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Review

Bone metastasis: Interaction between cancer cells and bone microenvironment

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ABSTRACT

Background: Bone is one of the most common target organs for cancer metastasis, especially in patients with advanced breast and prostate cancers. Despite recent advances in therapeutic approaches, bone metastases remain incurable and produce multiple complications called skeletal-related events, including hypercalcemia, pathological fractures, spinal compression, and bone pain, which are associated with poor prognosis.
Highlight: Although the precise mechanisms are yet to be fully elucidated, accumulating evidence suggests that bone provides a favorable microenvironment that enables circulating cancer cells to home, proliferate, and colonize, resulting in the formation of metastasis. Cancer cells that metastasize to bone also possess unique features, enabling them to utilize the bone microenvironment. Thus, communication between cancer cells and bone is believed to be critical for the development and progression of bone metastases.
Conclusion: Continued studies are warranted to understand the molecular mechanisms underlying bone metastases and to develop mechanism-based and effective therapeutic interventions.
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Contents

Abbreviations: BMSCs, bone marrow stromal cells; CD44s, the standard isoform of CD44; CD44v, the variant isoforms of CD44; COX-2, cyclooxygenase-2; CSCs, cancer stem cells; CXCL12, C–X–C motif chemokine 12; CXCR4, C–X–C chemokine receptor type 4; dnTβIIR, dominant-negative transforming growth factor β type II receptor; EpCAM, epithelial cell adhesion molecule; HA, hyaluronan; HAS, hyaluronan synthase; HIFs, hypoxia-inducible factors; HIV-TAT, human immunodeficiency virus-trans-activator of transcription; HSCs, hematopoietic stem cells; IGF-IR, insulin-like growth factor type I receptor; IGFs, insulin-like growth factors; IL, interleukin; NF-κB, nuclear factor-κB; OPCs, osteoclast precursor cells; OSFs, osteoclast-stimulating factors; PTHrP, parathyroid hormone-related protein; RANKL, receptor activator of nuclear factor-κB ligand; SDF-1, stromal-derived factor 1; TGFβ, transforming growth factor β

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1. Introduction

Bone is the third most preferred site of metastasis in patients with advanced cancer; however, some cancers show marked osteotropism [1]. Around 70% of patients with breast and prostate cancers and 30–40% of those with lung, kidney, and thyroid cancers eventually develop metastases in bone. Bone metastases are generally incurable using currently available treatments and cause devastating complications called skeletal-related events, including hypercalcemia, pathological fractures, spinal compression, and bone pain, which result in a poor prognosis. Although the mechanisms underlying bone metastasis are yet to be fully elucidated, growing evidence suggests that the bone microenvironment significantly contributes to cancer bone tropism. In addition, cancer cells that form bone metastases possess unique abilities. In this review, we will discuss the most recent reports on the interaction between cancer cells and the bone microenvironment and its role in the development and progression of bone metastases.

2. Cancer cell homing to bone

Cancer cell homing to a particular organ is a critical step in organ-specific metastasis. Among several candidates, C–X–C motif chemokine 12 (CXCL12), also known as stromal-derived factor 1 (SDF-1), which is produced by bone marrow stromal cells (BMSCs) and osteoblasts, is a key molecule for mediating cancer cell homing to bone. An *in vitro* study demonstrated that CXCL12 enhances transendothelial migration and invasion of cancer cells expressing the CXCL12 receptor, C–X–C chemokine receptor type 4 (CXCR4); this was inhibited by an anti-CXCR4 antibody [2]. Furthermore, *in vivo* studies using mouse models revealed that CXCR4 overexpression in cancer cells increased and administration of the neutralizing antibody to CXCR4 decreased the formation of bone metastases [3,4]. These findings suggest a critical role of the CXCL12/CXCR4 interaction in the development of bone metastases.

