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Osteoprotegerin, vascular calcification and atherosclerosis

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Abstract

The association of bone pathologies with atherosclerosis has stimulated the search for common mediators linking the skeletal and the vascular system. Since its initial discovery as a key regulator in bone metabolism, osteoprotegerin (OPG) has become the subject of intense interest for its role in vascular disease and calcification. Studies *in vitro* and in animal models suggest that OPG inhibits vascular calcification. Paradoxically however, clinical studies suggest that serum OPG levels increase in association with vascular calcification, coronary artery disease, stroke and future cardiovascular events. This has led to extensive debate on the potential of OPG as a biomarker of vascular disease. However the exact significance and mechanisms by which this bone-regulatory protein influences cardiovascular pathophysiology is still unclear. The need for a more complete picture is being addressed in increasing valuable research indicating OPG as not only a marker but also a mediator of vascular pathology modulating osteogenic, inflammatory and apoptotic responses. By integrating the results of recent experimental research, animal models and clinical studies, this review summarises the present understanding of the role of OPG in vascular disease and calcification.

Keywords

osteoprotegerin; vascular disease; calcification; inflammation; apoptosis

Introduction

A large number of studies have demonstrated a relationship between bone pathology and vascular disease. The coexistence of osteoporosis and features of atherosclerosis, particularly vascular calcification, has been consistently demonstrated and is most prevalent in postmenopausal women and elderly people^{1–5}. These observations suggest that there are common pathways which negatively affect bone metabolism and the vasculature. New insights in this field are emerging since the discovery of osteoprotegerin (OPG) in 1997 as a key regulator in bone turnover^{6–8}.

In a mouse model, deficiency of OPG (OPG^{-/-}) resulted in severe osteoporosis but also the unexpected phenotype of vascular calcification⁹. Since this combination of osteoporotic bone loss and arterial mineral accumulation mirrors similar associations seen in patients, OPG was suggested as a key link between bone and vascular disease¹⁰. Although most animal studies

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support a protective role for OPG in the vasculature ¹¹, observational studies in patients have paradoxically shown a positive association between serum OPG levels and clinical cardiovascular disease ¹². Whether elevated OPG is simply a marker of vascular damage, represents a counter-regulatory mechanism aimed to limit vascular disease or actively mediates disease progression is not immediately clear. To understand its complete mode of action on the interplay between bone and vascular disease, the pleiotrophic and reciprocal relations between OPG and calcification, inflammation and apoptosis as well as those of its ligands must be taken into consideration.

The OPG/ RANK/ RANKL/TRAIL system

OPG is a member of the tumor necrosis factor (TNF)-related family and part of the OPG/ receptor activator of NF- κ B ligand (RANKL)/ receptor activator of NF- κ B (RANK) triad. This cytokine network regulates the differentiation and activation of osteoclasts and hence the critical balance between bone formation (osteoblasts) and bone resorption (osteoclasts). RANKL expressed on osteoblastic, stromal and T cells binds to RANK on the surface of osteoclasts, monocytic and dendritic cells ¹³. RANKL-RANK interactions initiate intracellular signalling cascades including NF- κ B required for osteoclast differentiation and activity ¹⁴. OPG is a soluble glycoprotein widely expressed in most human tissues including the bone (osteoblasts) and the vasculature (endothelial and vascular smooth muscle cells, VSMC) ^{10, 15,16}. By acting as a soluble decoy receptor competing for RANKL, OPG prevents RANK-RANKL interactions and thus osteoclast differentiation and bone resorption ⁶. Besides RANKL, OPG is also able to bind and neutralise the pro-apoptotic actions of TNF-related apoptosis-inducing ligand (TRAIL) expressed by VSMC and T cells ¹⁷⁻¹⁹. Additional roles in immunological responses include the RANK-RANKL binding between dendritic and T cells which enhances the immunostimulatory capacity of dendritic cells and T cell proliferation ²⁰. Furthermore, OPG is critically involved in efficient antibody responses and B cell maturation ²¹.

