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KIDNEY AND BONE (S MOE AND I SALUSKY, SECTION EDITORS)

The Role of TGFβ in Bone-Muscle Crosstalk

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Abstract

Purpose of Review The role of bone-derived factors in regulation of skeletal muscle function is an important emerging aspect of research into bone-muscle crosstalk. Implications for this area of research are far reaching and include understanding skeletal muscle weakness in cancer, osteoporosis, cachexia, rare diseases of bone, and aging.

Recent Findings Recent research shows that bone-derived factors can lead to changes in the skeletal muscle. These changes can either be anabolic or catabolic, and we focus this review on the role of TGFβ in driving oxidative stress and skeletal muscle weakness in the setting of osteolytic cancer in the bone.

Summary The bone is a preferred site for breast cancer metastasis and leads to pathological bone loss. Osteolytic cancer in the bone leads to release of TGFβ from the bone via osteoclast-mediated bone destruction. Our appreciation of crosstalk between the muscle and bone has recently expanded beyond mechanical force-driven events to encompass a variety of signaling factors originating in one tissue and communicating to the other. This review summarizes some previously known mediators of bone-to-muscle signaling and also recent work identifying a new role for bone-derived TGFβ as a cause of skeletal muscle weakness in the setting of osteolytic cancer in the bone. Multiple points of potential therapeutic intervention are discussed.

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Introduction

Our understanding of bone-muscle crosstalk has been historically based on mechanical interactions between the bone and muscle. The bone is shaped by mechanical force applied by muscles, and the bone provides an attachment site for the muscle to maintain shape and drive locomotion. The mechanical aspects of bone-muscle interactions are critical for normal development and movement and play a large role in changes of these tissues in disease and aging, yet the interactions between the bone and muscle are more complicated. Just as our understanding of other organ system integrations has advanced, so too has our understanding of the complex endocrine-based crosstalk between the bone and muscle. Bone and muscle anabolism are tightly coupled during growth and development. Conversely, bone and muscle catabolism occur during aging. Compromising either the bone or muscle by disease, disuse or aging affects both tissues but the cellular and molecular mechanisms linking these are not well understood. It is in this context that we describe the role of transforming growth factor beta (TGFβ) in bone-muscle crosstalk and muscle weakness that occurs in osteolytic cancer in bone.

Bone-to-Skeletal-Muscle Signaling

The bone is a storehouse for minerals, collagenous, and non-collagenous proteins; the latter of which includes growth factors and cytokines [1]. The bone also acts as an active signaling mediator and endocrine organ [2, 3]. In addition,

osteoblasts and osteocytes in the bone secrete paracrine and endocrine factors that can influence the skeletal muscle. The consequences of bone-to-muscle signaling include changes in skeletal muscle mass and changes in skeletal muscle function [4].

Many of these bone-derived growth factors may have significant effects on muscle function. Osteocalcin is secreted by osteoblasts and signals via the G protein-coupled receptor, Gprc6a, in many cell types including the skeletal muscle. In humans, the level of active osteocalcin correlates with an increase in lower limb strength [5•]. Osteocalcin production is increased in response to insulin signaling in osteoblasts. Circulating osteocalcin then promotes a feed-forward loop by increasing insulin synthesis and increasing insulin sensitivity in adipose tissue and muscle [6, 7]. Additionally, osteocalcin signaling in the skeletal muscle increases mitochondrial content [8]. Interestingly, skeletal muscle mass, fiber number, abundance of contractile proteins, and specific force (i.e., normalized for size and weight of the muscle) were impacted using a bone-targeted connexin 43 in mice [9]. Osteocalcin levels were reduced in the connexin-43 mice but interestingly, insulin signaling was not affected. Using a synthetic osteocalcin, some of the abnormalities in muscle were rescued, suggesting that osteocalcin may have direct effects in the skeletal muscle [9].

Insulin-like growth factor 1 (IGF1) and bone morphogenetic protein 2 (BMP2), produced by osteoblasts, can be stored in the mineral bone matrix and released as a result of osteoclast-medicated bone resorption [10]. IGF1 promotes proliferation and differentiation of myogenic cells and is an important regulator of muscle mass during development [11]. In adult skeletal muscle, Akt activation downstream of IGF1 signaling causes significant hypertrophy that showed increased force but the specific force was unchanged [12]. BMP2 signaling in muscle has been shown to promote and maintain adult muscle mass [13••, 14, 15]. Interestingly, this model of growth factor signaling-induced hypertrophy also increased absolute muscle force, yet specific force was unchanged or even slightly decreased [13••].

