EXPERT OPINION

Osteoprotegerin expression during the micro- and macrometastatic phases of the osteoblastic metastasis in prostate cancer: therapeutic implications

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Introduction: Osteoprotegerin (OPG) acts as a soluble decoy receptor for the bone marrow stroma cell-derived and osteoblast-derived receptor activator of nuclear factor-kB ligand (RANKL), thus regulating the RANK-mediated osteoclastogenesis and osteoclast-mediated bone resorption at the metastatic niche of cancer in skeleton.

Areas covered: This article discusses the 'key' role of OPG expression during the early events of cancer cell invasion into the bone matrix and the subsequent events underlying the formation of osteoblastic metastasis, a unique event observed in human prostate cancer biology.

Expert opinion: Understanding the cellular and molecular events implicated in bone metastasis can facilitate designing new therapeutic strategies for targeting early and/or late events in the metastasis processes. The RANKL/RANK/OPG pathway is a key regulator of pathological bone metabolism in metastatic sites. Targeted manipulation of these molecules may provide sustainable antitumor responses.

Keywords: bone microenvironment, OPG, osteoblastic metastasis, osteoclastogenesis, RANK, RANKL

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1. The OPG/RANKL/RANK system in bone remodeling

Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily [1,2]. The expression of OPG has been documented in numerous tissues, including the prostate gland; however, its expression is significantly higher in bone marrow stroma cells and cells of the osteoblast phenotype [1]. In contrast to all other TNF receptor family members, OPG is a nonsignaling receptor molecule acting as a soluble decoy receptor for the receptor activator of nuclear factor-kB ligand (RANKL), thus indirectly modulating the cellular responses of RANK-expressing cells [3]. RANKL is mainly produced by osteoblasts, bone marrow stroma cells and activated T lymphocytes [3,4]. The expression of OPG and RANKL represents the most important targets for all the calcium-regulating hormones and proresorptive cytokines [3]. Indeed, the activation of RANK-signaling stimulates osteoclastogenesis and osteoclast-mediated bone resorption, thus increasing the serum levels of ionized calcium. The role of RANK-signaling has been documented in cells of the 'osteoclast lineage,' vascular endothelium and dendritic cells [4,5]. Therefore, its function is critical not only for bone remodeling but also for the immune system, contributing to T-cell activation and survival of dendritic cells [4,5]. Indeed,

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Article highlights.

- The variable expression of OPG and RANKL expression in bone marrow stroma cells regulates osteoclastogenesis, controls osteoclast function/survival and indirectly modulates bone formation at the active bone remodeling sites of the skeleton.
- Osteoblastic bone metastasis in prostate cancer is essentially incurable and only limited clinical response is seen by treatments such as androgen ablation and chemotherapy.
- Prostate cancer cells interacting with cells of the bone microenvironment can trigger the OPG 'on' – RANKL/RANK-signaling 'off' setting which suppresses osteoclast-mediated bone resorption thus producing an osteoblastic phenotype at the metastatic niche.
- Our evolving understanding of the role of the RANKL/RANK/OPG signaling pathway in bone metastasis is crucial to the development of novel strategies designed to successfully target metastatic prostate cancer tumors.
- Monitoring of OPG and RANKL levels may also be used to monitor metastatic disease progression in bones.

This box summarizes key points contained in the article.

RANK-signaling is a potent inducer of the fusion of preosteoclasts and the formation of mature osteoclasts and controls osteoclast survival [3,4]. Bone resorptive factors, such as 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2$VitD3], parathyroid hormone (PTH), PTH-related peptide (PTHrP), prostaglandin E_2 and interleukin 11 exert their actions mainly by increasing RANKL and decreasing OPG expression in the bone marrow stroma cells and the cell types of the 'osteoblast lineage,' such as osteoblasts, osteocytes and lining cells [1-6]. In summary, the differential regulation of the OPG and RANKL expression in bone marrow stroma cells and osteoblasts regulates the activation of RANK-signaling in the 'osteoclast lineage,' thus initiating osteoclastogenesis, controlling osteoclast function/survival and indirectly regulating bone formation at the active bone remodeling sites of the skeleton (**Figure 1**).

