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REVIEW

Osteoprotegerin and kidney disease

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Abstract Vascular calcification in chronic kidney disease (CKD) patients is associated to increased mortality. Osteoprotegerin (OPG) is a soluble tumor necrosis factor (TNF) superfamily receptor that inhibits the actions of the cytokines receptor activator of nuclear factor kappa-B ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL) by preventing their binding to signaling receptors in the cell membrane. OPG-deficient mice display vascular calcification while OPG prevented calcification of cultured vascular smooth muscle cells and protected kidney cells from TRAIL-induced death. OPG may be a biomarker in patients with kidney disease. Circulating OPG is increased in predialysis, dialysis and transplant CKD patients and may predict vascular calcification progression and patient survival. By contrast, circulating OPG is decreased in nephrotic syndrome. In addition, free and exosome-bound urinary OPG is increased in human kidney disease. Increased urinary OPG has been associated with lupus nephritis activity. Despite the association of high OPG levels with disease, experimental functional information available suggests that OPG might be protective in kidney disease and in vascular injury in the context of uremia. Thus, tissue injury results in increased OPG, while OPG may protect from tissue injury. Recombinant OPG was safe in phase I randomized controlled trials. Further research is needed to fully define the therapeutic and biomarker potential of OPG in patients with kidney disease.

Keywords Exosome · Kidney · Vascular calcification · Osteoprotegerin · TRAIL · Mortality

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Chronic kidney disease (CKD) is present in around 8–16 % of the worldwide population [1]. CKD may evolve to end-stage renal disease (ESRD) requiring dialysis and/or transplantation. In addition, CKD is a cardiovascular risk factor [2] and both cardiovascular and non-cardiovascular mortality are increased in CKD patients [3]. Understanding the cellular and molecular basis of the adverse outcomes of CKD patients is a research priority. CKD may be associated to disease-specific cardiovascular risk factors [2], such as CKD mineral bone disorders (CKD–MBD) [4]. CKD–MBD includes bone disease, hyperparathyroidism, altered calcium and phosphate metabolism, and cardiovascular calcification. Most recent attention has focused on phosphate metabolism and disposal, and its relationship with vascular calcification [5]. Indeed, one of the few medical interventions that impacts survival in CKD patients is the choice of oral phosphate binders [6, 7]. The survival advantage of non-calcium-based phosphate binders may be

related to protection from vascular calcification [6, 7]. In this regard, vascular calcification and hyperphosphatemia are features of the accelerated aging of Klotho deficient mice; and human CKD is also characterized by Klotho deficiency, vascular calcification and hyperphosphatemia [8, 9]. Furthermore, vascular calcification is also found in human progeria [10]. From this point of view, there is increasing interest in understanding the complex mechanisms regulating vascular calcification [11–13].

Osteoprotegerin (OPG) is a soluble protein from the tumor necrosis factor (TNF) receptor superfamily named after its best-characterized effect: bone protection [14, 15]. In addition, OPG protects from vascular calcification [16]. More recent evidence has linked OPG to kidney injury. We here review the biology of OPG and the role and biomarker potential of OPG in kidney disease.

TNF superfamily of ligands and receptors

Members of the TNF receptor superfamily are usually type I transmembrane glycoproteins, with some exceptions that include OPG [17]. TNF receptor superfamily proteins share a common extracellular cysteine-rich domain, which may be repeated up to six times in a receptor monomer chain. Some members of the family present a cytoplasmic ~180 amino acid interaction domain known as the death domain. TNF receptors bind proteins of the TNF superfamily, of which the best-known member is TNF-α [18]. Both ligands and receptors trimerize to trigger intracellular signals [19, 20]. TNF superfamily receptors are usually membrane-bound, whereas ligands can be membrane-bound or soluble [21]. Most TNF superfamily members bind to a unique receptor, but there is crosstalk between certain receptors and different ligands [17]. In addition TNF receptor superfamily members may be soluble either because they are encoded as soluble proteins or because of proteolytic processing yielding a soluble protein.

OPG structure and expression

Mature OPG is a 380-amino acid soluble glycoprotein which lacks transmembrane or cytoplasmic domains. OPG is found as either a 60-kDa monomer or 120-kDa dimer linked by disulfide bonds and by noncovalent interactions mediated by death domains and, to a lesser extent, by a C-terminal heparin-binding region [22]. The dimer is more bioactive than the monomer. Both OPG dimers and monomers circulate in serum [23]. Because of its size, under physiological conditions OPG is unlikely to be filtered at the glomerulus [24]. OPG has several structural regions. Four amino terminal cystein-rich domains provide binding sites for the TNF superfamily cytokines receptor activator of nuclear factor kappa-B ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL). Two death domains are typical of TNF superfamily receptors and a carboxy-terminal basic heparin-binding domain is a common feature of peptide growth factors [25–30] (Fig. 1a). Mouse and human OPG proteins are ~85 % identical (Fig. 1b), indicating a high conservation of the OPG gene throughout evolution, suggestive of an important function [31].

