Cell. Mol. Life Sci. 66 (2009) 841 – 851
1420-682X/09/050841-11
DOI 10.1007/s00018-008-8536-x


Cellular and Molecular Life Sciences

# Review

# Role of full-length osteoprotegerin in tumor cell biology

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Received 2 September 2008; received after revision 29 September 2008; accepted 13 October 2008
Online First 18 November 2008

**Abstract.** Osteoprotegerin (OPG) is a soluble tumor necrosis factor receptor family member, which potently inhibits RANKL-mediated osteoclastogenesis. Numerous constructs have been created for therapeutic purposes in which the heparin-binding and death homology domains of OPG were removed and the remaining peptide (amino acids 22–194) was fused to the Fc domain of human IgG1 (OPG-Fc). The administration of OPG-Fc efficiently counteracted bone loss in a variety of preclinical models of cancers. However, several *in vitro* studies have shown that native or recombinant full-length OPG not only neutralizes RANKL, but also the death-inducing ligand TRAIL, suggesting that OPG might potentially counteract the anti-tumor activity of TRAIL. Additional evidence suggests that full-length OPG possesses RANKL- and TRAIL-independent biological properties, mainly related to the promotion of endothelial cell survival and angiogenesis. Finally, breast tumor cells overexpressing OPG have shown increased bone metastatic potential *in vivo*. The relevance of these apparently conflicting findings in tumor cell biology is highlighted.

**Keywords.** Osteoprotegerin, osteoclasts, angiogenesis, TRAIL, apoptosis.

## Introduction

Osteoprotegerin (OPG) is a soluble member of the tumor necrosis factor (TNF) receptor superfamily, which was discovered in mice *via* a genomic-based approach. OPG transgenic mice were born with high bone mass and this phenotype progressed as animals aged [1]. High bone mass in these animals was associated with a marked decrease in the number and activity of osteoclasts [1]. OPG protein comprises 401 amino acids of which 21 form a signal peptide that is cleaved generating a mature form of 380 amino acids (Fig. 1). At the N terminus, there are four domains (D1–D4), which have cysteine-rich TNF receptor homologous motifs and are necessary and sufficient for binding to its major target, the receptor activator of nuclear factor (NF)-B ligand (RANKL), and for inhibiting osteoclastic differentiation and activity both *in vitro* and *in vivo* [2–4]. At the C terminus, there are tandem death-domain homologous regions (D5 and D6) followed by a heparin-binding site (D7) [2] and, at position 400, there is a cysteine required for homodimerization of the molecule (Fig. 1).

OPG represents an atypical member of the TNF receptor family since it is a secreted protein with no transmembrane domain. OPG is produced as a monomer (55–62 kDa), undergoes homodimerization, and is secreted as a disulfide-linked homodimeric glycoprotein with four or five potential glycosylation sites, generating a mature form of OPG of 110–120 kDa. The dimeric form of the protein exhibits a much greater (two to three log) higher affinity for

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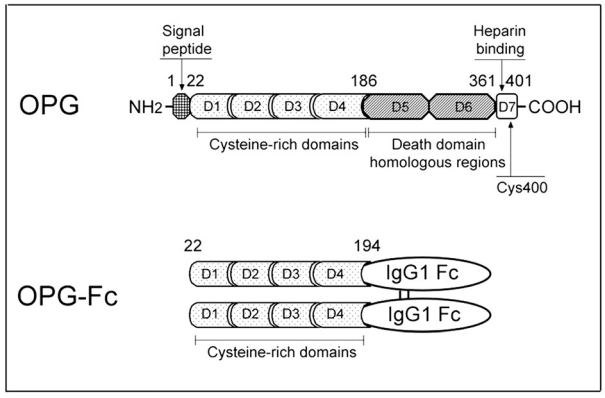


**Figure 1.** Schematic representation of the protein structure of osteoprotegerin (OPG) and OPG-Fc. Main domains and their biochemical and/or functional properties are indicated. Numbers in figure represent amino acids. $NH_2$ indicates N terminus, COOH, C terminus, Cys400, dimer formation site. Human OPG-Fc is a recombinant fusion protein that includes amino acids 22–194 of native OPG, comprising the minimal TNFR-like domains that mediate RANKL inhibition. This fragment lacks the signal peptide, death domain homologous regions and heparin-binding domain of native OPG. The Fc fragment of human IgG1 is fused to the C terminus of this 22–194 fragment to maintain a dimeric molecule with a sustained circulating half-life.