In addition to its roles in cancer cell homing, CXCL12 also plays a pivotal role in the homing to and maintenance of a niche for CXCR4-expressing hematopoietic stem cells (HSCs) [5]. Shiozawa et al. found that HSCs and bone-homed cancer cells co-localized to the same niche in the bone marrow [6]. More importantly, they clearly demonstrated that cancer cells compete with HSCs for occupancy of the HSC niche. Thus, it is likely that metastatic cancer cells home to bone and are maintained by the same mechanism as HSCs.

3. Cancer metastasis and bone remodeling

Once cancer cells colonize bone, they disrupt physiological coupling between bone formation and bone resorption maintained by osteoblasts and osteoclasts, respectively [7]. When osteoclast differentiation and function are enhanced by cancer cell-produced osteoclastogenic factors, including parathyroid hormone-related protein (PTHrP), which induce expression of the receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL) in BMSCs and osteoblasts [8,9], bone resorption-dominant osteolytic-type bone metastases are established. Conversely, when bone formation is stimulated by cancer cell-derived osteoblast-activating factors, osteoblastic (or osteosclerotic)-type metastases are formed. However, most solid tumors usually produce both types in one lesion, which is so-called mixed-type metastases.

It is rather surprising that osteoclastic bone resorption is enhanced in osteolytic and even in osteoblastic metastases. A clinical study showed that the bone resorption marker urinary N-terminal telopeptide of type I collagen and the bone formation marker serum bone alkaline phosphatase were increased in cancer patients with osteoblastic metastases more than in those with osteolytic metastases [10]. Yonou et al. reported that the number of osteoclasts was markedly increased in the early stage of osteoblastic metastatic development and decreased thereafter in a mouse model [11]. Of note, our study showed that stimulation of bone resorption in calvarial bones by repeated injections of interleukin-1β (IL-1β) before cancer cell inoculation markedly increased subsequent metastases to this local bone site, which was markedly suppressed by treatment with the bisphosphonate, zoledronic acid [12]. These findings, together with the fact that bisphosphonates and anti-RANKL antibody (potent inhibitors of osteoclastic bone resorption) inhibit bone metastases [13,14], suggest that bone resorption plays a critical role in bone metastases. The molecular mechanisms of how bone resorption contributes to bone metastases will be described in the next section.

4. Bone-derived growth factors

Bone is a storehouse of a variety of growth factors, including insulin-like growth factors (IGFs), transforming growth factor β (TGFβ), fibroblast growth factors, platelet-derived growth factors, and bone morphogenetic proteins [15], which are continuously released into the bone microenvironment by bone resorption. In physiological conditions, these bone-derived growth factors play a role in regulating bone remodeling [16,17]; however, they are also crucially involved in multiple aspects of bone metastasis.

4.1. Vicious cycle theory

The vicious cycle theory, a hypothesis proposed by Mundy and his colleagues [7], explains how communication between cancer cells and the bone microenvironment drives bone metastasis. In brief, bone-derived growth factors released through osteoclastic bone resorption stimulate the production of osteoclast-stimulating factors (OSFs), such as PTHrP, by cancer cells, which up-regulates RANKL
expression in BMSCs and osteoblasts. RANKL then promotes differentiation of RANK-expressing osteoclast precursor cells to mature osteoclasts, leading to further bone destruction (Fig. 1). Additionally, bone-derived growth factors enhance angiogenesis and cancer cell proliferation, invasion, and homing to bone.

