Role of osteoprotegerin (OPG) in cancer

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ABSTRACT

OPG (osteoprotegerin), a secreted member of the TNF (tumour necrosis factor) receptor superfamily, has a variety of biological functions which include the regulation of bone turnover. OPG is a potent inhibitor of osteoclastic bone resorption and has been investigated as a potential therapeutic for the treatment of both osteoporosis and tumour-induced bone disease. Indeed, in murine models of cancer-induced bone disease, inhibition of osteoclastic activity by OPG was also associated with a reduction in tumour burden. The discovery that OPG can bind to and inhibit the activity of TRAIL (TNF-related apoptosis-inducing ligand) triggered extensive research into the potential role of OPG in the regulation of tumour cell survival. A number of reports from studies using in vitro models have shown that OPG protects tumour cells from the effects of TRAIL, thereby possibly providing tumour cells that produce OPG with a survival advantage. However, the ability of OPG to act as a tumour cell survival factor remains to be verified using appropriate in vivo systems. A third area of interest has been the use of OPG as a prognostic marker in various cancer types, including myeloma, breast and prostate cancer. This review provides an overview of the role of OPG in cancer, both in cancer-induced bone disease and in tumour growth and survival.

OPG IN CANCER

OPG (osteoprotegerin), a novel secreted member of the TNFR [TNF (tumour necrosis factor) receptor] superfamily of proteins known to be involved in a large number of biological systems, plays a key role in the regulation of bone resorption (recently reviewed in [1]). The discovery that OPG is a potent inhibitor of osteoclast activity and maturation initiated research into the possibility of using this molecule as a therapeutic agent for the treatment of a variety of conditions associated with increased bone resorption, including tumour-induced bone disease [2]. A key discovery was the ability of OPG to bind to and inhibit the activity of TRAIL (TNF-related apoptosis-inducing ligand) and the suggestion that OPG production may provide cells with a survival advantage [3]. In vitro studies using a number of different tumour types have supported this hypothesis [4–8], but an undisputed functional link between OPG and cell survival in cancer remains to be established. The use of serum OPG measurements as a prognostic marker has also been investigated and, in several cancer types, elevated levels of serum OPG are found to be associated with poor prognosis [9–12].

In this review we summarize the published reports relating to the potential role of OPG in cancer, including data from in vitro and in vivo models, as well as clinical studies.

MOLECULAR STRUCTURE AND EXPRESSION OF OPG

OPG was identified by two independent groups in 1997, and initially also named OCIF (osteoclastogenesis...

Key words: apoptosis, bone turnover, metastatic bone disease, osteoprotegerin (OPG), receptor activator of nuclear factor κB ligand (RANKL), tumour cell survival, tumour-induced bone disease.

Abbreviations: BMSC, bone marrow stromal cell; ER, oestrogen receptor; HHM, humoral hypercalcaemia of malignancy; MGUS, monoclonal gammopathy of undetermined significance; OPG, osteoprotegerin; PSA, prostate specific antigen; PTHrP, parathyroid hormone-related protein; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; RT-PCR, reverse transcription–PCR; SCID, severe combined immunodeficient; sRANKL, soluble RANKL; TNF, tumour necrosis factor; TNFR, TNF receptor; TRAIL, TNF-related apoptosis-inducing ligand.

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inhibitory factor), TR-1 (TNF receptor-related molecule-1) and FDCR-1 (follicular dendritic cell receptor-1) [13–15]. As of 2000, the name osteoprotegerin (from Latin os for bone and protegere for to protect) has been used as agreed by the ASBMR (American Society for Bone and Mineral Research) Nomenclature Committee [16], reflecting the predominant function of OPG in bone metabolism. OPG is a member of the TNFR family, but differs from the other TNFRs due to the lack of a transmembrane domain, making this a soluble molecule with the ability to bind a number of different ligands. OPG has a wide tissue distribution and is found in vascular tissues, bone, prostate, testis, kidney, liver, lung, heart and a range of other tissues.

The human OPG gene is located at chromosome 8q23-24, and contains five different exons spread over a total of 29 kb [17]. Three main species of OPG have been identified, the most abundant being the 2.2–3 kb species, with two minor splice variant forms of 4.2–4.4 and 6.5–6.6 kb. OPG comprises 401 amino acids of which 21 are a signal peptide which is cleaved, generating a mature form of 380 amino acids (Figure 1). The molecular structure of OPG is as follows. At the N-terminus, there are four domains (D1–D4) which have cysteine-rich motifs involved in the formation of so-called ‘tethered loops’, and these domains are required for biological activity. At the C-terminus, there are tandem death-domain homologous regions (D5 and D6) followed by a heparin-binding site (D7). At position 400, there is a cysteine required for homodimerization of the molecule. OPG is produced as a monomer (55–62 kDa), but undergoes homodimerization and is secreted as a disulphide-linked homodimeric glycoprotein with four or five potential glycosylation sites, generating a mature form of OPG of 110–120 kDa. It is the dimeric form of the protein which has the highest heparin-binding capacity and also the highest hypocalcaemic ability.