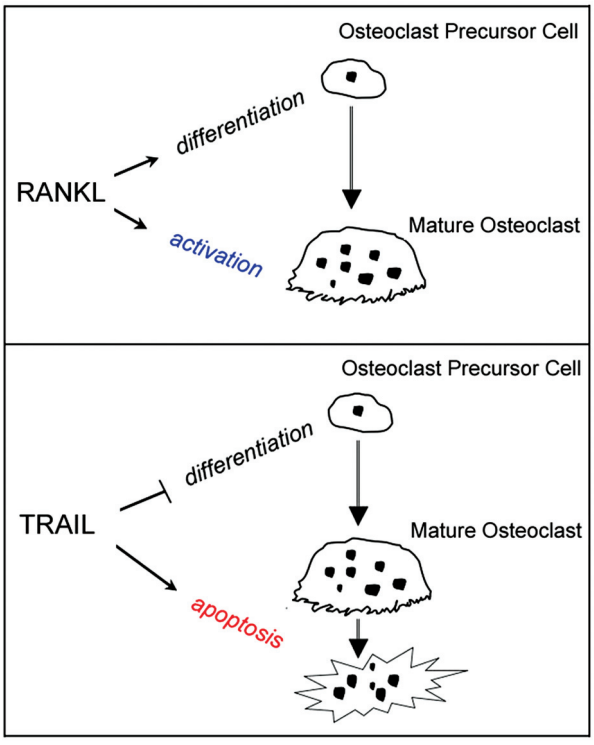


**Figure 2.** Comparison between the effects of RANKL and TRAIL in osteoclastogenesis. RANKL plays a key role in osteoclastogenesis by promoting the differentiation of pre-osteoclast precursors into mature multinucleated osteoclasts and the bone resorption activity of mature osteoclasts. On the other hand, recent studies have suggested a role of TRAIL as negative modulator of osteoclastic differentiation and inducer of apoptosis in mature osteoclasts.

RANKL than the monomeric form and a higher heparin-binding capacity [3]. Importantly, the heparin-binding domain (D7), besides being involved in the formation of homodimers [2], might regulate the release and the activity of OPG by mediating its binding to cell membrane-associated heparan sulfates, as suggested by several studies [5–7]. To enhance the pharmacological activity of native OPG, constructs have been created in which the signal peptide, the heparin-binding domain (D7) and the death-domain homologous regions (D5, D6) were removed, and the remaining amino acids 22–194 OPG peptide was fused to the Fc domain of human IgG1 (OPG-Fc) [4] (Fig. 1). OPG-Fc maintains the potent dimeric nature of full-length OPG, but shows a significantly increased circulating half-life [4]. For the topic of this review, it is particularly important to point out that the great majority of published studies describing the efficient anti-osteolytic activity of OPG in animal models of breast cancer, multiple myeloma, giant cell tumors and prostate cancers [8–14] have relied on the use of recombinant OPG-Fc.

## Cellular sources and anti-osteoclastic activity of OPG

Concerning the cellular sources of OPG, it has been shown that both bone marrow stromal cells and cells belonging to the osteoblastic lineage produce and release in culture detectable amounts of native full-length OPG (reviewed in [4, 15–18]). It is believed that the physical proximity of osteoblasts and osteoclasts during bone remodeling creates opportunities for osteoblast-mediated regulation of bone resorption. In addition, a more recent study has reported that cells of the B lineage are responsible for 64% of total bone marrow OPG production, with 45% derived from mature B cells [19].

