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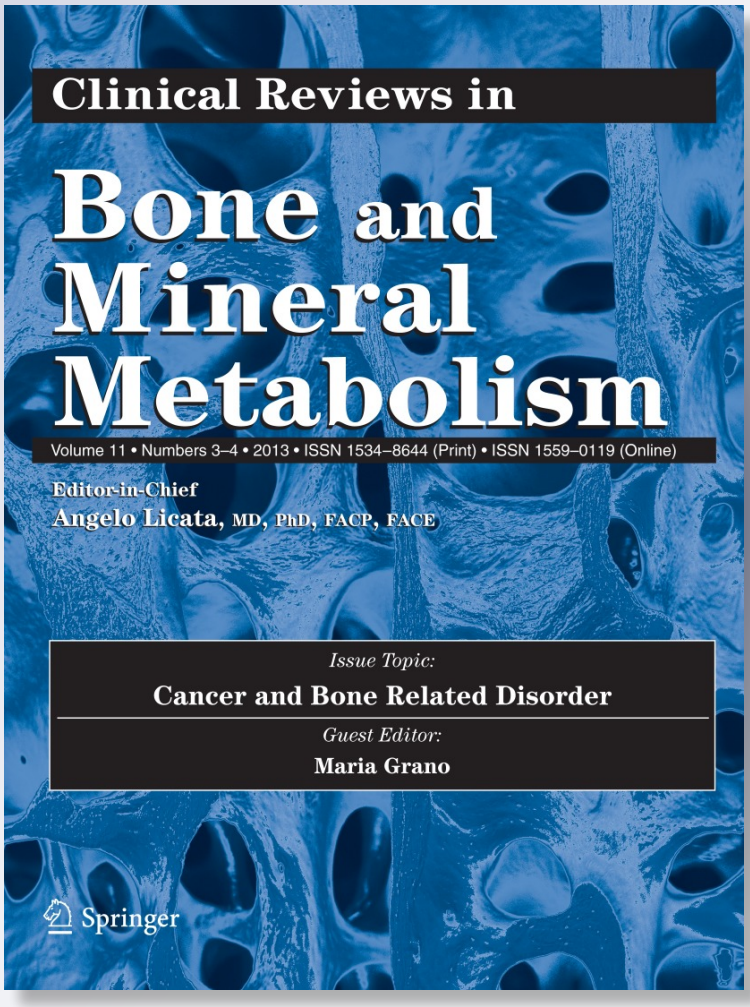
Clinical Reviews in Bone and Mineral Metabolism

ISSN 1534-8644
Volume 11
Combined 3-4

Clinic Rev Bone Miner Metab (2013)
11:87-93
DOI 10.1007/s12018-013-9144-3



Springer

Clinic Rev Bone Miner Metab (2013) 11:87–93
DOI 10.1007/s12018-013-9144-3

ORIGINAL PAPER

Solid Tumours Show Osteotropism: Mechanisms of Bone Metastases

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Published online: 13 October 2013

Abstract Bone metastases are a dismal consequence of cancer, causing severe morbidity and reducing the quality of life of patients. Solid tumours such as breast, prostate, lung and kidney cancer showed a marked osteotropism dependent on the special microenvironment provided by bone. Different cellular types are involved in the formation of bone metastases, indeed bone, immune system and tumour cells interact leading to bone lesions. During the bone resorption process, there is an intense cross-talk between immune system cells and osteoclasts (OCs). In particular, T cells release factors and cytokines, which rule osteoclastogenesis, and on the other hand, OCs produce factors that act on T cells, which are mediators of the tumour growth in bone. This review will summarize the main mechanisms of action in cancer-induced bone disease with particular regard to the cross-talk among cells of bone, tumour and immune system, focusing on factors and cytokines released by osteoclast, osteoblast, tumour cells and T cells.

Keywords Osteoclast · Osteolysis · Osteoblast · T cells

Background to Bone Metastases

Certain tumours such as breast, lung, kidney, thyroid and prostate cancer show a marked osteotropism, with a 70 % incidence of bone metastases [1]. Patients only affected by bone metastases can survive longer than ones with visceral metastases, but bone metastases cause severe morbidity in patients and reduce their quality of life [1]. Bone metastases are commonly classified in osteolytic and osteoblastic, even though this classification actually represents two extremes of a continuum in which dysregulation of the normal bone remodelling process occurs. Patients can show both osteolytic and osteoblastic metastases or mixed lesions containing both elements [2].

