Cell. Mol. Life Sci. 64 (2007) 2334 – 2350
1420-682X/07/182334-17
DOI 10.1007/s00018-007-7104-0

Cellular and Molecular Life Sciences

Review

RANKL, RANK, osteoprotegerin: key partners of osteoimmunology and vascular diseases

M. Baud'huin[a, b], F. Lamoureux[a, b], L. Duplomb[a, b], F. Rédini[a, b] and D. Heymann[a, b, c, *]

[a] Université de Nantes, Nantes Atlantique Universités, Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, EA3822, F-44035 Nantes (France), Fax: +33 240 412 845, e-mail: dominique.heymann@univ-nantes.fr
[b] INSERM, ERI 7, F-44035 Nantes (France)
[c] CHU de Nantes, F-44035 Nantes (France)

Received 27 February 2007; accepted 4 April 2007
Online First 29 May 2007

Abstract. 1997 saw the identification of a novel set of proteins within the tumor necrosis factor (TNF)/TNF receptor families that are required for the control of bone remodeling. Therefore, these receptors, receptor activator of nuclear factor kappa B (RANK), osteoprotegerin (OPG) and their ligand RANK ligand (RANKL) became the critical molecular triad controlling osteoclastogenesis and pathophysiologic bone remodeling. However, the establishment of the corresponding knock-out and transgenic mice revealed unexpected results, most particularly, the involvement of these factors in the vascular system and immunity. Thus, the OPG/RANK/RANKL molecular triad appears to be associated with vascular calcifications and plays a pivotal function in the development of the immune system through dendritic cells. OPG/RANK/RANKL thus constitute a molecular bridge spanning bone metabolism, vascular biology and immunity. This review summarizes recent knowledge of OPG/RANK/RANKL interactions and activities as well as the current evidence for their participation in osteoimmunology and vascular diseases. *In fine*, the targeting of the OPG/RANK/RANKL axis as novel therapeutic approaches will be discussed.

Keywords. RANKL, osteoprotegerin, bone remodeling, osteoclast, osteolysis, osteoimmunology, cardiovascular disease.

Introduction

Genomic and proteomic research over the past decade has identified a large number of cytokines, growth factors, transcriptional factors and enzymes controlling cell metabolism and/or participating in the establishment of the molecular network in each cell, into all tissues and between all organs. Such systems allow the coordination of cell differentiation during embryogenesis and coordinated cell activities throughout life [1], and a molecular cross-talk in bone between osteoblasts and osteoclasts to maintain bone mass. Thus, osteoclasts originating from hematopoietic multinucleated cells are specialized in calcified matrix resorption [2, 3], whereas osteoblasts derived from bone marrow mesenchymal stem cells are responsible for new bone formation [4]. It has been estimated that around 10% of total bone mass is renewed per year, participating in calcium and phosphorus homeostasis [5]. The communication networks between osteoclasts and osteoblasts are mainly com-

* Corresponding author.

prised of soluble cytokines and growth factors which modulate the expression of a wide range of genes through specific receptors and numerous transcription factors. However, it has been established that osteoblast-osteoclast contacts are required for the differentiation of osteoclast progenitors, thus demonstrating the pivotal role played by the membrane form of growth factors [6, 7]. These observations led the international scientific community to identify the soluble and membrane factors involved in osteoblast-osteoclast interactions.

Parathyroid hormone (PTH) [8], vitamin D3 [1,α(OH)$_2$D$_3$] [9] and calcitonin [10] have been considered for a long time as the principal regulators of calcium homeostasis in terrestrial vertebrates. Similarly, macrophage colony-stimulating factor (M-CSF) is a critical cytokine involved in osteoclastogenesis. M-CSF participates in the proliferation of osteoclast progenitors, maintaining a pool of progenitors in bone marrow, and it cooperates with receptor activator of nuclear factor kappa B (RANK) ligand (RANKL) to induce the fusion of osteoclast progenitors (pre-osteoclasts) into osteoclasts which will be secondarily activated into mature osteoclasts by RANKL (see below) [11, 12]. In contrast, interferons (IFNs), which play crucial roles in the regulation of a wide variety of innate and adaptive immune responses, interfere with RANKL-induced osteoclastogenesis and thus represent a critical mechanism for the suppression of pathological bone resorption associated with inflammation [13]. However, recent investigations in bone biology clearly identified a novel set of cytokines/cytokine receptors within the tumor necrosis factor (TNF) family that are required for the control of bone remodeling [14–18]. These molecules – RANKL and its receptors RANK and osteprotegerin (OPG) – appear as the final effectors of most of the osteotropic factors already identified [19]. RANKL is considered to be a powerful stimulator of bone resorption, while OPG is a soluble bone protector. In this context, pathologies characterized by deregulated bone remodeling are often associated with an imbalance between OPG and RANKL [18–21]. Furthermore, recent studies demonstrated that the OPG/RANK/RANKL molecular triad is also strongly implicated in the control of immune [22] and vascular [23] systems. Thus these three molecules constitute a molecular cross-talk between bone, vessels and immune cells, providing new strategies for the prevention and/or treatment of corresponding diseases.

The present review summarizes recent knowledge of OPG, RANK and RANKL interactions and roles in the pathophysiology of bone, vessels and immunity.

RANKL and its receptors OPG and RANK regulate bone metabolism: prognostic markers and therapeutic agents

Cytokines and growth factors can exist as different forms: expressed at the cell membrane, associated with the extracellular matrix, or in soluble forms, interacting with their membrane and soluble receptor to modulate cellular activities. *In fine,* the effects of a cytokine result in the balance between its own activities and the biological influence of its soluble receptors which act as agonist or antagonist agents. As the molecular partners OPG/RANK/RANKL thus include the RANKL cytokine, its membrane or soluble receptors, respectively RANK and OPG, it is necessary to take into consideration the potential duality of both receptors to determine RANKL activities.

OPG/RANK/RANKL: molecular and functional characteristics

Osteoclast activities are related to the bone resorption process in physiological as well as in pathological conditions. For many years, mesenchymal–derived stem cells including osteoblasts have been well known to modulate osteoclast differentiation and bone degradation [24–26]. However, the major inhibitor of osteoclastogenesis was only identified in 1997–1998 simultaneously by Tsudas group [27, 28] and Amgen Company [29]. They respectively named this novel negative regulator of osteoclast differentiation 'osteoclatogenesis inhibitory factor' (OCIF) and 'osteoprotegerin.' Its international name according to the TNF nomenclature is TNFRSF11B. The role of OPG has been clearly demonstrated by the development of transgenic and knock-out mice. Indeed, OPG knock-out mice exhibit a strong decrease in total bone density and bone volume and suffer from osteoporosis associated with a high incidence of fractures and vertebral deformities [30, 31]. Furthermore, this induced osteoporosis was totally reversed by intravenous injection of recombinant OPG [32]. In contrast, OPG transgenic mice suffer from a marked osteopetrosis characterized by a high bone turnover and an inhibition of osteoclastogenesis [29]. These data demonstrated that the presence of OPG is absolutely required to maintain bone mass in physiological situations. *In vitro* experiments confirmed the observations in the animal models and provided an explanation for the phenotype exhibited by the mice. Immediately after the identification of OPG, both laboratories identified within the TNF cytokine family, the ligand which bound OPG with high affinity, and called this molecular effector OPG ligand (OPGL) [33, 34]. OPGL was RANKL or the TNF

ligand superfamily member 11, TNFSF11, already known for its activity on the immune system. Indeed, RANKL is considered to be a dendritic cell-stimulating agent and acts as a survival factor for dendritic cells and for mature T cells, therefore regulating their proliferation [35–37]. These activities are associated with the activation of the nuclear factor kappa B (NF-κB) transcription factor after the binding of RANKL to its membrane receptor RANK (TNFRSF11A) [38, 39].

To determine the involvement of RANKL in bone metabolism, similar approaches based on genetically modified mice have been used by both research groups. In contrast to OPG-modified mice, RANKL transgenic mice exhibit a marked osteoporosis [33] and mice disrupted for RANKL are strongly osteopetrotic with a total absence of mature osteoclasts [40, 41]. Furthermore, severe bone loss and hypercalcemia are the main phenotypic characteristics of mice treated with recombinant RANKL. Overall, these data demonstrated that RANKL is a pro-resorptive factor while OPG is a powerful osteoprotective agent. *In vitro* experiments supported *in vivo* observations. Indeed, using a primary culture of osteoclast progenitors from bone marrow or a precursor cell line such as the murine monocytic cell line RAW264.7, RANKL induces osteoclast differentiation whereas OPG abolishes this phenomenon [16, 32–44]. Figure 1a illustrates RANKL/OPG activities in the RAW264.7 cells. After 4 days of culture in the presence of RANKL, RAW264.7 cells differentiate into large and multinucleated cells expressing osteoclastic markers (calcitonin receptor, cathepsin K, TRAP). When the cells are treated simultaneously with OPG and RANKL, osteoclast formation is abolished. RANKL is also able to activate mature osteoclasts, as determined by the stimulation of MMP and cathepsin K activities and the bone resorption capability of these cells [43, 44].

RANKL and OPG activities/functions were demonstrated in the years after their identification (Fig. 1b). In normal bone, RANKL is preferentially expressed on committed pre-osteoblastic cells, whereas its specific receptor RANK is expressed on osteoclast progenitors. In this system, RANKL is required for osteoclast differentiation and acts as a survival factor for osteoclast precursors [15–17]. OPG is a soluble factor produced by osteoblastic cells, and thus is considered as a decoy receptor for RANKL as confirmed by molecular binding experiments [45]. Indeed, OPG blocks the interaction between RANKL and RANK, inhibits the terminal stage of osteoclastic differentiation and then inhibits bone resorption (Figs. 1b, 2a) [15–18]. Furthermore, the inhibitory effect of OPG on bone resorption can be explained not only as a decoy receptor function but also as a modulator of the RANKL half-life [46]. Studies of OPG/RANKL in the serum of RANKL−/− and OPG−/− mice are in agreement with a potential role for OPG in the shedding of RANKL [47]. In turn, RANKL controls the bioavailability of OPG and its internalization and degradation [46]. In pathophysiological situations, OPG and RANKL must be considered as a molecular balance and be evaluated separately. OPG and RANKL expression are not restricted to bone tissue, as both factors can be produced by numerous cell types. OPG is a soluble factor, produced by a large number of cells, including immune cells, endothelial cells and osteoblasts [see review in ref. 18]. RANKL is expressed on the cell membrane and is also produced as a soluble factor by the same cells [40] (Fig. 2a). Three isoforms of RANKL have been identified in human and rodent, the first encoding a transmembrane form, the second, a soluble cytokine and the third, a cytoplasmic molecule [48, 49]. The soluble form of RANKL can also be produced by enzymatic shedding by the metalloprotease-disintegrin TNF-alpha convertase (TACE, also named a disintegrin and metalloproteinase, ADAM17) [50, 51], ADAM10 [52], ADAM19 [53], MMP-3 [54], MMP7 [54] and MMP-14 [52]. The exact function of each RANKL isoform must be clarified, but the literature clearly revealed the major relevance of the balance in RANKL-expressing cells between these three RANKL forms in osteoclastogenesis [55].