The human OPG gene is located on chromosome 8q and consists of five exons. OPG is expressed in multiple adult organs and cell types, including the lung, heart, kidney, liver, spleen, stomach, intestine, skin, thymus, lymph nodes, bone marrow, osteoblasts, vascular smooth muscle cells, dendritic cells, B-lymphocytes, articular chondrocytes, brain, spinal cord, and thyroid gland [25–29]. Vascular smooth muscle cells are thought to be the main source of OPG in the vascular wall, but endothelial cells also secrete OPG [29, 32]. The widespread expression of OPG suggests that it may have poorly understood roles beyond bone biology regulation. During murine embryogenesis (day 15) in situ hybridization localized OPG to the cartilaginous parts of developing bone, the aorta, the gastrointestinal tract, and the skin [28].

Many molecules positively or negatively regulate OPG expression. Cytokines and growth factors [interleukin (IL)-1α, IL-6, IL-11, IL-17, IL-18, TNF-α, TNF-β, bone morphogenetic protein (BMP)-2, transforming growth factor (TGF)-β1, angiotensin II, pigment epithelium-derived factor (PEDF), and platelet-derived growth factor (PDGF)], bone mineral metabolism molecules (calcium, vitamin D), hormones (17β-estradiol), and Wnt signaling upregulate OPG expression. Parathyroid hormone (PTH), glucocorticoids, prostaglandin E2, immunosuppressant drugs, peroxisome proliferator-activated receptor-gamma (PPAR-γ) activators, insulin-like growth factor (IGF)-1 and basic fibroblast growth factor (bFGF) downregulate OPG [25, 26, 29, 30]. It is yet unclear which of these multiple regulators is most important in vivo or how OPG contributes to disease modulation when the regulators are overexpressed.

Functions of OPG

Osteoprotegerin is a soluble, non-signaling decoy receptor that binds RANKL and TRAIL with an affinity in the same order of magnitude, although greater for RANKL [25–27, 29, 33]. In addition, OPG binds glycosaminoglycans such as heparin, heparan sulfate, chondroitin sulfate and dermatan sulfate [30]. Binding to these molecules regulates both OPG disposal and the function of the OPG ligand.