The initiation and progression of bone metastases is a complex multi-step process, involving a large number of cell–cell interactions coupled with many soluble factors. Skeletal metastases originate by local interactions between tumour cells and bone, which form a vicious cycle [3]. The presence of tumour cells causes a dysregulation of the fine balance regulating the normal bone remodelling, where bone resorption by osteoclasts (OCs) and bone formation by osteoblasts (OBs) are perfectly balanced. Tumour cells produce factors that facilitate binding to particular molecules expressed by cells residing in the bone microenvironment, and induce the bone cells to support their proliferation and expansion [4]. This generates the physical space for tumour expansion as bone is resorbed, and the release of tumour growth factors and cytokines supports tumour [2].

The skeleton is the preferred site for many tumour cells that can remain there in a dormant state for long periods of time. In the bone marrow of patients with primary cancer, the presence of disseminated tumour cells (DTCs) reveals the housing potential of the skeleton. Evidence exists that DTCs can persist in the bone marrow for years in a quiescent state and are resistant to cancer therapies. Patients with bone marrow DTCs at diagnosis are at a higher risk of both skeletal and extraskeletal metastasis [5, 6]. Among

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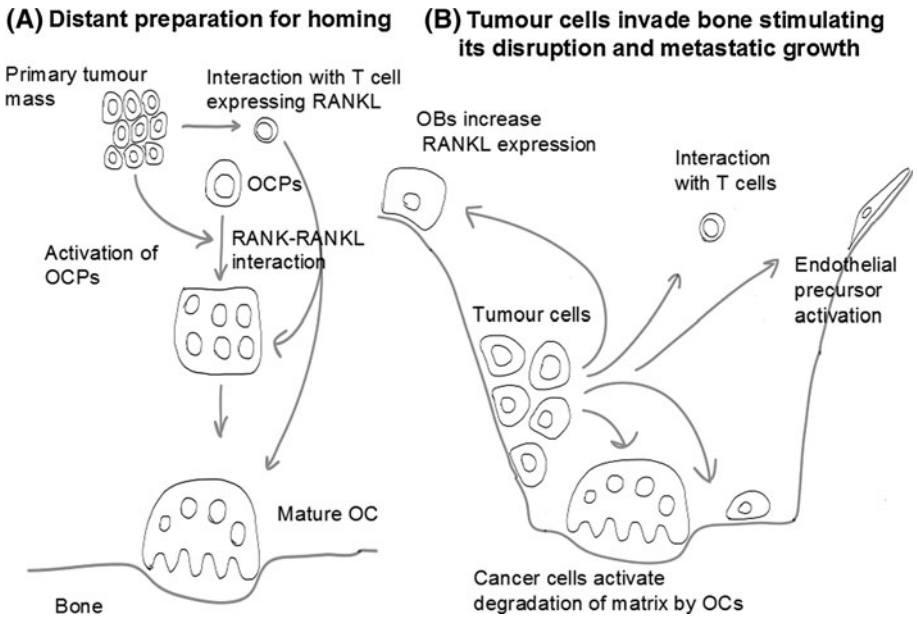

Fig. 1 **a** Bone metastasis formation is the product of a continuum of interactions between tumour cells, bone and circulating T cells and mesenchymal precursors. **b** These interactions result in a vicious circle sustaining tumour growth in bone and creating the most favourable conditions for the bone matrix disruption. *OCPs* osteoclast precursors, *OCs* osteoclasts, *OBs* osteoblasts

patients with prostate cancer who have had a radical prostatectomy, 72 % have DTCs in the bone marrow [7], and 30 % of patients with localized breast cancer have bone marrow DTCs at diagnosis [8]. It has been recently reported that cancer stem cell-like (CSCs-like), isolated from a primary breast cancer, directly metastasize to bone [9]. These data demonstrate that also breast CSCs-like have an important role in the development of bone lesions.

The contribution of the tumour microenvironment, the hemopoietic and the immune system for tumour cell growth in bone has been recently recognized, indeed these systems are in deep physical contact and share several common pathways. Both OC precursors and lymphocyte subsets such as T, B and NK cells arise from the same stem cell, and thus, some of the same receptors and ligands that mediate the immune process also rule the maturation of OC precursors and the ability of the mature cell to degrade bone [10]. OC precursors circulate within the mononuclear fraction of peripheral blood, and they act as a reservoir for replenishing the pre-OC pool in the bone marrow and as a source of pre-OCs that can be recruited into bone or joint tissue in response to reparative or pathological signals (Fig. 1a). On this basis, OCs can be considered as immune cells attracted in bone by stimulatory cytokines, expressed on accessory cells and undergoing specific differentiation.