OPG and vascular disease: lessons learned from animal models

Vascular medial calcification

A study of OPG^{-/-} mice generated on a mixed genetic background provided the first evidence for a role of OPG in the vasculature since surprisingly, 2/3rd of the animals displayed medial calcification of the renal arteries and aorta ⁹. According to the passive theory, such calcification could result from arterial accumulation of matrix products liberated from uncontrolled osteoclast degradation of bone. Interestingly however, these arteries are sites of endogenous OPG expression in normal mice suggesting that OPG has an additional protective role against pathological calcification within the vasculature ^{9,22}. The onset of arterial calcification in OPG^{-/-} mice could be completely prevented by transgenic OPG delivered from mid gestation through adulthood. In contrast, post-natal intravenous injection of recombinant OPG had no effect on the incidence of vascular calcification suggesting that OPG cannot reverse the calcification process once it had occurred ²². Further studies using OPG^{-/-} mice produced on a pure C57BL/6 background have suggested that aortic calcification is more limited than originally proposed (unpublished studies in our laboratory) and induced calcification models have been established to gain further insight into the role of OPG in vascular calcification ²³. Compared to OPG^{+/+} controls, high phosphate diet and 1 α ,25-dihydroxyvitamin D3 treatment in OPG^{-/-} mice augmented severe medial calcification which co-localised with vascular smooth muscle cells (VSMC) ²³. Neither apoptosis nor macrophage infiltration were observed in areas of calcification. The correlation between increased aortic alkaline phosphatase (ALP) activity, a crucial initiator of mineralisation, and the aortic calcification area in OPG^{-/-} mice suggested an anti-calcification role of OPG through downregulation of ALP activity. In

accordance with these findings, a rat model showed the ability of OPG administration to prevent vascular calcification induced by warfarin or high vitamin D doses ²⁴.

Atherosclerotic progression and calcification

Although the renal arteries and aorta are common sites of calcification in atherosclerosis patients, the calcification in OPG^{-/-} mouse arteries occurred in the absence of fat deposition or atherosclerotic-like plaques and involved medial calcium deposition rather than the intimal calcification observed in atherosclerosis ⁹. To examine the role of OPG in atherosclerosis, Bennet et al. generated mice deficient in both OPG and Apolipoprotein E (OPG^{-/-} ApoE^{-/-}). Compared to OPG^{+/+} ApoE^{-/-} mice, the additional inactivation of OPG resulted in increased atherosclerotic lesion size and calcification at 40 and 60 weeks. This suggests that OPG protects against advanced atherosclerotic lesion progression and calcification in older ApoE^{-/-} mice¹¹. In another recent study by Morony and colleagues, treatment of atherogenic diet-fed mice deficient in low density lipoprotein receptor (LDLR^{-/-}) with recombinant OPG significantly reduced the calcified lesion area and mineralisation marker osteocalcin but without affecting the atherosclerotic lesion size or vascular cytokines ²⁵. The disparate results of these study models might be explained by different influences of excessive exogenous OPG compared to endogenous OPG deficiency. If OPG plays a protective role against atherosclerosis, it is possible this effect only requires small endogenous concentrations without additional benefit from exogenous delivered OPG. Moreover, OPG deficiency only seemed to exert its vascular effects at older ages (>40 weeks) whereas the LDLR^{-/-} mice received OPG treatment for a maximum of 5 months. Moreover, the increased atherosclerotic progression in OPG deficient mice could be a consequence of enhanced calcification itself driving inflammatory processes in the absence of a direct role for OPG ²⁶.

The model of Morony and colleagues would appear more clinically relevant since excess OPG is typically present in patients with atherosclerosis. These investigators also noted that there were increased serum OPG levels in the untreated LDLR^{-/-} mice following commencing an atherogenic diet. The concentrations of OPG did not increase further with progression of atherosclerosis which suggested that OPG was a marker of onset rather than a mediator of progression of atherosclerosis. In conclusion, the data from animal models uniformly support an anti-calcification role for OPG, protecting against (induced) medial calcification as well atherosclerotic calcification. However, the role of OPG in the progression of atherosclerosis is less clear.

Human studies of OPG in vascular disease

Over recent years, numerous clinical studies have consistently reported higher serum levels of OPG in association with cardiovascular outcome including coronary artery disease (CAD), vascular calcification, advanced atherosclerosis, diabetic complications, heart failure, abdominal aortic aneurysm and cardiovascular mortality ¹². In addition to the reports outlined in an excellent review by Kiechl et al. ¹², here we summarise the main results and discuss the recent clinical studies investigating the vascular role of OPG (Table 1).

