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Mechanisms of Muscle Weakness Associated with Bone Metastases

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Abstract

Cancer-associated muscle dysfunction represents a deadly clinical problem, for which there is currently no treatment. Normal bone remodeling can be disrupted by tumor cells that metastasize to bone in certain stages of cancer, which results in increased morbidity including muscle weakness. The reason for this muscle weakness may be attributed to a reduction in muscle mass and/or a reduction in muscle function. In fact, it has been demonstrated that in advanced cancers, it is probably caused by a combination of reduced quantity and quality of muscle. This review focuses on the mechanisms that bone metastases promote skeletal muscle weakness in metastatic bone disease.

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Introduction

Cancer-associated muscle weakness represents a research challenge and a serious clinical problem[1-3]. The reason behind the problem of skeletal muscle weakness on advanced cancer patients relays on the fact that they also often have bone metastases and associated bone pain, fractures, hypercalcemia and nerve compression syndromes[4]. As a consequence, in the setting of bone fragility, muscle weakness is likely to increase the fracture risk even more than bone metastasis on its own.

Bone Metastases

Cancer metastasizes by completing a series of events known as the metastatic cascade. Tumor cells detach from the primary site, invade the adjacent extracellular matrix, and then enter the circulation through a process known as intravasation. Once at the target organ, tumor cells extravasate into the parenchyma to establish micro metastases, and the final outgrowth and colonization at the distant target. Circulating tumor cells from certain types of cancer like breast and prostate have particular affinity to grow in bone. This is because the bone microenvironment is a fertile soil for tumor growth as it houses abundant growth factors which act on tumor cells to fuel growth and other invasive behavior.

Bone is a dynamic organ and its homeostasis is maintained by a balanced production of osteoblast and osteoclasts. The relationship between osteoblastic bone formation and osteoclastic bone resorption is balanced in healthy individuals. In various bone diseases including malignancy, disruption of this balance results in the loss of the normal structural integrity of the skeleton[5]. Tumor cells excessively stimulate osteoclasts and osteoblasts, an interaction that is critical for tumor cell survival (Figure 1).

Patients with bone metastases have significant morbidity, including bone pain, fracture, hypercalcemia, muscle weakness and spinal cord compression. Cancer patients, who develop bone metastases, can survive for many years, during which they will suffer significant comorbidity; but once cancer metastasizes to bone the tumor is incurable. These comorbidities are known collectively as skeletal-related events (SREs). SREs are associated with impaired mobility, reduced quality of life[6], increased mortality, and higher healthcare costs. Standard antiresorptive

treatments, such as zoledronic acid or denosumab, decrease skeletal morbidity and delays SRE, but do not cause regression or cure the disease[4,7].

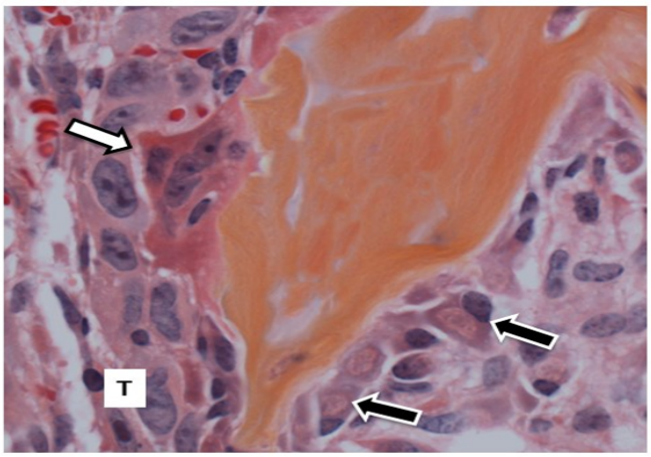

Figure 1: H&E stained sections from mouse with breast cancer bone metastases showing bone resorbing osteoclasts (white arrow) and bone forming Osteoblasts (black arrows). Both cells are interacting with tumor cells (T).

Bone metastases are classified according to the radiographic appearance into osteolytic or osteoblastic lesions. However, most bone metastases have components of osteolytic and osteoblastic activity. Certain types of cancer cause predominantly osteolytic lesion such as breast cancer while other cancers cause predominantly osteoblastic lesions like prostate cancer.

