RANK/RANKL/OPG: LITERATURE REVIEW

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Abstract

The discovery of the receptor activator of nuclear factor-kB (RANK)/RANK Ligand (RANKL)/osteoprotegerin (OPG) pathway contributed to the understanding of how bone formation and resorption were processed and regulated. RANKL and OPG are members of the tumor necrosis factor (TNF) and TNF receptor (TNFr) superfamilies, respectively, and binding to receptor activator of NF-kB (RANK) not only regulate osteoclast formation, activation and survival in normal bone modeling and remodeling, but also in several other pathologic conditions characterized by increased bone turnover. There is accumulating evidence of the potential role of OPG and RANKL in other tissues.

Looking beyond the RANK/RANKL/OPG axis, Wingless (Wnt) pathway emerged as the osteoblast differentiation way, and also as a bone mass regulator.

Researchers have been discovering new molecules and cytokines interactions. Altogether, data suggest that RANK/RANKL/OPG system could be targeted as a new treatment strategy in bone conditions. FREEDOM is the more recently published clinical trial about a RANKL-specific recombinant fully human monoclonal antibody (denosumab). OPG is also a potential innovative therapeutic option to be investigated.

Keywords: RANK; RANKL; Osteoprotegerin; Osteoclast; Bone Formation.

Introduction

Bone is a connective tissue made up of specific cells, osteoblasts (bone-forming), osteocytes (osteoblasts entrapped within lacunae) and osteoclasts (bone-reabsorbing), and an extracellular matrix of proteoglycans and collagen mineralized by the deposition of calcium hydroxyapatite[1]. Bone remodeling results from the balance between osteoblast and osteoclast activity, through four phases: activation, resorption, reversal and formation. This includes removal of trenches or tunnels of bone from the surfaces of trabecular and cortical bone, respectively, by osteoclasts, while osteoblasts subsequently fill in these trenches by laying down new bone matrix[2].

Formation matches resorption during normal bone remodeling. This remodeling becomes disturbed in a variety of pathologic conditions that affect the skeleton (osteoporosis, glucocorticoid-induced bone loss, multiple myeloma, and rheumatoid arthritis)[2,3]. Discovery of the receptor activator of nuclear factor-kB (RANK)/RANK ligand (RANKL)/ /osteoprotegerin (OPG) signaling pathway as a major regulatory system for osteoclast formation and action, showed the major role of the tumor necrosis factor (TNF) superfamily in bone metabolism[1,5].

Studies also revealed new functions of this triad in other pathologies and tissues, and suggest that in response to mechanical forces osteocytes regulate the osteoclasts recruitment to sites of bone resorption, by inducing the RANKL expression by osteoblastic cells in the local micro-environment[2-4]. Emerging treatments have been explored according to new molecules and mechanisms discoveries.

Osteoblasts differentiation and proliferation depends on Wingless (Wnt)/β-catenin pathway and mutations on some of their proteins lead to bone diseases (eg. loss-of-function mutation in the Wnt co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) is associated with osteoporosis)[6,7].

In this article, we will review RANK/RANKL/OPG triad, its role in the bone, and recent concepts.

RANK, RANKL and OPG signaling pathway

Ostoblasts are mononuclear cells responsible for

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the deposition of bone matrix and for osteoclasts regulation. They originate from mesenchymal stem cells (MSC) by the action of transcription factors like core binding factor α1 (Cbfa-1) also known as Runx2, osterix (Osx), activating transcription factor 4 (ATF4), and bone morphogenic proteins (BMP) as BMP4[6]. Osteoclasts are derived from mononuclear precursors in the myeloid lineage of hematopoietic cells that also originate macrophages[2]. Macrophage-colony stimulating factor (M--CSF) expression by osteoblastic stromal cells is required for progenitor cells to differentiate into osteoclasts, but is unable to complete this process by its own. In a 1997 publication OPG was identified, and its gene encoded a member of the TNF receptor family. In a 1998 publication RANKL was reported as a new member of the TNF family that could bind to OPG and RANK[8]. RANK/RANKL/ /OPG are closely linked with each other.

RANKL is synthesized in membranous or soluble form by the osteoblastic lineage cells, the immune cells, and some cancer cells. This factor links to the osteoclasts surface receptor, RANK, and stimulates bone resorption through osteoclastogenesis and the activation of multinucleated mature osteoclasts. OPG that is secreted by osteoblasts as a decoy receptor for RANKL, prevents RANKL from binding to RANK and bone resorption[1-6].

In the immune system RANKL in activated T cells binds to RANK expressed by the dendritic cells, regulating the function and survival of those cells. OPG is produced by B-lymphocytes and dendritic cells, maintaining an equilibrium in this system[1].

OPG

OPG belongs to the TNF receptor superfamily (TNFRS), preventing the biological effects of RANKL. Also known as TNFRS member 11B (TNFRS11B), osteoclastogenesis inhibitory factor (OCIF) and tropine reductase 1 (TR1), is highly expressed as a soluble protein, closely related to CD40 and able to bind to CD40 ligand (CD40L). Is produced in the adult lung, heart, kidney, liver, thymus, lymph nodes, bone marrow, osteoblasts, vascular smooth muscle cells, B-lymphocytes, and articular chondrocytes[1-3,6]. Over expression of OPG in the mice resulted in osteopetrosis and its deficiency determined osteoporosis[6]. The osteoprotective role of OPG is supported by the report of homozygous deletions of 100 kilobases of OPG in juvenile Paget's disease, and the inactivating deletion in exon 3 of OPG in idiopathic hyperphosphatasia[3].