Importantly, OPG is also produced by endothelial and vascular smooth muscle cells, which likely represent the major contributors to the circulating pool of OPG [20–22]. In this respect, although it is likely that OPG exerts its anti-osteoclastic activity mainly acting in the bone marrow microenvironment, it is also possible that circulating OPG might contribute to inhibit bone resorption by suppressing the differentiation of circulating monocytes, which represent the major reservoir of circulating pre-osteoclasts [23].

Inhibition of osteoclastogenesis by OPG occurs upon binding to its best-characterized ligand, RANKL, a member of the TNF family of cytokines, which exists either as a type II membrane or as a soluble protein [24]. RANKL, originally described as "OPG ligand", is predominantly expressed by activated immune cells and osteoblasts, and it shows the primary

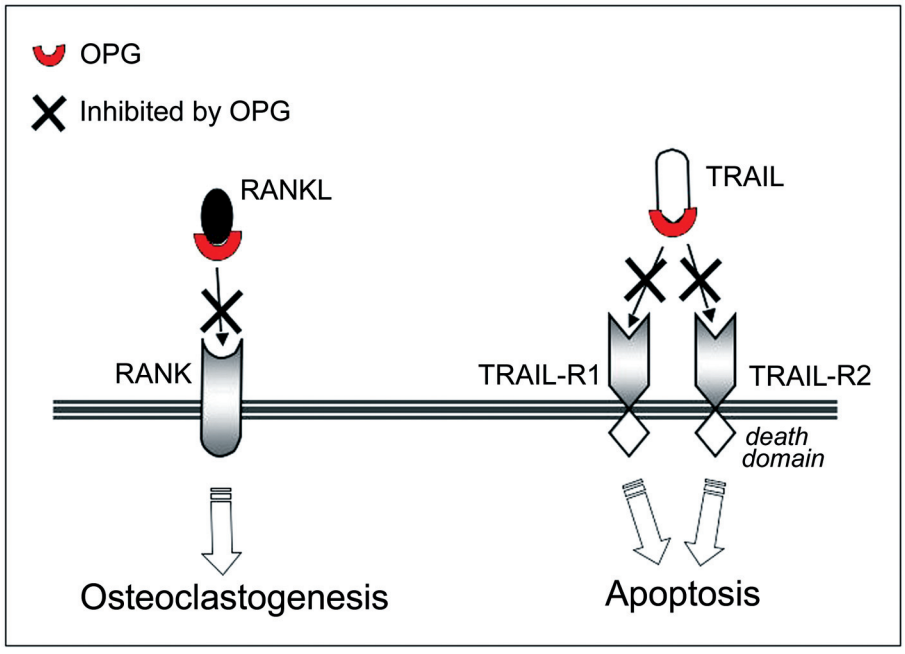


**Figure 3.** Mechanism of action of OPG on RANKL- and TRAIL-biological activities. RANKL exerts its pro-osteoclastic activity by interacting with its cognate high-affinity transmembrane receptor RANKL. The pro-apoptotic activity of TRAIL is mediated by two (TRAIL-R1 and TRAIL-R2) of its four membrane receptors. OPG, by efficiently binding RANKL and TRAIL, prevents their association with their transmembrane receptors and therefore counteracts both the RANKL-mediated osteoclastogenesis as well as the pro-apoptotic activity of TRAIL.

biological function of promoting the differentiation of monocytic precursor cells into mature osteoclasts and the bone resorption activity of mature osteoclasts (Fig. 2) [24–29]. RANKL exerts its pro-osteoclastic activity by interacting with its cognate high-affinity transmembrane receptor RANK [25–27], while both full-length OPG and chimeric OPG-Fc compete with transmembrane RANK for binding to RANKL (Fig. 3) [4, 7].