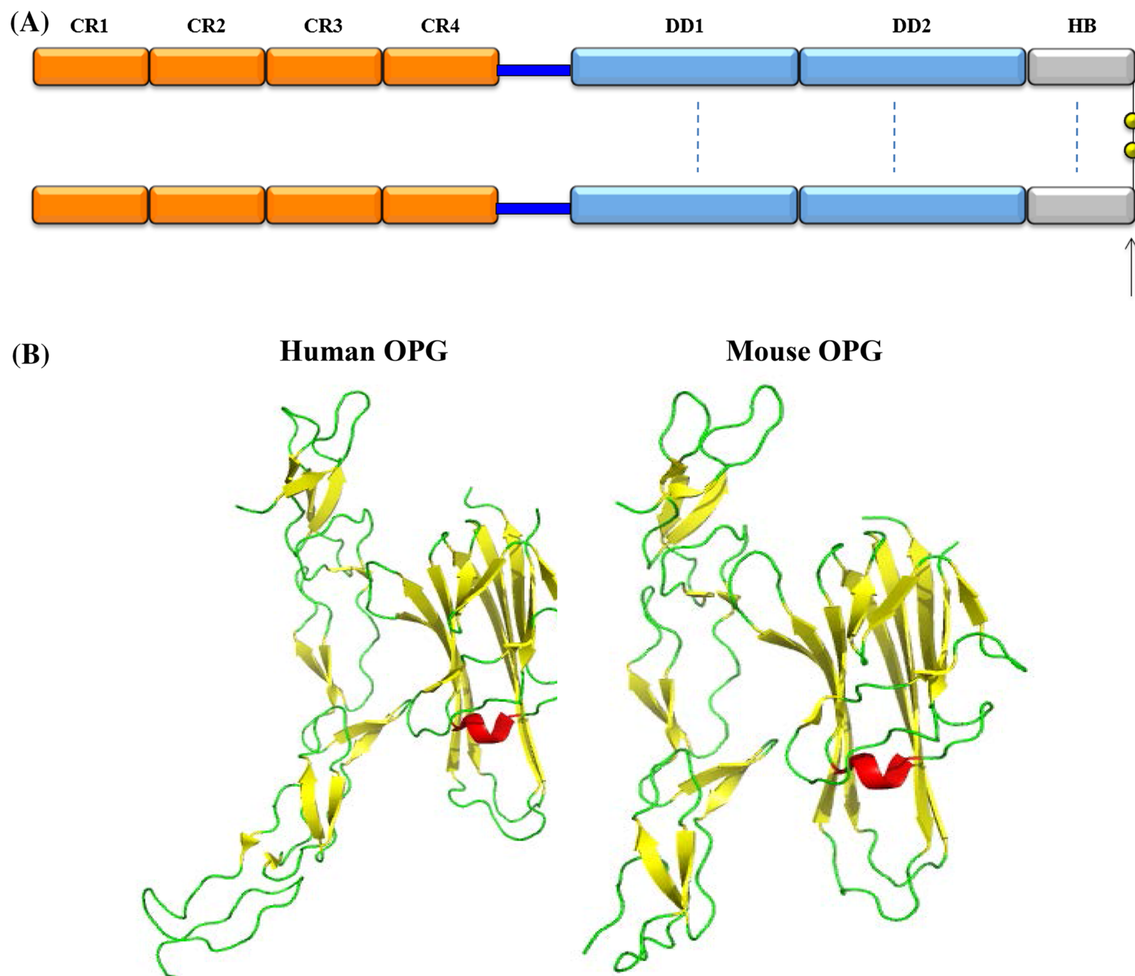

Fig. 1 Structure of osteoprotegerin (OPG). **a** Domain structure of 380-amino acid, mature OPG forming a dimer after shedding the signal peptide. Dimerization of OPG results from non-covalent interactions mediated by the death domains and to a lesser extent by a C-terminal heparin-binding region. A C-terminal intermolecular disulfide bond does not contribute to the formation or stability of OPG dimers [22]. *CR* cystein-rich domains, *DD* death domain, *HB* heparin binding region. **b** Conservation of the OPG gene throughout evolution, as seen in the similarity between mouse [35] and human [104] OPG proteins, according to http://www.rcsb.org/pdb/. Helix in *red*, loops in *green* and sheets in *yellow* (color figure online)

Thus, binding of OPG to any of these three ligands prevents binding to the others [34] providing a three-way interaction. OPG molecules lacking the glycosaminoglycan-binding domain are still active in inhibiting TRAIL binding to its cell membrane receptors [34] but are devoid of activity related to glycosaminoglycan-binding, such as adhesion of leukocytes to endothelium.

The OPG/RANKL complex is internalized either through lipid rafts by interaction with cell membrane syndecan-1 or by clathrin-coated pit formation. By contrast, binding to glycosaminoglycans competes with OPG/RANKL interaction and prevents OPG internalization [25].

The monomeric and the dimeric cytokine-binding regions of OPG bind RANKL with approximately 500-fold higher affinity than the cell membrane signaling receptor RANK [35]. Thus, OPG prevents RANKL activation of RANK and RANKL-induced activation of osteoclast maturation and function as well as bone loss [29, 36, 37].

The RANKL/RANK axis also regulates the crosstalk between the bone and the immune system. RANKL-expressing T cells can express RANKL and promote osteoclastogenesis, while OPG from B cells may counteract RANKL from T cells during the immune response [38]. OPG is also involved in the regulation of B cell maturation and efficient antibody responses [29, 39].

The affinity of TRAIL for OPG is weaker than that for other transmembrane TRAIL receptors [40]. Thus, it has been suggested that OPG interaction with TRAIL may be less physiologically significant than the interaction with RANKL. However, cell culture studies have clearly demonstrated that OPG protects from TRAIL cytotoxicity [25, 41, 42]. TRAIL is a potentially lethal TNF superfamily

protein normally expressed in many human tissues including the kidney [43]. Under physiological conditions normal parenchymal cells have protective mechanisms that prevent TRAIL-induced death. Both breast cancer cells and human kidney tubular cells release OPG that protects them from TRAIL cytotoxicity in culture [25, 41]. By contrast, in an inflammatory environment, parenchymal cells, such as kidney tubular cells, are sensitized to TRAIL-induced apoptosis [42]. However, conclusive demonstration of the functional relevance of OPG neutralization of TRAIL in vivo is lacking.

Binding of endothelial OPG to leukocyte RANKL or TRAIL may prevent endothelial cell apoptosis [44]. In addition, circulating OPG may directly interact with heparan sulfates in endothelium favoring leukocyte adhesion [45]. The pro-adhesive effect of OPG is prevented by heparin. It is unclear whether the pro-adhesive action of OPG is dependent on binding to TRAIL or RANKL in leukocytes, since OPG binding to glycosaminoglycans prevents binding to TRAIL or RANKL [34]. OPG also enhances the proangiogenic properties of endothelial cells and endothelial colony-forming cells, thus promoting vasculogenesis in vivo [46, 47]. OPG increased endothelial colony-forming cell viability and adhesion to activated endothelium under shear stress conditions. The proangiogenic properties of OPG appeared to require binding to syndecan-1, although OPG 1-194, that lacks the heparin-binding domain, still had pro-vasculogenic effects [47]. OPG also promoted pulmonary artery smooth muscle cell proliferation and migration and has survival factor activity for serum-deprived vascular smooth muscle cells, potentially contributing to vascular injury [48, 49].

