Minireview

Role of myokines and osteokines in cancer cachexia

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Impact statement

The concept that muscle and bone communicate not only mechanically, but also by exchanging soluble biochemical factors has triggered new investigations in musculoskeletal research. The idea that not just tumor-derived factors, but also the interaction of secreted myokines and osteokines can induce musculoskeletal loss paves the way to the development of new therapeutics. This review summarizes the most recent progress in the study of bone and muscle-derived factors in a setting of cancer cachexia. We highlight that soluble factors initially thought to derive exclusively from bones and muscles can instead be released also by tumors, suggesting a new concept of the trifecta of cancer-bone-muscle crosstalk. Lastly, we suggest the idea that future personalized therapeutic interventions should target not just the cancer soluble factors, but also bone and muscle to mitigate the negative effects of this triple "secretome" in order to improve outcomes and enhance retention and recovery of musculoskeletal function.

Abstract

Cancer-induced muscle wasting, i.e. cachexia, is associated with different types of cancer such as pancreatic, colorectal, lung, liver, gastric and esophageal. Cachexia affects prognosis and survival in cancer, and it is estimated that it will be the ultimate cause of death for up to 30% of cancer patients. Musculoskeletal alterations are known hallmarks of cancer cachexia, with skeletal muscle atrophy and weakness as the most studied. Recent evidence has shed light on the presence of bone loss in cachectic patients, even in the absence of bone-metastatic disease. In particular, we and others have shown that muscle and bone communicate by exchanging paracrine and endocrine factors, known as myokines and osteokines. This review will focus on describing the role of the most studied myokines, such as myostatin, irisin, the muscle metabolite β-aminoisobutyric acid, BAIBA, and IL-6, and osteokines, including TGF-β, osteocalcin, sclerostin, RANKL, PTHrP, FGF23, and the lipid mediator, PGE_2 during cancer-induced cachexia. The interplay of muscle and bone factors, together with tumor-derived soluble factors, characterizes a complex clinical scenario in which musculoskeletal alterations are amongst the most debilitating features. Understanding and targeting the "secretome" of cachectic patients will likely represent a promising strategy to preserve bone and muscle during cancer cachexia thereby enhancing recovery.

Keywords: Muscle, bone, cancer, cachexia, myokines, osteokines

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Cancer cachexia

Cachexia is defined as "ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment".[1] This condition is a comorbidity of cancer and other chronic diseases such as heart failure, chronic kidney disease, as well as chronic obstructive pulmonary disease, and is known to impair physical function, alter quality of life, reduce tolerance to anticancer therapy, and shorten patients' survival.[2] It is estimated that the prevalence of cachexia in the cancer population varies from 50% up to 80% depending on stage of the disease and tumor type. As such, cachexia is particularly common in pancreatic, esophageal, gastric, colorectal, lung, and liver cancer patients.[3] Moreover, it is estimated that cachexia will be the ultimate cause of death for up to 30% of all cancer patients.[4]

The etiology of cachexia is influenced by a complex interplay between the tumor burden and the consequent immune/inflammatory response of the host body. As such, several tumor-derived and host-derived humoral factors participate in the organ damage characterizing this syndrome.[5] New evidence from ours and other groups clearly shows the detrimental role of anticancer treatments (e.g. chemotherapy) in the pathogenesis of cachexia.[6,7] The progression of cachexia is a continuum composed of three stages of clinical relevance: pre-cachexia, cachexia, and refractory cachexia. Not all patients experience all phases and the progression depends on different individual factors.[1] This classification is critically important for the clinical management of cancer patients. Based on this, the identification of biomarkers of pre-cachexia is crucial to design clinical interventions targeting muscle and bone that, when administered in its earliest stages, may be able to correct this condition.