The third protagonist of the molecular triad is RANK. Like OPG and RANKL, transmembrane RANK is ubiquitously expressed, but in contrast to OPG/RANKL, it is considered to be an osteoclastic marker in physiological bone tissue [56] (Fig. 2a). Like the other members of the TNF receptor superfamily, RANK assembles into functional trimeric receptors after binding with RANKL [57]. This trimerization appears to be required to generate mature and functional osteoclasts. Indeed, RANK dimerization allows the formation of multinuclear TRAP-positive cells but without any osteoclastic markers and unable to induce pit resorption [57]. Similarly, using X-ray crystallographic analysis, Ito *et al.* [58] showed the ability of the RANKL ectodomain to constitute a trimer complex [58] and then confirmed that the formation of a 3:3 RANK-RANKL binary complex is necessary to generate optimal osteoclastogenesis (Fig. 1b). Although RANKL binding to RANK induced RANK trimerization, RANK is also able to self-assemble [59], similarly to other TNF receptors [60]. Thus, Kanazawa and Kudo [59] demonstrated that RANK is self-assembled through a restricted cytoplasmic domain (amino acid residues 534–539). Interestingly, overexpression of full-length RANK results in activation of NF-κB and osteoclastogenesis,

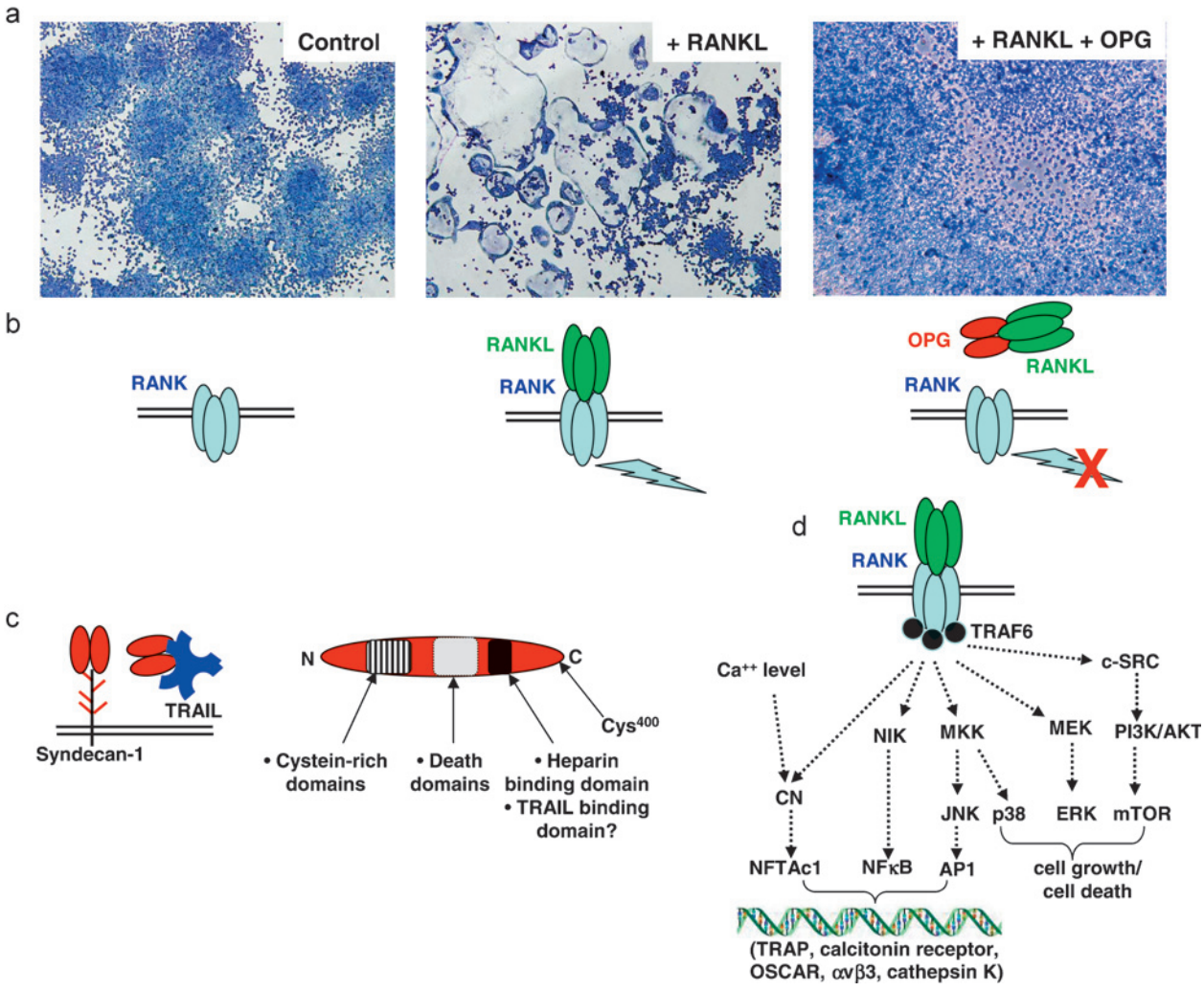

Figure 1. Characteristics of OPG/RANK/RANKL interactions. (*a*) After 96 h in the presence of 100 ng/ml human RANKL, the murine RAW264.7 monocytic cell line differentiates into osteoclasts while 100 ng/ml human OPG totally abolishes RANKL effects. (*b*) RANK is expressed on the osteoclast membrane and binds RANKL produced by osteoblast/bone marrow stromal cells. OPG acts as a decoy receptor and blocks the interaction between RANKL and RANK. (*c*) OPG possesses a heparin binding domain and interacts with two other ligands, TRAIL and syndecan-1; (*d*) RANKL binding to RANK transduces specific signals in osteoclasts corresponding to novel therapeutic targets of bone diseases; CN, calcineurin.

even in the absence of RANKL. It was earlier demonstrated that TNF receptor (TNFR)-I and II also selfassembled through a specific extracellular domain named the pre-ligand binding assembly domain (PLAD), thus avoiding the formation of mixed receptors TNFR-I and TNFR-II [60]. Overall, these data indicate that such a RANK domain could also be useful to allow the formation of specific receptor-activating signal transduction pathways (see below) and to avoid the formation of hybrid receptors.

OPG, belonging to the TNFR superfamily, is able to self-dimerize through a disulfide bond using Cys^{400} at the C-terminal portions [61, 62]. Indeed, the most active form of OPG is a homodimer that possesses higher affinity for the RANKL ectodomain compared to OPG monomer [63]. OPG contains seven domains: four cysteine-rich N-terminal domains (domains 1–4), two death domain-homologous regions (domains 5 and 6) and a C-terminal heparin-binding domain (domain 7) (Fig. 1c). Domains 1–4 are structurally related to the TNF receptor family and are sufficient to abolish osteoclast differentiation. Domains 5 and 6 can mediate a cytotoxic signal when they are included in a chimeric protein OPG-Fas [61], but their physiological functions remain to be elucidated. Domain 7 is composed of a heparin-binding domain and a Cys^{400} at its C-terminal portion (Fig. 1c). Although the affinity for heparin does not correlate with OPG inhibitory potential, this domain plays numerous key functions not all of which have been determined. Thus, OPG through its binding to syndecan-1 exerts a chemotaxis activity in human peripheral blood monocytes [64]. In

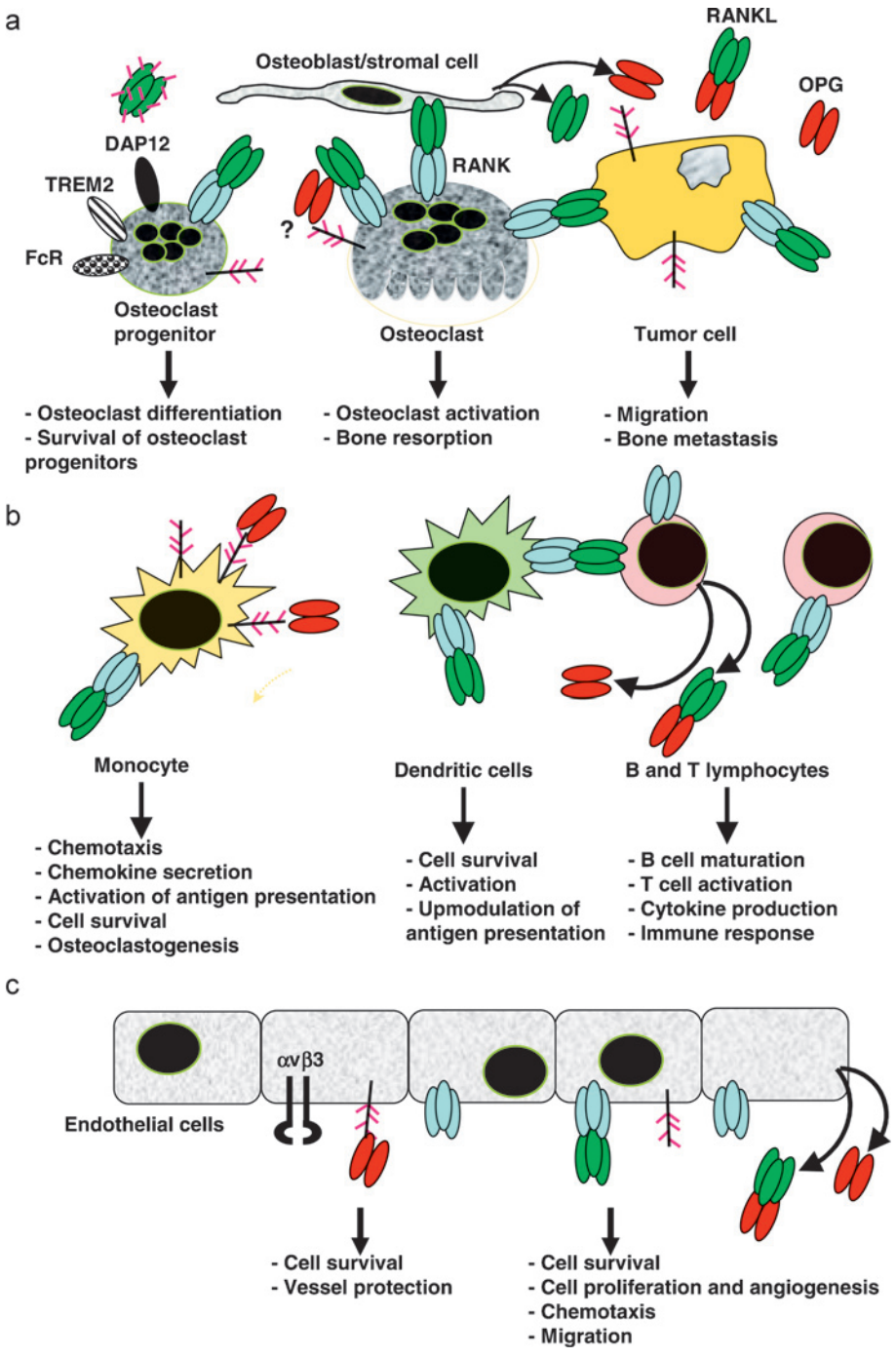

Figure 2. OPG/RANK/RANKL: key partners in osteoimmunology and vascular diseases. (*a*) OPG/RANK/RANKL participation in pathophysiological bone remodeling, their roles involving costimulatory molecules such as DAP12, TREM2, FcR and proteoglycans. (*b*) OPG/RANK/RANKL modulate the immune system via a pivotal activity in dendritic cells. (*c*) The third system affected by OPG/RANK/RANKL is the vascular compartment.

turn, syndecan-1 controls OPG bio-availability and is associated with an increase in local RANKL concentration and osteolysis in myeloma cells which overexpress this proteoglycan [65]. Furthermore, shedding of TNF-α proteoglycans and glycosaminoglycans strongly modulates OPG/RANK/RANKL interactions. Indeed, recent data demonstrated that RANK, RANKL, OPG and proteoglycans could form a very large complex 3:3:2:1 related to specific activities in osteoclasts such as MMP9 activities [66]. Such a very large complex involving protegoclycans can be compared to the cooperative dimerization of fibroblast growth factor (FGF)-1 upon a single heparin saccharide that may drive the formation of a 2:2:1 FGF1-FGFR2c-heparin ternary complex [67]. In this case, proteoglycans act as coreceptors for growth factors. Furthermore, depending on the bone remodeling step, OPG could be released from the bone matrix and thus modulate bone remodeling in a fashion similar to TGF-β [68]. In bone tissue, proteoglycans thus appear

critical for maintaining an appropriate number of osteoblasts and osteoclasts by modulating their proliferation and/or differentiation. OPG activities could involve the ras/MAPK pathways [45] but also PKC and PI3K/Akt [64].

Similarly to OPG/ RANK/RANKL, heparan sulfate proteoglycans appear crucial for other members of the TNF/TNFR superfamilies. While glycosaminoglycans mainly modulate OPG-RANKL interactions, dermatan sulfate also interacts with RANKL [69]. Indeed, Shinmyouzu *et al.* [69] showed that dermatan sulfate reduced the RANKL-induced levels of phosphorylation of p38 and ERK in osteoclast progenitors, thus abolishing osteoclastogenesis. Very recently, Bishof *et al.* [70] identified syndecan-2 as a new binding partner of the transactivator and calcium modulator and cyclophilin ligand interactor (TACI), a member of the TNFR family [71]. Similarly, heparin sulfate proteoglycan serves as a receptor for A proliferation-inducing ligand (APRIL), a member of the TNF family, promoting tumor cell proliferation [72]. OPG also has a third ligand, TNF-related apoptosis-inducing ligand (TRAIL; Fig. 1c) [73]. OPG binding to TRAIL results in the abolition of TRAIL apoptotic activity [74–82]. This cross-regulation has a strong impact in cancer biology. Indeed, these observations suggest that OPG is deleterious, acting as a survival factor in several cancer pathologies by the inhibition of TRAIL, a natural inducer of tumor cell apoptosis [76–82].