Raised serum OPG levels have been associated with the presence and the severity of coronary atherosclerosis in three cross-sectional studies of CAD patients undergoing coronary angiography ²⁷⁻²⁹. Moreover polymorphisms in the OPG gene have been linked to CAD ^{30, 31}. Elevated serum OPG concentrations have been found to correlate with the severity of peripheral artery disease ³² and heart failure ^{33,34}. In addition, circulating OPG levels appear to be higher in patients with symptomatic carotid stenosis ³⁵, unstable angina ³⁶, vulnerable carotid plaques ³⁷ and acute myocardial infarction ³⁸ compared to controls with stable atherosclerosis. Recent data from the Dallas Heart Study, a large-scale unselected population-based survey, demonstrate the increased prevalence of aortic plaque and coronary artery

calcification (CAC) across serum OPG quartiles³⁹. After adjustment for conventional risk factors, the upper OPG quartile was found to be independently associated with the prevalence and extent of CAC and aortic plaque. In addition to reports of serum OPG levels as a risk factor for the extent^{40,41} and progression⁴² of vascular calcification in hemodialysis patients, this is the first epidemiological evidence of a relation between serum OPG levels and artery calcification in an unselected population. Although not consistently found by all studies^{28, 43}, a large body of data supports the association between vascular calcification and serum OPG levels. In addition to most association studies concentrating on the coronary circulation, Clancy et al. recently reported an association between serum levels of OPG and infrarenal abdominal aorta calcification in patients with peripheral vascular disease⁴⁴. In patients with long-standing rheumatoid arthritis, a chronic inflammatory disease characterised by accelerated atherosclerosis, increased serum OPG concentrations have been independently associated with the severity of CAC⁴⁵. Interestingly, the association of elevated OPG with inflammation markers and arterial stiffness^{45,46} suggest that OPG may provide a mechanistic link between CAC and inflammation. The prospective report of Anand et al. found that of the range of biochemical markers assessed, only OPG predicted the extent of CAC and subsequent cardiovascular events in asymptomatic diabetic patients⁴⁷. In a further study, they found OPG to be a predictor of CAC progression in these patients⁴⁸.

The predictive value of serum OPG levels in the general population was first addressed in the Bruneck study. In this 10 year follow-up survey, serum OPG levels were found to represent an independent risk factor for the progression of atherosclerosis and the incidence as well as mortality from cardiovascular disease⁴⁹. The prognostic significance of increased serum OPG concentrations as a risk factor for cardiovascular mortality and morbidity has been confirmed in selected conditions of accelerated atherosclerosis such as elderly women⁵⁰, hemodialysis patients⁵¹ and diabetic subjects^{47,52,53}. In addition to predicting the survival of patients with post-infarction heart failure⁵⁴, serum OPG was found to be strongly and independent predictive for long-term mortality and heart failure development in patients with acute coronary syndromes⁵⁵. Another prospective study conducted by Moran and colleagues reported the positive association between serum OPG levels and the growth rate of abdominal aortic aneurysms⁵⁶. In patients on long-term hemodialysis⁴² as well as in asymptomatic diabetic patients⁴⁸, higher serum OPG concentrations predicted the extent and rapid progression of calcified plaques in the aorta.

To gain more insight the role of OPG in atherosclerotic development, subsequent studies have focused on its relation with endothelial dysfunction, an important early physiological event in atherosclerosis. The association between elevated serum OPG levels and impaired endothelial function, measured as decreased flow-mediated dilatation of the brachial artery (FMAD), was first demonstrated in a cross-sectional survey of type 2 diabetic patients⁵⁷. This was followed by prospective studies in newly diagnosed type 1 and type 2 diabetic patients which showed a significant correlation of the decreases in serum OPG levels with the improvements in FMAD endothelial function^{58,59}. Similar findings were recently reported in peripheral artery disease (PAD) patients by Golledge et al. which confirmed the correlation between increased serum OPG levels and decreased FMAD endothelial function⁶⁰. Also in postmenopausal women, elevated serum OPG levels were considered to be predictive for the presence of preclinical atherosclerotic damage measured as decreased FMAD and increased carotid intima media thickness⁶¹.

The possibility that OPG might be associated with ventricular function was suggested by the increased circulating and myocardial OPG concentrations found after heart failure due to ischemic cardiomyopathy^{34,53} or left ventricular (LV) pressure overload³³. In the general population, a strong and independent association was demonstrated between serum OPG levels

and indices of LV hypertrophy in male subjects and with indices of LV function in both sexes⁶².