2. Clinical aspects and cellular events during skeletal metastasis

In the clinical setting, the presence of extensive bone disease is firmly associated with cancer mortality. Moreover, skeletal metastases frequently cause serious clinical symptoms and complications, including pain, pathologic fractures, nerve-compression, hypercalcemia and anemia in cancer patients [7-10]. The architecture of the trabecular bone and the bone matrix microenvironment effectively attracts and supports the homing and metastatic growth of metastatic cancer cells. Consequently, during hematogenous cancer metastasis, circulating tumor cells (CTCs) are preferentially 'seeded' in the bone marrow where they develop metastatic foci [11]. Metastatic growth in bones requires the invasion/penetration of tumor cells from the bone marrow into the mineralized bone matrix, where they will provoke a host tissue reaction that can be either osteolytic or osteoblastic in nature. Notably, mixed lesions are also often observed [10,12,13]. Prostate cancer cells grown in bone matrix produce will most often trigger an osteoblastic reaction of the host tissue [8,14]. Osteoblastic bone metastasis in prostate cancer is practically incurable with only limited clinical response to androgen ablation therapies (medical or surgical castration) and chemotherapy [12,15,16].

The type of host tissue reaction to cancer cell growth in bones is determined by the local uncoupling of the bone remodeling process [9,10,13]. The lytic and blastic reactions of the host tissue are essentially the two extreme ends of the same metabolic process, which results in the local domination of either bone resorption or bone formation [10,14,15,17,18]. The lytic component of bone reaction is attributed to humoral factors secreted by cancer cells, and which can initially activate osteoclastogenesis an thereafter maintain active bone resorption at the metastatic niche [17,18]. The blastic component of host tissue reaction is mainly attributed to the activation of bone-related humoral factors that can both stimulate osteoblast differentiation and proliferation as well as block osteoclastogenesis and bone resorption in the metastatic foci [19-22].

It should be noted that the initial dissemination of circulating cancer cells in bone marrow results in a unique histological cross-talk between metastatic prostate cancer cells and the bone microenvironment. Of note, the metastastic cancer cells within the bone marrow are unable to penetrate into the calcified bone matrix [23,24]. Since the main function of osteoclasts is to resorb bone, the initial invasion of metastatic cancer cells into the mineralized bone matrix can be achieved only via the activation of osteoclastogenesis within the metastatic niche (**Figure 2**). This cancer cell-orchestrated osteoclastogenesis is mediated by cancer cell-secreted pro-osteoclastogenesis factors such as PTHrP and interleukin 6 (IL-6) (**Table 1**). Some of these factors may be related to the epithelial-mesenchymal transition (EMT) of circulating prostate cancer cells [11,23,25-27]. These pro-osteoclastogenesis factors can act on bone marrow stroma cells and cells of the osteoblast lineage at the metastatic niche and down regulate OPG expression while they stimulate RANKL expression. Thereby, they can activate the RANK-signaling in preosteoclast cells present in bone marrow. Thus, preosteoclasts are transformed to mature osteoclasts (fusion of pre-osteoclasts and maturation), thereby locally activating the bone resorptive process (**Figure 2**). Alternatively, the initial phase of bone metastasis (micrometastasic phase) would require the activation of osteoclast-mediated bone resorption, randomly at the sites of active bone remodeling most likely present in trabecular bones [11,23]. The micrometastasic phase of bone lesions is a common step for all types of 'osteophilic' cancers. Therefore, the ability of the disseminated prostate cancer cells to grown within the mineralized bone matrix is related to their ability to stimulate osteoclastogenesis at the metastatic niche [11,15,23].

