
Article:
Baud'huin, M.E., Duplomb, L., Teletchea, S. et al. (6 more authors) (2013) Osteoprotegerin: multiple partners for multiple functions. Cytokine and Growth Factor Reviews, 24 (5). pp. 401-409. ISSN 1359-6101

https://doi.org/10.1016/j.cytogfr.2013.06.001

Osteoprotegerin : multiple partners for multiple functions

BAUD'HUIN Marc[a,b,c,*], DUPLOMB Laurence[a,b,c], TELETCHEA Stéphane[a,b,c], RUIZ-VELASCO Carmen[a,b], MAILLASSON Mike[d], REDINI Françoise[a,b,e], HEYMANN Marie-Françoise[a, b, c,e], and HEYMANN Dominique [a, b, c,e*]

[a] INSERM, UMR957, Nantes, F-44035 France

[b] Université de Nantes, Nantes atlantique universités, Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, Nantes, F-44035, France

[c]CHU, Hôtel Dieu, Nantes, France

[d] INSERM, UMR892 and SFR Bonamy- OuestGenopole, F-44035 France

[e]Equipe labellisee Ligue Nationale Contre le Cancer 2012

Running title: OPG :a member of TNF family with multiple faces through multiple ligands

Key words: Osteoprotegerin, glycosaminoglycans, monocytes, cell adhesion

Correspondence and reprint request to

Pr. D. HEYMANNor Dr M. BAUD'HUIN

INSERM UMR 957

Faculté de Médecine

Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives

1 rue Gaston Veil 44035 Nantes Cedex 1, FRANCE

Tel: (011) 33 272 641 132

Fax: (011) 33 240 412 860

Email: dominique.heymann@univ-nantes.fr / marc.baudhuin@univ-nantes.fr

Abstract

Osteoprotegerin (OPG) is a secreted protein essential in bone turnover because of its role as a decoy receptor for the Receptor Activator of Nuclear Factor kB ligand (RANKL) in the osteoclasts, inhibiting their differentiation. However, there are additional ligands of OPG that confer multiple roles. Thus, OPG can promote cell survival, cell proliferation and facilitate migration through its binding to TNF-related apoptosis inducing ligand (TRAIL), glycosaminoglycans and proteoglycans. A large number of in vitro, in vivo and clinical studies provide evidences of the implication of OPG in vascular, bone, immune and tumor biology. This review aims to give an overview of the different ligands of OPG and their role in its biology.

Introduction

The Tumor Necrosis Factor (TNF)/TNF receptor superfamily is composed by more than 40 members, and controls a lot of genes essential for biological functions such as cellular differentiation, survival and death. Additionally TNF superfamily coordinates the development of numerous organs (Pfeffer 2003; Ware 2008). Among this family, Osteoprotegerin (OPG or TNFRSF11B according the international nomenclature) is soluble glycoprotein that exists as both a 60kDa monomer and a 120 kDa disulfide-linked dimer. OPG was originally characterized by two separate groups in 1997. Boyle and coworkers generated transgenic mice overexpressing various TNF receptor related cDNAs. A lineage of mice which over-expressed one specific cDNA developed a severe osteopetrosis due to a lack of osteoclasts. The protein encoded by this cDNA was therefore named osteoprotegerin, because it protected the bone from excessive resorption (Simonet et al. 1997). The second group in Japan reported a factor which was able to inhibit osteoclastogenesis (Yasuda et al. 1998). However, it was not until 1998 that both proteins were found to be equivalent.

Structurally, the mature form of OPG consists of 380 amino acids containing seven domains (Theoleyre et al. 2006; Baud'huin et al. 2007): 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). Domains 1-4 are structurally related to the TNF receptor family and are sufficient to abolish osteoclast differentiation and domains 5 and 6 can mediate a cytotoxic signal when they are included in a chimeric protein OPG-Fas (Yamaguchi et al. 1998) but their physiological functions remain unclear. The last domain possesses a heparin binding domain. Similar heparin binding domains has been observed in the structure of many other proteins such as fibroblast growth factors and their receptors in which it plays numerous key functions (activation, autophosphorylation, etc) (Eswarakumar et al. 2005; Guerrini et al. 2007). Additionally the presence of Cys-400 in the C-terminal

portionwithin domain 7 is essential for OPG dimerization with the formation of disulfide bound (Yamaguchi et al. 1998).

As OPG belongs to the TNF receptor family, it suggests that OPG has almost one ligand. The historical partner of OPG is RANKL which was identified few months following the description of OPG by the two same groups (Lacey et al. 1998; Yasuda et al. 1998). RANKL was initially discovered by two groups working on TNF-related cytokines thanks by its ability to sustain dendritic cell survival (Anderson et al. 1997; Wong et al. 1997). OPG is therefore a decoy receptor for the pro-osteoclastic cytokine RANKL (Theoleyre et al. 2004; Baud'huin et al. 2007) with a high affinity equivalent to RANKL for its receptor RANK (TNFRSF11A) (Table 1). The binding of OPG to RANKL prevents the interaction of RANKL for RANK expressed on the cell surface of osteoclast precursors, and thus inhibits the formation of osteoclasts (Boyle et al. 2003). Since then, numerous ligands for OPG have been identified: TRAIL (Emery et al. 1998), syndecan-1 (Standal et al. 2002), glycosaminoglycans (GAGs) (Theoleyre et al. 2006; Lamoureux et al. 2009), von Willebrand Factor (Zannettino et al. 2005) and Factor VIII-von Willebrand Factor complex (Baud'huin et al. 2009) (Table 1). This review focuses on the multiple biological functions achieved by OPG through the binding to its various ligands.

RANKL and TRAIL: ligands from the TNF family

RANKL is a transmembrane glycoprotein expressed on the surface of osteoblasts, stromal cells and T cells, however this molecule can be cleaved by proteases to give soluble RANKL with the same biological activity. The proteolytic cleavage is carried out by matrix metalloproteases or ADAMs (Georges et al. 2009). In bone, RANKL binds to its specific receptor RANK which is expressed on the membrane of osteoclast progenitors. Stoichiometrically, RANKL forms an homotrimeric protein and interacts with three monomers of RANK linked by disulfide bounds (Theoleyre et al. 2006). The binding of RANK to RANKL leads to the activation of several pathways involved in the differentiation, activation and survival of osteoclast. The RANK/RANKL is thus mandatory for the formation of mature osteoclasts. It has been shown that mice lacking Rankl did not have osteoclasts and suffered from severe osteopetrosis (Kong et al. 1999).

