Citation: BoneKEy Reports **4**, Article number: 689 (2015) | doi:10.1038/bonekey.2015.57

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REVIEW

Bone marrow as a metastatic niche for disseminated tumor cells from solid tumors

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Bone marrow is a heterogeneous organ containing diverse cell types, and it is a preferred metastatic site for several solid tumors such as breast and prostate cancer. Recently, it has been shown that bone metastatic cancer cells interact with the bone marrow microenvironment to survive and grow, and thus this microenvironment is referred to as the 'metastatic niche'. Once cancer cells spread to distant organs such as bone, the prognosis for the patient is generally poor. There is an urgent need to establish a greater understanding of the mechanisms whereby the bone marrow niche influences bone metastasis. Here we discuss insights into the contribution of the bone marrow 'metastatic niche' to progression of bone metastatic disease, with a particular focus on cells of hematopoietic and mesenchymal origin.

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Introduction

Primary tumors, with proper treatment, do not typically result in patient death. However, once tumors are established in other organs, the mortality of cancer patients increases markedly. Once detached from the primary tumor, a single tumor cell or a cluster of tumor cells can circulate throughout the body, and later take up residence in a distant site. Interestingly, each type of tumor has a distinct pattern of dissemination. It has been speculated that anatomical and mechanical structures in the human body result in organ preference of tumor metastasis.[1] This hypothesis, however, fails to explain all aspects of the metastatic behavior of disseminated tumor cells (DTCs).

Over a century ago, Stephen Paget famously stated in his 'seed and soil' theory that tumor cells seek a specific accommodating location to survive outside of the primary lesion.[2] That is, a hospitable microenvironment in the potential metastatic site selectively affects the dissemination route of DTCs. Consistent with this notion, recent studies have revealed that the communication between DTCs and the distant microenvironment, or 'metastatic niche', is crucial for the progression of DTCs.[3–5] A better understanding of the tumor-supportive aspects of this interaction is clearly needed for the development of more effective metastatic disease treatments.

Bone, or bone marrow, is a major target organ for metastasis, evidently providing a fertile 'soil' for DTCs. Prostate and breast cancers are particularly well known to metastasize to the bone. Bone marrow contains various cell types, including cells of hematopoietic origin and cells involved in bone formation and remodeling. One major function of the marrow is to regulate hematopoiesis. In the marrow, osteoblasts,[6–8] endothelial cells,[9,10] nerve cells,[11,12] adipocytes,[13] CXCL12-abundant reticular (CAR) cells[14] and mesenchymal stem cells[15,16] collectively serve as a specific 'niche' for hematopoietic stem cells (HSCs), maintaining the functions of HSCs including homing, self-renewal, quiescence and differentiation.[17–19]

It is now known that malignant cells that disseminate to and develop in the bone marrow do so by hijacking the bone marrow niche.[20] In fact, prostate and breast cancer both home to the marrow using mechanisms similar to HSC homing.[21,22] Not only are the DTCs supported by their chosen niche, but they can also instigate niche changes that preferentially cater to malignant cells. Indeed, myeloproliferative neoplasms remodel the normal osteoblastic HSC niche into a malignant niche that impairs normal hematopoiesis.[23] Thus, studying the cross talk between malignancy and the bone marrow microenvironment has rightfully become an area of great interest. However, detailed mechanisms underlying these interactions remain largely unknown. In this review, we will explore what is currently known about DTC-mediated bone marrow niche conversion and also suggest future directions for 'metastatic niche' research.

The Metastatic Niche in the Marrow

Bone marrow is a very heterogeneous organ, containing cells of hematopoietic origin (HSCs, osteoclasts, macrophages,

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Received 5 December 2014; accepted 20 March 2015; published online 20 May 2015

lymphocyte and so on), mesenchymal origin (mesenchymal stem cells (MSCs), osteoblasts, adipocytes and so on), endothelial cells and nerve cells. Osteoblasts, adipocytes, endothelial cells and nerve cells are well studied as the specific microenvironment, or niche, for HSCs. Osteoblasts and osteoclasts are also involved in bone remodeling directly or indirectly by interacting with HSCs. The cells in the marrow interact to support their unique functions and maintain bone structure. Recent studies have revealed that DTCs from primary tumors commandeer this supportive microenvironment, suggesting that DTCs may adapt to and alter a pre-existing niche (the 'HSC niche') to survive and grow as full-blown metastases (the 'metastatic niche').

