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Review Article

Sensory nerves: A driver of the vicious cycle in bone metastasis?

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HIGHLIGHTS

- Bone is densely innervated by sensory nerves.
- Excitation of the sensory nerve nociceptor TRPV1 induces cancer-associated bone pain and promotes cancer progression in bone.
- Sensory nerve TRPV1 is a therapeutic target for cancer progression and associated bone pain.

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ABSTRACT

Bone is one of the preferential target organs of cancer metastasis. Bone metastasis is associated with various complications, of which bone pain is most common and debilitating. The cancer-associated bone pain (CABP) is induced as a consequence of increased neurogenesis, reprogramming and axonogenesis of sensory nerves (SNs) in harmony with sensitization and excitation of SNs in response to the tumor microenvironment created in bone. Importantly, CABP is associated with increased mortality, of which precise cellular and molecular mechanism remains poorly understood. Bone is densely innervated by autonomic nerves (ANs) (sympathetic and parasympathetic nerves) and SNs. Recent studies have shown that the nerves innervating the tumor microenvironment establish intimate communications with tumors, producing various stimuli for tumors to progress and disseminate.

In this review, our current understanding of the role of SNs innervating bone in the pathophysiology of CABP will be overviewed. Then the hypothesis that SNs facilitate cancer progression in bone will be discussed in conjunction with our recent findings that SNs play an important role not only in the induction of CABP but also the progression of bone metastasis using a preclinical model of CABP. It is suggested that SNs are a critical component of the bone microenvironment that drives the vicious cycle between bone and cancer to progress bone metastasis. Suppression of the activity of bone-innervating SNs may have potential therapeutic effects on the progression of bone metastasis and induction of CABP.

Abbreviations: a3V-H$^+$-ATPase, a3 isoform vacuolar proton pump; AN, autonomic nerve; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; BMSC, bone marrow stromal cells; CABP, cancer-associated bone pain; CAP, cancer-associated pain; CCL2, C–C motif chemokine 2; CGRP, calcitonin gene-related peptide; CALCRL, calcitonin receptor-like receptor; CNS, central nervous system; COX, cyclooxygenase; CRPC, castration-resistant prostate cancer; CXCL1, C-X-C Motif Chemokine Ligand 1; CXCL2, C-X-C Motif Chemokine Ligand 2; DRG, dorsal root ganglion; G-CSF, granulocyte colony-stimulating factor; GDNF, glial-derived neurotrophic factor; HSCs, hematopoietic stem cells; HGF, hepatocyte growth factor; HIF-1α, hypoxia-inducible transcription factor-1α; HMGB-1, high mobility group box-1; HUVECs, human umbilical vein endothelial cells; IL-1β, interleukin 1β; MM, multiple myeloma; MOR, mu-opioid receptor; NE, norepinephrine; NGF, nerve growth factor; NI, nerve invasion; NSAIDs, nonsteroidal anti-inflammatory drugs; NPY, neuropeptide Y; OA, osteoarthritis; OPG, osteoprotegerin; PACAP, pituitary adenylate cyclase-activating peptide; CREB, cyclic AMP-responsive element-binding protein; PDAC, pancreatic ductal adenocarcinoma; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; ERK1/2, extracellular receptor kinase ½; PGE2, prostaglandin E2; PNI, perineural invasion; PanIN, pancreatic intraepithelial neoplasia; RAGE, receptor for advanced glycation end products; RANKL, receptor activator of NF-κB ligand; RAMP1, receptor activity modifying protein 1; RTX, resiniferatoxin; SN, sensory nerves; SP, substance P; SRE, skeletal-related event; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α; TrkA, tyrosine kinase receptor type 1; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide.

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1. Introduction

Majority of patients with advanced cancer suffer from cancer-associated pain (CAP), which is one of the most common and feared cancer symptoms [1] and has substantial psychological and physical impacts on cancer patients, insulting quality of life and increasing morbidity and mortality [2]. A most common CAP seen in patients with advanced cancer is cancer-associated bone pain (CABP) [3–5]. von Moos et al. [5] reported that more than 80% of patients with metastatic cancer show CABP, whereas only 23%, 11% and 8% of the same population of patients demonstrate pleuritic, neural and visceral pain, respectively. Therefore, determination of the mechanism of CABP in relation to cancer progression is important to design effective therapeutic interventions to improve outcomes of cancer patients.

It has been well-recognized that survival of cancer patients with CAP/CABP is poor compared to those without CAP/CABP [6,7]. However, the mechanism by which CAP/CABP is associated with poor survival remains unclear. CAP/CABP are basically induced as a consequence of sensitization and excitation in conjunction with electrophysiological changes of peripheral primary sensory nerves (SNs) in response to local noxious stimuli that are produced in the tumor microenvironment [8–10]. In turn, excited SNs secrete neurotransmitters and neurotrophins that may modulate cancer aggressiveness. In support of this notion, it has been demonstrated that metastasis of prostate cancer is decreased following spinal cord injury, suggesting an important role of nerves in prostate cancer progression [11–12]. Further, the association between stress-related psychosocial factors and higher lung cancer incidence in healthy populations or poorer survival in cancer patients implicates autonomic nerves (ANs) in cancer development and progression [13]. In addition, mounting clinical and preclinical studies have reported that nerves innervating the tumor microenvironment promote tumor growth and dissemination [14–19], and that surgical or chemical denervation (elimination of nerves) causes cancer regression [18–19]. It therefore seems likely that the nerves innervating tumor in turn facilitate cancer progression and dissemination via establishing reciprocal communications with tumor. However, most of these studies focused on the role of ANs in cancer progression, while understanding of the effects of SNs is limited [20], despite that SNs are a primary critical player in the pathophysiology of CABP, a most common and devastating complication of bone metastasis.

In this review, we will 1) briefly describe the role of SNs in the pathophysiology of CABP, 2) discuss the effects of SNs on cancer progression in bone, and 3) presents our recent findings that suggest that SNs facilitate not only CABP induction but also cancer progression in bone and cancer dissemination from bone.