The osteoclastogenesis is a tightly regulated process with the modulation of RANK/RANKL signaling by OPG which acts as a decoy receptor for RANKL. In bone, OPG is secreted by stromal cells and other cell types including osteoblastic lineage cells. The evidence showing the importance of OPG in bone biology was provided by studies based on genetic experiments. OPG-overexpressing mice exhibit severe osteopetrosis and a reduced number of mature osteoclasts (Simonet et al. 1997) on the other hand OPG-knockout mice are osteoporotic with a decreased bone density and a high incidence of fractures and bone deformities (Bucay et al. 1998; Mizuno et al. 1998). The interaction between RANKL and OPG consists of two anchoring points on OPG for RANKL by amino acids 68, 69, 82, and amino acids 88–91, 111, and 116–120 (Figure 1) (Baud'huin et al. 2009). However, it has been shown that the homodimeric form of OPG exhibits a much greater higher affinity for the homotrimeric form of RANKL (Schneeweis et al. 2005; Theoleyre et al. 2006). More recently, the crystal structure of the interaction between RANKL and OPG was described

(Luan et al. 2012). In this study, the authors showed that OPG (Domains 1 to 4) is able to interact with RANKL in a 1:1 ratio, and blocks the accessibilities of important biding sites of RANKL to RANK.

This competitive interaction within the triad RANK/RANKL/OPG suggests that OPG can have also an effect in tissues other than bone where RANKL is active. In this context, the RANKL-RANK system influences processes in the immune system. Both $RANK^{-/-}$ and $RANKL^{-/-}$ mice have a defect in the maturation of T and B cells suggesting by the phenotype of implicating these molecules in lymphocyte and lymph node development (Dougall et al. 1999; Kong et al. 1999). From this perspective, Yun and coworkers showed that OPG regulates B cell maturation and development; population of B cells was indeed increased in OPG-null mice. Moreover, the dendritic cells generated from these mice have an increased ability to stimulate T cells (Yun et al. 2001). This study suggests that OPG has a pivotal role in the immune response and can mediate cell survival.

This latter function is reinforced by the fact that OPG is able to bind to TNF-related apoptosis inducing ligand (TRAIL) and thus block TRAIL-induced apoptosis of Jurkat cells (Emery et al. 1998). TRAIL is a member of the TNF superfamily produced by immune cells within the tumor environment. TRAIL binds to its receptors as a homotrimer form, this trimeric form being biologically much more active than the monomeric one (Wiley et al. 1995). There are five TRAIL receptors described so far; two of these receptors (DR4 and DR5) contain death domain motifs and promote apoptosis. On the other hand, DcR1, DcR2 and OPG are unable to induce apoptosis and function as decoy receptors. OPG is binding to TRAIL with a comparable affinity to that of RANKL (Table 1) and the binding sites involve on OPG residues (Figure 1). The direct biological consequence of the interaction of OPG with TRAIL is the inhibition of TRAIL-induced apoptosis in numerous cancer cells (Holen et al. 2002; Shipman et al. 2003; Neville-Webbe et al. 2004). This mechanism could be important

in the release from cell death, as suggested by the production of OPG by tumor cells (Holen et al. 2002; Heymann 2012).

Additional studies have been performed using bone marrow stromal cells (BMSCs) derived from either breast or prostate cancer patients. In both cancer types, BMSCs were found to produce sufficient levels of OPG to protect tumor cells from TRAIL-induced apoptosis. This suggests that bone-derived OPG may be involved in promoting survival of these tumor cell types within the bone microenvironment (Neville-Webbe et al. 2004; Nyambo et al. 2004). In this context, a peptide-mimetic of OPG (OP3-4) was developed to treat bone disease in multiple myeloma and keep the TRAIL activity. OP3-4 was able to inhibit osteoclastogenesis of murine blood mononuclear cells but did not inhibit TRAIL-induced apoptosis of multiple myeloma cells (Heath et al. 2007). Moreover, recent in vivo experiment showed that anticancer efficacy of recombinant soluble Apo2L/TRAIL is retained in the bone microenvironment where biologically active OPG may be present at high concentrations (Zinonos et al. 2011). Taken together, OPG may have a dual function in tumor, promoting cancer cell survival through its binding to TRAIL and on the other hand limiting the tumor growth in cancer induced bone disease due to the inhibition of RANKL. Thus, in a model of osteosarcoma, OPG administered by gene transfer reduced tumor incidence and local tumor growth (Lamoureux et al. 2007). The mechanism suggested mentioned that OPG exerts indirect inhibitory effect on tumor progression through the inhibition of RANKL whose production is enhanced in bone tumor environment.

The ability of OPG to bind both to RANKL and TRAIL gives to him the potency to regulate bone biology and tumor biology through direct effect on osteoclasts or cancer cells. However, in vascular biology, these three partners play a pivotal role which has numerous outcomes in the physiopathology of vascular system. OPG is largely expressed in the vascular tissues including the heart, arteries and veins, and is constitutively secreted by endothelial

cells and vascular smooth muscle cells (Chikatsu et al. 2002; Collin-Osdoby 2004; Chollet et al. 2010). In oncologic context, endothelial cells closely located to tumor cells strongly express OPG, especially in metastatic foci (Heymann et al. 2008). The presence of arterial calcification of the media of the aorta and the renal arteries in OPG-deficient mice suggest the involvement of OPG in vascular biology (Bucay et al. 1998). In accordance with these data, Price and co-workers showed that administration of exogenous OPG prevent vascular calcification induced by warfarin or high vitamin D doses (Price et al. 2001). Paradoxically, clinical studies suggest that high serum OPG levels correlate with the severity of vascular calcification, coronary artery disease and heart failure. (Ueland et al. 2005; Ziegler et al. 2005; Helske et al. 2007). Very recently, it has been shown that OPG is more frequently expressed in atheromatous carotid plaques than femoral specimens, and correlates with the abundance of macrophages in the lesions (Heymann et al. 2012). These clinical findings are controversial with in vitro data. Indeed the inhibitory effect of OPG on vascular calcification in animal models could be passive or cellular. As mentioned above, OPG exerts an anti-apoptotic effect towards TRAIL and decreases in consequence the number of apoptotic bodies that may serve as nucleation sites for passive mineralization (Fleishman et al. 1990; Benslimane-Ahmim et al. 2011). Several studies have shown also that recombinant OPG can promote the survival/proliferation of mature vascular endothelial cells (Malyankar et al. 2000; Pritzker et al. 2004; Cross et al. 2006; Kobayashi-Sakamoto et al. 2006; McGonigle et al. 2009). Similarly, Lawrie et al showed OPG promotes the proliferation and the migration of pulmonary artery smooth muscle cell, and thus evidenced an important role of OPG in the pathogenesis of pulmonary arterial hypertension (Lawrie et al. 2008). However, the precise mechanism remains unclear and seems to involve multipartners. One study although suggests that OPG is blocking the apoptosis induced by TRAIL (Pritzker et al. 2004). It has been clearly demonstrated that OPG is able to stimulate endothelial colony-forming cells and thus

