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Osteoblasts Are the Centerpiece of the Metastatic Bone Microenvironment

Hyo Min Jeong[1,2], Sun Wook Cho[3], Serk In Park[1,2,4]

[1]Department of Biochemistry and Molecular Biology and [2]The BK21 Plus Program, Korea University College of Medicine; [3]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [4]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA

The tumor microenvironment is comprised of diverse stromal cell populations in addition to tumor cells. Increasing evidence now clearly supports the role of microenvironment stromal cells in tumor progression and metastasis, yet the regulatory mechanisms and interactions among tumor and stromal cells remain to be elucidated. Bone metastasis is the major problem in many types of human malignancies including prostate, breast and lung cancers, and the biological basis of bone metastasis let alone curative approaches are largely undetermined. Among the many types of stromal cells in bone, osteoblasts are shown to be an important player. In this regard, osteoblasts are a key target cell type in the development of bone metastasis, but there are currently no drugs or therapeutic approaches are available that specifically target osteoblasts. This review paper summarizes the current knowledge on osteoblasts in the metastatic tumor microenvironment, aiming to provide clues and directions for future research endeavor.

Keywords: Neoplasms; Neoplasm metastasis; Bone and bones; Microenvironment; Osteoblasts; Osteoclasts

INTRODUCTION

Patterns of tumor metastases are dependent on the recipient organ microenvironment, and each component of the metastatic tumor microenvironment plays distinct and unique roles in disease progression [1]. To better understand organ-specific metastases and to ultimately develop more effective therapeutics, the biology of key cellular components in the microenvironment must be clearly determined [2,3]. Among many types of solid tumors, prostate, breast and lung cancers predominantly develop bone-specific metastases [2,3]. Bone provides a unique microenvironment for the migrated tumor cells to survive and grow [4]. The normal physiology of the skeletal system requires a tightly-regulated balance between osteoblasts and osteoclasts. This balance is frequently tipped during the process of bone metastases [5]. The best characterized example is the vicious cycle hypothesis of bone metastasis [6]. Briefly, the migrated breast cancer cells express bone-modulatory factors such as

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Corresponding authors: Serk In Park
Department of Biochemistry and Molecular Biology, Korea University College of Medicine, 73 Inchon-ro, Seongbuk-gu, Seoul 02841, Korea
Tel: +82-2-2286-1305, **Fax:** +82-2-923-0480, **E-mail:** serkin@korea.edu

Sun Wook Cho
Department of Internal Medicine, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-4761, **Fax:** +82-2-764-2199, **E-mail:** swchomd@snu.ac.kr

parathyroid hormone-related peptide (PTHrP), leading to up-regulation of receptor activator of nuclear factor κB (RANKL) and monocyte-colony stimulating factor (M-CSF) in adjacent osteoblasts [6]. Subsequently, osteoclastogenesis occurs, followed by osteolysis and the release of bone matrix-embedded growth factors (e.g., transforming growth factor β [TGF-β]) [7]. These processes ultimately result in severely increased bone resorption and metastatic tumor growth.

The homeostasis of the human skeletal system is maintained by the coupling of bone resorption (by osteoclasts) and new bone formation (mainly by osteoblasts) [8]. Alterations in the bone homeostasis result in many types of bone diseases such as fractures and osteoporosis. In addition to the vicious cycle hypothesis described above, increasing lines of evidence now clearly support that alterations in the bone homeostasis contribute to the progression of bone metastases [9]. Among the many cell types in the metastatic bone microenvironment, including osteocytes, osteoblasts, osteoclasts, mesenchymal stem cells, and hematopoietic bone marrow cells in diverse stages of differentiation, the majority of research data concentrate on osteoclasts, and thus the current therapeutic approaches targeting the metastatic bone microenvironment are mostly osteoclast inhibitors (e.g., bisphosphonates, denosumab, and cathepsin K inhibitors) [10,11]. On the other hand, numerous research groups have demonstrated that other types of cells, particularly osteoblasts, are also important in the metastatic bone microenvironment, and also that osteoblasts play distinct and essential roles in metastatic progression. Accordingly, this review paper will summarize and highlight the recent publications supporting the role of osteoblasts in bone metastasis.

