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REVIEW

Cancer-associated muscle weakness: What's bone got to do with it?

David L Waning and Theresa A Guise

Department of Medicine, Division of Endocrinology, Indiana University School of Medicine, Indianapolis, IN, USA.

Cancer-associated muscle weakness is an important paraneoplastic syndrome for which there is currently no treatment. Tumor cells commonly metastasize to bone in advanced cancer to disrupt normal bone remodeling and result in morbidity that includes muscle weakness. Tumor in bone stimulates excessive osteoclast activity, which causes the release of growth factors stored in the mineralized bone matrix. These factors fuel a feed-forward vicious cycle of tumor growth in bone and bone destruction. Recent evidence indicates that these bone-derived growth factors can act systemically to cause muscle weakness. Muscle weakness can be caused by reduced muscle mass or reduced muscle function; in advanced disease, it is likely due to a combination of both reduced quantity and quality of muscle. In this review, we discuss possible mechanisms that lead to skeletal muscle weakness due to bone metastases.

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Introduction

Cancer-associated muscle dysfunction is a major paraneoplastic syndrome, the spectrum of which ranges from muscle weakness in the absence of weight loss to profound muscle wasting and cachexia.[1] Cancer-associated muscle dysfunction is a large research challenge and a deadly clinical problem; mortality is high (80%) and there is increased toxicity from cancer treatment.[1–3] Skeletal muscle weakness is a major clinical problem for advanced cancer patients as they also often have bone metastases and associated bone pain, fractures, hypercalcemia and nerve compression syndromes.[4] Muscle weakness in the setting of bone fragility likely increases the fracture risk even more than bone metastases alone.

Normal muscle contraction is dependent on precise calcium signaling in the muscle cell.[5] During excitation–contraction (E–C) coupling in skeletal muscle, sequestered calcium in the sarcoplasmic reticulum is released through activated ryanodine receptor/calcium release channel (RyR1) into the cytoplasm, permitting calcium-dependent actin–myosin cross-bridging and muscle contraction.[6] Cytosolic calcium is then transferred back to the lumen of the sarcoplasmic reticulum via a calcium-ATPase pump (SERCA) (**Figure 1**). Maladaptive modifications of RyR1 (nitrosylation and oxidation) resulting from chronic oxidative stress have been linked to pathologic sarcoplasmic reticulum calcium leak in diseases characterized by contractile dysfunction and muscle weakness, including heart failure,[7–9] muscular dystrophy[10] and age-related sarcopenia.[11] RyR1 oxidation disrupts a critical interaction between RyR1 and its stabilizing subunit calstabin1, resulting in leaky channels with impaired calcium handling and weakened muscle force production.[10,11] It is likely that similar mechanisms are involved in cancer-associated muscle weakness, as persistent increased oxidative stress is associated with cancer.[12] Further, transforming growth factor β (TGFβ), which is a critical factor for bone remodeling,[13] can mediate oxidative stress;[14] hence, it should be no surprise that bone metastases could be associated with muscle dysfunction.

Bone Metastases in Advanced Cancer

Bone remodeling, coordinately balanced by bone-destroying osteoclasts and bone-forming osteoblasts, maintains bone strength in healthy adults. This process is driven by the coupled activity of osteoclasts that resorb mineralized matrix and osteoblasts that lay down new bone.[15,16] Bone metastases are common in patients with advanced malignancy, especially those with breast, prostate and lung cancer. Tumor cells in the bone microenvironment disrupt normal bone remodeling to result in excess bone destruction or bone formation. Tumor cells produce factors that directly or indirectly stimulate osteoclastic bone resorption, which releases growth factors from the bone matrix, such as TGFβ that stimulate tumor invasion, growth and further osteolysis.[17] This reciprocal interaction between cancer cells and the bone microenvironment results in a feed-forward 'vicious cycle' that increases both bone destruction and the tumor burden (**Figure 2**).[17]

Correspondence: Professor TA Guise, Department of Medicine, Division of Endocrinology, Indiana University School of Medicine, 980W. Walnut, Walther Hall R3 / Rm C132, Indianapolis, IN 46202, USA.
E-mail: tguise@iu.edu