Factors Produced by Bone and Tumour Cells Modulate OC Activity

Bone marrow is a fertile soil for some tumour cells, which have a biological proclivity for this tissue. For instance, bone marrow produces factors, such as CXCL12, that attract cancer cells, which express the chemokine receptors, CXCR4 and CXCR7 [11, 12]. Moreover, activated OCs resorb bone and release growth factors enmeshed in the bone matrix, such as bone morphogenetic proteins (BMPs), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor and others that stimulate the growth of metastatic tumour cells in bone, and the production and release of bone resorbing factors from tumour cells [13, 14]. Indeed, cancer cells secrete molecules such as prostaglandins, parathyroid hormone, parathyroid hormone-related peptide (PTHrP), activated vitamin D and TNF that may stimulate RANKL expression on OBs and bone marrow stromal cells [15], increasing the OC number, survival and activity, thus promoting osteolytic metastases (Fig. 1b). The most prominent cause of bone destruction in metastases is the PTHrP, which stimulates OC bone resorption and is secreted by many cancer types [16–18]. In particular, PTHrP stimulates OBs and stromal cells to express receptor activator of nuclear factor-kB ligand (RANKL), which promotes OCs maturation [19, 20]. Cancer cells can express RANK, the RANKL receptor, indeed the use of antibodies blocking RANK/RANKL reduces bone metastases [21]. RANK may induce migration and homing of tumour cells to the RANKL-rich bone microenvironment [22].

Some cytokines such as interleukin-6 (IL-6), IL-8 and IL-11 are produced by malignant cells and activate OCs [23]. IL-6 can stimulate tumour cell proliferation, migration and invasion [24], and it may induce bone resorption or formation according to the interactions with other factors such as PTHrP, IL-1 and RANKL. IL-8 stimulates OCs maturation through binding with its receptor CXCR1 expressed on OCs precursor [25]. IL-11 stimulates

osteoclastogenesis, and it is a predictive factor for development of osteolytic bone metastases [26].

Notch–Jagged interactions in the bone marrow suggest direct activation of osteolysis by cancer cells through this unique interaction. Jagged1, a downstream mediator of the pro-metastatic TGF-β, promotes tumour growth through stimulation of IL-6 production from OBs, and it directly activates OC differentiation [27]. Moreover, Jagged is over-expressed by bone metastatic tumour cells [28], whereas its receptor Notch is frequently expressed by progenitors and mature cells in the bone marrow [29]. In breast cancer, Notch–Jagged interactions activate biological responses in OCs and OBs, which promote both tumour invasion of bone and tumour cell growth in the bone [27].

In addition to the cellular and molecular interactions described above, bone has physical properties such as low pH, hypoxia and high levels of extracellular calcium [30]. Proliferating tumour cells secrete high quantity of lactic acid, and thus, local area of bone has a low pH that stimulates OCs activity. On the other hand, tumour cells grow better with a low pH and release proteolytic enzymes that maintain the low pH, and thus, the tumour expansion is perpetuated [31]. Bone is a hypoxic tissue, and thus, it promotes the ability of cancer cells to growth under hypoxic conditions, which stimulate the expression of hypoxia inducible factor-1 (HIF-1). TGF-β potentiates HIF-1 signalling within bone, contributing to the vicious cycle driving the development of metastatic osteolysis [32]. Indeed, HIF-1 stimulates OCs formation and inhibits osteoblastogenesis in breast cancer [33]. The active bone resorption causes an increase in the extracellular calcium levels that stimulates the calcium-sensing receptors on surrounding cells and tumour cells, leading to an increased secretion of PTHrP, a potent stimulator of OCs [34, 35].