2. SN innervation in bone

Understanding of innervation of bone is important to explore the effects of peripheral nerves on cancer progression in bone. For details of bone innervation, please refer to recent outstanding review articles [21–22]. It has been reported that bone is innervated by extensive networks of both ANs and SNs [22–26]. Notably, 96% of nerves innervating the bone marrow are AN fibers and 4% SN fibers at the metaphysis [21]. Therefore, ANs are dominant nerves innervating the bone marrow in which metastatic cancer cells preferentially colonize, and thus have been implicated in cancer progression in the bone marrow [14–19]. On the other hand, our understanding of the contributions of SNs to cancer progression in bone is limited despite SNs play a central role in the pathophysiology of CABP. SNs are found to innervate the periosteum, cortical bone, trabecular bone and bone marrow [24],and regulate bone development, remodeling, metabolism, and repair to maintain bone homeostasis [22,24,27,28]. Notably, the density of calcitonin gene-related peptide (CGRP)-positive SNs is greater than that of tyrosine hydroxylase-positive ANs in the periosteum [24,29], providing the anatomical basis to the classical concept that CABP is essentially evoked following periosteal spread of cancer [30–31]. Another notable relevant feature of SN innervation in bone is that SNs distribute in the periosteum, bone marrow and cortical bone with a density ratio of 100:2:0.1 [9]. However, since the total area of the bone marrow is broader than that of the periosteum, the total number of SNs in the bone marrow is greater than that in the periosteum [24], consistent with the observation that patients with cancer that evidently colonizes only within the bone marrow cavity but not spread out on the periosteum often complain CABP. Recently, Lorenz et al. [32] created the detailed map of AN and SN innervation in and around bone and defined three distinct patterns of periosteal innervation that can be implicated in bone pain, fracture repair and bone homeostasis. It is expected that this comprehensive map will be of great help for further studies of the pathophysiology of CABP and the effects of SNs innervating bone on cancer progression.

3. Pathophysiology of CABP

To investigate the effects of excited SNs on cancer progression in bone, it is mandatory to understand the pathophysiology of CABP. The classical mechanism of CABP includes, 1) direct injury or damage of SN fibers by cancer invasion; 2) activation of periosteal SNs by mechanical stretching of the periosteum due to the expansion of cancer in the bone marrow cavity; and 3) hyperinnervation of SNs and neuroma formation in response to the presence of tumor [8,10,30,33]. Recent data showed that CABP is evoked in association with pathological neurogenesis, reprogramming and axonogenesis of SNs in concert with sensitization (a reduction in the threshold and an increase in the magnitude of a response to noxious stimulation) and excitation of SNs by a variety of neurotrophic factors, cytokines and chemokines that are produced in the tumor microenvironment in bone [8,10,33]. We and others found that cancer progression in bone promotes pathological sprouting and excitability of bone-innervating SNs, thereby eliciting CABP [34–37]. Thus, CABP is fundamentally a consequence of sensitization and excitation of SNs innervating bone. In fact, recent studies revealed that pain is initiated following sensitization and excitation of primary afferent SN receptors called “nociceptors” such as transient receptor potential vanilloid-1 (TRPV1) and acid-sensing ion channels (ASICs) [38]. These SN nociceptors perceive peripheral local noxious stimuli, get excited and convert the stimuli into electrochemical signals, which are subsequently transmitted to the spinal cord (secondary afferent neuron) via SN dorsal root ganglia (DRGs, primary afferent neuron), central nervous system (CNS) and brain [39] (Fig. 1). DRG is the cell body of SN fibers and plays as a gate way of peripheral noxious signals to CNS [40]. Thus, CABP is induced through an up-regulation of nociceptor activity of SNs innervating bone during the progression of bone metastasis.

3.1. Acidic microenvironment of bone metastasis

In the pathophysiology of CABP, bone provides a unique environment that facilitates CABP induction. Metastatic cancer cells develop a characteristic tumor microenvironment and metabolic activity in bone that is by nature hypoxic (oxygen concentration <1%) [41]. Under the hypoxic bone microenvironment, the expression of hypoxia-inducible transcription factor-1α (HIF-1α) is up-regulated to increase the secretion of protons and lactate via the

