucose concentration inhibits RANKL-induced osteoclastogenesis." *Bone* 42:1122-1130.
- Wong, B.R., Josien, R., Lee, S.Y., Vologodskaya, M., Steinman, R.M., Choi, Y. 1998. "The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor." *J. Biol. Chem.* 273: 28355-28359.
- Wong, B.R., Rho, J., Arron, J., Robinson, E., Orlinick, J., Chao, M., Kalachikov, S., Cayani, E., Bartlett, F.S., Frankel, W.N., Lee, S.Y., Choi, Y. 1997. "TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells." *J. Biol. Chem.* 272: 25190-25194.
- Yan, T., Riggs, B.L., Boyle, W.J., Khosla, S., 2000. "Regulation of osteoclastogenesis and RANK expression by TGF- $\beta$ 1." *J Cell Biochem* 83:320-325.
- Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinoshita, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaya, T., Higashio, K., Udagawa, N., Takahashi, N., Suda, T. 1998. "Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL." *Proc. Natl. Acad. Sci. (USA)* 95: 3597-3602.

- Yavropoulou, M.P., Yovos, J.G.2008. "Osteoclastogenesis—current knowledge and future perspectives". *J Musculoskelet Neuronal Interact* 8(3):204-16.
- You, L., Temiyasathit, S., Lee, P., Kimb, C.H., Tummala, P., Yao, W., Kingery, W., Malone, A.M., Kwon, R.Y., Jacobs, C.R. 2008. "Osteocytes as mechanosensors in the inhibition of bone resorption due to mechanical loading." *Bone* 42: 172–179.
- Zhang, Y., Heulsmann, A., Tondravi, M.M., Mukherjee, A., and Abu-Amer, Y. 2001. *The Journal of Biological Chemistry*. 276: 563–568.