Molecular Mechanisms of Bone Metastasis and Associated Muscle Weakness

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Abstract

Bone is a preferred site for breast cancer metastasis and leads to pathologic bone loss due to increased osteoclast-induced bone resorption. The homing of tumor cells to the bone depends on the support of the bone microenvironment in which the tumor cells prime the premetastatic niche. The colonization and growth of tumor cells then depend on adaptations in the invading tumor cells to take advantage of normal physiologic responses by mimicking bone marrow cells. This concerted effort by tumor cells leads to uncoupled bone remodeling in which the balance of osteoclast-driven bone resorption and osteoblast-driven bone deposition is lost. Breast cancer bone metastases often lead to osteolytic lesions due to hyperactive bone resorption. Release of growth factors from bone matrix during resorption then feeds a “vicious cycle” of bone destruction leading to many skeletal-related events. In addition to activity in bone, some of the factors released during bone resorption are also known to be involved in skeletal muscle regeneration and contraction. In this review, we discuss the mechanisms that lead to osteolytic breast cancer bone metastases and the potential for cancer-induced bone-muscle cross-talk leading to skeletal muscle weakness.

Introduction

Bone metastases are common in patients with advanced malignancy. Primary tumors exhibit metastatic tropism to particular organs and the skeleton is a preferred site for breast cancer metastasis. Breast cancer that is metastatic to bone causes a significant imbalance in normal bone remodeling through perturbation of osteoclast-mediated bone resorption and osteoblast-mediated bone formation (1). Bone metastases are classified on the basis of radiographic appearance as either osteolytic or osteoblastic (osteosclerotic). Breast cancer is typically associated with osteolytic lesions, but most cases involve uncoupled components of both bone destruction and new bone formation. Bone metastases from breast cancer affect 65% to 80% of patients with advanced malignancy (2). Bone metastases cause severe bone pain and increased risk of pathologic fracture, hypercalcemia, and nerve compression syndromes that significantly reduce the quality of life (1). Perhaps most devastating is the fact that once the primary tumor has spread to the bone, it is incurable. The current standard of care for patients with bone loss due to osteolytic bone metastases includes antiresorptive therapy aimed at reducing skeletal-related events but is not curative with regard to tumor burden (1, 2).

A significant comorbidity of osteolytic bone metastases is muscle weakness and fatigue that is often associated with cancer cachexia. Cachexia is a common paraneoplastic syndrome that is characterized by severe wasting due to loss of both fat and lean body mass (3, 4). Although the age and chemotherapeutic treatment regimens of patients with advanced disease and bone metastases make it difficult to assess the true incidence of malignancy-induced muscle weakness (5), a clinical perspective suggests that many patients do experience severe muscle weakness and fatigue. Improving muscle function and mobility of patients with cancer would have a positive impact on adherence to treatment regimens and overall health (5). Therefore, a better understanding of the mechanism(s) of muscle weakness associated with bone metastases and cancer cachexia will lead to targeted therapeutics. Moreover, refocusing attention to determine muscle quality in addition to improving muscle mass will likely provide the most beneficial treatment options for this devastating complication of malignancy.

Molecular mechanisms of bone metastasis

The initiation and progression of bone metastasis are complex multistep processes. Tumor cells must detach from the primary tumor and enter the systemic circulation (invasion), evade detection by the immune system, and adhere to capillaries in the bone marrow leading to extravasation into the bone marrow space (6). Tumor cells in the bone first form micrometastases that can either develop into overt metastatic lesions or lay dormant for long periods.
before reactivating in the bone microenvironment. In either case, it is believed that the invading tumor cells prime the bone microenvironment by enriching the premetastatic niche (local environment) for further colonization and growth of tumor cells (Fig. 1; refs. 2, 7–9).

The hematopoietic system plays an important role in development of the premetastatic niche. The bone marrow may serve as a protective milieu for dormant tumor cells to resist chemotherapeutic attack, and tumor cells may use the same physiologic mechanisms as those used by hematopoietic stem cells (HSC) homing to bone (10, 11). In the premetastatic niche, the invading tumor cells prime the stroma by production of factors that elicit responses in cells of the bone microenvironment and make it conducive to tumor colonization and growth (2). In addition, bone resorption also regulates HSC homing (12). Factors derived from tumor cells include osteopontin (OPN), which promotes bone marrow cell migration and tumor cell proliferation (13, 14); heparanase (HPSE), which acts in the extracellular matrix to reduce heparin sulfate chain length leading to increased bone resorption (15); and parathyroid hormone-related protein (PTHrP), which promotes bone resorption (16) and may also enhance production of bone marrow chemokines such as C-C motif ligand 2 (17). Recently, it has also been shown that the sympathetic nervous system is also capable of stimulating stromal cells, thus promoting breast cancer bone metastasis (18).

