Histol Histopathol (2008) 23: 497-506
DOI: 10.14670/HH-23.497

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Role of OPG/RANKL/RANK axis on the vasculature

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Summary. Vascular calcification, a degenerative process considered in the past to be a passive procedure, has now been suggested to be related to ossification. Many proteins responsible for bone formation have been identified on the arterial wall. The OPG/RANKL/RANK axis, responsible for ossification and bone mineralization, seems to play a major role in vasculature and atherosclerosis. Mice lacking OPG gene present osteoporosis and arterial calcification, while overexpression of OPG gene leads to osteopetrosis. In the present review the latest knowledge related to the effects of the OPG/RANKL/RANK axis on vasculature, including atherosclerosis, will be analyzed. The clinical significance of circulating OPG and RANKL levels in vascular diseases will also be referred.

Key words: OPG, RANKL, RANK, Vascular calcification

Introduction

It is now accepted that vascular calcification resembles embryonic osteogenesis. However, even some years ago, calcification was considered to be a passive, unregulated, degenerative process that normally occurs in an advanced stage of atherosclerotic plaque formation. Only during the last decade it has been suggested that vascular calcification is the result of regulated ossification (Demer et al., 1994; Otto et al., 1994). Ossification occurs in the restenotic aortic valve tissue after valvuloplasty (Feldman et al., 1993) and in some of the carotid atherosclerotic plaques (Hunt et al., 2002). Vascular calcification includes mostly osteogenic and, to a lesser extent chondrogenic differentiation of osteoblasts. It has been shown that many of the regulators of bone formation and some bone structural proteins, such as Bone Morphogenic Protein-2 (BMP-2) (Bostrom et al., 1993), Osteopontin (OPN) (Giachelli et al., 1993; Hirota et al., 1993; Ikeda et al., 1993), Matrix γ-Carboxyglutamic Acid Protein (MGP) (Shanahan et al., 1994), Osteoprotegerin (OPG) (Dhore et al., 2001) and Receptor Activator of Nuclear Factor κLigand (RANKL) (Dhore et al., 2001) are also expressed in atherosclerotic plaque. OPG, a protein homologue to Receptor Activator of Nuclear Factor κß (RANK) can bind to RANKL, blocking the binding of RANKL to RANK, resulting in inhibition of differentiation of preosteoclasts to mature osteoclasts (Lacey et al., 1998).

The first sign that calcification is subjected to regulatory mechanisms was observed by Anderson and Parker, who noticed elements of endochondral ossification in human calcified aorta specimens (Anderson and Parker, 1966; Tanimura et al., 1983). Later on, the idea was strengthened by the results of *in vitro* studies using cells of the medium tunic of human vessels. In a specific population of vascular smooth muscle cells (VSMCs), called "Calcifying Vascular Cells" (VSMCs that have been re-differentiated into osteoblast resembling cells), expression of osteoid (Giachelli et al., 1991) and osteogenic factors such as BMP and MGP (Bostrom et al., 1993; Shanahan et al., 1994) has been observed.

In regard to histological structure, the bone resembling formations of atherosclerotic lesions have sculpted features similar to resorptive sites in bone, called Howship's sulci. In bone, mineral resorption occurs by osteoclasts, large cells with multiple nuclei and abundant cytoplasm, deriving from monocytes of hematopoietic lineage and from differentiated macrophages exposed to inorganic minerals (Merkel et al., 1999). Atherosclerotic plaque, being rich in monocytes and macrophages (Gerrity and Naito, 1980)

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allocates an inexhaustible source of preosteoclasts. Maturation of these cells in osteoclasts requires the presence of two cytokines: the Monocytes Colony-Stimulating Factor (M-CSF) and the RANKL (Matsuzaki et al., 1998), being also detected in the atherosclerotic lesions (Clinton et al., 1992; Rosenfeld et al., 1992). Along with their presence in deconstruction of bone formations, macrophages might indirectly contribute to vascular calcification by producing cytokines and lipid oxidation products.