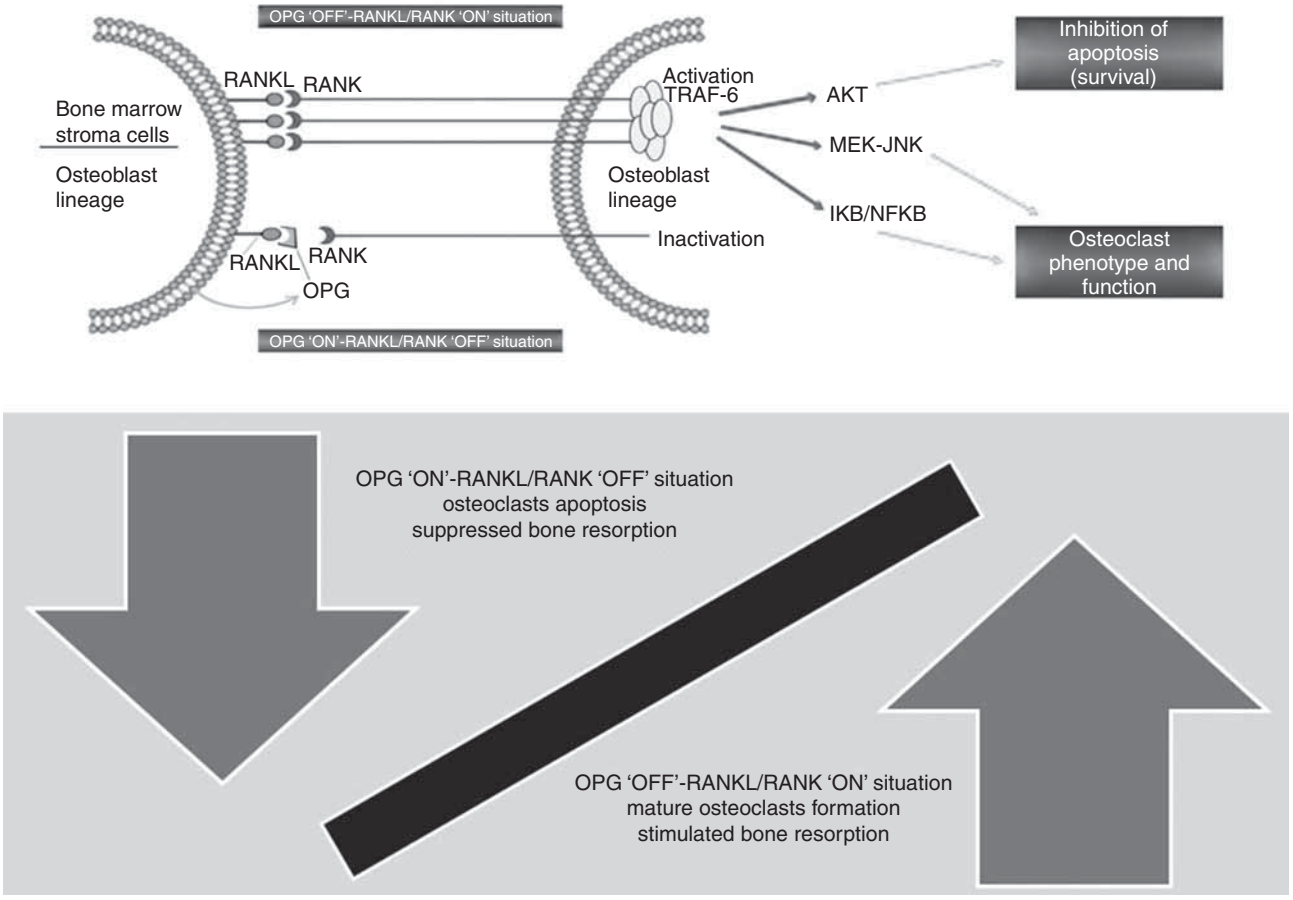

Figure 1. Schematic representation of the cellular events leading to osteoclastogenesis and osteoclast-mediated bone resorption. These events are produced by the functional interplay among cells of the osteoblast lineage and those of the osteoclast lineage. The differentiation of preosteoclasts, the formation of mature osteoclasts and osteoclast survival are maintained as long as RANKL (receptor activator of nuclear factor-Kβ ligand-mediated) /RANK-signaling is 'on' and the osteoprotegerin (OPG) expression of bone marrow stroma and osteoblast-like cells is 'off'. The reverse setting (RANK-signaling 'off' and OPG 'on') results in the induction of osteoclast apoptosis and the suppression of osteoclast-mediated bone resorption. The latter would enhance indirectly the bone forming process at any active bone remodeling site, including metastatic niche.