Mechanism underlying the vascular role of OPG

The data from clinical studies consistently report an association between OPG and the presence, severity and progression of a broad range of cardiovascular diseases. Whether OPG is a marker or rather plays a causal role in mediating or protecting against vascular injury is presently unclear. The mechanisms underlying the postulated role of OPG in atherosclerosis may involve endothelial and ventricular dysfunction, inflammation and calcification. A number of investigators have studied the expression pattern of OPG/RANK/RANKL in the normal and pathological vasculature and the cellular effects of OPG in an attempt to advance understanding of the role of OPG in arterial disease.

OPG/RANK/RANKL expression

Vascular cells producing OPG include endothelial cells and VSMC whereas RANKL is mainly expressed on infiltrating T cells, activated endothelial cells and RANK is expressed on monocytic osteoclast precursors as well as dendritic cells^{12,26,63}.

Although OPG is expressed in normal mice vessels, RANK and RANKL are not detected in the arteries of normal adult mice²². Interestingly, RANK and RANKL were detected in the calcified arteries of OPG^{-/-} mice and RANK expression coincided with the presence of multinuclear osteoclast-like cells. This may indicate that vascular OPG protects against RANK-RANKL induced osteoclast formation although the presence of functional osteoclasts in the calcified arteries and their potential significance are not clear²². In the atherosclerotic arteries of ApoE^{-/-} mice, OPG staining was most apparent adjacent to foam cells, while that of RANK and RANKL was located in areas rich in T cells³⁶. Tissue concentrations of these mediators were highest in vulnerable plaque phenotypes (ApoE^{-/-} x CD4dnTβRII mice)³⁶.

In line with the mouse models, RANKL and RANK are frequently undetected in the non-diseased human vessel while OPG is expressed in a wide range of tissues including normal arteries¹⁵. Early and advanced human atherosclerotic lesions of carotid arteries^{35,64} and abdominal aortas⁶⁵ have both RANKL and OPG immunoreactivity and mRNA expression.

OPG was mainly localized adjacent to calcified neointimal regions at the margins of lamellar bone-like structures^{64,65} while RANKL was only present in the extracellular matrix surrounding calcified deposits⁶⁵. Of note is the enhanced apoptosis surrounding calcified regions together with the similar spatial distribution pattern of OPG and TRAIL⁶⁴. OPG has also been detected in the calcified areas of the medial layer in Monckeberg's sclerosis⁶⁴ and found in increased concentrations in the aortic media of diabetic subjects⁶⁶. In contrast, human aortic valves of aortic stenosis patients showed significant lower OPG-positive cells in the calcified areas compared to non-calcified regions⁶⁷. Other locations of OPG expression in the cardiovascular system include the myocardium which shows increased OPG expression in cardiomyocytes of patients with heart failure due to dilated or ischaemic cardiomyopathy^{34,68}. Strong immunostaining for OPG/RANKL/RANK was also found within thrombus at the site of plaque rupture³⁶. The enhanced T-cell expression of RANKL in unstable atheroma³⁶ and the increased tissue OPG concentrations in symptomatic carotid atherosclerosis³⁵ could suggest that the OPG/RANK/RANKL axis plays an important role in plaque destabilisation. To translate these expression profiles to the mechanisms underlying the vascular role of OPG, osteogenic, inflammatory, apoptotic and hormonal pathways affecting cellular OPG production and actions must be taken into account.

OPG and Vascular Calcification

The mechanisms underlying the inhibitory effect of OPG on vascular calcification in animal models could be passive or cellular. The potential anti-apoptotic effect of OPG by binding TRAIL reduces the number of apoptotic bodies that may serve as nucleation sites for passive mineralisation^{69,70}. On the other hand, vascular calcification is believed to be an active cell-mediated process resembling osteogenesis and involving the expression of bone-related proteins such as ALP, a crucial initiator for bone mineralisation^{65,71–75}. In addition to the possible contribution of osteoblastic progenitor cells^{76–78} and myofibroblasts⁷⁹, it is widely accepted that VSMCs are the primary target of osteogenic differentiation^{75,80–82}. In OPG^{-/-} mice, increased serum ALP was found to normalise after recombinant OPG administration²² and aortic ALP activity correlated with the calcified lesion area²³. These results suggest that OPG may inhibit the active calcification process through downregulation of ALP activity. Moreover, OPG blocks the actions of RANKL which is found increased in diseased and calcified vessels^{22,25,67} and can *in vitro* induce ALP activity and calcification in vascular cells⁶⁷. OPG deficiency has been associated with decreased aortic PTHrP, a calcium-regulating hormone reported to inhibit VSMC calcification through depression of ALP. Therefore, downregulation of ALP by OPG could be mediated by blocking RANKL or via effects on calcium-regulating hormones.