**BIOLGICAL FUNCTIONS OF OPG**

**Role of OPG in bone remodelling**

The discovery of the OPG/RANK (receptor activator of nuclear factor κB)/RANKL (RANK ligand) system revolutionized our understanding of the molecular mechanisms responsible for the regulation of bone turnover. The binding of RANKL to RANK on pre-osteoclasts and osteoclasts is essential for their maturation and activity (Figure 2), and mice deficient in RANKL have extensive osteopetrosis due to a lack of functional osteoclasts [1]. OPG is a soluble decoy receptor for RANKL and thus prevents binding of RANKL to RANK and subsequent activation of osteoclast activity. OPG knockout mice have severe osteoporosis, with near total loss of cancellous bone [18]. Abnormal bone resorption due to local or systemic stimulation of osteoclast differentiation and activation is a hallmark of various benign and malignant bone diseases, identifying the molecules of the OPG/RANKL system as potential therapeutic targets. As discussed below, the use of OPG to treat tumour-induced...
bone disease has been successful in animal models of breast and prostate cancer, supporting the potential clinical use of this molecule. The role of OPG in bone remodelling has been the subject of excellent recent reviews [1,19].

**Role of OPG in cell survival**

Although much of the current interest in OPG is focussed on its ability to inhibit osteoclastogenesis, this protein was originally identified as a member of the TNFR family and has also been shown to be a soluble receptor for TRAIL [3]. TRAIL is present in tumours *in vivo* where it is produced by monocytes in response to interferon-γ or -α, and it is the principal mediator of acquired tumour cell killing activity [20,21]. Normal cells are found to be insensitive to the actions of TRAIL, forming the basis for the potential use of TRAIL as an anti-cancer drug [22]. Studies using TRAIL knockout mice have demonstrated a role for TRAIL in mediating the antitumour effects of the immune system, where TRAIL knockout mice are more susceptible to tumour initiation [23]. TRAIL exerts its effects through two classes of membrane-bound receptors (DR4 and DR5) carrying so-called death domains [24,25]. Activation of DR4/DR5 by TRAIL triggers the death-signalling cascade common to the TNF family. One potential strategy for cells to avoid TRAIL-induced apoptosis would be to express ‘decoy’ receptors for TRAIL that do not carry death-inducing domains and/or to secrete similar soluble factors that bind and inactivate TRAIL. At least two such cell-surface receptors have been identified: DcR1 and DcR2 [26,27]. However, a number of studies have failed to show a correlation between the expression of DcR1 and DcR2 on the cell surface and the sensitivity of the cells to TRAIL, suggesting that there are additional mechanisms controlling TRAIL resistance [28]. The ability of OPG to bind to TRAIL and prevent it from associating with death-inducing receptors suggests that OPG could act as an additional soluble decoy receptor for TRAIL, preventing induction of the apoptotic signalling cascade by TRAIL and thereby provide cells with a survival advantage (Figure 3).

**Additional biological functions of OPG**

In addition to the roles of OPG in bone metabolism and tumour cell survival described above, OPG has been reported to be associated with several other organ systems and pathologies, e.g. endometriosis [29], periodontal disease [30], thyroid disease [31] and coronary heart disease [32]. In most cases, the exact functions of OPG remain to be established, but the widespread expression of OPG suggests that this molecule may have multiple biological activities yet to be described. One example is that OPG is reported to be essential for normal B-cell development and function [33]. The most extensively studied functions of OPG outside bone metabolism and tumour cell survival have been related to a possible role in the vascular biology [34–40]. If OPG does have a significant function in the vasculature, this may ultimately have implications for tumour angiogenesis, a key process in cancer development.

**ROLE OF OPG IN DIFFERENT CANCERS**

OPG production is suggested to be part of a tumour cell survival strategy and a number of different tumour cells have been found to produce OPG consistent with this hypothesis [4–6]. In addition, dysregulation of the OPG/RANKL system is thought to be central to the bone disease associated with several cancers, including that of breast and prostate cancer, as well as in multiple myeloma [2]. Lipton et al. [41] found that OPG was not present at elevated levels in serum from patients with solid tumours compared with control subjects. When analysed according to site of the primary tumour, serum OPG was found to be higher in patients with colorectal cancer and pancreatic cancer compared with controls and lower in patients with myeloma. Interestingly, patients with bone metastases did not have increased serum OPG, whereas the opposite was true for patients with liver metastases. Serum measurements of OPG give an indication of the total circulating levels, but the key biological role of OPG in tumour development may be at local sites (tumour nests in bone) and therefore not reflected by circulating levels. There is increasing evidence of expression of OPG in tumours, and this information may need to be coupled with serum measurements in order to support conclusions about the role of OPG in tumorigenesis. The subsequent sections summarize the data describing
expression of OPG by tumour cells grown in vitro, effects of OPG in various in vivo models, as well as studies of OPG expression by human tumours and measurements of serum OPG from cancer patients.

