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The Role of Osteocytes in Pre-metastatic Niche Formation

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Abstract

Purpose of Review The formation of a pre-metastatic niche (PMN), in which primary cancer cells prime the distant site to be favorable to their engraftment and survival, may help explain the strong osteotropism observed in multiple cancers, such as breast and prostate. PMN formation, which includes extracellular matrix remodeling, increased angiogenesis and vascular permeability, enhanced bone marrow-derived cell recruitment and immune suppression, has mostly been described in soft tissues. In this review, we summarize current literature of PMN formation in bone. We also present evidence of a potential role for osteocytes to be the primary mediators of PMN development.

Recent Findings Osteocytes regulate the bone microenvironment in myriad ways beyond canonical bone tissue remodeling, including changes that contribute to PMN formation. Perilacunar tissue remodeling, which has been observed in both bone and non-bone metastatic cancers, is a potential mechanism by which osteocyte-cancer cell signaling stimulates changes to the bone microenvironment. Osteocytes also protect against endothelial permeability, including that induced by cancer cells, in a loading-mediated process. Finally, osteocytes are potent regulators of cells within the bone marrow, including progenitors and immune cells, and might be involved in this aspect of PMN formation.

Summary Osteocytes should be examined for their role in PMN formation.

Keywords Osteocyte · Bone metastasis · Pre-metastatic niche · Mechanical loading · Microenvironment

Introduction

Across multiple cancer types, bone is one of the most common sites of metastasis. In patients with metastatic breast or prostate cancers, the rate of skeletal metastasis is particularly high, with approximately 70% of patients experiencing secondary tumor formation in the bone [1]. The "seed and soil" hypothesis of cancer metastasis offers one explanation for such strong osteotropism that the skeleton is a desirable "soil" relative to other sites for disseminated cancer cells, the "seeds". The formation of a pre-metastatic niche (PMN) in bone tissue, in which primary cancer cells modify the distant site to favor engraftment and secondary tumor formation, may help explain why multiple types of cancer cells so readily engraft in the skeletal environment. PMN formation has been studied in soft tissues, including liver metastasis of pancreatic [2] and colorectal cancer [3] and lung metastasis of breast cancer [4], but the mechanisms of PMN formation in bone are less understood.

This focus on these soft tissues is understandable; both the lung and liver are frequent sites of metastasis and their essential, life-sustaining functions could easily be impaired by solid tumor formation. However, the importance of the skeleton in metastatic cancer should not be overlooked, as management of skeletal health is a crucial component of patient health. Skeletal metastases are common, and lesions associated with skeletal metastasis negatively impact patient mobility, cause painful skeletal-related events (SREs), and negatively impact patient prognosis [5]. Furthermore, bones can harbor dormant or quiescent tumor cells, increasing the likelihood of recurrence in patients long after the primary tumor has been eliminated [6]. Finally, skeletal metastasis has been correlated with decreased 5-year survival; while the National Cancer Institute SEER database currently indicates 5-year survival rate of 91% for all patients with breast cancer, the 5-year survival rate decreases to 13% for breast cancer patients who develop skeletal metastases

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[7]. Identifying contributing factors that promote skeletal metastasis and the subsequent development of preventative or treatment methods would certainly benefit patients.

The skeleton may be desirable due to (i) existing niches that cancer cells co-opt and (ii) its conduciveness to cancer cells creating their own PMNs, in addition to other factors (e.g., obesity) controlling bone dynamics that contribute to osteotropism. Several niches exist in bone that are critical for normal physiological processes. For example, niches for hematopoietic stem cells (HSCs) are located at the hard bone-bone marrow interface (the endosteal surface), primarily regulated by osteoblasts, and critical for functional blood cell formation (hematopoiesis), including homing, self-renewal, quiescence, and differentiation [8]. Disseminated prostate and breast cancer cells home to these niches using mechanisms similar to those of HSCs and compete with HSCs for occupancy therein [9, 10]. Cells in bone produce supporting growth factors and express adhesion molecules that may make it easy for the cancer cells to enter the marrow cavity and survive [11]. For example, osteopontin is a well-known matrix protein secreted by osteoblasts during bone formation, and it also facilitates better cell adhesion to tissues, including for tumor cells [12]. Another study demonstrated that breast cancer cells predominantly resided in niches with active osteogenesis in the pre-osteolytic phase [13]. Interestingly, while overt lesions ultimately developed, cathepsin K + osteoclasts were rarely associated with the breast cancer cells at the bone surface. Tumor cell proliferation was boosted via adherens junctions between tumor cells and early-stage osteogenic cells [13].

In this review, we will focus on the PMN in bone. The PMN arises from primary cancer cells initiating changes in the expression of extracellular matrix components and mobilization of bone marrow progenitor cells to create a conducive microenvironment for seeding and growth of disseminated cancer cells that subsequently arrive at the metastatic site [14]. Changes in the PMN include increased angiogenesis and vascular permeability, enhanced bone marrow-derived cell recruitment, immune suppression, and extracellular matrix remodeling [15]. These changes can be initiated by tumor-secreted factors [4] or cargo within extracellular vesicles [16•] released into the bloodstream by the primary tumor cells. Bone may be particularly susceptible to colonization by disseminated cancer cells because the skeleton, both the mineralized tissue and marrow tissue, is a depot of growth factors that support cancer cell survival and proliferation. Further, the high vascularity of bone tissue likely provides numerous opportunities for intravasation.

In the skeleton, PMN formation has mostly been associated with bone-resorbing osteoclasts. For example, one study found that breast cancer cells released pro-osteoclastic miRNA contained in exosomes that upregulated osteoclastogenesis and contributed to bone lesion formation prior to cancer cell engraftment [17•]. However, osteoclasts are unlikely to mediate all changes that occur during PMN formation, such as changes to vasculature. We hypothesize that osteocytes, "master orchestrators of bone" [18], are the primary recipients of cancer cell-derived signals and the primary directors of PMN formation as they have established roles in regulation of bone tissue remodeling, vasculature, and immune function. Given the key role of mechanical signals in directing bone remodeling via the osteocyte, we urther expect that mechanical signals would impact osteocyte-mediated PMN development. Though there is a paucity of data in this realm, we will discuss evidence of mechanical signals and PMN formation.