Recent data reveal that several membrane proteins cooperate with the RANK/RANKL complex to control bone remodeling. The signaling adapter protein DNAX-activating protein-12 (DAP12) is a homodimeric transmembrane molecule modulating cellular activation and maturation in myeloid lineage cells in association with the DAP12-associated receptors (DARs) which include triggering receptor expressed on myeloid cells (TREM), DAP12-associated lectin (MDL)-1 and NKG2D. In 2003, Kaifu *et al.* [83] showed that mice deficient for DAP12 exhibit a marked osteopetrosis owing to impaired osteoclastogenesis through a blockade of progenitor multinucleation. They also determined the molecular motif associated with this bone phenotype and provided evidence for the function of the immunoreceptor tyrosine-based activation motif (ITAM) in the regulation of osteoclastogenesis [84]: mice lacking ITAM-harboring adaptors such as Fc receptor common gamma subunit (FcRγ) and DAP12 are osteopetrotic [84, 85]. Similarly, Humphrey *et al.* [86] recently demonstrated the involvement of TREM2 in the regulation of osteoclast multinucleation, resorption and migration. In fact, DARs such as TREM2 associate with DAP12 or FcRγ and transduce costimulatory signals (especially calcium signaling through phospholipase Cγ) which cooperate with RANKL signaling during osteoclast differentiation (Fig. 2a) [87, 88]. In this context, any disturbance in the DAR-DAP12 signaling complex contributes to the development of bone diseases.

This section has described the main molecular and functional characteristics of OPG/RANK/RANKL. Overall, the abundant literature demonstrates that RANKL and OPG appear as final effectors of osteoclastogenesis and bone resorption.

OPG/RANK/RANKL and bone pathologies

Osteolytic processes are the main skeletal-related events in patients suffering from bone metastases and are associated with major sequelae and high levels of individual incapacity. In this context and according to the pivotal role of the OPG/RANK/RANKL triad in osteoclastogenesis, blocking osteoclastogenesis and osteoclast activities via RANKL pathway inhibition constitutes a potential novel approach to maintain skeletal integrity. It has been suggested that high levels of bone degradation could enhance cancer cell establishment and growth in bone tissue through biological factors such as growth factors sequestrated in bone extracellular matrix. Indeed, cancer cells express soluble or membrane factors promoting osteoclastogenesis and accentuating bone resorption. Thus, a vicious cycle is generated between bone and cancer cells [89]. The expression of RANKL has been demonstrated in a wide range of benign and malignant tumor cells [18, 20, 75, 89, 90] (Fig. 2a). Although RANKL released in the bone microenvironnement affects osteoclast activities, RANKL can also directly modulate the behavior of cancer cells. Indeed, very recently, several papers have reported the expression of functional RANK in human breast cancer cells, human prostate cancer and mouse melanoma cell lines [91–93]. These studies show that RANKL triggers RANK-positive cancer cell migration and growth in bone tissue. However, the development of bone metastases is probably not limited to the action of RANKL on cancer cells and cannot exclude the pivotal role of osteoclasts [94, 95]. We indicated above that several proteases produced in the bone microenvironment modulate the shedding of RANKL and then bone resorption. The following theory has been proposed: osteoclastogenesis and activation of immature osteoclasts could be mediated by a direct interaction with RANKL-expressing osteoblasts or/and by the protease-solubilized RANKL which can also participate in the recruitment of osteoclast progenitors [54]. Although the expression of RANKL by cancer cells is controver-

sial (especially for breast carcinoma cells), it is now hypothesized that the metastatic cells might express RANKL in an osseous context in contrast to the primary tumors [96]. Their migration to bone tissue is linked to the expression of membrane RANK and the chemoattractive function of RANKL. Based on these observations, current studies have disclosed that blocking RANKL-RANK interaction prevents the progression of prostate cancer in bone [97–100]. However, RANK expression is not restricted to non-bone cancer cells, because we recently showed that functional RANK is expressed at the surface of mouse [101] and human [102] osteosarcoma cell lines without modulation of cancer migration. RANK is also detected in more than 50% of human osteosarcoma specimens studied, with a preferential expression on osteosarcoma developed in pathological bone and bad responders to chemotherapy [102] (Fig. 2a). Thus RANKL may act as a 'soil' factor in primary and secondary bone cancer development dependently or independently of its direct effects on cancer cells. This fact is supported by several groups that have shown that inhibition of bone resorption using RANK-Fc or OPG-Fc blocks growth of tumor cells such as myeloma cells that do not express RANK [103, 104]. However, a wide range of benign and malignant tumor cells express OPG simultaneously with RANKL [18, 20, 75, 89, 90, 105] (Fig. 2a). Such an observation has led to evaluation of the clinical interest in measuring the RANKL/OPG ratio in bone pathologies. Thus, the RANKL/OPG balance is disturbed in severe osteolytic pathologies in favor of RANKL [106–108]. In such cases, the tumor microenvironment releases high levels of OPG to counterbalance the high concentration of RANKL. OPG may reflect a protective mechanism of the skeleton to compensate for increased bone resorption. This hypothesis is strengthened by the effect of bisphosphonate treatment which results in a decrease in the OPG level in contrast to RANKL which is not modified [109, 110]. *In fine*, as the RANKL/OPG ratio is significantly higher in patients with severe malignant osteolytic pathologies [106, 111—113], it could predict survival as demonstrated in multiple myeloma [114]. Moreover, the RANKL/OPG ratio may be used as a prognostic biological marker in non-malignant pathologies such as osteoporosis [108, 115], ankylosing spondylitis [116], rheumatoid arthritis [117], benign bone tumors, osteolysis associated with hip prosthetic loosening [106, 118, 119] or bone fractures [120]. For example, elderly women with hip fractures exhibit an increased RANKL/OPG mRNA content in iliac bone which is associated with increased fracture susceptibility. This ratio can also be considered as an important index for the evaluation of new drugs against bone diseases or as therapies for bone pathologies [121–129].

OPG/RANK/RANKL: the crossroads of immunity and bone metabolism

The immune phenotype of OPG transgenic mice demonstrated for the first time the relationship between the OPG/RANK/RANKL triad and the immune system [30]. OPG transgenic mice exhibit impaired thymocyte development. Consistent with these findings, mice with a disrupted RANKL gene show a lack of all lymph node organogenesis, normal splenic and Peyer's patches organization, and impaired thymocyte development [40, 41]. Moreover, RANK knock-out mice also lacked lymph nodes and produced defective B and T lymphocyte maturation while they exhibited normal thymic development [130]. RANKL expressed by thymic epithelial cells may be responsible for the development and maturation of RANK-positive lymphocytes [131]. In this context, the OPG/RANK/RANKL molecular triad constitutes a cross-talk between bone metabolism and the immune response, which has been labeled osteoimmunology [18, 132]. The OPG/RANK/RANKL triad exerts its activities on the different cell types involved in immunity. Thus, RANKL behaves as a chemotactic factor for monocytic cells through its binding to membrane RANK [133, 134], similar to its effect observed on osteoclasts [135], emphasizing the cross-talk between bone and immune systems (Fig. 2b). The RANKL-induced migration involved phosphatidylinositol 3-kinase, phosphodiesterase, and Src kinase signaling pathways. OPG also modulates the migration of monocytes via its binding to syndecan-1 and signaling involving protein kinase C, phosphatidylinositol 3-kinase/Akt and tyrosine kinase [64]. However, further experiments are needed to define the functional importance of the OPG/RANKL balance in the monocyte migration.

Complementary to their role in cell migration described above, recent data implicate the OPG/RANK/RANKL triad in inflammatory processes. A large number of cytokines are known to regulate many of the bone responses in inflammatory conditions [136]. Several cytokines are modulated by RANKL, thus affecting cell behavior [18, 137]. Thus, RANKL induced CCL22 (macrophage-derived chemokine) [138], monocyte chemotactic protein 1 (MCP-1) [139] and interleukin-8 [140]. In turn, the chemokine increased RANKL expression [18] leading to the establishment of a vicious cycle exacerbating the inflammatory process and the associated pathologies [141, 142]. Similarly, RANKL has been found to up-regulate RANK expression on monocytes, to activate

their capacity for antigen presentation through up-regulation of costimulatory molecule expression and to promote cell survival [143, 144]. Thus, RANKL enhanced the survival of macrophages and up-regulated the expression of CD86, and RANKL-treated macrophages show increased allogeneic T cell activation and phagocytic activity compared to control cells [144]. In a model of lipopolysaccharide (LPS)-induced endotoxic shock, administration of soluble RANK-Fc protects mice from death induced by sepsis, and in a model of inflammation-mediated arthritis, RANK-Fc ameliorates disease development and attenuates bone destruction [143]. Although lymphocytes are probably the major source of RANKL in inflammatory processes, mast cells [145], endothelial cells and platelets [146] also represent a potential origin of RANKL. Blockade of RANKL may allow treatment of human inflammatory disorders in which RANKL is overexpressed, such as periodontal diseases, rheumatoid arthritis or wear debris prosthesis [147–150]. However, very recently Maruyama et *al.* [151] found that a RANKL pre-treatment suppressed production of inflammatory cytokines in macrophages in response to LPS. In this model, prior administration of RANKL protects mice from LPS-induced death [151]. These data and those published by Seshasayee *et al.* [143] appear contradictory. In fact, RANKL may be produced to counterbalance the inflammatory process maintained by inflammatory cytokines. Unfortunately, when the inflammation is initiated, RANKL appears unable to block this process and may even contribute to maintain the pathology.

The RANKL/OPG molecular duo is also strongly associated with the biology of dendritic cells expressing RANK. Indeed, RANKL dramatically inhibits the apoptosis of dendritic cells via increased Bcl-xL expression [35] and induces T lymphocyte proliferation [36] (Fig. 2b). However, the core function of RANKL in dendritic cell biology is still not well understood. RANKL has been reported to activate intestinal dendritic cells, increase their survival *in vivo*, and inhibition of the RANK/RANKL axis by OPG-Fc results in reduced colitis [152]. On the other hand, in an inflammation-mediated transgenic mice model for type I diabetes, RANK-Fc treatment decreases the numbers of CD4+CD25+ regulatory T lymphocytes in pancreas-associated tissue that can exacerbate disease [153]. More recently, Loser *et al.* [154] reported that RANKL is expressed in keratinocytes of the inflamed skin and that RANKL can modify dendritic cell functions to maintain the number of peripheral CD4+CD25+ regulatory T lymphocytes. OPG might contribute to this control since it is expressed in dendritic cells and its expression is increased with their maturation [155]. Moreover, dendritic cells from homozygous OPG-deficient mice potentiate the mixed leukocyte reaction despite CD86, MHCII, and antigen presentation levels which are similar to heterozygous OPG-deficient mice [156]. Overall, these data demonstrated that RANKL is a key regulator of T lymphocyte-dendritic cell communication, modulating immunity and bone remodeling through dendritic cells [157].

OPG/RANKL and endothelial cells: a close functional relationship in vascular biology

The first clue that the OPG/RANK/RANKL triad might be the molecular system linking bone metabolism and vessel biology was provided by the vascular phenotype of OPG-deficient mice [30]. OPG-deficient mice exhibited medial calcification of the aorta and renal arteries but not of smaller vessels, suggesting that OPG and its molecular partners may play a role in the long-term observed association between osteoporosis and vascular calcification [30]. The lesions observed resemble human atherosclerotic lesions (RANKL and RANK expression, presence of osteoclast-like cells adjacent to the RANKL-expressing cells). OPG administration prevents calcification induced by warfarin or high doses of vitamin D in rat [158] but could not reverse this phenomenon once the mineralization process had occurred [32]. Moreover, OPG physically associated with the von Willebrand factor is localized in the Weibel-Palade bodies of endothelial cells and is rapidly secreted in response to inflammatory stimuli [158]. This observation definitively supports the notion that OPG/RANK/RANKL constitute a molecular bridge spanning skeletal disorders, vascular injury, inflammation and hemostasis [159, 160].

RANKL and OPG are differentially expressed in calcific aortic stenosis [161]. Indeed, while RANKL was not expressed at relevant levels in controls but detectable in aortic stenosis, OPG expression was marked in controls but significantly lower in this pathology. Furthermore, RANKL promotes matrix calcification and induces the expression of osteoblast-associated genes in cultured human aortic valve myofibroblasts, revealing a transition towards an osteogenic phenotype [161]. Microvascular endothelial cells produce an adapted microenvironment favorable to calcified tissue formation and thus stimulate the adhesion and transendothelial migration of monocytes that can differentiate into osteoclasts in the presence of RANKL [162]. These results suggest that the RANKL/OPG pathway may regulate valvular calcification in calcific aortic stenosis. Furthermore, Olesen *et al.* [163] suggested that OPG may play a special role in arterial disease in diabetes, since increased levels of OPG found in aortic tunica media

from diabetic patients are associated with calcification of human vascular smooth muscle cells [164]. In agreement with these findings, Anand *et al.* [165] revealed that among inflammatory biomarkers, only OPG predicted both subclinical disease and near-term cardiovascular events and may then be considered as a simple test for identifying high-risk type 2 diabetic patients. Similarly, Vik *et al.* [166] and Ziegler *et al.* [167] demonstrated the relationship between OPG serum levels and severity of artery disease.