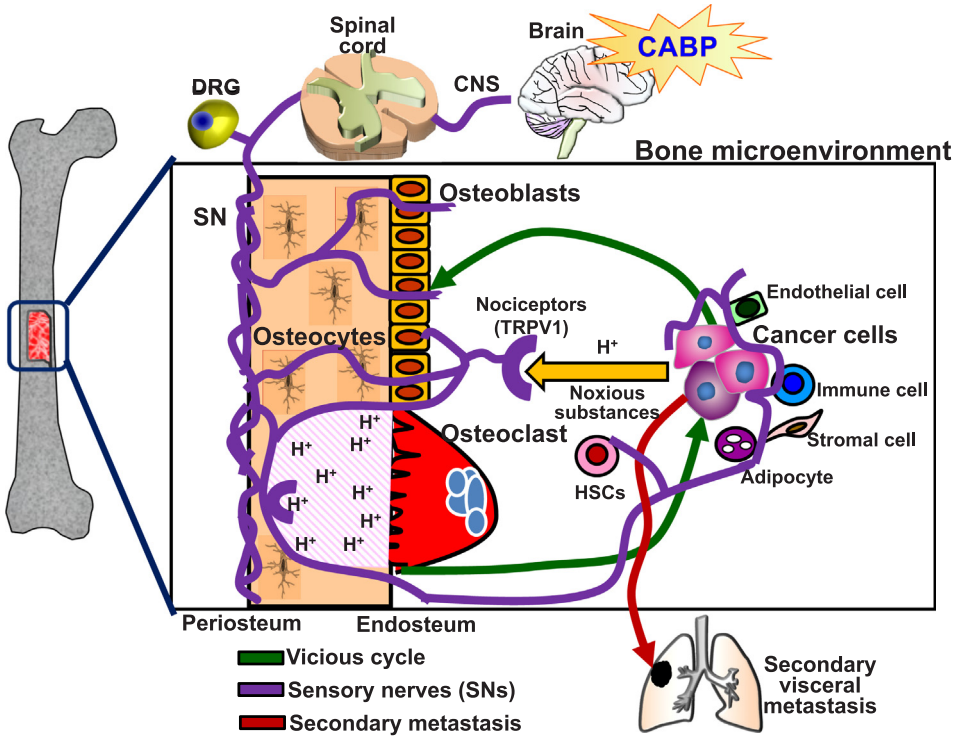

Fig. 1. SNs drive the vicious cycle of bone metastasis. Metastatic cancer cells releases bone-modifying factors for bone and bone releases bone-derived growth factors, cytokines and chemokines for metastatic cancer cells, establishing the vicious cycle (green). Tumor microenvironment in bone produces a variety of noxious substances such as protons (H^+) that hyper-excite sensory nerves (SNs) (purple), which widely distribute periosteal surfaces, cortical bone and bone marrow in bone to evoke cancer-associated bone pain (CABP) via activation of the SN nociceptors such as transient receptor potential vanilloid-1 (TRPV1). The SN nociceptors instantly convert the noxious stimuli into electrochemical signals and transmit them to brain through dorsal root ganglion (DRG) (primary afferent neuron), spinal cord (secondary afferent neuron) and central nervous system (CNS) to elicit CABP. Bone-resorbing-osteoclasts also secrete large amounts of H^+ to degrade bone minerals, aggravating local acidosis and thereby enhancing SN hyper-excitation and CABP. Excited SNs in turn stimulate cancer colonization in bone and secondary metastasis from bone (brown) by producing tumor-stimulating factors, developing reciprocal crosstalk with metastatic cancer cells. Further, recent mounting studies uncover that SNs also interact with the cellular components of the bone microenvironment including osteoblasts/stromal cells, osteocytes, immune cells, hematopoietic stem cells (HSCs), endothelial cells and adipocytes, which support and facilitate metastatic cancer progression in bone. SNs are a late-coming driver of the vicious cycle of bone metastasis and may be a unique therapeutic target for bone metastasis and CABP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plasma membrane proton/lactate transporters of cancer cells [42], making bone microenvironment acidic. Further, to survive and proliferate in the hostile hypoxic bone microenvironment, oxygen-independent aerobic glycolysis is promoted through Warburg effect [42] in cancer cells, leading to increased concentrations of cytoplasmic protons and lactate, which subsequently are excreted out of cancer cells, thereby generating an extracellular acidic tumor microenvironment. In addition, osteoclasts that are increased in the presence of metastatic cancer cells show elevated secretion of protons to degrade bone minerals [43–46]. Thus, protons secreted by bone-resorbing osteoclasts and bone-colonizing cancer cells together create aggravated acidic tumor microenvironment in bone.

3.2. TRPV1 perception of acidic microenvironment of bone metastasis

Protons are a potent inducer of pain [39]. It is therefore most likely that the acidic microenvironment of bone metastasis contributes to the induction of CABP. One of the SN nociceptors that are activated following perceiving protons is TRPV1. TRPV1 consists of 838 amino acids with a molecular size of 95KD and is almost exclusively expressed on the small unmyelinated c-fiber nociceptive afferent SNs [47]. TRPV1 expression is also detected in the gastrointestinal tract, and the epithelium of the bladder and skin [47]. Of interest, osteoblasts and osteoclasts are found to express TRPV1, although its precise function needs to be determined [48]. TRPV1 is a nonselective cation channel highly permeable to Ca^{2+} and activated by capsaicin, acid (<pH 6.0), and noxious heat (greater than43 °C) and pro-inflammatory mediators such as prostaglandins, bradykinin, ATP, and 5-hydroxytryptamine, and nerve growth factor (NGF). Notably, TRPV1 is sensitized to capsaicin, heat and inflammatory mediators under mild acidosis (pH 6 to 7), and conversely, inflammatory mediators can sensitize TRPV1 to protons [49]. Further, Ca^{2+} influx via the TRPV1 pore evokes membrane depolarization, followed by the activation of voltage-gated sodium channels and the generation of action potentials, increasing nociception [47]. In addition, the Ca^{2+} influx also propagates cytoplasmic signaling pathways including protein kinase C, protein kinase A, calmodulin, phosphatidylinositol 4, 5-bisphosphate [47], leading to the enhancement of pain. Since the bone microenvironment is constitutively rich in Ca^{2+}, CABP elicited following TRPV1 activation and SN excitation in bone may be more sustained and debilitating than CAP at non-bone sites.

Of particular interest, Riera et al. [49] described that $TRPV1^{-/-}$ mice live longer and exhibit more youthful metabolic profile at old age than wild-type mice of the same age. It is intriguing to determine whether the loss or suppression of TRPV1-mediated SN excitation and sensitization caused by noxious stimuli is beneficial to aging and metabolic activity.

We reported that inoculation of the JJN3 human myeloma cells in the bone marrow cavity of tibiae induced CABP and increased TRPV1 expression on $CGRP^+$ DRG SNs in mice [36]. Importantly, a single injection of a selective synthetic TRPV1 antagonist, SB366791, reduced SN excitation and CABP in these mice. Further, we found that excitation of DRG SNs innervating bone and CABP in $TRPV1^{-/-}$ mice intratibially injected with mouse Lewis lung cancer

cells were markedly decreased compared to those in wild-type mice [50]. In summary, these results suggest that CABP is associated with the excitation and sensitization of DRG SNs innervating bone following TRPV1 activation in response to the acidic bone microenvironment that is created by bone-colonizing cancer cells and bone-resorbing osteoclasts.