When RANKL expression is up-regulated OPG expression is down-regulated or not induced to the same degree as RANKL, and the RANKL/OPG ratio favors osteoclastogenesis[2]. OPG expression in osteoblasts is increased by vitamin D3, interleukin (IL)-1α, IL-1β, TNFα, TNFβ, BMP2, transforming growth factor β (TGFβ) and 17 -estradiol and Wnt signaling pathway. Its expression is decreased by prostaglandin E_2 (PGE_2), parathyroid hormone (PTH), glucocorticoids and insulin-like growth factor-1 (IGF-1) (Figure 1)[4].

Furthermore, the RANKL/OPG ratio expressed by pre-osteoblasts cells is higher than in mature osteoblasts, favoring osteoclasts maturation and action. Jagged1/Notch1 signaling negatively regulates osteoclast formation directly and indirectly by changing RANKL/OPG ratio in stromal cells. So, bone mass is regulated by osteoblasts through three signaling pathways: RANKL/RANK, Wnt/ /β-catenin and Jagged1/Notch1[2]. Jagged 1 is a 180 kDa type I transmembrane glycoprotein with an extracellular DSL (delta, serrate, lag-2 consensus sequence) domain that is necessary for bin-

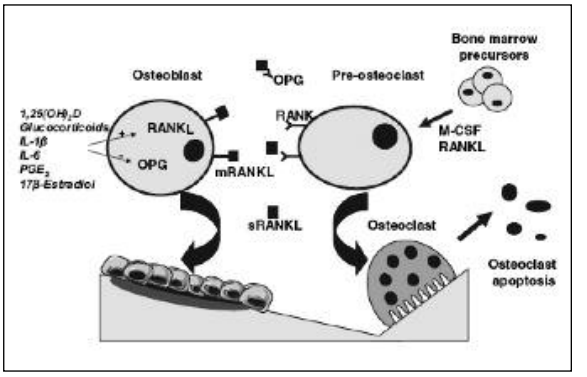

Figure 1. Regulatory mechanisms of bone remodeling: role of RANK, RANKL and OPG in osteoclast activation. OPG expression in osteoblasts is increased by vitamin D3, interleukin (IL) -1α, IL-1β, TNFα, TNFβ, BMP2, transforming growth factor β (TGFβ) and 17β-estradiol, and Wnt signaling pathway. Its expression is decreased by prostaglandin E2 (PGE2), parathyroid hormone (PTH), glucocorticoids and insulin-like growth factor-1 (IGF-1). From: Vega D, Maalouf NM, Sakhaee K. The role of receptor activator of nuclear factor-kB (RANK)/RANK ligand/Osteoprotegerin: clinical implications. The journal of clinical endocrinology and metabolism. 2007; 92: 4514-4521.

ding to Notch receptors. Jagged-Notch signaling specifies cell fate, modulates cell proliferation and differentiation, especially during hematopoiesis, myogenesis, neurogenesis and development of the vasculature. Direct cell-cell interactions are thought to be necessary for functional Notch signaling[2,6].

In mammals there are four Notch receptors (Notch 1-4). The canonical Notch signaling in skeletal biology is evolving while the non-canonical is poorly understood[9]. Suppression of Notch signaling by a selective g-secretase inhibitor or Notch2 short hairpin RNA suppressed RANKL-induced osteoclastogenesis. Induction of Notch signaling by Jagged1 or by ectopic expression of intracellular Notch2 enhanced nuclear factor of activated T cells 1 (NFATc1) promoter activity leading to the increase of osteoclastogenesis[10]. In a pathological context, aberration of Notch signaling is associated with osteosarcoma[9,10].

RANKL

RANKL belongs to the TNF superfamily, is expressed in bone, lung, bone marrow and lymphoid tissues, and exists as 3 isoforms: RANKL 1, 2 and 3. These three isoforms of this type II homotrimeric transmembrane protein can differentially regulate osteoclastogenesis and exists as a soluble and a membranous form. Soluble form has low capacity to generate osteoclasts[11]. Typically is expressed in a membrane-bound form in osteoblasts and activated T cells, and after a proteolytic cleavage by matrix metalloproteases (MMP3 or 7) or a disintegrin and metallopeptidase (ADAM) is secreted. Its expression by synovial cells and activated T cells in patients with rheumatoid arthritis contributes, with TNF, to joint destruction[1,2,8]. RANKL stimulates the release of immature osteoclasts progenitors into the circulation. Analysis of RANKL promoter revealed the presence of binding sites for vitamin D and glucocorticoids (stimulators)[6]. Clinical studies in mice showed RANKL expression in mammary epithelial cells during pregnancy and its effect in lactational hyperplasia of mammary epithelial cells and milk production. RANKL is also expressed by some malignant tumors cells, thus regulating tumor cell proliferation and probably migration[1,2]. Recently, the first report of a mutation in the RANKL gene was described in Canada. The affected individuals had osteopetrosis, without obvious defect in immunologic system[2].

MicroRNAs (miRs) are small non-coding RNAs that function in the spatiotemporal regulation of protein translation in animal cells. MiR-21 was identified as a miR expression signature of RANKL--induced osteoclastogenesis that down-regulates programmed cell death 4 (PDCD4) protein level, and RANKL-induced c-Fos up-regulates miR-21 gene expression[12].