Prostaglandin E_2 (PGE_2) is one of several factors released by osteocytes. This release occurs in the bone by exposure to fluid shear stress [16, 17]. PGE_2 promotes osteocyte survival [18] and induces new bone formation [19]. PGE_2 also accelerates myogenic differentiation in vitro [20]. The significance of PGE_2 signaling in the skeletal muscle is not completely understood and will require more studies [4].

In contrast to bone-derived factors leading to a hypertrophic response in the skeletal muscle, several osteokines are associated with reduced muscle mass or function. Fibroblast growth factor 23 (FGF-23) is produced in the bone by osteocytes and is critical for proper mineral metabolism. FGF-23 neutralizing antibody, which increases serum phosphate and 1,25 dihydroxyvitamin D3 levels, has been shown to improve murine grip strength in a model of rickets/osteomalacia (X-linked hypophosphatemic rickets/osteomalacia [XLH]) [21, 22]. In *Dmp1* null mice, a model of autosomal recessive hypophosphatemic rickets, skeletal muscle function was reduced (EDL and soleus muscles) but cardiac force production was not affected [23]. These data suggest that FGF-23, and in addition to vitamin D levels, could influence skeletal muscle function.

TGFβ and its family members myostatin and activin cause muscle atrophy or lead to reduced function. TGFβ and activin are made by osteoblasts and stored in the mineralized bone matrix [24, 25•, 26]. Activin and TGFβ are released into circulation from the bone matrix during osteoclast-mediated bone resorption. Both TGFβ and activin can affect the muscle, but their mode of action differs. Activin strongly induces skeletal muscle wasting in vivo using an adenovirus vector in mice. In these studies, there was a profound loss of skeletal muscle mass and decrease in peak force production yet no change in specific force [27]. In contrast, mice treated in vivo with TGFβ did not have altered muscle mass but did have a significant decrease in both raw force and specific force [28••].

Bone-Derived TGFβ Causes Skeletal Muscle Weakness

Cancer cells frequently metastasize to the bone, affecting some 450,000 patients in the USA each year. Osteolytic cancer in the bone causes decreased quality of life and decreased survival in patients [29, 30]. Osteolytic cancer metastases in the bone from breast cancer increase the risk of pathologic fractures. This significantly increases mortality in patients compared to patients without fractures [30]. From a clinical perspective, systemic muscle weakness is either unrecognized or under-appreciated by many clinicians. Systemic muscle weakness increases the incidence of falls that result in fractures, and this can develop into a vicious feed-forward cycle of increased impact to functional performance which further influences risk of falls and fractures. The end result is further eroded quality of life and decreased survival [4].

Bone metastasis is a complex process which begins with the detachment of primary tumor cells from the site of origin and systemic circulation (intravasation). The tumor cells must evade immune surveillance and enter the capillaries in the bone marrow [31]. Micrometastases of tumor cells in the bone develop into either overt metastatic lesions or can lay dormant for extended periods. In either case, invading tumor cells can prime the bone pre-metastatic niche that allows for further colonization of tumor cells [32–35].

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 [4]. This process is driven by

the coupled activity of osteoclasts that resorb mineralized matrix and osteoblast that lay down new bone [36, 37]. Bone strength is maintained in healthy adults by a coordinate balance of bone-destroying osteoclasts and bone-forming osteoblasts. Ultimately, tumor cells in the bone microenvironment disrupt this normal physiological process. 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 [35].

TGFβ plays a central role in tumor growth in the bone [38–41] and is released in high concentrations from the mineralized bone matrix during osteoclastic bone resorption [40•]. In addition, bone metastases are effectively decreased by TGFβ signaling blockade [41]. Our recent work has shown the novel idea that factors released from the bone during tumor-induced bone destruction exert systemic musculoskeletal effects beyond the immediate bone microenvironment (Fig. 1). We have shown significant skeletal muscle weakness in mice with osteolytic cancer in the bone, specifically in bone metastases from either prostate, lung, or breast cancers and also in multiple myeloma which affects the bone [42••]. These changes in the muscle occur without direct involvement of tumor cells in the muscle. In addition, muscle weakness is not observed when breast cancer cells (MDA-MB-231) are restricted to the primary site (i.e., mammary fat pad without bone metastases). Muscle weakness became more prominent with increasing osteolytic lesion area in mice. Furthermore, we found that muscle weakness was systemic. Tumor cells injected directly into one tibia and that led to local osteolytic lesions that caused muscle weakness in the contralateral limb [42••].