The precise mechanism of vascular calcification still remains unknown. However, *in vivo* and *in vitro* studies have shown that the OPG/RANKL/RANK axis participates in that process, but it has not been determined whether its contribution is beneficial or harmful. In the present review the latest knowledge regarding OPG/RANKL/RANK axis participation on vascular disease, including atherosclerosis, will be indicated.

Pathophysiology, structure and function of OPG and RANKL

OPG was isolated simultaneously by two different laboratories, and three synonyms, such as Osteoclastogenesis Inhibitory Factor (OCIF) (Yasuda et al., 1998), TNF-receptor Related Molecule-1 (TR1) (Kwon et al., 1998) and Follicular Dendritic Cell-derived Receptor-1 (FDCR) (Yun et al., 1998), have been eventually suggested. According to the American Society of Bone and Mineral Research Committee (ASBMRC), the use of the term OPG has been decided, as it implies its bone protective characteristics.

The human gene of OPG has been mapped and cloned, being located on chromosome 8q 23-24 and consisting of five exons (Hilton et al., 2001). Its activation begins between the 8th and 9th day of embryogenesis (Mizuno et al., 1998). OPG, member of the TNF-R superfamily, is a soluble glycoprotein consisting of 380 amino acids. It exists in two forms: as a monomeric of 60 KDa, and as homodimeric form linked with disulfide bond of 120 KDa, which is the active one (Yamaguchi et al., 1998). OPG consists of seven structural regions. (Fig. 1) Regions 1-4 of amino-terminal cystein rich domain resemble the extracellular structural regions of other members of the TNF-R family (Baker and Reddy, 1998). These regions are considered to be responsible for the downregulation of osteoclastogenesis. Regions 5 and 6 of carboxy-terminal (COOH) domain contain structures similar to those found in the cytoplasmic region of apoptosis mediators such as TNF-R1, DR3, CD95/Fas and TNF-related Apoptosis Inducing Ligand (TRAIL) receptors (Chinnaiyan et al., 1996; Itoh and Nagata, 1993; Walczak et al., 1997). Indeed, OPG regions 5 and 6 appear to transmit an apoptotic message when expressed in the protein complex of OPG/Fas, in which the transmembrane region of Fas enters between regions 4 and 5 of OPG (Yamaguchi et al., 1998). However, the members of TNF superfamily, which contain in their molecule regions that are related to apoptosis, have the ability to stimulate alternative signaling pathways, thus anticipating rather than promoting apoptosis (Lee et al., 1998). Finally, OPG region 7 includes a heparin-binding segment, a common feature of peptide growth factors (Leung et al., 1989; Eriksson et al., 1991; Zhang et al., 1991) and a cystein residue essential for disulfide bond formation and dimerization (Yamaguchi et al., 1998). (Fig. 1)

OPG is produced by an abundance of tissues, including the cardiovascular system, lungs, kidneys, bone, small and large intestine as well as by hematopoietic and immune cells (Simonet et al., 1997; Tan et al., 1997; Yun et al., 1998). In bone tissue, OPG is produced by osteoblasts, while in vessels by endothelial cells and VSMCs. Molecules that upregulate the excretion of OPG by osteoblasts are: cytokines IL-1α, IL-6, IL-11, IL-17, IL-18, TNF-α, TNF-ß, BMP-2, estrogens, calcium, vitamin D3, angiotensin II and platelet derived growth factor (PDGF) (Takai et al., 1998; Vidal et al., 1998; Hofbauer et al., 1999; Brandstrom et al., 2001; Collin-Osdoby et al., 2001; Makiishi et al., 2001; Saika et al., 2001; Wan et al., 2001). On the other hand, parathyroid hormone, glucocorticoids, prostaglandin E2, immunosuppressant medicines, peroxisome proliferators activated receptor (PPAR-γ) and basic fibroblast growth factor (bFGF) downregulate OPG production (Vidal et al., 1998; Nakagawa et al., 1999; Onyia et al., 2000; Brandstrom et al., 2001; Hofbauer et al., 2001).