4. Survival of cancer patients with CAP/CABP

To support the hypothesis that CAP/CABP promotes cancer progression in clinical settings, determination of survival of cancer patients with or without CAP/CABP is informative and can serve as a surrogate for the evaluation of the effects of SNs on cancer progression. Previous systematic reviews reported that survival is significantly shorter in advanced cancer patients with CAP than those without CAP [6,51–52]. In particular, CAP was found as an independent prognostic factor for overall survival in advanced prostate cancer patients [6], suggesting that CAP accelerates cancer progression leading to shorter survival.

Similar to CAP, CABP is also an indicator for poor survival. Most men with castration-resistant prostate cancer (CRPC) suffer from CABP associated with bone metastasis, and patients with low pain scores show significantly longer median survival times, lower prostate-specific antigen levels and slower progression of bone metastasis than do those with high pain scores [7,53–54]. Further, CABP at diagnosis of skeletal metastasis was associated with increased skeletal-related events (SRE) and cause-specific death in breast cancer patients [55]. These results are in good agreement with the notion that excited SN leading to CABP increases cancer aggressiveness in bone, thereby decreasing survival of cancer patients.

5. Effects of pharmacological alleviation of CAP/CABP on survival

As another approach to determine the effects of CAP/CABP and excited SNs on cancer progression, evaluation of the effects of analgesic agents on cancer progression in patients with CAP/CABP is worthwhile.

5.1. Effects of bone-targeted agents on survival

5.1.1. Effects of bisphosphonates

It has been shown that bone-targeted agents, bisphosphonates and denosumab, which have analgesic effects on CABP [4,56] but no direct anti-proliferative effects on cancer, improved overall and progression-free survival of lung cancer patients with bone metastasis [57]. Similarly, beneficial effects of zoledronic acid on overall survival is reported in breast cancer patients with advanced bone metastasis [58] and multiple myeloma patients [59], and adjuvant bisphosphonate therapy significantly improved overall survival in patients with metastatic castration-sensitive prostate cancer [60].

5.1.2. Effects of denosumab

Denosumab is also shown to significantly increase disease-free survival in patients with postmenopausal hormone receptor-positive early breast cancer receiving aromatase inhibitor therapy in the ABCSG-18 study [61], although it should be noted that the D-CARE study found no beneficial effects of denosumab on bone metastasis-free survival in women with high risk early breast cancer [62]. In addition, overall survival in patients with metastatic lung cancer [63] and bone metastasis-free survival in CRPC patients [64] were increased by denosumab treatment compared to zoledronic acid.

5.1.3. Effects of radium-223

Radium-223, which is the first recently-approved α-particle-emitting radiopharmaceutical for the treatment of CRPC with bone metastases and no evidence of visceral metastases [65], showed beneficial effects on overall survival and CABP in CRPC patients [65].

These results together show that suppression of CABP and decrease in cancer progression in bone and mortality are related, suggesting that CABP facilitates cancer progression.

5.2. Effects of non-steroidal anti-inflammatory drugs

Since inflammation is one of the hallmarks of cancer [66], the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on cancer progression and CABP are of interest. Earlier studies reported that low-dose aspirin given to cancer patients to manage CAP decreased metastasis and mortality [67]. Further, aspirin and other NSAIDs and cyclooxygenase-2 (COX-2) inhibitors have been shown to reduce a risk of lung, breast and esophageal cancer and inhibit the progression of colorectal cancer [67], suggesting that inflammatory CAP is associated with cancer progression and metastasis. However, since these agents inhibit prostaglandin-mediated tumor angiogenesis and induce apoptosis in cancer cells, contribution of alleviation of inflammatory CAP by NSAIDs to the inhibition of cancer progression and metastasis needs to be further investigated. Effects of NSAIDs on CABP are unclear. NSAIDs are not aggressively given to patients with CABP because of the lack of robust clinical evidence for the effectiveness on CABP.

5.3. Effects of opioids

Morphine/opioids, which are mainstay analgesic agents for the treatment of CAP/CABP, have been shown to have anti-cancer effects in preclinical studies, while most clinical studies describe pro-cancer effects of opioids, demonstrating conflicting results [68]. Analgesic effects of opioids are mediated via mu-opioid receptors (MORs) in the CNS, however, human prostate and lung cancer and endothelial cells also express MORs [68]. Thus, the effects of opioids on cancer progression are likely mediated via MORs and it appears unlikely that SN-related mechanisms are involved.

6. Perineural/nerve invasion (PNI/NI) and cancer

One relevant pathological process consistent with the hypothesis that CAP/CABP facilitates cancer progression is perineural invasion (PNI) or nerve invasion (NI). PNI or NI is defined as cancer cell invasion of the surrounding nerves or into the epineurial, perineurial and endoneurial spaces of the neuronal sheath, resulting in dense nerve innervation in the tumor microenvironment, respectively [19,69–70]. PNI/NI is commonly seen in cancers initiated in densely innervated organs such as pancreatic, head and neck, prostate, and colorectal, biliary tract and stomach cancer [19,69–70]. Cancers with PNI/NI disseminate along nerve fibers in the tumor, and concurrently promotes axonogenesis, reprogramming and neurogenesis of these nerves, which in turn facilitates cancer growth via the development of cancer-nerve cross-talk [19]. Liebl et al. [71] studied the prevalence of NI and its impact on survival by analyzing 16,000 HE-stained sections from 2,050 patients with various types of cancers, and found that NI prevalence is significantly associated with reduced survival. Likewise, other studies also reported the association of PNI/NI with CAP, metastasis, recurrence, morbidity and mortality, proposing that PNI/NI is an independent prognostic factor [19,69–70].

7. PNI and bone metastasis

7.1. PNI and bone metastasis of prostate cancer

PNI is demonstrated in bone metastasis as well. A clinicopathological study reported that PNI is detected in 449 (46%) out of 976 patients with prostate cancer by diagnostic biopsies and importantly prostate cancer with PNI is significantly associated with increased development of bone metastasis compared to prostate cancer without PNI over 10 year follow-up interval [72]. Further, it is also described that PNI detected in the biopsy specimens is the most reliable predictive histopathological feature for bone metastasis, increasing the risk of bone metastasis 11-fold [73]. These authors therefore propose that patients with PNI-positive prostate cancer should be closely followed up for future development of bone metastasis.