Overview of Bone Physiology

Bone Tissue Remodeling

Bone is a dynamic tissue that undergoes constant extracellular matrix (ECM) remodeling to replace old or damaged bone with new bone [19]. Remodeling is principally directed by osteocytes, which direct downstream bone removal by osteoclasts and bone replacement/formation by osteoblasts [20]. Osteoclasts originate from the hematopoietic lineage and resorb mineralized matrix after activation to their multinucleated state via receptor activator of nuclear factor kB ligand (RANKL) expression from osteocytes. Osteoblasts, which originate from the mesenchymal line, follow the osteoclasts and deposit new matrix in the voids produced by the osteoclasts. Osteocytes, the most abundant cells in the skeleton, are osteoblasts that have literally been buried alive in their own matrix. During this process, their morphology changes from cuboidal to stellate, which permits them to form an extensive interconnected network throughout the bone matrix called the lacunar-canalicular network (LCN) (Fig. 1a). Through the LCN, osteocytes sense and integrate mechanical and chemical signals and appropriately control the balance between osteoclast and osteoblast activities via secretions of signaling proteins. Osteocytes regulate osteoclastogenesis by secreting macrophage colony-stimulating factor and RANKL [18, 20]. They also express osteoprotegerin (OPG), a soluble RANKL inhibitor, and the RANKL to OPG ratio is a critical rheostat for the bone remodeling balance. Osteocytes constitutively secrete the potent anti-formation protein, sclerostin [18, 20], to regulate osteoblasts. Sclerostin, OPG, and RANKL are all highly sensitive to mechanical loading [18]. For example, increased mechanical loading reduces sclerostin expression [21]. Finally, osteocytes are also in direct cell–cell contact with cells in the bone marrow, such as stem cells and endothelial cells (Fig. 1b). Thus, the osteocyte syncytium regulates processes in bone beyond tissue remodeling.

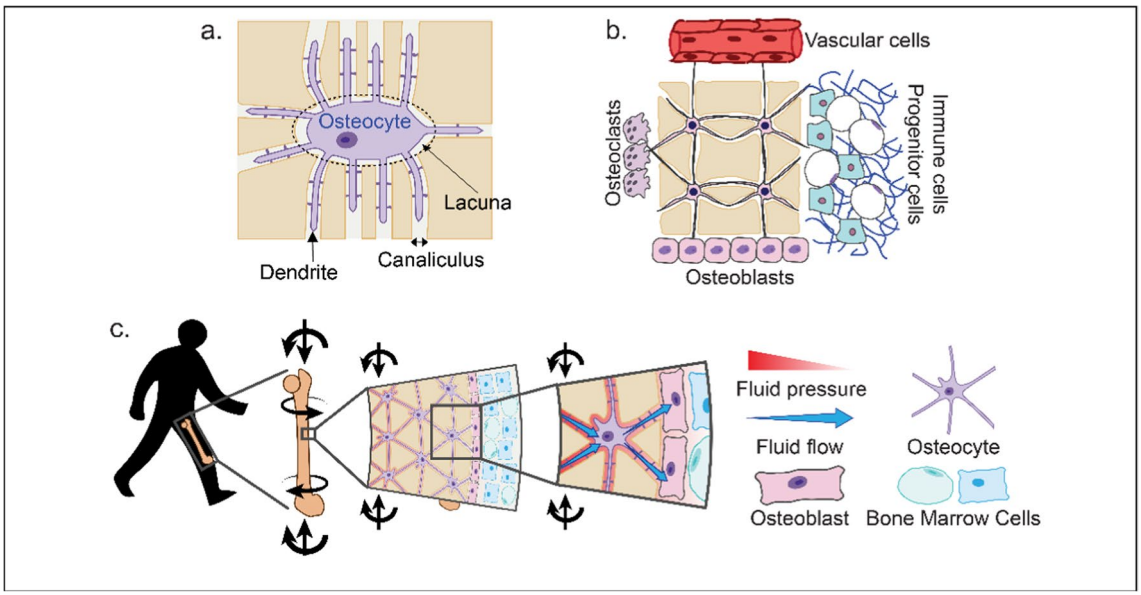

Fig. 1 Osteocyte microenvironment, communication, and mechanosensation. **a** Single osteocyte within the lacunar-canalicular network. **b** Osteocytes connect and communicate with cells from multiple physiological systems. **c** Loads cause tissue deformation, interstitial fluid pressurization, and fluid flow throughout the osteocyte lacunar-canalicular network

The Mechanostat: Mechanical Regulation of Remodeling by Osteocytes

In a healthy skeleton, the bone remodeling balance is primarily regulated by local mechanical signals, whereby the skeleton adapts to the mechanical environment to balance daily physical demands with metabolic cost (Fig. 1c). The "mechanostat" is managed by osteocytes, the primary mechanosensory cells in bone. Steady-state remodeling occurs continually in which formation and resorption are balanced; in this phase, remodeling takes place within a target physiological (non-zero) range of mechanical stimulus which is generally considered to be the strains created in bone due to daily physical activity. Reduced mechanical stimuli (e.g., due to bed rest, increased bone mass) shift remodeling to net bone loss via upregulated osteoclast number and activity. Conversely, increases in mechanical stimuli (e.g., due to sports, reduced bone mass, and/or stress-increasing architecture) promote net bone formation via osteoblast upregulation.

Perilacunar Remodeling by Osteocytes

Osteocytes can also directly remodel their local perilacunar bone tissue, a process called perilacunar remodeling (PLR). In fact, PLR may be more effective for mineral mobilization than canonical remodeling during times of intense need for calcium. Several conditions can induce physiological PLR, including lactation, unloading via sciatic neurectomy, or microgravity [22]. During PLR, osteocytes express bone-resorbing enzymes typically associated with osteoclasts (e.g., matrix metallopeptidases), and their lacunae become measurably larger. Osteocytes also have the ability to form new bone in their lacunae (e.g., post-lactation), resurrecting their former osteoblast function. While disuse activates PLR, the impact of increased mechanical loading on PLR is less clear [22]. In disease states, pathological PLR can occur, sometimes called osteocytic osteolysis, which leads to abnormal mechanosensation, hypercalcemia, and impaired bone quality [23].