Consistently with its role in the suppression of osteoclastogenesis, mutations in the human OPG/TNFRSF11B gene are associated with idiopathic hyperphosphatasia (also known as juvenile Paget's disease), an autosomal recessive bone disease characterized by deformities of long bones and kyphosis [30, 31]. Moreover, OPG-knockout mice display elevated bone turnover and vascular calcifications, which could be reversed by OPG-transgene overexpression [32, 33]. These observations have indicated that both the bone and vascular consequences, derived from endogenous OPG deficiency, can be prevented by systemic RANKL inhibition. A number of additional studies in animal models have shown that treatment of pathological conditions associated to high bone turnover, including bone lesions induced by direct injection of multiple myeloma, prostatic or breast tumor cells could be efficiently prevented by treatment with recombinant OPG-Fc constructs (reviewed in [34]).

## Interactions between OPG and TRAIL

It was shown a decade ago that, beside RANKL, OPG is also able to bind another TNF family member, the TNF-related apoptosis inducing ligand (TRAIL), whose extracellular domain shows approximately 25 % amino acid homology with RANKL (reviewed in [35]). Similarly to RANKL, TRAIL is also expressed either as a type II membrane protein or as a soluble protein (reviewed in [35]). Interestingly, the release of soluble TRAIL seems to be uncoupled by its expression at the cell membrane level. In fact, interferon (IFN)-α-activated neutrophils release relatively high levels of soluble TRAIL into the culture supernatant without expressing significant amounts of transmembrane TRAIL [36].

With respect to other members of the TNF ligand superfamily, TRAIL shows the most complex ligand-receptor interactions (reviewed in [35, 37]), since it can bind to four transmembrane receptors (TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4) as well as to soluble OPG [38]. Among the several putative physiological activities of TRAIL, its best-characterized function is the surveillance against tumors and, in particular, hematological malignancies [39–42]. Upon activation signals, virtually all cells of the immune systems (T lymphocytes, B lymphocytes, natural killer cells, dendritic cells, monocytes, granulocytes) up-regulate TRAIL expression and/or release [36, 42, 43].

After the initial study demonstrating that OPG efficiently binds to TRAIL [38], a subsequent study

[44] has questioned the role for OPG as a neutralizing receptor for TRAIL under physiological conditions (37°C). It is important to underline, however, that in this study [44] the affinity of recombinant TRAIL for the TRAIL receptors was measured using constructs in which the extracellular domains of TRAIL-R1, -R2, -R3 or OPG were fused with the Fc moiety of IgG1, assuming for OPG that the deleted D5, D6 and D7 domains of the native molecule were not relevant for the TRAIL binding activity. At 37°C, TRAIL affinity was maximal for TRAIL-R2-Fc ($K_d$ 2 nM), followed by TRAIL-R1-Fc ($K_d$ 70 nM), TRAIL-R3-Fc ($K_d$ 300 nM) and OPG-Fc ($K_d$ 400 nM) [44]. In contrast, it was subsequently shown that the affinity of TRAIL for the TRAIL-receptor-Fc constructs, including OPG-Fc, differed significantly from that reported for native TRAIL receptors [45, 46]. When a rationally designed small molecule mimic of OPG, termed OP3–4, was examined for association with either TRAIL or RANKL in binding assays, this peptide bound to RANKL with a $K_d$ of 3.89 nM and to TRAIL with a $K_d$ of 19.3 nM, thus showing only a four- to fivefold higher affinity for RANKL, as compared to TRAIL [46]. In line with these previous studies [38, 44–46], a more recent study [7] has confirmed that: (i) the affinity of native full-length OPG for TRAIL is only slightly (approximately twofold) lower than that for RANKL, and (ii) there are differences in ligand-receptor affinity when using OPG-Fc and native full-length OPG. In particular, native full-length OPG has shown a lower affinity for RANKL than the OPG-Fc form of the protein [7].