RANKL also belongs to the TNF superfamily. It is a transmembrane glycoprotein of 316 amino acids with a molecular mass of 38 KDa, and its extracellular region forms a trimer (Hofbauer and Heufelder, 2001; Schoppet et al., 2002; Walsh and Choi, 2003; Hofbauer and Schoppet, 2004; Sattler et al., 2004). RANKL also exists in a soluble form, which is either excreted by T-lymphocytes or is extracted proteolytically (through metalloproteinases action). RANKL was initially recognized as a cytokine with the ability to stimulate T-lymphocytes and dendritic cells, and for this reason the term TNF-Related Activation-Induced Cytokine (TRANCE) was suggested (Wong et al., 1997). RANKL

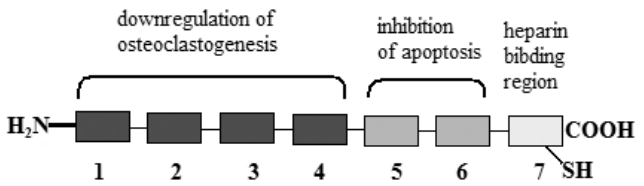

Fig 1. Functional structure of OPG: The regions 1-4 of amino-terminal cystein rich domain are responsible for the inhibition of osteoclastogenesis. The regions 5-6 of carboxy-terminal domain resembles cytoplasmic region of apoptosis mediators and region 7 includes a heparin-binding segment.

was cloned simultaneously by two different research groups, and due to its ability to promote differentiation, function and survival of osteoclasts, was named Osteoclasts Differentiation Factor (ODF) (Yasuda et al., 1998) and Osteoprotegerin Ligand (OPGL) (Lacey et al., 1998). The gene responsible for OPGL is located on chromosome 13q 14. The TNF family mediates an abundance of biological phenomena; it participates in the process of inflammation, in organogenesis, in the response of the immune system to exogenous and endogenous antigens and in the apoptotic process. RANKL is also involved in the development and the function of the bony tissue, the immune and, potentially, the cardiovascular system.

RANKL is produced by stromal cells, osteoblasts of the reticular bony tissue, cells of the cardiovascular system and activated T-lymphocytes (Hofbauer and Heufelder, 2001; Schoppet et al., 2002; Walsh and Choi, 2003; Hofbauer and Schoppet, 2004; Sattler et al., 2004). RANKL is upregulated in osteoblasts and stromal cells by cytokines IL-1_, IL-6, IL-11, IL-17, TNF-α, calcium, vitamin D3, parathyroid hormone, glucocorticoids, prostaglandin E_2, and immunosuppressive drugs and is downregulated by TGF-ß (Kong et al., 2000).

Actions of OPG and RANKL

Role of OPG and RANKL in skeletal and immune system

RANKL acts via its binding to RANK, a transmembrane receptor on the surface of monocytes/macrophages including dendritic cells, osteoclasts and their precursor cells. Post binding, RANKL induces intracellular signals that regulate cell differentiation, function and survival (Hofbauer and Heufelder, 2001; Schoppet et al., 2002; Walsh and Choi, 2003; Hofbauer and Schoppet, 2004; Sattler et al., 2004). RANKL actions are suspended by OPG, which acts as a soluble decoy receptor to prevent RANKL/RANK binding (Hofbauer and Heufelder, 2001; Schoppet et al., 2002; Walsh and Choi, 2003; Hofbauer and Schoppet, 2004; Sattler et al., 2004). Apart from RANKL, OPG binds and neutralizes the actions of pro-apoptotic factor TRAIL. In bone marrow, RANKL is produced by osteoblasts and osteoblastic fibroblastic stromal cells. Osteoclast precursor cells exposed to M-CSF and RANKL are able to differentiate into mature osteoclasts within a week (Lacey et al., 2000). The presence of M-CSF and RANKL is necessary and sufficient for this process (Lacey et al., 1998; Yasuda et al., 1998; Suda et al., 1999), while only RANKL is required to activate mature osteoclasts and bone resorption (Burgess et al., 1999). The RANKL/OPG ratio determines bone remodeling and bone mass. Mechanistically, most osteotropic signals act through changing the expression of RANKL and OPG (Hofbauer and Heufelder, 2001; Schoppet et al., 2002; Walsh and Choi, 2003; Hofbauer and Schoppet, 2004; Sattler et al., 2004). It has been shown by in vivo studies that injecting RANKL into normal adult mice can increase the size of osteoclasts and their bone-absorbent ability, resulting in systemic hypocalcaemia (Lacey et al., 1998). Disruption of the actions of RANKL inhibits osteoclast formation and function and results in extensive osteopetrotic phenotype (Kong et al., 1999). On the other hand, transgenic expression of OPG in mice causes osteopetrosis, while mice expressing $OPG^{-/-}$ genotype present extensive osteoporosis and catabolism of the bony tissue due to the unhindered action of RANKL to stimulate osteoclast formation, activity and survival (Bucay et al., 1998). The observation that these mice also present calcification of the medium tunic of aorta and renal arteries appeared interesting (Bucay et al., 1998). OPG administration in animals prevents bone loss due to lack of estrogens or tumor-induced osteolysis (Hofbauer and Schoppet, 2004).

