

Skeletal muscle Ca²⁺ mishandling: another effect of bone-to-muscle signaling

Jenna N. Regan¹, David L. Waning¹, and Theresa A. Guise¹

¹Indiana University School of Medicine, Indianapolis, IN 46202, USA

Disclosure of Potential Conflicts of Interest

T.A.G. has been a consultant for Novartis

No potential conflicts of interest were disclosed by J.N.R. or D.L.W.

Corresponding author:

Theresa A. Guise

980 W. Walnut

Walther Hall R3 / Rm C132

Indianapolis, IN 46202

e-mail: tguise@iu.edu

+1 317-278-7512

This is the author's manuscript of the article published in final edited form as:
Regan, J. N., Waning, D. L., & Guise, T. A. (2016, January). Skeletal muscle Ca²⁺ mishandling: Another effect of bone-to-muscle signaling. In *Seminars in cell & developmental biology* (Vol. 49, pp. 24-29). Academic Press. <http://dx.doi.org/10.1016/j.semcdb.2015.11.007>

Abstract

Our appreciation of crosstalk between 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. While the recent identification of new 'myokines' has shifted some focus to the role of muscle in this partnership, bone-derived factors and their effects on skeletal muscle should not be overlooked. This review summarizes some previously known mediators of bone-to-muscle signaling and also recent work identifying a new role for bone-derived TGF- β as a cause of skeletal muscle weakness in the setting of cancer-induced bone destruction. Oxidation of the ryanodine receptor/calcium release channel (RyR1) in skeletal muscle occurs via a TGF- β -Nox4-RyR1 axis and leads to calcium mishandling and decreased muscle function. Multiple points of potential therapeutic intervention were identified, from preventing the bone destruction to stabilizing the RYR1 calcium channel. This new data reinforces the concept that bone can be an important source of signaling factors in pathophysiological settings.

Keywords: bone, skeletal muscle, TGF- β , calcium handling

Introduction

Interactions between skeletal muscle and bone have long been considered primarily based on a simple mechanical understanding. Bone is shaped by mechanical force applied by muscles and gravity and bone provides an attachment site for muscle to maintain shape and drive locomotion. Recently, however, we have begun to understand an additional and more complex endocrine-based crosstalk between bone and muscle that goes beyond the mechanical connection. Given the close ties between bone and muscle, it is not surprising that development and maintenance of these two tissues are coordinated. Further, it would be expected that compromising either bone or muscle by disease, disuse/unloading or aging would affect both tissues. It is with this backdrop that we describe some previously-known bone-to-muscle signaling factors and then a newly identified cause of skeletal muscle weakness in osteolytic cancer in bone.

Developmental links between muscle and bone

Muscle and bone develop in close physical association and are interdependently regulated throughout development and adult life by mechanical strain, direct signaling crosstalk, and endocrine mechanisms. Physical factors such as exercise, aging, or disuse cause coordinated changes in bone and muscle mass in both experimental animal models and humans. While the anabolic effects of increased movement and loading and, conversely, the catabolic effects of immobilization or disuse have been well-documented, a clearer understanding of the molecular mechanisms by which bone and muscle are so tightly coupled is still very much a work in progress.

During embryonic development, mesenchymal precursors condense at sites of future bone formation. This is followed by differentiation to chondrocytes of the cartilage anlage in endochondral ossification or direct differentiation to osteoblasts in the case of intramembranous ossification [1]. The skeleton undergoes a modeling phase during postnatal growth and then remodels continuously throughout life. These processes are controlled by the activities of three major bone cells: osteoblasts, osteocytes, and osteoclasts. Osteoblasts line the surface of the bone and, when active, are responsible for the deposition of the mineralized matrix of the bone. Osteoclasts are bone-resorbing cells derived from the myeloid lineage. They secrete acid and proteases to dissolve the mineral component and degrade the collagen matrix, respectively. Osteocytes are terminally differentiated cells of the osteoblast lineage which are embedded within the bone matrix. They have a unique morphology with long dendritic processes that extend through canaliculi in the bone matrix. As such, they are considered the primary sensors of mechanical loading in bone, and they also play an important signaling role, both by secretion of paracrine factors that regulate osteoblasts and osteoclasts, as well as endocrine signals [2, 3]. This is, of course, a highly simplified overview. Each of these cell types has multiple other important roles in a biological context. Coordinated activity of the bone cells maintains a steady bone mass and controls calcium homeostasis, whereas unbalanced activity of osteoblasts or osteoclasts has pathological consequences.

