Role of the Bone Microenvironment in the Development of Painful Complications of Skeletal Metastases

Sun H. Park, Matthew R. Eber, D. Brooke Widner and Yusuke Shiozawa *

Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA; shpark@wakehealth.edu (S.H.P.); meber@wakehealth.edu (M.R.E.); dwidner@wakehealth.edu (D.B.W.)

* Correspondence: yshiozaw@wakehealth.edu

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Abstract: Cancer-induced bone pain (CIBP) is the most common and painful complication in patients with bone metastases. It causes a significant reduction in patient quality of life. Available analgesic treatments for CIBP, such as opioids that target the central nervous system, come with severe side effects as well as the risk of abuse and addiction. Therefore, alternative treatments for CIBP are desperately needed. Although the exact mechanisms of CIBP have not been fully elucidated, recent studies using preclinical models have demonstrated the role of the bone marrow microenvironment (e.g., osteoclasts, osteoblasts, macrophages, mast cells, mesenchymal stem cells, and fibroblasts) in CIBP development. Several clinical trials have been performed based on these findings. CIBP is a complex and challenging condition that currently has no standard effective treatments other than opioids. Further studies are clearly warranted to better understand this painful condition and develop more effective and safer targeted therapies.

Keywords: cancer-induced bone pain; bone marrow microenvironment; osteoclasts; osteoblasts; macrophages; mast cells; stromal cells

1. Introduction

Cancer-induced bone pain (CIBP) is the most common complication of bone metastases, and it significantly reduces the patient’s quality of life (QOL) [1]. CIBP poses a tremendous challenge to patients and their caregivers, in both managing it and identifying its underlying cause. In the quest for effective cancer therapy, maintaining QOL can be as crucial as treating the disease itself. Seventy-five percent of cancer patients experience pain throughout their disease [2]. Once cancer metastasizes to the bone, the first symptom is often acute bone pain. A full 80% of patients with bone metastases have CIBP [3].

CIBP remains a therapeutically challenging condition. It includes both spontaneous (ongoing) pain and breakthrough (movement-related) pain, which can present individually or in combination [4]. Unless each component of CIBP is appropriately treated, it cannot be managed. Analgesics for CIBP that target the central nervous system (e.g., opioids, nonsteroidal anti-inflammatory drugs or NSAIDs) are somewhat effective to reduce pain, but have severe side effects and are often extremely addictive [5–7]. External beam radiation, used for patients with bone metastases, is primarily palliative and only half of the patients receiving this therapy achieve partial or complete pain relief [4,8]. Alternatively, bisphosphonate and anti-receptor activator of nuclear factor κB ligand (RANKL) antibody treatments that decrease bone resorption in cancer patients with bone metastases [9,10], and radium-223 which forms complexes with hydroxypatite in bone [11–13] can also reduce the onset of pain. However,
even with these well-accepted clinical treatment modalities, 50% of patients with CIBP do not achieve controlled pain status [14].

CIBP is a very complex phenomenon that is uniquely distinct from other forms of chronic pain, such as inflammatory or neuropathic pain [15]. Bone is a richly innervated organ, and it has been suggested that bone metastatic cancer cells interact with sensory nerves in the bone microenvironment, resulting in CIBP development [15]. Mechanical stresses or mass effects from bone metastatic progression can directly induce CIBP, since sensory nerves that express mechanoreceptors reside throughout the interosseous membrane of long bones [16]. However, it has also been suggested that CIBP is not correlated with tumor type, location, number, or size of the metastases [17,18]. This may indicate that CIBP is developed not only during physical contact between bone metastatic cancer cells and sensory nerves, but also from signaling events initiated by cancer-derived factors [15,19–21]. Indeed, the acidic environment surrounding the tumor or cancer secreted growth factors, cytokines, or chemokines can all stimulate receptors on sensory nerves to induce CIBP [15,19,22,23].

The bone marrow provides a unique environment for both hematopoiesis and bone metastatic progression [24–26]. This microenvironment consists of several types of cells, including those that regulate bone remodeling, immune cells, stromal cells, and endothelial cells. It has been appreciated that the crosstalk between the bone microenvironment and bone metastatic cancer cells is crucial for bone metastatic progression [26–28]. However, little is known about how the molecular interactions between metastatic cancer and the bone marrow microenvironment affect CIBP.