Pre-metastatic Niche Hallmarks and Connections to Osteocytes

Extracellular Matrix Remodeling

Aberrant remodeling of the bone extracellular matrix (ECM) is associated with multiple pathologies, including cancer and metastasis. In PMN development, the initial phase or "priming phase" features ECM remodeling and stromal cell reprogramming by soluble factors and extracellular vesicles secreted by the primary tumor [24]. Changes to the ECM in the PMN share parallels with changes in primary tumor sites, including increased protein deposition and matrix stiffening. This "priming" phase has been studied most closely in the liver and lung, two common sites of metastasis with mortal consequences. Multiple cell types are involved in cancer-associated ECM remodeling in the lung, liver, and other soft tissues, including fibroblasts and tumor-associated

macrophages [4]. In breast-to-lung metastasis, early recruitment of M2 macrophages to the lung and protein secretions therein activated lung fibroblasts to deposit more ECM proteins such as collagen, contributing to PMN formation [25]. Similarly, in mice with orthotopic injections of triple-negative breast cancer cells, their lungs exhibited enhanced expression of ECM proteins (fibronectin, tenascin-c, and periostin) compared to mice with less aggressive luminal A breast cancer cells [26]. In vitro data supported distant preparation by breast cancer cells of the lung PMN. Extracellular vesicles collected from the triple-negative cells induced lung fibroblasts to secrete more ECM proteins, and breast cancer cell migration and proliferation was greater in response to conditioned media collected from triple-negative cell-bearing lungs [26].

ECM remodeling can also be influenced by expressed cellular factors, including matrix metalloproteinases that can degrade ECM molecules [4], as well as extracellular vesicles secreted by tumor cells. In mice with Lewis lung carcinoma (LLC), tumor-secreted extracellular vesicles upregulated hepatic stellate cell activation and increased ECM deposition as part of PMN formation in the distant liver [16•]. Specifically, alpha smooth muscle actin and collagen type 1 expression in liver was significantly upregulated in LLC-bearing mice compared to the non-tumor-bearing controls.

Factors expressed by tumor cells can also impact ECM remodeling in bone. For example, primary tumor cell secretion of parathyroid hormone-related protein (PTHrP) affected skeletal remodeling by upregulating osteoclastogenesis via the RANKL pathway in osteoblasts [27]. Osteoblasts are not the only bone cells affected by this signaling molecule, as osteocytes also respond to PTHrP signaling. In fact, PTH/PTHrP is crucial in the breast-bone axis, particularly during lactation, whereby osteocytes with the receptor for PTH/PTHrP are stimulated to liberate calcium and phosphate [28]. Osteocyte-specific deletion of the type 1 PTH/PTHrP receptor (PTHR1) reduced the amount of bone mineral density lost during lactation by 50%, with concomitant reduction in osteocyte PLR (discussed below) but also reduced osteoblast and osteoclast numbers and activity associated with lactation [29]. This indicates that PTH/PTHrP signaling from the breast acts on osteocytes and changes downstream bone remodeling. Further, women with early-stage breast cancer being treated with aromatase inhibitors had higher levels of sclerostin in their serum, which correlated with poorer bone health metrics (e.g., lower BMD, higher PINP) [30], pointing to the central role of osteocytes in regulating bone remodeling in breast cancer patients. As such, anti-sclerostin therapies, such as romosozumab, have shown promise in treating skeletal complications of breast cancer in preclinical studies. In mice with breast cancer bone metastases, treatment with an anti-sclerostin antibody reduced metastases by upregulating osteoblast-mediated bone formation and inhibiting osteoclast-dependent bone resorption [31•].

While these examples indicate a clear relationship between cancer-associated signaling and bone remodeling, changes in ECM remodeling during bone PMN formation remain largely unknown. Though osteoclasts are important contributors to PMN-associated ECM changes in bone, their function is largely confined to resorption, whereas osteocytes regulate many more processes in bone. Following intrailiac artery injection of breast cancer cells, the niches where cancer cells ultimately colonized were characterized by osteogenesis rather than osteoclastogenesis and were primarily comprised of cells positive for collagen type 1 [13]. This evidence, along with their role in guiding canonical remodeling, suggests that osteocytes are playing a role in the ECM remodeling phase of PMN development via their control over the actions of osteoblasts and osteoclasts.

An additional mechanism by which osteocytes could contribute to PMN formation is via PLR. To date, only a few studies have connected PLR and cancer. In one, a mouse model of bone metastatic melanoma, the osteocyte LCN was severely disrupted in tumor-bearing bones [32]. This resulted in compromised stiffness and disorganization of the collagen and apatite microstructure, as well as changes to osteocyte lacunae with evidence of both increased osteolytic activity and complete infilling with immature matrix, implicating aberrant perilacunar remodeling by osteocytes. Interestingly, Bonewald and colleagues recently reported exciting evidence of pathological PLR in non-bone metastatic cancers [33••]. Mice injected with colon, ovarian, or lung cancer cells displayed increased osteocyte lacunar area and TRAP expression (Fig. 2a), indicating pathological PLR, alongside significant bone loss, increased osteocyte apoptosis, and empty lacunae. In vitro studies corroborated the in vivo results. This study presents strong evidence that cancer cells elsewhere in the body are communicating with and altering osteocyte behavior and specifically affecting PLR. Thus, cancer cells could be leveraging PLR to liberate calcium and pro-tumorigenic factors from the bone matrix. However, whether PLR is involved in ECM remodeling during PMN development is wholly unknown at the present time.

Angiogenesis and Vascular Remodeling

Increased angiogenesis and vascular leakiness are characteristics of the earliest stages of PMN development [34]. Increased angiogenesis in the PMNs of secondary tumor sites has been identified in lung and brain metastasis of breast cancer [35], liver metastasis of colorectal cancer [3], and lung colonization of non-small-cell lung cancer [36], with cancer cell-secreted exosomes acting as the promoters. Increased vascular leakiness also promotes secondary