Apart from regulating bone density, RANKL and OPG are essential for dendritic cell functions, lymph node and T-lymphocytes development (Dougall et al., 1999; Kong et al., 1999; Walsh and Choi, 2003). Binding of T-cells' RANKL to dendritic cells' RANK promotes dendritic cell survival and their ability to produce cytokines, and stimulates T-cells' activation, proliferation and survival (Walsh and Choi, 2003). RANKL is involved in antigen surveillance, T-cell memory formation, immunogenic tolerance and autoimmune disease prevention. Increased RANKL production by activated T-lymphocytes is related to bone and joint destruction in an experimental model of adjuvant arthritis in mice (Kong et al., 1999). OPG deficient mice presented disturbance in B-cell maturation and antibody response (Yun et al., 2001). In addition, mice lacking RANKL or RANK have had defective maturation of T- and B-cells and appearance of lymph node development (Bucay et al., 1998; Kong et al., 1999; Dougall et al., 1999).

The OPG/RANKL/RANK axis is undoubtedly of central importance in regulating immune and skeletal tissue. Recent data revealed that the vascular system is also involved in this axis. Vascular endothelial cells are leading coordinators of inflammatory response, and immune-mediated mechanisms are involved in an abundance of vascular diseases, including vascular calcification. Moreover, vascular calcification may involve differentiation of osteogenic cells from VSMC or calcifying vascular cells, expression of multiple ossification-related molecules, formation of calcified structures resembling bone, and attendance of T-cells, macrophages and endothelial cells, which may constitute the source or target of OPG/RANKL/RANK actions. It seems possible that the OPG/RANKL/RANK axis exerts an important role in the vascular system through immunobiologic and osteogenetic mechanisms.

Cross-correlation of vascular calcification with ossification and osteoporosis

The idea that vascular calcification is related to

ossification was presumed to be due to the interactions of vascular and bone cells during the embryonic development of the skeletal system. Bone formation depends on an underlying vascular architecture. During embryonic development, endochondrial ossification is followed by invasion of neoangiogenic vessels into calcified cartilage parenchyma. At a later stage, preosteoblasts, emanating from vasculature pericytes or mesenchymal blood cells, initiate the mineralization of osteoid (Gerber and Gerrara, 2000). Neoangiogenesis is essential for fracture healing along with embryogenesis.

Vascular calcification appears to be closely related to chronic inflammatory atherosclerosis. The mechanism of ectopic ossification and chronic inflammation is not completely clarified. It is likely that activated immune cells migrate to sites of inflammation and release cytokines and generates free radicals, both participating in increased lipid oxidation in the arterial wall (Fiore and Serhan, 1990). In a later stage these factors may modify osteogenic regulatory genes, influencing the differentiation of mesenchymal cells. For example, cytokines and oxidized lipids promote the osteoblastic differentiation of calcifying vascular cells (CVCs) (Tintut et al., 2000, 2003; Romanov et al., 2003) (Fig. 2).