Therefore, in this review, we provide a concise overview of the known roles of the bone marrow microenvironment in the development of CIBP and discuss the future directions of research on this topic.

2. The Roles of the Bone Marrow Microenvironment in the Development of Cancer-Induced Bone Pain

2.1. Osteoclasts

During bone metastatic progression, osteolytic cancer cells—originating from primary tumors from breast cancer, lung cancer, renal cancer, sarcomas, and multiple myeloma, etc.—stimulate osteoblasts to release RANKL. The osteoblast-derived RANKL binds to its receptor RANK expressed on osteoclasts. This interaction induces osteoclast maturation and increases osteolytic activity, resulting in enhanced bone resorption. Resorption of the bone matrix then causes the release of growth factors such as transforming growth factor beta (TGF-β) and insulin-like growth factor 1 (IGF-1), leading to further bone metastatic progression. The process whereby bone metastatic cancer cells establish osteolytic lesions to enhance bone metastatic progression is called the “vicious cycle” [27,29–34]. It has been suggested that enhanced osteoclast activity can also lead to CIBP. Consistent with this notion, increased mechanical and thermal hyperalgesia are observed in rodents inoculated with osteolytic osteosarcoma into the bone, compared to animals without tumors [35–37]. Administration of the decoy RANKL receptor osteoprotegerin (OPG) to osteosarcoma-bearing animals significantly decreased spontaneous pain behaviors [35,36]. However, OPG treatments did not affect tumor size.

During the bone resorption process, osteoclasts acidify (pH 4.0–4.5) the extracellular space by releasing protons and chloride ions through membrane transport (V-type H⁺ ATPase) [38]. The low pH condition sensitizes sensory nerves to mechanical, thermal, and chemical stimuli by activating acid sensing receptors such as the acid-sensing ion channels (ASICs) and transient receptor potential cation channel subfamily V member 1 (TRPV1) expressed on sensory nerves [39,40]. In addition, osteoclast activity indirectly causes TRPV1 upregulation in sensory nerves through the release of TGF-β and IGF-1 derived from the resorbed bone matrix. These factors increase the expression of their receptors (TGF-βRI and IGF-1R) on sensory nerves. In a rat CIBP model using mammary gland carcinoma, the upregulation of these growth factor receptors correlates with the upregulation and sensitization of TRPV1 in sensory nerves. The tumor-bearing animals in these studies had significantly increased
mechanical and thermal sensitivity, and observed pain behaviors were reversed with treatments of TGF-βRI and IGF-1R antagonists [41,42].

Osteoclasts mediate bone resorption not only through the creation of acidic conditions, but also through adenosine triphosphate (ATP) production by mitochondrial cytochrome c oxidative activity [43]. Osteoclasts release accumulated intracellular ATP into the extracellular space, which can activate purinergic receptors such as P2X receptors, known as the ATP-gated ion channels [44,45]. It has been demonstrated that: (1) approximately 90% of the peripheral and central sensory neurons express P2X receptors; and (2) the subtypes of P2X receptors including P2X4, P2X3, and P2X7 regulate the development of neuropathic and inflammatory pain [46,47]. Specifically, P2X3 and P2X2/3 expressed on the terminal end of primary afferent neurons innervating bone have been shown to be involved in CIBP development [48–52]. For instance, in C3H/HeJ mice receiving intratibial inoculations of osteolytic fibrosarcoma, increased expression of P2X3 receptor in the peripheral nociceptive fibers is observed, and peri-tumoral injections of receptor antagonist naloxone-methiodide (A-317491) inhibited cancer-mediated thermal hyperalgesia [52]. Moreover, growing tumor cells also generate ATP [53,54], and ATP itself stimulates osteoclast activities through the P2X3 receptor [55,56]. Therefore, ATP can induce CIBP by directly interacting with P2X receptors on sensory nerves or indirectly through enhanced osteoclastic activity. Although further studies are warranted, pharmacologically targeting the ATP/P2X receptors axis may prove to be a potential therapeutic strategy for CIBP.