**OPG in prostate cancer**

There has been considerable interest in the role of OPG in prostate cancer, as overexpression of this molecule may partly explain why the associated bone lesions have a sclerotic phenotype. High levels of OPG associated with tumour sites would inhibit osteoclast activity and tilt the balance in favour of increased bone formation, and several studies have aimed to determine the relationship between OPG expression and bone formation in prostate cancer.

**Reports from in vitro systems**

Prostate cancer cells produce and release OPG when grown in vitro, and a number of studies have been carried out investigating the ability of prostate-cancer-cell-derived OPG to protect the cells from TRAIL-induced apoptosis. Holen et al. [4] found that the hormone-independent prostate cancer cell lines PC3 and DU 145 produced 10-fold more OPG than the hormone-dependent cell line LNCaP when grown under the same conditions. More detailed studies using PC3 cells showed that these cells produced sufficient OPG over a 2 day period to yield significant protection against TRAIL-induced apoptosis. This protective effect could be eliminated by addition of an excess of soluble RANKL, which has a higher affinity for OPG than TRAIL does. The authors [4] conclude that OPG production by tumour cells may provide a mechanism whereby they avoid elimination by the host immune system through TRAIL-induced apoptosis.

Nyambo et al. [7] extended these studies to determine whether OPG produced by BMSCs (bone marrow stromal cells) could protect prostate cancer cells from the effects of TRAIL. Human BMSCs isolated from bone biopsies taken from untreated prostate cancer patients were found to produce large amounts of OPG when grown in culture. When TRAIL-sensitive prostate cancer cells (PC3) were challenged with 50 ng/ml of TRAIL in the presence of BMSC-conditioned medium, the levels of apoptosis were substantially reduced compared with cells challenged in fresh medium (no OPG present). Removal of OPG from the conditioned medium by immunodepletion eliminated the protective effect against TRAIL. The authors [7] suggest that at least part of the survival advantage gained by prostate cancer cells when colonizing bone may be due to the production of OPG by BMSCs.

**Reports from in vivo models**

Investigations using in vivo models have focused on the role of OPG in prostate-cancer-induced bone disease, based on the hypothesis that overexpression of OPG would cause increased bone formation and lead to the primarily osteosclerotic bone lesions associated with prostate cancer bone metastases.
Corey and co-workers [42] have studied the effects of OPG overexpression on prostate cancer cell growth and on interactions between tumour cells and bone cells in vitro and in vivo. When OPG-C4-2 prostate cancer cells that overexpress OPG were implanted subcutaneously in mice, neither the tumour take rate nor the tumour growth rate was affected compared with those seen in mice receiving C4-2 cells that had been transfected with the empty vector. In contrast, when OPG-C4-2 cells were implanted in bone, the tumour volume was significantly decreased compared with that observed when using the parental C4-2 cells. The authors [42] conclude that OPG does not directly affect tumour cell growth, but does indirectly decrease growth of C4-2 tumours in bone by causing a decrease in tumour-associated osteolysis. This will, in turn, restrict the supply of growth factors and also limit the space tumours need to be able to expand within the bone marrow cavity. OPG expressed in prostate cancer bone metastases may contribute to the osteoblastic character of most prostate cancer bone lesions.

The effects of OPG administration on the growth of prostate cancer cells implanted in bone and at extra osseous sites in SCID (severe combined immunodeficient) mice have been investigated by Zhang et al. [43]. OPG did not affect the rate of proliferation of the prostate cancer cells used when they were grown in vitro. PSA (prostate serum antigen)-positive tumour infiltration was observed in the bones of all the vehicletreated animals, but not in any of the animals receiving OPG–Fc chimera. In this model, there was no effect of OPG administration on the growth of subcutaneous tumours, and the authors [43] conclude that OPG-mediated inhibition of osteoclastogenesis was associated with prevention of C4-2B cell growth in osseous, but not in extra-osseous, sites.

Yonou et al. [44] carried out a study on the effect of OPG on prostate cancer burden in human adult bone implanted into non-obese diabetic SCID mice. In this model, LNCaP cells were injected into the intramedullary space of human bone implanted subcutaneously on the back of the mice. Histomorphometrical analysis showed that OPG markedly reduced the number of osteoclasts and the size of the tumours in bone sites, but that it had no effect on the local growth of subcutaneous LNCaP tumours. The authors [44] conclude that this study shows the importance of osteoclastic bone resorption for the progression of prostate cancer in bone and that OPG can suppress both further progression of existing lesions, as well as the development of new ones. This work clearly demonstrates the usefulness of OPG as a tool in the studies of the molecular mechanisms involved in tumour-induced bone disease.

Further work by Kiefer et al. [45] has reported that administration of OPG decreases the growth of prostate cancer cells (LuCAP23.1) that had been injected intratibially into nude mice. OPG was found to be unable to prevent the establishment of prostate-derived tumours in bone, but did decrease the growth of the tumour cells as determined by measurements of PSA, following both a prevention and a treatment regimen. The authors conclude that OPG may be useful as a therapeutic agent in the treatment of tumour-induced bone disease from prostate cancer.