The RANKL/OPG system exerts its activities simultaneously on endothelial cells and vascular smooth muscle-related cells. Thus, OPG promotes endothelial cell survival through neutralizing pro-apoptotic TRAIL [73, 168] and must be considered as an αvβ3-induced and NF-κB-dependent survival factor for endothelial cells whose survival depends on OPG induction by NF-κB [169, 170] (Fig. 2c). The OPG survival effect has also been demonstrated in pathological conditions such as periodontitis [171]. Indeed, OPG produced by microvascular endothelial cells controls endothelial cell survival and bone remodeling during this pathology [172]. Cross *et al.* [173] also reported a pro-angiogenic effect of OPG and correlated OPG expression by tumor endothelial cells with clinical data in human tumors. However, while the role of OPG in vascular pathogenesis is not fully understood, OPG may reflect a protective mechanism of endothelial cells and bone during aggressive inflammation. RANKL has also been implicated in endothelial cell metabolism, as it induces angiogenesis *in vitro* and *in viv*o, acts as a chemotactic factor for endothelial cells and induces their migration [174, 175]. RANKL also promotes endothelial proliferation and survival [175, 176], suggesting a potential implication in tumor development and increased vascular permeability [177] (Fig. 2c). OPG and RANKL activities in endothelial cells are tightly regulated by cytokines and growth factors present in the microenvironment of endothelial cells [18, 178, 179].

RANK signal transduction pathway as a potential therapeutic target

RANKL has been named for its ability to activate NF-kB which constitutes one of the early molecular events induced by the binding of RANKL to RANK [26, 180, 181] (Fig. 1d). Similar to the other TNFR family members, RANK activation first recruits the TNR receptor-associated factor (TRAF) adaptor proteins associated with the intracytoplasmic domain of RANK and implicated in its oligomerization. Furthermore, TRAF6 acts as a pivotal adaptor leading to specific gene expression regulating osteoclast differentiation and activation. The downstream targets of TRAF6 include transcription factors such NF-κB, activator protein-1 (AP-1) and nuclear factor of activated T cells (NFAT), the cascades of mitogen-activated protein kinases (MAPKs) such as p38 stress kinase, c-Jun N-terminal kinase (JNK), ERK and the PI3K/AKT pathways which involve the mammalian target of rapamycin (mTOR) [18, 181].

The OPG/RANK/RANKL molecular triad constitutes a strategic therapeutic target for bone diseases and associated disorders. Thus, several approaches based on recombinant molecules (OPG-Fc, RANK-Fc) or specific antibodies against RANKL have been successfully reported in benign and malignant pathologies [89, 182–184] (Fig. 3). Recently, Cheng *et al.* [186] envisaged another strategy based on an OPG-like peptidomimetic. This peptide has been designed to avoid its binding to TRAIL and showed an effective activity in preventing myeloma bone disease [187]. Peptidomimetics selectively inhibiting RANKL but not TRAIL activity thus constitute novel therapeutic approaches to treat tumor-associated osteolysis. Similarly, TNFR loop peptides have been developed and abolish RANKL-induced signaling, bone resorption and bone loss [188]. Such peptidomimetics that mimic either cytokine receptors such as TNFR [188] or a 'decoy receptor' such as OPG [184, 187] may present various advantages, mainly a reduced immunogenicity, a more targeted effect and multiple applications in which these molecules are implicated (e.g. inhibition of bone resorption and inflammation) [189].

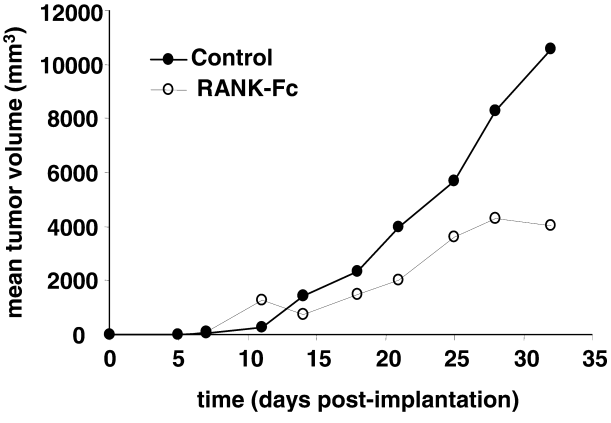

Figure 3. OPG/RANK/RANKL molecular pathway as a potential target for bone diseases. Sprague-Dawley rats with transplanted osteosarcoma [185] were treated or not with recombinant RANK-Fc (750 μg, once a week during 3 weeks). Representative mean tumor volumes of rats treated by RANK-Fc compared to the untreated control.

As another strategy to treat osteolysis relates to the blockade of specific signaling pathways currently activated by bone resorption effectors, the RANK signaling pathway has been identified as one of the

best approaches [190]. NF-κB plays a key role in RANK signaling and appears critical for osteoclastogenesis. This transcription factor is not necessary for the formation of osteoclast progenitors but is absolutely required for the differentiation steps of these progenitors [191, 192]. Furthermore, NF-κB bridges inflammation and bone homeostasis [193]. Indeed, NF-κB controls bone mass as demonstrated by the bone phenotype of knock-out mice [194] and contributes to the onset and progression of arthritis [195–198]. A role of NF-κB in osteolytic bone metastasis through granulocyte/macrophage colony-stimulating factor (GM-CSF) induction has also been very recently identified [199]. The authors identified the gene encoding GM-CSF as a target of NF-κB and showed that GM-CSF mediates osteolysis bone metastasis of breast cancer by stimulating osteoclastogenesis. In this respect, as NF-κB represents a potential target for the treatment of osteolysis of benign and malignant origin, therapeutic strategies based on inhibition of this transcription factor have recently emerged. Thus, Clohisy *et al.* [200] demonstrated that NF-κB signaling blockade abolished implant particle-induced osteoclastogenesis *in vitro*. They also demonstrated a significant decrease in bone erosion associated with inflammatory arthritis using dominant-negative IκB or mutated IκB proteins [201]. Similarly, synthetic double-stranded oligodeoxynucleotides demonstrated their efficacy in a mice model of intestinal carcinoma, acting as 'decoy' cis elements that block the binding of nuclear factors to promoter regions of targeted genes, resulting in the inhibition of gene transactivation *in vivo* as well as *in vitro* [202]. Penolazzi *et al.* [203, 204] developed peptide nucleic acid-DNA decoy chimeras targeting NF-κB which strongly induced osteoclast apoptosis and inhibited their differentiation. Umezawa's group chose the development of a synthetic NF-κB inhibitor [205] which abolishes osteoclast differentiation through down-regulation of NFATc1 [206], thus suggesting cross-talk between NFATc1 and NF-κB in RANKL-dependent osteoclastogenesis (Fig. 1d). TRAF6 actively participates in the signal transduction induced by the TNFR superfamily [18, 39] (Fig. 1) and thus represents a strategic therapeutic target of osteolytic pathologies. In this context, therapeutic use of antagonist peptides of RANK-TRAF6 interactions has been also envisaged [207].

Therapeutic targeting of OPG/RANK/RANKL interactions and signaling holds great promise for the treatment of malignant osteolysis (primary bone tumors, bone metastasis) and in inflammation-associated bone diseases (e.g. rheumatoid arthritis, prosthesis loosening). In the near future, additional studies are required to validate these therapies in pre-clinical models and to determine their clinical safety and efficacy.

Acknowledgements. This work was supported by the 'Comité des Pays de Loire de la Ligue Contre le Cancer'. M. Baud'huin received a fellowship from INSERM and The Région des Pays de la Loire. The authors wish to thank C. Bailly, M. N. Hervé and C. Le Corre from the Experimental Therapy Unit of IFR26 (Nantes, France) for their technical assistance.

1 Dempster, D. W. (2006) Anatomy and functions of the adult skeleton. In: Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism, edn 6 (Favus, M. J., Ed.), pp 7–11, American Society for Bone and Mineral Research, Durham.
2 Ross, F. P. (2006) Osteoclast biology and bone resorption. In: Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism, edn 6 (Favus, M. J., Ed.), pp. 30–35. American Society for Bone and Mineral Research, Durham.
3 Rousselle, A. V. and Heymann, D. (2002) Osteoclastic acidification during bone resorption. Bone 30, 533–540.
4 Aubin, J. E., Lian, J. B. and Stein, G. S. (2006) Bone formation maturation and functional activities of osteoblast lineage cells. In: Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism, edn 6 (Favus, M. J., Ed.), pp. 20–29, American Society for Bone and Mineral Research, Durham.
5 Favus, M. J., Bushinsky, D. A. and Lemann Jr, J. (2006) Regulation of calcium, magnesium, and phosphate metabolism. In: Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism, edn 6 (Favus, M. J., Ed.), pp. 76–83, American Society for Bone and Mineral Research, Durham.
6 Takahashi, N., Akatsu, T., Udagawa, N., Sasaki, T., Yamaguchi, A., Moseley, J. M., Martin, T. J. and Suda, T. (1988) Osteoblastic cells are involved in osteoclast formation. Endocrinology 123, 2600–2602.
7 Jimi, E., Nakamura, I., Amano, H., Taguchi, Y., Tsurukai, T., Tamura, M., Takahashi, N. and Suda, T. (1996) Osteoclast function is activated by osteoblastic cells through a mechanism involving cell-to-cell contact. Endocrinology 137, 2187–2190.
8 Friedman, P. A. and Goodman, W. G. (2006) PTH(1-84)/PTH(7-84): a balance of power. Am., J. Physiol. Renal Physiol. 290, F975–F984.
9 Holick, M. F. (2006) The role of vitamin D for bone health and fracture prevention. Curr. Osteoporos. Rep. 4, 96–102.
10 Findlay, D. M. and Sexton, P. M. (2004) Calcitonin. Growth Factors 22, 217–224.
11 Boyle, W. J., Simonet, W. S. and Lacey, D. L. (2003) Osteoclast differentiation and activation. Nature 423, 337–342.
12 Teitelbaum, S. L. (2007) Osteoclasts: what do they do and how do they do it? Am. J. Pathol. 170, 427–435.
13 Takayanagi, H., Sato, K., Takaoka, A. and Taniguchi, T. (2005) Interplay between interferon and other cytokine systems in bone metabolism. Immunol. Rev. 208, 181–193.
14 Tsuda, E., Goto, M., Michizuki, S., Yano, K., Kobayashi, F., Morinaga, T., Hisgashio, K. (1997) Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem. Biophys. Res. Commun. 234, 137–142.
15 Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, 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. and Boyle, W. J. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93, 165–176.