TUMOR-DERIVED HEDGEHOGS STIMULATE OSTEOBLASTS

Hedgehog (Hh) signaling is important in development, regulating cell growth, body pattern formation and organogenesis. Mammalians have three types of Hh proteins, i.e., Sonic, Indian, and Desert Hh with Sonic Hh (SHH) being the most thoroughly investigated in development and pathology. Several lines of evidence suggest that Hh is involved in organ-specific metastasis to bone, especially in breast and prostate cancers, as well as in bone tumors such as osteosarcomas. Lee et al. [12] demonstrated that SHH up-regulates RANKL expression in bone stromal cells and osteoblasts, leading to osteoclast formation. Subsequently, an increase in osteoclasts contributes to tumor-induced osteolysis and ultimatelys to increased tumor growth in bone. Recently, Shimo et al. [13] analyzed human oral squamous cell carcinoma patient samples and identified that SHH is expressed in bone-invasive tumor cells while patched receptor and Gli2 (a downstream transcription factor of HH signaling) are expressed in osteoclast progenitor cells, indicating that tumor-derived SHH stimulated osteoclast formation and bone resorption in tumor-induced jaw bone destruction. The above examples clearly show that Hh is a paracrine factor in the tumor microenvironment. Accordingly, metastatic tumor-derived Hh proteins stimulate the bone stromal cells to promote metastatic tumor growth. Indeed, Hh proteins reregulate RANKL expression in adjacent osteoblasts and bone marrow stromal cells, leading to osteoclastogenesis via activation of extracellular signal-regulated kinases (ERK), p38 mitogen-activated protein kinases (MAPK), increased expression of nuclear factor of activated T cells, cytoplasmic 1 (NFATc1). These changes ultimately induce RANKL-dependent osteoclastogenesis and tumor-induced osteolysis [14-17]. Further evidence has also come from studies on osteosarcoma. Chan et al. [18] demonstrated that osteoblast-specific Hh expression increased osteosarcoma development in p53 mutant mice. Furthermore, inhibition of Hh signaling reduced the expression of Yes-associated protein (Yap1), a potent oncogene, in osteosarcoma cells. Aberrant HH signaling induced Yap1 overexpression and also osteosarcoma development. They also showed that the long non-coding RNA H19, a highly relevant candidate for osteosarcoma development, is aberrantly expressed and induced by up-regulated Hh signaling and Yap1 overexpression, suggesting a molecular mechanism of HH-dependent osteosarcoma progression from osteoblasts.

Gli 2 is in the main signal transduction pathway downstream of Hh. Briefly, in the absence of Hh ligands, the patched (PTCH1) receptors accumulate and inhibit the function of Smoothened (Smo) protein, leading to degradation of the Gli transcription factor by proteasome. Upon activation, the PTCH1 receptors are degraded and thus Gli is activated, leading to its nuclear translocation and function as a transcriptional activator [19]. One important product of the transcriptional activation of Gli is PTHrP [20]. Johnson et al. [21] demonstrated that bone matrix-derived TGF-β increased the expression of Gli2 and PTHrP in metastatic breast cancer cells, leading to further bone destruction. Furthermore, the authors showed that Gli activity was not dependent on canonical Hh signaling, because MDA-MB-231 breast cancer cells do not express Smo [22]. Das et al. [23] demonstrated that Hh pathway is important in osteoblast maturation, particularly in the presence of breast cancer cells. Hh-mediated Gli2 stimulated secretion of bone morphogenetic protein

(BMP2), a potent osteogenic differentiation factor, as well as cyclin D, osteopontin, and insulin-like growth factor, suggesting that Hh is an important factor of osteoblastic functions. Collectively, the Hh proteins are important mediators between tumor and bone, and the signaling is bi-directional (i.e., tumor-to-stroma and stroma-to-tumor).