7.2. PNI and bone metastasis of breast cancer

Advanced breast cancers, which preferentially spread to bone, are also associated with PNI [74–75]. However, disease-free survival of PNI-positive breast cancer patients was not different from that of PNI-negative breast cancer patients. Further, in the breast, benign lesions such as atypical ductal hyperplasia and ductal carcinoma in situ are also associated with PNI [76]. Thus, prognostic importance of PNI in breast cancer remains to be determined.

7.3. PNI and bone metastasis of other type of cancers

Of interest, it is also shown that PNI is increased in the metastatic tumors in bone compared to the primary tumors in hepatocellular carcinoma [77], and that increased PNI in the primary colorectal cancer is associated with increased bone metastasis [78]. These results suggest that nerves may modulate bone metastasis of cancers with PNI that in nature have low predilection for spreading to bone. It is intriguing to determine if SNs confer the capacity to disseminate to bone to low bone-metastatic cancers through reciprocal cross-talk.

8. Interactions of nerves with cellular components of bone metastasis

8.1. Autonomic nerves and cancer

The role of autonomic nerves (ANs), which are composed of sympathetic (adrenergic) and parasympathetic (cholinergic) nerves, in cancer progression is briefly described here. For details, please refer to several excellent review articles [15–19].

8.1.1. Prostate cancer and ANs

A pioneering work of Magnon et al. [14] reported that catecholamine and acetylcholine secreted by sympathetic and parasympathetic nerves increase prostate cancer growth at early stages and bone metastasis at late stages, respectively. Further, they demonstrated that the density of AN innervation is significantly correlated with the Gleason score and cancer aggressiveness in prostate cancer patients. This study is the first to show that ANs are a critical component of the tumor microenvironment that regulates prostate cancer growth and bone metastasis.

8.1.2. Breast cancer and ANs

It is shown that activation of the sympathetic nervous system by chronic immobilization stress increased osteolytic bone metastases of breast cancer via upregulation of receptor activator of NF-κB ligand (RANKL) expression in osteoblasts. Importantly, the β-blocker propranolol, as well as osteoprotegerin (OPG), a natural inhibitor of RANKL, decreased bone metastases [79]. Conversely, stimulation of the β2-adrenergic receptors in osteoblasts promotes vascular endothelial growth factor (VEGF)-dependent neoangiogenesis, increasing bone vascular density and bone metastasis [80]. These results collectively indicate that sympathetic nerves and β-adrenergic receptors of osteoblasts facilitate breast cancer metastasis to bone in harmony with angiogenesis. Using a xenograft mouse model in which AN innervation in human breast cancer was genetically manipulated and a rat model of chemically-induced breast cancer, Kamiya et al. [81] showed that breast cancer growth and progression are promoted by sympathetic nerve stimulation and decreased by parasympathetic nerve stimulation. Further, they found that increased sympathetic and decreased parasympathetic nerve density in tumors are associated with poor clinical outcomes in breast cancer patients, suggesting that ANs regulate breast cancer aggressiveness.

8.1.3. Multiple myeloma and ANs

Of note, recent clinical studies have demonstrated that anti-adrenergic β-blockers decrease overall mortality in patients with multiple myeloma (MM), suggesting that sympathetic nerves promote the progression of MM [82]. Further, survival of MM patients with increased expression of cholinergic receptor mRNA is decreased, suggesting that parasympathetic input stimulates MM progression. Thus, ANs may regulate hematologic cancer as well as solid cancer.

8.2. SNS and cancer

Nerves and blood vessels develop under tight evolutionary relationship [83]. Mukouyama et al. [84] reported that SNs stimulate arteriogenesis *in vivo* and increase arterial marker expression in embryonic endothelial cells *in vitro*. These authors then demonstrated that peripheral SNs play as a template for the patterning of blood vessel branching and arterial differentiation via local secretion of VEGF, suggesting that angiogenesis and neurogenesis are regulated by the same principle and mechanism. Nerves and blood vessels exchange intimate and harmonious communications to regulate diverse physiological and pathological processes [85]. Since angiogenesis, a hallmark of cancer, is one of the most critical processes for the development, progression and dissemination of cancer [66], it is reasonable to assume that neurogenesis controls cancer aggressiveness as well.

8.2.1. SNS and pancreatic cancer

Three independent studies described an essential role of SNs in the development of pancreatic cancer. Bai et al. [86] determined the effects of chemical denervation of SNs by capsaicin on the development of chronic pancreatitis and pancreatic intraepithelial neoplasia (PanIN) in the mutant *Kras*-driven and caerulein-induced pancreatitis-associated carcinogenesis model in *LSL-KrasG12D/Pdx1-Cre* mice. They found that SN denervation by capsaicin significantly decreases the progression of chronic pancreatitis and PanIN-1 to high-grade PanIN-2 and -3. Using the same animal model, Saloman et al. [87] showed that SN ablation by capsaicin delays PanIN formation and prolongs survival of tumor-bearing mice compared to vehicle-treated control mice, and indicated that SNs innervating the tumor facilitate the initiation and progression of pancreatic ductal adenocarcinoma (PDAC). Sinha et al. [88] found that DRG SNs stimulate PDAC cell proliferation in co-cultures and that denervation of SNs by resiniferatoxin (RTX) slows down PanIN progression to PDAC in parallel with a decrease in SN density in the pancreas in KPC^{Pdx1} mice. Taken together, these results strongly suggest that SNs promote pancreatic carcinogenesis via a reciprocal cross-talk between the pancreas and SNs. They

also imply that capsaicin and RTX may be promising agents for suppressing chronic pancreatitis and pancreatic carcinogenesis through targeting SNs.