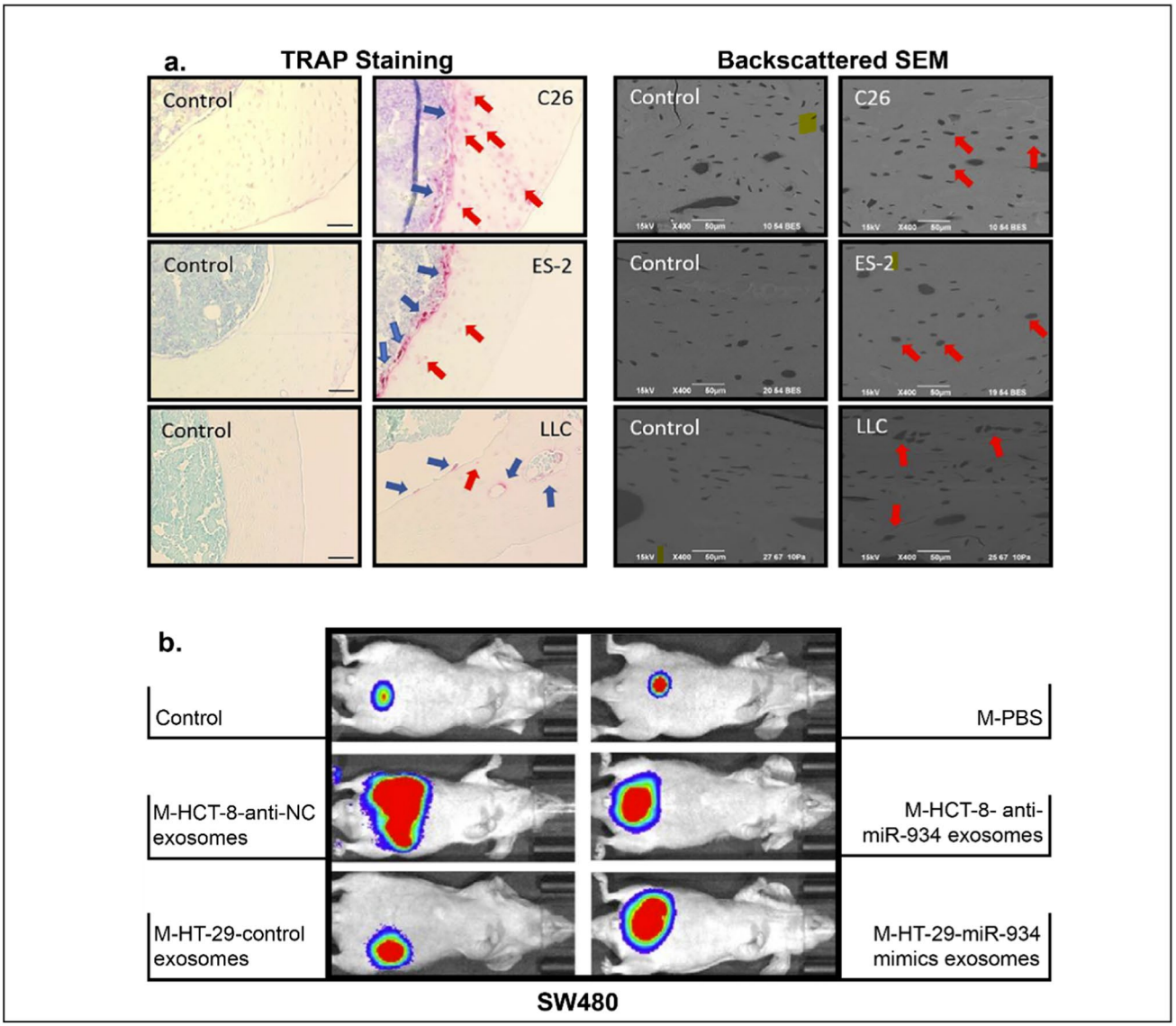

Fig. 2 Secondary tissue changes due to cancer extracellular vesicles. **a** Osteocytes in mouse models bearing non-bone metastasizing tumors show increased TRAP expression (left) and lacunar area (right) compared to non-tumor-bearing controls, demonstrating pathological perilacunar remodeling *(*adapted from [33••] with permission from Elsevier). **b** Mouse models showed increased liver metastasis when cancer cells were cocultured with conditioned media obtained from miR-934-overexpressing or downregulated CRC cells were increased compared to control groups, suggesting that M2 macrophage polarization enhances liver metastatic potential of colorectal cancer (adapted from [51•] with permission via the Creative Commons license)

tumor formation, with exosomes similarly identified as contributors [3, 36]. Common markers of vascular permeability are VEGF, RANKL, and OPG, which have a shared role in osteocytes as paracrine mediators of bone and vascular remodeling.

Osteocytes have connections to the vascular system that could support a role in PMN-associated changes in bone. Bone tissue remodeling and homeostasis involve osteocyte communication with vascular endothelial cells in the marrow, and osteocytes reciprocally play a role in bone angiogenesis and regulation of vascular permeability due to their direct contact with blood vessels in human cortical bone [37•]. This regulatory role of osteocytes in angiogenesis was observed in MLO-Y4s, where osteocyte-secreted VEGF activated angiogenesis in human vascular endothelial cells (HUVECs) [37•]. MLO-Y4 apoptosis also promoted angiogenesis in vitro via increased VEGF, with greater endothelial cell proliferation, migration, and tube formation [38]. Given that osteocyte apoptosis is often increased with cancer, this presents a potential mechanism by which osteocytes contribute to PMN development. Further, new vasculature is critical during the inflammatory, reparative, and remodeling stages

of bone fracture healing, and osteocytes are ideally situated to coordinate all the necessary processes, including ones involving the vasculature system (as reviewed in [39]). For example, osteocyte-derived factors (e.g., CYR61 cysteine-rich angiogenic inducer 61) were correlated with stimulation of angiogenesis and revascularization during healing [40]. Similarly, osteocytes express VEGF during healing and repair of critical size bone defects [41].

Additional evidence for the role of osteocytes in vasculature regulation includes angiogenic activity of proteins typically associated with osteocytes. RANKL and OPG, which are predominantly secreted by osteocytes, are key regulators of vascular permeability. For example, increased OPG expression decreased vascular permeability and maintained vascular health, while inactivation of OPG increased atherosclerosis [42]. Correspondingly, a higher RANKL to OPG ratio increased vascular permeability [43]. These proteins also act as a potential link between osteocytes, loading, and vascular remodeling. Mechanical loading increases OPG and decreases the RANKL to OPG ratio, which would protect vascular permeability, a potential mechanism by which loading is anti-tumorigenic. Additionally, mechanically flowed osteocytes reduced breast cancer cell-induced upregulation of endothelial permeability, cancer cell adhesion to endothelial monolayers, and their transendothelial migration [34, 44, 45••], providing evidence that the anti-tumorigenic effects of loading include protection of the bone vasculature. Finally, mechanical loading has been found to be protective against osteocyte apoptosis [38], suggesting that mechanical loading may similarly protect against the increased angiogenesis and vascular permeability associated with PMN formation.