TGF-β and bone metastases

The bone microenvironment is unique and may provide a fertile soil for cancer to thrive. The normal homeostasis between bone cells is disrupted by the release of growth factors and cytokines as a result of the presence of tumor cells within the bone microenvironment. The growth factors and cytokines are embedded in the mineralized bone matrix. Transforming growth factor-β (TGF-β) is the most abundant of these factors. TGF-β is part of a large family of polypeptide growth factors that includes activins, inhibins, and bone morphogenetic proteins (BMPs). TGF-β is released in a latent form and it is the proteolytic cleavage, interaction with integrins, or pH changes in the local environment that activates TGF-β. High local levels of liberated TGF-β result in increased tumor invasion, angiogenesis as well as immune suppression. In addition, TGF-β stimulates tumor production of osteolytic factors that stimulate further bone resorption[8,9]. This categorizes TGF-β as a crucial factor responsible for driving the feed-forward vicious cycle of tumor growth in bone. Therefore blocking TGF-β release, its production and/or signaling is a promising strategy to treat bone metastasis. Over the past two decades, several therapeutic strategies have been developed to inhibit TGF-β including TGF-β receptor I kinase inhibitors, TGF-β neutralizing antibodies, soluble receptor decoy-Fc fusions and TGF-β antisense oligonucleotides[10].

Muscle weakness and cachexia in cancer patients

Muscle weakness and fatigue is a significant co-morbidity of osteolytic bone metastases. This is often associated with cancer cachexia; a paraneoplastic syndrome associated with many types of cancer and is characterized by severe wasting due to loss of lean and fat mass[11]. Cachexia has long been recognized as a syndrome with an independent adverse effect on patients with cancer. Once cachexia has progressed to a clinically evident refractory stage, it is generally considered irreversible and is associated with an extremely high mortality[12,13]. Cancer cachexia is associated with high levels of interleukin (IL)-6, which is regulated by HIF-1α, NFκ B and TGF-β[11]. However, there are no effective therapies for muscle weakness or cachexia. Current clinical trials focus on improvement of muscle mass, but muscle function per se has not been adequately studied.

Normal muscle contraction and the role of RYR1 in muscle function

Excitation-contraction coupling is the process by which an electrical impulse of muscle fibers initiates muscle contraction. It is the activation of Ach nicotinic receptors that induces an end-plate potential and this electrical impulse continues along the muscle fiber as an action potential[14]. The action potential depolarization is generated primarily by a current through Nav1.4 sodium channels, the skeletal muscle voltage-gated sodium channel, localized at the sarcolemma and through the transverse (T)-Tubule system of the myofiber. Depolarization of the T-tubule membrane induces conformational changes in the T-tubule EC coupling voltage sensor, the voltage-gated Ca^{2+} channel, also known as the dihydropyridine receptor (DHPR). In skeletal muscle, the DHPR is mechanically coupled to the ryanodine receptor Ca^{2+} release channel type 1(RyR1), which in contrast to the DHPR, rests in the sarcoplasmic reticulum[14].

Ryanodine receptors (RyRs) are intracellular Ca^{2+} release channels on the sarcoplasmic and endoplasmic reticula required for fundamental cellular functions in most tissues, including skeletal and cardiac muscle excitation–contraction coupling, synaptic transmission and pancreatic beta cell function[15]. It is a Ca^{2+} permeable non-selective cation channel that releases Ca^{2+} stored in the sarco-endoplasmic reticulum of excitable and non-excitable cells. This ion channel is a tetramer and each sub unit contains approximately 5000 amino acids, so one single ion channel weights around 2MDa[16]. The type 1 Ryanodine Receptor (RyR1) mediates excitation–contraction coupling in skeletal muscle. Approximately 50% of RyR1 channels are mechanically activated by direct interaction with voltage-gated Ca^{2+} channels (VGCCs) on the plasma membrane[15]. Pathological oxidation of RyR1 results in leaky channels that contribute to muscle weakness[17,18].

Oxidative stress and Cancer

Oxidative stress is one of the most important events that gives rise to the conditions leading to tumor onset and progression[19]. Reactive oxygen species (ROS) is one of the most important species of free radicals. They are produced by various metabolic pathways, including mitochondrial aerobic metabolism. ROS play a critical role in the initiation and progression of various types of cancers. ROS controls many cellular processes, including cell proliferation, and thus stimulates the uncontrolled cell growth which may lead to tumor development[20].