RANK

RANK belongs to the TNFR superfamily, is synthesized as a type I homotrimeric transmembrane protein, and is expressed by different tissues such as skeletal muscle, thymus, liver, colon, mammary glands, prostate, pancreas, and cells of the monocyte/macrophage lineage (precursors and mature osteoclasts, B and T cells, dendritic cells, fibroblasts, and articular chondrocytes). RANKL produced by osteoblasts binds to RANK in the surface of osteoclasts, recruits the tumor necrosis factor receptor associated factor (TRAF) 2,5 and 6 that bind to RANK cytoplasmic domain (only TRAF6 seems to be essential in osteoclasts), leading to NF-kB activation and translocation to the nucleus. NF-kB increases c-Fos expression and c-Fos interacts with NFATc1 to trigger the osteoclastogenic genes transcription (Figure 2). At least seven signaling pathways are activated by RANK-mediated protein kinase signaling: four mediate osteoclastogenesis (inhibitor of NF-kB kinase/NF-kB, c-Jun amino-terminal kinase/activator protein-1, c-myc, and calcineurin/NFATc1) and three mediate osteoclast activation [Rous sarcoma oncogene (src) and mitogen-activated protein kinase kinase 6 (MKK6)/p38/microphthalmia-associated transcription factor (MITF)] and survival (src and extracellular signal-regulated kinase)[1,2,6,8].

On the basis of mice studies, NFATc1 was described as the master regulator of osteoclastogenesis (Figure 3). It is activated by a calcium-dependent calcineurin dephosphorylation. However some patients treated with cyclosporine A (NFATc1 inhibition) presented bone loss, what brought another explanation: NFATc1 also positively regulates expression of osterix, an essential transcription factor in osteoblast function, and the result of this net effect is reduced bone formation and osteoporosis[3].

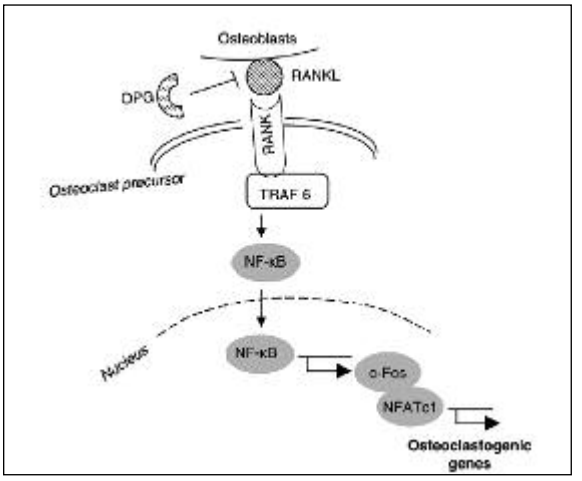

Figure 2. The essential signaling pathway for normal osteoclastogenesis. RANKL produced by osteoblasts binds to RANK in the surface of osteoclasts, recruits the tumor necrosis factor receptor associated factor (TRAF) 2,5 and 6 that bind to RANK cytoplasmic domain (only TRAF6 seems to be essential in osteoclasts), leading to NF-kB activation and translocation to the nucleus. NF-kB increases c-Fos expression and c-Fos interacts with FATc1 to trigger the osteoclastogenic genes transcription. From: Boyce BF, Xing L. Biology of RANK, RANKL, an osteoprotegerin. Arthritis research and therapy. 2007;9:1-7.

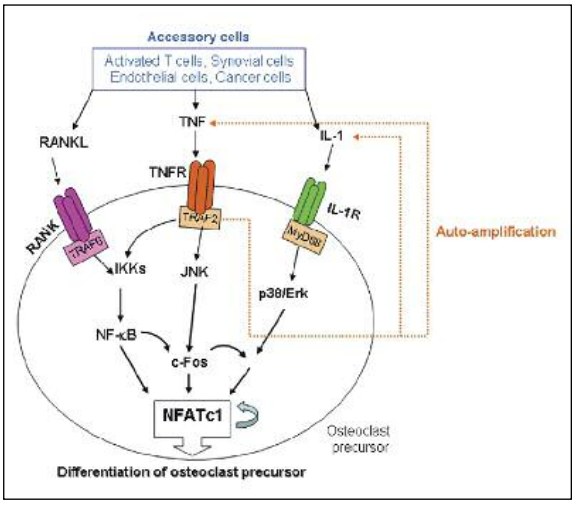

Figure 3. Signaling pathways involved osteoclastogenesis in diseases states with the activation of NFATc1. On the basis of mice studies, NFATc1 was described as the master regulator of osteoclastogenesis. From: Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Archives of biochemistry and biophysics. 2008; 473:139-146.

OPG/RANKL complex

The OPG/RANKL ratio is considered to better reflect the bone remodeling environment signs. A high ratio represents bone formation while a low ratio favors bone resorptio[1,4].

After OPG/RANKL complex formation, its internalization can be either through lipid rafts by membranous syndecan-1 or by the clathrin coat formation pathway. These two mechanisms control the bioavailability of extracellular OPG. In addition, glycosaminoglycans (GAGs) such as heparin, heparin sulfate, chondroitin sulfate and dermatan sulfate binds OPG via the heparin binding domains and compete with OPG/RANKL interaction, thus preventing OPG internalization through membranous RANKL. This internalization process is of particular importance for future therapeutic involvement of OPG[1].

The anti-resorptive effect of OPG can be explained by its properties of a decoy receptor and as a modulator of RANKL half-life. As RANKL and OPG controls each other bioavailability, the balance between RANKL and soluble OPG will be important for a curative application of OPG[1].

RANK/RANKL/OPG pathway in rheumatological conditions

Bone diseases are related to increased bone resorption, disturbed coupling between bone formation and resorption, and bone destruction[2].

GENETIC DISORDERS: *familiar expansile osteolysis* [activating 18-bp tandem duplication in the gene coding RANK (TNFRSF11A)]; *familiar form of early-onset Paget disease of bone* (similar 27-bp duplication of the previous gene); *expansile skeletal hyperphosphatasia* (15-bp tandem duplication in RANK); *idiopathic hyperphosphatasia* or *juvenile Paget disease* [homozygous complete deletion of OPG gene (TNFRSF11B)][5]. Sabacchi et al[13], reported mutations in the gene encoding RANKL in 6 patients with autossomal recessive osteopetrosis.