Concomitant with skeletal development during embryogenesis, myogenesis occurs as mesodermal precursors differentiate to myoblasts, which then fuse to form myofibers. Myofibers, which are post-mitotic, are renewed and repaired in response to injury or

growth stimulus by the activation of satellite cells. Satellite cells reside between the basal lamina and sarcolemma in resting muscles. Upon stimulation, they undergo a myogenic differentiation program and fuse into existing myofibers [4]. Adult muscle gains mass primarily through increased myofiber size (hypertrophy). Overall muscle force production is controlled both by the size and the contractile capabilities of individual myofibers. Measurements of the latter, termed muscle specific force, are corrected for differences in the size and weight of the muscle [5, 6].

The mechanical influence of muscle on bone begins during embryonic development [7]. Muscle contractions *in utero* are required for proper bone formation and growth [8-12], joint positioning and development [13], and bone morphology [11, 14]. Additional studies at early postnatal time points describe the role of muscle force in spontaneous fracture reduction [15] and development of the tendon-bone attachment site [16]. There are many studies describing the anabolic effects of physical activity and loading on both bone and muscle, some of which have been reviewed elsewhere [17, 18]. Indeed, for a detailed discussion of mechanotransduction and also the signaling mechanisms behind muscle-to-bone effects, we refer readers to several excellent recent reviews [19-22].

Bone-derived signals can affect muscle mass and function

Far from being a passive mineral storehouse, bone is increasingly recognized as an active signaling mediator and endocrine organ. Both osteoblasts and osteocytes secrete signaling molecules that can act in paracrine and endocrine fashions. Osteocalcin is a peptide secreted by osteoblasts that can signal to multiple cell types, including skeletal muscle, via the Gprc6a receptor. Osteocalcin production and activation in bone is increased in response to insulin signaling in osteoblasts. Circulating osteocalcin then promotes a feed-forward loop by increasing insulin synthesis in the pancreas as well as increasing insulin sensitivity in adipose tissue and skeletal muscle [23, 24]. There is some evidence for additional effects of osteocalcin on skeletal muscle, including increases in mitochondrial surface area [25]. In humans, undercarboxylated (active) osteocalcin (as percent of total osteocalcin) was positively correlated with lower limb strength [26]. Interestingly, skeletal muscle mass, specific force, fiber number and myosin heavy chain isoforms abundance were altered in mice with an osteoblast/osteocyte targeted deletion of connexin 43 [27]. Circulating levels of osteocalcin were reduced in these connexin 43 mutant mice but there was no evidence of alterations in insulin signaling or glucose homeostasis. Treating these mice with synthetic (undercarboxylated) osteocalcin rescued some of the muscle abnormalities, thus raising the possibility that osteocalcin may have other, more direct effects in skeletal muscle [27].

Other signaling molecules that can originate in bone and have anabolic/hypertrophic effects on skeletal muscle include insulin-like growth factor 1 (IGF1), bone morphogenetic protein 2 (BMP2), and prostaglandin E₂ (PGE₂). IGF1 and BMP2 produced by osteoblasts can either be freely secreted or incorporated into the bone matrix, to later be released through osteoclast-mediated bone resorption [28]. IGF1 is an important regulator of muscle mass during development by promoting both proliferation and differentiation of myogenic cells [29]. Akt activation (which can occur downstream of IGF1 signaling) in adult skeletal muscle has a rapid and dramatic hypertrophic effect. The hypertrophic muscles showed an increase in absolute force but

when the force measurements were normalized to cross-sectional area (i.e. specific force) the values were unchanged compared to control mice [30].

A role for BMP-Smad1/5/8 signaling in promoting and maintaining adult muscle mass has recently been elucidated [31-33]. Constitutively active BMP signaling promoted muscle hypertrophy and reduced muscle atrophy following denervation. Interestingly, this model of growth factor signaling-induced hypertrophy also increased absolute muscle force, yet specific force was unchanged or even slightly decreased [31]. Results from two independent groups strongly suggest that BMP signaling may influence muscle mass at least in part via competition with the activin/myostatin/TGF- β pathway (see below) for common cofactors and transcriptional targets.