Features of cachexia

Some of the principal systemic features of cachexia are reduced food intake, altered energy balance, metabolic abnormalities, and imbalance between anabolism and catabolism. These systemic derangements affect several tissues and organs, thus contributing to defining cachexia as a multi-organ syndrome. Among the most important drivers of cachexia are the systemic inflammatory response, the presence of high levels of tumor-derived factors, and the exacerbated protein catabolism that characterizes tumor metabolism *per se.*[4]

Systemic inflammation appears to be a hallmark of the majority of cancer patients, and proinflammatory cytokines, including IL-6, TNF-α, IFN-γ, and IL-1β are found chronically elevated in blood, peripheral tissues, and the central nervous system of subjects with cancer cachexia.[8] Pro-inflammatory cytokines can be produced by both the immune system and by tumor cells. Interestingly, the presence of tumor cells stimulates the body's innate immune system to produce an acute phase response, characterized by the production of acute phase proteins such as C-reactive protein, which in turn can negatively impact muscle mass, thus playing a critical role in the pathogenesis of cachexia.[9] Cytokines and chemokines are known to act in both paracrine and endocrine manners and together can generate a negative energy balance, altered metabolism, and a dramatic imbalance between anabolism and catabolism.[10] Moreover, it has been shown that inflammatory mediators can affect the central nervous system, thus promoting the occurrence of anorexia,[11] which, along with altered food taste, early satiety, pain and nausea, contributes to the reduction of food intake and to overall malnutrition,[4] thereby leading to the progressive body wasting that characterizes cachectic cancer patients.

Amongst the most relevant features of cancer cachexia are severe skeletal muscle loss and weakness. Skeletal muscle atrophy is due to an imbalance between protein synthesis and degradation rates that together determine a negative nitrogen balance.[12] During cancer cachexia, the increased skeletal muscle protein breakdown is primarily due to the hyperactivation of different proteolytic systems such as the ubiquitin-proteasome, Ca^{2+}-dependent calpains, caspases, and autophagy-lysosome systems.[13] However, whether alterations of protein synthesis rates also play a role in cancer-induced muscle wasting remains unknown. In this regard, we recently showed that impaired ribosomal production can determine anabolic deficit in cancer cachexia, although the causes of such impairment remain partially unclear.[14] In contrast, a reduced muscle regenerative process has also been found to contribute to muscle atrophy in a cancer setting.[13]

Fatigue and weakness (i.e. reduction of muscle strength and endurance) often accompany skeletal muscle atrophy and are both complications that severely compromise quality of life. The drivers of these functional impairments are not clearly defined. Interestingly, in combination with tumor burden, anticancer cytotoxic agents appear to play a detrimental role in muscle mass and function, and it has been shown that chemotherapy muscle toxicity can persist for long periods of time, even after tumor remission. In this regard, proper assessment of body composition (i.e. the proportion between lean and fat mass in a body) is becoming crucially important in choice of cancer treatment, especially considering that lower lean mass in cancer patients frequently associates with discontinuation, dose limitation of the therapy, and poor survival.[15]

While the alterations of skeletal muscle consequent to the onset of cancer are extensively studied, on the contrary, the bone loss occurring during cancer cachexia is poorly understood. Bone is the preferential site of metastasis for several types of malignancies, including breast and prostate cancer, and is a fairly common event in melanoma, lung, colorectal, and thyroid cancers. Cancer metastases can dramatically affect quality of life and ultimately reduce survival in cancer patients since once cancer has entered bone it is more difficult to treat.[16] Cancer metastases to bone, as well as anticancer agents are known to increase osteoclast number and activity, thus leading to reduced bone mass by enhanced bone resorption and formation of osteolytic lesions.[16] These events increase the risk of fracture, pain, and hypercalcemia.[16]

Interestingly, bone loss can occur also in the absence of bone metastases. Indeed, the incidence of vertebral fracture was found five times higher in women with breast cancer without bone metastases.[17] Similarly, cervical cancer patients were found to present lower bone mineral density in the absence of bone metastases,[18] whereas 40% of patients affected by non-small lung cancer without tumor dissemination to bone were shown to present with osteoporosis and osteopenia.[19] Several preclinical models for the study of cancer support the idea that bone alterations can occur in the absence of bone metastases. We showed that mice bearing the ES-2 ovarian cancer or the MC-38 colorectal cancer present with severe bone loss along with the development of skeletal muscle atrophy and weakness.[20,21] In addition, we also previously showed that routinely administered chemotherapy regimens can affect bone tissue and, for this reason, may play a critical role in driving the harmful musculoskeletal alterations that affect cancer patients.[22,23]