RHEUMATOID ARTHRITIS (RA): RANKL has been implicated as an important mediator of bone erosion[14]. Synovial T cells express RANKL and there is an over expression of RANKL messenger RNA (mRNA) and OPG in the RA patients synovium at the site of bone resorption, which contributes to osteoclast differentiation and activity[14-16]. OPG binding to soluble RANKL can better prevent osteoclast activation in non erosive arthritis than in RA[17]. Elevated serum levels of soluble RANKL normalize after anti-TNF therapy[4,8,14]. Assmann et al,

studied genetic variations of this pathway in the susceptibility to RA and showed the minor allele of the RANK SNP rs35211496 might be protective against RA[18]. Haynes et al, confirmed the hypothesis that successful treatment with modifying anti-rheumatic drugs (DMARDs) reduce RANKL/OPG ratio, suppressing osteoclast formation in the RA synovial tissue[19,20].

Spondyloarthropathies (SpA): the pattern of paraarticular bone tissue damage is different between different forms of peripheral arthritis. In SpA there is limited degradation of the paraarticular bone with new bone formation that can result in ankylosis[21]. In human SpA are described osteoclastic foci in the subchondral bone marrow of hip joints, which suggests a relation with cartilage-induced inflammation (the osteoclasts number is not increased at axial inflammation sites). The RANK/RANKL/OPG pathway contribute to bone erosions was demonstrated in RA, and also psoriatic arthritis (PsA), but only scarcely in peripheral joint inflammation in SpA[21]. Vandooren et al[22], demonstrated that both RANKL (mostly by cadherin 11-expressing synovial fibroblasts and CD3 T cells) and OPG were expressed in the inflamed synovium; the presence of osteoclasts precursors in the inflamed synovial tissue and that the factors needed to local osteoclastogenesis are present in the SpA synovium. There were no qualitative or quantitative differences in the expression of RANKL, OPG, and RANK between nonpsoriatic SpA, psoriatic SpA and RA synovium with the same degree of inflammation. They conclude that the relative protection against bone erosion in SpA cannot be explained by differences of RANK/RANKL/OPG synovial expression, and that these factors expression is disconnected from systemic and local inflammation[22].

Osteoporosis: in human osteoblastic cell lines have been shown a dose and time-dependent increase in OPG mRNA in response to 17-estradiol, which probably decreases the RANL-RANKL binding and osteoclastic bone resorption. Human bone marrow cells from untreated early postmenopausal women showed a greater expression of RANKL compared to the estrogen-treated group[4,8]. Ominsky et al, showed that ovariectomy in rats was associated with high levels of serum RANKL and osteoclast surface and reduced areal and volumetric BMD[23]. It was also showed that OPG reduced osteoclast surface and prevented ovariectomy-associated bone loss in the lumbar vertebrae, distal femur and femur neck[23]. In the glucocorticoid-induced osteoporosis the RANK/RANKL/OPG role was described: glucocorticoids stimulate RANKL expression by osteoclasts and inhibit OPG synthesis, favoring osteoclasts differentiation and proliferation (increased RANKL/OPG ratio and urinary and serum markers of bone resorption)[4,8].

Osteoarthritis (OA): OPG and RANKL have been found to be expressed and modulated in human OA subchondral bone, and by other articular chondrocytes. The OPG/RANKL ratio in the synovial fluid is greater in OA compared to AR. There are two different phenotypes of subchondral bone osteoblasts, L-OA (low endogenous levels of PGE_2) and H-OA (high endogenous levels of PGE_2). L-OA presents low PGE_2 level, low OPG/RANKL ratio, high osteoclastogenesis and a decreased subchondral bone thickness; while H-OA shows high PGE_2 level, high OPG/RANKL ratio, low osteoclastogenesis, and an increased subchondral bone thickness[1,24]. A recent *in vitro* study with human L-OA subchondral bone osteoblasts showed that the combination of glucosamine and chondroitin sulfate modulated OPG/RANKL ratio, decreasing bone resorption[25]. The addition of OPG or the inhibition of RANKL would be beneficial on the subchondral bone of the L-OA (resorptive phase), while in the H-OA patients the anti-resorptive agents are less effective as the subchondral bone seems to be in a formation phase[1]. Moreno-Rubio et al[24], showed that in patients with OA celecoxib decreased RANKL synthesis in the cartilage by increasing the OPG:RANKL ratio; in vitro, PGE2 regulated the expression and release of the mediators of bone metabolism by articular chondrocytes.

Polymialgia rheumatic (PMR): Pusatelli et al[26], found no significant differences in circulating OPG levels in PMR patients in the active phase of the disease or the follow-up compared to normal controls; the systemic RANKL (sRANKL) production is increased, is not modulated by corticosteroid treatment, and can be related to bone osteoporosis.

Systemic sclerosis (SS): microvascular damage is an early pathogenetic event in SS and RANK/RANKL/OPG system is involved in vascular biology. Dovio et al[27], showed that higher sRANKL levels and sRANKL/OPG ratio in patients with SS are a consequence of altered bone microenvironment, and showed dissociation between the well established activation/injury endothelial marker, soluble vascular cell adhesion molecule (sVAM), and OPG, as another vascular damage marker.

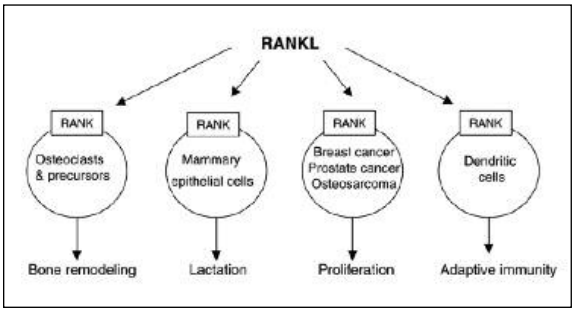

Figure 4. The role of the RANKL/RANK system in bone and other tissues. From: Boyce BF, Xing L. Biology of RANK, RANKL, an osteoprotegerin. Arthritis research and therapy. 2007;9:1-7.