Following anabolic loading, osteocytic VEGFA was not necessary for in vivo lamellar bone formation, suggesting that other signaling pathways are involved in vasculature regulation [46]. One potential signaling pathway could involve sclerostin, a potent anti-bone formation signal. Sclerostin has been implicated in promoting angiogenesis as it increased HUVEC proliferation and exerted angiogenic activity, in a manner similar to that of VEGF in vitro. Specifically, sclerostin induced the formation of a network of anastomosing tubules, a significant increase in the percentage of tubule number, total tubule length, and number of junctions [47]. LRP6, the receptor for sclerostin, was also found on the surface of HUVECs. Sclerostin is negatively regulated by mechanical loading, suggesting another mechanism by which loading protects bone vasculature. Osteocyte-mediated alterations in vascular porosity, though, seem to not be regulated via sclerostin. Vascular porosity, as measured by the number of canals and canal volume, in the cortical bone of sclerostin-deficient mice was similar to wild-type bones [48]. Administration of sclerostin antibodies acts as a strong anabolic signal, as does loading. Sclerostin antibody treatment enhanced bone fracture healing and was protective against tumor-induced bone disease [31•, 49, 50], alleviating bone destruction as well as displaying inhibition of tumor growth [31•]. Taken together, these studies suggest that osteocytes are the central cellular players in directing vascular changes, implicating their role in establishing the PMN. As such, the opportunity exists for the osteocyte-vascular cell signaling pathway to become exploited by the cancer metastatic pathway to promote PMN formation.

Bone Marrow-Derived Cell Recruitment and Immunosuppression

Bone marrow tissue is home to myriad cell types important to many physiological processes beyond bone homeostasis, including cells of hematopoietic origin [hematopoietic stem cells (HSCs), osteoclasts, macrophages, etc.), mesenchymal origin (mesenchymal stem cells (MSCs), osteoblasts, adipocytes, etc.], endothelial cells, and nerve cells. Macrophage and myeloid cell recruitment have been identified as key components in lung and liver PMN formation, particularly when facilitated by tumor-secreted extracellular vesicles. A study of pancreatic cancer liver metastasis showed that exosome treatment induced significant migration of macrophages to the liver; however, when the macrophages were depleted by knockout, no PMN formed [2]. Similarly, colorectal cancer cell-secreted extraceullar vesicles upregulated M2 macrophage polarization in the distant liver, and M2 macrophage polarization upregulated cancer cell colonization in the liver (Fig. 2b). Strikingly, pre-treatment of macrophages with tumor-derived exosomes also resulted in increased liver metastasis, even with inoculation of weakly metastatic colorectal cancer lines [51•]. These hepatic PMN features were paralleled in bone-to-lung osteosarcoma metastasis, in which mice pre-treated with osteosarcoma-conditioned media containing exosomes promoted PMN formation with subsequent overt lung metastases. Compared to control mice, lungs of the pre-treated mice had a larger population of myeloid cells and macrophages, which correlated to metastases [52]. Together, these results support the hypothesis that macrophage infiltration is a critical component in PMN formation.

In addition to macrophages, myeloid-derived suppressor cells (MDSCs) have been identified as having particular importance in PMN development. Among their many roles, MDSCs can independently promote angiogenesis, fibrosis, and endothelial permeability to support cancer cell migration and inhibit natural killer and T-cell activity to suppress the immune response to cancer cells [53]. Wang et al. highlighted the importance of MDSCs in PMN formation and metastasis using colorectal cancer mouse models. In vivo,

following cecal wall injection of primary tumor cells, mice experienced a significantly increased MDSC population in the liver and lung tissue. Critically, MDSCs isolated from pre-metastatic livers following primary tumor formation inhibited CD8+ T-cell activity more effectively than naive MDSCs. CXCL-1 expression was identified as a determining factor for MDSC infiltration into the liver and subsequent liver PMN formation [54]. Other factors involved in MDSC activity include M-CSF and numerous interleukins including IL-1, 4, 6, and 13, which stimulate MDSC differentiation and maturation. IL-6 also acts as a chemokine that mobilizes MDSCs along with transcriptional factors Snail and Twist1 [55].

The recruitment of bone marrow-derived cells, including macrophages and MDSCs, has close parallels with osteocyte activity in bone. As osteocytes recruit and direct osteoclasts and osteoblasts in the canonical remodeling pathway (described in section Bone Remodeling), a strong line of communication to bone marrow-derived progenitor cells (HSCs, MSCs, respectively) clearly exists. For example, signaling proteins (e.g., IL-6) are expressed by osteocytes to help recruit inflammatory cells, a mechanism that could be taken advantage of by cancer cells in preparing their PMN [39]. Osteocytes also express factors related to MDSC activity including IL-6, TNF, Snail, and Twist1, and CXCL1/2 [56••]. The direct relationship between osteocytes and MDSCs within the context of cancer is ambiguous, but the ability of osteocytes to express cytokines and cellular factors that are directly related to MDSC activity points to a pathway that could be exploited by tumor cells to induce PMN formation in bone.

Conclusions and Future Directions

The PMN is an increasingly well-documented phenomenon, the characteristics and hallmarks of which have thus far been studied in soft tissues, particularly the liver and lung. The common features of PMN formation include increased ECM deposition, increased vasculature and vascular permeability, recruitment of bone marrow-derived cells, and immune suppression. Between lung, liver, and bone tissues, parallels exist that support the investigation into the bone PMN using the same hallmarks as those established in soft tissue studies.

In lung tissue, lung fibroblasts are the most frequently investigated cell type with respect to PMN formation. Interestingly, lung fibroblasts share similarities with osteocytes; in addition to sharing a mesenchymal lineage, both osteocytes and lung fibroblasts are capable of collagen deposition and degradation. In the liver, hepatic stellate cells are largely responsible for liver remodeling and regeneration through the production and deposition of extracellular matrix molecules and the production of matrix metalloproteinases that degrade existing matrix [57]. Though their lineage has been controversial, accumulated evidence suggests that hepatic stellate cells have a mesenchymal, not hematopoietic, origin and may be a subset of mesenchymal stem cells, as hepatic stellate cells are capable of differentiation into other cell types, including osteocytes [58]. Finally, hepatic stellate cells, lung fibroblasts, and osteocytes all have mechanosensitive functions [59]. In addition to the similarities between osteocytes and other PMN-associated cell lines, osteocytes have several functions that could be exploited by cancer cells to support metastasis. Osteocyte PLR can be triggered by a heightened need for circulating calcium, such as during lactation [29]. To successfully metastasize, cancer cells at the primary site must first undergo the epithelial-to-mesenchymal transition (EMT), in which cells change their morphology and exhibit increased motility. EMT is initiated by multiple signaling cascades, which frequently operate through calcium-gated ion channels. This suggests the potential for primary tumor cells, including those that do not typically metastasize to bone, to stimulate calcium release via osteocyte PLR, which should be investigated.