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Bone metastases are classified on the basis of radiographic appearance as either osteolytic or osteoblastic (osteosclerotic). Breast cancer is typically associated with osteolytic or mixed lesions. Despite the radiographic appearance, most tumors in bone have uncoupled components of both bone destruction and new bone formation. Perhaps most devastating is the fact that once the primary tumor has spread to the bone it is almost always incurable.[4] The current standard of care for patients with bone metastases of any type includes bone-targeted anti-resorptive therapy, such as zoledronic acid or denosumab, in addition to chemotherapy, hormonal therapy, radiation and surgery. These effectively reduce skeletal-related events but do not cure the disease.[4,17]

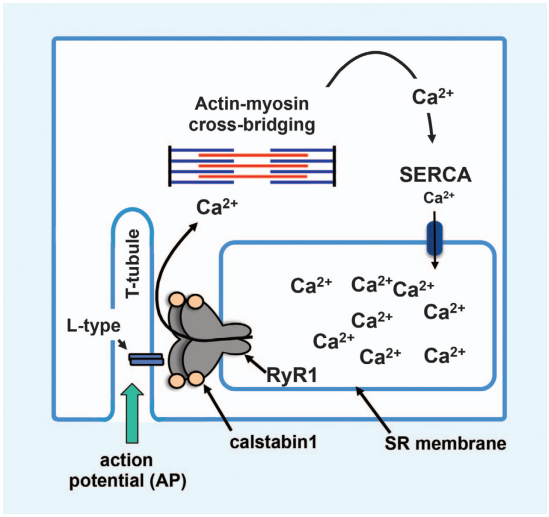

Figure 1 Skeletal muscle contraction. Skeletal muscle contraction begins with an action potential (AP) from the nervous system that activates L-type voltage-dependent calcium channels in the T-tubule system. Ryanodine receptor receptor/calcium release channel (RyR1) then releases calcium from the sarcoplasmic reticulum (SR). High cytosolic calcium causes actin–myosin cross-bridging and muscle contraction.[6] Cytosolic calcium is then transferred back to the lumen of the SR via calcium-ATPase pump (SERCA) during myocyte relaxation. Oxidation of RyR1 disrupts a critical interaction between RyR1 and its stabilizing subunit calstabin1 leading to calcium 'leak.' Pathologic calcium leak reduces tetanic calcium release, the key determinant of muscle contraction.

Cancer Cachexia

A significant co-morbidity of bone metastases is muscle weakness that is often associated with cancer cachexia. Cachexia is a common paraneoplastic syndrome that is characterized by severe wasting due to loss of skeletal muscle mass (with or without loss of fat mass) due to a negative protein balance caused by abnormal metabolism.[18,19] 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,[20] a clinical perspective suggests that many patients do experience severe muscle weakness and fatigue. Cancer cachexia is a multifactorial syndrome that is common in advanced malignancy occurring in ~80% of patients, which cannot be reversed by nutritional support and leads to significant function deficits. Cancer cachexia is estimated to be responsible for 20% of cancer-related deaths.[18,19] 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 to be greatly underestimated.[20] Reducing cachexia has been shown to extend life span even without affecting tumor growth in mice.[21] Improving muscle function and mobility of cancer

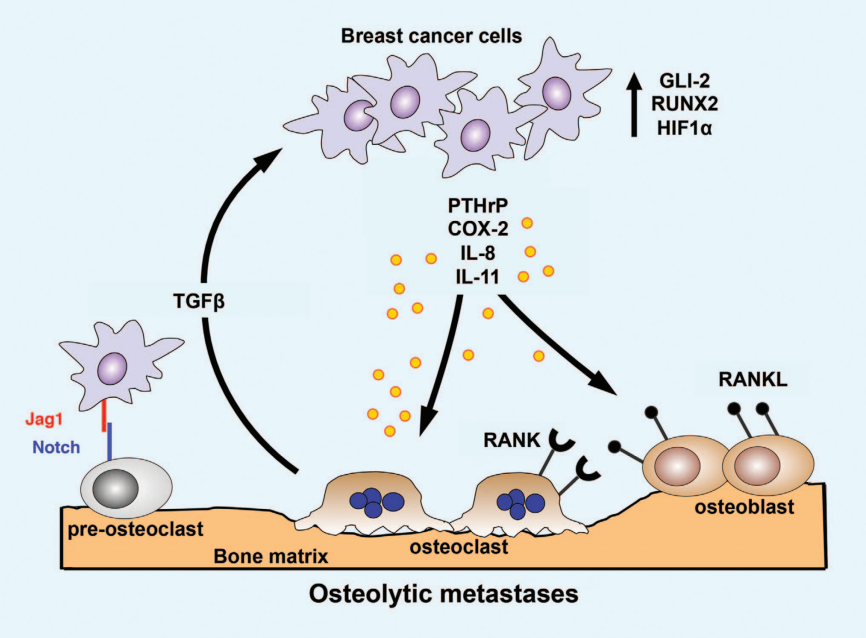

Figure 2 Vicious cycle of osteolytic bone metastasis. Osteolytic bone destruction due to dysregulation of normal bone remodeling is predominant in breast cancer metastasis. Breast cancer cells colonizing the bone secrete osteolytic factors: parathyroid hormone-related protein (PTHrP), cyclooxygenase-2 (COX-2), IL-8 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. Reviewed in.[17,65]