PGE₂ is one of several factors released by osteocytes in response to fluid shear stress [34]. It has been shown to promote osteocyte survival [35] and induce bone formation [36]. *In vitro*, PGE₂ accelerates myogenic differentiation [37]. Further studies will be required to test the significance of PGE₂ signaling in skeletal muscle *in vivo*, yet it has the potential to join a growing list of growth factors and “osteokines” that mediate bone-to-muscle communication in postnatal mammalian systems. Additional factors produced by bone that may affect muscle, including sclerostin, FGF23, and Wnt proteins, will not be discussed in detail here.

In contrast to bone-derived factors provoking a hypertrophic response in skeletal muscle, transforming growth factor beta (TGF- β) and its family members myostatin, activin, and GDF-11 reduce muscle function. Abundant amounts of TGF- β and activin are stored within the bone matrix following production by osteoblasts [38-40]. Activin, myostatin, and GDF11 all signal through the activin receptor type 2B to affect muscle [41]. Activin and TGF- β can be released from the bone matrix during osteoclastic bone resorption and be circulated throughout the body. While both factors cause muscle dysfunction, the mode by which they do so differs. Activin induces systemic muscle wasting and cachexia when expressed from muscle via an adeno-associated viral vector. In the muscle expressing elevated activin levels, there was profound muscle mass loss and decreases in peak force production, yet no change in specific force [42]. In contrast, muscles treated *in vivo* with TGF- β did not change in measured mass but did experience significant fibrosis and decreased cross-sectional area, leading to decreases in both peak force and specific force values [43].

Calcium handling in skeletal muscle

Proper calcium handling in muscle is critical for contraction. During excitation-contraction (E-C) coupling in skeletal muscle, sequestered calcium in the sarcoplasmic reticulum (SR) is released through activated ryanodine receptors (RyR1) into the cytoplasm, permitting calcium-dependent actin-myosin cross-bridging and muscle contraction [44]. Cytosolic calcium is then transferred back to the lumen of the SR via the calcium-ATPase pump (SERCA1) (**Figure 1**). Maladaptive oxidative modifications of RyR1 resulting from chronic oxidative stress have been linked to pathologic SR calcium leak and diseases characterized by contractile dysfunction and muscle weakness, including heart failure [45-48], muscular dystrophy [45] and age-related sarcopenia [49]. 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 [45, 49]. The RyR1 calcium release channel stabilizer, Rycal S107, is a small molecule in the 1,4-benzothiazepine family that fixes leaky RyR1 channels by inhibiting oxidation-induced depletion of the channel-stabilizing subunit calstabin1 from the RyR1 complex, thereby stabilizing the closed state of the channel and improving muscle strength.

Osteolytic cancer in bone and skeletal muscle weakness

Bone is a frequent site for cancer metastases, with more than 450,000 patients affected per year in the U.S. Osteolytic cancer in bone is a major contributor to decreased survival and quality of life for patients [50, 51]. Pathologic fractures caused by osteolytic cancer in bone in breast cancer patients increases risk of death compared to breast cancer patients without fractures [51]. Similarly, elevated serum bone resorption marker levels are highly predictive of negative outcomes in patients with osteolytic cancer in bone [52]. Systemic muscle weakness is under-appreciated or unrecognized by many clinicians and increases the risk of falls that result in fractures and further negatively impact performance status and survival.

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 [53]. 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 [54-57].

Bone strength is maintained in healthy adults by a coordinate balance of bone destroying osteoclasts and bone forming osteoblasts. Cancer in bone disrupts this normal remodeling process by producing factors which stimulate abnormal bone destruction and new bone formation, weaken bone and predispose to fractures [57]. Bone is a large storehouse for growth factors, such as TGF- β , which are deposited in bone matrix by osteoblasts. In fact, bone is the largest storehouse of TGF- β in the body [39]. TGF- β plays a central role in tumor growth in bone [58-61] and is released in high concentrations from the mineralized bone matrix during osteoclastic bone resorption [60]. TGF- β acts on tumor cells to enhance secretion of osteolytic factors [62] that increase bone destruction, driving a vicious cycle of skeletal metastases and bone destruction [57]. In addition, bone metastases are effectively decreased by TGF- β signaling blockade [61].

The idea that factors released from bone during tumor-induced bone destruction exert systemic musculoskeletal effects beyond the immediate bone microenvironment is new. Recent work from our lab has shown that a significant reduction in skeletal muscle function occurs in mice with bone metastases from breast, lung, and prostate cancer and in multiple myeloma in bone [63]. These changes in muscle function occur without direct involvement of tumor cells in muscle and are not observed when tumor growth is limited to the primary site (i.e. no bone metastases). The extent of bone destruction and muscle weakness were positively correlated, consistent with a causal relationship.