The studies described above show that increasing the levels of OPG, either by induction of overexpression or by administration of therapeutic doses of recombinant protein, results in reduced tumour-induced bone disease and accompanying tumour burden in bone, whereas tumours in extra-osseous sites are unaffected by OPG.

Reports from clinical studies

In humans, OPG is expressed both in the normal prostate, in prostate tumours and in bone metastases derived from prostate cancer, but the cells expressing OPG vary between the tissues. Brown et al. [46] determined the expression of OPG in prostatic tissues from 28 patients and four healthy donors by RT-PCR (reverse transcription–PCR) and immunohistochemistry. OPG was detected in normal prostate, in two out of ten primary prostate cancer specimens and increased in all bone metastases compared with non-osseous metastases and primary prostate tumours. These data suggest a role for OPG in tumour-associated bone disease in prostate cancer, but indicate that OPG is not highly expressed by prostate cancer cells within primary tumours. Similar results have been reported by Nyambo and co-workers [7] who found low OPG expression by the tumour cells in primary prostate cancer compared with that of the surrounding stromal cells in bone metastases. OPG produced by stromal cells in the bone microenvironment may support survival of tumour cells in the early stages of bone metastasis.

Several studies have been carried out measuring the levels of OPG in serum from prostate cancer patients in order to determine whether serum OPG levels change with progression of the disease. Brown et al. [47] measured OPG in serum from patients with benign prostatic hyperplasia, clinically localized prostate cancer, early recurring prostate cancer and advanced disease with evidence of bone metastases. They found serum OPG to be elevated in patients with bone metastases compared with all other groups, but OPG levels did not correlate with PSA levels. Interestingly, high levels of OPG were not associated with a decrease in the bone resorption marker sCTX (serum C-telopeptide of type I collagen), suggesting that endogenous OPG is not able to suppress bone resorption in the advanced prostate cancer setting. The authors [47] conclude that OPG is associated with the progression of prostate cancer in bone, but further studies are required to elucidate fully the effects of OPG on prostate cancer cells as well as in the development of bone metastases.
A potential role for serum OPG as a marker of early relapse in prostate cancer is suggested by the study of Eaton et al. [12], who compared serum OPG levels in 104 prostate cancer patients, ten cases of benign prostatic hyperplasia and ten healthy young men. The prostate cancer patients were divided into several groups: untreated patients with (i) organ confined or (ii) locally advanced disease, (iii) patients with advanced disease responding to androgen ablation, and (iv) patients with early signs of disease progression. Serum OPG was found to increase in patients who progressed following androgen ablation, and this increase was detectable prior to elevation of the classical marker PSA. The authors [12] suggest that serum OPG may not simply be a marker of advanced disease, but indicate changes in tumour cell survival and growth.

The findings from work reported by Jung et al. [48] are in agreement with the conclusions from the study by Eaton et al. [12]. Serum OPG was measured in 117 patients with prostate cancer, including 39 with stage pN0M0, 34 with stage pN1M0 and 44 with bone metastases, and compared with the levels measured in 35 healthy young men and 35 patients with benign prostatic hyperplasia. Serum OPG levels were found to be increased in patients with metastatic bone disease compared with patients with organ confined disease, but there was no positive correlation with tumour grade, tumour stage PSA or bone markers. Interestingly, ROC (receiver operator characteristic curve) analysis revealed that OPG had a better diagnostic accuracy than alkaline phosphatase or the bone resorption marker CTX for detecting bone metastases in prostate cancer patients, and the authors [48] suggested that OPG could be a useful marker of tumour induced bone disease.

### Summary (Table 1)

The studies in prostate cancer have revealed that OPG is expressed by prostate cancer cells in vitro and several reports have shown that this may lead to increased survival of tumour cells. In contrast, data from animal models of prostate cancer do not support that OPG affects the expansion of prostate tumours in vivo, and OPG is not found to be highly expressed by human prostate tumours. However, in models of tumour-induced bone disease, OPG treatment is able to prevent the development of lytic lesions, leading to reduced tumour volume in bone. Data from several studies measuring OPG in serum from prostate cancer patients suggest that OPG may be a prognostic marker and an indication of early disease recurrence.

### OPG in multiple myeloma

Multiple myeloma is a haematological malignancy characterized by clonal expansion of plasma cells within the local bone marrow microenvironment. Within this local microenvironment, a reciprocal relationship exists where myeloma cells promote osteoclastic bone resorption and, in turn, osteoclasts, osteoblasts and BMSCs promote myeloma cell growth and survival. Thus inhibitors of osteoclastic bone resorption have the potential to indirectly inhibit myeloma growth by making the bone marrow microenvironment less favourable for myeloma cell growth and survival. One of the major clinical features of multiple myeloma is the development of osteolytic bone disease and, over recent years, there is increasing evidence to suggest that the dysregulation of the RANK/RANKL/OPG system is important in the pathogenesis of myeloma bone disease. However, the high concentrations of OPG in the local bone marrow microenvironment and the susceptibility of myeloma cells to apoptosis induced by TRAIL raise the possibility that OPG may have additional roles and may be able to function as a paracrine survival factor in this local microenvironment in multiple myeloma.