Bone and muscle: A mutual interaction

Bone and muscle both derive from the paraxial mesoderm,[24] share common mesenchymal precursors, and develop synchronously during embryonic development.[25] Together, they are the most abundant tissues in the whole body. With the contribution of tendons, ligaments, joints, vascular and nervous systems, they constitute the support and the locomotion apparatus for the organism. While muscle contraction loads the bone, hence improving bone strain leading to maintenance or new bone formation, in contrast, immobility, and muscle atrophy induces bone loss due to unloading of bone.[26] It is well known that exercise is essential to maintain a healthy musculoskeletal system. However, different types of exercise have distinctive effects on bone and muscle. For example, endurance exercise, such as running, stimulates oxidative fibers can be associated with lower bone mineral density, BMD, compared with the resistance exercise, such as lifting weights, that increases muscle mass and is associated with higher BMD.[27] Dogma was that the mechanical interaction was the only interaction between the two tissues. More recently, several studies have suggested that muscle and bone can interact also in a biochemical and endocrine manner, in both physiological and pathological conditions.[28] Based on this idea, bone and skeletal muscle can function as secretory organs that can release paracrine and endocrine factors named "osteokines" and "myokines", respectively. This new concept of biochemical signaling is becoming an important area of study and the balance between myokines and osteokines likely plays a critical role in the maintenance of a healthy musculoskeletal system.

One example of the importance of bone and muscle interaction is in fracture healing. Using a murine open tibia fracture model in which skeletal muscle tissue was simultaneously damaged, faster bone recovery and a superior quality of the repair were observed.[29] The same observation can be appreciated also in human open tibia fractures.[26] A possible explanation of this event is that the muscle flaps adjacent to the bone fracture produce factors that improve bone repair.[26] Moreover, skeletal muscle stem cells have also been shown to act like osteoprogenitors, thus improving bone healing.[30] Overall, these findings show that access of skeletal muscle, i.e. "muscle flaps", to the fracture location can improve the fracture outcome.[31] Another example of bone-muscle communication was shown by using a murine model of osteogenesis imperfecta, a genetic connective tissue disorder characterized by "brittle bone" more susceptible to fractures. Surprisingly these mice also develop skeletal muscle weakness in spite of the absence of any muscle pathology. The investigators suggest that abnormal bone can release factors that compromise skeletal muscle function.[26,32]

The role of myokines in muscle-bone crosstalk

Myostatin

Myostatin (GDF-8) is a well-known myokine and member of the transforming growth factor (TGF) β superfamily. It was first described in double-muscled cattle, characterized by dramatically increased muscle mass due to a mutation in the myostatin gene.[33] Myostatin is a negative regulator of skeletal muscle mass; high levels induce muscle atrophy, whereas lower or lack of expression induces muscle hypertrophy in not only animals, but also in humans. Myostatin binds the activin receptor type-2 resulting in the phosphorylation of Smad2 and Smad3 leading to downstream signaling to inhibit the Akt-TORC1 anabolic pathway to ultimately impact muscle differentiation.[34] The skeletal muscle atrophy and adipose tissue wasting induced by overexpression of myostatin were the first indication of a potential role in the pathogenesis of cancer cachexia.[35] Several preclinical models of cancer cachexia, such as the Yoshida AH-130 hepatoma in rats and the C26 adenocarcinoma in mice, were shown to have increased myostatin levels in skeletal muscle, consistent with severe skeletal muscle wasting.[36,37] High levels of myostatin were also found to be elevated in the skeletal muscle of cancer patients.[38] However, the actual function of myostatin in human cancer-associated cachexia remains less clear.