Mesenchymal stem Cells

It has long been demonstrated that prostate and breast cancers have the potential to assume many properties indicative of osteoblast lineage cells.[24–26] This capacity for osteomimicry is thought to be a key feature of its bone metastatic potential. More recently, the differentiation potential of prostate cancer cells to assume an adipocyte lineage phenotype was revealed.[27] Human prostate cancer cell lines PC3 and DU145 reversibly differentiate into an adipocyte-like phenotype *in vitro* under adipogenic induction conditions, leading to growth arrest and apoptosis.[27] Similarly, human prostate cancer bone metastatic tissue microarray (TMA) samples exhibit a brown fat-specific marker, UCP1, suggesting that prostate cancer cells may also differentiate into an adipocyte phenotype *in vivo*.[27] As the differentiation potential of prostate cancer is likened to that of MSCs,[27] it is also possible that disseminated prostate cancer acquires an MSC-like phenotype to colonize the bone marrow.

In the bone marrow, MSCs may also be involved in establishing tumor dormancy. When the bone metastatic clone of human breast cancer cell line MDA-BM-231-BM2 is cocultured with human MSC line R14, the proliferation and migration of MDA-BM-231-BM2 cells are reduced, evidently by R14-derived exosomes.[28] The mechanism behind this transition is the high expression of microRNA mir-23b in the exosomes, which causes suppression of the *MARCKS* gene and subsequently its encoded protein, myristoylated alanine-rich C kinase substrate. As expected, exosome-treated or miR-23b-overexpressing MDA-BM-231-BM2 cells both exhibit dormancy in mice.[28]

It has been demonstrated that bone marrow-derived MSCs are capable of transforming into cancer-associated fibroblasts (CAFs) within the primary tumor,[29,30] whereupon CAFs promote lung metastases of human breast cancer cell line MDA-MB-231 through the Chemokine (C-C motif) ligand 5 (CCL5)/ C-C chemokine receptor type 5 (CCR5) axis,[29] and bone metastases of murine prostate cancer cell line RM-1 through the chemokine (C–X–C motif) ligand 16 (CXCL16)/ C-X-C chemokine receptor type 6 (CXCR6) axis.[30] In fact, MSC-derived CAFs may select for Src hyperactive, bone metastatic and triple negative breast cancer.[31] Specifically, CAF expression of CXCL12 and insulin-like growth factor-1 (IGF-1) was shown to strongly activate Src (PI3K-Akt pathway) in the triple negative human breast cancer cell lines MDA-MB-231 and CN34, which resulted in bone metastatic phenotype enrichment.[31] The bone metastases of Src hyperactive MDA-MB-231 and CN34 cells were prevented, however, when tumor-bearing mice were treated with AMD3100 and BMS754807, which are inhibitors of the receptors for CXCL12 and IGF-1 (CXCR4 and IGF1R), respectively.[31] These findings suggest that MSC-derived CAFs may have a crucial role in the initiation step of bone metastasis.

Adipocytes

Metabolic dysfunctions associated with obesity and aging are often recognized as risk factors for cancer progression. The marrows of these patients contain large numbers of adipocytes. Adipocytes are known to be involved in tumor progression and metastasis,[32,33] which may be partly owing to their negative effects on hematopoiesis;[13] however, little is known regarding the specific roles that these cells have in the metastatic process. A recent study demonstrated that marrow adipocytes support bone metastasis of prostate cancer,[34] as more PC3 cells, a prostate cancer cell line, grew in the bone marrow of animals receiving a high-fat diet than in that of animals on a regular diet.[34] Conditioned medium (CM) from adipocyte lineage cultures were obtained from differentiated mouse bone marrow stromal cells and found to increase gene expression of fatty acid binding protein 4 (FABP4), interleukin (IL)-1β and heme oxygenase (decycling) 1 (HMOX-1) in PC3 cells *in vitro*; however, this could be stopped by PPAR γ inhibitors.[34] In addition, this CM enhanced the proliferation and invasion of PC3 cells *in vitro*, whereas inhibitors of FABP4 and IL-1β prevented invasion.[34] Interestingly, in an *in vivo* study, the increase of *FABP4*, *IL-1β* and *HMOX-1* genes was observed in tumors within the marrow, but not in tumors grown subcutaneously.[34] Consistent with this, high levels of FABP4 expression are observed in human bone metastatic tumors near adipocytes.[34] Of added interest, aside from metabolic dysfunction, daily dietary intake may also alter the effects of adipocytes on metastatic niche development. Arachidonic acid, a polyunsaturated omega-6 fatty acid, stimulates adipogenesis of human primary bone marrow stromal cells.[35] When PC3 cells are cocultured with adipocytes, the uptake of arachidonic acid by PC3 cells is increased.[35] Simultaneously, arachidonic acid induces the migration of PC3 cells toward adipocytes, suggesting that high levels of arachidonic acid intake may facilitate prostate cancer bone metastasis.[35]