Genetically modified mice have provided further clues as to the key roles of OPG in vivo [16, 31, 50] (Table 1). Overexpression of OPG is associated to increased bone density (osteopetrosis), while targeted deletion of OPG results in severe premature osteoporosis [51]. Osteoporosis in OPG-deficient mice was explained by unrestricted RANKL promotion of osteoclast activity and bone resorption. Interestingly, OPG deficiency also results in calcification of the aorta and renal arteries. OPG−/−·ApoE−/− mice had more atherosclerotic plaques and calcified vascular lesions than OPG+/+·ApoE−/− controls [48, 49], indicating that OPG protects from atherosclerosis and vascular calcification. The molecular mechanisms of vascular calcification in these mice are unclear [16, 39, 52, 53]. Furthermore, human mutations that inactivate OPG cause juvenile Paget disease [54], an osteopathy characterized by rapidly remodeling woven bone, osteopenia and vascular injury in the form of aneurysms [55–57]. Thus, OPG also regulates vascular wall resistance to stress. As discussed above, vascular calcification is a key feature of CKD–MBD, while aortic aneurysms and CKD are frequent clinical companions in which CKD confers an increased risk of mortality [58, 59]. Thus, unraveling the role of OPG in kidney disease-associated vascular injury is of particular importance. While genetically modified mice lack a kidney phenotype beyond renal artery calcifications, their response to kidney injury has not been addressed.

OPG as a therapeutic agent

The role of OPG in bone mass regulation sparked research on its potential therapeutic role in human osteoporosis. *Escherichia coli*-expressed Fc-OPG is a recombinant protein with an N-terminal Fc fragment, while CHO-expressed OPG-Fc (AMGN-0007) has a C-terminal Fc fragment and is ten times more potent [60]. In animal models, OPG-Fc and soluble RANK-Fc fusion proteins inhibited RANKL-mediated osteoclastogenesis [39]. OPG-Fc lacks the signal-peptide, heparin-binding domain and death domain-homologous regions of native OPG, has a longer half-life and lower affinity for TRAIL, while affinity for RANKL is preserved [22, 61]. Administration of Fc-OPG reduced the area of calcified lesions without modulating atherosclerosis in atherogenic diet-fed ldlr (−/−) mice [62]. However, there is conflicting evidence on the potential of exogenous OPG to impact on disease. Secchiero et al. [61] in a series of papers have alerted on the potential risks of OPG administration by documenting adverse effects on pancreatic islet cells, endothelium and vascular fibrosis associated with up-regulation of arterial TGF-β1 in ApoE (−/−) mice [63–65]. Of particular interest to CKD, OPG inhibits vascular calcification induced by warfarin or by vitamin D in rats [66]. The use of oral anticoagulants targeting vitamin K is a key risk factor for calciphylaxis, the most severe form of vascular calcification [67].

Phase 1 clinical trials of two molecular forms of OPG (Fc-OPG and OPG-Fc) concluded that these constructs were safe in postmenopausal women or cancer patients and reduced biochemical markers of bone resorption [68, 69]. Despite the promising results, OPG was not further developed as a therapeutic agent since the anti-RANKL monoclonal antibody denosumab was more effective and there were concerns that OPG-mediated blockade of TRAIL might favor tumor development or growth [68–70]. Denosumab is now in clinical use for prevention of skeletal-related events in adults with bone metastases from solid tumors and for sex hormone-deprivation induced osteoporosis [71]. By contrast, no active clinical trials using OPG were found in the clinicaltrials.gov web page in November 2013 [72].

Table 1 In vivo role of OPG and OPG ligands according to the phenotype of genetically modified, non-stressed mice. No spontaneous kidney phenotype was observed in non-stressed mice, except for renal artery calcification in OPG KO mice

Mouse mutant	Bone phenotype	Vascular phenotype	Other
OPG KO [16]	Osteoporosis	Vascular calcification, including renal arteries	
	Scoliosis		
	↑osteoclast number		
	High bone remodeling		
OPG transgenic (overexpressing) [31]	High bone mass		
	Reduced or absent osteoclasts		
RANKL KO [50]	Osteopetrosis, no osteoclasts		Absent lymph nodes
	Absent tooth eruption		
RANK KO [102]	Osteopetrosis no osteoclasts		
	Absent tooth eruption		
TRAIL KO [103]	Normal bone density		No spontaneous tumors
			Increased susceptibility to autoimmune diseases

Biomarker potential of circulating OPG in kidney disease

There is increasing interest in biomarkers that allow risk stratification in CKD and eventually guide the use of new therapeutic approaches [73]. In the general population high serum OPG was associated with cardiovascular risk factors and/or predicted cardiovascular mortality [74]. Circulating OPG levels are increased in CKD patients [75–81] (Fig. 2). However there is not enough information on the in vivo role of OPG to consider OPG a uremic toxin [82]. In addition, circulating OPG is decreased in nephrotic syndrome [83]. Circulating OPG levels have been associated with adverse outcomes in CKD patients [74, 81, 84–88].