Moreover, the activation of the osteoclast-mediated bone resorption at the metastatic niche favors the release and/or activation of bone matrix-related growth factors, which are present in bone extracellular matrix (ECM), such as the latent transforming growth factor betas (TGFβs), bone morphogenetic proteins (BMPs), insulin-like growth factor 1 (IGF-1) and basic fibroblast growth factor (bFGF). Indeed, activation of such growth factors can provoke the establishment of a 'vicious cycle' of local cell–cell interactions within the metastatic microenvironment which results in the stimulation and growth of the metastatic cancer cells, and can also alter the biology of metastatic cancer cells to confer increased resistance to proapoptotic signals in bone lesions [23-25].

Osteoclast-mediated bone resorption, mediated by cancer cell-related proteases, such as the urokinase-type plasminogen activator (uPA), can turn plasminogen to plasmin and can activate other proteases, such as tissue metalloproteinases (MMPs) [7,28,29]. These cellular events result in an increased microenvironment proteolysis, which can further activate the bone bioavailability of the bone matrix-related growth factors that, in turn, can further stimulate the proliferation of metastatic cancer cells at the metastatic niche, induce changes in cancer cell biology, that facilitate cancer cell survival and inhibit apoptosis [7,11,15,23,29,30]. Of note, elucidation of the role of these bone metastasis microenvironment-related growth and survival factors (IGF-1, IL-6, TGFβ1, bFGF, etc.) allowed a better understanding of the mechanisms of chemotherapy- and castration-resistance of prostate cancer cells in bones [31-33]. Based on these findings, novel antisurvival factor (bone targeted) therapies in castration-resistant and chemotherapy-resistant prostate cancer patients were developed. Indeed, such bone microenvironment targeted therapies have produced significant objective clinical responses in castration–resistant and chemotherapy-resistant prostate cancer patients [18,30,32-39].