### Reports from in vitro systems

In contrast with breast cancer and prostate cancer, human myeloma cells do not express or release OPG. However, myeloma cells can interact with BMSCs and osteoblasts to reduce the concentration of OPG released from these cells, which is consistent with the hypothesis that decreased OPG may play a role in the pathogenesis of myeloma bone disease [6,49,50]. Myeloma cells can bind,
internalize and degrade OPG *in vitro*, mediated by an interaction between the proteoglycan syndecan-1, which is highly expressed on the surface of myeloma cells, and the heparin-binding domain of OPG [51]. More recently, Colucci et al. [52] have demonstrated *in vivo* that the formation of the OPG/TRAIL complex supports osteoclast formation in an *in vitro* osteoclastogenesis model using peripheral blood mononuclear cells from patients with multiple myeloma. In this study [52], T-cells from patients with multiple myeloma expressed increased RANKL, OPG and TRAIL and supported the formation of osteoclasts with longer survival. The role of the OPG/TRAIL complex was demonstrated by immunoprecipitation experiments, and a neutralizing antibody to TRAIL resulted in a decrease in osteoclast number. Shipman and Croucher [6] have demonstrated that both recombinant OPG and OPG released from osteoblasts and BMSCs can protect myeloma cells from apoptosis induced by TRAIL. In contrast, apoptosis induced by specific agonists of the TRAIL death receptors is not prevented by OPG, demonstrating the importance of the OPG/TRAIL interaction in protecting myeloma cells against apoptosis [53]. Although it is intriguing that myeloma cells could down-regulate the production of a tumour survival factor, *in vitro* studies demonstrated that the reduced concentration of OPG was still sufficient to inhibit apoptosis induced by TRAIL. OPG may have contrasting functions in multiple myeloma, as a key regulator of myeloma bone disease and as a paracrine survival factor in the local bone marrow microenvironment. Studies using TRAIL gene-deficient mice have demonstrated that these animals are more susceptible to tumour initiation, suggesting that TRAIL may be important in this process [23,54,55]. Therefore the interaction between OPG and TRAIL in multiple myeloma may be more important in the early stages of the disease, rather than in the advanced stages where the bone disease is more pronounced. The precise nature of the role of OPG in multiple myeloma remains to be determined, but will be dependent upon the relative concentrations, timing and location of expression of OPG, TRAIL and their respective ligands and receptors.

**Reports from in vivo models**

The key role of the RANK/RANKL/OPG system in the pathogenesis of myeloma bone disease is demonstrated by *in vivo* studies which target this system using murine models of multiple myeloma. The 5T33 murine model of myeloma is a spontaneously occurring model of murine myeloma which closely resembles the human form of the disease. This model of myeloma is characterized by an increase in serum paraprotein, which mirrors tumour burden, and by the development of osteolytic bone disease. In mice with established 5T2MM myeloma, treatment with recombinant Fc–OPG prevented the development of osteolytic bone lesions and was associated with a decrease in osteoclast number and an increase in cancellous bone volume and bone mineral density [56]. In a separate study, preventative treatment (from the time of tumour cell inoculation) of the 5T33 murine model of myeloma with recombinant Fc–OPG resulted in a significant decrease in serum paraprotein and tumour burden and an increase in time to morbidity. This effect was attributed to the inhibitory effect of OPG on myeloma bone disease altering the bone marrow microenvironment and thus indirectly inhibiting myeloma growth as, although osteoclast number was decreased *in vivo*, no antitumour effect of OPG was observed in parallel *in vitro* studies [57]. The significance of the RANK/RANKL/OPG system is highlighted further by *in vivo* studies using RANK–Fc, a recombinant RANKL antagonist which, in contrast with OPG, does not bind to TRAIL and therefore the effects reflect specific inhibition of RANKL. In studies using the 5TGM1 murine model of myeloma or the SCID-hu model of human multiple myeloma, where foetal bone xenografts are implanted in immunodeficient mice and injected with myeloma cells isolated from patients with multiple myeloma, RANK–Fc was shown to inhibit both the myeloma bone disease and to reduce serum paraprotein and tumour burden, an effect attributed to the inhibitory effect of RANK–Fc on myeloma bone disease [49,58,59].