Osteoblasts

Although the unique cell types of the HSC niche in the marrow remain controversial,[18,19] osteoblasts have been well studied as the 'niche' for HSCs,[6–8,17] and our recent studies using animal xenograft models have revealed that bone metastatic prostate cancer cells target this same niche during dissemination.[36] When prostate cancer cell lines (PC3 and C4-2B) reach the bone marrow, they prevent the engraftment of transplanted HSCs, suggesting that disseminated prostate cancer cells compete with HSCs for occupancy of the HSC niche.[36] This observation is confirmed when the niche sizes are manipulated: more dissemination is observed in animals that have more niches, and vice versa.[36] In addition, more tumor cells disseminate into the niche when this niche is vacated by stem cell mobilizing drugs (for example, granulocyte-colony stimulating factor (G-CSF) or CXCR4 inhibitor AMD3100).[36] As a result of this competition for the niche, disseminated prostate cancer cells displace HSCs from the marrow and induce the differentiation of HSCs into hematopoietic progenitor cells (HPCs).[36] Correspondingly, more HPCs are found in peripheral blood obtained from prostate cancer patients with bone

metastases compared with healthy controls or patients with local prostate cancer.[36]

Once DTCs establish residency in the niche, they frequently become dormant, probably in part through cell-to-cell contact with the niche. For example, when osteoblasts are cocultured with prostate cancer cell line, PC3 cells, the secretion of growth arrest-specific 6 (GAS6) by the osteoblasts is significantly enhanced.[37] Interestingly, GAS6 inhibits the proliferation of PC3 cells, while preventing apoptosis.[37] In addition, when PC3 cells are inoculated into murine skeletal tissues, the expression of Axl, one of three receptors (Axl, Tyro3, Mer) for GAS6, is markedly increased.[37] Along with this notion, in mice inoculated with prostate cancer cells, tumor growth is mainly observed in bones expressing low levels of GAS6 (forelimb), whereas tumors rarely grow in bones expressing abundant GAS6 (hindlimb).[38] Moreover, PC3 and DU145 cells, which are proliferating in the bones, express relatively low levels of Axl, compared with when they are in a dormant state.[39] These findings suggest that the osteoblastic niche controls dormancy of disseminated prostate cancer through a GAS6/Axl axis.

Hematopoietic Stem Cells

In the marrow, HSCs reside in their niche to maintain their phenotype; however, HSCs are not only influenced by the niche, but they are also active in the actual development of the niche.[40] Similarly, DTCs are involved in the development of a malignant niche, indirectly using HSCs. When HPCs, but not HSCs, obtained from the bone marrow of mice inoculated with osteoblastic prostate cancer cell line C4-2B cells are cocultured with murine bone marrow stromal cells, the osteoblastic differentiation of the bone marrow stromal cells is stimulated.[41] These HPCs express high levels of bone morphogenetic proteins (BMP)-2 and -6, and the osteoblastogenesis induced by C4-2B-bearing HPCs is inhibited by pan BMP inhibitor Noggin.[41] In contrast, HSCs isolated from the marrow of mice inoculated with osteolytic prostate cancer cell line PC3 express high levels of IL-6, and they differentiate into osteoclasts under the induction of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL).[41] This osteoclastogenesis is prevented by anti-IL-6 antibody treatment.[41] These findings suggest that targeting HSCs or HPCs may be a potential therapy for bone metastatic disease.