Overall, the biochemical studies have substantially confirmed a number of *in vitro* data demonstrating the ability of native OPG, produced by tumor cells or by bone marrow stromal cells, to efficiently counteract the pro-apoptotic activity of TRAIL in a variety of cell lines derived from prostate and breast cancers, ameloblastomas and multiple myeloma (Fig. 3) [47–51].

## Potential effects of OPG/RANKL/TRAIL interplay

In the context of the potential OPG/RANKL/TRAIL interplay in osteoclastogenesis, it should be mentioned that our studies [52, 53] and those of other groups [54–56] have suggested a role of TRAIL as a negative modulator of osteoclastic differentiation and an inducer of apoptosis in mature osteoclasts (Fig. 2). Of particular interest, it has been recently demonstrated that, *in vitro*, osteoclastic apoptosis is promoted by TRAIL endogenously expressed and released by end-stage osteoclasts in an autocrine/paracrine manner [56]. Thus, besides blocking RANKL-mediated osteoclastogenesis, OPG might paradoxically protect mature osteoclasts from apoptosis induced by native TRAIL produced by the same osteoclasts (Figs 2, 3) [56]. Although the net effect of TRAIL on osteoclastic differentiation and survival likely depends on the network of pro-survival and pro-apoptotic signals operating at a given time in the bone marrow microenvironment, the exact role of TRAIL in modulating osteoclastogenesis clearly requires further investigation.

Among cells of hematopoietic origin, mature megakaryocytes display the highest expression of OPG [57, 58], which is also found associated with von Willebrand factor in circulating platelets [59]. OPG locally released at the bone marrow level by either bone marrow stromal cells or mature megakaryocytes might also contribute to modulate the biological activity of TRAIL on hematopoietic cells [60–66] in both normal and pathological conditions.

## Evidence suggesting a potential role of OPG in favoring tumor development

### Promotion of endothelial cell survival and angiogenesis by OPG

A parallel line of research investigating the role of OPG in endothelial cell biology has suggested important implications for a potential role of OPG in favoring tumor development (Table 1). Initial studies have demonstrated that OPG mediates the integrin-dependent survival of serum-deprived microvascular endothelial cells [67]. Osteopontin engagement of αvβ3 on the microvascular endothelial cell surface triggered nuclear factor-κB (NF-κB)-dependent generation of OPG that was essential for conveying the anti-apoptotic actions of osteopontin-induced NF-κB activation in these endothelial cells. In addition, recombinant OPG has been shown to partially protect microvascular endothelial cells from apoptosis induced by TRAIL, used in combination with IFN-γ or c-flip inhibitors [68, 69].

Cross et al. [70] were the first to propose a link between the ability of OPG to promote endothelial cell survival and tumor angiogenesis. In their interesting study, these authors have documented the ability of OPG to induce the formation of cord-like capillary structures *in vitro*, using matrigel tubule formation assays [70]. In addition, Cross et al. have demonstrated endothelial OPG expression in approximately 60% of malignant tumors, but not in endothelium of normal tissues. Although the immunohistochemical analysis of OPG expression should be considered with caution, since the same group of investigators reported a cross-reactivity between OPG and carbonic

**Table 1.** Evidences supporting a role of full-length osteoprotegerin (OPG) in tumor development.

| Experimental context | Observations | Selected refs. |
|---|---|---|
| Endothelial cell biology | OPG promotes endothelial cell survival | [67–69] |
| | OPG induces angiogenesis | [70, 73] |
| | OPG promotes proliferation and migration of microvascular endothelial cells | [72] |
| | OPG induces leukocyte adhesion to endothelial cells | [79, 80] |
| Cancer cell cultures | OPG is overexpressed in colon cancer cell lines | [93, 94] |
| | OPG protects TRAIL mediated-apoptosis of tumor cells | [47–50, 93] |
| Preclinical studies | Overexpression of OPG by breast cancer cells correlated with bone-specific homing and colonization potential of the tumor | [99, 100] |
| Clinical studies | Endothelial OPG expression in malignant tumors, but not in normal tissues | [70] |
| | Elevated serum OPG levels in patients with cancer | [87–92] |
| | Correlation between serum OPG levels and tumor stage and aggressiveness | [91, 95] |
| | Association between a polymorphism of the OPG gene and an increased risk of advanced prostate cancer | [97] |