Not only does myostatin have a negative effect on muscle, but it also has negative consequences on bone. Myostatin deficiency was associated with increased osteogenic differentiation of bone marrow-derived mesenchymal stem cells, as well as with increased bone mass and strength.[39] More recently, it was shown that myostatin inhibits osteoblast differentiation by directly reducing the osteocyte-derived production of exosomal miR-218.[40] Myostatin was also found able to enhance the action of RANKL on osteoclast formation, both *in vitro* and *in vivo*.[41] In elderly subjects, the levels of mature myostatin were found to negatively correlate with BMD and positively with markers of bone resorption.[42]

Other members of the TGF-β superfamily such as activin are associated with muscle and bone alterations during cachexia. We recently showed that treatment with antagonists to the activin receptor type-2B (ACVR2B) was able to improve the cachectic phenotype. Indeed, the administration of the ACVR2B/Fc soluble receptor decoy was able to preserve body weight, bone mass, skeletal muscle mass, and strength both in models of chemotherapy and metastatic colorectal cancer-induced cachexia.[22,43]

Irisin

Irisin is the cleavage product of the transmembrane protein fibronectin type III domain-containing protein 5 (FNDC5) and was first described as released from skeletal muscle after exercise.[44] This protein can increase oxidative metabolism, promote myogenesis, and increase skeletal muscle mass.[45] Due to its potent action on regulating the browning of white adipose tissue, hence leading to reduced body weight and reduced adipose accumulation, a role for irisin in the regulation of obesity has also been intensely investigated.[46] In a model of atrophy induced by denervation, irisin was able to improve muscle mass by affecting myogenic signaling.[47] Interestingly, cortical bone mass was also modestly increased by the treatment with a low-irisin

dose.[48] In post-menopausal women with osteoporosis, as well as in patients with diabetes, liver and heart disease, irisin levels negatively correlated with the risk of fractures.[49] However, on the contrary, work from other groups showed that global FNDC5 knock-out mice show reduced RANKL levels in the circulation, consistent with increased trabecular bone.[50] Also, irisin was found to increase the expression of the negative bone regulator sclerostin, in MLO-Y4 osteocyte cultures and *in vivo*, thus possibly stimulating bone catabolism.[50] Based on these opposing observations, the role of irisin on bone regulation remains controversial.

In a model of gastric cancer-induced cachexia, the expression of FNDC5 was increased in adipose tissue, as were high circulating levels of irisin.[51] Moreover, irisin levels were found increased in the serum of patients suffering from cardiac cachexia, along with changes in the expression of markers of heart failure.[52] Interestingly, irisin protein expression was also recently shown to be highly expressed in lung, liver, and gastrointestinal tumors,[53] and in the tumor of cachectic patients, irisin levels were higher than cancer patients with stable body weight.[54–56] On the other hand, the levels of irisin were reduced in colorectal and breast cancer patients compared to normal subjects.[57,58] Again, as in bone metabolism, the role of irisin in cancer cachexia remains unclear.

Interleukin-6

Interleukin-6 (IL-6) is a pleiotropic proinflammatory cytokine. The main producer and regulator of this cytokine is the adipose tissue, but also other cells and tissues such as immune cells, hepatocytes and neoplastic cells can secrete IL-6 [59]. IL-6 plays an important role in the immune and acute phase responses.[59] The role of IL-6 as a myokine was first described by Pedersen and collaborators by showing evidence that IL-6 is a product of the skeletal muscle contraction, and that its levels are regulated by duration and intensity of the contraction.[60] On this regard, in exercised human muscle, the IL-6 receptor was described as increased, suggesting a autocrine role of IL-6 on muscle.[61] The signaling of IL-6 on skeletal muscle improves insulin-stimulated glucose uptake as well as fatty acid oxidation via AMPK.[62] This cytokine has also an important role in muscle stem cell-mediated hypertrophy. Indeed, it has been shown that IL-6 is produced by growing myofibers, whereas IL-6 deficiency reduces muscle hypertrophy by inhibiting muscle stem cell proliferation and fusion with preexisting myofibers.[63] A recent study by Chowdhury *et al.* reported that the bone-derived protein, osteocalcin, enhanced the effects muscle-derived IL-6 to enhance exercise capacity. They also showed that IL-6 from muscle induced osteoblasts to send signals to osteoclasts which is turn were responsible for the release of osteocalcin from bone. Therefore, bone and muscle crosstalk can occur through the release of osteocalcin from bone due to the action of Il-6 produced by contracted muscle.[64] However, high, pathologic levels IL-6 are able to directly inhibit osteoblast maturation and differentiation both *in vivo* and *in vitro*.[65,66] High IL-6 levels were also described in association with increased osteoclastogenesis and bone loss,[62] and IL-6 released by apoptotic osteocytes was found to improve the adhesion of osteoclast precursors, thus enhancing bone resorption.[67]