patients should thus have a positive impact on adherence to treatment regimens and overall health.[20] Therefore, a better understanding of the mechanisms of muscle weakness associated with bone metastases and cancer cachexia will lead to improved therapy. 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.

Muscle–bone Cross-talk

Muscle and bone anabolism are tightly coupled during growth and development.[22,23] Conversely, muscle and bone catabolism occur during aging.[24] Yet, the cellular and molecular mechanisms linking these two tissues are not well understood. Nor is it known which tissue influences the metabolism of the other.

Muscle secretes many factors that can act on other tissues, including bone. 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.[25] IGF-1 and FGF-2 at the muscle–bone interface stimulate bone formation.[26,27] Myostatin is a mediator of cachexia,[28] and myostatin deficiency increases bone density.[29] Conversely, bone-derived factors are known to modulate muscle. For example, Indian hedgehog (Ihh) promotes myoblast survival and myogenesis in both mouse and chick embryos,[22] thus indicating bidirectional bone–muscle cross-talk. It seems likely that, in cases of abnormal physiology, such as with the bone destruction that occurs from osteolytic bone metastases, the signals may also originate in bone and act on muscle.

Preclinical data from our laboratory show that mice with MDA-MB-231 breast cancer bone metastases have a significant reduction in forelimb grip strength and *ex vivo* maximum specific force generation of the extensor digitorum longus muscle due to improper calcium handling and that is independent of reduced muscle mass. *Ex vivo*-specific force calculations compensate for the 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. Mice with primary MDA-MB-231 breast tumors do not get bone metastases and do not have muscle dysfunction.[30] In a mouse model of osteolytic multiple myeloma, we observed systemic muscle dysfunction in the absence of cachexia.[31] In both of these mouse models of tumor-induced osteolytic bone destruction, the severity of muscle dysfunction correlated with increased osteolysis. In these mice with muscle weakness, there is evidence of oxidation of the calcium-handling protein, RyR1, and calcium leak in the skeletal muscle, which is responsible for the muscle weakness. Collectively, these observations suggest that the bone microenvironment could mediate these effects.

Bone-derived Factor(s) that may lead to Skeletal Muscle Weakness

Which bone-derived factors can induce systemic muscle dysfunction? Bone matrix is a rich storehouse of growth factors that have known effects on muscle, such as activin, TGFβ, IGF-1 and bone morphogenic protein 2 (BMP-2).[32,33] Some of these factors are released and activated as a consequence of osteoclastic bone resorption and have the potential to act systemically to promote muscle dysfunction. Skeletal muscle weakness is observed in other clinical conditions associated with bone loss, such as in patients with hyperparathyroidism. Patients with hyperparathyroidism fatigue quickly and have skeletal muscle atrophy, suggesting a link between bone loss and muscle weakness.[34]

The bone is a large storehouse for growth factors, such as TGFβ, which are deposited in the mineralized bone matrix by osteoblasts. In fact, bone is the largest storehouse of TGFβ in the body[13,35] and has a central role to promote tumor osteolysis due to bone metastases.[36–39] TGFβ is released in high concentrations from the mineralized bone matrix during osteoclastic bone resorption,[38] a process activated in all types of bone metastases. TGFβ acts on tumor cells to enhance secretion of osteolytic factors[40] that increase bone destruction and prometastatic factors, driving the feed-forward cycle of tumor growth and further bone destruction.[17] Human breast cancer bone metastases show active TGFβ signaling by nuclear accumulation of phosphoSMAD2/3[36] and TGFβ signaling blockade via stable expression of dominant-negative TGFβ receptor 2 (DNTβRII), or a using a TGFβ receptor 1 kinase inhibitor, suppresses bone metastasis in mice.[39,41]

