RANK Ligand and Osteoprotegerin
Paracrine Regulators of Bone Metabolism and Vascular Function

Michael Schoppet, Klaus T. Preissner, Lorenz C. Hofbauer

Abstract—In 1997, investigators isolated a secreted glycoprotein that blocked osteoclast differentiation from precursor cells, prevented osteoporosis (decreased bone mass) when administered to ovariectomized rats, and resulted in osteopetrosis (increased bone mass) when overexpressed in transgenic mice. Since then, the isolation and characterization of the protein named osteoprotegerin (OPG) has stimulated much work in the fields of endocrinology, rheumatology, and immunology. OPG functions as a soluble decoy receptor for receptor activator of nuclear factor-κB ligand (RANKL, or OPG ligand) and shares homologies with other members of the tumor necrosis factor receptor superfamily. OPG acts by competing with the receptor activator of nuclear factor-κB, which is expressed on osteoclasts and dendritic cells for specifically binding to RANKL. RANKL is crucially involved in osteoclast functions and bone remodeling as well as immune cell cross-talks, dendritic cell survival, and lymph node organogenesis. More recently, emerging evidence from in vitro studies and mouse genetics attributed OPG an important role in vascular biology. In fact, OPG could represent the long sought-after molecular link between arterial calcification and bone resorption, which underlies the clinical coincidence of vascular disease and osteoporosis, which are most prevalent in postmenopausal women and elderly people. (Arterioscler Thromb Vase Biol. 2002;22:549-553.)

Key Words: arterial calcification • dendritic cells • osteoporosis • osteoprotegerin • RANK ligand

Osteoprotegerin (OPG) was isolated independently by two laboratories,1,2 and synonyms such as osteoclastogenesis inhibitory factor (OCIF),3 TNF receptor-related molecule-1 (TR1),4 or follicular dendritic cell–derived receptor-1 (FDCR-1)5 have been coined. According to the American Society for Bone and Mineral Research Committee, the term osteoprotegerin (OPG) is now being recommended.6 The mouse7 and the human8 OPG genes have been cloned and characterized, and the human OPG gene represents a single-copy gene that contains 5 exons and spans 29 kb of the human genome located on chromosome 8.9 Murine OPG expression starts between days 8 and 9 during embryogenesis.7 Of note, the human OPG promoter sequence harbors binding elements for the osteoblast-specific transcription factor cbfa-1, which was found to increase OPG gene transcription.10

OPG is a member of the tumor necrosis factor receptor (TNFR) superfamily, and it represents a secretory basic glycoprotein that exists in a 60-kd monomeric form and a disulfide-linked homodimeric form of 120 kd.11 It has also been detected in a cell surface–associated form with some cell types,3 although sequence analysis failed to detect a classical hydrophobic transmembrane domain, which is typical for all other members of the TNFR superfamily.11 The molecule is composed of 401 amino acid residues as deduced from cDNA nucleotide sequencing with a signal peptide of 21 amino acids.3 OPG consists of 7 structural domains, of which the amino-terminal cysteine-rich domains 1 to 4 share some features with the extracellular domains of other members of the TNFR family (Figure 1).12

Mutation analyses have been used for functional characterization of OPG. Domains 1 to 4 are sufficient for conferring osteoclastogenesis inhibitory activity, which can be demonstrated by carboxy-terminal truncation mutants.11 Of note, monomeric and dimeric OPG were indistinguishable in their specific activity to inhibit osteoclastogenesis.13 The carboxy-terminal portion of the protein with domains 5 and 6 contains two death domain homologous regions, motifs that are found in the cytoplasmic region of mediators of apoptosis such as TNFR1, DR3, CD95/Fas, or TNF-related apoptosis-inducing ligand (TRAIL) receptors.14–16 In fact, domains 5 and 6 of OPG have been demonstrated to transduce an apoptotic signal when expressed as an OPG/Fas fusion protein in which the transmembrane region of Fas is inserted between domains 4 and 5 of OPG.11 However, death domain-containing members of the TNFR family are also able to stimulate alternative signaling pathways, thus preventing rather than triggering apoptosis.17 Finally, domain 7 harbors a heparin-binding region, a common feature of peptide growth factors and signal molecules,18–20 as well as an unpaired...
cysteine residue required for disulfide bond formation and dimerization (Figure 1).\textsuperscript{11,13}

**Expression and Regulation of OPG**

OPG is produced by a variety of tissues including the cardiovascular system (heart, arteries, veins), lung, kidney, intestine, and bone, as well as hematopoietic and immune cells.\textsuperscript{1,5,21} The expression and production of the protein is modulated by various cytokines, peptides, hormones, and drugs. Cytokines, including TNF-\(\alpha\), interleukin (IL)-1, IL-6, IL-11, TNF-\(\beta\), bone morphogenetic proteins, and steroid hormones such as 17\(\beta\)-estradiol are known to up-regulate OPG mRNA levels.\textsuperscript{22–29} In contrast, glucocorticoids (known to promote bone resorption) and the immunosuppressant cyclosporin A (which has the propensity to cause osteoporosis and vascular disease), parathyroid hormone (PTH), prostaglandin E\(_2\), and basic fibroblast growth factor all suppress the expression of OPG.\textsuperscript{30} Moreover, tensional force applied to bone surface is followed by enhanced OPG mRNA synthesis,\textsuperscript{36} whereas expression of OPG by bone marrow cells declines with aging,\textsuperscript{37} thus implicating OPG as a potential mediator of immobilization and senile osteoporosis.