2.2. Osteoblasts

Unlike osteolytic bone metastatic progression, the mechanisms driving osteoblastic bone metastatic progression are largely unknown. Prostate cancer is one of the primary cancer types that gives rise to developing osteoblastic lesions. It has been demonstrated that prostate cancer cells facilitate new bone formation by secreting parathyroid hormone-related protein (PTHrP) [57], urokinase-type plasminogen activator (uPA) [58], or prostate-specific antigen (PSA) [59]. Endothelin-1 (ET-1), a vasoconstrctor, is also known as an osteoblast inducing factor. It stimulates mitogenesis of osteoblasts when it binds to endothelin A receptor (ETAR) and endothelin B receptor (ETBR) expressed by osteoblasts [60,61]. When the osteolytic human breast cancer cell line ZR-75-1 was made to overexpress ET-1, osteoblastic metastases along with new bone formation were detected after inoculation into murine bone, and treatments with the ETAR selective antagonist (ABT-627) attenuated these osteoblastic lesions [62]. The ET-1/ETAR interaction also plays important roles in CIBP development [20,63]. In fact, the administration of ABT-627 into ET-1 expressing osteosarcoma-bearing mice attenuates CIBP behaviors, but the ETBR selective antagonist, A-192621, does not reduce CIBP behavior [20]. Consistently, phase II and III clinical trials in patients with hormone refractory prostate cancer showed that ABT-627 delayed the time to bone alkaline phosphatase progression and reduced bone pain, compared to those with placebo [64,65]. Additionally, local injection of ET-1 into the peri-tumoral area of a fibrosarcoma bone cancer murine model increased pain behaviors, and the ETAR antagonist BQ-123 inhibited these behaviors [66]. A meta-analysis of 9 clinical studies examining the effects of ETAR antagonists on castration-resistant prostate cancer patients, consistently showed that the ETAR antagonist Atrasentan reduces the relative risk of bone pain [67].

Newly formed bone or woven bone that is mediated by bone metastatic cancer cells is weaker than normal bone. While normal bone has a regular parallel alignment of collagen sheets and is mechanically strong, newly formed immature bone is made of a smaller number of randomly oriented collagen fibers and is mechanically weak, although it forms quickly [68,69]. Therefore, the weakening and instability of new tumor-bearing bone, easily leads to bone damage and CIBP. A canine prostate cancer cell line ACE-1 establishes osteoblastic bone metastatic lesions and develops spontaneous and mechanical pain behaviors in vivo [70]. Since the studies to reveal the impact of tumor-bearing newly formed bone on CIBP are underdeveloped, the use of this model can be helpful to further determine the detailed molecular mechanisms.
2.3. Immune Cells

Inflammation is also involved in the pathobiology of CIBP via several immune cell types (e.g., macrophages and mast cells) [71]. Macrophages are known to contribute to the tumor microenvironment, and they release inflammatory factors upon interacting with tumor cells. These inflammatory factors can induce both disease progression and cancer-related pain [72–74]. Some types of cancer cells that metastasize to the bone (e.g., breast cancer, prostate cancer) highly express and secrete the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [75,76]. Macrophages express the functional receptors for NGF [p75 neurotrophin receptor (p75NTR), tropomyosin receptor kinase A (TrkA)] and BDNF [tropomyosin receptor kinase B (TrkB)] [77]. NGF and BDNF derived from bone metastatic cancer cells activate macrophages to release pro-inflammatory cytokines [tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β] and inflammatory regulators [NGF, Prostaglandin E2 (PGE2)] that sensitize nociceptors [78,79]. In addition, as their names indicate, NGF and BDNF also directly regulate the survival, development, and function of neurons [80].