RUNT-RELATED TRANSCRIPTION FACTOR 2

Runt-related transcription factor 2 (RUNX2) is also known as core-binding factor subunit-α 1 (CBFα-1) and serves as a key transcription factor in osteoblast differentiation and gene expression. RUNX2 plays important roles in bone homeostasis and integrity of skeletal tissue [24-26]. RUNX2 binds to cognate DNA elements with the consensus nucleotide sequence 5'-ACCACA in the promoters/enhancers of target genes [27]. Franceschi and Xiao [28] extensively summarized the data supporting that RUNX2 is important in the responsiveness to multiple signal transduction pathways in osteoblasts. Indeed, the Franceschi group demonstrated that functional RUNX2 is essential to fibroblast growth factor 2 (FGF2)- and parathyroid hormone-induced osteocalcin expression in osteoblasts, and BMP2-stimulated osteoblast differentiation [29-32]. In this context, RUNX2 was shown to be a critical regulator of osteoblasts in the bone metastatic tumor microenvironment. For example, Zong et al. [33] showed that the RUNX2-PHTrP-RANKL pathway contributes to the progression of an experimental breast cancer bone metastases. Furthermore, Li et al. [34] demonstrated that RUNX 2 increased integrin β-like 1 expression, leading to up-regulation of TGF-β in the bone metastatic breast cancer stroma.

RUNX2 has been classically considered an osteoblast-specific transcription factor, but more recently, increasing evidence supports that RUNX2 is not only important in osteoblasts but also in tumor cells in the bone microenvironment. For example, Li et al. [35] recently showed that RUNX2 promotes breast cancer bone metastasis by increasing integrin α5-mediated colonization. In addition, Ge et al. [36] provided pivotal evidence that phospho-RUNX2 is proportionately expressed during the progression of prostate cancer bone metastasis with the highest expression in metastatic lesions using tissue microarray and *in vitro* prostate cancer cell line models. The authors also concluded that phospho-RUNX2 has a prognostic value for prostate cancer patients. RUNX2 is also involved in tumor-induced osteolysis by transcriptional suppression of TSSC1 via the BMP-TGF-β, Wnt, PTHrP, and Src pathways [26]. More specifically, expression of RUNX2 is associated with osteolysis, and up-regulated RUNX2 mediated direct interactions between the TGF-β/BMP and Smad signaling pathways, contributing to the formation of osteolytic- and osteoblastic- mixed bone lesions that are related with prostate cancer cells [37,38]. Moreover, tumor growth and distant metastasis of prostate cancer occur via the RUNX2-Smad complex. The RUNX2-Smad complex has functional activity that mediated TGF-β and BMP signaling through SMID (i.e., Smad interaction domain) [24]. Furthermore, the RUNX2-Smad complex increases the expression of cancer-associated genes. For example, vascular endothelial growth factor (VEGF), matrix metalloproteinases, interleukin 8 (IL-8), and PTHrP are up-regulated by RUNX2 in tumor cells, and mediated tumor growth and organ-specific metastasis to bone [24,39]. VEGF and IL-8 are both potent angiogenic factors and strong pro-tumorigenic factors [40]. PTHrP is an important mediator of the vicious cycle of bone metastasis and also plays diverse roles in tumor progression [2,6,41]. RUNX2 has an anti-mitogenic activity that contributes to drug resistance. RUNX2 physically and functionally interacts with the receptors for androgens and estrogens [42-44], which are the targets for many prostate cancer and breast cancer drugs [27].

In short, overexpression of RUNX2 is observed in the stromal compartment as well as in tumor cells and its phosphorylation is important when the tumor cells metastasize to bone. RUNX2 contacts with Smad, and build the RUNX2-Smad complex. The RUNX2-Smad complex regulates the activation of TGF-β/BMP signaling, stimulating osteolysis and bone destruction. In addition, genes that regulate osteoclast formation are expressed by the RUNX2-Smad complex. Lastly, RUNX2 inhibits the expression of TSSC1, a tumor suppressor that inhibits bone metastasis by blocking the promoter of the TSSC1 gene.

MICRORNAs IN THE BONE MICROENVIRONMENT

MicroRNAs (miRNAs) are non-coding RNAs composed of very short nucleotides sequences up to 22 nucleotides. miRNAs down-regulate mRNA-dependent translation of target genes by producing short hairpin RNAs. RNA polymerase II regulates the transcription of most miRNAs. miRNAs bind to partial complementary sequences of target genes to control their translation. One miRNA can affects dozens of mRNAs, and miRNAs have an important role as post-transcription regulators in bone formation [45-47].