JUVENILE DERMATOMYOSITIS (JDM): Rouster-Stevens et al[28], documented that at the time of diagnosis of JDM untreated patients have an elevated RANKL/OPG ratio compared to normal controls, and this ratio is related to lower bone mineral density (BMD)[29,30].

RANK/RANKL/OPG pathway in non-rheumatologic conditions

There is accumulating evidence of the potential role of OPG and RANKL in other tissues (Figure 4)[1].

BONE TUMORS: osteoclastic activating factors are produced by myeloma cells in response to IL-1, IL-6 and TNF-α. IL-7 may increase RANKL production in T cells, and there is also an increased lysosomal degradation of OPG. Although serum OPG levels correlated with World Health Organization multiple myeloma performance status, it have not be found to be associated with clinical stage or survival[4,29]. Myeloma cells release not only RANKL, but also dickkopff-1 (DKK-1), which suppresses bone formation, enhancing tumor growth. In metastatic bone diseases, tumor cells increase RANKL:OPG ratio directly and by T cells, osteoblast/stromal cells and endothelial cells, together with PTH related peptide, increasing bone removal and tumor growth[8].

VASCULAR CALCIFICATION: there are two main types of vascular calcification, depending if the calcium deposits are located in the intima (intimal calcification, related to atherosclerotic plaques) or in the medial layer (medial calcification, related to chronic kidney disease). An imbalance in the RANK/RANKL/OPG system was suggested as responsible for the calcification process of atherosclerotic plaques[4]. The identification of tissue-specific isoforms could increase the importance of sRANKL and OPG in predicting calcified plaque rupture[31,32]. However, direct evidence of a role of RANKL on vascular calcification is missing[33]. Panizo et al[33], showed that RANKL is able to induce vascular smooth muscle cells (VSMCs) calcification in vitro by binding to RANK; RANK activation will increase BMP4 expression by stimulating alternative NF-kB pathway. The inhibition of RANKL maybe is a possible target to treat vascular calcification[33,34].

INFLAMMATORY BOWEL DISEASE (IBD): Moschen et al[35], demonstrated that IBD is related to alterations in the RANKL/OPG system, and elevated RANKL/OPG ratio is associated to bone loss.

DIABETES MELLITUS (DM): Secchiero et al[36], showed that OPG but not the RANKL is significantly increased in type 2 DM patients compared to controls; serum OPG increases early after DM induction in mice, and showed a positive correlation with blood glucose levels and inverse correlation with free RANKL levels. Thus, increased OPG production represents an early event in DM and possibly is related to endothelial cell dysfunction.

CHRONIC ALCOHOLIC LIVER DISEASE: OPG is raised in alcoholics, especially in cirrhotics without relation with decreased BMD. Raised TNF and IL-6 levels were related with increased OPG levels, which support the protective effect of OPG in bone loss[37].

THYROID TUMORS: the role of RANK/RANKL/OPG in thyroid pathophysiology remains unclear. Heymann et al[38], showed that RANK/RANKL/OPG is expressed in the pathological thyroid gland by follicular cells, by malignant parafollicular cells, and in metastatic lymph node microenvironment. Thus this system might have a role in the pathogenesis of these tumors.

CHRONIC RENAL FAILURE: Fahrleitner-Pammer et al[39], demonstrated that RANK/RANKL/OPG system is associated with BMD in predialysis chronic renal failure. Serum OPG concentrations are lower in patients with adynamic bone disease, in contrast to those with increased bone turnover due to secondary hyperparathyroidism. It is possible that increased serum OPG in chronic kidney disease patients is an adaptative mechanism to attenuate PTH-induced bone loss[4].

BREAST AND PROSTATE CANCER: OPG production by breast cancer cells is a possible survival mechanism of the tumoral cells, because OPG inhibits TNF-related apoptosis-inducing ligand (TRAIL).

OPG is also a potential indicator for the diagnosis and early progression of prostate cancer (elevated levels)[4].

Wnt signaling pathway: interaction with RANK/RANKL/OPG

The Wnt proteins are a family of secreted growth factors found in all animal species that bind to cell--surface receptors and regulate cellular activities like cell fate, determination, proliferation, migration, polarity, and gene expression[6]. Genes encoding for Wnt proteins are highly conserved. At least four signaling pathways are described: Wnt/β-catenin; planar cell polarity; Wnt/Ca^{2+}; and protein kinase A.

The main biologic functions of the Wnt pathway in bone metabolism are: mesenchymal cell differentiation, implications in multiple myeloma and metastatic bone disease, bone mass regulation and bone response to mechanical loading.

The Wnt/β-catenin pathway involves the binding of Wnt proteins to LRP5 or 6 and a member of frizzled (Fz) family of proteins, increasing intracellular β-catenin levels which promote the transcription of target genes inside the nucleus. Its role in bone biology, RA and OA, has been highlighted. Wnt/receptor Fz is inhibited by members of the secreted frizzled-related protein family (sFRP) and Wnt inhibitory factor (WIF-1). Sclerostin (encoded by *SOST* gene) blocks LRP5 activity[6]. Inactivating mutation of Wnt co-receptor LRP5 and the lack of β-catenin, blocks the expression of transcription factors that determine osteoblastic phenotype and the mesenchymal cell achieves another phenotype (chondrocyte or adipocyte)[6,8], which results in reduced OPG expression and bone loss.