leads to the formation of new blood vessels in vivo (Benslimane-Ahmim et al. 2011). These findings confirmed the work of McGonigle and collaborators who described that OPG increases angiogenesis (McGonigle et al. 2009) but interestingly they suggest that OPG exerts its effect through the inhibition of the binding RANK/RANKL. This capacity of OPG to induce new blood vessels formation gives to OPG a potential proangiogenic role in tumors as suggested by different studies (Cross et al. 2006; Secchiero et al. 2008). More recently, Benslimane-Ahmin et al. (in press) revealed using proteomic approaches that the proangiogenic properties of OPG appeared to be mediated by the proteoglycan syndecan-1, although OPG 1-194 lacking its heparin-binding domain still had pro-vasculogenic effects in vitro and in vivo. All these effects were significantly attenuated by endothelial colony-forming cells incubation with the CXCR4 antagonist AMD-3100, and by prior heparan sulphate proteoglycan disruption. Taken together, these data suggest a central role of OPG in vascular biology. The mechanism of action remains unclear and seems to involve RANKL and TRAIL but the interplay between these molecules is not sufficient to explain the different functions of OPG in vasculature.

Glycoaminoglycans and proteoglycans : ligands from the extracellular matrix.

Glycoaminoglycans (GAGs) are linear polymers of repeated disaccharidic units, bearing sulfate residues. The number and the position of sulfation are extremely variable, depending on the tissue, cell and metabolic context, ensuring structural variability in these polysaccharides (Bernfield et al. 1999). GAGs have an extended diversity of functions and are implicated in various biological processes such as morphogenesis, tissue structuring and organization, blood clotting. All GAGs, apart from hyaluronic acid (cartilage, vitreous humor) and heparin (mast cells), are present in the body as proteoglycans (PGs), covalently attached

to a core protein. PGs are present in many tissues, in the extracellular matrix or associated to the cell membrane.

The full length OPG binds to a wide variety of GAGs through its heparin binding domain in contrast to OPG-Fc in which the heparin-binding domain is lacking (Theoleyre et al. 2006; Lamoureux et al. 2009). The capacity of GAGs to bind to OPG can be evaluated by BIAcore analysis (Figure 2). Among the different GAGs, heparin presents the highest affinity for OPG (Kd: $1.3.10^{-10}$M for heparin, see Table 1).The dissociation constant is higher (hundred fold) for dermatan sulfate (DS) and chondroitin sulfate (CS) ($3.7.10^{-8}$ and 2.10^{-8} M respectively, Table1). This lower affinity is mainly due to the degree of sulfation as recently confirmed by Robinson et al. and by Salbach et al. (Robinson et al. 2012; Salbach et al. 2012). Heparin, indeed, is the most sulfated GAGs compared to CS and DS (Velasco et al. 2010). Moreover, a highly sulfated exopoly-saccharide derived from Altermonasinfernus (Velasco et al. 2011) has a binding affinity comparable to heparin (2.10^{-10} M, Table 1). The length of GAGs is another property of GAGs that govern their binding to OPG. As shown in Figure 2 and Table 1, oligosaccharide made of 4 sugar units (dp4) is much less affine for OPG than an oligosaccharide made of 18 sugar units (dp18) (Kd of 7.10^{-7} and $2.8.10^{-8}$ M respectively). As represented in Figure 2F, heparin is able to shift the binding of OPG to heparin immobilized on the sensorchip. The other GAGs studied are also able to shift the binding but a concentration 100 fold greater is required. This competition binding confirmed the importance of sulfation and size of GAGs for their biological activities. Thus, we have recently showed that GAGs act directly on osteoclastogenesis (Baud'huin et al. 2011). We found that heparin and others GAGs are able to inhibit the differentiation of osteoclast precursors into osteoclasts through an inhibition of cell adhesion. Our study confirmed also that the length and the sulfation are critical parameters in the inhibition of osteoclastogenesis. On the contrary, Irie and co-workers (Irie et al. 2007) showed that GAGs by binding to OPG

prevent OPG-mediated inhibition of osteoclastic differentiation, and therefore enhance bone resorption. Furthermore, Lamoureux et al. showed that GAGs inhibit full length OPG binding to TRAIL or to RANKL but not OPG truncated form lacking its heparin binding domain (Lamoureux et al. 2009). Thus, GAGs may represent potent regulators of OPG availability and anti-tumor activity in bone tumor microenvironment (Lamoureux et al. 2008).

The binding of OPG with GAGs implies also activities through interaction with PGs. The first role of the OPG heparin-binding domain has been revealed by Standal (Standal et al. 2002) who demonstrated that myeloma cells internalize and degrade OPG through its binding to syndecan-1 and consequently increase the local RANKL concentration andso induce osteolysis in patients. Moreover, OPG induces osteopontin expression via syndecan-1 and activates phosphoinositol 3-kinase/Akt in human periodontal ligament cells (Yongchaitrakul et al. 2009). PGs are also involved in OPG-induced chemotaxis of monocytes (Mosheimer et al. 2005). Indeed, OPG can interact with syndecan-1 expressed by monocytes (potential osteoclast precursors) and can stimulate the cell migration. In this context, OPG is a chemotactic factor for monocytes which can be recruited in inflammatory context or during osteolysis process.OPG seems also to modulate cell adhesion. Indeed, OPG stimulates the expression of the cell adhesion molecule angiopoietin-2 in TNF-α activated cells (Mangan et al. 2007), increases leucocyte adhesion to endothelial cells (Zauli et al. 2007) and stimulates proliferation and migration of human microvascular endothelial cells (Kobayashi-Sakamoto et al. 2008). Proteoglycans and GAGs have been suspected to initiate these OPG activities.