Vascular calcification and osteoporosis usually occur at the same time, presenting similar risk factors, such as age, chronic renal failure, inflammatory diseases, use of glucocorticoids and estrogen deficiency (Parhami et al., 1997; Min et al., 2000; Glass and Witztum, 2001; Hofbauer and Schoppet, 2001, 2004; Kiel et al., 2001; Sattler et al., 2004). Osteoporotic patients present more often with vascular calcification and, in plain radiographs, calcium deposits in the aortic wall can be noted in the presence of osteopenic vertebrae (Parhami et al., 1997; Min et al., 2000; Glass and Witztum, 2001; Hofbauer and Schoppet, 2001; Kiel et al., 2001). Epidemiologic studies confirmed an increased rate of osteoporosis in individuals presenting atherosclerosis, cardiovascular diseases or calcified aorta. In fact, the degree of atherosclerotic plaque calcification approaches bone loss, leading to the transport of resorbed bone calcium into the arterial wall (Hak et al., 2000; Hofbauer and Schoppet, 2001). This process seems to involve OPG as a basic ligand between bone tissue and the vascular system (Hofbauer and Heufelder, 2001; Hofbauer and Schoppet, 2001).

In vivo observations

Experimental animal studies

Besides osteoporosis, two thirds of OPG$^{-/-}$ mice also presented medial calcification of renal and aortic arteries in sites where endogenous OPG is normally expressed (Bucay et al., 1998; Min et al., 2000). This implies that OPG exerts a certain protective role *in vivo* and represents the link between osteoporosis and vascular calcification (Bucay et al., 1998; Hofbauer and

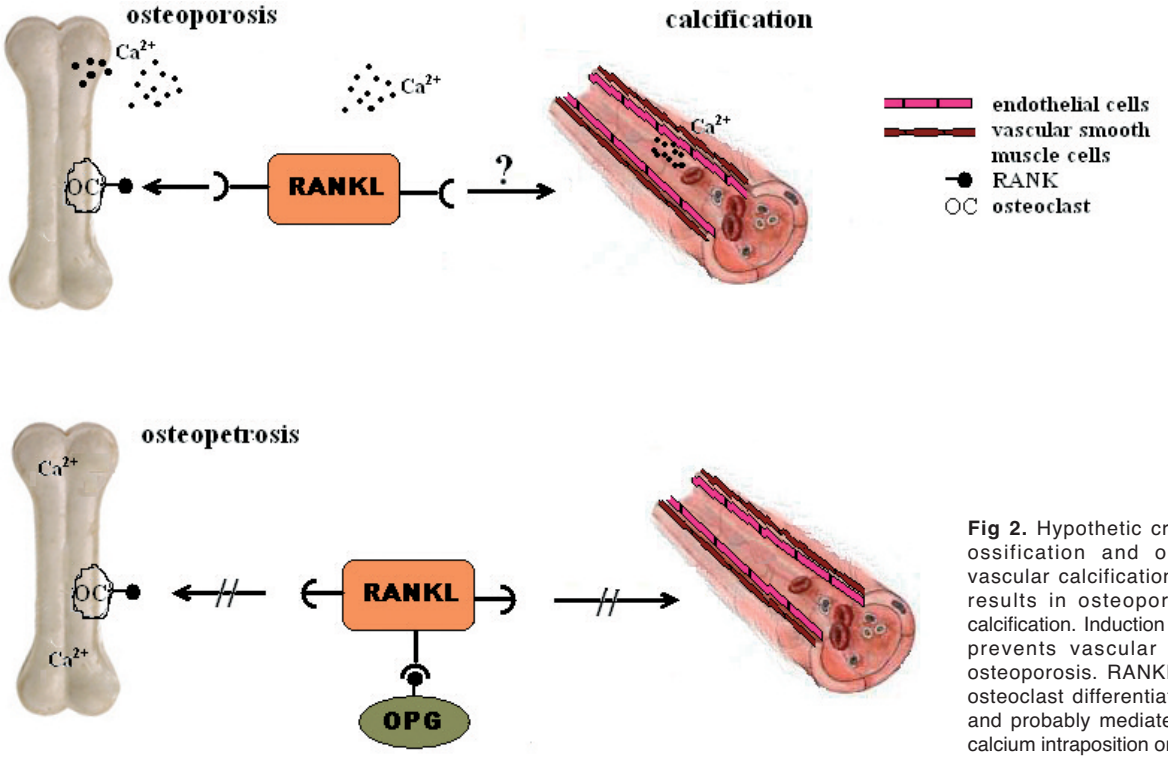

Fig 2. Hypothetic cross-correlation of ossification and osteoporosis with vascular calcification. OPG deficiency results in osteoporosis and arterial calcification. Induction of OPG expression prevents vascular calcification and osteoporosis. RANKL is necessary for osteoclast differentiation and activation and probably mediates or even induces calcium intraposition on arterial wall.