**Reports from clinical studies**

Several studies have demonstrated that serum concentrations of OPG are decreased in patients with multiple myeloma and that the ratio of sRANKL (soluble RANKL)/OPG is altered to reflect both a decrease in OPG and an increase in sRANKL. Seidel et al. [60] demonstrated that serum OPG was significantly decreased in myeloma patients compared with healthy age- and sex-matched controls. In addition, this study [60] demonstrated an association of OPG concentrations with the degree of osteolytic bone disease as assessed by radiography. Politou and co-workers [61] have demonstrated the significance of the ratio of serum concentration of sRANKL to OPG in patients with multiple myeloma. OPG was significantly decreased in the serum of patients with multiple myeloma compared with healthy individuals or to patients with MGUS (monoclonal gammopathy of undetermined significance). In contrast, sRANKL was increased significantly in the serum of patients with multiple myeloma and MGUS compared with healthy individuals. The overall conclusion was that the sRANKL/OPG ratio is increased in patients with MGUS and increased further in patients with multiple myeloma, and may represent a tool to distinguish cases of MGUS and early myeloma. More recently, serum OPG was found to be increased in patients with multiple myeloma following autologous stem cell transplantation, an increase which was mirrored by a decrease in markers of bone resorption and which preceded an increase in markers of bone formation [62].
Table 2 OPG in multiple myeloma at a glance

<table>
<thead>
<tr>
<th>Studies</th>
<th>Findings</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>In vitro systems</strong></td>
<td>Myeloma cells do not express OPG, but down-regulate OPG release from osteoblasts and BMSCs</td>
<td>[6,49–51]</td>
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<td></td>
<td>Myeloma cells can bind, internalize and degrade OPG</td>
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<td></td>
<td>OPG released from osteoblasts can inhibit TRAIL-induced apoptosis of myeloma cells</td>
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<tr>
<td><strong>In vivo models</strong></td>
<td>Fc–OPG can prevent the development of myeloma bone disease, reduce tumour burden and increase survival</td>
<td>[56,57]</td>
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<tr>
<td>Clinical studies</td>
<td>Serum concentrations of OPG are decreased in patients with multiple myeloma compared with healthy controls or patients with MGUS</td>
<td>[60,61]</td>
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Summary (Table 2)
Studies have shown that, in contrast with breast cancer and prostate cancer cells, human myeloma cells do not express or release OPG, but have the capacity to down-regulate OPG release from other cell types and thus decrease local concentrations of OPG. Clinical studies have shown that the levels of OPG in the serum of myeloma patients are reduced and that there is a resulting imbalance in the OPG/RANKL ratio. There is increasing evidence for a disruption of the OPG/RANKL/RANK system associated with myeloma bone disease and these molecules represent potential therapeutic targets for the treatment of myeloma bone disease.

OPG in breast cancer
The expression and/or function of OPG in breast cancer has been reported, and the potential involvement of OPG both in tumour induced bone disease and also in tumour cell survival has been described by several groups. As the metastatic bone lesions associated with advanced breast cancer are mainly osteolytic, it is likely that the involvement of OPG in this setting is different from that of the mainly osteoblastic lesions of prostate cancer.

Reports from in vitro systems
OPG is expressed by a number of breast cancer cell lines in vitro and studies by Holen et al. [5] have shown that hormone-independent MDA-MB-231 and MDA-MB-436 cells produce sufficient OPG to be protected from TRAIL-induced apoptosis in vitro. Taken together with data published for prostate cancer cells [4], the production of OPG may be a part of a survival strategy used by tumour cells in their quest to avoid elimination by the host immune system. In a separate study, this group has also shown [63] that OPG produced by BMSCs isolated from patients with breast cancer was able to inhibit TRAIL-induced apoptosis of MDA-MB-436 breast cancer cells. If OPG is able to support tumour cell survival, the high levels of OPG in the bone microenvironment may be a contributing factor to the propensity of breast cancer cells to metastasize to bone.

Reports from in vivo models
As for prostate cancer, in vivo studies of OPG in breast cancer have focused on the effects on tumour-induced bone disease. Morony et al. [64] investigated the ability of recombinant OPG to inhibit tumour-induced osteoclastogenesis, osteolysis and tumour burden in an SCID mouse model. Human MDA-MB-231 breast cancer cells were injected intracardially into nude mice, a procedure normally resulting in the development of extensive tumour-induced bone disease. Following tumour inoculation, mice were treated with recombinant OPG (25 mg·kg⁻¹·3 days⁻¹ for 4 weeks) and the bones and soft tissues processed for histological analysis. OPG treatment completely prevented the formation of tumour-associated lytic lesions and this was accompanied by a 75% reduction in skeletal tumour burden. Tumour-associated osteoclasts were also eradicated, but there was no effect of the treatment on soft tissue tumours. The data support the notion that inhibition of bone resorption prevents tumour growth at skeletal sites, leading to the observed reduced tumour burden. Based on this study, OPG may be a therapeutic option for the treatment of tumour-induced bone disease in breast cancer.

Reports from clinical studies
Thomas et al. [65] have reported the expression of OPG mRNA both by a series of breast cancer cell lines in vitro and also by primary breast tumours. The hormone-dependent MCF7 cells and the hormone-independent MDA-MB-231 cells, as well as the 12 breast tumour samples from infiltrating ductal carcinomas, had expression of OPG, suggesting that OPG gene expression is a common feature of this type of breast tumour. In this study [65], no data were provided regarding the expression of OPG protein or which cell types within the tumours expressed OPG, as mRNA extraction was done from whole-tissue samples.