In the case of chronic inflammation, the secretion of ROS/reactive nitrogen species RNS may lead to the amplification of dysregulated processes and eventually to the development of a preneoplastic condition. If the amount of cellular ROS/RNS produced is high enough to overcome an endogenous antioxidant response, than irreversible oxidative damage can

occur nucleic acids, lipids, and proteins, which may result in genetic and/or epigenetic alterations leading to the dysregulation of oncogenes and tumor suppressor genes. Hence, the oxidative stress and chronic inflammation processes are tightly coupled and the failure to block these processes can result in genetic/epigenetic changes that drive the initiation of carcinogenesis[21]. Low antioxidant status and increased oxidative stress levels are detected in cancer patients, even before oncology treatment starts. Hence the evaluation of tissue redox status has a diagnostic potential in oncology[22].

Muscle weakness and RyR1 oxidation in bone metastases

To study the role of oxidation in muscle weakness associated with osteolytic bone metastases, we examined mice with bone metastases resulting from different human cancers, including breast cancer (MDA-MB-231, MCF-7and, ZR-75-1), lung cancer (A549 and RWGT2), prostate cancer (PC-3) and multiple myeloma (JJN3). We compared the results obtained from each tumor group of mice with those from non-tumor bearing mice. All tumor-bearing mice presented with a reduction in forelimb grip strength and their Extensor Digitorum Longus (EDL) muscle showed lower *ex vivo* muscle-specific force. We tested for peak tetanic Ca^{2+} to determine muscle force, and we found that this was also lower in the MDA-MB-231–inoculated mice than in the non–tumor-bearing control mice. Thus, in addition to loss of muscle mass, mice with breast cancer bone metastases had loss of muscle function[23]. This observation was extended in several other murine models of cancers that cause osteolytic lesion including lung cancer, prostate cancer and multiple myeloma as well as in breast cancer models with osteoblastic bone metastases including the ZR-75-1 and MCF-7.

Since our data indicate that muscle weakness strongly correlated with bone destruction and remodeling, we further investigated whether the interaction between the tumor and-bone microenvironment plays a role in cancer-associated muscle weakness. Using a mammary fat pad breast cancer mouse model, we inoculated MDA-MB-231 breast cancer cells (10^6) into the mammary fat pad. In this model tumor grows locally and does not metastasize to the bone. We found that, in contrast to mice with bone metastases, mice with primary MDA-MB-231 mammary tumors had normal muscle function. These mice showed no difference in body weight, body composition or muscle mass, compared to non-tumor bearing controls. Notably, these mice did not exhibit the biochemical signature of leaky RyR1 channels, in contrast to mice with bone metastases. These data suggest that the invasion of the bone-microenvironment with tumor cells has a critical role in the development of cancer-associated muscle weakness.

Skeletal muscle from mice and humans with bone metastases exhibited higher skeletal muscle protein oxidation as compared to muscle from mice and humans without bone metastases. These included sarcomeric proteins (tropomyosin and myosin and others) and the RyR1 Ca^{2+} release channel, which was identified as being both nitrosylated and oxidized. Our data indicated that, oxidation of RyR1 channels in skeletal muscle results in a pathological Sarcoplasmic Reticulum SR Ca^{2+} leak that is associated with muscle weakness in the mouse model of bone metastases. Skeletal muscle RyR1 channels from mice with bone metastases were oxidized, nitrosylated and depleted of the stabilizing subunit calstabin1 (also known as FKBP12) when compared to that from non-tumor control mice. Oxidation, nitrosylation and depletion of the calstabin1 stabilizing subunit are considered a biochemical signature for leaky RYR1[17,24]. To determine whether the RyR1 modifications observed in murine models of bone metastases are relevant to human cancer, we examined skeletal muscle samples from patients with breast and lung cancer–associated bone metastases for RyR1 and compared them to those from humans who did not have cancer. We found the same post-translational modifications as we observed in the murine models, which we hereafter refer to as the biochemical signature of leaky RyR1 channels.

Inhibiting RyR1 Ca^{2+} leak improves muscle strength

S107 is a small molecule in the 1,4-benzothizepine family. It inhibits the oxidation-induced depletion of the channel-stabilizing subunit calstabin1 from the RyR1 complex. This action stabilizes the closed state of the channel and prevents aberrant intracellular Ca^{2+} leak, thereby improving the Ca^{2+} signal for muscle force production and enhancing muscle strength and exercise capacity in rodents[25]. Significant improvement in forelimb grip strength and *ex vivo* muscle-specific force of the EDL in mice with breast cancer–associated bone metastases was achieved when mice were treated with S107 compared to vehicle-treated mice. In mice with bone metastases, S107 treatment prevented depletion of calstabin1 from the skeletal muscle RyR1 complex but, as previously reported, did not prevent oxidation or nitrosylation of RyR1[26]. In Mice with bone metastases, S107 treatment also led to higher peak tetanic Ca^{2+} in muscle fibers and lower skeletal muscle RyR1 open probability (Po), which is consistent with decreased SR Ca^{2+} leak, compared to vehicle treated mice.