Osteoclasts

Bone resorption, one of the key features of bone metastasis, is mediated by DTCs inducing the formation and activation of osteoclasts.[42] Therefore, targeting osteoclastogenesis with bisphosphonates[43] or the RANKL inhibitor denosumab[44] is a treatment strategy used in bone metastatic disease. Human breast cancer coexpresses high levels of metalloproteinase (MMP)-13 and its activator MT1-MMP in bone metastatic lesions.[45] The bone metastatic breast cancer cell line MDA-MB-231 expresses higher levels of MMP-13 than the less aggressive cell line MCF-7, and the MMP-13 expression in MDA-MB-231 cells is enhanced by IL-8 and parathyroid hormone-related protein (PTHrP) treatments.[45] CM obtained from IL-8- or PTHrP-treated MDA-MB-231 cells increases tartrate-resistant acid phosphatase (TRAP)-positive osteoclastic differentiation and the bone resorptive ability of human preosteoclasts. Yet this osteoclastogenesis is inhibited by the pan-MMP inhibitor GM6001, and partially by the MMP-13-specific inhibitor CL-82197 or MMP-13 knockdown in cancer cells using shRNA.[45] MMP-13-induced osteoclastogenesis may be explained in part by the activation of pro-MMP-9 and cleavage of an osteoclastogenesis suppressor galectin-3.[45] When shMMP-13 MDA-MB-231 cells are implanted into the bones of mice, bone resorption and osteoclastogenesis are reduced when compared with control, although there are no significant differences in tumor size.[45] When murine breast cancer cell line Cl66 cells are implanted into the subcutaneous spaces of mice near the calvarial bone, MMP-13, MMP-9, transforming growth factor (TGF)-β and phosphorylated Smad2 are expressed greater in the area where tumor interfaces with bone (TB interface), compared with areas of tumor alone.[46] It is in this TB interface where larger numbers of osteoclasts and bone resorption areas are found.[46] In addition, osteoclast number, osteolytic lesion frequency and MMP-13 expression at the TB interface are all significantly reduced when mice are treated with MMP-13 antisense oligonucleotides.[46] Furthermore, MMP-13 antisense oligonucleotide treatments also impair the expression of MMP-9, TGF-β and phosphorylated Smad2 in the TB interface.[46] Increased expression of placental growth factor (PIGF) is observed when MDA-MB-231 cells reach the marrow, and PIGF induces migration of MDA-MB-231 cells via the ERK pathway.[47] Anti-murine PIGF antibody 5D11D4 treatments prevent metastasis to bone and bone resorption in mice inoculated with either MDA-MB-231 cells or murine melanoma cell line B16/F10 cells.[47] 5D11D4 treatment inhibits osteoclastogenesis by reducing RANKL expression in murine bone marrow cells without affecting angiogenesis.[47] Heptapeptide hormone angiotensin-(1–7) (Ang-(1–7)) decreases the secretion of vascular endothelial growth factor (VEGF) and PIGF from PC3 and DU145 prostate cancer cells, and it prevents cancer cell proliferation and migration, inhibiting bone metastasis and osteoclastogenesis.[48]

Although some recent studies caution against the paradigm of a purely hypoxic niche,[10,19] bone marrow has long been thought to be a hypoxic environment. When treated with TGF-β under hypoxic conditions, the breast cancer cell line MDA-MB-231 increases the expression of angiogenesis factor VEGF and homing receptor CXCR4 through hypoxia-inducible factor (HIF)-1α.[49] This effect may be via the cyclooxygenase-2 (COX-2) signaling pathway, as bone metastatic clone 1833 MDA-MB-231 cells enhance COX-2 expression under hypoxia and TGF-β treatment.[50] When the TGF-β pathway and HIF-1α are knocked down in MDA-MB-231 cells, tumor growth and osteolytic lesions in tumor-inoculated mice are diminished.[49] Moreover, when parental MDA-MB-231 cell-bearing mice are treated with HIF-1α inhibitor 2ME2 and/or TGF-β type I kinase inhibitor SD-208, which is also known to inhibit melanoma bone metastasis,[51] 2ME2 and SD-208 synergistically prevent bone metastasis and inhibit osteoclastogoenesis, all while enhancing osteoblastogenesis.[49] Furthermore, TGF-β-induced factor 2 (Tgif2) induces osteoclastogenesis owing to the down-regulation of microRNA miR-34a expression in osteoclasts.[52] Consequently, when mice were treated with miR-34a-carrying chitosan nanoparticles, the blockage of osteoclastgenesis prevented bone metastasis of human breast cancer cell line MDA231-BoM-1833 and B16/F10, whereas more metastases of these cell lines were observed in bones of miR-34a–knouckout (KO) mice.[52] These findings suggest that the bone

resorption mediated by the interaction between DTCs and osteoclasts is a key step for establishing bone metastasis.