Reinholz and co-workers [66] examined gene expression patterns of a number of TNF family members, including RANKL and OPG, in normal, non-invasive, invasive and metastatic human breast cancer specimens. OPG expression was unchanged between normal breast tissues...
Table 3  OPG in breast cancer at a glance

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<thead>
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<th>Studies</th>
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<th>References</th>
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<td><em>In vivo</em> models</td>
<td>OPG administration inhibits tumour growth in bone</td>
<td>[64]</td>
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<td>OPG is expressed in primary breast tumours and in metastases in various tissues</td>
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<td>OPG expression correlates with ER/PR status of the tumour</td>
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and non-invasive breast tissues. In metastatic tissues, there was increased OPG expression in one case of liver metastases compared with normal breast tissue, but none of the other metastases had elevated OPG expression. So far the study by Van Poznak et al. [67] is the only report that has examined the expression patterns of OPG protein in breast tumours and normal breast tissues, describing which cell types express this protein within tumours. OPG protein localization was determined in a series of invasive breast cancers and in healthy controls by immunohistochemistry. OPG was detected in 22/40 (55%) of the breast tumours and there was a significant positive correlation between OPG expression and ER (oestrogen receptor) status with 75% of the ER+/PR+ (progesterone receptor) tumours expressing OPG. In normal breast tissues, OPG was only present in epithelial cells in areas of ducts having undergone columnar changes. There was no correlation between the tumour cell expression of OPG with that of either RANKL or TRAIL detected in the same samples. The authors [67] speculate that there could be a role for OPG in the regulation of tumour cell survival in breast cancer, but further studies are needed in order to confirm this.

There is limited published information relating to serum OPG levels in breast cancer patients, but the study by Lipton et al. [41] includes a subset of breast cancer patients (n = 61). No increase in serum OPG levels was detected in patients with breast cancer compared with that in healthy control subjects (n = 112), but the range of OPG levels was wider in the breast cancer group. No breakdown of the OPG levels according to diagnosis within the breast cancer group was provided in this study and no information regarding the diagnosis was given. Further studies including larger numbers of well-characterized patients following rigorous protocols for sample collection, processing, storage and measurements of OPG are required to clarify the usefulness of serum measurements of OPG in breast cancer.

Summary (Table 3)

No clear role for OPG in the development of breast cancer has been identified yet, but there is convincing evidence that breast tumour cells express OPG both *in vitro* and *in vivo*. Measurements of serum levels of OPG have only been reported from a very limited number of breast cancer patients and have not been related to precise diagnosis, stage of disease or prognosis. To date there is no information available relating to the expression of OPG in bone metastases from breast cancer, and it is not known whether OPG expression by the primary tumour affects the formation of bone metastases. On the other hand, there is good evidence that pharmacological doses of OPG prevent the development of lytic bone disease in animal models of breast cancer. Further investigations that include larger numbers of patients will be required in order to establish the potential role of OPG in breast cancer development and progression.

**OPG in other cancers**

Reports from *in vitro* systems implicating OPG as a survival factor for tumour cells have led to investigations of OPG expression in a number of different cancers. Studies have been carried out in patients with tumour-induced bone disease, as well as cancers that have no propensity to metastasize to bone.

Ito et al. [9] examined the expression of OPG by gastric carcinoma cell lines and material from 103 cases of primary gastric carcinomas by RT-PCR and immunohistochemistry, and related OPG expression to clinicopathological information, such as tumour stage, depth of invasion, presence of lymph node metastasis and prognosis. They report a significant correlation between OPG expression and depth of tumour invasion, nodal metastasis and tumour stage, with strong OPG expression more frequent in stages III and IV than stages I and II. The authors [9] speculate that high levels of OPG expression could possibly affect the levels of TRAIL-induced apoptosis in the tumours, and suggest that OPG expression may be a marker of aggressive gastric carcinomas.

Bladder carcinoma cells have been found to be sensitive to TRAIL-induced apoptosis, and OPG production by these cells may therefore interfere with immune-mediated...
antitumour toxicity [11]. In patients with bladder carcinoma, serum OPG was found to be elevated compared with the mean concentration in healthy individuals, and OPG levels were found to be associated with high tumour stage and grade. After a follow up period of 5 years, patients who had low serum OPG levels had a longer post-operative tumour-free interval and increased survival compared with patients with high levels of serum OPG. The authors [11] suggest that serum OPG correlates with tumour stage and is also predictive of early recurrence of bladder carcinoma.

OPG has also been investigated as one of the potential mediators of the extensive bone disease associated with giant cell tumours (GCT) of bone, where disruption of the normal balance of bone remodelling is one of the hallmarks [68,69].