Taken together, these data show that PGs and GAGs are key partners for biological activities of OPG (inhibition of osteoclastogenesis in vitro, bioavailability of OPG and monocyte chemotaxis in favour of pro-osteolytic activity). In this context, we investigate the role of OPG on human monocytes adhesion and its control by GAGs. After 5 days of culture, human $CD14^+$ cells cultured in the presence of human OPG (100 ng/mL) strongly adhere to

the plastic, whereas very few cells adhere in culture medium alone (0.3% of the initial monocytes number) (Figure3A). This is confirmed by the cell counting, OPG increased by 25 fold the number of adherent monocytes (Figure 3B). When 125 µg/mL of heparin was added to the OPG-containing culture medium, the effect of OPG on $CD14^+$ cell adhesion was completely abolished (Figure 3A, $p < 0.001$). The sulfation and the size of the GAGs chains are involved in this inhibition of OPG-mediated adhesion. Thud, DS and dp18 added to the culture are able to inhibit OPG effect with the same intensity as heparin (99% of inhibition, Figure 3C). On the contrary, the OPG-induced $CD14^+$ cell adhesion was inhibited by 50%in the presence of dp4 and chondroitin sulfate (Figure 3C).We then investigated the signaling pathway involved in the adhesion of $CD14^+$ cells induced by OPG. We found that OPG is able to activate the PI3k / Akt pathway. As shown in Figure 2D, OPG induces phosphorylation of Akt after 10 minutes which is maintained at 45 minutes. The induction of phosphorylation was inhibited by heparin. Our data suggest that OPG could promote monocyte adhesion through an Akt/PI3-Kinase signaling pathway and recruit monocytes where OPG is produced (bone, endothelial cells). However, this phenomenon is a long process during around 5 days, compare to the ability of OPG to induce leucocyte adhesion to endothelial cells in 5 to 15 minutes (Zauli et al. 2007). This data suggests that OPG facilitates the recruitment of monocytes where OPG is produced, such as bone microenvironment, vasculature and tumor context.

von Willebrand factor/factor VIII complex : ligand from the vascular compartment

Factor VIII (FVIII) associated with the von Willebrand factor (vWF) is a key protagonist of the coagulation process as evidenced in patients suffering from hemophilia A (Bolton-Maggs et al. 2003). vWF enables the recruitment of platelets while factor VIII acts as a catalysor of

the blood clotting. In the circulation, FVIII is non-covalently complexed with vWF to form the FVIII/vWF complex.

In 2005, Zannettino and co-workers demonstrated that vWF is associated with OPG in Weibel Palade bodies of endothelial cells (Zannettino et al. 2005). More recently, it has been shown that OPG is also colocalised with vWF in alpha granules of platelets and megakaryocytes (Chollet et al. 2010). OPG interacts with the A1 domain of vWF and remains associated after secretion from endothelial cells (Shahbazi et al. 2007). The binding site of OPG to vWF has common domain with the binding site of GP1Ba protein to vWF which facilitates initial platelet adhesion (Figure 4). The binding affinity of vWF for OPG ($3{,}5.10^{-9}$ M) is comparable with that of OPG for RANKL as mentioned in Table 1. Taken together, these data support a possible role of vWF-OPG complex in regulation of endothelial cells survival, osteoclast differentiation, vascular injury and inflammation such as thrombus formation (Zannettino et al. 2005; Lenting et al. 2010).

The complex FVIII/vWF is also able to bind to OPG (Kd = $7{,}2.10^{-8}$ M, Table 1), blocking the interaction of OPG with TRAIL suggesting a potential function ofFVIII/vWF complex in cancer development (Baud'huin et al. 2009). Moreover, the binding of OPG to FVIII/vWF complex reinforces the OPG-inhibition of RANKL-induced osteoclastogenesis making a direct link between vascular and bone biology (Baud'huin et al. 2009). Recently, Melchiorre et al. showed that the synovium in hemophilic arthropathy highly expressed RANK and RANKL in contrast to OPG which decreased suggesting an activation of the resorptive process. They concluded that low tissue expression of OPG paralleled its serum levels and the severity of hemophilic arthropathy (Melchiorre et al., 2012). .These data are consistent with a randomized study showing that the prophylaxis with recombinant Factor VIII prevents joint damages in young haemophilic patients (Manco-Johnson et al. 2007), possibly through a decrease in osteoclastogenesis.

Conclusions

OPG has mainly been described as an anti-resorptive cytokine, as the first ligand reported was RANKL. However since that time, numerous ligands were described and studied in the last fifteen years giving to OPG an important role in vascular, tumor and immune biology (Figure 5). Clinical outcomes confirm that OPG is an active cytokine in a wide range of pathologies (osteoporosis, arthritis, vascular calcification, cancer bone-related disease) and can be considered as a biological marker or/and therapeutic targets for these pathologies. But the mechanisms by which OPG and its multiple partners act together and/or sequentially remain uncertain and need further investigation.

Acknowledgments:

This work was supported by the Région des Pays de la Loire [Program entitled “Ciblage Moléculaire et Applications Thérapeutique” (CIMATH)].

Figure legends

Figure 1: Structure of OPG in interaction with RANKL or TRAIL. OPG (red, model as described previously (Baud'huin et al. 2009)), was superposed on RANK (PDB id:3ME2, (Liu et al. 2010)) or DR5 (PDB id: 1D0G, (Hymowitz et al. 1999)) to conserve the receptor orientation observed in the crystallographic structures and the position of their respective ligands RANKL (green) or TRAIL (blue). Monomers only are presented for clarity. All proteins are represented with a transparent van der Walls surface and their secondary structure formed by an anti-parallel beta-sheets scaffold for RANKL and TRAIL or successive anti-parallel beta sheets regrouped in cystein-rich domains (CRD) for OPG are displayed in ribbon. The superposition was made using Discovery Studio 3.1 (Accelrys Inc., San Diego, USA) and the final rendering using POV-Ray (http://www.povray.org) and The GIMP (http://www.gimp.org).

Figure 2: GAGs bind to OPG with various affinities. Full-length human OPG (1 µg/mL in 5 mM maleate, pH 6.0) was covalently immobilized to dextran matrix of a CM5 sensor chip (BIAcore) at a flow rate of 5 µl/min. Immobilisation level of 4000 RU was then obtained. Binding assays were performed at 25°C in 10 mMHepes buffer, pH 7.4, containing 0.15 M NaCl and 0.005% P20 surfactant (HBS-P buffer, BIAcore). Increasing concentrations of **(A)** heparin (rage from 0.5 nM to 20 nM), **(B)** dermatan sulphate, **(C)** chondroitin sulphate (range from 100 nM to 3200 nM), **(D)** dp 4 (range from 1.6 µM to 12.8 µM) or **(E)** dp 18 (range from 100 nM to 3200 nM) were injected over the immobilized OPG at a flow rate of 60 µl/min, with 1 min association and 8 min dissociation. Kds of OPG for GAGs were determined using single cycle kinetics by ½ dilutions. The resulting sensorgrams were fitted using BiaEval 4.1 software (BIAcore). All experiments were performed at 25˚C. **(F)** Biotinylated heparin

was immobilized on a SA chip, and OPG was incubated for 2 hours with different GAGs at several concentrations. The couple OPG-GAGs was then injected on the immobilized heparin and the fixation of OPG to heparin was measured.