Tumor cells invading the bone also express factors that facilitate further recruitment to the bone microenvironment, a process called osteotropism (19). αvβ3 integrin promotes adhesion of breast cancer cells in bone and is associated with bone metastasis (20). αvβ3 integrin also cooperates with bone sialoprotein and matrix metalloproteinase (MMP)-2 to promote tumor cell colonization in bone (21, 22). Receptor activator of NF-κB (RANK) mediates osteoclast-induced bone resorption and supports tumor cell colonization (23). The chemokine CXC ligand 12 (CXCL12; also known as stromal cell-derived factor 1 (SDF-1)) is a potent chemoattractant for HSCs and is highly expressed on osteoblasts and bone marrow stromal cells. Expression of its receptor, CXC receptor 4 (CXCR4), on cancer cells plays an important role in bringing tumor cells to bone (24). In addition, interactions between CXCL12 and CXCR4 in the bone microenvironment lead to an upregulation of αvβ3 integrin, facilitating additional cell adhesion. CXCR4 was identified as one of a set of proteins highly overexpressed in breast cancer cells (MDA-MB-231) of high bone metastatic potential by serial selection in vivo (10). Kang and colleagues also found that MMP-1, interleukin (IL)-11, and connective tissue growth factor (CTGF) were highly expressed in in vivo serially selected tumor cells that exhibited increased homing to bone compared with parental cells. IL-11 and MMP-1 stimulate bone resorption by increasing osteoblast production of RANK ligand (RANKL). Increased expression of MMP-1 and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS1) suppresses osteoprotegerin (OPG) expression in osteoblasts, which leads to osteoclast differentiation (25). CTGF stimulates osteoblast proliferation, which leads to further osteoclast activation and increased osteolysis (2).

Figure 1. Premetastatic niche and bone homing. Modulation of the bone microenvironment by circulating breast cancer cells results in priming of the bone marrow as a premetastatic niche through tumor cell secretion of OPN, HPSE, and PTHrP. Colonization of the bone and recruitment of HSC occur by tumor cell expression of integrins (αvβ3), RANK, CXCR4, MMP-1, IL-11, and CTGF. CXCL12 (SDF-1) expression on osteoblasts facilitates homing of tumor cells to bone.

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Following osteotropism, the tumor cells adapt to the bone marrow space by expressing factors that allow growth in their new microenvironment. Osteolytic lesions are the most common type observed from breast cancers metastatic to bone. PTHrP secreted by breast cancer cells was the first characterized tumor-derived mediator of bone destruction (16). In mice without detectable circulating PTHrP or hypercalcemia, neutralizing antibodies to PTHrP blocked breast cancer bone metastases-associated bone loss and tumor growth. It was then shown that TGF-β in the bone microenvironment induced the expression of PTHrP by metastatic breast cancer cells that led to increased bone destruction (26). Breast cancer cells in bone also express COX-2, which supports the development and progression of bone metastases controlled by prostaglandin leading to bone resorption (27). The osteolytic factors IL-8 and IL-11 are also expressed by tumor cells in the bone microenvironment and directly support osteoclast maturation (28, 29). In addition to secreted factors, tumor cells express transcription factors that support growth in bone. The transcription factors, GLI2, runt-related transcription factor 2 (RUNX2), and hypoxia-inducible growth factor-1α (HIF-1α) promote osteolysis. GLI2, part of the Hedgehog signaling network, induces PTHrP expression leading to bone destruction (30). RUNX2 regulates MMP-9 transcription leading to increased tumor cell invasion by breaking down the extracellular matrix (31). HIF-1α expression inhibits osteoblast differentiation and promotes osteoclastogenesis, thus supporting bone resorption and tumor growth (32, 33). Tumor cells also express Jagged1 (Jag1) that activates the Notch pathway, which activates osteoclast differentiation (34).

In the normal adult, setting bone is constantly remodeled to adjust for functional demands or to repair microfractures that occur as a part of normal activity. This process is driven by the coupled activity of osteoclasts that resorb mineralized matrix and osteoblasts that lay down new bone (35, 36). Ultimately, tumor cells in the bone microenvironment disrupt this normal physiologic process and skew balance toward either bone destruction or bone formation. In most breast cancers metastatic to bone, the tumor cells produce factors that directly or indirectly induce the formation of osteoclasts. In turn, bone resorption releases growth factors from bone matrix (e.g., TGF-β) that stimulate tumor growth and further osteolysis. This reciprocal interaction between breast cancer cells and the bone microenvironment results in a “vicious cycle” that increases both bone destruction and the tumor burden (Fig. 2; ref. 2).