8.3. SNS and osteoclasts

Osteoclasts play a central role in the pathophysiology of bone metastasis via establishing so-called "the vicious cycle" with cancer cells [43–46]. Reduction of CABP in patients with bone metastasis of solid cancers [4–5,56] and MM bone disease [89] by the treatment with the specific inhibitors of osteoclasts, such as bisphosphonates and denosumab, indicates that osteoclasts are responsible for inducing CABP. Consistent with these clinical results, several preclinical studies including ours also reported that inhibition of osteoclastic bone resorption by OPG [90] and zoledronic acid [36,91] reduced CABP as well. There are comprehensive reviews for details of the effects of osteoclasts on SNs and CABP [22,92].

8.3.1. Role of protons

Bone-resorbing osteoclasts secrete protons via the a3 isoform vacuolar proton pumps (a3V-H^+-ATPase) on plasma membrane to degrade bone minerals [93], acidifying the resorption lacunae to the pH value ~4.5 [94]. We showed that $CGRP^+$ SNs are running in the close proximity of the osteoclast resorption lacunae or contacting osteoclasts in cancer-colonized bone, and that inhibition of proton release by osteoclasts using the selective V-H^+-ATPase inhibitor, bafilomycin A1, blocked the development of acidic microenvironment in cancer-colonized bone and significantly reduced CABP in a MM animal model [36]. Of interest, we found that the selective p-type proton pump inhibitor, rabeprazole, which is prescribed for gastric pain [95], did not reduce CABP (unpublished data), indicating a specific contribution of the V-H^+-ATPase to CABP. These results indicate that bone-resorbing osteoclasts activate pH-sensitive nociceptors of SNs innervating bone by releasing the noxious protons via the plasma membrane V-H^+-ATPase to evoke CABP, in collaboration with the protons derived from metastatic cancer cells.

8.3.2. Role of Netrin-1

Netrin-1 has been known to regulate the development of nervous system as an axon guidance molecule [96]. Recent studies revealed that Netrin-1 also modulates tumorigenesis and metastasis of various types of cancers [96]. More recently, it is shown that osteoclasts secrete Netrin-1 to promote axonogenesis of SNs innervating subchondral bone in mice with osteoarthritis (OA), and inhibition of osteoclastogenesis decreased sprouting of SNs in subchondral bone, hyper-excitability of DRG SNs and pain behaviors in OA mice [97]. Further, deletion of Netrin-1 in osteoclasts reduced OA pain. These authors also reported that knockout of Netrin-1 in osteoclasts abrogates SN innervation into porous endplates and PGE2-induced spinal pain [98]. Thus, osteoclasts modulate SN excitability by releasing Netrin-1.

8.3.3. Role of PD-1

Programmed cell death protein 1 (PD-1) of activated T cells bound with its ligand programmed death-ligand 1 (PD-L1) is a co-inhibitory checkpoint signal that regulates T cell activity [99]. Many types of cancers show increased expression of PD-L1, forming PD-L1/PD-1 complex to evade T cell immunity. Interestingly, Wang et al. determined the role of PD-1 in CABP associated with mouse Lewis lung cancer and demonstrated that the binding of soluble tumor PD-L1 to PD-1 of pre-osteoclasts leads to increased osteoclastogenesis, bone destruction and CABP induction [100]. Further, they showed that anti-PD-1 monoclonal antibody, nivolumab, reduced CABP and bone destruction via inhibiting osteoclastogenesis. Thus, it is suggested that PD-L1/PD-1 axis is a novel molecular target in the treatment of bone metastasis and CABP. Effects of nivolumab on bone metastasis and CABP in cancer patients need to be investigated.

8.3.4. Role of neurotransmitters and neurotrophins released from SNs

SNs in turn may regulate osteoclast differentiation and function. Since true synapses are likely absent in bone, peripheral SNs release a variety of neurotransmitters and neurotrophins into the extracellular space through non-synaptic vesicular fusion in axon varicosities [22]. These neurotransmitters and neurotrophins then diffuse to bind to cognitive receptors on neighboring osteoclasts at local sites [22]. The SN neurotransmitters such as CGRP, substance P (SP), vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating peptide, and neurotrophins such as NGF, brain-derived neurotrophic factor, semaphorin 3A and PGE2 have been implicated in the modulation of the differentiation and function of osteoclasts. Details of the effects of these factors on osteoclasts are found elsewhere [22,28].

8.4. SNs and osteoblasts/stromal cells

Osteoblasts are also under the influences of neurotransmitters and neurotrophins released from peripheral SNs innervating bone to maintain bone homeostasis [22]. However, convincing data that support that osteoblasts participate in the pathophysiology of CABP are limited. As described above, osteoclasts play a central role in the pathophysiology of CABP and osteoblasts/stromal cells are the cells that control the differentiation and function of osteoclasts via RANKL expression. Therefore, it is likely that osteoblasts/stromal cells at least indirectly regulate SN excitation and CABP induction via osteoclasts. Further, as a piece of *in vitro* evidence for direct regulation of SN sprouting by osteoblasts, Neto et al. [101] established and characterized a co-culture system of osteoblasts and DRG SNs mimicking a bone microenvironment in which SN fibers innervate. Using this compartmentalized microfluidic platform system, they found that DRG SNs increase and extend CGRP-positive axons toward osteoblasts, suggesting that osteoblasts release neurotrophic axon guidance molecules for SNs. These *in vitro* results suggest that osteoblasts may directly control SN activity and CABP induction. In agreement with these *in vitro* data, ultrastructural examination of the periosteal cellular layer and the mineralizing osteo-chondral junction revealed that peptidergic SN fibers contact osteoblasts [102], demonstrating intimate spatial relationship between SNs and osteoblasts.

As another piece of evidence for a direct effect of osteoblasts/stromal cells on SN activity, bone marrow stromal cells (BMSCs) were found to have potent analgesic effects in animal models of inflammatory pain, neuropathic pain, and CABP by inhibiting monocyte infiltration and glial activation, and cytokine/chemokine production in the DRG and spinal cord via secretion of transforming growth factor β (TGFβ) [103].

