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 pathological 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 pre-metastatic niche. The colonization and growth of tumor cells then depends on adaptations in the invading tumor cells to take advantage of normal physiological 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 based on 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 65-80% of patients with advanced malignancy (2). Bone metastases cause severe bone pain, increased risk of pathological 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 anti-resorptive therapy aimed at reducing skeletal related events but is not curative with regard to tumor burden (1, 2).

A significant co-morbidity 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 makes it is 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 cancer patients 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 is a complex multistep process. Tumor cells must detach from the primary tumor and enter the systemic circulation (intravasation), 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 micro-metastases 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 pre-metastatic niche (local environment) for further colonization and growth of tumor cells (Figure 1) (2, 7-9).

The hematopoietic system plays an important role in development of the pre-metastatic 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 physiological mechanisms as those used by hematopoietic stem cells (HSCs) homing to bone (10, 11). In the pre-metastatic 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) that promotes bone resorption (16) and may also enhance production of bone marrow chemokines such as C-C motif ligand 2 (CCL2) (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 (BSP) and matrix metalloproteinase-2 (MMP-2) to promote tumor cell colonization in bone (21, 22). Receptor activator of nuclear factor k-B (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 chemo-attractant 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 up-regulation 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 et al., also found that matrix metalloproteinase 1 (MMP-1), 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 to 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).

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 TGFβ 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 cyclooxygenase-2 (COX-2) which supports the development and progression of bone metastases controlled by prostaglandin (PGE$_2$) 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-induced growth factor 1 α (HIF1α) 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). HIF1α 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 osteoblast that lay down new bone (35, 36). Ultimately tumor cells in the bone microenvironment disrupt this normal physiological process and skew balance either toward bone destruction or bone formation. In the case of 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. TGFβ) 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 (Figure 2)(2).

Pre-clinical data suggests that reducing bone resorption prevents the development of bone metastases. Osteoclast inhibitors are useful agents to slow or reverse bone loss (2) while anti-resorptive therapy (bisphosphonates), osteoprotegerin (OPG) and other RANKL antagonists reduce growth of bone metastases (37, 38). TGFβ antagonism is another mechanism for reducing tumor growth in bone. TGFβ is abundant in the mineralized bone matrix and is released from the matrix during osteoclastic bone resorption (39). Blocking the TGFβ pathway reduces bone metastasis and tumor burden (40-43). Blocking bone resorption especially through modulating TGFβ 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 1 (IGF-1), fibroblast growth factor 2 (FGF-2), Myostatin (also called growth and differentiation factor 8 [GDF8]) 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 (Ihh) 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, that the signals are co-opted and lead to a shift in the homeostatic signaling balance (Figure 3).

Data from our laboratory using a pre-clinical model of breast cancer bone metastases (MDAMB-231 cells) shows 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 percent of patients. There is no effective treatment for cancer cachexia and it has been estimated to be responsible for 20 percent 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 lab 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). In 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, TGFβ, IGF-1 and bone morphogenic protein 2 (BMP-2) (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 TGFβ family members (Activin A, Myostatin, GDF-11) and is important in regulating muscle mass (52). Pharmacological 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) (54). However in these studies it is not possible to determine if 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 since 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.

TGFβ 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 TGFβ signaling in muscle also inhibits satellite cell activation and impairs myocyte differentiation (59, 60). Increased TGFβ signaling is also associated with skeletal muscle dysfunction in many of the muscular dystrophies (61, 62). In a direct assessment of the effect of TGFβ on muscle function the contractile properties of the extensor digitorum longus muscle (EDL) were examined from limbs exposed to recombinant TGFβ. Muscle function from limbs receiving TGFβ treatment exhibited a significant reduction in specific

force (63). These experiments suggest that TGFβ is a capable factor in reducing muscle function independent of changes in muscle mass.

In contrast to the negative effects possible from Activin A, Myostatin and TGFβ signaling in muscle, IGF-1 and BMP-2 signaling results in muscle hypertrophy (58, 64, 65). IGF-1 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 breast cancer patients with either osteoporosis or metastatic bone disease and receiving bisphosphonate therapy (67). Vitamin D deficiency has been studied in rodent models using vitamin D receptor knock-out (VDRKO) mice. Functional muscle tests in VDRKO mice exhibited and 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 (TUG), 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. Fibroblast growth factor-23 (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 [XLH]) 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.