Preclinical data suggest that reducing bone resorption prevents the development of bone metastases. Osteoclast inhibitors are useful agents to slow or reverse bone loss (2), whereas antiresorptive therapy (bisphosphonates), OPG, and other RANKL antagonists reduce growth of bone metastases (37, 38). TGF-β antagonism is another mechanism for reducing tumor growth in bone. TGF-β is abundant in the mineralized bone matrix and is released from the matrix during osteoclastic bone resorption (39). Blocking the TGF-β pathway reduces bone metastasis and tumor burden (40–43). Blocking bone resorption, especially through modulating TGF-β signaling, offers a promising area for therapeutic intervention in bone metastasis and potentially its comorbidities.

Muscle dysfunction associated with breast cancer bone metastasis

Muscle and bone anabolism are tightly coupled during growth and development. Conversely, muscle and bone catabolism occur during aging. Yet the cellular and molecular mechanisms linking these two tissues are not well understood.

Muscle is known to secrete many factors capable of affecting other tissues. These factors, collectively termed myokines, include the bone active molecules insulin-like growth factor-I (IGF-I), fibroblast growth factor (FGF)-2, myostatin (also called growth and differentiation factor; GDF-8), and IL-6 (44). Our current understanding of bone and muscle cross-talk seems to show a predominant role of signaling in the direction of muscle to bone. Yet bone-derived factors are also known to modulate muscle. For example, Indian hedgehog promotes myoblast survival and myogenesis in both mouse and chick embryos (45) thus indicating bidirectional bone–muscle cross-talk. It seems likely that in cases of abnormal physiology, such as osteolytic bone metastases, the signals are co-opted and lead to a shift in the homeostatic signaling balance (Fig. 3).

Data from our laboratory using a preclinical model of breast cancer bone metastases (MDA-MB-231 cells) show significant reduction in forelimb grip strength and ex vivo maximum specific force generation of the extensor digitorum longus (EDL) muscle that cannot be explained by reduction in muscle mass. Ex vivo specific force calculations compensate for differences in size and weight of individual muscles. Further muscle dysfunction is systemic and dependent on tumor-induced osteolytic bone resorption without tumor cell involvement in the muscle. Primary MDA-MB-231 tumors (mammary fat pad injection site) do not elicit muscle dysfunction (46). Our investigation into muscle function in mice with breast cancer bone metastases was borne out of the observation that these mice develop cachexia with advancing bone destruction. Cancer cachexia is one of the most common paraneoplastic conditions in advanced malignancy, occurring in approximately 80% of patients. There is no effective treatment for cancer cachexia, and it has been estimated to be responsible for 20% of cancer-related deaths (3, 47). However, there is a large heterogeneity in clinical presentation of cachexia that can vary according to tumor type, site, and individual patient factors. In fact, the true incidence of cancer cachexia is likely greatly underestimated (5).

Many well-established models of cancer cachexia have used reduction of muscle size to imply muscle dysfunction. However, this does not take into account the loss of muscle quality. Our laboratory has shown that mice with bone metastases exhibited a primary defect (in addition to loss of muscle weight) that is independent of cachexia; although mechanisms of cancer cachexia may also be at work (48).
a mouse model of multiple myeloma that leads to osteolytic bone lesions but without measurable cachexia, we observed systemic muscle dysfunction (49). In both of these mouse models of osteolytic bone loss, the severity of muscle dysfunction correlated with an increase in bone destruction.

The salient question therefore is what factor(s) derived from bone matrix during resorption is capable of inducing systemic muscle dysfunction? Bone matrix is a rich storehouse of growth factors that have known effects on muscle, such as activin A, TGF-β, IGF-I, and bone morphogenic protein 2 (BMP-2; refs. 50, 51). It is useful to begin by
considering these as potentiators of muscle dysfunction due to bone destruction.

The high-affinity activin type 2 receptor, ActRIIB, mediates signaling of a small group of TGF-β family members (activin A, myostatin, GDF-11) and is important in regulating muscle mass (52). Pharmacologic blockade of ActRIIB prevents muscle wasting, induces muscle satellite cell mobilization and differentiation, and significantly prolongs survival in murine models of cachexia (53). In addition, blockade of ActRIIB dramatically improves muscle function in a Duchenne muscular dystrophy model (mdx mice; ref. 54). However, in these studies it is not possible to determine whether the effect is due to blocking activin A, myostatin, or GDF-11 signaling due to receptor usage overlap. Myostatin signaling antagonism has been investigated as a way to improve muscle wasting due to cachexia because myostatin is a potent inhibitor of skeletal muscle differentiation and growth (55). Activin A has also been shown to function with myostatin to reduce muscle size (56). GDF-11 shares 90% sequence homology with myostatin and in skeletal muscle inhibits myoblast differentiation (57), suggesting that GDF-11 may act in a very similar manner as myostatin.