The SR/ER membrane is endowed with Ca^{2+} release channels and SERCA pumps. Ca^{2+} release channels (RYR1) allow Ca^{2+} to diffuse out of the store on opening of the channels whereas the SERCA accumulate Ca^{2+} in the lumen against its electrochemical gradient[27] and lower SERCA activity could contribute to decreased tetanic Ca^{2+}. In our studies, we observed no difference in SERCA activity in skeletal muscle from non-tumor mice as compared to that from mice with bone metastases whether or not mice were treated with S107.

To see if S107 treatment had any effect on tumor growth in mice, we looked by x-ray at the extend of bone destruction and histologically on tumor burden as well as the number of osteoclasts at the tumor bone interface as markers of tumor aggression and development. S107 had no effect on the development and progression of bone metastases in our mouse model. S107 has no effect on body weight, or the distribution of fat and lean composition, muscle mass or affect muscle fiber diameter or mid-calf cross-sectional area as compared to vehicle-treated mice. S107 treatment did eliminate the correlation between higher bone destruction and lower muscle function.

TGF-β inhibition improves muscle strength

As mentioned earlier, TGF-β stimulates tumor production of osteolytic factors that stimulate further bone resorption[8,9]. Our data showed that TGF-β–induced phosphorylation of the signaling factor SMAD3 was higher in skeletal muscle from mice bearing MDA-MB-231 breast cancer bone metastases compared to in non-tumor control mice. This was not observed in mice bearing MDA-MB-231 primary breast cancer

tumor without bone metastases. This observation is consistent with a systemic effect of bone-derived TGF-β on skeletal muscle.SMAD3 phosphorylation was also higher in skeletal muscle from patients with breast and lung cancer bone metastases than in skeletal muscle from patients without bone metastases. TGF-β released from the sites of bone destruction and circulate in the blood, measurements of serum TGF-β concentrations was higher in tumor-bearing mice with bone metastases, but not in those with primary breast cancer compared to non-tumor mice. Higher SMAD3 phosphorylation was also confirmed in skeletal muscle samples from mouse models of human cancers with osteolytic or mixed osteolytic/osteoblastic bone metastases due to A549 and RWGT2 lung cancer, MCF-7 breast cancer, PC-3 prostate cancer, and JJN-3 multiple myeloma compared to non-tumor controls.In contrast, the osteoblastic ZR75-1 breast cancer bone metastases did not exhibit higher SMAD3 phosphorylation in skeletal muscle compared to non-tumor control mice.

TGF-β released as a consequence of osteolytic bone destruction was determined to play a role in cancer-associated muscle weakness when TGF-β release from bone was inhabited using various pharmacological agents. Using the TGF-β receptor I kinase inhibitor SD-208, anti-resorptive bisphosphonate therapy or a combination of the two drugs, in murine model of breast cancer with bone metastases, to reduce TGF-β release from the bone matrix had a significant reduction in SMAD3 phosphorylation, indicating a block to TGF-β signaling and improved muscle function as indicated by significant improvement in forelimb grip strength and EDL muscle specific force. Treatment also reduced RyR1 oxidation and nitrosylation and preserved calstabin1 binding to the RyR1 complex compared with vehicle-treated mice. *In vitro*, SD-208 reduced SMAD3 phosphorylation in C2C12 myotubes treated with TGF-β. These data suggest that bone-derived TGF-β plays a critical role in cancer-associated muscle weakness.

RyR1 oxidation in response to TGF-β is mediated by NADPH oxidase 4 (Nox4)