Acknowledgments

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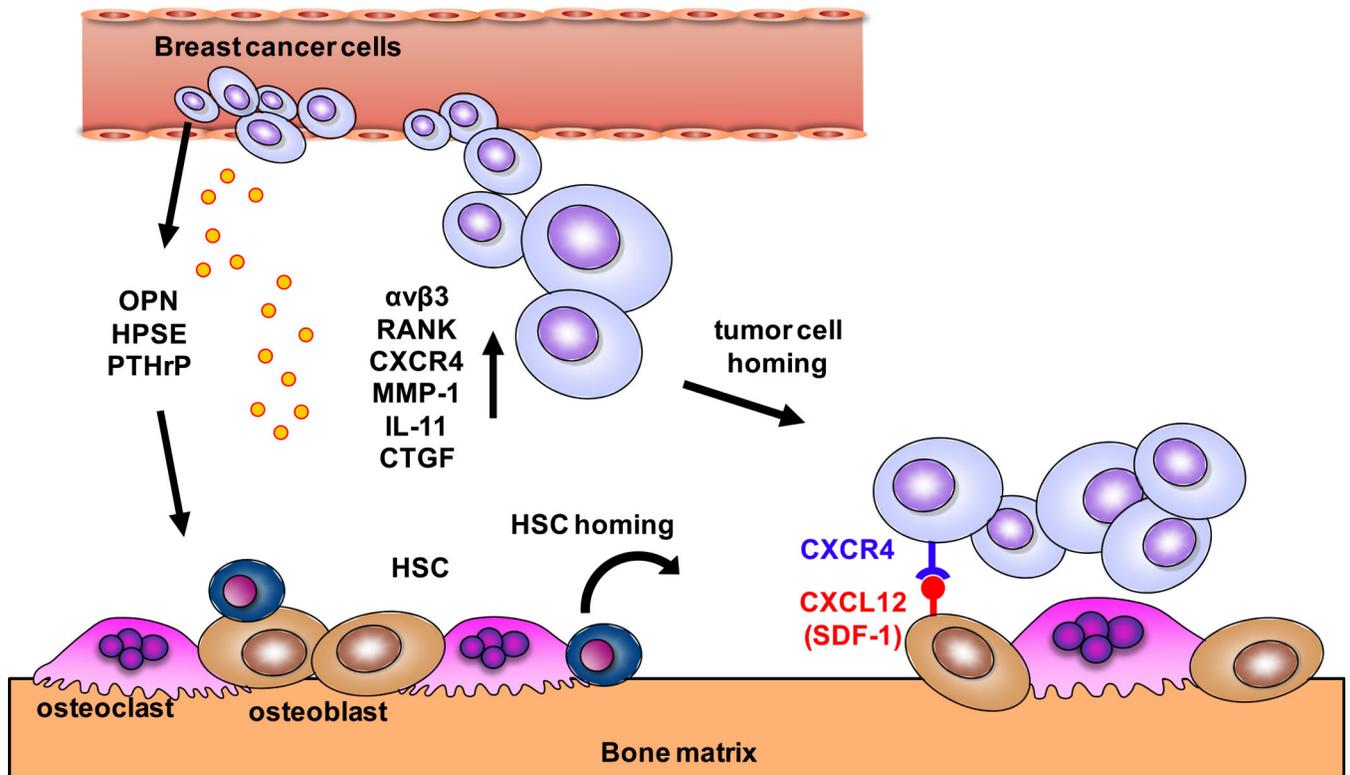**Figure 1. Pre-metastatic niche and bone homing**
Modulation of the bone microenvironment by circulating breast cancer cells results in priming of the bone marrow as a pre-metastatic niche through tumor cell secretion of osteopontin (OPN), heparanase (HPSE) and parathyroid hormone related protein (PTHrP). Colonization of the bone and recruitment of hematopoietic stem cells (HSCs) occurs by tumor cell expression of integrins (αvβ3), receptor activator of NF-kB (RANK), CXCR4, matrix metalloproteinase (MMP-1), IL-11 and connective tissue growth factor (CTGF). CXCL12 (SDF-1) expression on osteoblasts facilitates homing of tumor cells to bone.
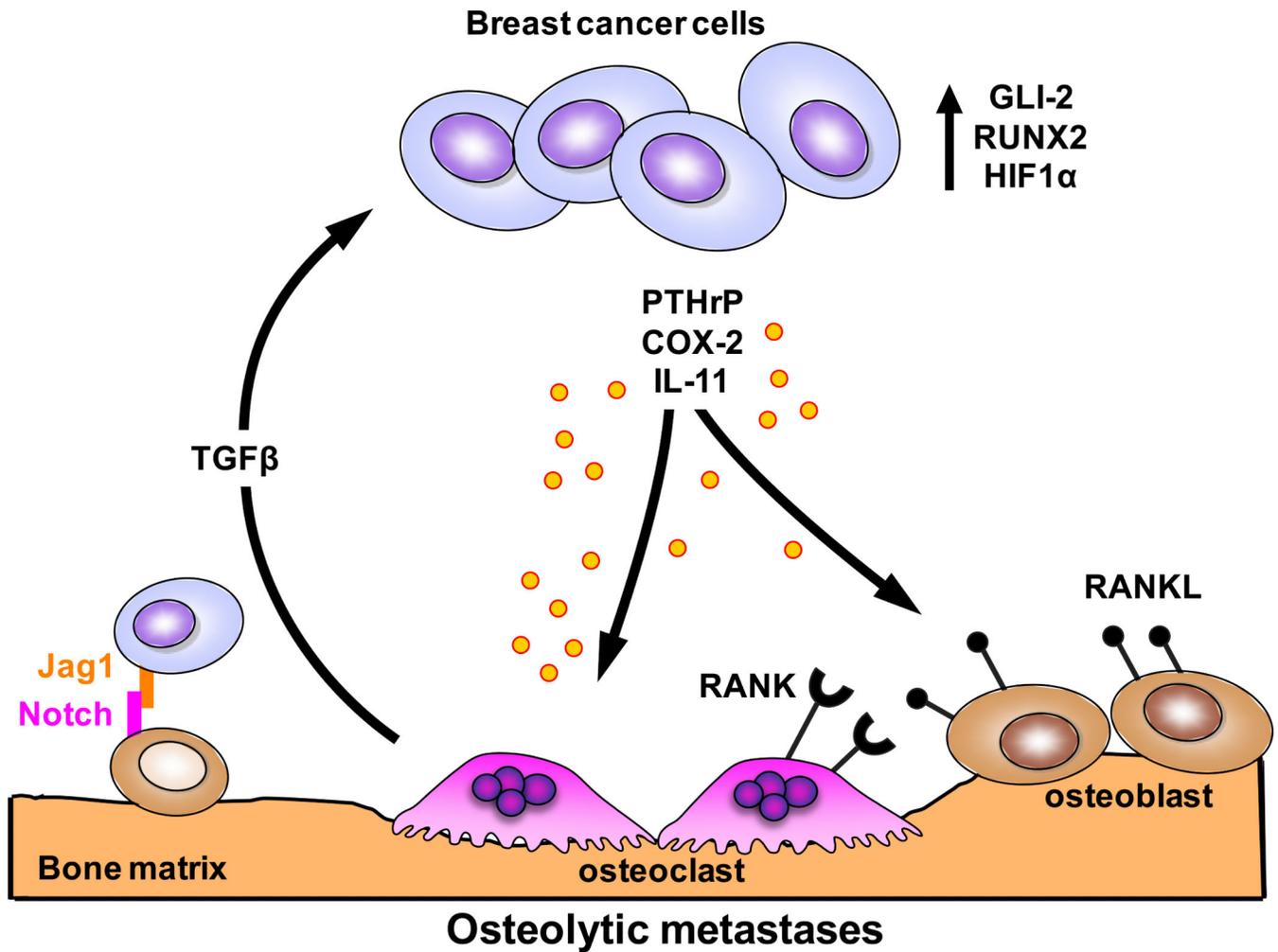