IL-1β can stimulate expression of cyclooxygenase 2 (Cox-2), and Cox-2 facilitates synthesis of prostaglandins in macrophages [81]. Prostaglandins can sensitize or activate the sensory nerves by binding to prostanoid receptors, resulting in CIBP [82]. The administration of a Cox-2 inhibitor into an osteosarcoma CIBP model inhibits pain behaviors as well as bone destruction without affecting tumor burden [82–86]. Since most NSAIDs inhibit both Cox-1 and Cox-2, which are required for PGE2 synthesis [87], and the major adverse events mediated by NSAIDs are thought to be Cox-1 dependent [88,89], blocking Cox-2 release from macrophages may be a better option for CIBP treatment than NSAIDs. However, a randomized, Phase III trial between a selective Cox-2 inhibitor (rofecoxib) and a non-selective NSAID (naproxen) in patients with rheumatoid arthritis revealed that chronic administration of rofecoxib causes more myocardial infarction than naproxen, while there is no significant difference in treatment efficacy between the two [90]. A more recent pre-clinical study using a lung carcinoma CIBP mouse model demonstrated that when microsomal PGE synthase-1 (mPGES-1), another prostaglandin synthesizing enzyme, is deleted, the onset of pain behaviors mediated by the growth of lung carcinoma in the tibia is delayed [91]. These findings suggest that targeting Cox-2 expression and PGE2 synthesis in macrophages can be potential therapeutic strategies for CIBP. However, further studies are needed to elucidate the pathophysiology of adverse events before moving into the clinic.

Pre-clinical murine CIBP studies of osteosarcoma, breast cancer, and prostate cancer have demonstrated that blockage of NGF significantly attenuates pain behaviors and bone destruction mediated by bone tumors [92–94]. Additionally, an anti-NGF monoclonal antibody tanezumab was clinically tested in patients with osteoarthritis and diabetic peripheral neuropathy, and overall significant pain relief is observed in patients treated with tanezumab compared to those treated with placebo [95–98]. Although larger clinical trials will be needed, a recent clinical study in patients with bone metastatic cancer, breast cancer, renal cell carcinoma, or multiple myeloma (placebo n = 30, tanezumab n = 29) demonstrated greater efficacy in pain relief in patients treated with tanezumab than placebo treated patients [99].

Protease-activated receptors (PAR-2), a sub-family of G protein-coupled receptors that are highly expressed on sensory nerves, are known to be involved in the development of inflammatory and neuropathic pain in rodent models [100–102]. PAR-2 is mainly activated by mast cell tryptase and trypsin [103–105]. Mast cells are located near sensory neurons; contain cytoplasmic granules that store inflammatory mediators; and their release of pain transmitters causes pain [106–108]. When the conditioned medium obtained from human squamous cell carcinoma is injected into the mouse hind paw, the skin mast cells are activated and increased pain behaviors are observed [109]. However, this cancer-associated mechanical allodynia is reversed with treatments of the trypside inhibitor APC-366 or soybean trypsin inhibitor (SBTI) [109]. Activation of PAR-2 has been shown to increase levels of neuropeptides such as calcitonin gene-related peptide (CGRP) and substance
P (SP). Sensory nerve sprouting from CGRP expressing neurons is known to be associated with skeletal pain behaviors [110–113], and levels of plasma CGRP directly correlate with the pain intensity experienced in several pain related conditions [114,115]. It has been demonstrated that bone tumor enhances the PAR-2 expression in sensory nerves [116]. In addition, recent studies have revealed that tumor-infiltrating mast cells in bone metastatic tumors of gastric cancer promote bone metastatic growth, osteolytic lesions, and CIBP by stimulating angiogenesis [117,118].

2.4. Stromal Cells

Bone is a hypoxic tissue (pO2: 8.1–26.7 mmHg) [119,120], and this hypoxic environment is crucial for controlling angiogenesis [121], bone repair [122], osteoblastogenesis [123], osteoclastogenesis [124], and hematopoiesis [125]. Moreover, under hypoxia, tumor cells generate large amounts of lactate through elevated levels of aerobic glycolysis, leading to a lowering of intracellular pH (pH 6.8). This is known as the Warburg effect [53,126]. To prevent cell death mediated by this intracellular acidification, tumor cells actively pump out the protons and lactate to the extracellular space. This extracellular acidic environment surrounding tumor cells can stimulate the cells of stromal origin in the marrow, such as mesenchymal stem cells (MSCs) and fibroblasts [127]. Bone marrow MSCs and fibroblasts are known to express high levels of acid sensing receptors [acid-sensing ion channel 3 (ASIC3), ASIC4, G protein-coupled receptor 4 (GPR4), and GPR65] [128], and become activated by the acidic environment created by bone metastatic tumor cells. This interaction leads to expression and secretion of inflammatory cytokines [IL-6, IL-8, IL-15, chemokine (C-C motif) ligand 5 (CCL5), IL-1ß] as well as nociceptive mediators such as NGF and BDNF [128]. Therefore, it has been suggested that bone metastatic tumor cells induce CIBP by interacting with bone marrow stromal cells.