anhydrase II, at least with some anti-OPG monoclonal antibodies [71], nevertheless the study of Cross et al. strongly suggests a link between OPG overexpression and tumor angiogenesis. In keeping with a potential biological role for OPG in the development and/or maintenance of the tumor vasculature, recent studies have demonstrated that recombinant full-length OPG directly promotes both the proliferation and the migration of microvascular endothelial cells [72] and induces angiogenesis in an *in vivo* implanted matrigel assay [73].

Consistently with the idea that OPG might exert direct biological activities independently of its neutralizing effects towards TRAIL or RANKL, the ability of OPG to promote angiogenesis was regulated by heparin, strongly suggesting the involvement of the heparin-binding domain of OPG (D7) in the pro-angiogenic activity of OPG [73]. The biological importance of the OPG heparin-binding domain (D7) was underscored by additional reports indicating its role also in controlling the release of OPG by vascular cells [5] as well as in the monocyte chemotaxis triggered by OPG [74]. Indeed, both angiogenesis and monocyte attraction induced by full-length OPG were abolished by pre-treating cells with heparinases and chondroitinases, suggesting that full-length OPG likely promotes these biological effects interacting with cell surface heparan sulfates, as recently demonstrated for well-characterized pro-angiogenic cytokines, such as basic fibroblast growth factor [75].

## OPG and inflammation

Several studies have shown that inflammatory cytokines promote the expression and release of OPG by endothelial cells [76–79]. In turn, we and other authors have demonstrated that recombinant full-length OPG promotes leukocyte adhesion to endothelial cells [79, 80], and increased OPG levels have been clearly linked to an increased cardiovascular risk (reviewed in [81]). In this context, it has also been shown that one single nucleotide polymorphism in the promoter region of the human gene for OPG is related to vascular morphology and function [82].

Although the above-mentioned studies point on a ligand-independent pro-inflammatory effect of OPG, it should also be mentioned that previous studies of our group have demonstrated that soluble TRAIL displays an anti-inflammatory activity on endothelial cells obtained from large vessels, such as human umbilical vein endothelial cells or aortic endothelial cells [83–85]. Thus, it is possible that part of the pro-inflammatory activity of OPG is mediated by an inhibitory activity on TRAIL, although at present this conclusion is speculative since *in vivo* studies demonstrating that OPG is able to prevent the anti-inflammatory activity of TRAIL on endothelial cells are still lacking.

Overall, these findings pointing to a potential role of OPG in promoting/modulating inflammation are noteworthy since inflammation represents an initial key step in promoting tumor angiogenesis [86].

## OPG serum levels and tissue expression in cancer patients

A potential role of OPG in tumor cell biology is supported by different studies that have investigated the OPG serum levels, OPG tissue expression and OPG polymorphisms in cancer patients (Table 1). The majority of these studies [87–92] have revealed that OPG serum levels were significantly elevated in patients with more advanced cancer. Moreover, it is particularly noteworthy that OPG levels were in-

creased in the serum of patients with cancer metastasized to the bone, with the remarkable exception of patients affected multiple myeloma, who usually show decreased OPG serum levels with respect to age- and sex-matched normal controls [87–92]. Surprisingly, however, OPG serum levels were elevated also in other types of tumors that do not show a preferential tropism for bone, such as bladder and colon cancer [91, 93–95]. In patients affected by bladder carcinoma [91], after a follow-up period of 5 years, patients who had low serum OPG levels had a longer post-operative tumor-free interval and increased survival compared with patients with high levels of serum OPG, suggesting that serum OPG correlates with tumor stage and it is also predictive of early recurrence of bladder carcinoma.