Chronic kidney disease

Diabetes mellitus is the most frequent cause of CKD that progresses to ESRD. In addition, diabetes is associated to macrovascular and microvascular disease, including a high prevalence of vascular calcification [85]. By contrast, there is a negative association between diabetes and abdominal aortic aneurysms [89]. Serum OPG has been associated to the presence of diabetes. There is an independent positive correlation between circulating OPG and basal glycemia [86] or hemoglobin A1c levels [90]. Diabetic patients with CKD have higher OPG levels than diabetics without CKD. In 1,939 non-dialysis adult type 1 diabetics, those with macroalbuminuria and/or renal impairment had higher OPG concentrations than those without overt kidney disease [32].

Circulating OPG levels increase as estimated glomerular filtration rate (eGFR) decreases in diabetic and non-diabetic CKD patients [32, 75–78, 81, 90] (Fig. 2a). However different authors have reported very different values. This may be related to differences in enzyme-linked immunosorbent assay (ELISA) kits, conversion factors, technical issues, or to real differences between patient populations.

Renal replacement therapy

Serum OPG levels are increased in patients undergoing renal replacement therapy (hemodialysis, peritoneal dialysis or kidney transplantation) [23, 80, 87, 88, 91, 92]. In hemodialysis patients, OPG values were similar in three reports, but much lower in a fourth one [87]. The conversion factor to International System (SI) units indicated in the datasheet of the Immunodiagnostik kit used this fourth report (MW 19.9 kD) and it is very different from the molecular weight of OPG and the values used by other assays (MW 120 kD) (Fig. 2b). There was a significant correlation between OPG levels, dialysis vintage and age in hemodialysis patients [23]. It was suggested that serum OPG might help discriminate hemodialysis patients with biopsy-proven low turnover bone disease from those with high turnover renal osteodystrophy when intact PTH (iPTH) is $\leq$300 pg/ml [87]. Within this PTH range, mean OPG was lower in patients with adynamic bone disease than in those with hyperparathyroidism and mixed osteodystrophy. However, no formal assessment of test performance was reported. Two studies [80, 88] measured serum OPG levels in peritoneal dialysis (Fig. 2c).

Two studies measuring serum OPG levels in renal transplant recipients were very discordant (Fig. 2d) [23, 92]. The reason for the discrepancy is unclear. Renal function may have contributed, but was not presented in one of the reports.

Nephrotic syndrome

Nephrotic syndrome represents the most severe form of proteinuric kidney disease. As a result of protein loss