16 Yasuda, H., Shima, N., Nakagawa, N., Mochizuki, S. I., Yano, K., Fujise, N., Sato, Y., Goto, M., Yamaguchi, K., Kuriyama, M., Kanno, T., Murakami, A., Tsuda, E., Morinaga, T. and Higashio, K. (1998) Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis *in vitro*. Endocrinology 139, 1329–1237.
17 Blair, J. M., Zheng, Y., Dunstan, C. R. (in press) RANK ligand. Int. J. Biochem. Cell Biol.
18 Théoleyre, S., Wittrant, Y., Kwan Tat, S., Fortun, Y., Redini, F. and Heymann, D. (2004) The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev. 15, 457–475.
19 Kwan Tat, S., Padrines, M., Théoleyre, S., Heymann, D. and Fortun, Y. (2004) IL6, RANKL, TNF-alpha/IL1: interrelations in bone resorption pathology. Cytokine Growth Factor Rev. 15, 49–60.
20 Blair, J. M., Zhou, H., Seibel, M. J. and Dunstan, C. (2006) Mechanisms of disease: roles of OPG, RANKL and RANK in the pathophysiology of skeletal metastasis. Nat. Clin. Pract. Oncol. 3, 41–49.
21 Rogers, A. and Eastell, R. (2005) Circulating osteoprotegerin and receptor activator of nuclear factor κB ligand: clinical utility in metabolic bone disease assessment. J. Clin. Endocr. Metab. 90, 6323–6331.
22 Walsh, M. C., Kim, N., Kadono, Y., Rho, J., Lee, S. Y., Lorenzo, J. and Choi, Y. (2006) Osteoimmunology: interplay between the immune system and bone metabolism. Annu. Rev. Immunol. 24, 33–63.
23 Kiechl, S., Werner, P., Knoflach, M., Willeit, J. and Schett, G. (2006) The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease. Expert Rev. Cardiovasc. Ther. 4, 801–811.
24 Roodman, G. D. (1996) Advances in bone biology: the osteoclast. Endocr. Rev. 17, 308–332.
25 Abu-Amer, Y. (2005) Advances in osteoclast differentiation and function. Curr. Drug Targets Immune Endocr. Metab. Disord. 5, 347–355.
26 Roodman, G. D. (2006) Regulation of osteoclast differentiation. Ann. N. Y. Acad. Sci. 1068, 100–109.
27 Yasuda, H., Shima, N., Nakagawa, N., Mochizuki, S. I., Yano, K., Fujise, N., Sato, Y., Goto, M., Yamaguchi, K., Kuriyama, M., Kanno, T., Murakami, A., Tsuda, E., Morinaga, T. and Higashio, K. (1998) Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis *in vitro*. Endocrinology 139, 1329–1337.
28 Tsuda, E., Goto, M., Mochizuki, S., Yano, K., Kobayashi, F., Morinaga, T. and Higashio, K. (1997) Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem. Biophys. Res. Commun. 234, 137–142.
29 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., Pattison, W., Campbell, P., Sander, S., Van, G., Tarpley, J., Derby, P., Lee, R., Amgen EST Program and Boyle, W. J. (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89, 309–319.
30 Bucay, N., Sarosi, I., Dunstan, C. R., Morony, S., Tarpley, J., Capparelli, C., Scully, S., Tan, H. L., Xu, W., Lacey, D. L., Boyle, W. J. and Simonet, W. S. (1998) Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev. 12, 1260–1268.
31 Yun, T. J., Tallquist, M. D., Aicher, A., Rafferty, K. L., Marshall, A. J., Moon, J. J., Ewings, M. E., Mohaupt, M., Herring, S. W. and Clarck, E. A. (2001) Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function. J. Immunol. 166, 1482–1491.
32 Min, H., Morony, S., Sarosi, I., Dunstan, C. R., Capparelli, C., Scully, S., Van, G., Kaufman, S., Kostenuik, P. J., Lacey, D. L., Boyle, W. J. and Simonet, W. S. (2000) Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. J. Exp. Med. 192, 463–464.
33 Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, 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. and Boyle, W. J. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93, 165–176.
34 Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N. and 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.
35 Wong, B. R., Josien, R., Lee, S. Y., Sauter, B., Li, H. L., Steinman, R. M. and Choi, Y. (1997) TRANCE (tumor necrosis factor TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J. Exp. Med. 186, 2075–2080.
36 Anderson, D. M., Maraskovsky, E., Billingsley, W. L., Dougall, W. C., Tometsko, M. E., Roux, E. R., Teepe, M. C., DuRose, R. F., Cosman, D. and Galibert, L. (1997) A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390, 175–179.
37 Wong, B. H., Rho, J., Arron, J., Robinson, E., Orlinick, J., Chao, M., Kalachikov, S., Cayani, E., Barlett, F. S.3rd, Frankel, W. N., Lee, S. Y. and 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, 25190–2514.
38 Damay, B. G., Haridas, V., Ni, J., Moore, P. A. and 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 NF-kappab and c-Jun N-terminal kinase. J. Biol. Chem. 273, 20551–20555.
39 Wong, B. R., Josien, R., Lee, S. Y., Vologodskaia, M., Steinman, R. M. and Choi, Y. (1998) The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. J. Biol. Chem. 273, 28355–28359.
40 Kong, Y. Y., Feige, U., Sarosi, I., Bolon, B., Tafuri, A., Morony, S., Capprelli, 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. and Penninger, J. M. (1999) Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402, 304–309.
41 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. and Penninger, J. M. (2000) The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell 103, 41–50.
42 Burgess, T. L., Qian, Y., Kaufman, Ring, B. D., Van, G., Capparelli, M., Kelley, M., Hsu, H., Boyle, W. J., Dunstan, C., Hsu, S. and Lacey, D. L. (1999) The ligang for osteoprotegerin (OPGL) directly activates mature osteoclasts. J. Cell Biol. 145, 527–238.
43 Wittrant, Y., Théoleyre, S., Couillaud, S., Dunstan, C., Heymann, D. and Rédini, F. (2004) Relevance of *in vitro* osteoclastogenesis system to study receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) biological activities? Exp. Cell Res. 293, 292–301.
44 Wittrant, Y., Théoleyre, S., Couillaud, S., Dunstan, C., Heymann, D. and Rédini, F. (2003) Regulation of osteoclast

protease expression by RANKL. Biochem. Biophys. Res. Commun. 310, 774–778.
45 Théoleyre, S., Wittrant, Y., Couillaud, S., Vusio, P., Berreur, M., Dunstan, C., Blanchard, F., Rédini, F. and Heymann, D. (2004) Cellular activity and signalling induced by osteoprotegerin in osteoclasts: involvement of receptor activator of NF-κB ligand and MAPK. Biochim. Biophys. Acta 1644, 1–7.
46 Kwan Tat, S., Padrines, M., Théoleyre, S., Bataglia, S., Heymann, D., Redini, F. and Fortun, Y. (2006) OPG/membranous-RANKL complex is internalized *via* the clathrin pathway before a lysosomal and a proteasomal degradation. Bone 39, 706–715.
47 Nakamichi, Y., Udagawa, N., Kobayashi, Y., Nakamura, M., Yamamoto, Y., Yamashita, T., Mizoguchi, T., Sato, M., Mogi, M., Penninger, J. M. and Takahashi, N. (2007) Osteoprotegerin reduces the serum level of receptor activator of NF-KB ligand derived from osteoblasts. J. Immunol. 178, 192–200.
48 Ikeda, T., Kasai, M., Utsuyama, M. and Hirokawa, K. (2001) Determination of three isoforms of the receptor activator of nuclear factor-κB ligand and their differential expression in bone and thymus. Endocrinology 142, 1418–1426.
49 Suzuki, J., Ikeda, T., Kuroyama, H., Seki, S., Kasai, M., Utsuyama, M., Tatsumi, M., Uematsu, H. and Hirokawa, K. (2004) Regulation of osteoclastogenesis by the three human RANKL isoforms expressed in NIH3T3 cells. Biochem. Biophys. Res. Commun. 314, 1021–1027.
50 Lum, L., Wong, B. R., Josien, R., Becherer, J. D., Erdjument-Bromage, H., Schlondorff, J., Temps, P., Choi, Y. and Blodel, C. P. (1999) Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. J. Biol. Chem. 274, 13613–13618.
51 Boissy, P., Lenhard, T. R., Kirgegaard, T., Peschon, J. J., Black, R. A., Delaisse, J. M. and del Carmen Ovejero, M. (2003) An assessment of ADAMs in bone cells: absence of TACE activity prevents osteoclast recruitment and the formation of the marrow cavity in developing long bone. FEBS Lett. 553, 257–261.
52 Hikita, A., Yana,, I., Wakeyama, H., Nakamura, M., Kadono, Y., Oshima, Y., Nakamura, K., Seiki, M. and Tanaka, S. (2006) Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. J. Biol. Chem. 281, 36846–36855.
53 Chesneau, V. (2001) Catalytic properties of ADAM19. J. Biol. Chem. 278, 22331–22340.
54 Lynch, C. C., Kikosaka, A., Acuff, H. B., Martin, M. D., Kawai, N., Singh, R. K., Varfo-Gogola, T. C., Begtrup, J. L., Peterson, T. E., Fingleton, B., Shirai, T., Matrisian, L. M. and Futakuchi, M. (2005) MMP-7 promotes prostate cancer-induced osteolysis via the solubilisation of RANKL. Cancer Cell 7, 485–496.
55 Ikeda, T., Kasai, M., Kuroyama, H., Seki, S., Utsuyama, M., Hirokawa, K. (2003) Multimerisation of the RANKL isoforms and regulation of osteoclastogenesis. J. Biol. Chem. 278, 47217–47222.
56 Nakagawa, N., Kinosaki, M., Yamaguchi, K., Shima, N., Yasuda, H., Yano, K., Morinaga, T. and Higashio, K. (1998) RANK is essential signalling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem. Biophys. Res. Commun. 253, 396–400.
57 Iwamoto, K., Miyamoto, T., Sawatani, Y., Hosogane, N., Hamaguchi, I., Takami, M., Nomiyama, K., Takagi, K. and Suda, T. (2004) Dimer formation of receptor activator of nuclear factor κB induces incomplete osteoclast formation. Biochem. Biophys. Res. Commun. 325, 229–234.
58 Ito, S., Wakabayashi, K., Ubukota, O., Hayashi, S., Okada, F. and Hata, T. (2002) Crystal structure of the extracellular domain of mouse RANK ligand at 2.2-A resolution. J. Biol. Chem. 277, 6631–6636.
59 Kanazawa, K. and Kudo, A. (2005) Self-assembled RANK induces osteoclastogenesis ligand-independently. J. Bone Miner. Res. 20, 2053–2060.
60 Chan, F. K., Chun, H. J., Zheng, L., Siegel, R. M., Bui, K. L. and Lenardo, M. J. (2000) A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science 288, 2351–2354.
61 Yamaguchi, K., Kinosaki, M., Goto, M., Tsuda, E., Morinaga, T. and Higashio, K. (1998) Characterization of structural domains of human osteoclastogenesis inhibitory factor. J. Biol. Chem. 273, 5117–5123.
62 Takahashi, N., Udagawa, N. and Suda, T. (1999) A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. Biochem. Biophys. Res. Commun. 256, 449–455.
63 Schneeweis, L. A., Willard, D. and Milla, M. E. (2005) Functional dissection of osteoprotegerin and its interaction with RANKL. J. Biol. Chem. 280, 41155–41164.
64 Mosheimer, B. A., Kaneider, N. C., Feistritzer, C., Djanani, A. M., Sturn, D. H., Patsch, J. R. and Wiedermann, C. J. (2005) Syndecan-1 is involved in osteoprotegerin-induced chemotaxis in human peripheral blood monocytes. J. Clin. Endocrinol. Metab. 90, 2964–2971.
65 Standal, T., Seidel, C., Hjertner,, O., Plesner, T., Sanderson, R. D., Waage, A., Borset, M. and Sundan, A. (2002) Osteoprotegerin is bound, internalized, and degraded by multiple myeloma cells. Blood 100, 3002–3007.
66 Théoleyre, S., Kwan Tat, S., Vusio, P., Blanchard, F., Gallagher, J., Ricard-Blum, S., Fortun, Y., Padrines, M., Rédini, F. and Heymann, D. (2006) Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: role in the interactions with receptor activator of nuclear factor κB ligand (RANKL) and RANK. Biochem. Biophys. Res. Commun. 347, 460–467
67 Stringer, S. E., Forster, M. J., Mulloy, B., Bishop, C. R., Graham, G. J. and Gallagher, J. T. (2002) Characterization of the binding site on heparan sulfate for macrophage inflammatory protein 1α. Blood 100, 1543–1550.
68 Fox, S. W. and Lovibond, A. C. (2005) Current insights into the role of transforming growth factor-beta in bone resorption. Mol. Cell Endocrinol. 243, 19–26.
69 Shinmyouzu, K., Takahashi, T., Ariyoshi, W., Ischimiya, H., Kanzaki, S. and Nishihara, T. (2007) Dermatan sulphate inhibits osteoclast formation by binding to receptor activator of NF-κB ligand. Biochem. Biophys. Res. Commun. 354, 447–452.
70 Bishof, D., Elsawa, S. F., Mantchev, G., Yoon, J., Michels, G. E., Nilson, A., Sutor, S. L., Platt, J. L., Ansell, S. M., von Bulow, G. and Bram, R. J. (2006) Selective activation of TACI by syndecan-2. Blood 107, 3235–3242.
71 Sakurai, D., Hase, H., Kanno, Y., Kojima, H., Okumura, K. and Kobata, T. (2007) TACI regulates Ig1 production by APRIL in collaboration with HSPG. Blood 109, 2961–2967.
72 Hendriks, J., Planelles, L., de Jong-Odding, J., Hardenberg, G., Pals, S. T., Hahne, M., Spaargaren, M. and Medema, J. P. (2005) Heparan sulfate proteoglycan binding promotes APRIL-induced tumor cell proliferation. Cell Death Differ. 12: 637–648.
73 Emery, J. G., McDonnell, P., Burke, M. B., Deen, L. C., Lyn, S., Silverman, C., Dul, E., Appelbaum, E. R., Eicman, C., DiPrinzio, R., Dodds, R. A., James, I. E., Rosenberg, M., Lee, J. C. and YounG, P. R. (1998) Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J. Biol. Chem. 273, 14363–14367.
74 Cross, S. S., Harrison, R. F., Balasubramanian, S. P., Lippitt, J. M., Evans, C. A., Reed, M. W. and Holen, I. (2006) Expression of receptor activator of nuclear factor kappabeta ligand (RANKL) and tumour necrosis factor related, apoptosis inducing ligand (TRAIL) in breast cancer, and their relations with osteoprotegerin, oestrogen receptor, and clinicopathological variables. J. Clin. Pathol. 59, 716–720.
75 Holen, I. and Shipman, C. M. (2006) Role of osteoprotegerin (OPG) in cancer.
Clin. Sci. 110, 279–291.