Furthermore, muscle weakness developed in the contralateral limb in mice with tumor in a single tibia, indicating the systemic nature of muscle weakness due to bone-destruction [63].

In our study, mice with osteolytic bone lesions had reduced forelimb grip strength *in vivo* and also decreased *ex vivo* specific force of the extensor digitorum longus (EDL) muscle. Notably, the difference in specific force suggested an internal defect in the contractile capability of individual myofibers. An unbiased proteomics approach identified RyR1 as being oxidized and nitrosylated in skeletal muscle from mice with breast cancer bone metastases compared to muscle from non-tumor bearing mice. RyR1 oxidation and loss of its stabilizing subunit, calstabin1, is a unique biochemical signature of leaky RyR1 channels. This biochemical signature was present in muscle from mice with osteolytic bone metastases and multiple myeloma, but not from mice with primary breast cancer (no bone metastases). Importantly, the biochemical signature of RyR1 calcium leak was also evident in skeletal muscle samples taken from patients with breast cancer that also had bone metastases, validating the clinical relevance of the mouse data. Rycal S107 improved *in vivo* forelimb grip strength and *ex vivo* specific force (EDL) in mice with breast cancer bone metastases. Rycal S107 prevented dissociation of calstabin1 from the RyR1 complex even in the presence of RyR1 oxidation as previously reported [45, 49], and without directly reducing tumor progression or bone destruction. These data showed that RyR1 calcium leak plays a role in skeletal muscle weakness in osteolytic cancer in bone.

Bone-derived TGF- β leads to skeletal muscle weakness via increase in oxidative stress

TGF- β has been implicated in muscle weakness [43] and TGF- β is released from bone as a consequence of bone metastases [60]. SMAD3 phosphorylation was increased in muscle of mice and humans with bone metastases, implicating TGF- β signaling in weakness. To investigate the contribution of this signaling pathway, we blocked TGF- β in mice with breast cancer bone metastases using: 1) TGF- β receptor I kinase inhibitor (SD-208) [64], 2) anti-TGF- β ligand monoclonal antibody (1D11), or 3) bisphosphonate (zoledronic acid, ZA) to inhibit release of TGF- β from bone [60]. All three interventions significantly improved *in vivo* forelimb grip strength and *ex vivo* EDL muscle specific force. Importantly, significant improvements of muscle function in mice receiving anti-TGF- β monoclonal (1D11) therapy confirms the specificity of TGF- β as a mediator of the muscle weakness and blockade of bone resorption (ZA) confirms that bone is the source of TGF- β .

Treatments that blocked TGF- β release or signaling also reduced RyR1 oxidation and nitrosylation and stabilized calstabin1-RyR1 complexes. Because there was a reduction in RyR1 oxidation, we investigated sources of oxidative stress linked to TGF- β . NADPH oxidase 4 (Nox4) is a constitutively active oxidase and TGF- β target that generates reactive oxygen species (ROS) [65]. We found Nox4 expression was increased in muscle from mice with breast cancer bone metastases, whereas Nox4 expression was reduced when mice were treated with either anti-TGF- β (SD-208 and 1D11) or anti-resorptive (ZA) agents. In cultured myotubes, TGF- β increased Nox4 expression, RyR1 oxidation and loss of calstabin1 binding. Nox4 silencing was able to reduce RyR1

oxidation and prevent dissociation of calstabin1 from the RyR1 complex. TGF- β also increased the direct interaction between Nox4-RyR1 *in vitro*, an association also found in muscle from mice and humans with breast cancer bone metastases. Finally, using a Nox4 inhibitor (GKT137831 [66]) *in vivo*, we showed significantly improved *ex vivo* EDL specific force in mice with breast cancer bone metastases and reduction in RyR1 oxidation. These data describe a novel TGF- β -Nox4-RyR1 axis responsible for skeletal muscle weakness in osteolytic cancer in bone [63].