**Role of RANKL and OPG in Bone Metabolism**

The RANKL gene encodes a protein of 316 amino acids with a molecular mass of 38 kd, of which the extracellular domains self-associate as a trimer. Its expression is also modulated by various cytokines (IL-1, IL-6, IL-11, TNF-\(\alpha\)), glucocorticoids, and PTH.\textsuperscript{38} RANKL is produced by osteoblastic lineage cells and activated T cells and promotes osteoclast formation, fusion, differentiation, activation, and survival, leading to enhanced bone resorption and bone loss.\textsuperscript{39,40}

Except for a primary secreted form produced by T cells and some cancer cell lines, RANKL exists either in a cell-bound form or a truncated ectodomain variant derived from enzymatic cleavage of the cellular form by a TNF-\(\alpha\)-converting enzyme-like protease (TACE) (Figure 2).\textsuperscript{41} RANKL stimulates its specific receptor RANK, which is expressed by a restricted number of cell types, including progenitor and mature osteoclasts, activated T cells, and myeloid-derived dendritic cells (DCs) (Figure 2).\textsuperscript{42–46} RANK activation by RANKL initiates intracellular signaling cascades that involve c-Jun, NF-\(\kappa\)B, and serine/threonine kinase Akt/PKB pathways.\textsuperscript{47}

The biological effects of OPG are opposite of the RANKL-mediated effects, because OPG acts as a soluble inhibitor that prevents RANKL interaction and subsequent stimulation with its receptor, RANK (Figure 2).\textsuperscript{48} Therefore, mice with excessive or defective production of RANKL, RANK, or OPG display both extremes of skeletal phenotypes, ie, osteoporosis (OPG knockout) and osteopetrosis (OPG transgenic, RANKL knockout, RANK knockout).\textsuperscript{1,49–51} In conclusion, RANKL, RANK, and OPG represent a novel cytokine network and act as key regulators of bone metabolism and osteoclast biology (for review, see Suda et al\textsuperscript{52} and Teitelbaum\textsuperscript{53}).

**OPG and the Immune System**

A number of studies have highlighted the involvement of OPG and its cognate ligand, RANKL, in immune responses. Binding of RANKL to RANK augments DC survival via Bel-x\(_L\) induction, enhances the immunostimulatory capacity of DCs, and modulates activated T cells.\textsuperscript{54–58} Thus, in addition to its osteotropic effects, RANKL exerts important immunomodulatory functions, as evident from the phenotype of RANKL-deficient mice, which display lymph node agenesis as well as impaired splenic structures and Peyer’s patches.\textsuperscript{51} Interestingly, deletion of the RANK gene leads to the exact phenocopy of RANKL-deficient mice,\textsuperscript{59} thus establishing the importance of the RANKL-RANK pathway in immune regulation. By studying OPG-deficient mice, OPG was found to be critically involved in B cell maturation and the generation of efficient antibody responses.\textsuperscript{60} Furthermore, bone marrow–derived DCs are more potent in stimulating allogeneic T cells in OPG-deficient mice as compared with...
endothelial cells have been implicated as cellular sources of mineral deposits in the vascular system. Interestingly, affected workers reported multinucleated osteoclast-like cells in the calcified vascular lesions of OPG-deficient mice with the concomitant detection of RANK transcripts, which are indicative of the osteoclastic and the DC lineage.

The hypothesis that the RANKL/OPG system could link osteoporosis and arterial calcification is underlined by the high clinical prevalence and coincidence of arterial calcification and cardiovascular disease in postmenopausal women and elderly people with osteoporosis. Interestingly, a recent study in elderly women found a significant correlation of elevated OPG serum levels and cardiovascular mortality. Similarly, an earlier study detected increased serum concentrations of OPG in osteoporotic and postmenopausal women as compared with age-matched women without osteoporosis, and OPG levels were highest in those with the highest bone turnover and the most severe osteoporosis. The seeming paradox of increased serum levels of OPG in patients with active osteoporosis and vascular disease has been interpreted as an incomplete regulatory mechanism to counteract disease progression.

Finally, OPG (used in concentrations known to block bone resorption) was found to inhibit warfarin- and vitamin D-induced vascular calcification in rats in vivo, which was similar to the effects of bisphosphonates, another established drug class known to inhibit osteoclastic bone resorption and bone loss (Figure 3).

**Summary**

The novel TNF superfamily members RANKL and OPG are essential paracrine mediators of bone metabolism and immune functions and have been clearly implicated in various skeletal and immune disorders and diseases at the interface between bone metabolism and the immune system such as rheumatoid arthritis. In the vascular system, OPG is produced by smooth muscle and endothelial cells in vitro and acts as a survival factor for endothelial cells. One recent study demonstrated RANKL and OPG immunoreactivity in the nondiseased vessel wall and in early atherosclerotic lesions in human tissues, whereas in advanced calcified lesions, only RANKL was detected in the extracellular matrix surrounding calcium deposits. Moreover, systemic administration of OPG has been found to prevent vitamin D-induced vascular calcification in rodents. Finally, a recent clinical study has demonstrated that OPG administration is a safe and effective therapy in reducing biochemical markers of bone turnover in postmenopausal women. In summary, emerging evidence indicates that OPG is not merely a protective factor for bone, but may, in fact, act as a protective factor for the vascular system. Further studies are required to assess the contribution of RANKL and OPG in vascular disease, to analyze their role as biochemical markers of vascular diseases, and to evaluate the potential of OPG as a new “vasculoproteogranin” in therapeutic studies.

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