The Wnt signaling in osteoprogenitors promotes new bone formation by functioning as a positive regulator and upregulating OPG and down-regulating RANKL. Kamiya et al[7], found that osteoblasts respond to BMP signaling to support differentiation of osteoclasts through RANKL/OPG pathway, possibly by downregulating *Opg* gene and upregulating *Rankl*. It was also showed in mice that BMP signaling via BMP1A receptor directs osteoblasts to reduce bone mass by upregulating sclerostin expression as a Wnt inhibitor, and supporting osteoclastogenesis through the RANKL/OPG pathway.

Dkk-1 is a soluble inhibitor of Wnt pathway and a negative regulator of osteoblastogenesis in vivo (in mice)[40]. Diarra et al[41], proposed that Dkk-1 is a master regulator of joint remodeling, shifting the balance from bone resorption (increased Dkk-1 expression) to bone formation (decreased Dkk-1 expression). Wnt inhibitors Dkk-1 and 2 can induce osteoclastogenesis by changing the RANKL/OPG pathway in vitro[42] (Figure 5).

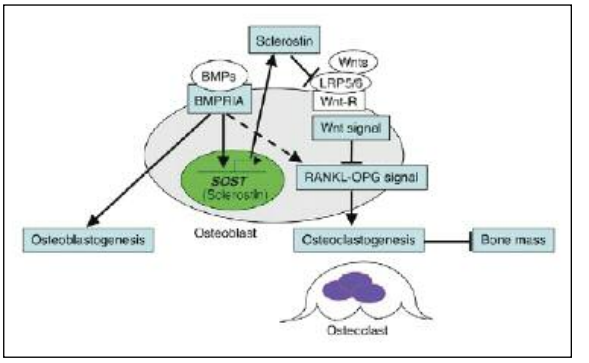

Figure 5. A model of the relationship between BMPR1A and canonical Wnt signaling in mouse bone. Wnt inhibitors Dkk-1 and 2 can induce osteoclastogenesis by changing the RANKL/OPG pathway in vitro.
From: Kamiya N, Ye L, Kobayashi T, Mochida Y, Yamauchi M, e tal. BMP signaling negatively regulates bone mass through sclerostin by inhibiting the canonical Wnt pathway. Development 2008;135:3801-3811.

Wnt system activation seems to be responsible for syndesmophyts growth in SpA.

New hypothesis

IL-6 is a mechano-sensitive cytocine and probably a key factor to the biomechanical control of bone remodeling in OA, possibly decreasing OPG/RANKL ratio[43,44].

TGFβ inducible early gene-1 (TIEG) directly binds to and inhibits OPG promoter activity in osteoblasts, explaining the possible inability of TIEG knockout osteoblasts to support osteoclast differentiation[45].

Leukotriene B4 is capable of inducing osteoclast differentiation by a RANKL-dependent mechanism[46].

Pigment epithelium-derived factor (PEDF), the most potent inhibitor of angiogenesis, up-regulates OPG and thus inhibits osteoclast function by regulating OPG expression[47].

MSCs can differentiate into adipocytes, osteoblasts, and other cells. There are a reciprocal relation between adipogenesis and osteogenesis. Der-Chih et al[48], identified cAMP/PKA signaling, that

regulates bone homeostasis, as a via controlling cyto-differentiation of MSCs (adipocytogenesis, osteogenesis, osteoclastogenesis) by controlling the release of leptin and altering RANKL/OPG gene expression.

The leucine-rich repeat-containing 17 (LRRc17) is a member of the LRR superfamily that acts as a negative regulator of RANKL-induced osteoclast differentiation (by decreasing NFATc1 expression depending on phospholipase C signaling), and thus, is a specific inhibitory molecule for osteoclastogenesis. Recombinant LRRc17 did not affect the differentiation of other myeloid precursors. The regulation of LRRc17 expression in oteoblasts by $1,25(OH)_2D_3$ suggests that this molecule is produced by osteoblasts and regulates its interaction with osteoclasts[49].

Emerging treatments

RANKL-SPECIFIC RECOMBINANT FULLY HUMAN MONOCLONAL ANTIBODY (DENOSUMAB): clinical trials showed its effectiveness in suppressing bone resorption, with an increase in BMD in postmenopausal women with osteoporotic low BMD[50], and have the potential to prevent progression of erosions in RA and metastatic bone disease. The recently published FREEDOM study[51] assessed the effects on fracture reduction in postmenopausal osteoporosis, and achieved a reduction of vertebral and hip fractures to 2,3% and 0,7% respectively, compared to 7,2% and 1,2% in the placebo group. As in the other trials, adverse events (infections or neoplasm) were similar to placebo[4,8].

OPG: beside its ability to inhibit osteoclastic activity, OPG can promote cell survival by inhibiting TRAIL-induced apoptosis[52]. A randomized controlled trial was conducted in postmenopausal women to determine the effect of a single subcutaneous dose of OPG on bone resorption (by urinary N-telopeptide and seric alkaline phosphatase). It concluded that OPG acted primarily on osteoclasts to decrease bone resorption and that a single OPG subcutaneous dose (3mg/Kg) was effective to reduce the bone turnover for a sustained period[52,53]. However, OPG has also been reported as a potential survival factor for several different cell types, through the TRAIL activity inhibition. Breast cancer cells produce OPG in order to be protected from the TRAIL effects in vitro[54]. Holen et al, demonstrated that OPG can act as an endocrine survival factor for breast cancer cells[55]. This new unexpected role of OPG discouraged investigators to further studies of the OPG administration bone effects. OPG might be a therapeutic option for bone lysis in metastatic breast cancer and in multiple myeloma. OPG is a potential marker of prostate cancer progression or relapse, and a potential marker of bone disease in renal osteodystrophy[52].

