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Mechanisms of cancer cell metastasis to the bone: a multistep process

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Abstract

For metastasis to occur, tumor cells must first detach from their tissue of origin. This requires altering both the tissue of origin and the cancer cell. Once detached, cancer cells in circulation must also acquire survival mechanisms. Although many may successfully disseminate, variation exists in the efficiency with which circulating tumor cells home to and invade the bone marrow as metastatic seeds. Disseminated tumor cells that do successfully invade the marrow are secured by cell–cell and cell–extracellular matrix adhesion. However, establishing a foothold in the marrow is not sufficient for disseminated tumor cells to create metastases. A significant latent phase must be overcome by either rescuing cellular proliferation or attenuating micrometastatic mass dormancy programs. Finally, growing metastases fuel osteolysis, osteoblastogenesis and T-cell differentiation, creating a variety of tumor phenotypes. Each step in the metastatic cascade is rich in biological targets and mechanistic pathways.

Keywords

anoikis; autophagy; circulating tumor cells; disseminated tumor cells; dormancy; metastasis; osteoblastogenesis; osteolysis; survival

Lung, breast and prostate cancer share the characteristic of being among the most frequent sites of primary tumor development in human beings. Tumor cells from these three sites also share another characteristic: they all disseminate to and form metastatic lesions in the bone. The result is a high prevalence of bone metastases, which, by some estimates, are present in

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nearly 350,000 people dying annually in the USA alone [1]. Furthermore, bone metastases can cause chronic bone pain, hypercalcemia, pathologic fractures and, in some cases, debilitating nerve compression, which contributes significantly to the morbidity of patients with advanced disease [2]. The presence of bone metastases is also indicative of a poor prognosis, with only 20% of breast cancer patients presenting with bone metastasis surviving more than 5 years [3] and a significant elevation in the 1-year mortality rate among prostate cancer patients with bone disease as compared with patients with no skeletal involvement [4].

Given the clear prevalence and clinical significance of bone metastases, understanding of the mechanisms exploited by cancer to form these pernicious lesions is the topic of intense research focus among cancer and bone biologists. While advances have been made, much of the process remains an unsolved puzzle. This is partly because metastasis to the bone is not so much a single process as it is a series of sequential processes requiring cancer cells to employ new mechanisms at each stage [5]. In this article, we review the existing understanding of the mechanisms involved in the metastasis of cancer to bone while highlighting questions that will require further inquiry.

Dissemination

The first step in metastasis formation is the successful escape of cancer cells from their primary tumor. This requires both extravasation of cancer cells from the tumor and survival in the circulation. While these events occur far from the eventual site of bone metastasis formation, they are critical steps in the metastatic process that impose significant selective pressure on tumor cells. Therefore, the mechanisms employed during these early stages of metastasis are fundamental to the biology of tumor cells that eventually serve as bone metastatic seeds.

A variety of factors contribute to the ability of metastatic cancer cells to escape that can be placed into two categories: changes in the stromal tissue associated with tumors and changes in the tumor cells themselves. Fibroblasts associated with solid tumors (or cancer-associated fibroblasts [CAFs]) frequently acquire an activated myofibroblastic phenotype in response to physical contact with tumor cells, elevated levels of secreted growth factors including EGF, FGF and IGF, and hypoxia resulting from aberrant tumor metabolism. In turn, the activated fibroblasts produce elevated levels of matrix metalloproteinases and remodel the extracellular matrix (ECM) of tumors. Cancer cells and activated fibroblasts also secrete elevated levels of VEGF- and CXCL-family chemokines, such as CXCL12 and CCL2, which results in the creation of gradients that actively recruit leukocytes and endothelial cells into the tumor microenvironment [6]. Similar to CAFs, leukocytic infiltrates frequently differentiate into a tumor-associated macrophage (TAM) phenotype that produces angiogenic growth factors, vascular progenitor-recruiting chemokines and matrix metalloproteinases contributing to ECM remodeling [7]. Matrix remodeling in tumors is believed to enhance both the development of tumor vasculature and the dissociation of tumor cells from their native tissue [6]. At the same time, recruited vascular cells form an aberrant tumor vasculature, with vessels that demonstrate elevated permeability and express cell adhesion molecules (CAMs) that allow tumor cell intravasation [8,9]. It has also been suggested that tumors develop new and atypical lymphatic vessels mediated, in part, by VEGF-C produced by tumor cells, CAFs and TAMs [10]. Although controversial, this would suggest a mechanism may exist for tumor cells to metastasize through the lymphatic as well as systemic circulation (Figure 1).

While tissue remodeling creates a microenvironment permissive of cancer cell escape, changes in the tumor microenvironment may also combine with changes in the genetic

landscape of transformed cells to promote metastasis. Through a combination of genetic [11] and epigenetic alterations [12], the transcriptomes of metastatic cancer cells are altered such that molecular phenotypes permissive of tissue detachment, cytoskeletal motility and chemotaxis emerge. This promigration phenotype is enhanced by TAMs, which produce the tumor cell migration-stimulating factors CXCL12, IL-6 and TNF [13]. In addition, CAFs produce high levels of TGF- β [6] – a potent inducer of the epithelial–mesenchymal transition, which confers significant invasive potential to metastatic cells [14].

Survival in transit

Cancer cells that disseminate but die in circulation do not constitute metastatic seeds. The mechanical and biochemical environment of the circulation is vastly different from the epithelial tissues in which metastatic carcinomas originate and represent a significant challenge for cancer cells to survive. In addition, the epithelial tissues in which carcinomas originate demonstrate anoikis, the unique property of inducing programmed cell death in cells that successfully become fully detached from their native tissue. Therefore, activation of survival mechanisms is a critical pre-requisite to efficient bone metastasis formation.