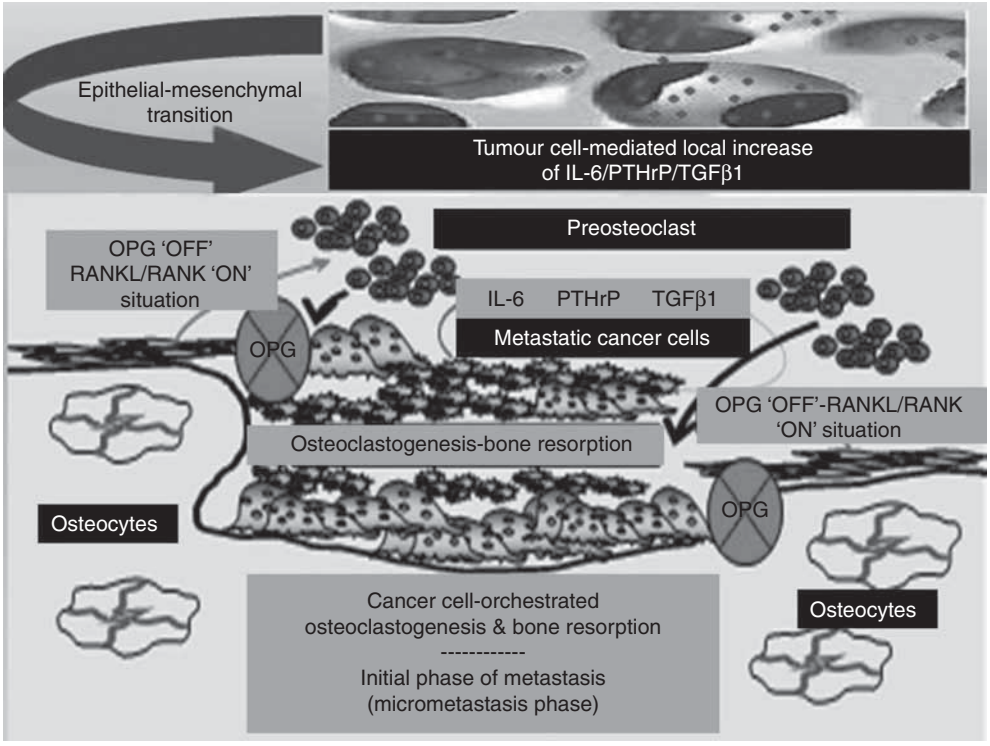

Figure 2. Schematic representation of the cellular events taking place at the initial phase of bone metastasis (micrometastasis phase). Circulating prostate cancer cells after their initial dissemination into the bone marrow come to an anatomical 'close up' with mineralized bone architecture. However, cancer cells cannot expand within the mineralized bone unless they would be capable of stimulating osteoclastogenesis, locally. The latter is achievable by the parathyroid hormone related protein (PTHrP), interleukin 6 (IL-6) and transforming growth factor beta 1 (TGFβ1) produced by the metastatic cancer cells and are acting on host both tissue cells and tumor cells. These pro-osteoclastogenesis factors inhibit the osteoprotegerin (OPG) expression in the bone marrow stroma cells and cells of the osteoblast lineage, while they stimulate the expression of the receptor activator of nuclear factor-kB ligand (RANKL) stimulating the differentiation of the RANK-expressing preosteoclasts present in bone marrow. The setting of the OPG 'off' – RANKL/RANK-signaling 'on' at the metastasis niche activates osteoclastogenesis and bone resorption (demineralization of bone matrix & bone resorption), thus enabling the initial penetration of the disseminated prostate cancer cells from the bone marrow into the mineralized bone matrix.

Although the initial phase of bone lesions (micrometastatic phase) is a common step for all the types of 'osteophilic' cancers (**Figure 2**), the second phase of bone metastasis (macrometastatic phase) consists of the local growth and evolution of the micrometastasis phase within the bone matrix, resulting in either lytic or blastic macrometastasis [11,15]. Tumor cells that maintain the initial setting of OPG 'off' – RANKL/RANK-signaling 'on' at the metastatic niche induce an osteolytic reaction during the macrometastatic phase. On the other hand, prostate cancer cells interacting with cells of the bone microenvironment can apparently trigger the OPG 'on' – RANKL/RANK-signaling 'off' setting which can suppress osteoclast-related bone resorption thus facilitating the development of the osteoblastic macrometastasis at the metastatic niche (**Figure 3**). Even at that stage, local cell–cell interactions always maintain a basal osteoclastic activity, albeit significantly reduced, which supports the active bone remodeling process at the metastatic site [11,23,24,27].

Recently, a novel theory introduced the concept of 'gradient and spatial' expression and distribution of receptors, decoy molecules and ligands associated with the OPG-RANKL/RANK system within the microenvironment of osteoblastic metastasis, which can explain the transition from initial lytic to blastic reaction of the host tissue in prostate cancer metastasis [40]. In addition, other studies have demonstrated that human prostate cancer cells can secrete OPG [41], which in turn can inhibit osteoclastogenesis at the metastatic niche, thus preventing tumor establishment in bones of experimental animals [42]. Moreover, OPG was able to optimize the survival of prostate cancer cells, *in vitro* [41]. This antiapoptotic action of OPG was attributed to the blockage of the TRAIL death-activating receptors, an action related to the D5 and D6 domains present at the C-terminal region of OPG [2,41].

In addition, OPG can prevent the development of skeletal lesions by inoculated prostate cancer cells in experimental animals, whereas it fails to prevent the establishment and growth

Table 1. Cancer cell-secreted pro-osteoclastogenesis factors during the initial phase of bone metastasis (micrometastasis phase).