Of interest, it is shown that the progenitor cells present within the endoneurial compartment of SNs are the major source of osteogenic precursor cells detected in the heterotopic ossification site in mice [104]. This observation may raise the possibility that cells of osteoblast lineage may be involved in SN excitation and CABP induction following re-differentiation into endoneurial SN progenitors depending on the surrounding environment.

Below, some recent topics related to the role of osteoblast in CABP induction are presented.

8.4.1. Role of semaphorins

Semaphorins were initially identified as an axon guidance molecule [105]. However, later it was shown that semaphorins regulate the development and maintenance of many organs and

tissues including nervous, cardiovascular, immune, endocrine, hepatic, renal, reproductive, respiratory and musculoskeletal systems, and cancer through their receptor plexin.

Fukuda et al. [26] reported that SN-derived semaphorin 3A regulates bone remodeling indirectly through the modulation of SN innervation in bone rather than through the direct effects on osteoblasts in mice. As a clinically relevant case to this finding, familial dysautonomia patients, who are characterized by the loss of unmyelinated SN axons, are known to have a predisposition to osteoporosis, reconfirming an important role of SNs in bone homeostasis maintained by osteoblasts [106].

8.4.2. Role of PGE2

Chen et al. [107] showed that PGE2 released from osteoblasts activates EP4 receptors of SNs to increase bone formation via inhibiting adrenergic sympathetic activity, proposing that SNs sense bone mass through local concentrations of PGE2. These authors subsequently showed that the differentiation of bone marrow mesenchymal stromal cells into osteoblast lineage, but not adipocyte lineage, is regulated by PGE2/EP4 axis in SNs [108]. These results suggest that bone homeostasis is maintained by the harmonious functional balance between osteoblasts and ANs/SNs innervating bone.

8.5. SNS and osteocytes

Osteocytes are terminally-differentiated osteoblasts and most abundant cells in bone, playing multifunctional roles in the regulation of osteoblasts and osteoclasts to maintain bone and mineral homeostasis [109]. Recently, data are emerging that osteocytes interact with metastatic cancer cells to progress bone metastasis, suggesting that osteocytes are also an important component of the bone microenvironment that participates in the regulation of the vicious cycle of bone metastasis [110]. Although the role of osteocytes in SN excitation and CABP induction during the progression of bone metastasis is currently unknown, morphological and anatomical features of osteocytes suggest that osteocytes may be involved in the pathophysiology of CABP induction. Osteocytes are embedded in mineralized bone extending dendritic processes, which resemble SN axons, to communicate with other osteocytes. Meanwhile SNs that enter bone at the periosteal surfaces densely innervate cortical bone where numerous osteocytes exist extending the networks of dendritic processes. Therefore, osteocytes and SNs may have chances to physically interact via dendritic processes and axons, which may excite SNs and evoke CABP, and in turn change metabolic activity and functions of osteocytes.

8.6. SNS and immune cells

Lines of recent studies support the concept that in neuroinflammation in the CNS and PNS, neuronal functions are influenced by associated infiltrating satellite and immune cells, such as astrocytes, microglia, macrophages, monocytes, neutrophils, T cells and mast cells [111]. Immune cells at peripheral SN terminals and within the spinal cord release mediators that control mechanical and thermal sensitivity of SN nociceptors to modulate SN excitation. These mediators are, for example, pro-inflammatory cytokines (tumor necrosis factor, interleukin-1β (IL-1β), IL-6 and IL-17), chemokines (C–C motif chemokine 2: CCL2, and C-X-C Motif Chemokine Ligand 1: CXCL1), NGF, PGE2, serotonin, and histamine that act on receptors expressed on peripheral SN axon terminals including cytokine receptors, chemokine receptors, G-protein-coupled receptors, and tyrosine kinase receptor type 1 (TrkA). In turn, SN nociceptors release neuropeptides and neurotransmitters from SN terminals that regulate vascular, innate, and adaptive immune cell responses [112]. Thus, it seems likely that SNs and immune cells develop crosstalk to modulate CABP. In fact, as described above, PD-L1/PD-1 axis, which is a check point signal for T cells, is implicated in the pathophysiology of CABP associated with osteoclasts. It is expected that involvement of additional immune cells and novel molecules will be identified in CABP induction and progression of cancer colonization in bone.

8.7. SNS and hematopoietic stem cells

Hematopoietic stem cells (HSCs) give rise to bone-resorbing osteoclasts, which play an important role in the pathophysiology of SN excitation and CABP. Therefore, HSCs likely possess the potential to modulate SN excitation and CABP induction during the differentiation to osteoclasts. Further, SNs innervating the bone marrow cavity may encounter and crosstalk with HSCs that reside in the endosteal niche in the bone marrow.

Recently, Gao et al. [113] have published an exciting article reporting the relationship of HSCs with nociceptive SNs in the bone marrow. They showed that SNs, which occupy approximately 80 % of the nerves in the bone marrow, promote HSC mobilization from bone marrow niches into blood vessels via increased secretion of the neurotransmitter CGRP. Interestingly, CGRP, of which secretion from SNs is increased in the presence of a natural component of chilli pepper capsaicin and granulocyte colony-stimulating factor (G-CSF), directly activates a receptor dimer comprising the calcitonin receptor-like receptor (CALCRL) and receptor activity modifying protein 1 (RAMP1) expressed on HSCs. Although sympathetic nerves are known to regulate the HSC niche [114], this is the first study that demonstrates that SNs directly regulate the mobilization of HSCs as well. These authors propose that SNs are a target in designing a strategy to improve the yield of HSCs for stem cell-based therapy such as autologous stem cell transplantation.

8.8. SNS and bone marrow adipocytes

Adipocytes, which occupy 15 to 40% of the bone marrow space in early adulthood and increasing up to 60% with age [46], are found to increase cancer cell growth and osteolysis in mice fed with high-fat diet and directly promote cancer cell proliferation and invasion *in vitro* [46]. In addition to their direct effects on cancer cells, bone marrow adipocytes also promote osteoclast differentiation and activity through adipocyte-derived RANKL, CXCL1, and CXCL2 and conversely suppress osteoblast differentiation by inhibiting bone morphogenic protein (BMP) signaling, thereby advancing osteolytic bone metastasis.