4.2. IGFs

IGFs, the most abundant growth factors stored in bone [15], have been implicated in the development, progression, and aggressiveness of many cancer types [18]. Our study using clinical specimens revealed that, in the majority of cases (86.7%), metastatic cancer cells in bone were positive for IGF type I receptor (IGF-IR), the primary receptor for IGFs [12]. Furthermore, a preclinical study using a mouse model of bone metastasis demonstrated that inhibition of IGF signaling in cancer cells through introduction of dominant-negative IGF-IR and IGF-IR knockdown significantly inhibited bone metastasis formation with decreased mitosis and increased apoptosis in metastatic breast cancer cells in bone [12]. Similar results were also obtained in mouse models of multiple myeloma [19], neuroblastoma [20], and prostate cancer [21]. Inhibition of Akt and NF-κB pathways, which are activated through IGF-IR, also suppressed bone metastases [12]. These results suggest that bone-derived IGFs promote bone metastases with increased proliferation and decreased apoptosis in metastatic cancer cells through the IGF-activated IGF-IR, Akt, and NF-κB pathway.

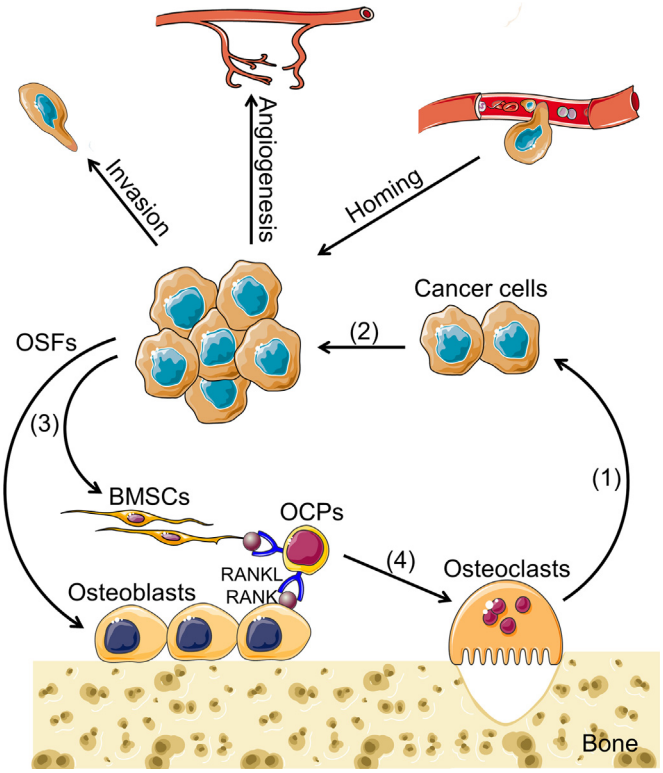

Fig. 1. "Vicious cycle" in bone metastases. Bone-derived growth factors such as IGFs and TGFβ, which are released continuously from the bone matrix due to osteoclastic bone destruction (1), promote cancer cell proliferation (2) and the production of OSFs (3). OSFs upregulate RANKL expression by BMSCs and osteoblasts, which interacts with RANK expressed by osteoclast precursor cells (OPCs). This interaction promotes osteoclastogenesis and, further, bone destruction (4), resulting in the establishment of a "vicious cycle." In addition, bone-derived growth factors play a variety of roles in cancer progression, including cell invasion, angiogenesis, and homing. Images have been adapted from Servier Medical Art (https://smart.servier.com/).

4.3. *TGFβ*

TGFβ, the second most-abundant growth factor stored in bone [15], has been shown to promote the production of osteolytic factors, including PTHrP and IL-11 [3,22]. These factors induce RANKL expression and inhibit osteoprotegerin expression in BMSCs and osteoblasts, thereby stimulating osteoclastic bone destruction and progression of bone metastases. In support of this notion, the introduction of dominant-negative TGFβ type II receptor (dnTβIIR) to cancer cells and administration of TGFβ receptor kinase inhibitors were found to suppress bone metastases in mice [22,23]. Our previous study revealed that TGFβ also increases the expression of cyclooxygenase-2 (COX-2) and prostaglandin E2, which is a major metabolite of COX-2 and an OSF, in cancer cells [24]. Overexpression of dnTβIIR decreased bone metastases along with impaired COX-2 expression in cancer cells in mice. Additionally, COX-2 inhibitors suppressed bone metastases with the reduced number of osteoclasts. Furthermore, Sethi et al. demonstrated that TGFβ enhances Jagged1 expression in cancer cells [25]. Jagged1-Notch signaling induced the release of IL-6 from osteoblasts and activated bone destruction, thereby promoting bone metastases. These findings collectively suggest the critical involvement of bone-derived TGFβ in bone metastases.