PTHrP: upregulates RANKL expression and suppresses OPG expression (stimulation of osteoclastogenesis)
IL-6: increases the effects of PTHrP on osteoclastogenesis and on osteoclast-mediated bone resorption
IL-1: increases osteoclasts differentiation and survival & enhances bone resorption
IL-8: increases osteoclastogenesis and bone resorption
IL-11: increases osteoclastogenesis and bone resorption
TGFβ1: stimulates osteoclasts function (at RANKL/RANK 'off' setting)
bFGF: regulates osteoclasts differentiation and RANKL & MCSF expression
uPA/plasmin/MMPs system: assists the osteoclast-mediated bone resorption and activates/increases the local bioavailability of growth factors in bone ECM, such as IGFs, TGFβs, bFGF, BMPs

bFGF: Basic fibroblast growth factor; BMPs: Bone morphogenetic proteins; IL-1, -6, -8, -11: Interleukin-1, -6, -8,-11; MCSF: Macrophage colony stimulating factor; MMPs: Metalloproteases; PTHrP: Parathyroid hormone related peptide; TGFβ1: Transforming growth factor 1; uPA: Urokinase type plasminogen activator.

of such cancer cells in subcutaneous tissues [2,41,42]. Further, the coculture of PC-3 cells with MG-63 osteoblast-like cells, using a three-dimensional type I coculture system, did significantly enhance the expression of OPG by PC-3 cells (**Figure 3**). This increase of OPG expression, both at the mRNA and protein levels, is positively correlated with the duration of the coculture of the PC-3 cells with MG-63 osteoblast-like cells [43]. Thus, after the initial penetration within bone matrix, prostate cancer cells establish new cell–cell interactions with cells of the osteoblast phenotype present in bone matrix. This complex dialogue results in increased OPG expression, which in turn, can block RANKL/RANK-mediated activation of bone resorption during the macrometastasis phase. A positive association exists between OPG serum levels with disease progression [41,44]. More specifically, RANK/RANKL/OPG serum levels have been shown to positively correlate with Gleason's score, tumor stage and serum PSA levels [45]. These data also indicate that increasing OPG levels and OPG/RANKL ratio can be used to monitor disease progression in bones. It should be noted that a large number of other local mediators have been implicated in this process, including endothelin 1 (ET-1), TGFβs, IL-1, IL-8, IL-11 and BMPs [24,46-48]. Each of these modulators may play variable roles within the bone metastasis microenvironment, depending on the phase of the metastatic process (micrometastasis or macrometastasis phase).

3. Conclusion

Bone metastasis is the single most catastrophic event in the progression of prostate cancer. Elucidating the cellular and molecular mechanisms implicated in the different phases of the metastatic process (micrometastasis and macrometastasis phases) will be crucial for the development of effective therapeutic strategies that can alter disease progression. The currently available treatment strategies in advanced prostate cancer patients include androgen ablation therapies (surgical and medical castration; with or without the use of antiandrogens), biphosphonates, external beam irradiation therapy, radiopharmaceuticals and cytotoxic chemotherapy. However, the impact on overall survival achieved thus far is limited [49,50]. The high complexity of cell–cell crosstalk among cancer cells, bone cells and the immune system, as well as the numerous molecular mediators involved in such interactions at the metastatic niche, indicate that combinational and multitargeted anticancer therapies may be required to achieve significant benefits in survival and quality of life of patients with advanced prostate cancer [11,15,30,34,36,39,51].

4. Expert opinion

An important goal in advanced prostate cancer management will be the prevention of bone metastasis. This, however, would require the acquisition of new and reliable diagnostic techniques, which could enable the detection of such catastrophic events very early during disease progression of the otherwise clinically localized prostate cancer patients. Therapeutic blockade of the molecular setting [OPG 'off' – RANKL/RANK-signaling 'on'] during the initial phase of cancer cells dissemination within the bone marrow of such patients may be warranted. To this end, several molecular methods have been successfully employed for the detection of circulating tumor cells (CTCs) in the peripheral blood and bone marrow of patients with clinically localized prostate cancer [52-55]. It is expected that in the near future the optimization of such molecular techniques, employing multiple and specific tumor markers and molecular markers of the EMT process, such as the PTHrP expression [54], would enable the selection of prostate cancer patients of 'high risk' for developing bone metastasis. In these patients the administration of bone microenvironment targeted therapies could potentially prevent osteoclastogenesis and bone resorption, and thus block the expansion of invading tumor cells within bone matrix. These therapies should therefore be employed early on at the evolution of prostate cancer.