Macrophages

Macrophages are hematopoietic cells that are well known to be involved in tumor progression and metastasis through the expression of inflammatory cytokines and proteases, especially in primary tumor sites.[53] These macrophages are referred to as tumor-associated macrophages (TAMs). Two types of macrophages are known to exist: M1 tumor-inhibiting macrophages and M2 tumor-initiating macrophages (known as TAMs). Macrophages are frequently found in the marrow, where they help establish a favorable tumor microenvironment. For example, the cysteine protease cathepsin K (CTSK) is one of the proteases that osteoclasts and macrophages secrete in the marrow. When PC3 cells are implanted directly into the bone marrow of CTSK-KO mice, less growth is observed compared with wild-type mice.[54] However, there are no significant differences in the growth of subcutaneously implanted tumors between CTSK-KO and wild-type mice.[54] Compared with wild type, CTSK-KO mice have fewer, less invasive macrophages and more osteoclasts, but functionally less bone resorption.[54] When normal macrophages directly interact with tumor cells, expression of inflammatory factors COX-2 and CCL2 is increased. However, macrophages from CTSK-KO mice decrease the expression of COX-2 (a 22-fold decrease) and CCL2 (a 17-fold decrease).[54] Therefore, it appears that marrow macrophages positively control tumor progression through the CTSK/COX-2/CCL2 pathway.

Marrow macrophages may promote metastatic growth by suppressing tumor-induced inflammation. When mouse bone marrow macrophages ($F4/80^+$) are cocultured with apoptotic mouse prostate cancer RM-1 cells, macrophages show more phagocytosis of apoptotic tumor cells (specifically termed efferocytosis) than nonapoptotic tumor cells, through the STAT3/SOCS3 pathway, by increasing the expression of a phagocytosis promoter, milk fat globule-EGF factor 8 (MFG-E8).[55] Consistent with this, high levels of MFG-E8 were found colocalized with macrophages in human prostate TMA samples.[55] In addition, these macrophages enhance the secretion of M2 macrophage-related proteins, IL-6, CCL2 and CCL1, and increase the expression of M2 macrophage-related genes, *IL-10*, *TGF-b1*, *Ym-1* and *arginase 1*, suggesting that MFG-E8-mediated efferocytosis promotes M2 polarization of macrophages.[55]

In contrast, myeloid-derived suppressor cells (MDSCs), which are progenitors of macrophages and osteoclasts, can induce tumor growth by differentiating into osteoclasts. MDSCs ($CD11b^+Gr\text{-}1^+$) obtained from the bone marrow of mice with murine breast cancer cell line 4T1 bone metastases ($MDSCs^{+bone\ mets}$) differentiate into TRAP-positive, bone resorptive osteoclasts *in vitro* and express CTSK, carbonic anhydrase-2, MMP-9 and NO.[56] However, MDSCs from the bone marrow of mice without bone metastases, regardless of other metastatic sites (lung, lymph node, spleen or blood), do not differentiate into osteoclasts, suggesting that the osteoclast differentiation of MDSCs is unique to the bone metastatic environment.[56] In addition, when $MDSCs^{+bone\ mets}$ are transplanted, osteoclast differentiation of $MDSCs^{+bone\ mets}$ and bone distraction are observed in recipient mice.[56] Interestingly, both *in vitro* and *in vivo* osteoclastogenesis mediated by $MDSCs^{+bone\ mets}$ are inhibited by blocking NO.[56] The high NO production in $MDSCs^{+bone\ mets}$ enhances HIF-1α expression through the PIK3 or ERK pathway.[56]