Apoptosis is regulated by a combination of cytochrome-C-driven mitochondrial effectors and FADD-driven death-receptor effectors. In both cases, regulation is mediated by a combination of proapoptotic and antiapoptotic proteins. As reviewed by Igney and Krammer, cancer cells often overexpress the antiapoptotic mitochondrial effectors Bcl2, Bcl-XL and Mcl1 while underexpressing or developing inactivating mutations of the proapoptotic mitochondrial effectors Bax, Apaf1 and caspases [15]. The result is a molecular state that favors survival over apoptosis under conditions that would otherwise induce programmed cell death in untransformed cells. Cancer cells have also demonstrated the ability to alter proteosomal degradation of Mcl-1, leading to accumulation of this antiapoptotic protein as a mechanism for diminishing anoikis sensitivity [16]. With respect to the FADD-driven death-receptor pathway, malignant cells demonstrate inactivation of this arm of apoptosis signaling by overexpression of FLIP [17], a protein that prevents downstream caspase activation by binding and sequestering FADD (Figure 2).

In addition to apoptosis and anoikis resistance, cancer cells in circulation may also use autophagy as a survival mechanism. While nutrients do travel through circulation, they are also unloaded to metabolically active tissues. Therefore, it is expected that cancer cells in circulation will experience regions of venous circulation where nutrients are deficient enough to cause death by metabolic starvation. Autophagy is activated by diminished activity of mTOR-signaling and other amino acid signaling pathways [18], which constitutes a mechanism by which cancer cells can detect and respond to nutrient-deficient conditions to extract critical nutrients from endocytic contents when extracellular resources may not suffice. However, autophagic death is also activated by the same process and, therefore, requires cancer cells to inhibit a second form of death machinery. It has been demonstrated that cancer cells can utilize monocyte chemokines to activate Survivin-driven survival programs under autophagic conditions [19]. Although not fully explored, other mechanisms for attenuating autophagic death, while exploiting its survival benefits under nutrient-deficient conditions, are also likely to contribute to cancer cell survival in circulation.

Homing to & seeding the bone marrow

Not all cancer cells that successfully escape their native tissue and survive in circulation go on to seed metastases. Instead, variations in metastatic efficiency exist that determine whether a cell that survives in circulation goes on to seed tumors at particular distant sites. Advances in circulating tumor cell enumeration have provided a quantitative assessment of metastatic efficiency. For instance, the number of circulating tumor cells per milliliter of

blood in ovarian cancer patients may reach nearly ten-times the number in prostate cancer patients [20]. Yet, bone metastases are rare in ovarian cancer and frequent in patients with prostate cancer. This suggests that there are tumor cells that are capable of escaping and surviving in circulation but incapable of efficiently invading and seeding secondary growths in the bone marrow.

At the turn of the 20th century Paget and Ewing proposed two seemingly contradictory views of how tumor cells seed secondary sites to form metastases. Ewing proposed that the process was primarily physical, causing highly perfused tissues with dense vasculatures favoring embolization to be more likely to serve as metastatic sites [21]. Paget hypothesized that both the seed (cancer cells) and the soil (the secondary site) contributed to the proclivity of cancer cells to take root in specific tissues for metastasis formation [22]. While bone marrow is the site of blood production and possesses an accordingly well perfused and dense vasculature, there are, in fact, biological attributes of the bone marrow that make it a 'favorable soil' for some cells but not necessarily others. The seeding of bone metastases accordingly transpires in a manner consistent with Paget's notion that cancer cells metastasizing to this tissue do so because they have a unique biological proclivity for the tissue. This proclivity is controlled by a variety of factors that determine the extent to which cancer cells are adept at engaging and communicating with the bone marrow (Figure 3).

An important first step in establishing communication between metastasizing cancer cells and the bone marrow is the exit of cancer cells from the vasculature once in the marrow cavity. The chemokine, CXCL12, and its receptors CXCR4 and CXCR7, have demonstrated a driving role in this process. While CXCL12 is also known for its role as a ligand promoting chemotaxis of endothelial cells [23], it is a potent regulator of homing between blood and bone marrow for hematopoietic progenitors [24]. Bone metastatic cancer cells appear to mimic this process by expressing CXCR4 and CXCR7, which allows them to respond to chemotactic gradients of CXCL12 and mimic the vascular exit strategy employed by hematopoietic progenitors returning to the bone marrow from circulation [25,26].

As reviewed recently by Schneider [27], expression of the integrin $\alpha 5 \beta 3$ on bone metastatic cancer cells mediates their adhesion to the vitronectin, osteopontin, bone sialoprotein, fibronectin and thrombospondin components of the ECM in bone marrow while $\alpha 4 \beta 1$ integrin expression permits cancer cell engagement of fibrinogen, ICAM and VCAM expressed by the vascular and stromal cells of bone marrow. In addition to serving as physical anchors permitting metastatic cells to establish footholds in the bone marrow, integrin engagement prompts focal adhesion complex formation, which activates the focal adhesion kinase and src kinase pathways upstream of survival and proliferation programs [28].