Denosumab, a fully humanized monoclonal antibody that specifically binds to RANKL and thus inhibits osteoclast activity, was developed in order to specifically target the OPG pathway [56]. When compared to intravenous administration of the third-generation biphosphonate zoledronic acid, subcutaneous denosumab significantly increased time to first skeletal-related events in patients with metastatic

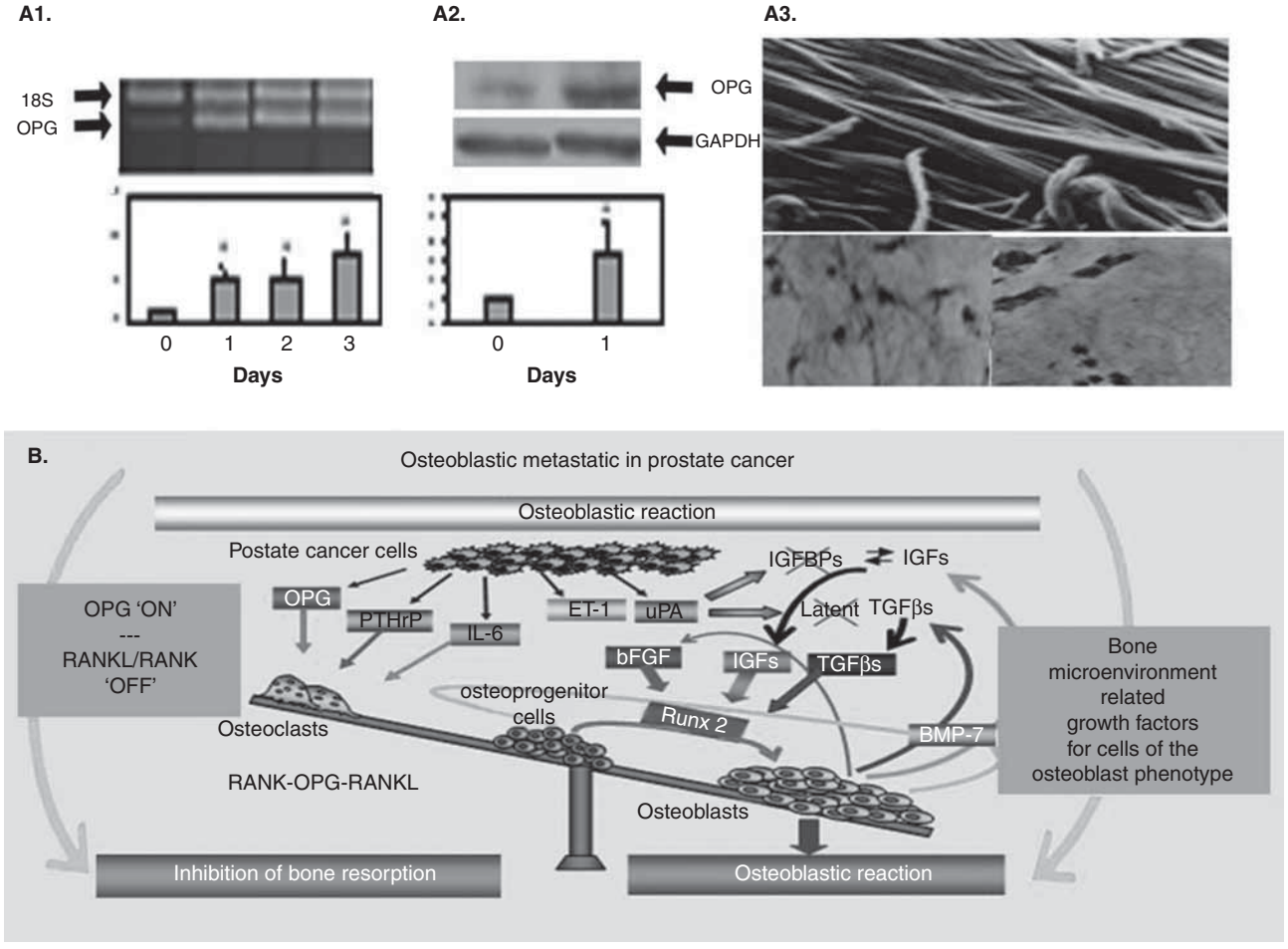

Figure 3. The macrometastasis phase of bone lesions. Upper panel: PC-3 human prostate cancer cells enhance their osteoprotegerin (OPG) expression during coculture with MG-63 human osteoblast-like cells. This was dependent on the duration of coculture (days) of the PC-3 cells with MG-63 osteoblast-like cells (**A1**: mRNA expression; **A2**: protein expression) in three dimensional type I collagen gel system. **A3**: The three-dimensional type I collagen system used for coculturing PC-3 cells and MG-63 cells, *in vitro*. **A3**: upper panel: type I collagen fibers; **A3**: lower panel (left): MG-63 osteoblast-like cells growing in collagen gels; **A3**: lower panel (right): PC-3 prostate cancer cells growing in collagen gels. Lower panel: Schematic representation of the cell–cell interactions leading to the blastic reaction of bone. By setting 'on' the osteoprotegerin (OPG) expression and switching 'off' the RANKL (receptor activator of nuclear factor-Kβ ligand)/RANK-signaling in host tissue microenvironment, bone resorption is suppressed. This setting of the local interactions enables the activation of the osteoblast-derived growth factors at the metastatic niche, which in their turn, stimulate the growth of host tissue cellular components, thus resulting in the blastic reaction of the host tissue.

castration-resistant prostate cancer [57]. In contrast to biphosphonates, denosumab lacks renal toxicity and thus does not require renal dosing in patients with chronic kidney disease. However, denosumab demonstrates a significantly stronger association with hypocalcemia and osteonecrosis of the jaw compared to zolendronic acid [57]. However, as with all osteoclasts-targeting agents tested thus far [58,59], no survival benefit has