Although experimental and animal studies suggest OPG exerts anti-calcification potential, OPG concentrations are elevated in patients with calcified vessels. Possible explanations can be found in interacting pathways altering the production of OPG, for example inflammation. Pro-inflammatory mediators such as TNF- α are able to induce OPG expression in VSMC and endothelial cells^{15,66,83,84,85} but are also implicated in the development of vascular calcification by inducing ALP^{75,86,87}. In other words, agents that stimulate VSMC calcification may also stimulate OPG production, possibly as a protective mechanism attempting to counteract osteogenic or pro-apoptotic calcification mechanisms. Moreover, calcification itself in the form of calcium crystals internalised by macrophages stimulate the secretion of pro-inflammatory cytokines capable of simultaneously increasing OPG production and further enhancing osteogenic differentiation²⁶. Interestingly, for some factors such as oxidized lipids and cytokines, the net effect on calcification is positive in the artery wall while negative in bone by respectively stimulating and inhibiting the osteogenic differentiation of vascular and osteoblastic cells^{88,90}. In accordance, statins, which are reported to decrease TNF-induced OPG production in vascular cells⁹¹, inhibit the calcification of vascular cells but paradoxically stimulate bone cell calcification⁹². The mechanisms for these reverse effects on vascular and osteogenic cells are unclear but the involvement of the OPG/RANK/RANKL axis has been suggested⁹⁰. In contrast to bone, the vascular OPG/RANKL ratio increased in response to ovariectomy with a corresponding fourfold increase in arterial calcification⁹³. This suggests the importance of endocrine effects in the association of bone destruction and arterial mineralisation in postmenopausal women. Moreover since calcification is a well-known feature of atherosclerosis, the complex interactions involved will contribute to the net effect of the expression and actions of OPG in atherosclerotic calcification.

OPG and Vascular Disease

The origin of elevated OPG concentrations—The main potential sources of elevated OPG are summarised in figure 1. Since VSMC produce much higher amounts of OPG than endothelial cells⁶⁶ and are thought to play a significant role in the development of vascular calcification^{64,65,94}, VSMC are thought to be the major vascular cell producing OPG in the diseased arterial wall. However the enormous surface of the endothelium lining the circulation suggests endothelial cells are additional key cells involved in the production and release of circulating OPG in human serum. Reported close correlations between OPG and major cardiovascular risk factors such as age, HbA1c, waist-hip ratio, lipid profile, hypertension,

homocysteine and hormone levels have been suggested to provide a partial explanation for the elevated serum OPG concentrations in cardiovascular disease^{39,45,47,52,59,95–99}. However even after correction for conventional cardiovascular risk factors, serum OPG levels remained an independent risk factor for the incidence and severity of cardiovascular disease, vascular mortality, atherosclerosis, LV dysfunction and coronary calcification^{39,47,49,62}. The majority of clinical studies have reported the association of elevated serum OPG with higher levels of inflammation (C-reactive protein, erythrocyte sedimentation rate, fibrinogen) in the general population^{39,49} as well as in CAD²⁸, diabetic^{46,58,59} and rheumatoid arthritis patients⁴⁵. The ability of pro-inflammatory mediators such as TNF α , interleukin-1 and platelet-derived growth factor to enhance OPG expression and production in vascular cells may explain the association of OPG concentrations and cardiovascular diseases^{15,66,83–85}. The suggestion that OPG is a marker of inflammation is supported by its downregulation by anti-inflammatory agents such as immunosuppressants, PPAR- γ ligands and anti-TNF therapy^{63,100,101}. As inflammation affects a whole range of other organs, it is likely that OPG production in other tissues is also influenced and may contribute to elevated serum OPG in chronic inflammatory diseases such as atherosclerosis.