Heufelder, 2001; Hofbauer and Schoppet, 2001). Calcified arteries of OPG-/- mice express RANKL and RANK, proteins that were not detectible in normal vasculature, whereas close to calcifying vascular cells that produce RANKL, multinucleated RANK+ osteoclast-like cells were noted (Min et al., 2000).

Retrospective studies have shown that transgenic administration of OPG to OPG-/- mice from midgestation through adulthood prevents vascular calcification, while postnatal administration of OPG cannot reverse it once it has occurred (Min et al., 2000). However, both treatments were sufficient in reversing osteoporosis (Min et al., 2000).

It has been reported that parenteral administration of OPG prevents vascular calcification caused by warfarin or high doses of vitamin D treatment, without reducing serum calcium or phosphate levels (Price et al., 2001). Vascular calcification was also observed in the matrix GLA protein (MGP) knockout mice. MGP is a protein produced by VSMCs and chondrocytes and is found in vessel and cartilage extracellular matrix. It has been suggested that MGP may protect the arterial wall by inhibiting BMP-2 (Price et al., 2000; Wallin et al., 2000; Rutsch and Terkeltaub, 2003).

Additionally, mice with mutation in gene klotho, responsible for glycolipid breakdown, developed arterial medial calcification and osteopenia, presenting increased serum OPG levels (Yamashita et al., 2002). Endogenous klotho protein provides cardiovascular protection mediated by nitric oxide (Saito et al., 1998).

Clinical studies

Endothelial cells and VSMCs normally produce OPG, whereas its levels are particularly increased in aorta and renal arteries. On the other hand, RANKL and RANK are not detected in normal vasculature (Min et al., 2000; Saika et al., 2001; Schoppet et al., 2002, 2004; Kaden et al., 2004). Small quantities of RANKL have been detected in initial stages of atherosclerosis, whereas in advanced lesions and calcified vessels and valves OPG remained constant, or decreased slightly, while the expression of RANKL and RANK was simultaneously increased. In atherosclerotic lesions, OPG was located around calcified bone-like structures, and RANKL was associated with the adjoining matrix (Dhore et al., 2001) (Table 1).

Blood serum OPG levels are supposed to increase in individuals with vascular calcification and coronary disease (Schoppet et al., 2003), myocardial infraction (Ueland et al., 2004), diabetic microangiopathy (Browner et al., 2001; Knudsen et al., 2003; Schoppet et al., 2003; Anand et al., 2006), peripheral arterial disease (PAD) (Ziegler et al., 2005; Moran et al., 2005) and postmenopausal women with osteoporosis. In individuals undergoing myocardial infarction, serum OPG levels were increased in the acute phase, whereas in the follow-up a progressive reduction of these OPG levels was reported. On the contrary, OPG levels remained increased even after the acute phase in those who passed away (Jono et al., 2002; Ueland et al., 2004; Avignon et al., 2005). Higher serum OPG levels were also detected in symptomatic carotid artery stenosis patients compared to asymptomatic ones (Golledge et al., 2004; Kiechl et al., 2004; Vik et al., 2007). In patients with complications of diabetic microangiopathy significantly increased plasma OPG concentrations have been referred (Browner et al., 2001; Knudsen et al., 2003; Schoppet et al., 2003; Anand et al., 2006). Patients with clinical stage III or IV PAD present increased OPG plasma concentrations in comparison to those without ischemic ulcerations (Moran et al., 2005; Ziegler et al., 2005). Statistical analysis of serum OPG levels *in vivo*, proved serum OPG as an independent prognostic factor

Table 1. Clinical data regarding OPG and RANKL in vascular diseases.