TGF-β is a potent regulator of wound healing in muscle, and persistent exposure leads to altered extracellular matrix architecture and formation of fibrotic tissue in muscle (58). Increased TGF-β signaling in muscle also inhibits satellite cell activation and impairs myocyte differentiation (59, 60). Increased TGF-β signaling is also associated with skeletal muscle dysfunction in many of the muscular dystrophies (61, 62). In a direct assessment of the effect of TGF-β on muscle function, the contractile properties of the EDL muscle were examined from limbs exposed to recombinant TGF-β. Muscle function from limbs receiving TGF-β treatment exhibited a significant reduction in specific force (63). These experiments suggest that TGF-β is a capable factor in reducing muscle function independent of changes in muscle mass.

In contrast with the negative effects possible from activin A, myostatin, and TGF-β signaling in muscle, IGF-I and BMP-2 signaling results in muscle hypertrophy (58, 64, 65). IGF-I is a major regulator of muscle mass due to its effect on myogenic cell proliferation and differentiation (66). Likewise, BMP signaling leads to muscle hypertrophy, but, interestingly, specific force (corrected for muscle mass) is significantly lower when BMP signaling is constitutively activated (64). This result demonstrates the importance of interpreting muscle-specific function, not merely muscle mass, in murine models of skeletal muscle weakness.

In addition to factors released from bone matrix during osteoclast-driven resorption, other factors present in patients with malignancy involving bone may play important roles in muscle weakness. Serum vitamin D levels are low among patients with breast cancer who are receiving bisphosphonate therapy (67). Vitamin D deficiency has been studied in rodent models using vitamin D receptor knockout (VDRKO) mice. Functional muscle tests in VDRKO mice exhibited an increase in sinking episodes in a forced swim test, reduced “time on” in a rotarod test (68, 69), and reduced time before falling from a vertical screen test (70). These results indicate an overall defect in motor performance in mice lacking proper vitamin D metabolism. In human studies, rickets and osteomalacia are associated with muscle weakness. In addition to general weakness, more specific muscle deficits are also commonly reported, including reduced timed up and go, 6-minute walk, stair climbing, and object lifting (71, 72). It should be noted that myopathies reported with vitamin D deficiency might also involve calcium and phosphate deficiencies, thus complicating the assessment of individual factors. FGF-23 neutralizing antibody, which increases serum phosphate and vitamin D levels, has been shown to improve murine grip strength in a model of rickets/osteomalacia (X-linked hypophosphatemic rickets/osteomalacia), suggesting that vitamin D levels could influence muscle function (73).

MicroRNA (miRNA) profiling of tumors has identified signatures associated with diagnosis and progression. Human miRNA Let-7 was recently shown to be elevated in serum of mice harboring breast cancer bone metastases (74). miRNA Let-7 is also elevated in serum of elderly patients with muscle weakness and has been suggested to reduce regenerative capacity in aging (75).

Another intriguing possibility is the role of the sympathetic nervous system in muscle weakness due to bone metastases. The sympathetic nervous system modulates skeletal muscle metabolism, ion transport, and contractility. Recent evidence has shown that the sympathetic nervous system is capable of promoting breast cancer bone metastasis through stimulation of marrow stromal cells (18), yet a connection to muscle weakness has not been investigated.

Summary

Bone and muscle functions are tightly coupled in normal physiology. Recent studies have focused on muscle as an endocrine organ with a predominant role over bone in bone–muscle cross-talk. Osteolytic bone metastases from breast cancer represent a severe divergence from normal bone physiology by tipping the balance of remodeling. Bone is a rich storehouse of growth factors that have activity in bone (as a part of normal remodeling) and in other organs, including muscle. It is therefore possible that during hyperactive bone resorption, bone might have a predominant role over muscle in bone–muscle cross-talk and become a source of “osteokines” that affect muscle function. Likewise, factors released from muscle may play an important role in bone metabolism that could further exacerbate the role of bone in muscle dysfunction. Identification and characterization of such factors would provide new possibilities for therapeutic intervention in muscle weakness associated with malignancy and perhaps cancer cachexia.

Disclosure of Potential Conflicts of Interest

T.A. Guise reports receiving commercial research grants from AstraZeneca and Exelexis, is a consultant/advisory board member for Novartis, and has provided expert testimony on bisphosphonates and osteonecrosis of the jaw.
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