Improvements in endothelial function of treated diabetic patients were associated with the decrease in serum OPG levels and C-reactive protein supporting a link between endothelium, inflammation and OPG^{58,59}. Strong OPG immunostaining in the failing myocardium³⁴ as well as increased serum OPG levels in association with LV mass and function⁶² and after heart failure^{34,53} suggest an additional contribution of the myocardium to circulating OPG concentrations. Conversely, a recent study raised the possibility of a bidirectional OPG exchange by suggesting that elevated serum OPG levels augment OPG extraction by the heart and peripheral tissues³³.

Mediating role of elevated OPG in vascular disease—The role of OPG in cardiovascular disease is debated. The suggestion that increased OPG levels resulting from vascular damage represent a protective counter-regulatory mechanism is challenged by the fact that some of the actions of OPG/RANK/RANKL support a role in stimulating atherosclerosis progression and destabilisation (summarised in figure 2).

In support of a beneficial vascular role for OPG is the recent finding of accelerated atherosclerotic lesion progression in OPG deficient mice¹¹. An athero-protective effect of OPG could be related to its anti-calcification function as described above. By interrupting the positive feedback loop between osteogenic differentiation and inflammatory cytokine production, OPG could help by retarding atherosclerotic progression²⁶. Moreover, acting as a soluble decoy receptor for RANKL and TRAIL, OPG blocks binding of these mediators to their cognate receptors and subsequent pro-inflammatory and pro-apoptotic events (reviewed by Collin-Osdoby et al.¹⁵). For example, OPG has been identified as an *in vitro* survival factor for endothelial cells in part by blocking TRAIL-induced apoptosis^{102,103}. Although this may protect against endothelial injury, the role of OPG and TRAIL in VSMC apoptosis is less clear and controversial. Apoptotic death of VSMC has been proposed as a mechanism of plaque destabilisation due to VSMC depletion and insufficient apoptotic cell clearance^{19,12,104,105}. Some studies support a protective role for OPG against VSMC apoptosis by acting as a VSMC survival factor¹¹ and blocking induced VSMC apoptosis by TRAIL expressing T-cells¹⁹. In contrast, other studies report an opposite effect of OPG enhancing VSMC apoptosis⁵⁶ and TRAIL stimulating VSMC survival^{106–108}. In addition, OPG may inhibit the potential anti-inflammatory activity of TRAIL-induced apoptosis by targeting infiltrating macrophages¹⁰⁷.

OPG also influences production of other agents important in plaque stability such as matrix metalloproteinases. Incubation of VSMC and monocytic cells with OPG stimulated the expression of matrix metalloproteinases associated with matrix degradation as well as the

production of interleukin-6^{36,56}. Other mechanisms supporting a pro-atherosclerotic role for OPG include its ability to *in vitro* enhance the expression of endothelial cell adhesion molecules and subsequent infiltration of leukocytes and monocytic cells^{109,110,111}. Furthermore, OPG might contribute to endothelial dysfunction by blocking RANKL signalling which is able to activate protective intracellular endothelial pathways such as the nitric oxide synthase pathway¹¹². The early impairment of nitric oxide release is a key feature of endothelial dysfunction which invariably precedes permanent vascular alterations¹¹³. These findings could at least partly account for the relation between increased OPG serum levels with symptomatic and unstable cardiovascular disease.

Concluding remarks

Clearly, the vascular role of OPG is multifaceted and depends on the interplay with its ligands, RANKL and TRAIL, and a bidirectional modulation involving osteogenic, inflammatory and apoptotic responses.

Animal studies generally favour a protective role of OPG, particularly in terms of vascular calcification. At least a minimal amount of endogenous arterial OPG seems necessary to protect against vascular calcification. In addition to inhibiting apoptotic passive calcification, the ability of OPG to inhibit ALP-mediated osteogenic differentiation of vascular cells is also likely to contribute to the protective role of OPG.

On the other hand, elevated serum concentrations of OPG are found in a range of cardiovascular pathologies, suggesting the potential value of OPG as a biomarker of vascular risk and prognosis. Cellular studies have suggested a number of mechanisms by which such elevated concentrations of OPG could promote the progression and instability of atherosclerosis. Further animal studies of tissue-specific ablation or over-expression of OPG would be helpful to clarify the dilemma whether OPG has a dual, protective or detrimental effect on atherosclerosis development and progression.