Lymphoid Cells

Bone marrow is also a lymphoid organ. There are many subclasses of T cells, some of which home to the marrow and support tumor progression. For example, an increased number of $CD4^+Foxp3^+$ regulatory T (Treg) cells can be found in the bone marrow of prostate cancer patients with bone metastases compared with prostate cancer patients with local disease or healthy controls.[57] Treg cells express high levels of homing receptor CXCR4, and treatment with an anti-CXCR4 antibody inhibits the migration of Treg cells to CXCL12-expressing bone marrow fluid obtained from prostate cancer patients with bone metastases, suggesting that Treg cells home to bone marrow through the CXCR4/CXCL12 axis.[57] These Treg cells can interfere with the immune response by preventing T-cell proliferation, decreasing the expression of interferon (IFN)-γ and IL-2 production and by suppressing osteoclastogenesis through activation by $RANK^+$ dendritic cells.[57] When Treg cells are co-inoculated with murine prostate cancer cells (RM1) into mice, less osteolytic metastases are observed owing to T-cell-mediated suppression of osteoclastogenesis.[57] In contrast, when Treg cells are depleted from these tumor-bearing animals, bone densities in the animals are reduced.[57] This suggests that Treg cells act within the metastatic niche in an immunosuppressive manner.

Similarly, when murine melanoma cell line B16 is injected into PLCγ2-KO mice, which have reduced myeloid cell function and numbers of osteoclasts, the bone tumor burden is significantly increased compared with wild-type mice.[58] When B16 cells are inoculated into wild-type mice transplanted with bone marrow cells from PLCγ2-KO mice, similar tumor growth is observed, further implicating myeloid cells with tumor growth in the bone.[58] In contrast, when the experiments are repeated using Lyn-KO mice in which the number of osteoclasts and myeloid cell function are enhanced, the growth of B16 cells in the bone is inhibited.[58] Interestingly, tumor growth in the marrow is impaired when the function of $CD8^+$ T cells is enhanced in tumor-bearing mice, whereas inoculated tumor grows in the marrow when $CD8^+$ T cells are diminished.[58] In addition, the inhibitive effects of zoledronic acid (ZA) on tumor growth are prevented in the $CD8^+$ T cell–deficient condition.[58] Importantly, when $CD8^+$ T cells are supplemented into PLCγ2-KO mice or when $CD8^+$ T cells are depleted from Lyn-KO mice, tumor growth in bone is normalized.[58] These findings suggest that $CD8^+$ T cells have important roles in tumor growth in the marrow independent of osteoclast condition.

When mice are inoculated with metastatic murine breast cancer cell line 4T1, more systemic bone loss is observed compared with mice inoculated with non-metastatic cell line 67NR.[59] Accordingly, more pro-osteoclastic cytokines (IL-1β, IL-6, IL-17F, RANKL, tumor necrosis factor (TNF)-α) but less osteoprotegerin (OPG), a decoy receptor for RANKL, are found in the serum obtained from 4T1-bearing animals.[59] Interestingly, production of these cytokines parallels what is seen during tumor dissemination. When $CD3^+$ T cells are isolated from 4T1- or 67NR-bearing mice and transplanted into nude mice, bone loss is observed only in recipients of T cells derived from 4T1-bearing donors, and this is reversed by inhibiting RANKL in