Preclinical (in vivo) models naturally include integrated physiological systems that exist in organisms but also have significant limitations in their ability to reproduce breast-to-bone tropism, resulting in limited knowledge of PMN formation in bone. Typically, immunocompromised mice are injected with human cells (xenograft), either from immortalized cell lines or patient-derived cells, in an effort to better mimic the human disease [60]. For bone metastasis, orthotopic injection into the mammary fat pad typically does not result in bone metastasis, limiting the study of intravasation and extravasation. Thus, cells are more commonly injected into circulation via the heart, the tail vein/caudal artery, or directly into the medullary space of long bones, all routes that more accurately represent tissue colonization rather than all steps of metastasis. These approaches also do not reliably reflect "true" bone metastasis. For example, the tail vein injection mostly results in lung metastasis, and intracardiac infusion results in broad organ targeting [61]. Further, the obvious drawback of using immunocompromised mice is that they do not have intact immune systems, which precludes their use in understanding the role of the immune system in cancer, including that in the PMN. An alternative is using genetically engineered mice that spontaneously develop tumors, such as overexpression of oncogenes or tumor suppressor knockouts or using syngeneic mice that require mouse-derived cancer cells from the same strain background. These latter approaches better reflect human mammary carcinogenesis but mostly result in lung

metastases rather than bone (nicely collated in Table 2 found in [61]). In vivo models of anabolic mechanical loading, such as tibial compression, low-magnitude high-frequency vibration, and treadmill running, are widely used to investigate the influence of mechanical signals on bone in a variety of settings: aging, type I osteoporosis, development, and more [62, 63]. These loading models have revealed that increased mechanical signals in the anabolic range are osteoprotective with anti-tumorigenic effects [63]. However, without preclinical models that capture the full metastatic cascade, elucidating the role of loading via osteocytes on PMN development will likely be challenging.

Another barrier to studying skeletal PMN formation is a need for physiological in vitro models. When considering the hallmarks of PMN formation, multiple physiological systems are active and acting in concert, including but not limited to the immune system, the cardiovascular system, and the muscular system. Recapitulating this in vitro is incredibly challenging, even with the most sophisticated systems available to date. Organs-on-a-chip is a potential path forward, as there are recent advances in platforms that model multiple physiological systems, such as bone/cartilage [64] and the cardiovascular system [65]. Work is ongoing to develop body-on-a-chip platforms to recapitulate interactions across multiple organs. For example, an exciting recent study by the Vunjak-Novakovic group established a multi-organ chip incorporating matured human heart, liver, bone, and skin tissue niches that were linked by recirculating vascular flow [66] and could recapitulate the clinical effects of doxorubicin in humans.

Metastatic tropism to bone is a significant clinical problem, as bone metastasis remains incurable. Understanding the mechanisms by which bone is—or becomes—a desirable distant site will help identify potential avenues for therapeutic interventions. In this review, we have summarized current knowledge of PMN formation by primary tumor cells and provided evidence that tumor cells are preparing their PMN in bone via osteocytes, the principal regulators of skeletal homeostatic processes. We hypothesize that investigations of this connection will open the door to novel methods of halting metastasis and improving patient outcomes.

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Declarations

Conflict of Interest The authors declare no conflict of interest with the contents of this article.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Jiang W, Rixiati Y, Zhao B, Li Y, Tang C, Liu J. Incidence, prevalence, and outcomes of systemic malignancy with bone metastases. https://doi.org/10.1177/2309499020915989.
2. Costa-Silva B, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2014;17(6):816–26. https://doi.org/10.1038/ncb3169.
3. Zeng Z, et al. Cancer-derived exosomal miR-25–3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. Nat Commun. 2018;9(1). https://doi.org/10.1038/S41467-018-07810-W.
4. Paolillo M, Schinelli S. Extracellular matrix alterations in metastatic processes. Int J Mol Sci. 2019;20(19). https://doi.org/10.3390/IJMS20194947.
5. So A, Chin J, Fleshner N, Saad F. Management of skeletal-related events in patients with advanced prostate cancer and bone metastases: incorporating new agents into clinical practice. Can Urol Assoc J. 2012;6(6):465. https://doi.org/10.5489/CUAJ.12149.
6. Mayhew V, Omokehinde T, Johnson RW. Tumor dormancy in bone. 2019. https://doi.org/10.1002/cnr2.1156.
7. Svensson E, Christiansen CF, Ulrichsen SP, Rørth MR, Sørensen HT. Survival after bone metastasis by primary cancer type: a Danish population-based cohort study. BMJ Open. 2017;7(9). https://doi.org/10.1136/BMJOPEN-2017-016022.
8. Fröbel J, et al. The hematopoietic bone marrow niche ecosystem. Front Cell Dev Biol. 2021;9:1958. https://doi.org/10.3389/FCELL.2021.705410/BIBTEX.
9. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, Mccauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone 1. CANCER Res. 2002;62:1832–1837. Accessed: Apr. 20, 2023. [Online]. Available: http://aacrjournals.org/cancerres/article-pdf/62/6/1832/2501673/1832.pdf.
10. Müller A, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410(6824):50–6. https://doi.org/10.1038/35065016.
11. David Roodman G. Mechanisms of bone metastasis. N Engl J Med. 2004:350:1655–64. Accessed: Apr. 20, 2023. [Online]. Available: https://www.nejm.org.
12. Furger KA, Menon RK, Tuck AB, Bramwell VH, Chambers AF. The functional and clinical roles of osteopontin in cancer and metastasis. Curr Mol Med. 2001;1(5):621–32. https://doi.org/10.2174/1566524013363339.
13. Wang H, et al. The Osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. Cancer Cell. 2015;27(2):193–210. https://doi.org/10.1016/J.CCELL.2014.11.017.
14. Wels J, Kaplan RN, Rafii S, Lyden D. Migratory neighbors and distant invaders: tumor-associated niche cells. Genes Dev. 2008;22(5):559–74. https://doi.org/10.1101/GAD.1636908.
15. Dong Q, Liu X, Cheng K, Sheng J, Kong J, Liu T. Pre-metastatic niche formation in different organs induced by tumor extracellular vesicles. Front Cell Dev Biol. 2021;9. https://doi.org/10.3389/FCELL.2021.733627.
16. • Hsu YL, et al. Bone-marrow-derived cell-released extracellular vesicle miR-92a regulates hepatic pre-metastatic niche in lung cancer. Oncogene. 2020;39(4):739–53. https://doi.org/

10.1038/S41388-019-1024-Y. **Activation of hepatic stellate cells, which play a similar in liver as osteocytes in bone, results in extracellular matrix remodeling as part of pre-metastatic niche formation.**

17.• Yuan X, et al. Breast cancer exosomes contribute to pre-metastatic niche formation and promote bone metastasis of tumor cells. Theranostics. 2021;11(3):1429–45. https://doi.org/10.7150/thno.45351. **Breast cancer-derived exosomes travel to and affect osteoclast activity to initiate pre-metastatic niche formation.**

18. Schaffler MB, Cheung WY, Majeska R, Kennedy O. Osteocytes: master orchestrators of bone. Calcif Tissue Int. 2014;94(1):5. https://doi.org/10.1007/S00223-013-9790-Y.