thus far been demonstrated by denosumab in patients with metastatic castration-resistant prostate cancer [57]. Immune therapies can selectively target the pathways of interaction between prostate cancer cells, the bone stroma and the immune system. The RANKL/RANK/OPG molecules are key regulators of pathological bone metabolism in metastatic sites. Of note, RANKL can suppress apoptosis of dendritic cells and facilitate T-cell growth [60]. In addition, OPG serves as a weak decoy receptor for TRAIL [61], a molecule that has been implicated in multiple immunoregulatory pathways that are indispensable for the immune surveillance of tumors and metastases [62]. Thus, targeted manipulation of immune regulators affecting the RANKL/RANK/OPG pathway within the bone microenvironment could potentially produce potent antitumor responses. A major challenge for such tumor immunotherapy strategies will be to fully suppress tumor-induced immunologic tolerance in order to produce a sustained anticancer response.

The bone metastasis microenvironment is composed of multiple distinct cell types that engage in a complex dialogue with one another. Our understanding of these heterotypic interactions is continuously evolving. Indeed, the signaling circuitries associated with OPG within the bone metastasis niche will be charted in far greater detail and clarity in the coming years. However, it appears that these interactions evolve during bone metastasis progression from the micrometastasis to the macrometastasis phase and beyond. This can further complicate the design of effective therapeutic strategies aimed at modulating OPG signaling pathways. A better understanding of these dynamic variations, perhaps via the establishment and refinement of a limited set of organizing principles with potent explanatory power, will be crucial for the development of such novel therapies. Ultimately, as our understanding of the complex biology of bone metastasis grows, future strategies targeting the bone microenvironment will be tailored to the individual phenotypic characteristics of each patient.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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