Figure 2. Vicious cycle of osteolytic bone metastasis
Osteolytic bone destruction due to dysregulation of normal bone remodeling is predominant
in breast cancer metastasis. Breast cancer cells colonizing the bone secrete osteolytic factors:
parathyroid hormone related protein (PTHrP), cyclooxygenase-2 (COX-2) and IL-11. Tumor
cells also express transcription factors GLI2, runt related transcription factor 2 (RUNX2)
and hypoxia-induced growth factor 1α (HIF1α) that promote osteolysis. Jagged1 (Jag1)
expressed on tumor cells activates osteoclast differentiation by inducing Notch signaling in
pre-osteoclasts. Bone resorption releases TGFβ from the bone matrix, which enhances tumor
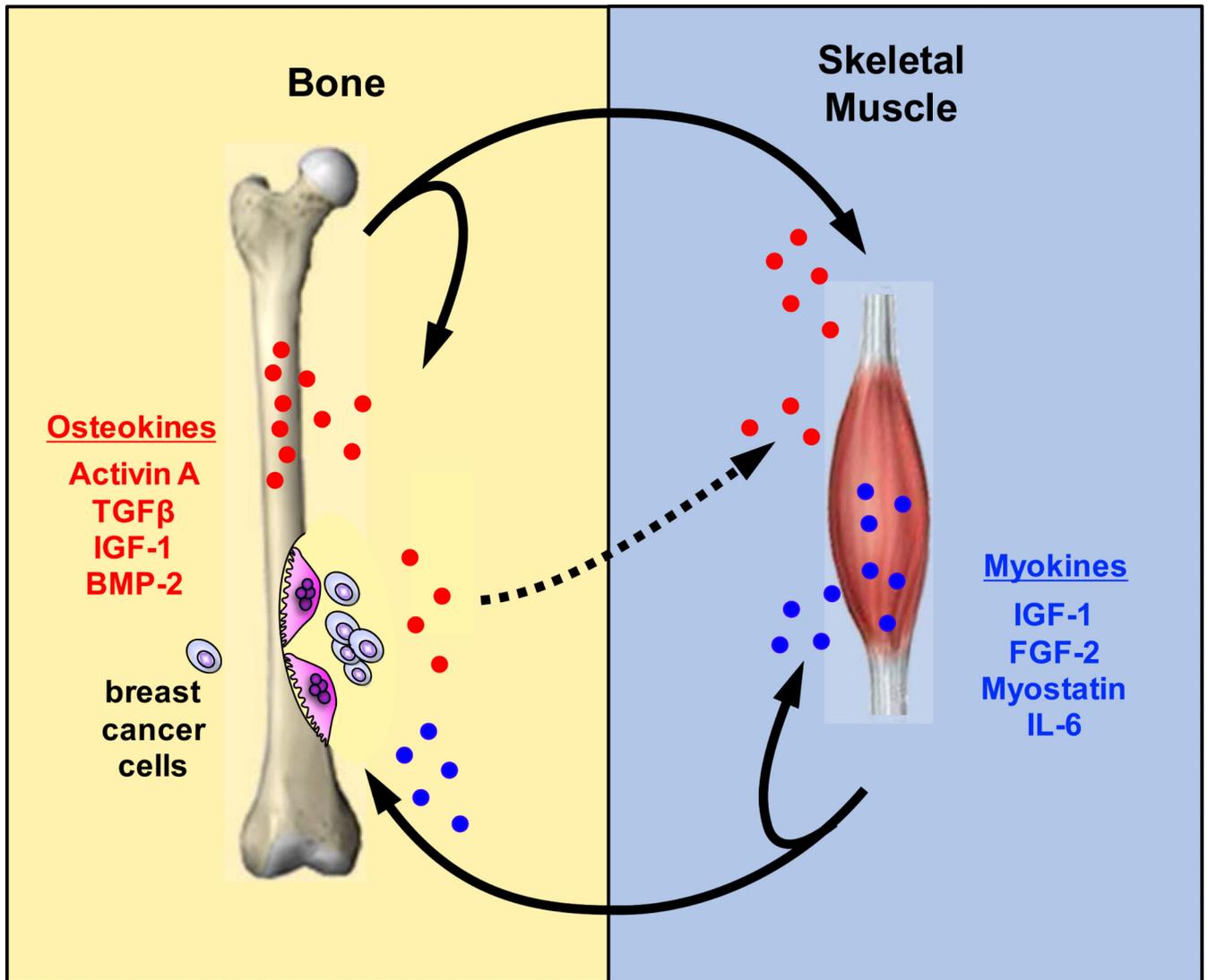cell proliferation and survival thus feeding a vicious cycle leading to further bone
destruction.

Figure 3. Bone-muscle cross-talk

Bone and muscle are physically and functionally tightly coupled. Insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), Myostatin and IL-6 are factor released from muscle that have functions in muscle as well as bone. These factors have been collectively termed myokines. Likewise Activin A, TGFβ, IGF-1 and bone morphogenic protein 2 (BMP-2) released from bone affect bone as well as muscle and thus we have called these osteokines. During osteolytic bone resorption due to breast cancer bone metastases osteokines are released and may be responsible for systemic skeletal muscle weakness. In certain settings tumor-derived factors could also lead to modulation of muscle activity (dotted line).