The role of IL-6 in cancer-induced cachexia is extensively described. Several murine preclinical models for the study of cancer cachexia showed elevated levels of IL-6 in the circulation.[20,43,68] In human cancer cachexia, elevated IL-6 levels are predictors of body weight loss,[69] as well as a negative prognostic factor especially in cachectic lung cancer patients.[70] In cancer, IL-6 is often directly produced by cancer cells, and both IL-6 levels and body mass return to normal values when IL-6-producing tumors are removed from cachectic mice.[71] Similarly, the use of specific neutralizing antibodies against IL-6 was effective in reducing muscle protein hypercatabolism and preserving muscle mass.[72] The mechanisms of IL-6-dependent muscle atrophy are well characterized. In particular, IL-6 has been shown to drive muscle atrophy during cancer cachexia by activating the JAK/STAT3 pathway, thereby stimulating muscle protein degradation and the activation of an acute phase response in skeletal muscle.[9]

β-aminoisobutyric acid

β-aminoisobutyric acid (BAIBA), a novel small molecule metabolite, has been described to participate in several bone and muscle processes. BAIBA was first described as being produced by skeletal muscle during exercise in humans and rodents via the regulation of the transcription factor PGC-1α.[73] BAIBA promotes the browning of white adipose tissue by increasing the expression of specific genes such as uncoupling proteins, and improves glucose tolerance and enhances hepatic fatty acid β-oxidation.[73] In skeletal muscle, L-BAIBA has an autocrine function and was found to improve insulin resistance and inflammation, and to stimulate fatty acid β-oxidation through the regulation of the AMP and PPARδ pathways.[74] BAIBA was also shown to improve muscle contraction and strength in a sex-dependent manner.[45] The L/S enantiomer of BAIBA is a natural catabolite of valine and the D/R enantiomer is produced from thymine. Normally, one enantiomer is active and the other inactive, although there are examples of both enantiomers being concurrently active. In the first study to test the different potential functions of L and D BAIBA, L-BAIBA was found to play a role in the maintenance of bone mass by protecting osteocytes from reactive oxygen species.[75] Trabecular bone loss resulting from hindlimb unloading was attenuated along with osteocyte cell death in mice receiving drinking water supplemented with L-BAIBA.[75] L-BAIBA exerts its function by binding the receptor Mas-related G-protein receptor type D.[75] In osteocytes, the expression of this receptor is reduced with aging, and this could explain the involvement of this pathway in the osteoporotic process.[75] The ability of L-BAIBA to increase muscle function and maintain bone volume suggests the potential use of this molecule for the detrimental effects of cancer cachexia on musculoskeletal health.

Osteokines in muscle-bone crosstalk

Transforming growth factor β

Cancer invasion of bone activates the latent bone transforming growth factor β (TGF-β) leading to the release of active TGF-β, which through a vicious cycle stimulates tumor growth, cancer cell invasion, and more bone destruction.[76] In addition to the devastating effects of this autocrine factor on bone, TGF-β released from the bone matrix was also described to induce muscle weakness by decreasing Ca^{2+}-induced muscle force production. Breast, lung and prostate metastatic cancer, as well as multiple myeloma are potent activators of TGF-β leading to not only devasting effects on bone but also on muscle.[77] Moreover, body mass, skeletal muscle atrophy, and weakness were improved by blocking TGF-β signaling by using the TGF-β receptor I kinase inhibitor SD-208 or the bone-targeting bisphosphonate zoledronic acid.[77] Using a similar approach, treatment with the bisphosphonate pamidronate was found to reduce bone loss and improve muscle atrophy in pediatric burn patients,[78] and we recently demonstrated that pamidronate exerts its beneficial effect in pediatric burn patients by reducing the release of TGF-β by the bone matrix.[79]