miRNAs potentially contribute to the proliferation and metas-

tasis of tumor cells by regulating target gene expression. Many miRNA function as tumor suppressors; however, some miRNAs aid in tumor growth and metastasis [48]. miRNAs affect each step in the multistep process of hematogenous metastasis including bone metastasis. For example, miR103/107 contributes to tumor progression by down-regulating Dicer, which has an important role in processing miRNA precursors. Up-regulated Dicer promotes the expression of miRNAs that inhibit tumor progression and metastasis, and therefore miR103/107 inhibits the expression of Dicer, contributing to tumor progression [49]. miR-154, miR-379, and miR-409-3p/5p work as oncogenes in bone metastasis of prostate cancer [50,51]. miR-154, miR379 and miR-409-3p/5p are members of δ-like 1 homolog-deiodinase, iodothryonine3 (DLK1-DIO3), and are activated in the epithelial to mesenchymal transition (EMT) of prostate cancer cells [50]. miRNAs in the DLK1-DIO3 cluster have an important role in totipotency during embryogenesis. Furthermore, these miRNAs play a role in inducing pluripotent stem cell formation [52]. The miRNAs of the DLK1-DIO3 cluster are up-regulated in bone metastatic prostate and breast cancer cells. Among those, miR-379 and miR-154 located in the DLK1-DOI3 cluster are specifically up-regulated in prostate cancer cells with the mesenchymal phenotype (i.e., more metastatic). miR-379 and miR-154 are expressed by embryonic stem cells and pluripotent stem cells, and these miRNAs regulate bone-specific prostate cancer cells by inducing EMT [50]. miR-154 has two main functions. First, miR-154 functions as a negative regulator of tumor suppressor. Second, miR-154 regulates the expression of EMT- and stemness-related genes to mediate downstream convergent signal pathways. When miR-154 is inhibited, E-cadherin expression is up-regulated, suggesting decreased invasion and metastasis [50]. In addition, miR-379 is located in the upstream of the miR-154 gene, and has a similar role as miR-154 [50]. In bone metastatic prostate cancer cells, the expression of miR-409-3p/5p is high. miR-409-3p/5p inhibits tumor suppressor genes in prostate cancer cells, for example stromal antigen 2 (STAG2), Ras suppressor protein 1 (RSU1), retinoblastoma-like 2 (RBL2), and nitrogen permease regulator-like 2 (NPRL2) [51,53]. miR-409-3p is predicted to activate the Ras signaling pathway and the hypoxia inducible factor-1α pathway, as well as regulate polycomb group proteins and osteoblastic pathways. miR-409-5p is predicted to activate the E2F pathway, Ras signaling pathway, Akt pathway, and aneuploidy [53]. Taken together, multiple lines of evidence demonstrate that miR-409-3p/5p is elevated in the bone metastatic EMT cell models and this microRNA functions by repressing several tumor suppressor genes [51].

DISCUSSION

Osteoblasts are derived from mesenchymal stem cells in bone marrow, and serves as the major cell type essential to bone anabolic processes. Differentiation of osteoblasts is determined by multiple steps via many growth factors, receptors, and transcriptional factors such as Hh/Ptch, BMP, fibroblast growth fators (FGFs), parathyroid hormone (PTH), RUNX2, WNT, and TGF-β-SMAD. The pathways have a high degree of cross-talk among them, with RUNX2 being a major point of convergence. Osteons, a functional unit of osteoblasts, mediate the bone anabolic reaction by producing organic matrices of bone tissue including cross-linked collagen, and osteocalcin, osteopontin. Subsequently, the matrices become mineralized, forming hard tissue. The roles of osteoblasts in the tumor microenvironment have been a focus of extensive research efforts thus far, with the vicious cycle hypothesis being the most well investigated by Dr. Gregory Mundy's group [3]. The hypothesis was later clinically and experimentally proven to be legitimate and provided a solid scientific rationale for the development of multiple bone-targeted agents. However, the over-simplified cycle comprised of tumor, osteoblasts, osteoclasts, and TGF-β, does not perfectly explain the complex interactions among the extremely diverse stromal cells in the bone microenvironment or the pathologic progression of bone metastasis. For example, we previously demonstrated that megakaryocytes, previously considered to have no clear roles in bone metastasis, are the first cell type that encounter extravasating tumor cells in the bone sinusoidal vessel architecture, and induce apoptosis of tumor cells thus protecting from bone metastasis [54]. In addition, Jung et al. [55] and Shiozawa et al. [56] provided pivotal evidence supporting that the osteoblastic hematopoietic stem cell niches are the main sites of tumor cell localization, and metastatic tumor cells compete with stem cells for the occupancy of the stem cell niche. We further developed experimental approaches to elucidate the interactions between tumor and stromal cells. Most notably, we demonstrated that primary tumor-derived PTHrP circulates to stimulate osteoblasts, leading to up-regulation of myeloid-derived suppressor cells (MDSC). These pre-activated MDSCs in the tumor hosts then enter the systemic circulation to increase primary tumor growth and angiogenesis [6]. Fig. 1 demonstrates that osteoblasts are stimulated to grow (bracket) and form new woven bones (solid arrow), indicating that osteoblasts are one of the first responder cells in bone metastasis, potentially playing critical functions other than expressing osteoclastic factors (RANKL and M-CSF) [57,58]. This review paper summarizs