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 crosstalk. Osteolytic cancer in bone represents a divergence from normal bone physiology by tipping the balance of remodeling. Our recently published work shows the bone destruction driven by osteolytic tumor cells also directly causes skeletal muscle weakness. We have identified the TGF- β -Nox4-RyR1 axis as the mechanism by which a factor released from the bone matrix (TGF- β) leads to oxidation of RyR1 and calcium mishandling in skeletal muscle that severely compromises muscle function [63] (**Figure 2**). Pharmacological blockade of: 1) RyR1 calcium leak, 2) TGF- β release from bone, 3) TGF- β signaling, or 4) Nox4 activity, all improved muscle function in mice with osteolytic cancer in bone [63]. This identification of new potential therapeutic targets illustrates the importance of considering the bone as a source of signaling factors in disease states. Furthermore, this represents a new mechanism of bone-muscle crosstalk where bone plays a predominant role over muscle and becomes a source of 'osteokines' that affect muscle function. The continued identification and characterization of such factors will provide new possibilities for therapeutic targets in muscle weakness associated with malignancy and other diseases.

Acknowledgements

This work was supported by NIH grants (U01CA143057 from the NCI Tumor Microenvironment Network; R01CA69158), Susan G. Komen Foundation, the Jerry and Peggy Throgmartin Endowment of the IU Simon Cancer Center, IU Simon Cancer Center Breast Cancer Program, American Cancer Society and IU Simon Cancer Center (IRG-84-002-28), the IU Health Strategic Research Initiative in Oncology, and a generous donation from the Withycombe family.

Figure 1. Excitation-contraction coupling. Contraction begins with an action potential that propagates through the T-tubule system. Calcium is released from the sarcoplasmic reticulum (SR) via interaction of the dihydropyridine receptor (DHPR) and the ryanodine receptor 1 (RyR1). Calcium release from the SR store enables actin-myosin cross-bridging and muscle contraction. Myoplasmic free calcium concentration is restored to resting levels primarily by pumping calcium back into the SR via the sarco/endoplasmic reticulum ATPase (SERCA).

Figure 2. Skeletal muscle weakness due to osteolytic cancer in bone. Activation of the TGF- β -Nox4-RyR1 axis in the skeletal myocyte begins with release of TGF- β from the bone matrix during osteoclast-mediated bone resorption. TGF- β signaling leads to increased oxidation of ryanodine receptor 1 (RyR1) via the constitutively active NADPH oxidase 4 (Nox4). RyR1 oxidation causes sarcoplasmic reticulum (SR) calcium leak and muscle weakness.

References

- [1] Long F, Ornitz DM. Development of the endochondral skeleton. *Cold Spring Harb Perspect Biol* 2013;5:a008334.
- [2] Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. *Endocr Rev* 2013;34:658-90.
- [3] Bellido T. Osteocyte-driven bone remodeling. *Calcif Tissue Int* 2014;94:25-34.
- [4] Dumont NA, Wang YX, Rudnicki MA. Intrinsic and extrinsic mechanisms regulating satellite cell function. *Development* 2015;142:1572-81.
- [5] Fauconnier J, Meli AC, Thireau J, Roberge S, Shan J, Sassi Y, et al. Ryanodine receptor leak mediated by caspase-8 activation leads to left ventricular injury after myocardial ischemia-reperfusion. *Proceedings of the National Academy of Sciences of the United States of America* 2011;108:13258-63.
- [6] Yamada T, Place N, Kosterina N, Ostberg T, Zhang SJ, Grundtman C, et al. Impaired myofibrillar function in the soleus muscle of mice with collagen-induced arthritis. *Arthritis Rheum* 2009;60:3280-9.
- [7] Nowlan NC, Sharpe J, Roddy KA, Prendergast PJ, Murphy P. Mechanobiology of embryonic skeletal development: Insights from animal models. *Birth Defects Res C Embryo Today* 2010;90:203-13.
- [8] Hosseini A, Hogg DA. The effects of paralysis on skeletal development in the chick embryo. I. General effects. *J Anat* 1991;177:159-68.
- [9] Shwartz Y, Farkas Z, Stern T, Aszodi A, Zelzer E. Muscle contraction controls skeletal morphogenesis through regulation of chondrocyte convergent extension. *Dev Biol* 2012;370:154-63.
- [10] Rot-Nikcevic I, Reddy T, Downing KJ, Belliveau AC, Hallgrimsson B, Hall BK, et al. *Myf5*^{-/-} :*MyoD*^{-/-} amyogenic fetuses reveal the importance of early contraction and static loading by striated muscle in mouse skeletogenesis. *Dev Genes Evol* 2006;216:1-9.
- [11] Nowlan NC, Murphy P, Prendergast PJ. A dynamic pattern of mechanical stimulation promotes ossification in avian embryonic long bones. *J Biomech* 2008;41:249-58.
- [12] Blitz E, Viukov S, Sharir A, Shwartz Y, Galloway JL, Pryce BA, et al. Bone ridge patterning during musculoskeletal assembly is mediated through SCX regulation of *Bmp4* at the tendon-skeleton junction. *Dev Cell* 2009;17:861-73.
- [13] Kahn J, Shwartz Y, Blitz E, Krief S, Sharir A, Breitel DA, et al. Muscle contraction is necessary to maintain joint progenitor cell fate. *Dev Cell* 2009;16:734-43.
- [14] Sharir A, Stern T, Rot C, Shahar R, Zelzer E. Muscle force regulates bone shaping for optimal load-bearing capacity during embryogenesis. *Development* 2011;138:3247-59.
- [15] Rot C, Stern T, Blecher R, Friesem B, Zelzer E. A mechanical Jack-like Mechanism drives spontaneous fracture healing in neonatal mice. *Dev Cell* 2014;31:159-70.
- [16] Tatara AM, Lipner JH, Das R, Kim HM, Patel N, Ntouvali E, et al. The role of muscle loading on bone (Re)modeling at the developing enthesis. *PLoS One* 2014;9:e97375.
- [17] DiGirolamo DJ, Kiel DP, Esser KA. Bone and skeletal muscle: neighbors with close ties. *J Bone Miner Res* 2013;28:1509-18.