Figure 3: OPG induced adhesion of human CD14$^+$cells which is reversed by GAGs.10^6 cells were seeded in 6-wells plate, and cultured for 3 days in the presence or the absence of full length- OPG (100 ng/mL) and/or heparin (125 µg/mL) (**A**,**B**) or different glycosaminoglycans (**C**) (5 µM). After 3 days, cells were washedwith PBS, and observed under light microscope (Leica DM IRB, Camera: Olympus D70, Analysis software: Olympus DP controller/Manager) (original magnification x100) **(A),** then detached by trypsin-EDTA dissociation and counted **(B)** or after washing, 50 µL of α-MEM supplemented with 10% FCS was added as well as 50µL of XTT and OD was read at 490nm after 4h incubation at 37°C**(C)**. Experiments were performed three times in triplicate. ### P<0.001 compared to the control; *** P<0.001 compared to OPG.CD14$^+$ cells were stimulated for 10 or 45 min with 100 ng/mL OPG, in presence or not of 125 µg/mL heparin and (**D**) western blot analysis was performed on cell lysates to determine the level of Akt phosphorylation. Actin informed about equal loading charge Experiments were performed three times, and a representative blot is shown.

Figure 4: Binding modes comparison of various vWF partners. Top left: amino acids involved in OPG recognition (green). Top middle: amino acids involved in heparin recognition (Adachi et al. 2006). Top right: amino acids involved in GPIba recognition (orange, PDB id: 1SQ0, (Dumas et al. 2004)). On the opposite face of the A1 domain, bitiscetin (cyan, PDB id: 1UEX, (Maita et al. 2003)) and botrocetin (purple, PDB id: 1IJK,

(Fukuda et al. 2002)) binding domains. N-terminal (brown) and C-terminal (blue) ends of the A1 vWFdomain are in tube representation.

Figure 5: Schematic representation of the involvement of OPG in vascular, bone and cancer biology. OPG is biological active on several tissues mainly as a decoy receptor for ligands thus blocking their activity. As a result, OPG is inhibiting activities of RANKL and TRAIL in bone, vascular, tumor and immune biology. However, OPG can acts directly on cells by interacting with syndecans and promoting adhesion or migration

References

Adachi, T., T. Matsushita, Z. Dong, A. Katsumi, T. Nakayama, T. Kojima, H. Saito, J. E. Sadler and T. Naoe (2006). "Identification of amino acid residues essential for heparin binding by the A1 domain of human von Willebrand factor." Biochem Biophys Res Commun**339**(4): 1178-1183.

Anderson, D. M., E. Maraskovsky, W. L. Billingsley, W. C. Dougall, M. E. Tometsko, E. R. Roux, M. C. Teepe, R. F. DuBose, D. Cosman and L. Galibert (1997). "A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function." Nature**390**(6656): 175-179.

Baud'huin, M., L. Duplomb, S. Teletchea, C. Charrier, M. Maillasson, M. Fouassier and D. Heymann (2009). "Factor VIII-von Willebrand factor complex inhibits osteoclastogenesis and controls cell survival." J Biol Chem**284**(46): 31704-31713.

Baud'huin, M., F. Lamoureux, L. Duplomb, F. Redini and D. Heymann (2007). "RANKL, RANK, osteoprotegerin: key partners of osteoimmunology and vascular diseases." Cell Mol Life Sci**29**: 29.

Baud'huin, M., C. Ruiz-Velasco, G. Jego, C. Charrier, N. Gasiunas, J. Gallagher, M. Maillasson, A. Naggi, M. Padrines, F. Redini, L. Duplomb and D. Heymann (2011). "Glycosaminoglycans inhibit the adherence and the spreading of osteoclasts and their precursors: role in osteoclastogenesis and bone resorption." Eur J Cell Biol**90**(1): 49-57.

Benslimane-Ahmim, Z., D. Heymann, B. Dizier, A. Lokajczyk, R. Brion, I. Laurendeau, I. Bieche, D. M. Smadja, I. Galy-Fauroux, S. Colliec-Jouault, A. M. Fischer and C. Boisson-Vidal (2011). "Osteoprotegerin, a new actor in vasculogenesis, stimulates endothelial colony-forming cells properties." J Thromb Haemost**9**(4): 834-843.

Bernfield, M., M. Gotte, P. W. Park, O. Reizes, M. L. Fitzgerald, J. Lincecum and M. Zako (1999). "Functions of cell surface heparan sulfate proteoglycans." Annu Rev Biochem**68**: 729-777.

Bolton-Maggs, P. H. and K. J. Pasi (2003). "Haemophilias A and B." Lancet**361**(9371): 1801-1809.

Boyle, W. J., W. S. Simonet and D. L. Lacey (2003). "Osteoclast differentiation and activation." Nature**423**(6937): 337-342.

Bucay, N., I. Sarosi, C. R. Dunstan, S. Morony, J. Tarpley, C. Capparelli, S. Scully, H. L. Tan, W. Xu, D. L. Lacey, W. J. Boyle and W. S. Simonet (1998). "osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification." Genes Dev**12**(9): 1260-1268.

Chikatsu, N., Y. Takeuchi, S. Fukumoto, K. Yano, N. Fujita, T. Tsuruo and T. Fujita (2002). "Clonal endothelial cells produce humoral factors that inhibit osteoclast-like cell formation in vitro." Endocr J**49**(4): 439-447.

Chollet, M. E., J. P. Brouland, C. Bal dit Sollier, F. Bauduer, L. Drouet and S. Bellucci (2010). "Evidence of a colocalisation of osteoprotegerin (OPG) with von Willebrand factor (VWF) in platelets and megakaryocytes alpha granules. Studies from normal and grey platelets." Br J Haematol**148**(5): 805-807.

Collin-Osdoby, P. (2004). "Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin." Circ Res**95**(11): 1046-1057.

Cross, S. S., Z. Yang, N. J. Brown, S. P. Balasubramanian, C. A. Evans, J. K. Woodward, H. L. Neville-Webbe, J. M. Lippitt, M. W. Reed, R. E. Coleman and I. Holen (2006). "Osteoprotegerin (OPG)--a potential new role in the regulation of endothelial cell phenotype and tumour angiogenesis?" Int J Cancer**118**(8): 1901-1908.

Dougall, W. C., M. Glaccum, K. Charrier, K. Rohrbach, K. Brasel, T. De Smedt, E. Daro, J. Smith, M. E. Tometsko, C. R. Maliszewski, A. Armstrong, V. Shen, S. Bain, D. Cosman, D. Anderson, P. J. Morrissey, J. J. Peschon and J. Schuh (1999). "RANK is essential for osteoclast and lymph node development." Genes Dev**13**(18): 2412-2424.