Disease states	Results	References
Diabetic microangiopathy	• increased plasma OPG levels in diabetic type II patients • more intense increased plasma OPG levels in patients with microvascular complications	Knudsen et al., 2003; Schoppet et al., 2003; Anand et al., 2006; Browner et al., 2001
CAD and AMI	• the increased plasma OPG values were higher in the CAD than in the non-CAD group of patients with persistently higher plasma OPG levels in non-survivors	Ueland et al., 2004; Avignon et al., 2005; Jono et al., 2002
Mönckeberg sclerosis	• OPG mRNA was evident in the vicinity of calcified areas in the medial layer • RANKL protein and mRNA were barely or not detectable	Schoppet et al., 2004
Carotid stenosis	• increased OPG and OPN expression levels on endarterectomy specimens removed from symptomatic patients • no difference in RANKL expression in relation with patients' symptoms	Kiechl et al., 2004; Golledge et al., 2004; Vik, 2007
PAD	• increased plasma OPG levels in patients with PAD with clinical stages III–IV compared to those noted in patients without ischemic ulcerations	Ziegler et al., 2005; Moran et al., 2005

OPG: osteoprtegerin, CAD: carotid artery disease, AMI: acute myocardial infarction, OPN: osteopontin, RANKL: receptor activator of nuclear factor κB ligand, PAD: peripheral arterial disease.