5. Hypoxia

Intratumoral hypoxia is a hallmark of the cancer microenvironment, which is caused by increased oxygen consumption and/or insufficient blood supply [26]. The recent study using a two-photon phosphorescence lifetime microscopy technique clearly demonstrated that the bone marrow is a hypoxic tissue [27]. Hypoxia signals are mainly transduced by activation of hypoxia-inducible factors (HIFs), which act as regulators of cancer progression [26]. Hypoxia was also shown to affect both bone and cancer cells. Osteoclast-like cell formation was increased and osteoblastic differentiation was inhibited under hypoxic conditions [26].

Although the precise oxygen concentrations in bone metastases of cancer patients have not yet been determined, our immunohistochemical study using the hypoxia marker, pimonidazole, revealed the presence of hypoxic regions in mouse bone metastases, in which nuclear accumulation of HIF-1α in cancer cells was observed [28]. Furthermore, the stable expression of either constitutively active or dominant-negative HIF-1α increased or decreased the development of bone metastases, respectively [28].

A hypoxia-activated pro-drug, TOP3, is a fusion protein comprised of a protein transduction domain embedded in the human immunodeficiency virus-trans-activator of transcription (HIV-TAT) protein, oxygen-dependent degradation domain, and procaspase-3 [29]. TOP3 induces caspase-3-mediated cell death, specifically under hypoxic conditions. We reported that TOP3 selectively induced apoptosis in hypoxic tumor cells *in vitro* and significantly reduced bone metastases in mice [28]. These results collectively suggest that intratumoral hypoxia and the resulting activation of HIF-1 promote bone metastases. It is also likely that hypoxia leads to the development of osteolytic bone metastases by suppressing osteoblast differentiation and promoting osteoclastogenesis.

6. Cancer stem cells (CSCs)

The CSC hypothesis is a concept that cancers are sustained by a small population of cancer cells with tumor-initiating potential [30]. CSCs are supposed to have the capabilities of self-renewal and differentiation, which lead to the development of cancers composed of heterogeneous cell populations. Because these stem-cell-like properties are required to initiate secondary tumor formation in distant organs, CSCs are expected to play a central role in the development of bone metastases.

CD44 is an adhesion molecule that binds to the extracellular matrix, mainly hyaluronan (HA), and has been implicated in cancer cell migration, invasion, and metastasis [31]. CD44 is also currently recognized as a representative marker for stem cells of several cancer types. Consistent with this notion, our study showed that CD44 knockdown in cancer cells significantly inhibited tumor sphere formation, cell migration and invasion *in vitro,* and tumor formation in orthotopic sites in mice [32]. More importantly, downregulation of CD44 markedly suppressed bone metastases with a decreased number of osteoclasts. Of interest, the expression of HA synthase 2 (HAS2), one of the HASs, was downregulated in CD44-knockdown cells, and HA localization in bone metastatic lesions was markedly reduced. Moreover, 4-methylumbelliferone, an inhibitor of HA synthesis, decreased tumor sphere formation and osteoclast-like cell differentiation *in vitro* and suppressed bone metastasis formation with a reduced number of osteoclasts in mice. These results suggest that CD44 expression in cancer cells promotes bone metastases by enhancing tumorigenicity, cell migration and invasion, and HA production. It may also be possible that HA provides a niche for bone-metastatic CSCs.