As a consequence of its potent antiresorptive activities, OPG is suggested to be useful as a therapeutic agent in the treatment of HHM (humoral hypercalcaemia of malignancy). In initial studies of induced hypercalcaemia, Morony and co-workers [64] used recombinant OPG to treat mice with HHM. Concurrent OPG treatment reduced osteoclast numbers and bone resorption, and prevented hypercalcaemia. These studies were extended by Capparelli et al. [70], who used a syngeneic mouse model of HHM. Treatment with recombinant OPG prior to the onset of HHM resulted in a dose-dependent decrease in HHM as determined by blood calcium measurements. In mice with established HHM, administration of 2.5 mg of OPG·kg\(^{-1}\)·day\(^{-1}\) for 4 days resulted in complete normalization of serum calcium within 48 h. OPG treatment did not affect plasma PTHrP (parathyroid hormone-related protein) levels, tumour size, tumour PTHrP mRNA levels or proliferation of colon-26 tumour cells in vitro, suggesting that OPG does not have direct antitumour effects. The authors [70] suggest that OPG may be a potential therapeutic agent for use in treatment of HHM.

Summary

OPG is expressed by different tumour types, but the information available is somewhat fragmented with studies either measuring circulating OPG or describing OPG expression by tumour tissues, but not both. There are considerable variations in the way tissues and serum samples have been obtained, stored, processed, stained, measured, scored, evaluated and interpreted, and this makes it impossible to draw firm overall conclusions regarding the role of OPG in cancers. In many cases the sources of the OPG measured are unknown, and it could originate from tumour cells, cells of the vasculature, the cells of the bone microenvironment or from a combination of various sources. The roles of tumour-generated OPG may vary according to tumour burden, with dense tumour masses being able to secrete substantial amounts of OPG due to the high cell number rather than the high expression by individual cells. Many of the published clinical studies have included low numbers of patients, and would require confirmation from larger cohorts.

**THERAPEUTIC APPLICATIONS OF OPG**

As described above, OPG has been used in animal models to successfully treat tumour-induced bone disease both in the context of breast and prostate cancer and multiple myeloma. To date, only one study [71] has been reported from a clinical trial in cancer patients receiving a recombinant OPG construct (AMGN-0007).

Owing to its ability to inhibit bone resorption, OPG was first tested in the clinical setting for the treatment of post-menopausal osteoporosis. Bekker et al. [72] conducted a randomized double-blind placebo-controlled sequential dose-escalation study in 52 postmenopausal women diagnosed with osteoporosis. Patients received a single subcutaneous dose of OPG (0.1, 0.3, 1 and 3 mg/kg of body weight) and measurements of bone turnover markers were carried out at regular intervals for up to 84 days following OPG treatment. The single dose of OPG caused a rapid and sustained decrease in the levels of bone resorption and a delayed reduction in bone formation markers thought to be due to a general depression of bone turnover. OPG was well tolerated and the authors [72] suggest that OPG may be an effective treatment of bone disease involving excessive bone resorption.

In 2003, Bod and co-workers [71] reported the results from a randomized double-blind double-dummy active-controlled sequential dose-escalation study to determine the safety and effect on bone resorption of a single dose of OPG (AMGN-0007) in patients with multiple myeloma (\(n=28\)) or breast carcinoma (\(n=26\)) with confirmed lytic bone lesions. Patients were randomized to receive AMGN-0007 or pamidronate and followed for 56 days. The biological activity of AMGN-0007 was determined by measurements of markers of bone resorption. As in the osteoporosis study, AMGN-0007 was well tolerated and caused a rapid and sustained dose-dependent decrease in bone resorption marker [NTX (N-telopeptide fragment)] levels, comparable with that obtained with pamidronate. The authors [71] conclude that OPG may be a suitable therapeutic agent in the treatment of tumour-induced bone disease. However, recent developments of specific antibodies to RANKL have lead to a shift in the therapeutic approach to inhibition of bone resorption away from the use of recombinant OPG.

**OVERALL CONCLUSIONS**

The reported role of OPG in cancer contradicts in vitro evidence for OPG acting as a tumour cell survival factor, whereas studies from in vivo models have so far failed to confirm effects of OPG on tumour growth at
extra-osseous sites. These discrepancies may be partly explained by the limitations of some of the available in vivo models, which commonly involve implantation of large numbers of tumour cells at single sites, rather than development of tumours from circulating tumour cells. Increased tumour cell survival through the inhibition of TRAIL-induced apoptosis may be a mechanism involved in very early tumour development when there are only a low number of cancer cells present.

In conclusion, there is emerging evidence for a role of OPG in malignancy, but a direct functional role of OPG in cancer development remains to be proven. Therapeutic doses of OPG are able to reduce substantially tumour-induced bone disease, whereas the ability of endogenously produced OPG to affect this process is less well established. The role of OPG in vivo will be dependent upon the relative concentrations, timing and location of expression of OPG, TRAIL and their ligands, particularly in the local bone marrow microenvironment. The extent of ‘crosstalk’ between tumour cells and the surrounding stroma will also influence the expression levels of the molecules involved in the OPG/RANK system, both in the primary tumour and in bone metastases. As highlighted throughout this review, there are still many unanswered questions relating to the role of OPG and associated molecules in cancer development that require our continued research efforts.

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