Dumas, J. J., R. Kumar, T. McDonagh, F. Sullivan, M. L. Stahl, W. S. Somers and L. Mosyak (2004). "Crystal structure of the wild-type von Willebrand factor A1-glycoprotein Ibalpha complex reveals conformation differences with a complex bearing von Willebrand disease mutations." J Biol Chem**279**(22): 23327-23334.

Emery, J. G., P. McDonnell, M. B. Burke, K. C. Deen, S. Lyn, C. Silverman, E. Dul, E. R. Appelbaum, C. Eichman, R. DiPrinzio, R. A. Dodds, I. E. James, M. Rosenberg, J. C. Lee and P. R. Young (1998). "Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL." J Biol Chem**273**(23): 14363-14367.

Eswarakumar, V. P., I. Lax and J. Schlessinger (2005). "Cellular signaling by fibroblast growth factor receptors." Cytokine Growth Factor Rev**16**(2): 139-149.

Fleishman, D. G., S. P. Gambaryan, E. A. Lavrora, V. A. Nikiforov, A. A. Saulus and V. F. Vasilieva (1990). "Secretion of endogenous lithium in teleost kidneys as a probable reflection of Na+ and water secretion in their renal proximal tubules." C R Acad Sci III**311**(1): 37-42.

Fukuda, K., T. A. Doggett, L. A. Bankston, M. A. Cruz, T. G. Diacovo and R. C. Liddington (2002). "Structural basis of von Willebrand factor activation by the snake toxin botrocetin." Structure**10**(7): 943-950.

Georges, S., C. Ruiz Velasco, V. Trichet, Y. Fortun, D. Heymann and M. Padrines (2009). "Proteases and bone remodelling." Cytokine Growth Factor Rev**20**(1): 29-41.

Guerrini, M., M. Hricovini and G. Torri (2007). "Interaction of heparins with fibroblast growth factors: conformational aspects." Curr Pharm Des**13**(20): 2045-2056.

Heath, D. J., K. Vanderkerken, X. Cheng, O. Gallagher, M. Prideaux, R. Murali and P. I. Croucher (2007). "An osteoprotegerin-like peptidomimetic inhibits osteoclastic bone resorption and osteolytic bone disease in myeloma." Cancer Res**67**(1): 202-208.

Helske, S., P. T. Kovanen, K. A. Lindstedt, K. Salmela, J. Lommi, H. Turto, K. Werkkala and M. Kupari (2007). "Increased circulating concentrations and augmented myocardial extraction of osteoprotegerin in heart failure due to left ventricular pressure overload." Eur J Heart Fail**9**(4): 357-363.

Heymann, M. F., F. Herisson, J. M. Davaine, C. Charrier, S. Battaglia, N. Passuti, G. Lambert, Y. Goueffic and D. Heymann (2012). "Role of the OPG/RANK/RANKL triad in calcifications of the atheromatous plaques: comparison between carotid and femoral beds." Cytokine**58**(2): 300-306.

Holen, I., P. I. Croucher, F. C. Hamdy and C. L. Eaton (2002). "Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells." Cancer Res**62**(6): 1619-1623.

Hymowitz, S. G., H. W. Christinger, G. Fuh, M. Ultsch, M. O'Connell, R. F. Kelley, A. Ashkenazi and A. M. de Vos (1999). "Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5." Mol Cell**4**(4): 563-571.

Irie, A., M. Takami, H. Kubo, N. Sekino-Suzuki, K. Kasahara and Y. Sanai (2007). "Heparin enhances osteoclastic bone resorption by inhibiting osteoprotegerin activity." Bone**5**: 5.

Kobayashi-Sakamoto, M., K. Hirose, M. Nishikata, E. Isogai and I. Chiba (2006). "Osteoprotegerin protects endothelial cells against apoptotic cell death induced by Porphyromonas gingivalis cysteine proteinases." FEMS Microbiol Lett**264**(2): 238-245.

Kobayashi-Sakamoto, M., E. Isogai, K. Hirose and I. Chiba (2008). "Role of alpha(v) integrin in osteoprotegerin-induced endothelial cell migration and proliferation." Microvasc Res**4**: 4.

Kong, Y. Y., H. Yoshida, I. Sarosi, H. L. Tan, E. Timms, C. Capparelli, S. Morony, A. J. Oliveira-dos-Santos, G. Van, A. Itie, W. Khoo, A. Wakeham, C. R. Dunstan, D. L. Lacey, T. W. Mak, W. J. Boyle and J. M. Penninger (1999). "OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis." Nature**397**(6717): 315-323.

Lacey, D. L., E. Timms, H. L. Tan, M. J. Kelley, C. R. Dunstan, T. Burgess, R. Elliott, A. Colombero, G. Elliott, S. Scully, H. Hsu, J. Sullivan, N. Hawkins, E. Davy, C. Capparelli, A. Eli, Y. X. Qian, S. Kaufman, I. Sarosi, V. Shalhoub, G. Senaldi, J. Guo, J. Delaney and W. J. Boyle (1998). "Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation." Cell**93**(2): 165-176.

Lamoureux, F., G. Picarda, L. Garrigue-Antar, M. Baud'huin, V. Trichet, A. Vidal, E. Miot-Noirault, B. Pitard, D. Heymann and F. Redini (2009). "Glycosaminoglycans as potential regulators of osteoprotegerin therapeutic activity in osteosarcoma." Cancer Res**69**(2): 526-536.

Lamoureux, F., G. Picarda, V. Trichet, B. Pitard, D. Heymann and F. Rédini (2008). "Proteoglycans are potential regulators of osteoprotegerin (OPG) antitumoral and anti-bone resorption activities in osteosarcoma [abstract]." Cancer Treat Rev**34/S1**(S1): P46.

Lamoureux, F., P. Richard, Y. Wittrant, S. Battaglia, P. Pilet, V. Trichet, F. Blanchard, F. Gouin, B. Pitard, D. Heymann and F. Redini (2007). "Therapeutic relevance of osteoprotegerin gene therapy in osteosarcoma: blockade of the vicious cycle between tumor cell proliferation and bone resorption." Cancer Res**67**(15): 7308-7318.

Lawrie, A., E. Waterman, M. Southwood, D. Evans, J. Suntharalingam, S. Francis, D. Crossman, P. Croucher, N. Morrell and C. Newman (2008). "Evidence of a role for osteoprotegerin in the pathogenesis of pulmonary arterial hypertension." Am J Pathol**172**(1): 256-264.