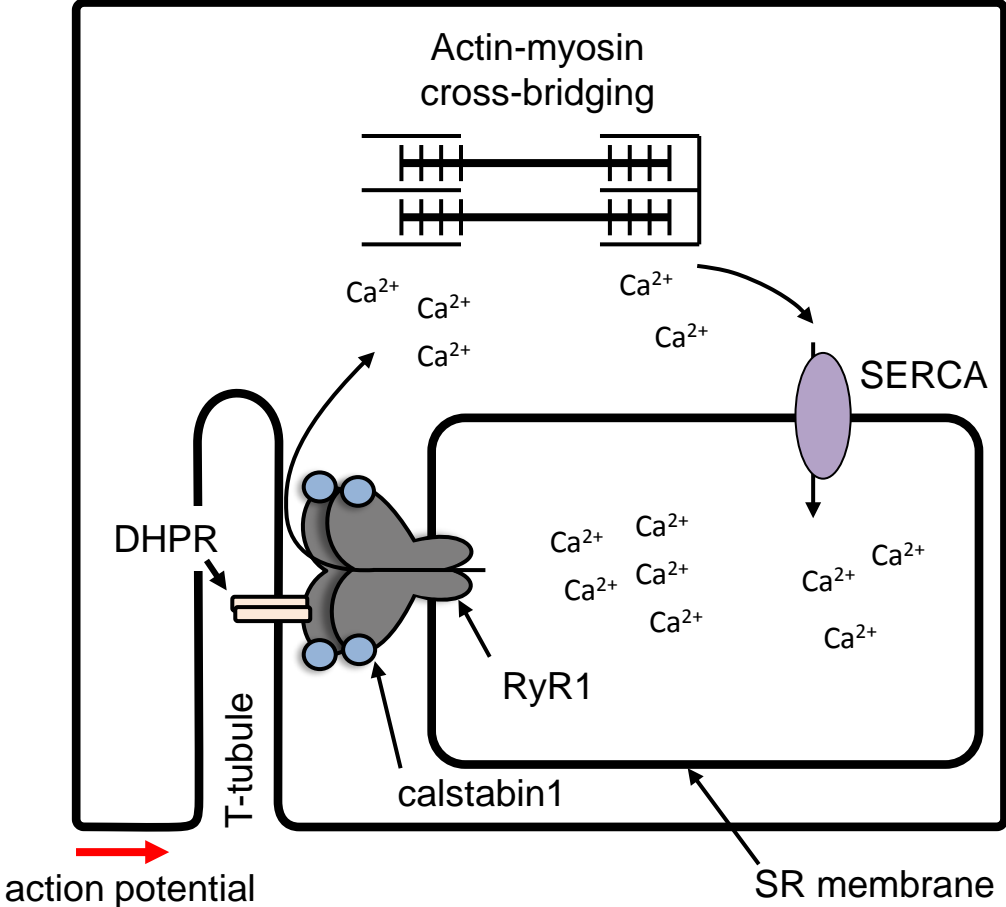
- [18] Tagliaferri C, Wittrant Y, Davicco MJ, Walrand S, Coxam V. Muscle and bone, two interconnected tissues. *Ageing Res Rev* 2015;21:55-70.
- [19] Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol* 2012;8:457-65.
- [20] Robling AG. The interaction of biological factors with mechanical signals in bone adaptation: recent developments. *Curr Osteoporos Rep* 2012;10:126-31.
- [21] Laurent MR, Dubois V, Claessens F, Verschueren SM, Vanderschueren D, Gielen E, et al. Muscle-bone interactions: from experimental models to the clinic? A critical update. *Molecular and cellular endocrinology* 2015.
- [22] Brotto M, Johnson ML. Endocrine crosstalk between muscle and bone. *Curr Osteoporos Rep* 2014;12:135-41.
- [23] Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* 2010;142:296-308.
- [24] Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell* 2010;142:309-19.
- [25] Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007;130:456-69.
- [26] Levinger I, Scott D, Nicholson GC, Stuart AL, Duque G, McCorquodale T, et al. Undercarboxylated osteocalcin, muscle strength and indices of bone health in older women. *Bone* 2014;64:8-12.
- [27] Shen H, Grimston S, Civitelli R, Thomopoulos S. Deletion of connexin43 in osteoblasts/osteocytes leads to impaired muscle formation in mice. *J Bone Miner Res* 2015;30:596-605.
- [28] Wildemann B, Kadow-Romacker A, Haas NP, Schmidmaier G. Quantification of various growth factors in different demineralized bone matrix preparations. *Journal of biomedical materials research Part A* 2007;81:437-42.
- [29] Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skeletal muscle* 2011;1:4.
- [30] Blaauw B, Canato M, Agatea L, Toniolo L, Mammucari C, Masiero E, et al. Inducible activation of Akt increases skeletal muscle mass and force without satellite cell activation. *FASEB J* 2009;23:3896-905.
- [31] Sartori R, Schirwis E, Blaauw B, Bortolanza S, Zhao J, Enzo E, et al. BMP signaling controls muscle mass. *Nature genetics* 2013;45:1309-18.
- [32] Winbanks CE, Chen JL, Qian H, Liu Y, Bernardo BC, Beyer C, et al. The bone morphogenetic protein axis is a positive regulator of skeletal muscle mass. *J Cell Biol* 2013;203:345-57.
- [33] Sartori R, Gregorevic P, Sandri M. TGFbeta and BMP signaling in skeletal muscle: potential significance for muscle-related disease. *Trends Endocrinol Metab* 2014;25:464-71.
- [34] Ajubi NE, Klein-Nulend J, Nijweide PJ, Vrijheid-Lammers T, Alblas MJ, Burger EH. Pulsating fluid flow increases prostaglandin production by cultured chicken osteocytes--a cytoskeleton-dependent process. *Biochem Biophys Res Commun* 1996;225:62-8.