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Figure 1.

The origin of increased OPG. The production of OPG in VSMC and endothelial cells is enhanced by inflammatory cytokines and may reflect endothelial dysfunction. Additionally, the failing myocardium, plaque rupture and other inflamed tissues could contribute to elevated circulating OPG concentrations. LV, left ventricular; MMP, matrix metalloproteases; OPG, osteoprotegerin; VSMC, vascular smooth muscle cells.

Figure 2. OPG as a mediator in vascular disease progression

ALP, alkaline phosphatase; eNOS, endothelial nitric oxide synthase; MMP, matrix metalloproteinases; OPG, osteoprotegerin; TRAIL, TNF-related apoptosis-inducing ligand; RANKL, receptor activator of NF-κB ligand; VSMC, vascular smooth muscle cells.

Table 1
Recent epidemiological studies assessing the association between serum OPG concentrations and cardiovascular pathology.

Author and year	Cohort characteristics	n	Study Design	Outcome measure	Main findings	Ref
Clancy et al. 2006	Peripheral vascular disease	109	Prospective	Abdominal aortic calcification	Significant association of OPG with abdominal aortic calcification	44
Gogo et al. 2006	Percutaneous coronary intervention	100	Prospective	Quantitative coronary angiography	No association of OPG with coronary angiographic calcification	43
Sandberg et al. 2006	Angina	100	Cross-sectional	Stable versus unstable angina	OPG was increased in unstable but not in stable angina patients	36
Shin et al. 2006	Type 2 diabetes	104	Cross-sectional	Endothelial function by FMAD	Significant association of OPG with endothelial dysfunction measured as decreased FMAD	57
Rasmussen et al. 2006	Type 1 Diabetes	291	Cross-sectional	CV disease (CHD/ stroke/ intermittent claudication)	Significant association of OPG with CV disease, systolic blood pressure and glycaemic status	52
Xiang et al. 2006, 2007	Type 1 and type 2 diabetes	50/86	Prospective	Endothelial function by FMAD	After 6 months of insulin therapy, serum OPG levels were markedly decreased. Changes in OPG showed significant correlation with the changes in FMAD which improved markedly during treatment.	58 59
Anand et al. 2006, 2007	Type 2 diabetes	510	Prospective	CAC and CV events	OPG is an independent predictor of CAC severity and significantly associated with CAC progression (30±5 months) and short term CV events (18±5 months).	47 48
Avignon et al. 2007	Type 1 and type 2 diabetes	465	Cross-sectional	SMI by stress myocardial perfusion images	Independent association of OPG with SMI in asymptomatic diabetic patients	53
Asanuma et al. 2007	Rheumatoid arthritis	244	Cross-sectional	CAC	Independent association of OPG with CAC	45
Abedin et al. 2007	Unselected population	3386	Cross-sectional	CAC and aortic plaque	Independent association of OPG with CAC and aortic plaque	39
Helske et al. 2007	Aortic stenosis	131	Cross-sectional	HF	Independent association of OPG with heart failure	33
Omland et al. 2007	General population	2715	Cross-sectional	LV structure and systolic function	Independent association of OPG with decreased LV function (both sexes) and with LV hypertrophy (males)	62
Omland et al. 2008	Acute coronary syndromes	897	Prospective	Mortality, myocardial infarction, HF, stroke	OPG is a strong predictor of long-term mortality and HF development	55
Kadoglou et al. 2008	carotid stenosis >50%	164	Cross-sectional	Carotid plaque vulnerability assessed by ultrasound gray-scale median score	OPG is independently associated with carotid plaque vulnerability	37

Author and year	Cohort characteristics	n	Study Design	Outcome measure	Main findings	Ref
Siepi et al. 2008	Postmenopausal women	195	Cross-sectional	FMAD and carotid IMT	Significant association of OPG with IMT and decreased FMAD	61
Golledge et al. 2008	Peripheral artery disease	60	Cross-sectional	Endothelial function by FMAD	Significant association of OPG and decreased FMAD	60

CAC, coronary artery calcification; CHD, coronary heart disease; CV, cardiovascular; FMAD, flow mediated arterial dilatation; HF, heart failure; IMT, intima media thickness; LV, left ventricular; OPG, osteoprotegerin; SMI, silent myocardial ischemia.