Lenting, P. J., J. N. Pegon, E. Groot and P. G. de Groot (2010). "Regulation of von Willebrand factor-platelet interactions." Thromb Haemost**104**(3): 449-455.

Liu, C., T. S. Walter, P. Huang, S. Zhang, X. Zhu, Y. Wu, L. R. Wedderburn, P. Tang, R. J. Owens, D. I. Stuart, J. Ren and B. Gao (2010). "Structural and functional insights of RANKL-RANK interaction and signaling." J Immunol**184**(12): 6910-6919.

Luan, X., Q. Lu, Y. Jiang, S. Zhang, Q. Wang, H. Yuan, W. Zhao, J. Wang and X. Wang (2012). "Crystal structure of human RANKL complexed with its decoy receptor osteoprotegerin." J Immunol**189**(1): 245-252.

Maita, N., K. Nishio, E. Nishimoto, T. Matsui, Y. Shikamoto, T. Morita, J. E. Sadler and H. Mizuno (2003). "Crystal structure of von Willebrand factor A1 domain complexed with snake venom, bitiscetin: insight into glycoprotein Ibalpha binding mechanism induced by snake venom proteins." J Biol Chem**278**(39): 37777-37781.

Malyankar, U. M., M. Scatena, K. L. Suchland, T. J. Yun, E. A. Clark and C. M. Giachelli (2000). "Osteoprotegerin is an alpha vbeta 3-induced, NF-kappa B-dependent survival factor for endothelial cells." J Biol Chem**275**(28): 20959-20962.

Manco-Johnson, M. J., T. C. Abshire, A. D. Shapiro, B. Riske, M. R. Hacker, R. Kilcoyne, J. D. Ingram, M. L. Manco-Johnson, S. Funk, L. Jacobson, L. A. Valentino, W. K. Hoots, G. R. Buchanan, D. DiMichele, M. Recht, D. Brown, C. Leissinger, S. Bleak, A. Cohen, P. Mathew, A. Matsunaga, D. Medeiros, D. Nugent, G. A. Thomas, A. A. Thompson, K. McRedmond, J. M. Soucie, H. Austin and B. L. Evatt (2007).

"Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia." N Engl J Med**357**(6): 535-544.

Mangan, S. H., A. V. Campenhout, C. Rush and J. Golledge (2007). "Osteoprotegerin upregulates endothelial cell adhesion molecule response to tumor necrosis factor-alpha associated with induction of angiopoietin-2." Cardiovasc Res**76**(3): 494-505.

McGonigle, J. S., C. M. Giachelli and M. Scatena (2009). "Osteoprotegerin and RANKL differentially regulate angiogenesis and endothelial cell function." Angiogenesis**12**(1): 35-46.

Mizuno, A., N. Amizuka, K. Irie, A. Murakami, N. Fujise, T. Kanno, Y. Sato, N. Nakagawa, H. Yasuda, S. Mochizuki, T. Gomibuchi, K. Yano, N. Shima, N. Washida, E. Tsuda, T. Morinaga, K. Higashio and H. Ozawa (1998). "Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin." Biochem Biophys Res Commun**247**(3): 610-615.

Mosheimer, B. A., N. C. Kaneider, C. Feistritzer, A. M. Djanani, D. H. Sturn, J. R. Patsch and C. J. Wiedermann (2005). "Syndecan-1 is involved in osteoprotegerin-induced chemotaxis in human peripheral blood monocytes." J Clin Endocrinol Metab**90**(5): 2964-2971.

Neville-Webbe, H. L., N. A. Cross, C. L. Eaton, R. Nyambo, C. A. Evans, R. E. Coleman and I. Holen (2004). "Osteoprotegerin (OPG) produced by bone marrow stromal cells protects breast cancer cells from TRAIL-induced apoptosis." Breast Cancer Res Treat**86**(3): 269-279.

Nyambo, R., N. Cross, J. Lippitt, I. Holen, G. Bryden, F. C. Hamdy and C. L. Eaton (2004). "Human bone marrow stromal cells protect prostate cancer cells from TRAIL-induced apoptosis." J Bone Miner Res**19**(10): 1712-1721.

Pfeffer, K. (2003). "Biological functions of tumor necrosis factor cytokines and their receptors." Cytokine Growth Factor Rev**14**(3-4): 185-191.

Price, P. A., H. H. June, J. R. Buckley and M. K. Williamson (2001). "Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D." Arterioscler Thromb Vasc Biol**21**(10): 1610-1616.

Pritzker, L. B., M. Scatena and C. M. Giachelli (2004). "The role of osteoprotegerin and tumor necrosis factor-related apoptosis-inducing ligand in human microvascular endothelial cell survival." Mol Biol Cell**15**(6): 2834-2841.

Robinson, D. E., D. J. Buttle, R. D. Short, S. L. McArthur, D. A. Steele and J. D. Whittle (2012). "Glycosaminoglycan (GAG) binding surfaces for characterizing GAG-protein interactions." Biomaterials**33**(4): 1007-1016.

Schneeweis, L. A., D. Willard and M. E. Milla (2005). "Functional dissection of osteoprotegerin and its interaction with receptor activator of NF-kappaB ligand." J Biol Chem**280**(50): 41155-41164.

Secchiero, P., F. Corallini, E. Rimondi, C. Chiaruttini, M. G. di Iasio, A. Rustighi, G. Del Sal and G. Zauli (2008). "Activation of the p53 pathway down-regulates the osteoprotegerin expression and release by vascular endothelial cells." Blood**111**(3): 1287-1294.

Shahbazi, S., P. J. Lenting, C. Fribourg, V. Terraube, C. V. Denis and O. D. Christophe (2007). "Characterization of the interaction between von Willebrand factor and osteoprotegerin." J Thromb Haemost**5**(9): 1956-1962.

Shipman, C. M. and P. I. Croucher (2003). "Osteoprotegerin is a soluble decoy receptor for tumor necrosis factor-related apoptosis-inducing ligand/Apo2 ligand and can function as a paracrine survival factor for human myeloma cells." Cancer Res**63**(5): 912-916.

Simonet, W. S., D. L. Lacey, C. R. Dunstan, M. Kelley, M. S. Chang, R. Luthy, H. Q. Nguyen, S. Wooden, L. Bennett, T. Boone, G. Shimamoto, M. DeRose, R. Elliott, A.

Colombero, H. L. Tan, G. Trail, J. Sullivan, E. Davy, N. Bucay, L. Renshaw-Gegg, T. M. Hughes, D. Hill, W. Pattison, P. Campbell, S. Sander, G. Van, J. Tarpley, P. Derby, R. Lee and W. J. Boyle (1997). "Osteoprotegerin: a novel secreted protein involved in the regulation of bone density." Cell**89**(2): 309-319.