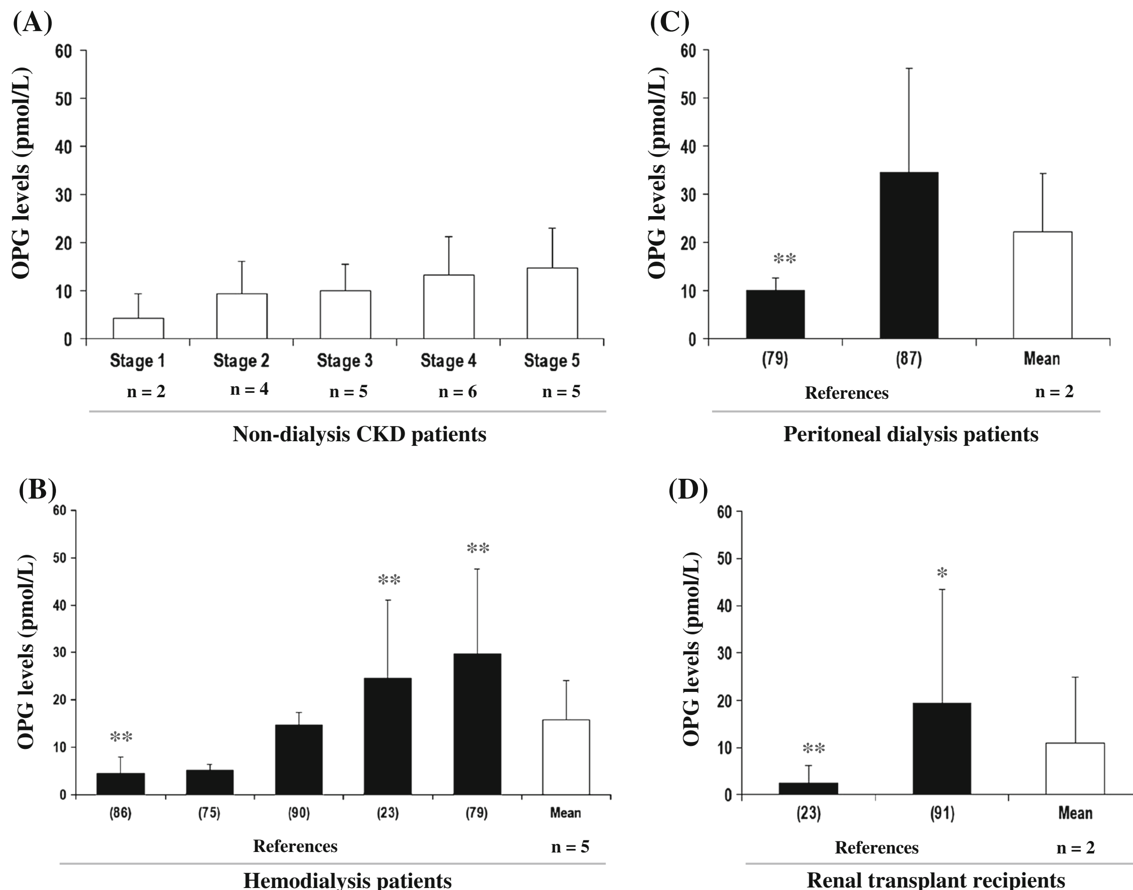

Fig. 2 Serum osteoprotegerin (OPG) levels in chronic kidney disease (CKD) patients. **a** Non-dialysis CKD patients. Four different ELISA kits were used: Biovendor (Laboratory Medicine Inc., Brno, Czech Republic) in references [75, 77, 78, 81]; Rapid Bio Lab (West Hills, CA, USA) in [76]; ALPCO (Salem, NH, USA) in [79] and Immunodiagnostic (UK) in [80]. **b** Hemodialysis patients. Data as reported in references [23, 76, 80, 87, 91]. **c** Peritoneal dialysis patients. Data as reported in [80, 88]. **d** Renal transplant recipients. Data as reported in [23, 92]. The n represents the number of studies used to calculate the mean. Data presented as mean and SD of several studies (*empty columns*) or mean or median and SD of individual studies (*black columns*). Conversion from conventional to International System (SI) units was performed if needed according to instructions in each ELISA datasheet (*Conversion factor 1 pmol/l = 120 pg/ml and **Conversion factor 1 pg/ml = 0.05 pmol/l according to datasheet)

through proteinuria and a compensatory increase in protein production mainly by the liver, nephrotic syndrome is characterized by a wide spectrum of changes in serum protein concentrations. In childhood nephrotic syndrome, serum OPG was lower, even in newly diagnosed patients (prior to steroid therapy) than in normal controls (0.84 ± 0.18 vs. 1.1 ± 0.22 pg/ml) [83]. OPG was even lower in steroid-dependent frequent relapsers (0.24 ± 0.14 pg/ml). There was a negative correlation between OPG and markers of disease severity such as serum cholesterol and urinary protein excretion. Low OPG was hypothesized to result from urinary losses and to potentially contribute to bone resorption. Steroids, which are known to increase RANKL and to reduce OPG expression, were thought to further decrease OPG values [83].

Vascular calcification

Higher circulating OPG levels have been associated with vascular calcification in CKD patients [16, 77, 78]. High circulating OPG levels were associated with the presence of coronary artery calcifications (CAC) in non-dialyzed CKD patients, peritoneal dialysis, and renal transplant patients [77, 78, 80, 88, 89, 92–95]. Circulating OPG levels also correlated with factors associated with vascular calcification. In hemodialysis patients OPG levels correlated with dialysis vintage and age [23]. In adults with type 1 diabetes, high circulating OPG was associated to peripheral vascular events such as revascularization procedures or amputation of lower extremities [32]. OPG levels increased rapidly in association with early vascular calcification stages and then reached a plateau. It was hypothesized that

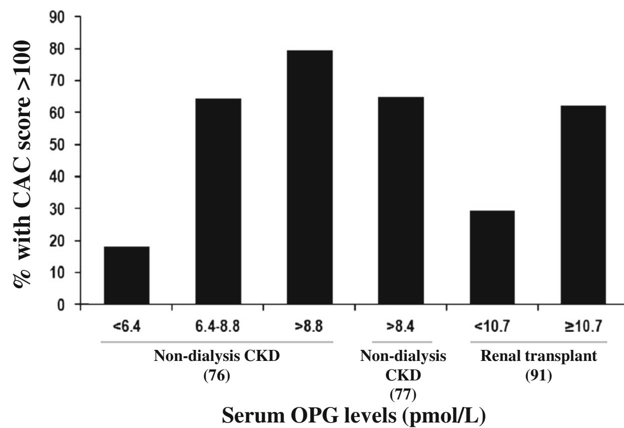

Fig. 3 Relation between coronary artery calcification (CAC) score and serum osteoprotegerin (OPG) levels. Data are summarized from [77, 78, 92] and correspond to non-dialysis chronic kidney disease (CKD) and transplantation CKD patients. Patients with score 100–400 and ≥400 from Ref. [78] were grouped together (score ≥ 100)

OPG may be a marker of atherosclerosis/vascular calcification onset rather than its severity or progression [78].