Conclusion

The RANK/RANKL/OPG pathway mediates the effects of the calciotropic hormones in different tissues and their imbalance contribute to several clinical rheumatologic and non-rheumatologic conditions. Multiple molecular discoveries gave rise to different mechanisms of interaction between signaling pathways that tried to explain bone formation/resorption. According to this development, new emerging treatments have been studied, like denosumab already approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of postmenopausal osteoporosis and the potential role of OPG as an osteoclastic inhibitor and a cell survival promoter.

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References

1. Tat ST, Pelletier JP, Velasco CR, Padrines M, Pelletier JM. New perspective in osteoarthritis: the OPG and RANKL system as a potential therapeutic target? Keio J Med 2009;58:29-40.
2. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Archives of biochemistry and biophysics 2008;473:139-146.
3. Boyce BF, Xing L. Biology of RANK, RANKL, an osteoprotegerin. Arthritis research and therapy 2007:1-7.
4. Vega D, Maalouf NM, Sakhaee K. The role of receptor activator of nuclear factor-kB (RANK)/RANK ligand/Osteoprotegerin: clinical implications. The Journal of Clinical Endocrinology and Metabolism 2007;92:4514-4521.
5. Whyte MP, Mumm S. Heritable disorders of the RANKL/OPG/RANK signaling pathway. J Musculoskel Neuron Interact 2004;4:254-267.
6. Caetano-Lopes J, Canhão H, Fonseca JE. Osteoblasts and bone formation. Acta Reumatológica Portuguesa 2007;32:103-110.

7. Kamiya N, Ye L, Kobayashi T et al. BMP signaling negatively regulates bone mass through sclerostin by inhibiting the canonical Wnt pathway. Development 2008;135:3801-3811.
8. Geusens P. Emerging treatments for postmenopausal osteoporosis – focus on denosumab. Clinical intervention in aging 2009;4:241-250.
9. Tao J, Chen S, Lee B. Alteration of Notch signaling in skeletal development and disease. Ann N Y Acad Sci 2010;1192:257-268.
10. Engin F, Lee B. NOTCHing the bone: Insights into multi-functionality. Bone 2010; 46:274-280.
11. Nakashima T, Kobayashi Y, Yamasaki S, et al. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. Biochem Biophys Res Commun 2000;275:768-775.
12. Sugatani T, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. Blood 2011; 117:3648-3657.
13. Sabacchi C, Frattini A, Guerrini MM, et al. Osteoclast--poor human osteopetrosis due to mutations in the gene encoding RANKL. Nat Genet 2007;39:960-962.
14. Pettit AR, Walsh NC, Manning C, Goldring SR, GRavallese EM. RANKL protein is expressed at the pannus-bone interface at sites of articular bone erosion in rheumatoid arthritis. Rheumatology 2006;45:1068-1076.
15. Ainola M, Mandelin J, Liljeström M, Konttinen YT, Salo J. Imbalanced expression of RANKL and osteoprotegerin mRNA in pannus tissue of rheumatoid arthritis. Clin Exp Rheumatol 2008;26:240-246.
16. Kim YG, Lee CK, Oh JS et al. Effect of interleukin-32 gamma on differentiation of osteoclasts from CD14+ monocytes. Arthritis Rheum 2010; 62:515-23.
17. Hein GE, Meister M, Oelzner P, Franke S. sRANKL and OPG in serum and synovial fluid of patients with rheumatoid arthritis in comparision to non-destructive chronic arthritis. Rheumatol Int 2008;28:765--769.
18. Assmann G, Koenig J, Pfreundschuh M, et al. Genetic variations in genes encoding RANK, RANKL, and OPG in rheumatoid arthritis: a case-control study. J Rheumatol 2010;37:900-904.
19. Haynes D, Crotti T, Weedon H et al. Modulation of RANKL and Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis in response to disease-modifying antirheumatic drug treatment and correlation with radiologic outcome. Arthritis and Rheumatism 2008;59:911-920.
20. Haynes DR, Barg E, Crotti TN et al. Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology 2003;42:123-134.
21. Walsh NC, Gravallese EM. Bone remodeling in rheumatic diseases: a question of balance. Immunol Rev 2010;233:301-312.
22. Vandooren B, Cantaert T, Noordenbos T, Tak PP, Baeten D. The abundant synovial expression of the RANK/RANKL/Osteoprotegerin system in peripheral spondylarthritis is partially disconnected from inflammation. Arthritis and Rheumatism 2008;58:718--729.
23. Ominsky MS, Li X, Asuncion FJ et al. RANKL inhibition with osteoprotegerin increases bone strength by improving cortical and trabecular bone architecture in ovariectomized rats. Journal of Bone and Mineral Research 2008;23:672-682.
24. Moreno-Rubio J, Herrero-Beaumont G, Tardio L, Alvarez-Soria MA, Largo R. Nonsteroidal antiinflammatory drugs and prostaglandin E(2) modulate the synthesis of osteoprotegerin and RANKL in the cartilage of patients with Sever knee osteoarthritis. Arthritis Rheum 2010;62:478-488.
25. Tat K, Pelletier JP, Vergés J et al. Chondroitin and glucosamine sulfate in combination decrease the proresorptive properties of human osteoarthritis subchondral bone osteoblasts. Arthritis Res Ther 2007;9: R117.
26. Pulsatelli L, Dolzani P, Silvestri T et al. Circulating RANKL/OPG in polymyalgia rheumatic. Clin Exp Rheumatol 2007;25:621-623.
27. Dovio A, Data V, Carignola R et al. Circulating osteoprotegerin and soluble RANK ligand in systemic sclerosis. J Rheumatol 2008;35:2206-2213.
28. Rouster-Stevens KA, Langman CB, Price HE et al. RANKL:Osteoprotegerin ratio and bone mineral density in children with untreated juvenile dermatomyositis. Arthritis and Rheumatism 2007;56:977-983.
29. Zdzisinnska B, Bjoarska-Junak A, Walter-Croneck A, Kandefer-Szerszen M. Dysregulation of the receptor activator of NF-kappaB ligand and osteoprotegerin production influence the apoptosis of multiple myeloma patients' bone marrow stromal cells cocultured with myeloma cells. Arch Immunol Ther Exp 2010;58:153-163.
30. Spelling P, Bonfá E, Caparbo VF, Pereira RM. Osteoprotegerin/RANKL system imbalance in active polyarticular-onset juvenile idiopathic arthritis: a bone damage biomarker? Scand J Rheumatol 2008;37:439--444.
31. Montecucco F, Steffens S, Mach F. The immune response is involved in atherosclerotic plaque calcification: could the RANKL/RANK/OPG system be a marker of plaque instability? Clin Dev Immunol 2007;2007:75805.
32. D'Amelio P, Isaia G, Isaia GC. The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease. J Endocrinol Invest 2009;32:6-9.
33. Panizo S, Cardus A, Encinas M et al. RANKL increases vascular smooth muscle cell calcification through a RANK-BMP4 dependent pathway. Journal of the American Heart Association- Circulation Research 2009; 104:1041-1048.
34. Bakhireva LN, Laughlin GA, Bettencourt R, Barrett--Connor E. Does Osteoprotegerin or receptor activa-