76 Sandra, F., Hendarmin, L. and Nakamura, S. (2006) Osteoprotegerin (OPG) binds with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL): suppression of TRAIL-induced apoptosis in ameloblastomas. Oral Oncol. 42, 415–420.
77 Van Poznak, C., Cross, S. S., Saggese, M., Hudis, C., Panageas, K. S., Norton, L., Coleman, R. E. and Holen, I. (2006) 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. Pathol. 59, 56–63.
78 Neville-Webbe, H. L., Cross, N. A., Eaton, C. L., Nyambo, R., Evans, C. A., Coleman, R. E. and Holen, I. (2004) Osteoprotegerin (OPG) produced by bone marrow stromal cells protects breast cancer cells from TRAIL-induced apoptosis. Breast Cancer Res. Treat. 86, 269–279.
79 Nyambo, R., Cross, N., Lippitt, J., Holen, I., Bryden, G., Hamdy, F. C. and Eaton, C. L. (2004) Human bone marrow stromal cells protect prostate cancer cells from TRAIL-induced apoptosis. J. Bone Miner. Res. 19, 1712–1721.
80 Miyashita, T., Kawakami, A., Nakashima, T., Yamasaki, S., Tamai, M., Tanaka, F., Kamachi, M., Ida, H., Migita, K., Origuchi, T., Nakao, K. and Eguchi, K. (2004) Osteoprotegerin (OPG) acts as an endogenous decoy receptor in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis of fibroblast-like synovial cells. Clin. Exp. Immunol. 137, 430–436.
81 Atkins, G., J., Bouralexis, S., Evdokiou, A., Hays, S., Labrinidis, A., Zannettino, A. C., Haynes, D. R. and Findlay, D. M. (2002) Human osteoblasts are resistant to Apo2L/TRAIL-mediated apoptosis. Bone 31, 448–456.
82 Holen, I., Croucher, P. I., Hamdy, F. C. and Eaton, C. L. (2002) Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells. Cancer Res. 62, 1619–1623.
83 Kaifu, T., Nakahara, J., Inui, M., Mishima, K., Momiyama, T., Kaji, M., Sugahara, A., Koito, H., Ujike-Asai, A., Nakamura, A., Kanazawa, K., Tan-Takeuchi, K., Iwasaki, K., Yokoyama, W. M., Kudo, A., Fujiwara, M., Asou, H. and Takai, T. (2003) Osteopetrosis and thalamic hypomyelinosis with synaptic degeneration in DAP12-deficient mice. J. Clin. Invest. 111, 323–332.
84 Koga, T., Inui, M., Inoue, K., Kim, S., Suematsu, A., Kobayashi, E., Iwata, T., Ohnishi, H., Matozaki, T., Kodama, T., Taniguchi, T., Takayanagi, H. and Takai, T. (2004) Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. Nature 428, 758–763.
85 Mocsai, A., Humphrey, M. B., Van Ziffle, J. A., Hu, Y., Burghardt, A., Spusta, S. C., Majumdar, S., Lanier, L.L and Lowell, C. A. (2004) The immunomodulatory adapter proteins DAP12 and Fc receptor gamma-chain (FcRgamma) regulate development of functional osteoclasts through the Syk tyrosine kinase. Proc. Natl. Acad. Sci., U.S.A. 101, 6158–6163.
86 Humphrey, M. B., Lanier, L. L. and Nakamura, M. C. (2005) Role of ITAM-containing proteins and their receptors in the immune system and bone. Immunol. Rev. 208, 50–65.
87 Humphrey, M. B., Ogasawara, K., Yao, W., Spusta, S. C., Daws, M. R., Lane, N. E., Lanier, L. L. and Nakamura, M. C. (2004) The signalling adapter protein DAP12 regulates multinucleation during osteoclast development. J. Bone Miner. Res. 19, 224–234.
88 Humphrey, M. B., Daws, M. R., Spusta, S. C., Torchia, J. A., Lanier, L. L., Seaman, W. E. and Nakamura, M. C. (2006) TREM2, a DAP12-associated receptor, regulates osteoclast differentiation and function. J. Bone Miner. Res. 21, 237–245.
89 Wittrant, Y., Théoleyre, S., Chipoy, C., Padrines, M., Blanchard, F., Heymann, D. and Rédini, F. (2004) RANKL/RANK/OPG: new therapeutic targets in bone tumors and associated osteolysis. Biochim. Biophys. Acta 1704, 49–57
90 Dougall, W. C. and Chaisson, M. (2006) The RANKL/RANK/OPG triad in cancer-induced bone diseases. Cancer Metastasis Rev. 25, 541–549.
91 Jones, D. H., Nakashima T., Sanchez, O. H., Kozieradzki I., Komarova, S. V., Sarosi I., Morony S., Rubin E., Sarao R., Hojilla, C.V., Komnenovic V., Kong, Y. Y., Schreiber M., Dixon, S. J., Sims, S. M., Khokha R., Wada T. and Penninger, J. M. (2006) Regulation of cancer cell migration and bone metastasis by RANKL. Nature 440: 692–696.
92 Mori K, Le Goff, B., Charrier, C., Battaglia, S., Heymann, D. and Rédini, F. (2007) DU145 human prostate cancer cells express functional receptor activator of NFκB: new insights in the prostate cancer bone metastasis process. Bone 40, 981–990.
93 Tometsko, M., Armstrong, A., Miller, R., Jones, J., Chaisson, M., Branstetter, D. and Dougall, W. (2004) RANK ligand directly induces osteoclastogenic, angiogenic, chemoattractive and invasive factors on RANK-expressing human cancer cells MDA-MB-231 and PC3. J. Bone Miner. Res. 19: S25
94 Martin, T. J. and Mundy, G. R. (2007) Bone metastasis: can osteoclasts be excluded? Nature 445, E19.
95 Jones, D.H., Nakashima T., Sanchez, O.H., Kozieradzki I., Komarova, S.V., Sarosi I., Morony S., Rubin E., Sarao R., Hojilla, C.V., Komnenovic V., Kong, Y.Y., Schreiber M., Dixon, S.J., Sims, S.M., Khokha R., Wada T. and Penninger, J.M. (2007) Jones et al. reply. Nature 445, E19-E20.
96 Brown J. M., Corey, E., Lee, Z. D., True, L. D., Yun, T. J., Tondravi, M. and Vessella, R. L. (2001) Osteoprotegerin and RANK ligand expression in prostate cancer. Urology 57: 611–616.
97 Zhang J., Dai J., Qi Y., Lin, D.L., Smith P., Strayhorn C., Mizokami A., Fu Z., Westman J. and Keller, E.T. (2001) Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. J. Clin. Invest. 107, 1235–1244.
98 Zhang, J., Dai, J., Yao, Z., Lu, Y., Dougall, W. and Keller, E. T. (2003) Soluble receptor activator of nuclear factor κB Fc diminishes prostate cancer progression in bone. Cancer Res. 63, 7883–7390.
99 Corey E., Brown, L.G., Kiefer, J.A., Quinn, J.E., Pitts, T.E., Blair, J.M. and Vessella, R.L. (2005) Osteoprotegerin in prostate cancer bone metastasis. Cancer Res. 65, 1710–1718.
100 Whang, P.G., Schwarz, E.M., Gamradt, S.C., Dougall, W.C. and Lieberman, J.R. (2005) The effects of RANK blockade and osteoclast depletion in a model of pure osteoblastic prostate cancer metastasis in bone. J. Orthop. Res. 23: 1475–1483.
101 Wittrant, Y., Mori, K., Riet, A., Kamijo, A., Heymann, D. and Rédini F (2006) RANKL directly induces bone morphogenetic protein-2 expression in RANK-expression POS-1 osteosarcoma cells. Int. J. Oncol. 28, 261–269
102 Mori, K., Berreur, M., Le Goff, B., Riet, A., Moreau, A., Blanchard, F., Chevalier, C., Guisle-Marsollier, I., Léger, J., Gouin, F., Rédini, F. and Heymann, D. (2007) Human osteosarcoma cells express functional receptor activator of nuclear factor-kappa, B. J. Pathol. 211, 555–562.
103 Pearse, R. N., Sordillo, E. M., Yaccoby, S., Wong, B. R., Liau, D. F., Colman, N., Michaeli, J., Epstein, J. and Choi, Y. (2001) Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc. Natl. Acad. Sci. USA 98, 1581–1586.
104 Vanderkerken, K., De Leenheer, E., Shipman, C., Asosingh, K., Willems, A., Van Camp, B. and Croucher, P. (2003) Recombinant osteoprotegerin decreases tumor burden and increases survival in a murine model of multiple myeloma. Cancer Res. 63, 287–289.
105 Brown, J. M., Vessella, R. L., Kostenuik, P. J., Dunstan, C. R., Lange, P. H. and Corey, E. (2001) Serum osteoprotegerin levels are increased in patients with advanced prostate cancer. Clin. Cancer Res. 7, 2977–2983.
106 Grimaud, E., Soubigou, L., Couillaud, S., Coipeau, P., Moreau, A., Passuti, N., Gouin, F., Rédini, F. and Heymann,

D. (2003) Receptor activator of NF-kB ligand (RANKL)/ osteoprotegerin (OPG) ratio is increased in severe osteolysis. Am. J. Pathol. 163, 2021–2031.
107 Giuliani, N., Bataille, R., Mancini, C., Lazzaretti, M. and Barille, S. (2001) Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. Blood 98, 3527–3533.
108 Voskaridou, E. and Terpos E (2005) Osteoprotegerin to soluble receptor activator of nuclear factor kappa-B ligand ratio is reduced in patients with thalassaemia-related osteoporosis who receive vitamin D3. Eur. J. Haematol. 74, 359–361.
109 Alvarez, L., Peris, P., Guanabens, N., Vidal, S., Ros, I., Pons, F., Filella, X., Monegal, A., Munoz-Gomez, J. and Ballesta, A. M. (2003) Serum osteoprotegerin and its ligand in Paget's disease of bone: relationship to disease activity and effect of treatment with bisphosphonates. Arthritis Rheum. 48, 824–828.
110 Dobnig, H., Hofbauer, L. C., Viereck, C., Obermayer-Pietsch, B. and Fahrleitner-Pammer A . (2006) Changes in the RANK ligand/osteoprotegerin system are correlated to changes in bone mineral density in bisphosphonate-treated osteoporotic patients. Osteoporosis Int. 17, 693–703.
111 Chen, G., Sircar, K., Aprikian, A., Potti, A., Goltzman, D. and Rabbani SA (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.
112 Milkosch, P., Igerc, I., Kidlacek, S., Woloszczuk, W., Gallowithsch, H. J., Kresnik, E., Stettner, H., Grimm, G., Lind, P. and Pietschmann, P. (2006) Receptor activator of nuclear factor kappaB ligand and osteoprotegerin in men with thyroid cancer. Eur. J. Clin. Invest. 35, 566–573.
113 Granchi, D., Garaventa, A., Amato, I., Paolucci, P. and Baldini, N. (2006) Plasma levels of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in patients with neuroblastoma. Int. J. Cancer. 119, 146–151.
114 Terpos, E., Szydlo, R., Apperley, J. F., Hatjiharissi, E., Politou, M., Meletis, J., Viniou, N., Yataganas, X., Goldman, J. M. and Rahemtulla, A. (2003) Soluble receptor activator of nuclear factor kappaB ligand-osteoprotegerin ratio predicts survival in multiple myeloma: proposal for a novel prognostic index. Blood 102, 1064–1069.
115 D'Amore, M., Fanelli, M., D'Amore, S., Fontana, A. and Minenna, G. (2006) Receptor activator of NF(kappa)B ligand/osteoprotegerin (RANKL/OPG) system and osteopontin (OPN) serum levels in a population of Apulian postmenopausal women. Panminerva Med. 48, 215–221.
116 Kim, H. R., Kim, H. Y. and Lee, S. H. (2006) Elevated serum levels of soluble receptor activator of nuclear factors-kappaB ligand (sRANKL) and reduced bone mineral density in patients with ankylosing spondylitis (AS). Rheumatology 45, 1197–1200.
117 Vanderborght, A., Linsen, L., Thewissen, M., Geusens, P., Raus, J. and Stinissen, P. (2004) Osteoprotegerin and receptor activator of nuclear factor-kappaB ligand mRNA expression in patients with rheumatoid arthritis and healthy controls. J. Rheumatol. 31, 1483–1490.
118 Veigl, D., Niederlova, J. and Krystufkova, O. (in press). Periprosthetic osteolysis and its association with the molecule RANKL expression. Physiol. Res.
119 Granchi, D., Pellacani, A., Spina, M., Cenni, E., Savarino, L. M., Baldini, N. and Giunti, A. (2006) Serum levels of osteoprotegerin and receptor activator of nuclear factor-kappaB ligand as markers of periprosthetic osteolysis. J. Bone Joint Surg. Am. 88, 1501–1509.
120 Abdallah, B. M., Stilgren, L. S., Nissen, N., Kassem, M., Jorgensen, H. R. and Abrahamsen, B. (2005) Increased RANKL/OPG mRNA ratio in iliac bone biopsies from women with hip fractures. Calcif. Tissue Int. 76, 90–97.
121 Martini, G., Gennari, L., Merlotti , D., Salvadori, S., Franci, M. B., Campagna, S., Avanzati, A., De Paola, V., Vallegi, F. and Nuti R (2007). Serum OPG and RANKL levels before and after intravenous bisphosphonate treatment in Paget's disease of bone. Bone 40, 457–463.
122 Terpos, E., Politou, M., Szydlo, R., Nadal, E., Avery, S., Olavarria, E., Kanfer, E., Goldman, J. M., Apperley, J. F. and Rahemtulla, A. (2004). Autologous stem cell transplantation normalizes abnormal bone remodeling and sRANKL/osteoprotegerin ratio in patients with multiple myeloma. Leukemia 18, 1420–1426.
123 Di Carlo, C., Tommaselli, G. A., Gargano, V., Sammartino, A., Bifulco, G., Tauchmanova, L., Colao, A. and Nappi, C. (2007) Effects of estrogen-progestin therapy on serum levels of RANKL, osteoprotegerin, osteocalcin, leptin, and ghrelin in postmenopausal women. Menopause 14, 38–44.
124 Dovio, A., Data, V. and Angeli, A. (2005) Circulating osteoprotegerin and soluble RANKL: do they have a future in clinical practice? J. Endocrinol. Invest. 28, 14–22.
125 Humphrey, E. L. Williams, J. H. H., Davie, M. W. J. and Marshall, M. J. (2006) Effects of dissociated glucocorticoids on OPG and RANKL in osteoblastic cells. Bone 38, 652–661.
126 Palmqvist, P., Lundberg, P., Persson, E., Johansson, A., Lundgren, I., Lie, A., Conaway, H. H. and Lerner, U. H. (2005) Inhibition of hormone and cytokine stimulated osteoclastogenesis and bone resorption by interleukin-4 and interleukin-13 is associated with increased OPG and decreased RANKL and RANK in a STAT6 dependent pathway. J. Biol. Chem. 281, 2414–2419.
127 Kaji, H., Kanatani, M., Sugimoto, T. and Chihara, K. (2005) Statins modulate the levels of osteoprotegerin/receptor activator activatot of NFkappa B ligand mRNA in mouse bone-cell cultures. Horm. Metab. Res. 37, 589–592.
128 Li, Y., Kucuk, O., Hussain, M., Abrams, J., Cher, M. L. and Sarkar, F. H. (2006) Antitumor and antimetastatic activities of docetaxel are enhanced by genistein through regulation of osteoprotegerin/receptor activator of nuclear factor kappaB (RANK)/RANK ligand/MMP-9 signaling in prostate cancer. Cancer Res. 66, 4816–4825.
129 Kiviranta, R., Morko, J., Alatalo, S. L., NicAmhlaoibh, R., Risteli, J., Laitala-Leinonen, T. and Vuorio, E. (2005) Impaired bone resorption in cathepsin K-deficient mice is partially compensated for by enhanced osteoclastogenesis and increased expression of other proteases via an increased RANKL/OPG ratio. Bone 36, 159–172.
130 Li, J., Sarosi, I., Yan, X. Q., Morony, S., Capparelli, C., Tan, H. L., McCabe, S., Elliott, R., Scully, S., Van, G., Kaufman, S., Juan, S. C., Sun, Y., Tarpley, J., Martin, L., Christensen, K., McCabe, J., Kostenuik, P., Hsu, H., Fletcher, F., Dunstan, C. R., Lacey, D. L. and 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.
131 Lee, H. W., Kim, B. S., Kim, H. J., Lee, C. W., Yoo, H. J., Kim, J. B. and Yoon, S. (2005) Upregulation of receptor activator of nuclear factor-kappaB ligand expression in the thymic subcapsular, paraseptal, perivascular, and medullary epithelial cells during thymus regeneration. Histochem. Cell Biol. 123, 491–500.
132 Walsh, M. C., Kim, N., Kadono, Y., Rho, J., Lee, S. Y., Lorenzo, J. and Choi, Y. (2006) Osteoimmunology: interplay between the immune system and bone metabolism. Annu. Rev. Immunol. 24, 33–63.
133 Mosheimer, B. A., Kaneider, N. C., Feistritzer, C., Sturn, D. H. and Wiedermann, C. J. (2004) Expression and function of RANK in human monocyte chemotaxis. Arthritis Rheum. 50, 2309–2316.
134 Breuil, V., Schmid-Antomarchi, H., Schmid-Alliana, A., Rezzonico, R., Euller-Ziegler, L. and Rossi, B. (2003) The receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) is a new chemotactic factor for human monocytes. FASEB J. 17, 1751–1753.
135 Henrisksen, K., Karsdal, M., Delaisse, J. M. and Engsig, M. T. (2003) RANKL and vascular endothelial growth factor