Osteocalcin

Osteocalcin (Ocn) is a non-collagenous protein involved in bone mineralization. Ocn is produced by mature osteoblasts and stored in the bone matrix.[28] The carboxylated form of Ocn has affinity for hydroxyapatite, and its release from bone into the circulation as an undercarboxylated bioactive form is due to osteoclast-mediated pH decrease on the bone surface.[28] Ocn acts in target tissues by binding to the Gprc6a receptor, and primarily impacts the regulation of glucose uptake and energy metabolism.[80] It has been shown that Ocn levels are increased in both humans and murine models after aerobic exercise.[64,81] In this regard, Ocn was shown to play an important role in the adaptation to exercise. Indeed, mice with deletion of Ocn or its receptor Gprc6a displayed reduced exercise capacity and reduced muscle mass, accompanied by enhanced uptake of energy substrates, including glucose and fatty acid by myofibers.[80] Interestingly, Ocn levels were reduced with aging in both mice and humans, and exogenous administration of Ocn was able to restore exercise capacity and muscle mass in old mice and increase exercise performance in young animals.[80,81] Whether Ocn plays a role in cancer cachexia remains to be elucidated. These findings highlight the potential role of Ocn/Gprc6a to improve muscle atrophy and weakness associated with cancer and other chronic conditions.

Sclerostin and Wnts

Sclerostin is a glycoprotein predominantly expressed by mature osteocytes, although also some tumors were found to be a source of this factor.[82] Sclerostin is an antagonist of the Wnt/β-catenin signaling pathway and considered to be the most important bone-derived negative regulator of bone mass and osteoblast differentiation.[83] Interestingly, high sclerostin levels were found associated with lower muscle mass, but not lower bone mass in female and male subjects.[84] In another study, low skeletal muscle mass index was correlated with higher serum levels of sclerostin in hemodialysis patients.[85] Moreover, type 2 diabetic patients undergoing high-intensity interval training exercise showed improved muscle mass, along with reduced levels of circulating sclerostin.[86] Hesse *et al.* showed that tumor-derived sclerostin inhibits bone formation in breast cancer-induced cachexia, hence suggesting sclerostin as an important driver of both bone and muscle loss.[82] Interestingly, treatment with anti-sclerostin antibodies in breast cancer-bearing mice not only was able to improve bone mass, but also reduced skeletal muscle atrophy by acting on the NF-kB pathway and on the differentiation process. More importantly, in this study, sclerostin inhibition also improved muscle strength, reduced tumor mass, and prolonged survival in tumor-bearing mice.[82] These data provide evidence that sclerostin directly contributes to maintain muscle homeostasis and its levels may be used as predictors of low skeletal muscle mass. Of note, it was recently shown that sclerostin can be produced and released by C2C12 and primary myoblasts, and the conditioned media derived from muscle cells was able to inhibit the differentiation of 2T3 osteogenic cells.[87] These findings were further confirmed by evidence that sclerostin is also produced *in vivo* by muscle, regardless of age, muscle type, or phenotype (i.e. glycolytic vs. oxidative).[87] Altogether, these observations would seem to suggest that also muscle-derived sclerostin can affect bone mass.[87]

Whereas sclerostin is an inhibitor of the Wnt/β-catenin signaling pathway, the Wnts are agonists of this pathway and are also made by bone cells. Brotto *et al*. showed that osteocyte-derived Wnt3a was able to play a role in muscle-bone crosstalk. Specifically, Wnt3a released in the conditioned media of MLO-Y4 osteocyte-like cells stimulated C2C12 myoblast differentiation, as well as improved muscle contractility *ex vivo* by modulating intracellular Ca^{2+} signaling.[88] Also Wnt7a, another member of the Wnt family of ligands, was shown to improve muscle atrophy induced by the cachexiogenic C26 colon adenocarcinoma cells, both *in vitro* and *in vivo*, mainly by reactivating the AKT/mTOR anabolic pathway and by stimulating muscle stem cell differentiation.[89] Therefore, the Wnts appear to be positive regulators of both bone and muscle in contrast to sclerostin.