19. Florencio-Silva R, Rodrigues Da G, Sasso S, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. 2015. https://doi.org/10.1155/2015/421746.

20. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011;26(2):229–38. https://doi.org/10.1002/JBMR.320.

21. Galea GL, Lanyon LE, Price JS. Sclerostin's role in bone's adaptive response to mechanical loading. 2016. https://doi.org/10.1016/j.bone.2016.10.008.

22. Tsourdi E, Jähn K, Rauner M, Busse B, Bonewald LF. Physiological and pathological osteocytic osteolysis. J Musculoskelet Neuronal Interact. 2018;18(3):292. Accessed: Apr. 20, 2023. [Online]. Available: /pmc/articles/PMC6146198/.

23. Jähn-Rickert K, Zimmermann EA. Potential role of perilacunar remodeling in the progression of osteoporosis and implications on age-related decline in fracture resistance of bone. Curr Osteoporos Rep. 2021;19(4):391–402. https://doi.org/10.1007/S11914-021-00686-8.

24. Liu Y, Cao X. Characteristics and significance of the pre-metastatic niche. Cancer Cell. 2016;30(5):668–81. https://doi.org/10.1016/J.CCELL.2016.09.011.

25. Qi Y, Zhao T, Li R, Han M. Macrophage-secreted S100A4 supports breast cancer metastasis by remodeling the extracellular matrix in the premetastatic niche. Biomed Res Int. 2022;2022. https://doi.org/10.1155/2022/9895504.

26. Medeiros B, et al. Triple-negative primary breast tumors induce supportive premetastatic changes in the extracellular matrix and soluble components of the lung microenvironment. Cancers (Basel). 2020;12(1). https://doi.org/10.3390/CANCERS12010172.

27. Martin TJ, Johnson RW. Multiple actions of parathyroid hormone-related protein in breast cancer bone metastasis. Br J Pharmacol. 2021;178(9):1923–35. https://doi.org/10.1111/BPH.14709.

28. Athonvarangkul D, Wysolmerski JJ. Crosstalk within a brain-breast-bone axis regulates mineral and skeletal metabolism during lactation. Front Physiol. 2023;14. https://doi.org/10.3389/FPHYS.2023.1121579.

29. Qing H, et al. Demonstration of osteocytic perilacunar/canalicular remodeling in mice during lactation. J Bone Miner Res. 2012;27(5):1018. https://doi.org/10.1002/JBMR.1567.

30. Kyvernitakis I, Rachner TD, Urbschat A, Hars O, Hofbauer LC, Hadji P. Effect of aromatase inhibition on serum levels of sclerostin and dickkopf-1, bone turnover markers and bone mineral density in women with breast cancer. J Cancer Res Clin Oncol. 2014;140(10):1671–80. https://doi.org/10.1007/S00432-014-1726-Z/TABLES/3.

31.• Hesse E, Schröder S, Brandt D, Pamperin J, Saito H, Taipaleenmäki H. Sclerostin inhibition alleviates breast cancer-induced bone metastases and muscle weakness. JCI Insight. 2019;5(9). https://doi.org/10.1172/JCI.INSIGHT.125543. **Inhibition of sclerostin, primarily secreted by osteocytes, reduced breast cancer cell trafficking to bone, growth of cells that did engraft, and subsequent tumor-induced bone loss.**

32. Sekita A, Matsugaki A, Ishimoto T, Nakano T. Synchronous disruption of anisotropic arrangement of the osteocyte network and collagen/apatite in melanoma bone metastasis. J Struct Biol. 2017;197(3):260–70. https://doi.org/10.1016/J.JSB.2016.12.003.

33.•• Pin F, et al. Non-bone metastatic cancers promote osteocyte-induced bone destruction. Cancer Lett. 2021;520:80–90. https://doi.org/10.1016/J.CANLET.2021.06.030. **Demonstrates that osteocytes and distant tumor cells signal to each other, with resulting aberrant osteocyte perilacunar remodeling.**

34. Peinado H, et al. Pre-metastatic niches: organ-specific homes for metastases. Nat Rev Cancer. 2017;17. https://doi.org/10.1038/nrc.2017.6.

35. Zhou W, et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. Cancer Cell. 2014;25(4):501–15. https://doi.org/10.1016/J.CCR.2014.03.007.

36. Ma Z, et al. Tumor-derived exosomal miR-3157–3p promotes angiogenesis, vascular permeability and metastasis by targeting TIMP/KLF2 in non-small cell lung cancer. Cell Death Dis. 2021;12(9). https://doi.org/10.1038/S41419-021-04037-4.

37.• Prasadam I, Zhou Y, Du Z, Chen J, Crawford R, Xiao Y. Osteocyte-induced angiogenesis via VEGF-MAPK-dependent pathways in endothelial cells. https://doi.org/10.1007/s11010-013-1840-2. **Osteocytes regulate angiogenesis through signaling to endothelial cells via VEGF.**

38. Cheung WY, Liu C, Tonelli-Zasarsky RML, Simmons CA, You L. Osteocyte apoptosis is mechanically regulated and induces angiogenesis in vitro. J Orthop Res. 2011;29(4):523–30. https://doi.org/10.1002/JOR.21283.

39. Choy MHV, et al. How much do we know about the role of osteocytes in different phases of fracture healing? A systematic review. J Orthop Transl. 2019;21:111–21. https://doi.org/10.1016/J.JOT.2019.07.005.

40. Hadjiargyrou M, Ahrens W, Rubin CT. Temporal expression of the chondrogenic and angiogenic growth factor CYR61 during fracture repair. J Bone Miner Res. 2000;15(6):1014–23. https://doi.org/10.1359/JBMR.2000.15.6.1014.

41. Rocha CA, Cestari TM, Vidotti HA, De Assis GF, Garlet GP, Taga R. Sintered anorganic bone graft increases autocrine expression of VEGF, MMP-2 and MMP-9 during repair of critical-size bone defects. J Mol Histol. 2014;45(4):447–61. https://doi.org/10.1007/S10735-014-9565-4.

42. Bennett BJ, et al. Osteoprotegerin inactivation accelerates advanced atherosclerotic lesion progression and calcification in older ApoE-/- mice. Arterioscler Thromb Vasc Biol. 2006;26(9):2117–24. https://doi.org/10.1161/01.ATV.0000236428.91125.E6/FORMAT/EPUB.