The Notch–Jagged receptor–ligand pair may also be involved in the interaction between metastatic cancer cells and bone marrow cells derived from progenitors of both the mesenchymal and hematopoietic lineages. It has been shown that the ligand, Jagged, is overexpressed by bone metastatic tumor cells [29] whereas Notch is a receptor frequently expressed by progenitors and mature cells in the bone marrow [30]. Notch–Jagged interactions initiates signal transduction pathways in addition to mediating cell–cell adhesion, causing this interaction to serve as both a facilitator of engagement and communication. A recent study demonstrates that metastatic cancer cells exploit Notch–Jagged interactions to activate biological responses in both hematopoietic lineage-derived osteoclasts and mesenchymal lineage-derived osteoblasts to promote both tumor cell invasion of ossified bone and tumor cell growth in the bone [31]. The intracellular pathways regulating these activities are likely to emerge as Notch–Jagged interactions between metastatic cancer and bone marrow cells are studied further.

It has also been demonstrated that bone metastasis-forming cancer cell lines express the receptor for annexin II, a protein expressed on the surface of and utilized by osteoblasts adhering to hematopoietic stem cells [32]. This study also demonstrated that neutralization of annexin II with monoclonal antibodies significantly diminished the take of bone-homing prostate cancer cells in an intracardiac injection murine model of metastasis and that cancer cells treated with annexin II activate mitogenic ERK signaling. Together, these findings suggest that annexin II interaction mediates adhesion of tumor cells to osteoblasts in the bone marrow while supporting tumor cell proliferation.

Given the number of engagement factors involved in homing and seeding of bone metastatic cancer cells, the notion of spatial distribution is one worthy of consideration. Specifically, bone marrow as a tissue is less a homogenous mix than it is a heterogeneous topology in which some locations may possess a plurality of the factors described while other locations may possess few or none. Viewing the bone marrow this way gives rise to the notion that unique spaces, or niches, that are distinctly susceptible and targeted by tumor cells to establish metastatic footholds may exist within the bone marrow. This concept of a premetastatic niche was recently tested using preclinical models of prostate cancer metastasis, which demonstrate that prostate cancer cells may target and invade niches that otherwise would be occupied by hematopoietic stem cells [33]. Implicit in this observation is the prospect of competition between blood progenitors and bone metastatic prostate cancer. Therefore, mechanisms used by prostate cancer to subvert hematopoietic stem cells – although not well understood – may also contribute to the seeding of bone metastasis. It also remains to be seen whether metastatic cell invasion of the stem cell niche is a feature that is unique to prostate cancer or a common attribute among bone marrow-disseminating cancer cells, regardless of their origin.

Metastasis formation by disseminated tumor cells

Disseminated tumor cells (DTCs) have been observed and extracted from the bone marrow of breast [34] and prostate [35] cancer patients. In both diseases, DTCs are present in the bone marrow of patients with early-stage disease. From a selection point of view, these cells represent a unique subset of tumor cells that successfully disseminate, survive in circulation, home in on and invade the bone marrow, and manage to survive after establishing a foothold. Therefore, from an evolutionary perspective, one may expect these cells to aggressively form tumors within the bone marrow. However, this is not the case with recurrence after curative treatment of primary disease taking years to develop [34,35]. The prolonged development of overt metastatic disease also contrasts with biochemical serum biomarker evidence suggesting that, once developed, metastatic disease doubles in size at rates that are comparable to, if not greater than, primary disease [36]. This suggests a biphasic process of tumor formation by DTCs with an initial latent or dormant phase followed by an aggressive active phase representing what is seen in patients with end-stage disease. A total of two explanations have emerged that help to explain the apparent biphasic kinetics, referred to as proliferative dormancy and mass dormancy, respectively [37].

The proliferative dormancy concept is predicated on the notion that early DTCs exist as metabolically active survival-driven cells that have somehow suppressed or exited the cell cycle [37]. This results in the observed latent phase of disseminated disease as dormant DTCs survive and persist but do not proliferate. Mechanisms contributing to the proliferative dormancy of DTCs are poorly understood, as are mechanisms contributing to dormancy abrogation. In the context of bone-engaged DTCs, it has been demonstrated that GAS6 is secreted by human primary osteoblasts and functions to induce cell cycle arrest, leading to diminished proliferation in prostate cancer cell lines expressing the cell surface receptor Axl [38]. Recently, SMAD-4 signaling downstream of TGF- β , a morphogenetic

factor richly expressed by bone marrow stromal cells, was demonstrated to suppress cyclin D in PTEN-deficient prostate cancer models [39]. The same study demonstrated aggressive cellular proliferation and metastatic tumor growth in models with *SMAD-4* deletion, nominating SMAD signaling as a functional regulator of TGF- β -induced dormancy. In addition, in a recent study, cocultures of breast cancer cells with bone marrow stromal cells (BMSCs) demonstrated the formation of connexin-43-mediated gap junctions allowing the exchange of cytosolic molecules between BMSCs and breast cancer cells [40]. Breast cancer cells in these coculture experiments demonstrated G0/G1 cell cycle arrest that may be mediated by miRNAs transferred to cancer cells through the gap junctions that they form with BMSCs [41].

Each of these examples suggests a role for the cells and soluble factors present in bone marrow stroma as drivers of proliferative dormancy. This suggests that DTCs must adapt to the dormancy-inducing microenvironment of the marrow in order to form overt metastatic lesions. Work in melanoma cell lines suggests that tumor cells enter proliferative arrest in response to environmental stress when activation of the mitogenic ERK kinase is outpaced by p38-mediated stress-response pathways [42]. As reviewed by Horak *et al.*, a number of metastasis suppressor genes including *KISS1*, *CSRP3*, *Kail*, *MKK4*, *MKK7*, *MKK6*, *Nm23* and *RKIP* also suppress tumor cell proliferation by inhibiting ERK-activity, activating p38 signaling, or both [43]. The implication of these findings is that proliferative states can be rescued by DTCs that adapt methods for activating ERK or suppressing p38 in the bone microenvironment. Certainly, further inquiry into the genetic and epigenetic molecular basis for the development, persistence and eventual abrogation of proliferative dormancy in DTCs is needed (Figure 4).