tor of nuclear factor-kappa B ligand mediate the association between and coronary artery calcification? The J of Clin Endocrin and Metabolism 2008;93:2009--2012.
35. Moschen AR, Kaser A, Enrich B et al. The RANKL/ /OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut 2005; 54:479-487.
36. Secchiero P, Corallini F, Pandolfi A et al. An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction. Am J Pathol 2006;169: 2236-2244.
37. Garcia-Valdecasas-Campelo E, González-Reimers E, Santolaria-Fernández F et al. Serum osteoprotegerin and RANKL levels in chronic alcoholic liver disease. Alcohol 2006;41:261-266.
38. Heymann MF, Riet A, Le Goff B et al. OPG, RANK and RANK ligand expression in thyroid lesions. REgul Pept 2008;148:46-53.
39. Fahrleitner-Pammer A, Dobnig H, Dimai HP et al. The effect of RANKL and OPG on bone mineral density in predialysis chronic renal failure. Clin Nephrol 2009;71:652-659.
40. Daoussi D, Andonopoulos AP, Liossis SNC. Wnt pathway and Il-17: novel regulators of joint remodeling in rheumatic diseases. Looking beyond the RANK-RANKL-OPG axis. Semin Arthritis Rheum 2010;39:369--383.
41. Diarra D, Stolina M, Polzer K et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med 2007;13:156-163.
42. Fujita KK; Janz S. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. Molecular Cancer 2007;6:71:1-13.
43. Sanchez C, Gabay O, Salvat C, Henrotin YE, Berenbaum F. Mechanical loading highly increases IL-6 production and decreases OPG expression by osteoblasts. Osteoarthritis Cartilage 2009;17:473-481.
44. Kim CH, Kim KH, Jacobs CR. Effects of high frequency loading on RANKL and OPG mRNA expression in ST-2 murine stromal cells. BMC musculoskeletal disorders 2009;109:1-7.
45. Subramaniam M, Hawse JR, Bruinsma ES et al. TGF--beta inducible early gene-1 directly binds to, and represses, the OPG promoter in osteoblasts. Biochem Biophys Res Commun 2010;392:72-76.
46. Chen ZK, Lv HS, Jiang J. LTB4 can stimulate human osteoclast differentiation dependent of RANKL. Artif Cells Blood Substit Immobil Biotechnol 2010;38:52--56.
47. Akiyama T, Dass Cr, Shinoda Y et al. PEDF regulates osteoclasts via osteoprotegerin and RANKL. Biochem Biophys Re Commun 2010; 391:789-794.
48. Yang DC, Tsay HJ, Lin SY et al. cAMP/PKA regulates osteogenesis, adipogenesis and ratio of RANKL/OPG mRNA expression in mesenchymal stem cells by suppressing leptin. PLoS ONE 2008;3:1-10.
49. Kim T, Kim K, Lee SH et al. Identification of LRRc17 as a negative regulator of receptor activator of NF-kB ligand (RANKL)-induced osteoclast differentiation. Journal of Biological Chemistry 2009;284:15308--15316.
50. Moen MD, Kean SJ. Denosumab: a review of its use in the treatment of postmenopausal osteoporosis. Drugs Aging 2011;28:63-82.
51. Cumming SR, Martin JS, McClung MR et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. NEJM 2009;361:756-765.
52. Fili S, Karalaki M, Schaller B. Therapeutic implications of osteroprotegerin. Cancer cell international 2009;26:1-8.
53. Bekker PJ, Holloway D, Nakanishi A, et al. The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 2001;16:348-360.
54. Van Poznak C, Cross SS, Saggese M, et al. Expression of osteoprotegerin (OPG), TNF related apoptosis inducing ligand (TRAIL), and receptor activator of nuclear factor kappaB ligand (RANKL) in human breast tumours. J Clin Patholog 2006;59:56-63.
55. Holen I, Cross SS, Neville-Webbe HL, Cross NA, Balasubramaniam SP, et al. Osteoprotegerin (OPG) expression by breast cancer cells in vitro and breast tumours in vivo – a role in tumours cell survival? Breast Cancer Res Treat 2005;92:207-215.