43. Min J-K, et al. Receptor activator of nuclear factor (NF)-B ligand (RANKL) increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice. 2007. https://doi.org/10.1182/blood-2006-06-029298.

44. Ma YHV, et al. Mechanical regulation of breast cancer migration and apoptosis via direct and indirect osteocyte signaling. J Cell Biochem. 2018;119(7):5665–75. https://doi.org/10.1002/JCB.26745.

45.•• Ma YHV, Xu L, Mei X, Middleton K, You L. Mechanically stimulated osteocytes reduce the bone-metastatic potential of breast cancer cells in vitro by signaling through endothelial cells. J Cell Biochem. 2019;120(5):7590–601. https://doi.org/10.1002/JCB.28034. **Fluid flow-stimulated osteocytes reduced endothelial cell permeability, and breast cancer cell adhesion to and migration through endothelial layers. This result was mediated in part by reducing cancer cell secretion of proteases, such as MMP9.**

46. McKenzie JA, Galbreath IM, Coello AF, Hixon KR, Silva MJ. VEGFA from osteoblasts is not required for lamellar bone formation following tibial loading. Bone. 2022;163. https://doi.org/10.1016/J.BONE.2022.116502.
47. Hadji P, et al. Management of aromatase inhibitor-associated bone loss (AIBL) in postmenopausal women with hormone sensitive breast cancer: joint position statement of the IOF, CABS, ECTS, IEG, ESCEO IMS, and SIOG. J Bone Oncol. 2017;7:1–12. https://doi.org/10.1016/J.JBO.2017.03.001.
48. Mosey H, et al. Sost deficiency does not alter bone's lacunar or vascular porosity in mice. Front Mater. 2017;4:27. https://doi.org/10.3389/FMATS.2017.00027/BIBTEX.
49. McDonald MM, et al. Inhibition of sclerostin by systemic treatment with sclerostin antibody enhances healing of proximal tibial defects in ovariectomized rats. J Orthop Res. 2012;30(10):1541–8. https://doi.org/10.1002/JOR.22109.
50. McDonald MM, et al. Inhibiting the osteocyte-specific protein sclerostin increases bone mass and fracture resistance in multiple myeloma. Blood. 2017;129(26):3452–64. https://doi.org/10.1182/BLOOD-2017-03-773341.
51.• Zhao S, et al. Tumor-derived exosomal miR-934 induces macrophage M2 polarization to promote liver metastasis of colorectal cancer. J Hematol Oncol. 2020;13(1). https://doi.org/10.1186/S13045-020-00991-2. **Colon cancer-secreted exosomes modulated cross-talk with the distant liver via macrophage polarization, of which osteocytes are also capable, and contributed formation of the pre-metastatic niche.**
52. Zhong L, et al. Rab22a-NeoF1 fusion protein promotes osteosarcoma lung metastasis through its secretion into exosomes. Signal Transduct Target Ther. 2021;61(1):1–16. https://doi.org/10.1038/s41392-020-00414-1.
53. Wang Y, Ding Y, Guo N, Wang S. MDSCs: key criminals of tumor pre-metastatic niche formation. Front Immunol. 2019;10(FEB):172. https://doi.org/10.3389/FIMMU.2019.00172/BIBTEX.
54. Wang D, Sun H, Wei J, Cen B, DuBois RN. CXCL1 is critical for premetastatic niche formation and metastasis in colorectal cancer. Cancer Res. 2017;77(13):3655–65. https://doi.org/10.1158/0008-5472.CAN-16-3199.
55. Tie Y, Tang F, Wei Yq, Wei Xw. Immunosuppressive cells in cancer: mechanisms and potential therapeutic targets. J Hematol Oncol. 2022;15(1). https://doi.org/10.1186/S13045-022-01282-8.
56.•• Dwivedi A, Kiely PA, Hoey DA. Mechanically stimulated osteocytes promote the proliferation and migration of breast cancer cells via a potential CXCL1/2 mechanism. Biochem Biophys Res Commun. 2021;534:14–20. https://doi.org/10.1016/J.BBRC.2020.12.016. **Fluid flow-stimulated osteocytes stimulated secretion of CXCL1 and CXCL12, cytokines that contribute to breast cancer homing to and engraftment in bone.**
57. Sato M, Suzuki S, Senoo H. Hepatic stellate cells: unique characteristics in cell biology and phenotype. Cell Struct Funct. 2003;28(2):105–12. https://doi.org/10.1247/CSF.28.105.
58. Kordes C, Bock HH, Reichert D, May P, Häussinger D. Hepatic stellate cells: current state and open questions. Biol Chem. 2021;402(9):1021–32. https://doi.org/10.1515/HSZ-2021-0180.
59. Kang N. Mechanotransduction in liver diseases. Semin Liver Dis. 2020;40(1):84–90. https://doi.org/10.1055/S-0039-3399502/ID/JR1900042-23/BIB.
60. Sakamoto K, Schmidt JW, Wagner KU. Mouse models of breast cancer. Methods Mol Biol. 2015;1267:47–71. https://doi.org/10.1007/978-1-4939-2297-0_3.
61. Fantozzi A, Christofori G. Mouse models of breast cancer metastasis. Breast Cancer Res. 2006;8(4). https://doi.org/10.1186/BCR1530.
62. Main RP, Shefelbine SJ, Meakin LB, Silva MJ, van der Meulen MCH, Willie BM. Murine axial compression tibial loading model to study bone mechanobiology: implementing the model and reporting results. J Orthop Res. 2020;38(2):233–52. https://doi.org/10.1002/JOR.24466.
63. Falk CH, Liu B, Lynch ME. Bone mechanics in cancer. Encycl Bone Biol. 2020:445–457. https://doi.org/10.1016/B978-0-12-801238-3.11256-5.
64. Hu Y, et al. Bone/cartilage organoid on-chip: construction strategy and application. Bioact Mater. 2023;25:29–41. https://doi.org/10.1016/J.BIOACTMAT.2023.01.016.
65. Liu Y, Lin L, Qiao L. Recent developments in organ-on-a-chip technology for cardiovascular disease research. Anal Bioanal Chem. 2023. https://doi.org/10.1007/S00216-023-04596-9.
66. Ronaldson-Bouchard K, et al. A multi-organ chip with matured tissue niches linked by vascular flow. Nat Biomed Eng. 2022;6(4):351–71. https://doi.org/10.1038/S41551-022-00882-6.