By contrast, the ‘mass dormancy’ explanation of latency is an attempt to explain the latent phase through the existence of multicellular micrometastases that persist but do not grow [44]. The central tenet of this hypothesis is that micrometastatic foci, formed by DTCs, experience cell death at a rate equivalent to the proliferation rate of their constituent DTCs. The result is a stable but minimal residual disease burden that, at some point, must overcome its cell death rate, develop enhanced proliferation rates, or both, to form clinically overt metastases. Seminal work by Folkman and colleagues demonstrated the viability of this idea in a murine model of spontaneously disseminating Lewis lung cancer [45]. In their study, dormant micrometastases formed in animals when subcutaneous primary tumors were left intact whereas overt tumors grew in animals whose tumors were removed. Dormant micrometastases in this model had equivalent cellular proliferation rates as overt tumors when assayed for bromodeoxyuridine uptake. At the same time, nearly three-times the amount of apoptosis was observed in micrometastases, suggesting latency in this model was consistent with mass dormancy.

This seminal study also demonstrated that overt metastases in mice, whose primary tumors had been resected, were significantly more vascular than micrometastases in mice whose primary tumors remained intact. It is in this context that the concept of an angiogenic switch for dormancy abrogation has been proposed, which claims that mass dormancy in micrometastases is overcome by neovascularization [46]. However, the perioperative activation of tumor angiogenesis demonstrated in murine models [45] would suggest that micrometastases experiencing mass dormancy through this mechanism are likely to escape shortly after primary tumor resection. This is contradictory to what is seen in clinical micrometastatic disease, where periods of latency generally last several years following excision of primary tumors [34,35,37,44]. The discrepancy may be due to employment of a combination of dormancy mechanisms by clinical micrometastases.

More broadly, DTC dormancy creates a variety of clinical challenges to understanding and treating bone metastases. It is not standard practice to evaluate bone marrow aspirates prior to biochemical relapse for the presence of early disseminated cells. It is also not clear whether doing so to identify patients with dormant DTCs will help identify patients at higher risk for overt metastatic disease. Meanwhile, not looking may lead to a missed window of opportunity to target disseminated disease prior to manifestation of the lethal phenotype. However, much of what we have learned regarding dormancy biology is dependent on the context of the preclinical models employed. The dearth of molecular pathology studies comparing clinical DTCs from patients in the latent phase of disseminated disease to overt secondary growths found in patients with active metastatic disease severely confounds the discovery and development of functional targets that are present in *Homo sapiens* to exploit for therapeutic benefit during this unique window. Future clinical endeavors targeting this question will likely shed much needed light on the mechanistic underpinnings of clinical DTC dormancy and perhaps nominate therapeutic strategies to target metastatic disease before the lethal phenotype sets in.

Clonality & stem cells in metastasis formation

In addition to the challenge of overcoming dormancy, the efficiency of metastasis formation may also be limited by the need for DTCs to come from a clonal origin capable of forming tumors. While high-resolution genomic analysis of DTCs is encumbered by the small number of cells obtained from clinical marrow aspirates, a modified comparative genomic hybridization-array approach has been successfully employed by Holcomb and associates to identify copy number variations (CNVs) present in DTCs [47]. The authors found that DTCs were isolable from patients clinically diagnosed with metastatic as well as organ-confined prostate cancer. In addition, CNVs, in the DTCs of both patient populations, were highly concordant with CNVs found in patient-matched primary tumor cells, suggesting that the DTCs did, in fact, originate from the primary tumor. The modified comparative genomic hybridization array analysis also revealed that DTCs from patients diagnosed with metastatic disease had a wider panel of CNV aberrations than those obtained from patients with organ-confined disease. This suggests that, while less aberrant tumor cells may disseminate to and lodge in the bone marrow, the ability to form metastases is limited to a clonal subset of cancer cells that disseminate after having accumulated enough genetic lesions for outgrowth at the secondary site. Consistent with this possibility is the observation by Holcomb that regions containing known metastasis suppressor genes *KAI1* and *MKK4* were frequently deleted in DTCs from patients diagnosed with metastatic disease but not in DTCs from patients diagnosed with clinically localized primary tumors.

A separate study by Liu and colleagues analyzed the CNV profiles of 94 anatomically distinct tumors obtained at autopsy from 30 men who succumbed to prostate cancer [48]. The study found that unsupervised hierarchical clustering of CNV profiles for 80 samples obtained from 24 subjects led to a perfect clustering of samples by the subjects from which tumors were obtained in 63% of cases. The implication of this finding is that anatomically distinct tumors within patients may have monoclonal origins. This is particularly remarkable when considering that primary prostate cancers present are multi-focal [49] and possess a heterogeneous collection of genetic clones [50]. Consistent with Holcomb's analysis of DTCs, these findings in overt metastatic lesions also suggests that only a unique clonal subset of primary cancer cells are capable of forming overt lesions following dissemination.