Aortic pulse wave velocity is an indirect, noninvasive method to assess aortic stiffness and increases in the presence of vascular calcification. CKD patients with the highest serum OPG levels had a 10 % higher aortic pulse wave velocity compared to those with the lowest levels [79].

Figure 3 integrates data from three studies that related serum OPG levels to coronary artery calcium (CAC) score in CKD patients. In non-dialysis CKD patients, serum OPG levels in the higher tertile (>8.8 pmol/l, conversion 1 pmol/l = 120 pg/ml) were associated with the presence of CAC [77]. In 195 non-dialysis CKD patients, OPG ≥ 10.71 pmol/l was the only variable significantly associated with moderate CAC (100–400) [odds ratio (OR) 2.73 [1.03;7.26]; $p = 0.04$] [78]. In renal transplant recipients, receiver operating characteristic (ROC) curve analysis yielded an optimal plasma OPG cutoff value for predicting a CAC score of 8.3 pmol/l. The percentage of calcified (CAC > 100) subjects with OPG > 8.3 pmol/l was 75 % (OR 5.72 [1.94;16.8], $p = 0.002$) [92]. Baseline CAC, but not baseline or 1-year OPG, was associated with CAC progression. In a different set of 107 transplanted patients, vascular calcification progression was again observed almost exclusively in patients with a prior vascular calcification, but in a multivariate analysis, serum calcium, OPG, and eGFR were independently associated with progression at 1 year [96].

Mortality

Cardiovascular mortality is the leading cause of death in patients with CKD even after successful transplantation [92, 95]. For pre-dialysis CKD patients, the risk for cardiovascular death may be higher than the risk for ESRD requiring renal replacement therapy [75]. In patients with vascular calcification the risk of cardiovascular death is particularly high [91]. Serum OPG has been associated with cardiovascular mortality in pre-dialysis CKD patients [76].

In renal transplant recipients, baseline OPG levels were associated with long-term (7–9 years) cardiovascular or all-cause mortality [93]. Circulating OPG was higher in non-survivors and in patients who died from cardiovascular disease than in survivors and patients who died from other causes [93].

In a prospective cohort study of 1,157 elderly women, multivariable-adjusted linear regression models showed that elevated OPG levels at baseline were associated with faster decline in eGFR at 5- and 10-year follow-up and a higher risk of hospitalizations or death [97]. However, baseline OPG levels were higher in patients with lower baseline eGFR.

In summary, circulating OPG is a potential biomarker for vascular calcifications and mortality in CKD. However, a causal role of OPG in these associations remains uncertain. In mice, OPG deficiency is associated to diffuse medial calcification of the aorta and renal arteries. By contrast, in humans with CKD, increased OPG is associated with vascular calcification. Several hypotheses may explain this potential discrepancy [79, 95]. The high OPG levels in patients with vascular disease may be triggered by inflammation or by the presence of osteoblast-like cells in calcified vessels. OPG might thus be a marker of injury while playing a protective role in vascular injury by inhibiting vascular wall calcification. This concept is supported by observations in OPG-deficient mice and by the demonstration that OPG dose-dependently within a pathophysiologically relevant range (100–10,000 pg/ml) reduces vascular smooth muscle calcification in culture [16, 98]. Alternatively, medial calcification in OPG-deficient mice might be secondary to severe osteoporosis and not a direct result of OPG deficiency in the vasculature. In this regard, information is missing on the temporal sequence of events. Thus, no prospective study has determined whether serum OPG levels rise in response to the development of vascular calcification or whether elevated levels of serum OPG precede the development of vascular calcification [79, 95].

Kidney and urinary OPG

There is little information on OPG expression and function during kidney injury. A transcriptomics analysis identified TRAIL and OPG as the apoptosis-related genes most

highly expressed in human diabetic nephropathy of the tubulointerstitium [41]. Diabetic nephropathy is the most frequent form of CKD [99]. The findings were interpreted as disclosing local renal synthesis of OPG mRNA during tissue injury. Human kidney OPG mRNA levels directly correlated with serum creatinine, proteinuria and histological injury scores, indicating a relationship between OPG expression and severity of kidney injury. While the increased expression of TRAIL was confirmed at the protein level and localized to podocytes and tubular cells by immunohistochemistry, OPG protein was not studied [41]. In more recent studies the Nephromine database of published kidney transcriptome datasets disclosed that OPG mRNA was increased in human kidneys with IgA nephropathy and that renal cortex OPG mRNA levels correlated with chronicity index quartiles in aging humans [100]. OPG was increased in tubular epithelial cells in CKD biopsies and in epithelial cells lining kidney cysts in another form of kidney injury, autosomal dominant polycystic kidney disease [100]. Furthermore, while in normal kidney OPG was present at the basolateral side of tubular cells, a diffuse staining pattern was found in injured kidney suggesting that directional secretion of OPG might be affected by kidney disease. Specifically, immunostaining suggested that during kidney disease tubular cells might secrete OPG to the tubular lumen, thus favoring the presence of OPG in urine [100].