The mechanism by which TGF-β mediates oxidation of RyR1 was shown to be through Nox4. Nox4 is expressed in skeletal muscle and contributes to oxidative stress in cardiomyocytes and Nox4 protein is increased in muscle in response to TGF-β signaling[28,29]. Previously published data show that Nox4 protein interacts with RyR1 channels[30]. Our current data demonstrated an increase in Nox4 co-immuno precipitation with RyR1 in skeletal muscle from mice with bone metastases as well as in muscle samples from patients with breast and lung cancer bone metastases. This observation of an increase in Nox4-RyR1 binding was reported in bone metastases from murine models of breast cancer (MDA-MB-231, MCF-7), prostate cancer (PC-3), lung cancer (RWGT2 and A549), and multiple myeloma (JJN-3). No increase in Nox4-RyR1 binding in muscle from mice with MDA-MB-231 primary breast cancer without bone metastases was observed and no increase Nox4-RyR1 binding was reported in bone metastases from breast cancer osteoblastic line ZR-75-1. *In-vitro* studies demonstrated that TGF-β treatment increased Nox4 protein expression in myotubes. This observed increase in Nox4 protein expression was blocked using the TGF-β RI kinase inhibitor, SD208, but not S107. When myotubes were treated with TGF-β Nox4 binding to RyR1 channels was increased. When we knocked down Nox4 in myotubes TGF-β treatment did not induce RyR1 remodeling or reduce of calstabin1 binding to RyR1 compared to scrambled control samples. This data confirms that Nox4 mediates TGF-β induced oxidation of RyR1.

Summary

Despite the fact that many important advances have occurred in the field of cancer cachexia over the past decade, including progress in understanding the mechanisms of cancer and the development of promising pharmacologic drugs, no approved agents for cancer cachexia currently exist. Cachexia, weight loss and muscle weakness are associated with important clinical outcomes such as decreased survival, fewer completed cycles of chemotherapy, more treatment side effects, and poorer health-related quality of life.

Different types of cancer including breast, prostate, lung and multiple myeloma shows evidence for the role of the tumor-bone microenvironment in the generation of muscle weakness. This observation suggests that a common mechanism may be broadly applicable to different types of cancer that tend to metastasize to bone or other disease states associated with bone loss. Our observations suggest that different types of therapy including blocking TGF-β using a TGFBRI kinase inhibitor or by antiresorptive drugs (bisphosphonates or denosumab) or by blocking RyR1 calcium leakage can all lead to improvement in muscle strength associated with cancer. It is now evident that a link has been established between bone and skeletal muscle whereby factors elaborated from bone can profoundly affect muscle function (Figure 2).

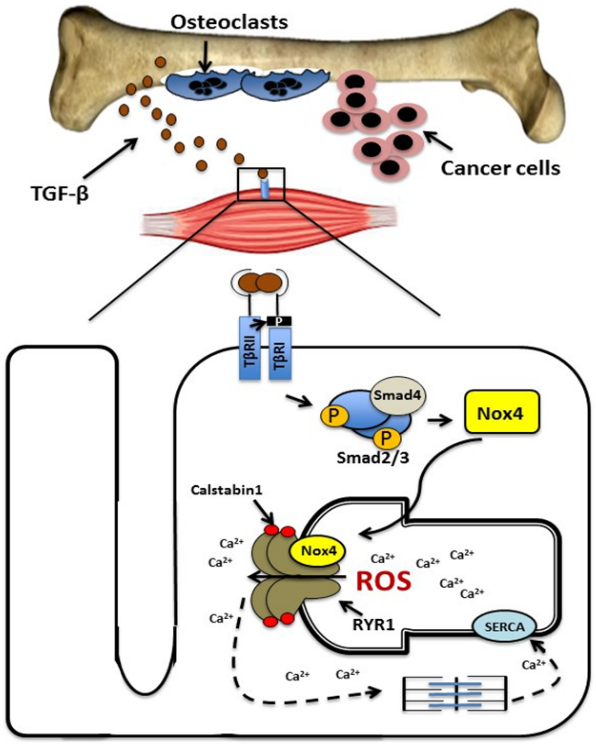

Figure 2: TGF-β is released from bone as a result of bone destruction by an osteoclast that is stimulated by a cancer cell. TGF-β drives the expression of Nox4 in muscle through SMAD2/3 signaling and Nox4 produces (ROS) which cause oxidation of the RyR1 on the (SR). Oxidation of RyR1 leads to binding of the RyR1 stabilizing unit (calstabin1) leading to Ca^{2+} leakage from the SR. Thus depleting the Ca^{2+} store and causing reduction in muscle force.

Thus, targeting skeletal muscle weakness caused by the TGF-β–Nox4–RyR1 axis represents a novel therapeutic approach to improving the quality of life in cancer patients with muscle weakness associated with increased bone destruction. Our observations indicate that muscle dysfunction can occur before the loss of muscle mass (as seen with cachexia) and suggest that a spectrum of muscle dysfunction, ranging from muscle weakness to profound cachexia, exists in patients with bone metastases.

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