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.[42] Increased TGFβ signaling in muscle also inhibits satellite cell activation, impairs myocyte differentiation[43,44] and is associated with skeletal muscle dysfunction in many of the muscular dystrophies.[45,46] In a direct assessment of the effect of TGFβ on muscle function, the contractile properties of the extensor digitorum longus muscle were examined from limbs exposed to recombinant TGFβ via subcutaneous injection directly into the lower hindlimb. Muscle function in TGFβ-treated limbs showed a significant reduction in specific force without changes in muscle mass[47] in contrast to other TGFβ family members that lead to reduced muscle mass (see below). These experiments suggest that TGFβ can reduce muscle function via a variety of mechanisms, which include fibrosis, myocyte differentiation and alteration in calcium-handling proteins in the sarcoplasmic reticulum, such as RyR1 and SERCA, which is independent of changes in muscle mass.

Other TGFβ family members may have a role in cancer-associated muscle dysfunction. The high-affinity activin receptor type 2B, ActRIIB, mediates signaling of a small group of TGFβ family members, including activin, myostatin and GDF-11, and is important in regulating muscle mass.[48] Pharmacological blockade of ActRIIB prevents muscle wasting, induces muscle satellite cell mobilization and differentiation and significantly prolongs survival in murine models of cachexia.[21] In addition, blockade of ActRIIB markedly improves muscle function in a Duchenne muscular dystrophy model (mdx mice).[49] However, in these studies, it is not possible to determine whether the effect is due to blocking activin, myostatin or GDF-11 signaling because of receptor promiscuity. Myostatin antagonist has been investigated as a way to improve muscle wasting due to cachexia, as myostatin is a potent inhibitor of skeletal muscle differentiation and growth.[50] GDF-11 shares 90% sequence homology with myostatin and in skeletal muscle inhibits myoblast differentiation,[51] suggesting

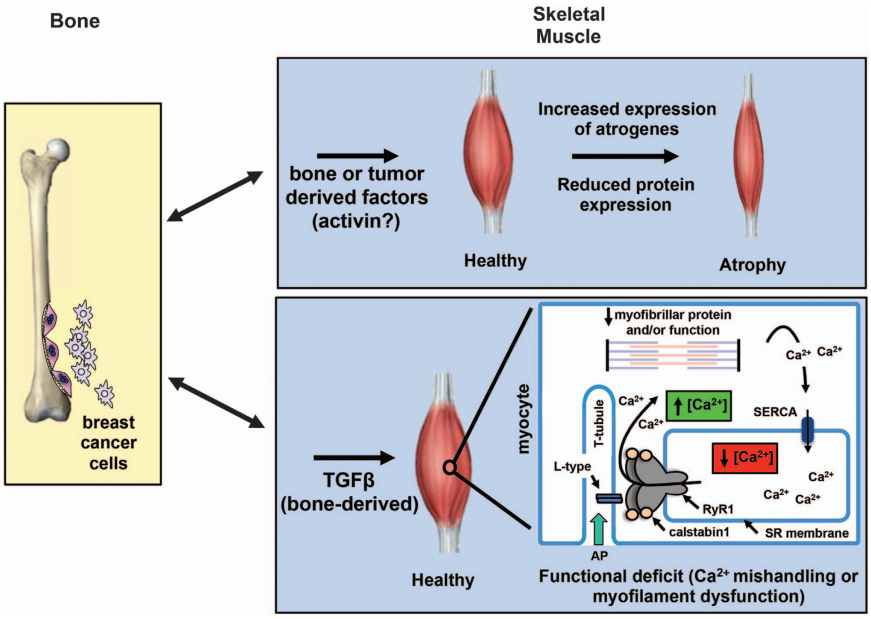

Figure 3 Bone–muscle cross-talk. Bone and muscle are physically and functionally tightly coupled. During osteolytic bone resorption due to tumor metastases in bone, bone-derived factors may be responsible for reduced skeletal muscle mass, whereas TGFβ is responsible for systemic muscle dysfunction via oxidation of RyR1.[30] Muscle weakness occurring via atrophy involves induction of skeletal muscle-specific ubiquitin ligase expression (atrogin-1/MAFbx and MuRF1/TRIM63) and reduced protein synthesis.[66] Muscle weakness can also occur through disruption of calcium signaling (RyR1 or SERCA oxidation) that reduces SR calcium and increases cytosolic calcium[30,31] or by interference with actin-myosin cross-bridging.[67] Myofibrillar protein oxidation has also been shown to lead to contractile dysfunction in heart failure and represents another possible mechanism of muscle weakness in cancer.[68] In certain settings, muscle atrophy and muscle dysfunction can occur together furthering overall weakness.

that GDF-11 may act in a very similar manner as myostatin. It is likely that these proteins promote the muscle wasting characteristic of cachexia, whereas other mediators contribute to muscle dysfunction by altering calcium handling (RyR1 and SERCA) or myofibrillar proteins in muscle cells (**Figure 3**).