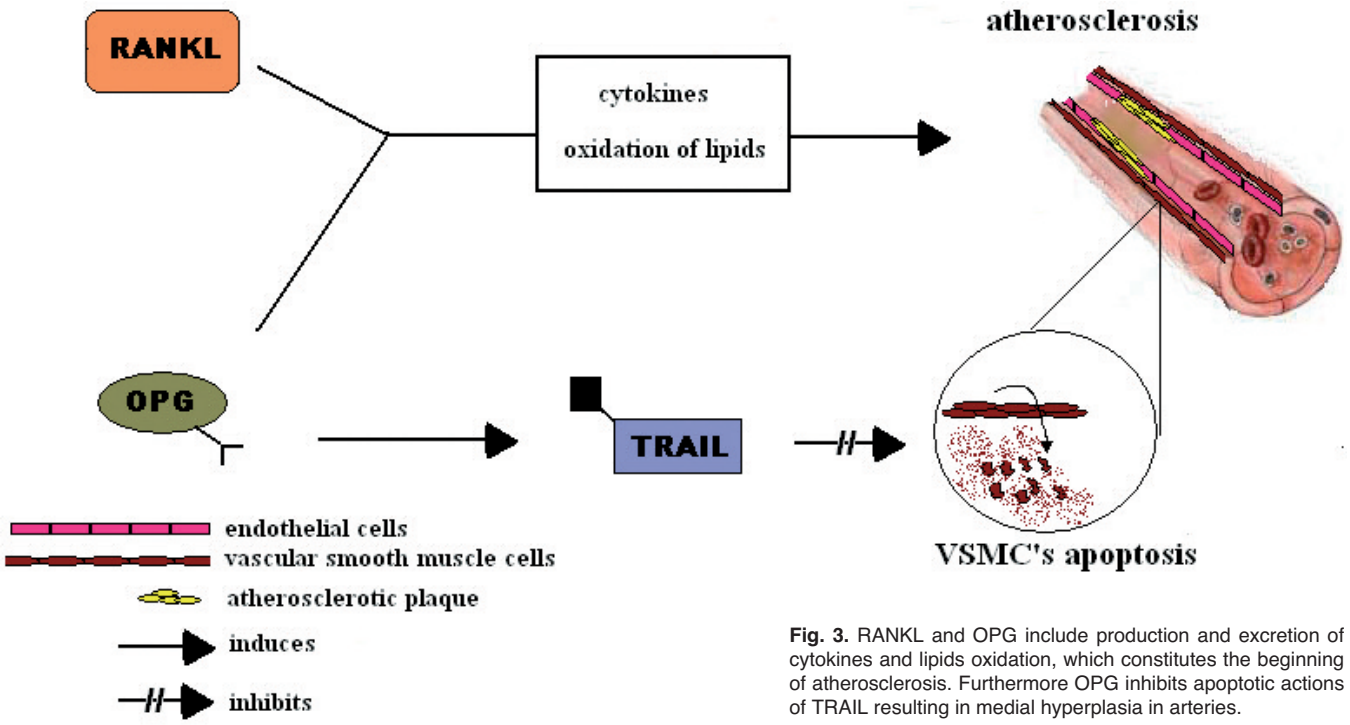

Fig. 3. RANKL and OPG include production and excretion of cytokines and lipids oxidation, which constitutes the beginning of atherosclerosis. Furthermore OPG inhibits apoptotic actions of TRAIL resulting in medial hyperplasia in arteries.

for cardiovascular disease (Schoppet et al., 2003).

As has been mentioned above, individuals with cardiovascular diseases present elevated serum OPG levels, and this is a predictor of lesion advance. Elevation of OPG is in contrast to its protective role upon the arterial wall by inhibiting osteoclast and endothelial cell apoptosis. Is the increase of serum OPG levels the cause or the result of vascular calcification?

Serum OPG levels' elevation may be the result of the human organism's effort to protect the vascular tissue after the initiation of the atherosclerotic process - measurements in experimental studies concern total serum OPG level and not the free one, therefore this increase is probably due to the binding of OPG to RANKL and TRAIL. OPG levels may also be increased secondarily, due to the inflammatory cytokines produced in atherosclerosis (Fig. 3). The simultaneous determination of OPG and RANKL levels in both atherosclerotic lesions and serum should help to delineate the participation of these molecules in atherosclerotic process.

Conclusions

The TNF superfamily members OPG/RANKL/RANK are undoubtedly mediators of bone mineralization. In the vasculature, OPG is normally produced by ECs and VSMCs, and RANKL is only present at calcified vessels, at high concentrations. $OPG^{-/-}$ mice present arterial medial calcification and osteoporosis, while transgenic administration of OPG to them reverses this process. In addition, administration of OPG prevents experimentally induced vascular calcification. It becomes evident that the mechanisms related to osteoporosis resemble those of arterial calcification, both presenting a common participation of the axis OPG/RANKL/RANK.

In most osteoporotic patients with vascular calcification, radiographs reveal that calcium deposits in the aortic wall are observed exactly in front of osteoporotic and degenerated vertebrae.

In vascular diseases, serum OPG levels are increased and seem to correlate to symptoms and advance of lesions. Additionally, after acute myocardial infraction, in symptomatic carotid artery stenosis and degenerative aortic aneurysm an increased level of OPG has been noted. OPG concentrations on arterial atherosclerotic plaque are proportional to histological lesion advance. Serum OPG levels seem to be an independent prognostic factor for cardiovascular diseases.

Moreover, OPG effects may differ depending on the stage of atherosclerotic lesion. In early stages OPG may be increased in order to protect vessels, by activating inflammatory pathways, in the effort to compensate vasculature damage. As the lesion progresses, OPG may become injurious to the vessels or is just unable to reverse the procedure of calcification. The role of OPG in plaque stability remains still unknown. Localized calcification process results in areas of different stiffness across the plaque that renders it more fragile.

The anti-apoptotic functions of OPG also seem to be contradictory. Factors that contribute to the process of atherosclerosis are apoptotic elements that constitute the nucleus around which inorganic minerals are deposited. Binding of OPG to the anti-apoptotic factor TRAIL inhibits the above mechanism. On the other hand, supression of apoptosis in VSMCs can reinforce the arterial medial calcification.

Existing data suggest the beneficial effects of the OPG on the arterial wall. However, there are still numerous queries that need to be solved. Why do serum OPG levels increase in vascular calcification? Are they the result or the cause of the process? How can that affect clinical assessment, treatment or even prevention? Further studies and experimental models are required to clarify the participation of each molecule of the OPG/RANK/RANKL axis in vascular atherosclerotic calcification, especially the measurement of free OPG serum levels.

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Accepted September 28, 2007