Osteoclasts and Immune System Talk

After the discovery of RANKL and its receptor RANK, the molecular links between the immune system and bone have emerged, creating the new field of study called osteoimmunology. RANKL and RANK were first identified as factors expressed on T cells and dendritic cells (DCs), respectively. They modulate the immunity through DCs because they increase the ability of DCs to stimulate naïve T-cell proliferation and enhance DC survival. They were later identified as the key osteoclastogenic molecules, and now, it is clear that a host of immune factors including costimulatory receptors, cytokines such as IFNγ and TNF, and T and B lymphocytes regulate bone cell development and bone turnover and are important in pathogenesis of bone disease [36]. IFNγ has a controversial role in osteoclastogenesis because it has an antiosteoclastogenic effect in vitro [37] and in vivo in animal studies [38], whereas in humans, it increases in oestrogen deficiency and in rheumatoid arthritis with bone loss [39, 40]. IFNγ influences osteoclastogenesis both directly and indirectly: it targets maturing OCs, thus blocking OC formation [41], and it stimulates T-cell activation, thus increasing pro-osteoclastogenic factors [42].

Recently, investigators focused on the OC modulatory activity of T cells, showing that OCs are able to present antigenic peptides to T cells and to induce FoxP3 expression in CD8+ T cells [43]. In this way, CD8/FoxP3+ cells act as T lymphocyte regulators, able to rule inappropriate activation of the immune response [43]. The cellular responses in cell–cell interactions between T cells and OCs are regulated through reciprocal CD137/CD137L and RANK/RANKL interactions [44]. CD137 is a co-stimulatory member of the TNF receptor induced by T-cell receptor activation, and it is characterized by the ability to transduce signals in both directions, through the receptor and into the cell that expresses the ligand. Its ligand CD137L is expressed on DCs and OC precursors: in vitro CD137L ligation suppresses osteoclastogenesis through the inhibition of multi-nucleation. On the other hand, RANKL expressed on T cells binds to RANK on OCs, producing a reverse signal in T cells able to enhance apoptosis.

T Cells Regulate Tumour Growth in Bone

Recently, it has been demonstrated that T cells are additional regulators of bone tumour growth, regardless of the OC status [45], and thus, the concept of vicious cycle has been enlarged. Blockade of OC activity efficiently decreases tumour burden as well as associated bone lesions in immune-compromised animals bearing human osteolytic cancers. Some patients treated with antiresorptive therapies develop further skeletal metastases within 2 years from the beginning of the treatment, suggesting that additional cells modulate bone tumour growth. These cells are T lymphocytes: tumour cell-derived IL-6, IL-1 and TGF-β can drive T-cell differentiation towards a Th17 secretory helper-cell phenotype capable to induce RANKL production by OB and OC activation through IL-17 production [46]. In breast cancer patients, memory T cells have been found in the bone marrow, suggesting their role in cancer immune surveillance [47]. Moreover, the RANKL–RANK interaction between helper T cells and breast cancer cells promotes invasion, dissemination and metastasis formation from orthotopic syngeneic mouse mammary tumour virus–Erbb2 tumours in immunocompetent mice [48].

Noteworthy, some antibone metastatic therapies also showed immunomodulatory effects: the blockade of TGF-β at metastatic sites may locally activate an antitumour T-cell

response, because normally TGF-β, released in bone marrow by OC activity, inhibits T-cell proliferation [49]. Zoledronic acid, used as antiresorptive agent, can activate cytotoxic γ/δ-T cells and inhibit populations of myeloid-derived cells with T-cell-suppressor capabilities [50]. Zhang and colleagues provide compelling evidence that a condition of immune deficiency can interfere with the antitumour effects of OC blockade [45]. Modulation of antitumour T-cell responses alters tumour growth in bone, indeed Lyn(−/−) mice, which have more numerous OCs and a hyperactive myeloid population with an increased T-cell responses, had reduced tumour growth in bone despite enhanced osteolysis [45]. Lyn is a member of the Src family tyrosine kinases, and it down regulates different intracellular pathways, including the PLCγ2 activation, which regulates the OC formation and function; thus, Lyn inhibits OC differentiation [51]. PLCγ2(−/−) mice, which have dysfunctional OCs [52] and impaired T-cell activation, had increased bone tumour burden despite protection from bone loss. Importantly, injection of antigen-specific wild-type cytotoxic CD8(+) T cells in both these mice models reduces the growth of tumour cells in the bone, regardless of OC functionality [45]. According to these data, T cells act as critical regulators of tumour growth in bone, in particular their activation diminishes bone metastases, whereas their depletion enhances them, even in the presence of zoledronic acid [45].