In contrast to the negative effects possible from activin, myostatin and TGFβ signaling in muscle, IGF-1 and BMP-2 signaling results in muscle hypertrophy.[42,52,53] IGF-1 is a major regulator of muscle mass because of its effect on myogenic cell proliferation and differentiation.[54] Likewise, BMP signaling leads to muscle hypertrophy, but interestingly specific force (corrected for muscle mass) is significantly lower when BMP signaling is constitutively activated.[52] This result demonstrates the importance of interpreting muscle-specific function (quality) not merely muscle mass (quantity) in murine models of skeletal muscle weakness. The contribution of BMP and TGFβ signaling in skeletal muscle that ultimately leads to a positive or a negative protein balance is unclear and remains to be determined.

In the setting of cancer cachexia without bone metastases, tumor-derived factors may also lead to muscle wasting. Tumor-derived parathyroid hormone-related protein has been shown to have a role in cancer cachexia and muscle weakness in a model of Lewis lung carcinoma (LLC).[55] In bone metastases from breast cancer, blocking TGFβ prevents parathyroid hormone-related protein secretion and inhibits bone destruction[39] that could theoretically lead to improved muscle function. However, in mice with bone metastases and cachexia the skeletal muscle-specific force (which takes into account the reduction in muscle size) is reduced. As noted above, TGFβ, when injected directly into the hindlimb of mice, has been shown to reduce skeletal muscle-specific force and indicate a direct role for TGFβ to reduce muscle function independent of muscle mass.[47] These data show that careful consideration must be given to studies of muscle force and muscle mass.

In addition to factors released from bone matrix during osteoclast-driven resorption, other insults that affect muscle and bone in patients with malignancy may promote muscle weakness. Serum 25-hydroxyvitamin D concentrations are often low in breast cancer patients with osteoporosis or bone metastases who receive bisphosphonate therapy.[56] Functional muscle tests in vitamin D receptor knock-out (VDRKO) mice showed an increase in sinking episodes in a forced swim test[57] and reduced time before falling from a vertical screen test.[58] These results indicate an overall muscle weakness in mice lacking vitamin D receptor that may involve reduced muscle mass, as well as reduced muscle function. In humans, the bone mineralization defects associated with rickets and osteomalacia are associated with muscle weakness measured by reduced timed up and go, 6-minute walk, stair climbing and object lifting.[59,60] 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.[61] Despite these studies, the exact mechanism by which vitamin D deficiency causes muscle weakness is unknown.

MicroRNA (miRNA) profiling has identified signatures associated with cancer, bone and muscle. Human miRNA Let-7 was recently shown to be increased in serum of mice with breast cancer bone metastases.[62] miRNA Let-7 is also increased in serum of elderly patients with muscle weakness and has been suggested to reduce regenerative capacity in aging.[63] Further studies are needed to show the mechanism by which Let-7 may affect muscle.

Other unexplored mechanisms of muscle weakness include the role of the sympathetic nervous system 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;[64] yet, a connection to muscle weakness has not been investigated.

Summary

Bone and muscle functions are tightly coupled in normal physiology. Most studies have focused on muscle as an endocrine organ with a predominant role in bone cell function. However, recent evidence suggests that events in bone may alter muscle function as well. The example discussed here, bone metastases, represents a severe disruption of normal bone 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 accelerated bone resorption, such as that which occurs in bone metastases, bone might have a predominant role to alter muscle function and become a source of 'osteokines' that affect muscle function. Likewise, factors released from muscle may have an important role in bone metabolism that could further exacerbate the role of bone as a driver of muscle dysfunction.

Whatever factors are identified that transmit signals between bone and muscle, it is clear that bone-derived factors are capable of impacting muscle and that the effects can manifest as reduction in muscle mass (quantity) or reduction in myocyte function (quality) or both, as is likely in advanced disease (**Figure 3**). Identification and characterization of factors involved in bone–muscle cross-talk will provide new possibilities for therapeutic intervention in muscle weakness and cachexia associated with malignancy.

Conflict of Interest

T.A. Guise was a consultant/advisory board member of Novartis. D.L. declares no conflict of interest.

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