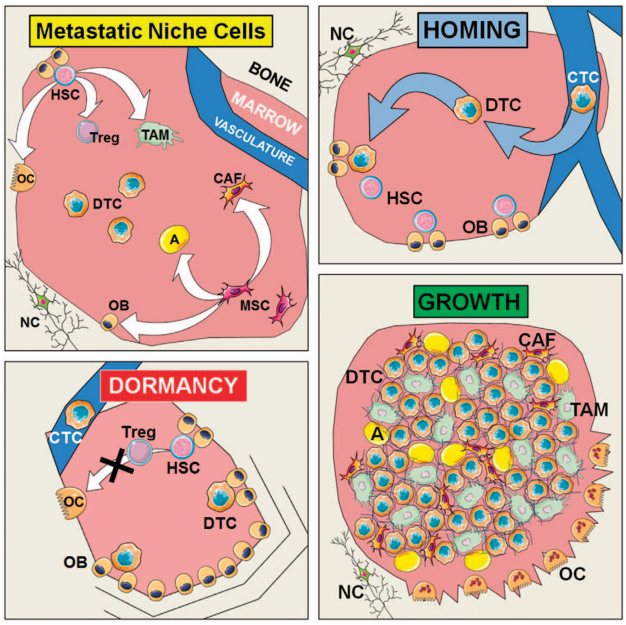

Figure 1 Development of the 'metastatic niche' by the interaction of disseminated tumor cells with the bone marrow microenvironment. Growing evidence supports the idea that tumor cell behavior is dependent on the surrounding microenvironment. This suggests that the microenvironment in distant tissues, such as bone, is essential for disseminated tumor cell (DTC) survival and metastatic growth. However, DTCs are not passive in the marrow—they also put pressure on their surroundings to create a more advantageous microenvironment (metastatic niche). Top left: bone marrow contains many cell types that directly influence one another and DTC fate. Hematopoietic stem cells (HSCs) can differentiate into macrophages, osteoclasts (OCs), T cells and other lymphocytes, whereas mesenchymal stem cells (MSCs) differentiate into adipocytes (A), osteoblasts (OB) and fibroblasts (white arrows). All of these cell types make up the deadly 'metastatic niche.' Top right: once in the marrow, DTCs home to where HSCs and OBs reside, or the 'HSC niche' (blue arrows). Bottom left: DTCs are kept dormant in the HSC niche through OB bone formation and regulatory T-cell (Treg) inhibition of osteoclastogenesis. In addition, endothelial cells that line the vasculature also help regulate circulating tumor cell (CTC) and DTC dormancy. Bottom right: DTCs can be allowed to grow with help from MSC-derived cancer associated fibroblasts (CAFs) and HSC-derived tumor-associated macrophages (TAMs), adipocytes and OC bone resorption. Nerve cells (NCs) also participate in the progression of DTCs.

the T cells before transplantation, but not with inhibition of IL-17F.[59] When 4T1 cells are implanted into control or RANKL-knockdown T-cell-transplanted mice, 95% of lymph node metastases and 100% of bone metastases are eliminated in the RANKL-knockdown T-cell-transplanted mice.[59] These findings suggest that bone metastatic cancer recruits T cells to the marrow to facilitate future bone metastasis by regulating osteoclastogenesis.

Endothelial Cells

During dissemination, cancer cells have constant contact with endothelial cells or pericytes, as DTCs must intravasate into and extravasate out of the blood stream to reach the secondary site. When the orthotopic injection of highly metastatic breast cancer cell line MDA-MB-231 cells or intracardiac injection of weakly metastatic line T4-2 cells into mice was performed, Ki67-negative dormant cells were observed near the thrombospondin-1 (TSP-1)-expressing perivascular niche in the lung and bone marrow.[4] Furthermore, a dormant population is maintained when cancer cells are cocultured with the mixture of MSCs and primary human umbilical vein endothelial cells (HUVECs), whereas coculture with MSCs alone does not.[4] The dormancy supportive effects of endothelial cells are canceled with an anti-TSP-1 antibody.[4] *In vivo* mouse and zebrafish models revealed that sprouting neovascular tips facilitate tumor growth, whereas dormant cancer cells are associated with the stable microvasculature, and *in vitro* culture studies demonstrated that this might be owing to the the high levels of TGF-β1 and periostin (POSTN) expressed by the sprouting endothelial tip cells.[4] These findings suggest that endothelial cells or pericytes serve as a potential metastatic niche that regulates tumor dormancy.

Nerve cells

It is accepted that the nervous system is also involved in tumor progression and metastasis. When PC3 cells are orthotopically implanted into immunocompromised mice, the development of nerves within the primary tumor is observed.[60] Chemical (6-hydroxydopamine, 6OHDA) or surgical denervation prevents development of the primary tumor and metastasis.[60] Moreover, when β_2- and β_3-adrenergic receptors are knocked down in mice, both primary tumor development and dissemination of PC3 and LNCaP cells are significantly impaired.[60] When sympathetic nerve ablation is initiated at a young age, tumor progression is inhibited in a spontaneous model of prostate cancer in which c-Myc is highly expressed in the prostate.[60] Interestingly, when cholinergic receptor muscarinic 1 (Chrm1) is chemically upregulated with carbamoylcholine chloride (carbachol), tumor dissemination to lymph node and bone is increased in both xenograft and spontaneous prostate cancer models, whereas if Chrm1 is blocked by a nonselective muscarinic antagonist, scopolamine, or is genetically ablated, tumor dissemination is inhibited, resulting in longer survival.[60] More importantly, the density of nerve fibers in the primary tumor is correlated with the clinical progression of human prostate cancer.[60]