A possible explanation for the clonality of metastasis is the cancer stem cell hypothesis. The ability to self-renew, differentiate and regrow organs are characteristics that define somatic stem cells. As reviewed by Visvader and Lindeman, rare subpopulations of cells may exist in somatic cancers that possess three characteristics; self-renewal, an ability to develop into

any cell constituting a member of the tumor cell population and the proliferative potential to grow tumors [51]. Therefore, the cancer stem cell hypothesis suggests that metastatic lesions result from DTCs possessing the ‘stemness’ necessary to regenerate lesions. Accordingly, only those genetic aberrations present in the cancer stem cell population of the primary tumor will propagate to anatomically distinct overt metastases, which may explain the monoclonal origins observed by Liu. However, this contrast with the implication of Holcomb’s findings, which suggest that primary tumors will form and disseminate clones containing few genomic aberrations but only those with more significant alterations form clinically overt metastases. Certainly, more work is needed to resolve whether apparent clonality of metastases results from dissemination of stem cells, selection favoring outgrowth of DTCs possessing a minimum threshold of unique genetic alterations, or both.

Types of bone metastatic tumors

Leo Tolstoy began his 1873 work; ‘Anna Karenina’, with the line, “Happy families are all alike; every unhappy family is unhappy in its own way” [52]. While the subject of Tolstoy’s work is vastly different from that of this article, the underlying premise nevertheless applies. Normal bone marrow exists as a tissue with a defined architecture that is maintained by regulated turnover of its blood-forming and bone-forming compartments. By contrast, tumors developing from metastatic disseminations to the marrow exhibit a range of phenotypes with each type being driven by a unique set of mechanistic drivers.

A clear case for distinct phenotypes of bone metastatic lesions is presented by the existence of osteolytic (bone resorbing) and osteoblastic (bone forming) tumors. A third category of lesions is clinically evident in which a mixture of the two phenotypes is seen. The existence of mixed lesions suggests that the processes that regulate tumor-associated osteolysis and tumor-induced bone formation may occur together in bone metastasis and are not mutually exclusive activities. Furthermore, the relative activity of these two coexisting processes defines the global phenotype that a metastatic lesion ultimately adopts. Nevertheless, the mechanisms for driving tumor-associated osteolysis and tumor-induced bone formation are presented separately (Figure 5).

Osteolytic metastases are a consequence of tumor-induced activation of bone-matrix resorption. Resorption of mineralized bone matrix is the natural function of osteoclasts, a multinucleated cell of hematopoietic origin residing in the bone. As reviewed by Roodman, tumor cell production of IL-1, IL-6, MIP1 α or RANK ligand (RANKL) can activate osteoclastogenesis, a process involving the fusion of mononuclear macrophage-like osteoclast precursors into multinucleated cells [6]. These stimuli are transduced in osteoclast precursors by a combination of JUN kinase, NF- κ B and calcineurin–NFAT pathways, which attenuate downstream transcriptional networks that regulate osteoclast maturation [53]. It has also been shown that IL-8 and CCL2 can induce precursors to undergo osteoclastogenesis through a RANKL-independent mechanism [54].

In addition to stimulating the production of new osteoclasts, bone metastatic cancer cells co-opt cells of the bone marrow into activating bone resorption programs in existing osteoclasts. Specifically, the production of prostaglandins, parathyroid hormone, parathyroid hormone-related peptide, activated vitamin D, IL-6 and TNF by cancer cells may lead to tumor-induced increases in RANKL expression on osteoblasts and bone marrow stromal cells [6]. A less studied possibility involves the co-opting of T cells, which are ubiquitous in bone marrow but not present in immune-deficient animal models, to produce these osteoclast-activating secretions. In particular, tumor cell-derived IL-6, IL-1 and TGF- β can drive T-cell differentiation towards a Th17 secretory helper-cell phenotype capable of inducing osteoblastic RANKL and osteoclast activation through IL-17 production [55]. T

cells may also be under-appreciated direct inducers of metastasis. Specifically, it was recently demonstrated that RANKL–RANK interaction between helper T cells and breast cancer cells promotes invasion, dissemination and metastasis formation from orthotopic syngeneic mouse mammary tumor virus–ErbB2 tumors in immunocompetent mice [56]. Recent work on Notch–Jagged interactions in the bone marrow suggests direct activation of osteolysis by cancer cells through this unique interaction [31].

As reviewed by Logothetis and Lin, osteoblastic metastases are prevalent in advanced prostate cancer patients and induced by cancer cell interactions with osteoblasts and their progenitors by production of TGF- β , bone morphogenetic protein, IGF, FGF and WNTs [57]. Osteoblasts respond to morphogenetic factors by activating SMAD signaling, growth factors by MAPK and PKC signaling; and to WNT by acting β -catenin-regulated pathways. These pathways converge on and interact with the RUNX2 transcriptional network, which drives osteoblastic differentiation and proliferation. Cancer-induced WNT signaling in osteoblasts may also be mediated by lymphoid enhancer binding/T-cell factor transcriptional networks downstream of β -catenin dimerization with lymphoid enhancer binding/T-cell factor [58,59]. Prostate cancer cells also demonstrate osteomimicry by responding to growth factor stimulation via activation of Cbfa, MSX and other osteoblastic transcription factors [60]. This would suggest that bone-forming tumors may also occur through differentiation of the cancer cells towards an osteoblastic bone-forming phenotype, which is a phenomenon that has been observed in the bone metastatic prostate cancer cell-line, C4-2b [61].

A minimally studied category of cancer and bone interactions likely to contribute to metastatic tumor phenotype are those driven by steroid hormones. Given the predilection of prostate and breast cancers – both steroid-sensitive diseases – to form bone metastasis, there may be great value in understanding the extent to which mesenchymal and hematopoietic lineages of the bone marrow participate in metabolic interconversion and *de novo* synthesis of steroid hormones. In addition, it has been shown that hormone-sensitive prostate cancer cells can respond to steroid deprivation by activating *de novo* synthesis [62], which implies that bone cells interacting with metastatic cancer may be stimulated by steroids produced locally by tumor cells. Research targeting the steroid-driven responses of blood-forming and bone-forming cells in the marrow may accordingly represent a largely unexplored area of high-value investigation.