Regarding the relationship with SNs, recent studies have revealed that adipose tissue modulates systemic metabolism through secretion of endocrine and paracrine factors such as leptin and TNFα from adipocytes, immune cells and endothelial cells within adipose tissue to local SN fibers to transmit these adipose signals to CNS, which subsequently initiates output to other tissues [115]. Clinical observations that patients with lipedema suffer from pain and that liposuction significantly reduces pain [116] suggest that adipocytes may secrete noxious signals to SNs to evoke pain such as CABP. SN fibers in turn release neurotransmitters such as CGRP and SP to regulate the differentiation and function of adipocytes. Therefore, there is a reciprocal crosstalk between bone marrow adipocytes and SNs that likely plays an important role in driving the vicious cycle.

8.9. SNS and endothelial cells

As described above, nerves and blood vessels co-develop under tight evolutionary relationship through the same principle and mechanism [83]. To maintain the harmonious relationship, nerves

and blood vessels establish a crosstalk to share one pathway. For example, VEGF, in addition to stimulating angiogenesis, promotes nerve survival and axonal growth [117]. Further, endothelial cells release glial-derived neurotrophic factor (GDNF) to increase nerve survival and axonal growth. Neurotrophic NGF also stimulates angiogenesis, demonstrating that there are significant overlaps between these signaling pathways. Recently, Grasman and Kaplan [118] reported that human umbilical vein endothelial cells (HUVECs) stimulate axonal growth of DRG SNs via secreting brain-derived neurotrophic factor (BDNF). Thus, although *in vivo* results are not shown here, it is feasible to suggest that endothelial cells in the bone marrow are a member of the vicious cycle driver and may contribute to SN excitation and CABP induction in the tumor microenvironment.

9. Role of SNs in cancer progression in bone and secondary metastasis from bone

We have recently reported that CABP is induced following inoculation of 4T1 mouse breast cancer cells into the bone marrow cavity of tibiae of female BALB/c mice [37]. The 4T1 breast cancer cells aggressively grew with extensive osteolytic lesions in bone. Further, as tumor grew in bone, these mice exhibited progressive CABP evaluated by the hind-paw mechanical hypersensitivity and increased expression of phosphorylated extracellular receptor kinase 1/2 (pERK1/2) and cyclic AMP-responsive element-binding protein (pCREB), two molecular markers of pain, in DRG SNs innervating bone. Using this model, we demonstrated that high mobility group box 1 (HMGB1) secreted by 4T1 breast cancer cells induces CABP via binding to the receptor for advanced glycation end products (RAGE) expressed on SNs [37]. Further, we found 4T1 breast cancer cells in tibiae create an acidic microenvironment, and promote sprouting and excitation of SNs in tibiae with increased expression and activation TRPV1 of SNs, thereby evoking CABP. Blocking TRPV1 activation by a synthetic (SB366791) and a natural (iodo-resiniferatoxin) TRPV1 antagonist or genetic ablation of TRPV1, alleviated CABP and decreased 4T1 breast cancer colonization in tibiae and pulmonary metastasis from bone (manuscript in preparation). Notably, 4T1 breast cancer cells did not express TRPV1, suggesting that the effects of TRPV1 antagonists and genetic ablation seen here are unlikely due to direct effects on 4T1 breast cancer cells but likely through inhibition of activation of TRPV1 of SNs. From these results, it is proposed that activation of TRPV1 of SNs facilitates 4T1 breast cancer colonization in bone, driving the vicious cycle of bone metastasis.

We also found that 4T1 breast cancer cells colonized in bone subsequently disseminated to lung, which was inhibited by the treatment with TRPV1 antagonists. It has been known that cancer cells in bone metastasis often spread to distant visceral organs after a persistent period of dormancy, developing secondary metastasis that further increases the mortality of cancer patients [119–121]. However, the mechanism of secondary visceral metastasis from bone remains poorly understood. Our results suggest that excited SNs play a role in facilitating migration of bone metastatic cancer cells to next distant organs and may provide a clue for elucidating the mechanism of secondary metastasis from bone.[122].

10. Conclusion

Poor survival of cancer patients with CABP compared to those without CABP raises the notion that SN excitation has direct impacts on cancer aggressiveness. Here clinical and preclinical studies that are consistent with this notion are presented and discussed. However, the results of these studies virtually provide circumstantial but not compelling evidence for the biological effects of SNs on cancer progression and metastasis. Using a preclinical model of intratibial inoculation of mouse 4T1 breast cancer cells, we found that the activation of the SN nociceptor TRPV1 induces CABP and stimulates 4T1 breast cancer progression in bone and metastasis to lung. Importantly, suppression of SN excitation and resultant CABP by the administration of selective synthetic and natural TRPV1 antagonists decreases the progression and pulmonary metastasis of 4T1 breast cancer cells that express little TRPV1. These results provide experimental evidence for SN control of cancer progression and metastasis. Obviously, however, further preclinical experiments and clinical investigations are needed to prove the notion and elucidate the mechanism by which excited SN promotes cancer progression and metastasis. It is also intriguing to test newer TRPV1 antagonists without the thermoregulatory adverse effects for SN excitation and CABP induction and cancer progression in clinical settings. Along this line, several TRPV1 antagonists were on clinical trials (NCT00461682 for rectal pain, NCT00269022 for migraine, NCT01006304 for pain, NCT02712957 for OA pain, NCT00281684 for dental pain). In conclusion, SNs are an important component of the bone microenvironment that induces CABP and drives the vicious cycle of bone metastasis by promoting cancer progression in cooperation with osteoclasts, osteoblasts/stromal cells, osteocytes, immune cells, HSCs and yet-unidentified bone marrow cells (**Fig. 1**). SNs may have therapeutic potential for not only CABP but also cancer progression in bone and secondary dissemination from bone.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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