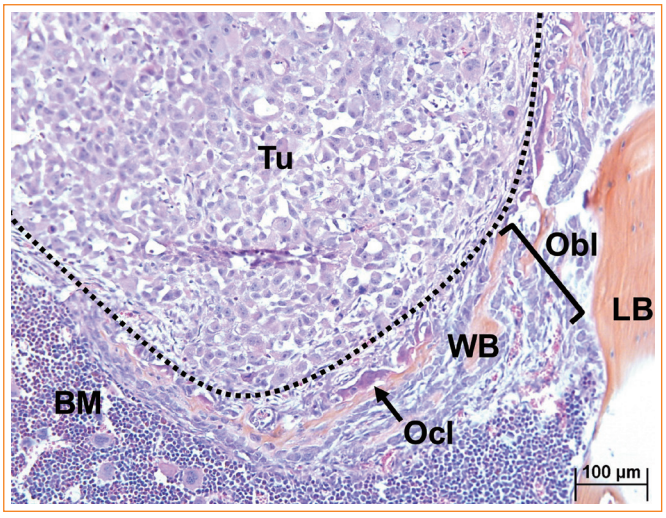

Fig. 1. PC-3, metastatic human prostate cancer cells, were injected in to the proximal tibia of male athymic nude mice [57]. Tumors (Tu) were harvested after 3 weeks, followed by fixation, decalcification, sectioning, and modified H&E staining (showing bone matrices in orange) [58]. Osteoblasts (Obl), physiologically unilayer cells, formed multiple layers (indicating proliferation; bracket), with woven bone (WB; newly formed bone; solid arrow) formation around the tumor tissue (dotted line). Lamellar bone (LB; remodeled bone) and osteoclasts (Ocl) are clearly visible. This data supports that osteoblasts are actively respond to metastatic tumor cells, and potentially play important roles in bone metastasis. BM, bone marrow.

the current evidence supporting how osteoblasts are regulated and what roles they play in the progression of bone metastasis. In particular, we examined the three most significant mediators including Hh and patched receptor signaling (i.e., an extracellular stimulant and the cell surface receptor), the RUNX2 transcription factor and miRNAs (i.e., post-transcriptional level regulation). All of this evidence clearly warrants extensive further research, potentially providing an important foundation for novel and advanced therapeutics specific to bone metastasis.

CONCLUSIONS

Beyond the conventional concept of tumor-initiative and bone-responsive process, recent data are focused on how the cells in the bone environment (i.e., osteocytes, osteoblasts, and osteoclasts) initiate the dormant disseminates tumor cells in bone. The current evidence clearly demonstrates that tumor cells engage with osteoblastic lineage cells on the endosteal surface and enter the long-term dormancy. On the other hand, osteoclast-mediated bone remodeling that is activated by estrogen withdrawal can release dormant tumor cells from the active control of the endosteal niche, facilitating reactivation and tumor growth. Instead of osteoclasts passively responsing to tumor-derived products, bone cells may be crucial orchestrators that dominate bone metastasis development. In this point of view, the osteoblastic niche can be a potential therapeutic target for the treatment of bone metastasis. Further intensive studies focusing on bone cell responding to tumor cells are required to overcome bone metastasis, one of the leading causes of cancer mortality.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Hyo Min Jeong *http://orcid.org/0000-0002-2600-7986*
Sun Wook Cho *http://orcid.org/0000-0002-7394-3830*
Serk In Park *http://orcid.org/0000-0001-8643-5096*

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