(VEGF) induce osteoclast chemotaxis through an ERK1/2-dependent mechanism. J. Biol. Chem. 278, 48745–48753.
136 Lee, S. K. and Lorenzo, J. (2006) Cytokines regulating osteoclast formation and function. Curr. Opin. Rheumatol. 18, 411–418.
137 Kwak, H. B., Lee, S. W., Jin, H. M., Ha, H., Lee, S. H., Takeshita, S., Tanaka, S., Kim, H. M., Kim, H. H. and Lee, Z. H. (2005) Monokine induced by interferon-gamma is induced by receptor activator of nuclear factor kappa B ligand and is involved in osteoclast adhesion and migration. Blood 105, 2963–2969.
138 Nakamura, E. S., Koizumi, K., Kobayashi, M., Saitoh, Y., Arita, Y., Nakayama, Y., Sakurai, H., Yoshie, O. and Saiki, I. (2006) RANKL-induced CCL22/macrophage-derived chemokine produced from osteoclasts potentially promotes the bone metastasis of lung cancer expressing its receptor CCR4. Clin. Exp. Metastasis. 23, 9–18.
139 Kim, M. S., Day, C. J., and Morrison, N. A. (2005) MCP-1 is induced by receptor activator of nuclear factor-{kappa}B ligand, promotes human osteoclast fusion, and rescues granulocyte macrophage colony-stimulating factor suppression of osteoclast formation J. Biol. Chem. 280, 16163–16169.
140 Secchiero, P., Corallini, F., Barbarotto, E., Melloni, E., di Iasio, M. G., Tiribellu, M. and Zauli, G. (2006) Role of the RANKL/RANK system in the induction of interleukin-8 (IL-8) in B chronic lymphocytic leukemia (B-CLL) cells. J. Cell Physiol. 207, 158–164.
141 Terpos, E., Politou, M., Viniou, N. and Rahemtulla, A. (2005) Significance of macrophage inflammatory protein-1 alpha (MIP-1alpha) in multiple myeloma. Leuk. Lymphoma 46, 1699–1707.
142 Bendre, M., Gaddy, D., Nicholas, R. W. and Suva, L. J. (2003) Breast cancer metastasis to bone: it is not all about PTHrP. Clin. Orthop. Relat. Res. 415 Suppl: S39–S45.
143 Seshasayee, D., Wang, H., Lee, W. P., Gribling, P., Ross, J., Wan Bruggen, 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.
144 Park, H. J., Park, O. J. and Shin, J. (2005) Receptor activator of NF-kappaB ligand enhances the activity of macrophages as antigen presenting cell. Exp. Mol. Med. 37, 524–532.
145 Ali, A. S., Lax, A.S, Lijestrom, M., Paakkari, I., Ashammakhi, N., Kovanen, P. T. and Konttinen, Y. T. (2006) Mast cells in atherosclerosis as a source of the cytokine RANKL. Clin. Chem. Lab. Med. 44, 672–674.
146 Maitz, P., Kandler, B., Fischer, M. B., Watzek, G. and Gruber, R. (2006) Activated platelets retain their potential to induce osteoclast-like cell formation in murine bone marrow cultures. Platelets 17, 477–483
147 Kawai, T., Matsuyama, T., Hosokawa, Y., Makihira, S., Ski, M., Karimbux, N. Y., Gooncalves, N. Y., Goncalves, R. B., Valcerde, P., Dibart, S., Li, Y. P., Miranda, L. A., Ernst, C. W., Izumi, Y. and Taubman, M. A. (2006) B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. Am. J. Pathol. 169, 987–998.
148 Kotake, S., Udagawa,, N., Hakoda, M., Mogi, M., Yano, K., Tsuda, E., Takahashi, K., Furuya, T., Ishiyama, S., Kim, K. J., Saito, S., Nishikawa, N., Togari, A., Tomastsu, T., Suda, T. and Kamatani, N. (2001) Activated human T cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone destruction in rheumatoid arthritis patients. Arthritis Rheum. 44, 1003–1012.
149 Schett, G., Hayer, S., Zwerina, J., Redlich, K., and Smolen, J. S. (2005) Mechanisms of disease: the link between RANKL and arthritic bone disease. Nat. Clin. Pract. Rheumatol. 1, 47–54.
150 Masui, T., Sakano, S., Hasegawa, Y., Warashina, H. and Ishiguro, N. (2005) Expression of inflammatory cytokines, RANKL and OPG induced by titanium, cobalt-chromium and polyethylene particles. Biomaterials 26, 1695–1702.
151 Maruyama, K., Takada, Y., Ray, N., Kishimoto, Y., Penninger, J. M., Yasuda, H. and Matsuo, K. (2006) Receptor activator of NF-kappa B ligand and osteoprotegerin regulate proinflammatory cytokine production in mice. J. Immunol. 177, 3799–3805.
152 Ashcroft, A. J., Cruickshank, S. M., Croucher, P. I., Perry, M. J., Rollinson, S., Lippit, J. M., Child, J. A., Dunstan, C., Felsburg, P. J., Morgan, G. J. and Carding, S. R. (2003) Colonic dendritic cells, intestinal inflammation, and T cell-mediated bone destruction are modulated by recombinant osteoprotegerin. Immunity19, 849–861.
153 Green, E. A., Choi, Y. and Flavell, R. A. (2002) Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. Immunity 16, 183–191.
154 Loser, K., Mehling, A., Loeser, S., Apelt, J., Kuhn, A., Grabbe, S., Schwarz, T., Penninger, J. M. and Beissert, S. (2006) Epidermal RANKL controls regulatory T-cell numbers via activation of dentritic cells. Nat. Med. 12, 1372–1379.
155 Schoppet, M., Henser, S., Ruppert, V., Stubig, T., Al-Fakhri, N., Maisch, B. and Hofbauer, L. C. (2007) Osteoprotegerin expression in dendritic cells increases with maturation and is NF-kappaB-dependent. J. Cell. Biochem. 100, 1430—1439.
156 Yun, T. J., Tallquist, M. D., Aicher, A., Rafferty, K. L., Marshall, A. J., Moon, J. J., Ewings, M. E., Mohaupt, M., Herring, S. W. and Clark, E. A. (2001) Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function. J. Immunol. 166, 1482–1491.
157 Grevic, D. V., Lukic, K., Kovacic, N., Ivcevic, S., Katavic, V. and Marusic, A. (2006) Activated T lymphocytes suppress osteoclastogenesis by diverting early monocyte/macrophage progenitor lineage commitment towards dendritic cell differentiation through down-regulation of receptor activator of nuclear factor-kappaB and c-Fos. Clin. Exp. Immunol. 146, 46–58.
158 Zannettino, A. C., Holding, C. A., Diamond, P., Atkins, G. J., Kostakis, P., Farrugia, A., Gamble, J., To, L. B., Findlay, D. M., Haynes, D. R. (2005) Osteoprotegerin (OPG) is localized to the Weibel-Palade bodies of human vascular endothelial cells and is physically associated with von Willebrand factor. J. Cell Physiol. 204, 714–723.
159 Collin-Osdoby, P. (2004) Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. Circ. Res. 95, 1046–1057.
160 Collin-Osdoby, P., Rothe, L., Anderson, F., Nelson, M., Maloney, W. and Osdoby, P. (2001) Receptor activator of NF-kappa B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. J. Biol. Chem. 276, 20659–20672.
161 Kaden, J. J., Bickelhaupt, S., Grobholz, R., Haase, K. K., Sarikoc, A., Kilic, R., Brueckmann, M., Lang, S., Zahn, I., Vahl, C., Hagl, S., Dempfle, C. E. and Borggrefe, M. (2004) Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulate aortic valve calcification. J. Mol. Cell Cardiol. 36, 57–66.
162 Kindle, L., Rothe, L., Kriss, M., Osdoby, P. and Collin-Osdoby, P. (2006) Human microvascular endothelial cell activation by IL-1 and TNF-alpha stimulates the adhesion and transendothelial migration of circulating human CD14+ monocytes that develop with RANKL into functional osteoclasts. J. Bone Miner. Res. 21, 193–206.
163 Olesen, P., Ledet, T. and Rasmussen, L. M. (2005) Arterial osteoprotegerin: increased amounts in diabetes and modifiable synthesis from vascular smooth muscle cells by insulin and TNF-alpha. Diabetologia 48, 561–568.
164 Olesen, P., Nguyen, K., Wogensen, L., Ledet, T. and Rasmussen, L. M. (2007) Calcification of human vascular smooth muscle cells: associations with osteoprotegerin expression and acceleration by high-dose insulin. Am. J. Physiol. Heart Circ. Physiol. 292, H1058-H1064.