Future perspective

It was Socrates who first observed that, “The more I learn, the more I learn how little I know.” This certainly holds with respect to our understanding of the molecular underpinnings of bone metastasis. An appreciation for the multistep nature of this process permits tackling the research challenges associated with bone metastatic disease in a structured manner. However, the more we learn about the processes involved at each step, the more apparent the intricacies of the course taken by cancer cells to form bone metastases become. As more processes and mechanisms are identified, significant efforts will be needed to determine which constitute truly limiting steps to the efficiency of metastasis formation. Doing so will permit translational research efforts to better target experimental therapeutics against the drivers of metastatic disease.

Discovery-driven research will also be needed to fill substantial gaps in our understanding. This is particularly evident with respect to the molecular biology of premetastatic latency observed in early bone marrow disseminations and the eventual abrogation of dormancy programs leading to overt bone disease. Animal models recapitulating progression from primary disease by early dissemination, a prolonged latent phase and spontaneous bone metastasis formation will likely assist in identifying and testing the mechanistic

underpinnings of this process. Molecular pathology studies comparing overt bone lesions with micrometastatic disease obtained from clinical marrow biopsies will likely be informative. Similarly, little is presently known about the nature of cancer–bone interactions from a steroid-driven point of view. An understanding of steroid-driven interactions between tumor cells and both the bone-forming and blood-forming compartments of the bone marrow is likely to play a substantial role in our emerging understanding of the mechanisms of bone metastasis.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

1. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer*. 1987; 55(1):61–66. [PubMed: 3814476]
2. Gralow JR, Biermann JS, Farooki A, et al. NCCN Task Force Report: bone health in cancer care. *J Natl Compr Canc Netw*. 2009; 7(Suppl 3):S1–S32. [PubMed: 19555589]
3. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev*. 2001; 27(3):165–176. [PubMed: 11417967]
4. Norgaard M, Jensen AO, Jacobsen JB, Cetin K, Fryzek JP, Sorensen HT. Skeletal related events, bone metastasis and survival of prostate cancer: a population based cohort study in Denmark (1999 to 2007). *J Urol*. 2010; 184(1):162–167. [PubMed: 20483155]
5. Pienta KJ, Loberg R. The “emigration, migration, and immigration” of prostate cancer. *Clin Prostate Cancer*. 2005; 4(1):24–30. [PubMed: 15992458]
6. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med*. 2004; 350(16):1655–1664. [PubMed: 15084698]
7. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory microenvironment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol*. 2008; 66(1):1–9. [PubMed: 17913510]
8. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science*. 2005; 307(5706):58–62. [PubMed: 15637262]
9. Blood CH, Zetter BR. Tumor interactions with the vasculature: angiogenesis and tumor metastasis. *Biochim Biophys Acta*. 1990; 1032(1):89–118. [PubMed: 1694687]
10. Pepper MS. Lymphangiogenesis and tumor metastasis: myth or reality? *Clin Cancer Res*. 2001; 7(3):462–468. [PubMed: 11297234]
11. Nguyen DX, Massague J. Genetic determinants of cancer metastasis. *Nat Rev Genet*. 2007; 8(5):341–352. [PubMed: 17440531]
12. Rodenhiser DI. Epigenetic contributions to cancer metastasis. *Clin Exp Metastasis*. 2009; 26(1):5–18. [PubMed: 18386135]
13. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory microenvironment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol*. 2008; 66(1):1–9. [PubMed: 17913510]
14. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev*. 2009; 28(1–2):15–33. [PubMed: 19169796]
- 15▪. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer*. 2002; 2(4):277–288. A comprehensive review of cell death pathways and survival mechanisms exploited by cancer. [PubMed: 12001989]
16. Woods NT, Yamaguchi H, Lee FY, Bhalla KN, Wang HG. Anoikis, initiated by Mcl-1 degradation and Bim induction, is deregulated during oncogenesis. *Cancer Res*. 2007; 67(22):10744–10752. [PubMed: 18006817]
17. Mawji IA, Simpson CD, Hurren R, et al. Critical role for Fas-associated death domain-like interleukin-1-converting enzyme-like inhibitory protein in anoikis resistance and distant tumor formation. *J Natl Cancer Inst*. 2007; 99(10):811–822. [PubMed: 17505076]