In our study, mice with osteolytic cancer in the bone had reduced grip strength in vivo (forelimb) and also decreased whole muscle contractility measured as the specific force of the extensor digitorum longus (EDL) muscle. The difference in specific force suggested a defect in the contractile machinery in muscle. An unbiased proteomics screen was used to identify myocyte proteins that were modified in mice with osteolytic cancer in the bone. We identified the ryanodine receptor (RyR1) as being oxidized in the skeletal muscle from these mice compared to muscle from non-tumor bearing controls. RyR1 oxidation and loss of its stabilizing subunit, calstabin1, are unique biochemical signatures of RyR1 channel calcium leak that leads to muscle weakness [43, 44]. These biochemical signatures were present in the muscle from mice with osteolytic cancer in the bone and multiple myeloma, but not from mice with primary breast cancer. Also, the

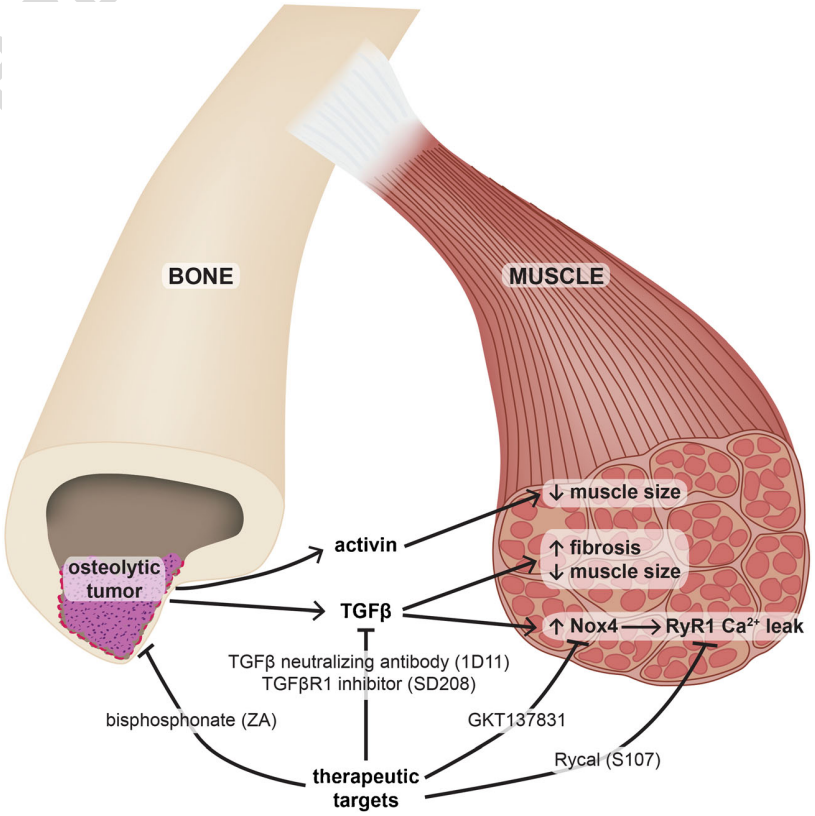

Fig. 1 TGFβ and activin signal from bone to muscle during tumor-induced osteolytic bone destruction. Upon resorption of the mineralized bone matrix, active TGFβ and activin are released into circulation and act upon skeletal muscle. Activin causes significant reductions in muscle size while TGFβ reduces muscle size, increases fibrosis, and leads to muscle contractile dysfunction. In addition, TGFβ upregulates the expression of NADPH oxidase 4 (Nox4), leading to oxidation of ryanodine receptor (RyR1) which causes calcium leak and muscle weakness. Opportunities for therapeutic intervention include (1) blocking bone destruction using bisphosphonates such as zoledronic acid (ZA), (2) blocking TGFβ activity with a neutralizing antibody (1D11) or TGFβR1 kinase inhibitor (SD-208), (3) blocking Nox4 activity (GKT137831), or (4) reducing RyR1 calcium leak using a Rycal (S107)

biochemical signature of RyR1 calcium leak was evident in skeletal muscle samples taken from patients with breast cancer that had bone metastases. This data was essential to validate the clinical relevance of our pre-clinical mouse data.