It should be noted that CD44 has a variety of alternatively spliced isoforms [31]. The standard isoform of CD44 (CD44s) and the variant isoforms (CD44v) have been suggested to play distinct functional roles in cancer cell biology. However, so far, we have not been able to find differential roles between CD44s and CD44v, particularly CD44v8–10, in bone metastases [33].

Epithelial cell adhesion molecule (EpCAM), which is expressed in a wide variety of cells of epithelial origin, is also defined as a marker for some types of CSCs [30]. Our study using EpCAM-negative and -positive ($EpCAM^{neg}$ and $EpCAM^{pos}$) cell populations isolated from cancer cell lines showed that $EpCAM^{pos}$, but not $EpCAM^{neg}$, cells possessed CSC-like properties, including self-renewal, differentiation, and tumorigenic abilities [34]. Furthermore, the development of bone metastases was markedly increased in mice inoculated with $EpCAM^{pos}$ cells. These results again suggest that cancer stem-like properties contribute to the enhancement of bone metastases.

7. Conclusion

As described, in part, above, preclinical studies to date have shown the involvement of a variety of molecules in the development and progression of bone metastases. Recent advances in our understanding of the mechanisms underlying bone metastases have led to the development of a variety of novel therapeutic agents. Some of them, represented by bisphosphonates and the anti-RANKL antibody denosumab, are widely and successfully being used for bone metastasis treatment, whereas others are under clinical investigation [35]. It is hoped that these efforts will lead to new and innovative therapeutic strategies for blocking and curing bone metastases.

CRediT authorship contribution statement

Toru Hiraga: Conceptualization, Funding acquisition, Supervision, Validation, Writing - original draft, Writing - review & editing.

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Ethical approval

Ethical approval is not required for this review.

Conflicts of interest

The author declares no conflict of interest.