- 18*. Codogno P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. *Cell Death Differ.* 2005; 12(Suppl 2):1509–1518. Describes survival responses triggered by nutrient deprivation and autophagy regulation. [PubMed: 16247498]
19. Roca H, Varsos ZS, Mizutani K, Pienta KJ. CCL2, survivin and autophagy: new links with implications in human cancer. *Autophagy.* 2008; 4(7):969–971. [PubMed: 18758234]
20. He W, Kularatne SA, Kalli KR, et al. Quantitation of circulating tumor cells in blood samples from ovarian and prostate cancer patients using tumor-specific fluorescent ligands. *Int J Cancer.* 2008; 123(8):1968–1973. [PubMed: 18661519]
21. Ewing J. *Neoplastic Diseases: A Treatise on Tumors.* WB Saunders Co; Philadelphia & London: 1928.
22. Paget S. The distribution of secondary growths in cancer of the breast. *Lancet.* 1889; 133(3421): 571–573.
23. Salcedo R, Oppenheim JJ. Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. *Microcirculation.* 2003; 10(3–4):359–370. [PubMed: 12851652]
24. Broxmeyer HE, Orschell CM, Clapp DW, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med.* 2005; 201(8):1307–1318. [PubMed: 15837815]
25. Wang J, Shiozawa Y, Wang Y, et al. The role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer. *J Biol Chem.* 2008; 283(7):4283–4294. [PubMed: 18057003]
26. 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. *Cancer Res.* 2002; 62(6):1832–1837. [PubMed: 11912162]
27. Schneider JG, Amend SR, Weilbaecher KN. Integrins and bone metastasis: integrating tumor cell and stromal cell interactions. *Bone.* 2011; 48(1):54–65. [PubMed: 20850578]
28. Giancotti FG, Ruoslahti E. Integrin signaling. *Science.* 1999; 285(5430):1028–1032. [PubMed: 10446041]
29. Santagata S, Demicheli F, Riva A, et al. *JAGGED1* expression is associated with prostate cancer metastasis and recurrence. *Cancer Res.* 2004; 64(19):6854–6857. [PubMed: 15466172]
30. Chiba S. Notch signaling in stem cell systems. *Stem Cells.* 2006; 24(11):2437–2447. [PubMed: 16888285]
31. Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell.* 2011; 19(2): 192–205. [PubMed: 21295524]
32. Shiozawa Y, Havens AM, Jung Y, et al. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J Cell Biochem.* 2008; 105(2):370–380. [PubMed: 18636554]
- 33*. Shiozawa Y, Pedersen EA, Havens AM, et al. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. *J Clin Invest.* 2011; 121(4):1298–1312. Comprehensive preclinical study demonstrating that prostate cancer targets the stem cell niche of hematopoietic stem cells *in vivo* when seeding the bone marrow and uses similar mechanisms as hematopoietic stem cells to home to and invade the niche. [PubMed: 21436587]
34. Janni W, Vogl FD, Wiedswang G, et al. Persistence of disseminated tumor cells in the bone marrow of breast cancer patients predicts increased risk for relapse – a European pooled analysis. *Clin Cancer Res.* 2011; 17(9):2967–2976. [PubMed: 21415211]
35. Morgan TM, Lange PH, Porter MP, et al. Disseminated tumor cells in prostate cancer patients after radical prostatectomy and without evidence of disease predicts biochemical recurrence. *Clin Cancer Res.* 2009; 15(2):677–683. [PubMed: 19147774]
36. Abouassaly R, Paciorek A, Ryan CJ, Carroll PR, Klein EA. Predictors of clinical metastasis in prostate cancer patients receiving androgen deprivation therapy: results from CaPSURE. *Cancer.* 2009; 115(19):4470–4476. [PubMed: 19637339]
37. Vessella RL, Pantel K, Mohla S. Tumor cell dormancy: an NCI workshop report. *Cancer Biol Ther.* 2007; 6(9):1496–1504. [PubMed: 17881897]

38. Shiozawa Y, Pedersen EA, Patel LR, et al. GAS6/AXL axis regulates prostate cancer invasion, proliferation, and survival in the bone marrow niche. *Neoplasia*. 2010; 12(2):116–127. [PubMed: 20126470]
39. Ding Z, Wu CJ, Chu GC, et al. SMAD4-dependent barrier constrains prostate cancer growth and metastatic progression. *Nature*. 2011; 470(7333):269–273. [PubMed: 21289624]
40. Moharita AL, Taborga M, Corcoran KE, Bryan M, Patel PS, Rameshwar P. SDF-1 α regulation in breast cancer cells contacting bone marrow stroma is critical for normal hematopoiesis. *Blood*. 2006; 108(10):3245–3252. [PubMed: 16857992]
41. Lim PK, Bliss SA, Patel SA, et al. Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Res*. 2011; 71(5):1550–1560. [PubMed: 21343399]
42. Ranganathan AC, Adam AP, Zhang L, Aguirre-Ghiso JA. Tumor cell dormancy induced by p38SAPK and ER-stress signaling: an adaptive advantage for metastatic cells? *Cancer Biol Ther*. 2006; 5(7):729–735. Describes dormancy regulation as the balance of stress and proliferative pathway activity. [PubMed: 16861922]
43. Horak CE, Lee JH, Marshall JC, Shreeve SM, Steeg PS. The role of metastasis suppressor genes in metastatic dormancy. *APMIS*. 2008; 116(7–8):586–601. [PubMed: 18834404]
44. Wikman H, Vessella R, Pantel K. Cancer micrometastasis and tumour dormancy. *APMIS*. 2008; 116(7–8):754–770. [PubMed: 18834417]
45. Holmgren L, O'Reilly MS, Folkman J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat Med*. 1995; 1(2):149–153. Seminal study demonstrating dormant micrometastases displaying mass-dormancy occurring in the Lewis lung carcinoma murine model of spontaneous metastasis. [PubMed: 7585012]
46. Naumov GN, Akslen LA, Folkman J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch. *Cell Cycle*. 2006; 5(16):1779–1787. [PubMed: 16931911]
47. Holcomb IN, Grove DI, Kinnunen M, et al. Genomic alterations indicate tumor origin and varied metastatic potential of disseminated cells from prostate cancer patients. *Cancer Res*. 2008; 68(14):5599–5608. [PubMed: 18632612]
48. Liu W, Laitinen S, Khan S, et al. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. *Nat Med*. 2009; 15(5):559–565. Clinical molecular pathology study demonstrating anatomically disperse metastases are derived from monoclonal origins in patients who succumb to prostate cancer. [PubMed: 19363497]
49. Miller GJ, Cygan JM. Morphology of prostate cancer: the effects of multifocality on histological grade, tumor volume and capsule penetration. *J Urol*. 1994; 152(5 Pt 2):1709–1713. [PubMed: 7933231]
50. Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancers reveals genotypic heterogeneity. *Cancer Res*. 1998; 58(1):23–28. [PubMed: 9426051]
51. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer*. 2008; 8(10):755–768. [PubMed: 18784658]
52. Tolstoy, L. Anna Karenina. John Lane Company; NY, USA: 1911.
53. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. *Nat Rev Genet*. 2003; 4(8):638–649. [PubMed: 12897775]
54. Mizutani K, Sud S, Pienta KJ. Prostate cancer promotes CD11b positive cells to differentiate into osteoclasts. *J Cell Biochem*. 2009; 106(4):563–569. [PubMed: 19170075]
55. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med*. 2009; 361(9):888–898. Reviews the underappreciated topic of T-cell interactions with osteoclasts and osteoblasts as regulators of bone biology. [PubMed: 19710487]
56. Tan W, Zhang W, Strasner A, et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL–RANK signalling. *Nature*. 2011; 470(7335):548–553. [PubMed: 21326202]
57. Logothetis CJ, Lin SH. Osteoblasts in prostate cancer metastasis to bone. *Nat Rev Cancer*. 2005; 5(1):21–28. [PubMed: 15630412]
58. Behrens J, Von Kries JP, Kuhl M, et al. Functional interaction of β -catenin with the transcription factor LEF-1. *Nature*. 1996; 382(6592):638–642. [PubMed: 8757136]