TGFβ Leads to Increased Oxidative Stress in the Skeletal Muscle

TGFβ has previously been directly implicated in muscle weakness [28••] and we have shown that with osteolytic cancer in the bone; TGFβ signaling in muscle leads to an increase in oxidative stress [42••]. In mice and humans with osteolytic cancer in the bone, SMAD3 phosphorylation was increased, which implicated a role for TGFβ signaling in skeletal muscle weakness. To further investigate the role of the TGFβ signaling pathway, we blocked TGFβ in mice with osteolytic breast cancer in the bone with (1) SD-208 (TGFβ receptor I kinase inhibitor) [45], (2) 1D11 (anti-TGFβ neutralizing antibody), or (3) zoledronic acid (bisphosphonate that blocks the release of TGFβ from the bone matrix) [40•]. All three therapeutic interventions improved our measures of muscle function, in vivo forelimb grip strength, and whole muscle contractility of the EDL muscle. Importantly, anti-TGFβ monoclonal (1D11) therapy in vivo confirms the specificity of TGFβ as a mediator of skeletal muscle weakness whereas zoledronic acid (to block bone resorption) confirms the bone as the source of TGFβ.

In addition, therapeutic treatments that blocked TGFβ release or TGFβ signaling also reduced oxidation of RyR1 and also stabilized the interaction between calstabin1 and RyR1 in a complex necessary for proper calcium handling in the skeletal muscle [4]. Due to the observed reduction in RyR1 oxidation, we began to investigate the possible sources of skeletal muscle oxidative stress. NADPH oxidase 4 (Nox4) is a constitutively active enzyme that generates reactive oxygen species (ROS); Nox4 is also a TGFβ target gene [46]. We found that Nox4 expression increased in skeletal muscle from mice with osteolytic cancer in the bone. Nox4 expression was reduced in mice treated with anti-TGFβ (SD-208 and ID11) therapies or when mice were treated with zoledronic acid. In cultured C2C12 myotubes, TGFβ was found to increase Nox4 expression and increase RyR1 oxidation and leads to reduced calstabin1-RyR1 binding. Silencing Nox4 reduced RyR1 oxidation and prevented the dissociation of calstabin1 from the RyR1 complex. Interestingly, TGFβ also caused an increase in the direct interaction between Nox4 and RyR1 in vitro. This effect was recapitulated in the skeletal muscle from mice and humans with osteolytic cancer in the bone. Finally, a Nox4 inhibitor (GKT137831 [47]) in mice, showed a significant improvement in skeletal muscle function by whole muscle contractility in mice with osteolytic cancer in the bone. GKT137831 also caused a reduction in RyR1 oxidation in mice. Taken together, these data describe an important and novel TGFβ-Nox4-RyR1 axis that is responsible for skeletal muscle weakness in cases of osteolytic cancer in the bone [42••].

Conclusions

The functions of bone and muscle are tightly coupled in normal physiology. Many recent studies have focused on the endocrine role of muscle and its interactions in bone-muscle crosstalk [4]. Osteolytic cancer in the bone significantly diverges from normal bone physiology. Our recently published work shows the bone destruction driven by osteolytic cancer in the bone directly causes skeletal muscle weakness via muscle oxidative stress and calcium mishandling [4, 42••]. We have identified the novel TGFβ-Nox4-RyR1 axis as a critical mechanism that causes significant skeletal muscle weakness [42••]. These findings have large translational potential and clinical implications. Therapeutic treatments with agents that block RyR1 calcium leak, release of TGFβ from the bone, TGFβ signaling, or Nox4 activity all significantly improved muscle function in mice with osteolytic cancer in the bone [42••]. These findings are in addition to recent studies that have shown that TGFβ blockade, via long-term treatment with losartan, inhibited muscle destruction and promoted regeneration in the *mdx* mouse model of Duchenne muscular dystrophy [48]. It has also been shown that TGFβ blockade, using suramin, prevented exercise-induced skeletal muscle damage in *mdx* mice [49].

New therapeutic targets for the debilitating complications of skeletal muscle weakness in cancer and other myopathies are needed. Studies that demonstrate new and novel mechanisms of bone-muscle crosstalk and identification and characterization of more factors that influence bone-muscle communication will make a dramatic impact on possible therapeutic targets. These studies will lead to therapeutics to treat muscle weakness in cancer, as well as other bone diseases, and even aging.

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Compliance with Ethical Standards

Conflict of Interest Jenna Regan, Trupti Trivedi, Theresa Guise, and David Waning declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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