Several studies have investigated the OPG expression/release in epithelial carcinomas of the gastroenteric tract [93–95]. Overall, it has been demonstrated that (i) OPG is overexpressed in colon cancer cell lines through the Wnt/beta-catenin signaling [93], (ii) OPG accumulates in the medium of cultivated colon cancer cell lines and confers resistance to TRAIL-mediated apoptosis [93, 94], and (iii) OPG levels are significantly increased in serum of patients with advanced disease [93, 95]. In particular, Ito et al. [95] reported a significant correlation between the OPG expression and the depth of primary gastric tumor invasion, nodal metastasis and tumor stage, with strong OPG expression more frequent in stages III and IV than in stages I and II. Overall, these data suggest that OPG expression may be a marker of aggressive gastric carcinomas and that expression of OPG, acting as a survival factor, might provide colorectal cancer cells with an essential growth advantage and might contribute to cell invasion and metastasis. A gene expression profiling study has demonstrated that OPG is able to prevent anoikis, a form of apoptosis induced by substrate detachment [96], providing a potential mechanism able to explain the biological advantage of epithelial cancer cells to express OPG.

Finally, a recent study has demonstrated the association between a polymorphism of the OPG gene and an increased risk of advanced prostate cancer [97].

## Pro-metastatic potential of full-length OPG

The data illustrated above underline that an apparent paradox exists between (i) the ability of native or full-length recombinant OPG to protect different types of cancer cells from TRAIL-mediated apoptosis and/or to directly promote tumor cell survival and tumor-associated angiogenesis (reviewed in [51]) and (ii) the promising therapeutical potential of recombinant OPG-Fc to limit hypercalcemia and to reduce the burden or osteolytic lesions associated to different types of tumors in animal models as well as to reduce tumor establishment in bone (reviewed in [34]). In this respect, it is noteworthy that different studies have reported the ability of recombinant OPG-Fc or of adeno-associated virus (AAV)-expressing OPG-Fc to counteract the bone metastatic potentials of different types of tumors, including breast cancer [34, 98]. In this context, the study of Fisher et al. [99] is particularly noteworthy since these authors have on one hand confirmed the anti-metastatic potential of recombinant OPG-Fc but, on the other hand, have shown that full-length OPG overexpressed by breast cancer cells displays a clear pro-metastatic activity. Moreover, another study showed that overexpression of full-length OPG gene directly correlated with bone-specific homing and colonization potential of the tumor [100], acting primarily by increasing cellular proliferation in a ligand (TRAIL and RANKL)-independent manner, a phenomenon proposed for other members of the TNF receptor superfamily [101].

The ability of native OPG to promote angiogenesis [70, 72, 73] and to worsen bone-associated metastases, at least in some breast cancer models [99, 100], might represent more than a simple association. In fact, it should be taken into account that angiogenesis is closely associated with bone resorption and bone formation [102], and angiogenic factors, such as vascular endothelial growth factor, are important regulators also of osteoclast and osteoblast activity [102]. In fact, it has also been shown that bone remodeling takes place in specialized vascular structures, the "bone remodeling compartments", which are lined by cells exhibiting osteoblastic staining characteristics on the outside, while the inner wall of the compartment is made up of the bone surface with either resorptive or formative cells, depending on the state of the remodeling cycle [103]. Importantly, close contacts between vascular endothelium and bone remodeling compartments were reported [103], further suggesting that the interface between vascular endothelium and specialized bone areas is extremely important in the physiopathology of the bone.