- [35] Kitase Y, Barragan L, Qing H, Kondoh S, Jiang JX, Johnson ML, et al. Mechanical induction of PGE2 in osteocytes blocks glucocorticoid-induced apoptosis through both the beta-catenin and PKA pathways. *J Bone Miner Res* 2010;25:2657-68.
- [36] Harada SI, Balena R, Rodan GA, Rodan SB. The role of prostaglandins in bone formation. *Connect Tissue Res* 1995;31:279-82.
- [37] Mo C, Romero-Suarez S, Bonewald L, Johnson M, Brotto M. Prostaglandin E2: from clinical applications to its potential role in bone- muscle crosstalk and myogenic differentiation. *Recent Pat Biotechnol* 2012;6:223-9.
- [38] Bonewald LF, Mundy GR. Role of transforming growth factor-beta in bone remodeling. *Clin Orthop Relat Res* 1990:261-76.
- [39] Dallas SL, Rosser JL, Mundy GR, Bonewald LF. Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. *The Journal of biological chemistry* 2002;277:21352-60.
- [40] Sakai R, Eto Y. Involvement of activin in the regulation of bone metabolism. *Molecular and cellular endocrinology* 2001;180:183-8.
- [41] Lee SJ, Reed LA, Davies MV, Girgenrath S, Goad ME, Tomkinson KN, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:18117-22.
- [42] Chen JL, Walton KL, Winbanks CE, Murphy KT, Thomson RE, Mankanji Y, et al. Elevated expression of activins promotes muscle wasting and cachexia. *FASEB J* 2014;28:1711-23.
- [43] Mendias CL, Gumucio JP, Davis ME, Bromley CW, Davis CS, Brooks SV. Transforming growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of atrogen-1 and scleraxis. *Muscle & nerve* 2012;45:55-9.
- [44] Bellinger AM, Reiken S, Dura M, Murphy PW, Deng SX, Landry DW, et al. Remodeling of ryanodine receptor complex causes "leaky" channels: a molecular mechanism for decreased exercise capacity. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105:2198-202.
- [45] Bellinger AM, Reiken S, Carlson C, Mongillo M, Liu X, Rothman L, et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat Med* 2009;15:325-30.
- [46] Lehnart SE, Wehrens XH, Reiken S, Warriar S, Belevych AE, Harvey RD, et al. Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell* 2005;123:25-35.
- [47] Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblyt N, et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 2000;101:365-76.
- [48] Reiken S, Gaburjakova M, Guatimosim S, Gomez AM, D'Armiento J, Burkhoff D, et al. Protein kinase A phosphorylation of the cardiac calcium release channel (ryanodine receptor) in normal and failing hearts. Role of phosphatases and response to isoproterenol. *The Journal of biological chemistry* 2003;278:444-53.
- [49] Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, et al. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. *Cell Metab* 2011;14:196-207.