Rank/RANKL/OPG

Receptor activator of nuclear factor β ligand, RANKL, is the most important regulator of bone resorption. Though made by immune cells, in bone the production of RANKL is carried out mostly by late osteoblasts and osteocytes.[90] RANKL binds its receptor, RANK, on bone monocytes/macrophage osteoclast precursors inducing fusion and activation into bone resorptive osteoclasts. The activity of RANKL is regulated by its decoy receptor osteoprotegerin (OPG), that binds to RANKL and inhibits its osteoclastogenic activity.[90] The ratio and relative abundance of each determine the degree of bone resorption/bone formation.

Interestingly, RANK receptor was found also in skeletal muscle and in C2C12 myotubes, thereby suggesting a regulatory role of the RANK/RANKL/OPG axis on skeletal muscle. It was subsequently discovered that in denervated fast-twitch fibers, the RANK/RANKL pathway affects skeletal muscle function by modulating SERCA activity and Ca^{2+} storage.[91] RANK/RANKL muscle levels were found elevated in *mdx* mice, a murine model of Duchenne muscular dystrophy, and the use of neutralizing antibodies against RANKL was able to improve muscle histology and function.[92] The anti-human RANKL antibody (i.e. Denosumab) is an FDA-approved drug for the treatment of bone loss in patients who have risk of bone fracture. Bonnet *et al*. showed that the treatment of osteoporotic post-menopausal women with Denosumab not only improved BMD, but also increased appendicular lean mass and muscle handgrip strength.[93] Furthermore, alterations of the RANKL/OPG balance using $OPG^{-/-}$ mice were sufficient to cause bone loss, as well as atrophy and weakness in fast-twitch myofibers.[92] Though developed for the treatment of osteoporosis, anti-RANKL therapies clearly have potential to benefit muscle in addition to bone with regard to cancer cachexia

Parathyroid hormone-related protein

Parathyroid hormone-related protein (PTHrP) is highly expressed in the lactating breast and in the placenta to insure calcium uptake but has also been shown to be produced by osteoblasts.[94,95] Abnormal expression in tumors is well described resulting in hypercalcemia. In cancer, this factor is an important driver of cancer-induced osteolysis and calcium release.[96] In particular, hypercalcemia due to elevated PTHrP can induce neuromuscular symptoms and muscle weakness.[97] Onuma *et al.* were the first to show that in a model of cancer cachexia, treatment with anti-PTHrP antibody was able to reduce hypercalcemia, as well as restore locomotor activity, along with attenuation of body weight loss, fat wasting, and skeletal muscle atrophy.[98] PTHrP was also shown to stimulate browning of the white adipose tissue (WAT), hence inducing the thermogenic program in mice implanted with Lewis lung carcinomas (LLC).[99] Conversely, *in vivo* neutralization of PTHrP was able to prevent WAT browning and reduce energy wasting, as well as improve weight loss and skeletal muscle wasting in tumor hosts.[99] Interestingly, it was shown that LLC tumors are able to release extracellular vesicles (EVs) containing PTHrP, and this event induces lipolysis in 3T3-L1 adipocytes *in vitro*.[100] In *in vivo* conditions, the same EVs-containing PTHrP were found to be important drivers of fat loss and WAT browning in LLC bearers.[100] Altogether, these observations support PTHrP as a new target to counteract muscle, WAT, and bone loss during cancer cachexia.

Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF23) was the first osteocyte-derived hormone described to have a systemic regulatory function.[28] The most important role of FGF23 is the regulation of phosphate reabsorption by the kidney.[101] FGF23 has

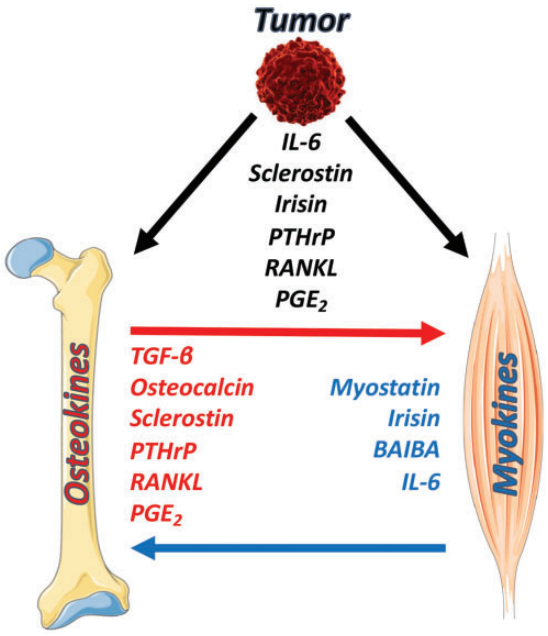

Figure 1. Schematic representation of the bone-muscle crosstalk in a context of cancer cachexia. Osteokines and myokines contribute to the bone-muscle derangements associated with tumor burden. Some of the factors produced by muscle and bone can be released also from the tumor, thus complicating the bone-muscle interaction. Images adapted from Servier Medical Art (https://smart.servier.com). (A color version of this figure is available in the online journal.)

negative effects on cardiac muscle by inducing left ventricular hypertrophy;[102] however, even though FGF23 receptors are expressed in skeletal muscle, *ex vivo* treatment with recombinant FGF23 did not significantly alter muscle function.[28,103] Further studies are needed to elucidate a direct role of FGF23 in skeletal muscle homeostasis other than phosphate uptake by muscle.

Prostaglandin E_2

Prostaglandin E_2 (PGE_2), an arachidonic acid metabolite, secreted by mechanically stimulated osteocytes was described to improve myogenic differentiation in primary myoblasts.[104] This molecule is produced by various cell types, including osteocytes, when stimulated by fluid-flow stress or under bone loading.[104] For example, it is estimated that osteocytes produce 100-fold more PGE_2 when compared to the muscle tissue.[28] Regardless of the source of the PGE_2, a recent study showed increased activity of 15-PDGH, an enzyme that degrades PGE_2, in aging skeletal muscle, suggesting a critical role of PGE_2. In 24- to 28-month-old mice muscle, specific inhibition of 15-PGDH using adeno-associated virus containing the short hairpin RNA to 15-PGDH resulted in increased PGE_2 to levels similar to those found in young mice and this was sufficient to preserve muscle mass and function.[105] The question remains as to the major source of PGE_2 targeting muscle in young animals, an autocrine source or from a distant organ-such as bone.

Conclusions

Skeletal muscle and bone have a long-lasting relationship that starts with embryogenesis and, through a harmonized development, reaches completion in the adult organism. Together bone and muscle share the decline that characterizes aging, resulting in the loss of bone and muscle known as osteoporosis and sarcopenia, respectively. The two tissues are regulated in tandem and can be concomitantly compromised in different pathological conditions. The reasons for this close association not only include the mechanical interactions but also the biochemical crosstalk. In this review, we emphasized the importance of the communication between muscle and bone during cancer cachexia. We summarized some of the most studied myokines and osteokines that are known to be directly involved in the musculoskeletal pathologies associated with cancer cachexia, representing a clinical scenario in which the biochemical exchanges between muscle and bone are complicated by the ability of the tumor to produce and release factors normally made by bone or muscle to target and compromise bone and muscle function (Figure 1). Given that different tumors are known to secrete soluble factors and to affect muscle and bone tissue homeostasis, we propose that personalized therapeutic interventions targeting myokines and osteokines should be taken into account and combined with routinely used anticancer strategies to better preserve bone and muscle in a context of cancer cachexia.

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FP, LFB, and AB conceived the contents of the review; FP and AB wrote the review; LFB edited the paper. All authors have read and agreed to the published version of the manuscript.

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