165 Anand, D. V., Lahiri, A., Lim, E., Hopkins, D. and Corder, R. (2006) The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. J. Am. Coll. Cardiol. 47, 1850–1857.

166 Vik, A., Mathiesen, E. B., Noto, A. T., Sveinbjornsson, B., Brox, J. and Hansen, J. B. (2007) Serum osteoprotegerin is inversely associated with carotid plaque echogenicity in humans. Atherosclerosis 191, 128–134.

167 Ziegler, S., Kudlacek, S., Luger, A. and Minar, E. (2005) Osteoprotegerin plasma concentrations correlate with severity of peripheral artery disease. Atherosclerosis 182, 175–180.

168 Pritzker, L. B., Scatena, M. and Giachelli, C. M. (2004) The role of osteoprotegerin and tumor necrosis factor-related apoptosis-inducing ligand in human microvascular endothelial cell survival. Mol. Biol. Cell. 15, 2834–2841.

169 Malyankar, U. M., Scatena, M., Suchland, K. L., Yun, T. J., Clark, E. A. and Giachelli, C. M. (2000) Osteoprotegerin is an alpha vbeta 3-induced, NF-kappa B-dependent survival factor for endothelial cells. J. Biol. Chem. 275, 20959–20962.

170 Scatena, M., Giachelli, C. (2002) The αvβ3 integrin, NF-κB, osteoprotegerin endothelial cell survival pathway. Trends Cardiovasc. Med. 12, 83–88.

171 Kobayashi-Sakamoto, M., Hirose, K., Nishikata, M., Isogai, E. and Chiba, I. (2006) Osteoprotegerin protects endothelial cells against apoptotic cell death induced by *Porphyromonas gingivalis* cysteine proteinases. FEMS Microbiol. Lett. 264, 238–245.

172 Kobayashi-Sakamoto, M., Hirose, K., Isogai, E. and Chiba, I. (2004) NF-kappaB-dependent induction of osteoprotegerin by *Porphyromonas gingivalis* in endothelial cells. Biochem. Biophys. Res. Commun. 315, 107–112.

173 Cross, S. S., Yang, Z., Brown, N. J., Balasubramanian, S. P., Evans, C. A., Woodward, J. K., Neville-Webbe, H. L., Lippitt, J. M., Reed, M. W., Coleman, R. E. and Holen, I. (2006) Osteoprotegerin (OPG) – a potential new role in the regulation of endothelial cell phenotype and tumour angiogenesis? Int. J. Cancer 118, 1901–1908.

174 Kim, Y. M., Kim, Y. M., Lee, Y. M., Kim, H. S., Kim, J. D., Choi, Y., Kim, K. W., Lee, S. Y. and Kwon, Y. G. (2002) TNF-related activation-induced cytokine (TRANCE) induces angiogenesis through the activation of Src and phospholipase C (PLC) in human endothelial cells. J. Biol. Chem. 277, 6799–6805.

175 Min, J. K., Kim, Y. M., Kim, Y. M., Kim, E. C., Gho, Y. S., Kang, I. J., Lee, S. Y., Kong, Y. Y. and Kwon, Y. G. (2003) Vascular endothelial growth factor up-regulates expression of receptor activator of NF-kappa B (RANK) in endothelial cells: concomitant increase of angiogenic responses to RANK ligand. J. Biol. Chem. 278, 39548–39557.

176 Kim, H. H., Shin, H. S., Kwak, H. J., Ahn, K. Y., Kim, J. H., Lee, H. J., Lee, M. S., Lee, Z. H. and Koh, G. Y. (2003) RANKL regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. FASEB J. 17, 2163–2165.

177 Min, J. K., Cho, Y. L., Choi, J. H., Kim, Y., Kim, J. H., Yu, Y. S., Rho, J., Mochizuki, N., Kim, Y. M., Oh, G. T. and Kwon, Y. G.. (2007) Receptor activator of nuclear factor-kB ligand increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice. Blood 109, 1496–1502.

178 Zhang, J., Fu, M., Myles, D., Zhu, X., Du, J., Cao, X. and Chen, Y. E. (2002) PDGF induces osteoprotegerin expression in vascular smooth muscle cells by multiple signal pathways. FEBS Lett. 521, 180–184.

179 Yao, S., Liu, D., Pan, F. and Wise, G. E. (2006) Effect of vascular endothelial growth factor on RANK gene expression in osteoclast precursors and on osteoclastogenesis. Arch. Oral Biol. 51, 596–602.

180 Asagiri, M. and Takayanagi, H. (2007) The molecular understanding of osteoclast differentiation. Bone 40, 251–264.

181 Wada, T., Nakashima, T., Hiroshi, N. and Penninger, J. (2006) RANKL-RANK signaling in osteoclastogenesis and bone disease. Trends Mol. Med. 12, 17–25.

182 Body, J. J., Facon, T., Coleman, R. E., Lipton, A., Geurs, F., Fan, M., Holloway, D., Peterson, M. C. and Bekker, P. J. (2006) A study of the biological receptor activator of nuclear factor-kappa B ligand inhibitor, Denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin. Cancer Res. 12, 1221- 1228.

183 Kim, H. K.W., Morgan-Bagley, S. and Kostenuik, P. (2006) RANKL inhibition: a novel strategy to decrease femoral head deformity after ischemic osteonecrosis. J. Bone Miner. Res. 21, 1946–1954.

184 Feeley, B. T., Liu, N. Q., Conduah, A. H., Krenek, L., Roth, K., Dougall, W. C., Huard, J., Dubinett, S. and Lieberman, J. R. (2006) Mixed metastatic lung cancer lesions in bone are inhibited by noggin overexpression and rank: Fc administration. J. Bone Miner. Res. 21, 1571–1580.

185 Heymann, D., Ory, B., Blanchard, F., Heymann, M. F., Coipeau, P., Charrier, C., Couillaud, S., Thièry, J. P., Gouin, F. and Rédini, F. (2005) Enhanced tumor regression and tissue repair when zoledronic acid is combined with ifosfamide in rat osteosarcoma. Bone 37, 74–86.

186 Cheng, X., Kinosaki, M., Takami, M., Choi, Y., Zhang, H. and Murali, R. (2004) Disabling of receptor activator of nuclear factor-kappaB (RANK) receptor complex by novel osteoprotegerin-like peptidomimetics restores bone loss in vivo. J. Biol. Chem. 279, 8269–8277.

187 Heath, D. J., Vanderkerken, K., Cheng, X., Gallagher, O., Prideaux, M., Murali, R. and Croucher, P. I. (2007) An osteoprotegerin-like peptidomimetic inhibits osteoclastic bone resorption and osteolytic bone disease in myeloma. Cancer Res. 67, 202–208.

188 Aoki, K., Saito, H., Itzstein, C., Ishiguro, M., Shibata, T., Blanque, R., Mian, A. H., Takahashi, M., Suzuki, Y., Yoshimatsu, M., Yamaguchi, A., Deprez, P., Mollat, P., Murali, R., Ohya, K., Horne, W. C. and Baron, R. (2006) A TNF receptor loop peptide mimic blocks RANK ligand-induced signaling, bone resorption, and bone loss. J. Clin. Invest. 116, 1525–1534.

189 Heymann, D., Fortun, Y., Rédini, F. and Padrines, M. (2005) Osteolytic bone diseases: physiological analogues of bone resorption effectors as alternative therapeutic tools to the standard bisphosphonates. Drug Discov. Today 10, 242–247.

190 Xing, L., Bushnell, T. P., Carlson, L., Tai, Z., Tondravi, M., Siebenlist, U., Young, F. and Boyce, B. F. (2002) NF-kappaB p50 and p52 expression is not required for RANK-expressing osteoclast progenitor formation but is essential for RANK- and cytokine-mediated osteoclastogenesis. J. Bone Miner. Res. 17, 1200–10

191 Xing, L., Carlson, L., Story, B., Tai, Z., Keng, P., Siebenlist, U. and Boyce, B. F. (2003) Expression of either NF-kappaB p50 or p52 in osteoclast precursors is required for IL-1-induced bone resorption. J. Bone Miner. Res. 18, 260–269.

192 Chen, T. and Feng, X. (2006) Cell-based assay strategy for identification of motif-specific RANK signaling pathway inhibitors. Assay Drug Dev. Technol. 4, 473–492.

193 Jimi, E. and Ghish, S. (2005) Role of nuclear factor-kappa B in the immune system and bone. Immunol. Rev. 208, 80–87.

194 Iotsova, V., Caamano, J., Loy, J., Yang, Y., Lewin, A. and Bravo, R. (1997) Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. Nat. Med. 3, 1285–1289.

195 Eguchi, J., Koshino, T., Takagi, T., Hayashi, T. and Saito, T. (2002) NF-kappa B and I-kappa B overexpression in articular chondrocytes with progression of type II collagen-induced arthritis in DBA/1 mouse knees. Clin. Exp. Rheumatol. 20, 647–652.

196 Cho, M. L., Kang, J. W., Moon, Y. M., Nam, H. J., Jhun, J. Y., Heo, S. B., Jin, H. T., Min, S. Y., Ju, J. H., Park, K. S., Cho, Y. G., Yoon, C. H., Park, S. H., Sung, Y. C. and Kim, H. Y. (2006) STAT3 and NF-kappaB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice. J. Immunol. 176, 5652–5661.

197 Amos, N., Lauder, S., Evans, A., Feldmann, M. and Bondeson, J. (2006) Adenoviral gene transfer into osteoarthritis synovial cells using the endogenous inhibitor IkappaBalpha reveals that most, but not all, inflammatory and destructive mediators are NFkappaB dependent. Rheumatology 45, 1201–1209.
198 Okamoto, T. (2006) NF-kappaB and rheumatic diseases. Endocr. Metab. Immune Disord. Drug Targets 6, 359–372.
199 Park, B. K., Zhang, H., Zeng, Q., Dai, J., Keller, E. T., Giordano, T., Gu, K., Shah, V., Zarbo, R. J., McCauley, L., Shi, S., Chen, S. and Wang, C. Y. (2007). NF-kB in breast cancer cells promotes osteolytic bone metastasis by inducing osteoclastogenesis via GM-CSF. Nat. Med. 13, 62–69.
200 Clohisy,, J. C., Hirayama, T., Frazier, E., Han, S. K. and Abu-Amer, Y. (2004) NF-κB signaling blockade abolishes implant particle-induced osteoclastogenesis. J. Orthop. Res. 22, 13–20.
201 Clohisy,, J. C., Roy, B. C., Biondo, C., Frazier, E., Willis, D., Teitelbaum, S. L. and Abu-Amer, Y. (2003) Direct inhibition of NF-kappa B blocks bone erosion associated with inflammatory arthritis. J. Immunol. 171, 5547–5553
202 Kawamura, I., Morishita, R., Tomita, N., Lacey, E., Aketa, M., Tsujimoto, S., Manda, T., Tomoi, M., Kida, I., Higaki, J., Kaneda, Y., Shimomura, K. and Ogihara, T. (1999) Intratumoral injection of oligonucleotides to the NF kappa B binding site inhibits cachexia in a mouse tumor model. Gene Ther. 6, 91–97.
203 Penolazzi, L., Borgatti, M., Lambertini, E., Mischiati, C., Finotti, A., Romanelli, A., Saviano, M., Pedone, C., Piva, R. and Gambari, R. (2004) Peptide nucleic acid-DNA decoy chimeras targeting NF-kappaB transcription factors: induction of apoptosis in human primary osteoclasts. Int. J. Mol. Med. 14, 145–152.
204 Penolazzi, L., Lambertini, E., Borgatti, M., Piva, R., Cozzani, M., Giovannini, I., Naccari, R., Siciliani, G. and Gambari, R. (2003) Decoy oligodeoxynucleotides targeting NF-kappaB transcription factors: induction of apoptosis in human primary osteoclasts. Biochem. Pharmacol. 66, 1189–1198.
205 Suzuki, Y., Sgiyama C, Ohno, O. and Umezawa, K. (2004). Preparation and biological activities of optically active dehydroxymethylepoxyquinomycin, a novel NF-kB inhibitor. Tetrahedron 60, 7061–7066.
206 Takatsuna, H., Asgiri, M., Kubota, T., Oka, K., Osada, T., Sugiyama, C., Saito, H., Aoki, K., Ohya, K., Takayanagi, H. and Umezawa, K. (2005) Inhibition of RANKL-induced osteoclastogenesis by (–)-DHMEQ, a novel NF-kappaB inhibitor, through downregulation of NFATc1. J. Bone Miner. Res. 20, 653–662.
207 Ye, H., Arron, J. R., Lamothe, B., Cirilli, M., Kobayashi, T., Shevde, N. K., Segal, D., Dzivenu, O. K., Vologodskaia, M., Yim, M., Du, K., Singh, S., Pike, J. W., Darnay, B. G., Choi, Y. and Wu, H. (2002) Distinct molecular mechanism for initiating TRAF6 signalling. Nature 418, 443–447.