59. Qiang YW, Hu B, Chen Y, et al. Bortezomib induces osteoblast differentiation via Wnt-independent activation of β -catenin/TCF signaling. *Blood*. 2009; 113(18):4319–4330. [PubMed: 19196662]
60. Koeneman KS, Yeung F, Chung LW. Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. *Prostate*. 1999; 39(4):246–261. [PubMed: 10344214]
61. Lin DL, Tarnowski CP, Zhang J, et al. Bone metastatic LNCaP-derivative C4-2B prostate cancer cell line mineralizes *in vitro*. *Prostate*. 2001; 47(3):212–221. [PubMed: 11351351]
62. Cheng J, Wu Y, Mohler JL, Ip C. The transcriptomics of *de novo* androgen biosynthesis in prostate cancer cells following androgen reduction. *Cancer Biol Ther*. 2010; 9(12):1033–1042. [PubMed: 20404538]

Executive summary

Dissemination

- Stromal fibroblasts acquire an activated myofibroblastic phenotype, recruit tumor-associated macrophages and endothelial cells, and remodel the extracellular matrix.
- Tumor cells acquire genetic and epigenetic lesions that promote escape from their tissue of origin.
- Remodeling of the native tissue and formation of aberrant tumor vasculature facilitates tumor cell escape.

Survival in transit

- Overexpression of antiapoptotic effectors Bcl2, Bcl-XL and Mcl1 and underexpression of proapoptotic effectors Bax, Apf1 and caspase 8 empower the cell to survive.
- Reduced mTOR and amino acid signaling triggers autophagy for cancer cell survival.
- Autophagic death is attenuated by chemokines in circulation, surviving signaling and possibly other mechanisms.

Bone migration & engagement

- Chemotactic gradients of CXCL12 illuminate a vascular exit path.
- Integrin expression provides disseminated tumor cells (DTCs) with a foothold in the bone marrow.
- Notch–Jagged and Annexin II interactions also play a role in adhesion of tumor cells to osteoblasts.
- Interactions providing DTCs with a foothold also support their mitogenic activity.

Metastasis formation by disseminated tumor cells

- A latent phase exists after seeding but prior to bone tumor formation.
- Proliferative dormancy is a state in which cells are metabolically active but nonproliferative.
- Mass dormancy describes latent growth in micrometastases where a population of DTCs has equal cell death and proliferation rates.
- An angiogenic switch may abrogate dormancy perioperatively.
- Further investigation is very much needed in this area.

Types of bone metastatic tumors

- Cytokines secreted by DTCs stimulate osteoclastogenesis.
- DTCs also co-opt stromal and blood cells of the marrow to induce osteolysis.
- DTCs can activate osteomimicry pathways driven by CfbA, MSX and other osteoblastic transcription factors.
- DTCs also induce osteoblastogenesis resulting in bone formation.

- The balance of osteolytic and osteoblastic processes defines the overall phenotype adopted by bone metastatic lesions.

Figure 1. Dissemination is regulated by a combination of changes in the primary tumor microenvironment.

Figure 2. Cancer cells suppress cell death mechanisms and overcome nutrient deprivation to survive in circulation.

Figure 3. Bone metastatic cells use chemotaxis and cellular and extracellular adhesive interactions to home to and seed the bone marrow

AXII: Annexin II; AXIIR: Annexin II receptor; HSC: Hematopoietic stem cell.

Figure 4. Proliferative and mass dormancy models attempt to explain prolonged periods of latency prior to overt metastasis formation by disseminated tumor cells
DTC: Disseminated tumor cell.

Figure 5. A complex web of cancer–bone–T-cell interactions mediated by soluble factors, cell–cell interactions and downstream transcriptional networks that regulates osteolysis, osteoblastogenesis and the ultimate phenotype of bone metastatic lesions

BMP: Bone morphogenetic protein; DTC: Disseminated tumor cell; LEF: Lymphoid enhancer binding; PKC: Protein kinase C; PTH: Parathyroid hormone; PTHrP: Parathyroid hormone-related peptide; SMAD: Mothers against decapentaplegic homolog; TCF: T-cell factor; Vit D: Vitamin D; WNT: Wingless-type MMTV integration site family.