## Therapeutic regiments able to circumvent the potential pro-tumoral activity of full-length OPG

In recent years, a fully human anti-RANKL antibody (denosumab) has been developed and is currently being tested in clinical trials (reviewed in [104]). Of note, preclinical studies have demonstrated that denosumab is more effective than OPG-Fc since it decreases bone turnover markers at lower doses than OPG-Fc, and when used at equivalent doses, denosu-

mab has a longer anti-resorptive effect than OPG-Fc (reviewed in [104]). Denosumab was converted to a non-cytotoxic IgG2 mAb, known as AMG 162, which has an extremely high affinity for human RANKL ($K_d$ approximately $10^{-12}$ M) and has no TRAIL-binding properties. Currently, there are several ongoing Phase III clinical trials with denosumab in patients with metastatic lytic bone lesions, such as multiple myeloma, prostate and breast cancer.

An important consequence of the availability of a RANKL inhibitor such as denosumab is that it renders conceptually feasible the association with a therapeutic approach aimed to down-regulate endogenous OPG expression/release. In this respect, the first demonstration that OPG expression/release can be pharmacologically down-modulated was provided by Fu and colleagues [105], who have shown that activation of peroxisome proliferator-activated receptor (PPAR)-γ inhibits OPG expression and release in cell culture. More recently, we have shown that activation of the p53 pathway by Nutlin-3, a small non-genotoxic activator of the p53 pathway, attenuates the expression and release of OPG by endothelial cells [106]. These findings are particularly noteworthy in an oncological perspective, taking into account that Nutlin-3 exerts a direct cytotoxic effect in various types of both solid tumors and hematological malignancies possessing a wild-type p53 [107–111]. The ability of Nutlin-3 to inhibit OPG expression/release is noteworthy also for indirect anti-tumor activity deriving from the ability of Nutlin-3 to inhibit endothelial cell proliferation and migration [112]. Obviously, a potential drawback in the ability of either PPAR-γ activators or Nutlin-3 to down-regulate OPG expression/release is the promotion of osteoclastogenesis, which is already a major complication associated with a variety of cancers. Therefore, the clinical association of denosumab with either PPAR-γ activators or Nutlin-3 might efficiently counterbalance the potential side effect (i.e., increase of bone turnover) associated to the down-modulation of endogenous OPG release. Anyhow, it should also be mentioned that Nutlin-3 itself displays a direct anti-osteoclastic activity when added *in vitro* to pre-osteoclasts [113]. Although the net effect of Nutlin-3 on osteoclastogenesis needs to be tested in animal models, the *in vitro* studies suggest the direct anti-osteoclastic activity of Nutlin-3 could overcome its ability to down-regulate OPG expression/release.

## Concluding remarks

The primary aim of this review has been to discuss the apparently conflicting data on the potential role of OPG in relationship to tumor cell biology (Table 1). While little doubts exist on the ability of pharmacological concentrations of recombinant chimeric OPG-Fc to efficiently counteract bone turnover associated to different pathological conditions, including bone metastases, as evaluated in preclinical animal models, the overall effects of full-length OPG are much less clear. In fact, although the demonstration of meaningful interactions between OPG and TRAIL *in vivo* are still lacking, accumulating evidence of ligand-independent pro-angiogenic and pro-survival activities of full-length OPG start to accumulate (Table 1). Careful experiments aimed at a better characterization of the potential biological differences between OPG-Fc and full-length OPG in terms of pro-survival, pro-angiogenic, anti-TRAIL activities are urgently needed to elucidate this issue. Once, and if, it is confirmed that OPG plays a direct pathogenetic role in tumor cell biology and tumor-associated angiogenesis, it will be of interest to explore therapeutical approaches capable of inhibiting endogenous OPG expression/release. The recent availability of RANKL inhibitor denosumab as a more selective molecule able to inhibit osteoclastogenesis will aid the application of these therapeutical approaches (in a combination therapeutic setting) in a variety of pathologies associated with elevated bone turnover, including bone metastases.

*Acknowledgements.* This work was supported by grants from AIRC (Associazione Italiana per la Ricerca contro il Cancro) to G. Z. and *CariFe* Foundation to P. S.

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