- [50] Lam K, Chow E, Zhang L, Wong E, Bedard G, Fairchild A, et al. Determinants of quality of life in advanced cancer patients with bone metastases undergoing palliative radiation treatment. *Support Care Cancer* 2013;21:3021-30.
- [51] Saad F, Lipton A, Cook R, Chen YM, Smith M, Coleman R. Pathologic fractures correlate with reduced survival in patients with malignant bone disease. *Cancer* 2007;110:1860-7.
- [52] Brown JE, Cook RJ, Major P, Lipton A, Saad F, Smith M, et al. Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer, and other solid tumors. *Journal of the National Cancer Institute* 2005;97:59-69.
- [53] Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell* 2011;147:275-92.
- [54] Lu X, Mu E, Wei Y, Riethdorf S, Yang Q, Yuan M, et al. VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging alpha4beta1-positive osteoclast progenitors. *Cancer Cell* 2011;20:701-14.
- [55] Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, et al. Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *The American journal of pathology* 1998;153:865-73.
- [56] Watson MA, Ylagan LR, Trinkaus KM, Gillanders WE, Naughton MJ, Weilbaecher KN, et al. Isolation and molecular profiling of bone marrow micrometastases identifies TWIST1 as a marker of early tumor relapse in breast cancer patients. *Clin Cancer Res* 2007;13:5001-9.
- [57] Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nature reviews Cancer* 2011;11:411-25.
- [58] Kang Y, He W, Tulley S, Gupta GP, Serganova I, Chen CR, et al. Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:13909-14.
- [59] Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, et al. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 2003;3:537-49.
- [60] Korpel M, Yan J, Lu X, Xu S, Lerit DA, Kang Y. Imaging transforming growth factor-beta signaling dynamics and therapeutic response in breast cancer bone metastasis. *Nat Med* 2009;15:960-6.
- [61] Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, et al. TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *The Journal of clinical investigation* 1999;103:197-206.
- [62] Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, et al. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. *The Journal of clinical investigation* 1996;98:1544-9.
- [63] Waning DL, Mohammad KS, Reiken S, Xie W, Andersson DC, John S, et al. Excess TGF-beta mediates muscle weakness associated with bone metastases in mice. *Nat Med* 2015.
- [64] Dunn LK, Mohammad KS, Fournier PG, McKenna CR, Davis HW, Niewolna M, et al. Hypoxia and TGF-beta drive breast cancer bone metastases through parallel signaling pathways in tumor cells and the bone microenvironment. *PLoS One* 2009;4:e6896.

- [65] Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, et al. Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. *Circulation* 2004;109:227-33.
- [66] Laleu B, Gaggini F, Orchard M, Fioraso-Cartier L, Cagnon L, Houngninou-Molango S, et al. First in class, potent, and orally bioavailable NADPH oxidase isoform 4 (Nox4) inhibitors for the treatment of idiopathic pulmonary fibrosis. *J Med Chem* 2010;53:7715-30.

Skeletal Myocyte



RyR1 calcium leak and muscle weakness