Evidence further supporting local synthesis of OPG by tubular epithelium was obtained in cultured cells. Neutralization of OPG sensitized cultured human tubular cells to TRAIL-induced death, suggesting that OPG is an autocrine cytoprotective factor [41]. OPG was found in tubular cell-secreted exosome-like particles [101]. Exosomes and other microvesicles actively secreted by cells may play a variety of roles, including disposal of unwanted material, intercellular communication and regulation of cell death and of vascular calcification. Thus OPG in exosomes might contribute to any of these functions. OPG was also found in urinary exosomes [100]. In an exploratory study OPG was increased in urinary exosome-like vesicles in CKD patients, including those with diabetic nephropathy, IgA nephropathy and polycystic kidney disease, all of them diseases characterized by increased kidney OPG mRNA or protein [100].

Urinary OPG may also be a potential biomarker of lupus nephritis activity. In 87 lupus nephritis patients urinary OPG levels were associated with evidence of active nephritis such as hematuria, urine protein/creatinine ratio, and the presence of circulating anti-dsDNA antibodies [102]. The authors hypothesized that microvascular endothelial cells from inflamed kidneys were the source of urinary OPG. However, an alternative hypothesis is that urinary OPG originated in stressed tubular cells (Fig. 4). Indeed, as commented above, tubular cell OPG might more readily access the urinary space than endothelial cell-derived OPG, and immunohistochemistry data and the presence of OPG in exosomes suggest a tubular origin of urinary OPG.

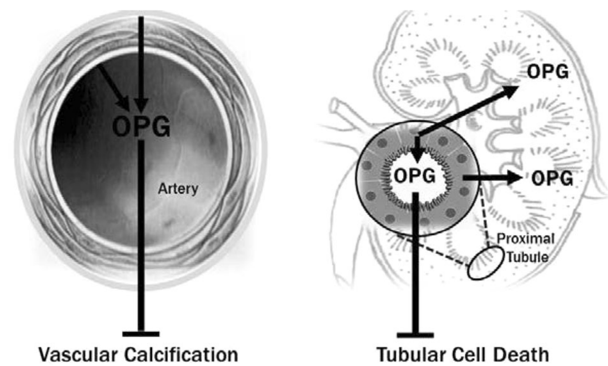

Fig. 4 Osteoprotegerin (OPG) origin and functions in the kidney and potential systemic functions. Vascular smooth muscle cells are thought to be the main source of OPG in the vascular wall, but endothelial cells also secrete OPG. In the vascular wall the best characterized effect of OPG is prevention of vascular calcification. Tubular cells are a source of OPG in the kidneys. OPG from tubular cells may appear in urine, at least in part associated to exosomes. Tubular cell OPG may be an autocrine response that protects from TRAIL-induced apoptosis

Conclusions and required research

In conclusion, functional animal studies, cell culture studies and human genetic defects suggest that OPG has a key role in downregulating vascular calcification, preventing arterial aneurysms and protecting kidney cells from inflammation-induced death (Fig. 4). In addition increased circulating, urinary and kidney OPG levels are observed in different forms of human CKD. Increased circulating OPG was associated with vascular calcification and mortality in CKD patients. An integration of experimental and clinical data suggests that the increase in OPG observed in vivo may be compensatory and protective, but that it fails to fully prevent injury. OPG may contribute to slower progression of tissue injury as suggested by the more severe vascular injury observed in atherosclerosis-prone OPG-deficient mice. Alternatively, the forms of OPG accumulated in CKD may be dysfunctional and not able to provide tissue protection. There is plenty of experience with this notion. As a clear example, the high PTH levels in CKD patients are in part due to accumulation of non-functional peptides. Still, some authors support the notion that despite preventing calcification, OPG may contribute to injury.

Specific areas require further research in order for OPG to be incorporated into daily clinical practice. Recent

studies concluded that the discriminatory capacity of current assays for OPG is not enough for clinical use at least to screen for fractures or vascular calcification in CKD [84, 103]. Thus, OPG may have a role as a biomarker of risk. However, better standardization of assays and definition of cutoff points is required to validate its potential use as a biomarker.

There are great gaps in our understanding of the role of OPG in kidney diseases and the role and meaning of urinary OPG. Despite the promising findings, further studies are needed to establish the role of urinary OPG, either free or associated with exosomes, in monitoring or staging of human kidney injury.

Finally, recombinant OPG has been successfully tested and verified to prevent calcification in mice and shown to be safe in phase I human clinical trials. Although research into OPG as a therapeutic agent in osteoporosis has been abandoned, an improved understanding of its pathophysiology may identify new potential uses in cardiovascular and kidney protection.

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Conflict of interest None.

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