Standal, T., C. Seidel, O. Hjertner, T. Plesner, R. D. Sanderson, A. Waage, M. Borset and A. Sundan (2002). "Osteoprotegerin is bound, internalized, and degraded by multiple myeloma cells." Blood**100**(8): 3002-3007.

Theoleyre, S., S. Kwan Tat, P. Vusio, F. Blanchard, J. Gallagher, S. Ricard-Blum, Y. Fortun, M. Padrines, F. Redini and D. Heymann (2006). "Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: role in the interactions with receptor activator of nuclear factor kappaB ligand (RANKL) and RANK." Biochem Biophys Res Commun**347**(2): 460-467.

Theoleyre, S., Y. Wittrant, S. K. Tat, Y. Fortun, F. Redini and D. Heymann (2004). "The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling." Cytokine Growth Factor Rev**15**(6): 457-475.

Ueland, T., A. Yndestad, E. Oie, G. Florholmen, B. Halvorsen, S. S. Froland, S. Simonsen, G. Christensen, L. Gullestad and P. Aukrust (2005). "Dysregulated osteoprotegerin/RANK ligand/RANK axis in clinical and experimental heart failure." Circulation**111**(19): 2461-2468.

Velasco, C. R., M. Baud'huin, C. Sinquin, M. Maillasson, D. Heymann, S. Colliec-Jouault and M. Padrines (2011). "Effects of a sulfated exopolysaccharide produced by Altermonas infernus on bone biology." Glycobiology**21**(6): 781-795.

Velasco, C. R., S. Colliec-Jouault, F. Redini, D. Heymann and M. Padrines (2010). "Proteoglycans on bone tumor development." Drug Discov Today**15**(13-14): 553-560.

Ware, C. F. (2008). "The TNF Superfamily-2008." Cytokine Growth Factor Rev**19**(3-4): 183-186.

Wiley, S. R., K. Schooley, P. J. Smolak, W. S. Din, C. P. Huang, J. K. Nicholl, G. R. Sutherland, T. D. Smith, C. Rauch, C. A. Smith and et al. (1995). "Identification and characterization of a new member of the TNF family that induces apoptosis." Immunity**3**(6): 673-682.

Wong, B. R., J. Rho, J. Arron, E. Robinson, J. Orlinick, M. Chao, S. Kalachikov, E. Cayani, F. S. Bartlett, 3rd, W. N. Frankel, S. Y. Lee and Y. Choi (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**(40): 25190-25194.

Yamaguchi, K., M. Kinosaki, M. Goto, F. Kobayashi, E. Tsuda, T. Morinaga and K. Higashio (1998). "Characterization of structural domains of human osteoclastogenesis inhibitory factor." J Biol Chem**273**(9): 5117-5123.

Yasuda, H., N. Shima, N. Nakagawa, S. I. Mochizuki, K. Yano, N. Fujise, Y. Sato, M. Goto, K. Yamaguchi, M. Kuriyama, T. Kanno, A. Murakami, E. Tsuda, T. Morinaga and K. Higashio (1998). "Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro." Endocrinology**139**(3): 1329-1337.

Yasuda, H., N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, E. Tsuda, T. Morinaga, K. Higashio, N. Udagawa, N. Takahashi and T. Suda (1998). "Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL." Proc Natl Acad Sci U S A**95**(7): 3597-3602.

Yongchaitrakul, T., J. Manokawinchoke and P. Pavasant (2009). "Osteoprotegerin induces osteopontin via syndecan-1 and phosphoinositol 3-kinase/Akt in human periodontal ligament cells." J Periodontal Res**44**(6): 776-783.

Yun, T. J., M. D. Tallquist, A. Aicher, K. L. Rafferty, A. J. Marshall, J. J. Moon, M. E. Ewings, M. Mohaupt, S. W. Herring and E. A. Clark (2001). "Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function." J Immunol**166**(3): 1482-1491.

Zannettino, A. C., C. A. Holding, P. Diamond, G. J. Atkins, P. Kostakis, A. Farrugia, J. Gamble, L. B. To, D. M. Findlay and D. R. Haynes (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**(2): 714-723.

Zauli, G., F. Corallini, F. Bossi, F. Fischetti, P. Durigutto, C. Celeghini, F. Tedesco and P. Secchiero (2007). "Osteoprotegerin increases leukocyte adhesion to endothelial cells both in vitro and in vivo." Blood**110**(2): 536-543.

Ziegler, S., S. Kudlacek, A. Luger and E. Minar (2005). "Osteoprotegerin plasma concentrations correlate with severity of peripheral artery disease." Atherosclerosis**182**(1): 175-180.

Zinonos, I., A. Labrinidis, M. Lee, V. Liapis, S. Hay, V. Ponomarev, P. Diamond, D. M. Findlay, A. C. Zannettino and A. Evdokiou (2011). "Anticancer efficacy of Apo2L/TRAIL is retained in the presence of high and biologically active concentrations of osteoprotegerin in vivo." J Bone Miner Res**26**(3): 630-643.

Salbach J, Kliemt S, Rauner M, Rachner TD, Goettsch C, Kalkhof S, von Bergen M, Möller S, Schnabelrauch M, Hintze V, Scharnweber D, Hofbauer LC (2012). The effect of the degree of sulfation of glycosaminoglycans on osteoclast function and signaling pathways. Biomaterials 33(33):8418-29.

Benslimane-Ahmim, Z., Poirier, F., Delomenie, C., Lokajczyk, A., Grelac, F., Galy-Fauroux, I., Mouhamed, A., Fisher, A.M., Heymann, D., Lutomski, D., Boisson-Vidal, C. “Mechanistic study of the proangiogenic effect of osteoprotegerin”. Angiogenesis, in press

Heymann, M.F., Riet, A., Le Goff, B., Battaglia, S., Paineau, J., Heymann D. (2008). “OPG, RANK and RANK ligand expression in thyroid lesions”. Regul Pept. 148(1-3):46-53.

Heymann, D. (2012). “Anti-RANKL therapy for bone tumours: Basic, pre-clinical and clinical evidences”. J Bone Oncology, 1(1) :2-11.

Melchiorre D, Milia AF, Linari S, Romano E, Benelli G, Manetti M, Guiducci S, Ceccarelli C, Innocenti M, Carulli C, Civinini R, Morfini M, Matucci-Cerinic M, Ibba-Manneschi L. (2012) RANK-RANKL-OPG in hemophilic arthropathy: from clinical and imaging diagnosis to histopathology. J Rheumatol. 39(8):1678-86.