References

[1] Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. Clin Cancer Res 2006;12:6243s–9s.
[2] Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 2002;62:1832–7.
[3] Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordón-Cardo C, et al. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 2003;3:537–49.
[4] Sun Y-X, Schneider A, Jung Y, Wang J, Dai J, Wang J, et al. Skeletal localization and neutralization of the SDF-1(CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites in vivo. J Bone Miner Res 2004;20:318–329.
[5] Crane GM, Jeffery E, Morrison SJ. Adult haematopoietic stem cell niches. Nat Rev Immunol 2017;17:573–90.
[6] Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J, et al. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. J Clin Invest 2011;121:1298–312.
[7] Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2002;2:584–93.
[8] Hiraga T, Tanaka S, Ikegame M, Koizumi M, Iguchi H, Nakajima T, et al. Morphology of bone metastasis. Eur J Cancer 1998;34:230–9.
[9] Nakamura H, Hiraga T, Ninomiya T, Hosoya A, Fujisaki N, Yoneda T, et al. Involvement of cell-cell and cell-matrix interactions in bone destruction induced by metastatic MDA-MB-231 human breast cancer cells in nude mice. J Bone Miner Metab 2008;26:642–7.
[10] Demers LM, Costa L, Lipton A. Biochemical markers and skeletal metastases. Cancer 2000;88:2919–26.
[11] Yonou H, Ochiai A, Goya M, Kanomata N, Hokama S, Morozumi M, et al. Intraosseous growth of human prostate cancer in implanted adult human bone: relationship of prostate cancer cells to osteoclasts in osteoblastic metastatic lesions. Prostate 2004;58:406–13.
[12] Hiraga T, Myoui A, Hashimoto N, Sasaki A, Hata K, Morita Y, et al. Bone-derived IGF mediates crosstalk between bone and breast cancer cells in bony metastases. Cancer Res 2012;72:4238–49.
[13] Hiraga T, Williams PJ, Mundy GR, Yoneda T. The bisphosphonate ibandronate promotes apoptosis in MDA-MB-231 human breast cancer cells in bone metastases. Cancer Res 2001;61:4418–24.
[14] Sousa S, Clézardin P. Bone-targeted therapies in cancer-induced bone disease. Calcif Tissue Int 2018;102:227–50.
[15] Hauschka P, Mavrakos A, Iafrati M, Doleman S, Klagsbrun M. Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-Sepharose. J Biol Chem 1986;261:12665–74.
[16] Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, et al. TGF-β1–induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med 2009;15:757–65.
[17] Xian L, Wu X, Pang L, Lou M, Rosen CJ, Qiu T, et al. Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. Nat Med 2012;18:1095–101.
[18] Maki RG. Small is beautiful: insulin-like growth factors and their role in growth, development, and cancer. J Clin Oncol 2010;28:4985–95.
[19] Araki K, Sangai T, Miyamoto S, Maeda H, Zhang S-C, Nakamura M, et al. Inhibition of bone-derived insulin-like growth factors by a ligand-specific antibody suppresses the growth of human multiple myeloma in the human adult bone explanted in NOD/SCID mouse. Int J Cancer 2006;118:2602–8.
[20] van Golen CM, Schwab TS, Kim B, Soules ME, Su Oh S, Fung K, et al. Insulin-like growth factor-I receptor expression regulates neuroblastoma metastasis to bone. Cancer Res 2006;66:6570–8.
[21] Kimura T, Kuwata T, Ashimine S, Yamazaki M, Yamauchi C, Nagai K, et al. Targeting of bone-derived insulin-like growth factor-II by a human neutralizing antibody suppresses the growth of prostate cancer cells in a human bone environment. Clin Cancer Res 2010;16:121–9.
[22] Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, et al. TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J Clin Invest 1999;103:197–206.
[23] Ehata S, Hanyu A, Fujime M, Katsuno Y, Fukunaga E, Goto K, et al. Ki26894, a novel transforming growth factor-b type I receptor kinase inhibitor, inhibits in vitro invasion and in vivo bone metastasis of a human breast cancer cell line. Cancer Sci 2007;98:127–33.
[24] Hiraga T, Myoui A, Choi ME, Yoshikawa H, Yoneda T. Stimulation of cyclooxygenase-2 expression by bone-derived transforming growth factor-beta enhances bone metastases in breast cancer. Cancer Res 2006;66:2067–73.
[25] Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived Jagged1 promotes osteolytic bone metastasis of breast cancer by engaging Notch signaling in bone cells. Cancer Cell 2011;19:192–205.
[26] Hiraga T. Hypoxic microenvironment and metastatic bone disease. Int J Mol Sci 2018;19:3523.
[27] Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. Nature 2014;508:269–73.
[28] Hiraga T, Kizaka-Kondoh S, Hirota K, Hiraoka M, Yoneda T. Hypoxia and hypoxia-inducible factor-1 expression enhance osteolytic bone metastases of breast cancer. Cancer Res 2007;67:4157–63.
[29] Harada H, Hiraoka M, Kizaka-Kondoh S. Antitumor effect of TAT-oxygen-dependent degradation-caspase-3 fusion protein specifically stabilized and activated in hypoxic tumor cells. Cancer Res 2002;62:2013–8.
[30] Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. Cell Stem Cell 2012;10:717–28.
[31] Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol 2018;11:64.
[32] Hiraga T, Ito S, Nakamura H. Cancer stem-like cell marker CD44 promotes bone metastases by enhancing tumorigenicity, cell motility, and hyaluronan production. Cancer Res 2013;73:4112–22.
[33] Hiraga T, Nakamura H. Comparable roles of CD44v8-10 and CD44s in the development of bone metastases in a mouse model. Oncol Lett 2016:2962–9.
[34] Hiraga T, Ito S, Nakamura H. EpCAM expression in breast cancer cells is associated with enhanced bone metastasis formation. Int J Cancer 2016;138:1698–708.
[35] Hiraga T. Targeted agents in preclinical and early clinical development for the treatment of cancer bone metastases. Expert Opin Investig Drugs 2016;25:319–34.