3. Discussion

Despite the improvement of current cytotoxic treatments, these treatments may not provide survival benefits to all advanced cancer patients. However, most of these patients suffer from symptoms that negatively impact their QOL, such as CIBP. CIBP is a very complex symptom since bone metastatic cancer, sensory nerves, and the bone microenvironment interact together to cause such a painful condition. Therefore, revealing the detailed mechanisms whereby the components that are responsible for bone metastatic progression are involved in the CIBP development will be very important in furthering understanding of this painful symptom and possibly for the development of effective therapies. In this review, we discussed the roles of the cells controlling bone remodeling, immune cells, and stromal cells in the development of CIBP (Figure 1). However, these findings are based on limited evidence. Further studies are therefore clearly needed in this area.

It has been suggested that higher patient QOL may indicate a survival benefit [129]. Along with this notion, recent research suggests that CIBP may be both a reason for decreased QOL and an indicator of survival [130–133]. For instance, the ALSYMPCA trial [134], which investigated the role of radium-223 in patients with prostate cancer and bone metastases, demonstrated that decreased pain levels correlated with increased overall survival. Additionally, the importance of nerves for cancer development has been appreciated [135]. Recent studies revealed that the sympathetic nervous system regulates the metastatic process of prostate cancer to bone [136], and that denervation can suppress tumorigenesis and metastasis [136–139]. However, it remains unclear whether sensory nerves that innervate bone, which are responsible for bone pain, also promote metastatic progression to bone. Therefore, revealing the mechanisms of CIBP may provide a strong foundation for much-needed treatments for bone metastases. Stopping pain signals may be useful to improve both morbidity and mortality.
Figure 1. Mechanisms of bone microenvironment involvement in cancer-induced bone pain. Bone-disseminated tumor cells release factors (e.g., ET1) to stimulate the proliferation of osteoblasts (e.g., endothelin A/B receptors), resulting in new bone formation, which is structurally weak and prone to fracture. Active osteoblasts release RANKL to promote osteoclast activity, resulting in increased bone resorption which also weakens bone. During bone resorption, nociceptors become sensitized and activated through osteoclast mediated acidification and ATP accumulation, which activates the acid sensing TRPV1 and ASICS receptors, or the ATP-gated P2X receptors expressed on sensory neurons, respectively. Tumor cell derived H+ directly induces nociception via activation of the acid sensing receptors expressed on the sensory neurons. Stromal cells (e.g., fibroblasts and mesenchymal stem cells) also express acid sensing receptors, and acidification of the bone marrow space stimulates release of stromal cell derived pro-inflammatory cytokines (IL-6, IL-8, IL-15, CCL5, IL-1β) and nociceptive mediators (NGF and BDNF). Tumor cells also express NGF and BDNF, which activate macrophages to release pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and inflammatory regulators (NGF and Prostaglandins) which directly induce pain via binding to their receptors on sensory neurons. Finally, tumor cells interact with peri-neural and tumor-infiltrated mast cells, releasing mast cell derived proteases (trypsin and tryptase) which activate sensory neurons by binding to PAR-2 receptor, resulting in pain and upregulation of pain-related neuropeptides (CGRP and SP).

4. Conclusions

The current first-line treatment for CIBP is still opioids. Opioids are somewhat effective, but can have serious side effects, and abuse and addiction of these analgesics are a growing concern. Therefore, more effective and safer alternative treatment options for CIBP are urgently needed. Unfortunately, there is currently no better treatment for CIBP than opioids, when administered alone. However, several lines of evidence suggest that the combination of non-opioid analgesics with opioids provides synergistic or additive analgesic effects that can lead to decreased opioid dose. In the case of CIBP, the combination treatment of PAR-2 antagonists with morphine can allow the use of significantly lower doses of morphine (1 mg/kg) while maintaining the same levels of analgesia as single high doses of morphine (3 or 10 mg/kg) in a CIBP rat model [140]. Although it might be difficult to immediately
replace opioids with other treatment modalities, we still need to continue efforts to reduce opioid use by discovering potential therapeutic targets for CIBP within the bone marrow microenvironment.

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