Further proofs of the direct dialogue between T cells and OCs derived by studies conducted on the PBMCs of patients affected by solid tumours with bone metastases, which showed an increase in circulating OC precursors compared with both healthy controls and cancer patients without bone metastases [53]. These OC precursors differentiate into mature, multi-nucleated and bone resorbing OCs in vitro, without adding pro-osteoclastogenic factors. This osteoclastogenesis is dependent on T cells that release TNF-α and RANKL, which act synergistically in promoting the OC differentiation. On the other hand, T-cell depletion does not allow the differentiation of PBMCs into OCs without adding M-CSF and RANKL [53]. A similar mechanism of T cell-dependent osteoclastogenesis also occurs in multiple myeloma [54], confirming the role of T cells in the cancer vicious cycle with bone involvement.

As previously reported, IL-7 is an important regulator of the interaction between bone and immune system. Many studies report a role of IL-7 in bone homeostasis, in particular to bone loss in oestrogen deficiency conditions [55–57], psoriatic arthritis [58] and periodontitis [59]. Other works support an active role of IL-7 in promoting bone lesions from solid tumours and multiple myeloma [60–63]. In culture of PBMCs derived from patients with bone metastases, IL-7 is mainly released by B cells, and it directly sensitizes T cells to produce pro-osteoclastogenic factors, such as TNF-α and RANKL, which enhance spontaneous osteoclastogenesis [60, 64]. Moreover, in bone metastatic patients, IL-7 sera levels were found significantly higher than in non-bone metastatic patients and in healthy controls [60, 62]. This serum IL-7 increase directly depends on tumour production, indeed a strong IL-7 expression was detected in tumour masses originated in a human-in-mice model of bone metastases and in human bone metastatic biopsies [65].

Factors Regulating OB Activity

Since cancer-induced bone lesions depend on a dysregulation of the balanced activity between OCs and OBs, cancer cells also release factors that affect osteoblastogenesis and bone formation. In the bone marrow, mesenchymal stem cells differentiate in OBs through local factors, such TGFβ, BMPs, IGF and WNT proteins. The OB-stimulating activity of metastatic tumour cells is thought to be due to the ability of these cells to express many of the factors listed above that can drive OB formation and activation. Several BMPs are stored in the bone matrix and released during resorption, stimulating the proliferation of both OBs and cancer cells; thus, they contribute to the osteoblastic lesion [66, 67].

The IGF system stimulates OB activity and bone formation [68]. Moreover, IGF is also a mitogen for prostate cancer cells, and its expression resulted in up-regulation by tumour cells, indicating a potential role for this system in osteoblastic lesions [69].

Prostate cancer cells express endothelin-1 (ET-1), which stimulates OBs and inhibits OCs activity, mediating the formation of osteoblastic metastases [70, 71]. Recently, ET-1 was found to decrease transcription of DKK-1, a Wnt signalling inhibitor, thus stimulating bone formation by inhibition of Wnt pathway [72].

Prostate cancer is typically characterized by the presence of osteoblastic lesions with underlying osteolytic area [73]. The osteolytic activity is explained by an increase in serum RANKL/OPG ratio in prostate cancer patients; thus, an enhanced OC formation plays an active role in bone-forming lesions. The RANKL increment depends at least in part by the increased IL-7 production from T and B cells, but unlike other solid tumours, IL-7 expression is not significantly different in prostate cancer patients and in normal controls [61]. In the early phase of prostate cancer bone metastases, the increased OC activity also depends on Wnt agonist Dickkopf-1 (DKK-1) that inhibits OBs, favouring lytic lesions [74]. In a subsequent phase, there is an increase in endothelin-1 (ET-1), which stimulates OBs and inhibit OCs, by decreasing the synthesis of DKK-1 [72]. ET-1 is also expressed by breast

cancer cells, explaining the presence of mixed bone lesions in advanced disease.

Conclusion

The past decade of research provided fundamental understanding of the interactions among tumour cells, bone microenvironment and immune system cells, such as T cells, which release many factors and cytokines activating OCs and OBs, thus promoting the formation of bone metastases. This interplay leads to the activation of multiple signalling that provide different potential molecular targets for the treatment of bone metastases.

Acknowledgments We thank the organizers of the Benish Trophy and the Italian Ministry of Health: Ricerca Sanitaria Finalizzata e Giovani Ricercatori 2009 (GR 2009-1584485) for supporting this work.

Disclosures

Conflict of interest The authors Ilaria Roato and Riccardo Ferracini declare that they have no conflict of interest.

Animal/Human studies This review does not contain any studies with human or animal subjects performed by the any of the authors.

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