Conclusions

Once cancer cells spread to distant organs such as bone, survival rates of cancer patients drastically decline. Each year, many patients who had been predicted to be cured of their cancer by surgery or radiation therapy present with incurable metastatic disease manifested as metastatic lesions in the bone, often years after primary treatment. DTCs derived from epithelial cancers, including prostate, breast, glioma and gastrointestinal cancer, have been detected in the bone marrow.[61–64] The presence of bone marrow DTCs has been correlated with a poor prognosis,[65] as DTCs frequently lead to lethal bone metastases.[66] This progression may also be environmentally influenced by pressures from the bone marrow microenvironment, or the 'metastatic niche'. Although the mechanisms are yet to be defined, it is generally believed that DTCs can become overt and clinically relevant metastases[63] that lead to disease recurrence even after treatment.[67] Therefore, new approaches to treat bone metastasis are urgently needed. For example, HSC mobilizing drug (for example, G-CSF and AMD3100) can be used to mobilize the niche-engaged dormant DTCs to re-enter the cell cycle.[68] Indeed, AMD3100 enhances the susceptibility to chemotherapy of acute myeloid leukemia[69] and multiple myeloma.[70] Recent clinical trials of adjuvant ZA have revealed that, when

DTCs only exist in the bone marrow of breast cancer patients, ZA improved disease-free survival and overall survival, suggesting that ZA interferes with the interaction of DTCs and the niche.[71,72] A recent study using xenograft breast cancer mouse models demonstrated that the hormonal status of the bone marrow microenvironment also influences tumor progression and ZA treatment effects on bone metastatic disease.[73] DTCs in mice given ovariectomies (OVX) grew better than those in sham-surgery-treated mice.[73] Moreover, ZA prevented OVX-induced tumor growth in the marrow, reflecting that antiresorptive therapy may be beneficial for postmenopausal breast cancer patients.[73] In contrast, ZA did not affect tumor growth in the marrow of sham-operated mice, suggesting that adjuvant ZA alone may not be a sufficient treatment in the premenopausal breast cancer setting.[73] Although further studies are clearly warranted, targeting the metastatic niche may be a promising treatment strategy for bone metastatic disease.

The concept that DTCs parasitize the harsh bone marrow microenvironment to grow and survive (**Figure 1**) is better understood, yet it remains an active area of investigation where some critical questions remain unanswered:

1. Do DTCs really parasitize the HSC niche?
2. Is the metastatic niche in the marrow the same as the HSC niche?
3. Can targeting the HSC niche serve as a potential therapy for bone metastatic disease?
4. Is it realistic to target a single cell type of the metastatic niche, especially in the marrow?
5. Do all cell types of the marrow interact to form the metastatic niche to support DTC survival? If so, how?
6. Does the metastatic niche control the chemoresistance of DTCs in the marrow?
7. How does the marrow metastatic niche influence tumor dormancy and recurrence?
8. Do the metabolic states of the metastatic niche affect the growth of DTCs?

To answer these questions, we must continue to place importance on the efforts to advance our understanding and technological capabilities, while developing a system or database to share new ideas and information with other experts in this field worldwide. Further multi-institutional research collaborations are absolutely paramount.

'Why does cancer recur even after a long disease-free interval?' This is a crucial question to answer if our goal is to cure cancer. DTCs shed from a primary tumor may lie dormant in distant tissues for long periods of time, all while retaining the potential to explode into metastatic growth with help from the bone marrow microenvironment. Therefore, it is vital to understand the interactions between DTCs and the bone marrow 'metastatic niche.' This review focuses on the fundamental mechanisms behind the provocative concept that the bone marrow microenvironment has a supportive role in bone metastasis. Observations discussed here are relevant to a more complete understanding of how this microenvironment functions in establishing DTCs, and what circumstances lead to dormancy and reactivation of DTCs in the marrow. Ultimately, it should be possible to develop targeted therapy for eradication of currently incurable bone metastatic disease by developing a deeper understanding of the cancer/niche interaction.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work is directly supported by the National Cancer Institute (CA093900 to R.S. Taichman, and CA163124 to Y. Shiozawa and R.S. Taichman), the Department of Defense (W81XWH-11-1-0636 to R.S. Taichman, and W81XWH-14-1-0403 to Y. Shiozawa and R.S. Taichman) and the Prostate Cancer Foundation (Y. Shiozawa and R.S. Taichman). Graphics adapted from Servier Medical Art (www.servier.com).

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