

# Mechanisms of Muscle Weakness Associated with Bone Metastases

Khalid S. Mohammad<sup>1</sup>, Theresa A. Guise<sup>1</sup>, Miguel Manzano<sup>2,3</sup>, Maria Vallet-Regí<sup>2,3\*</sup>

<sup>1</sup>Department of Medicine, Division of Endocrinology, Indiana University School of Medicine, IN, USA

<sup>2</sup>Departamento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense de Madrid. Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12, Spain

<sup>3</sup>Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain

**\*Corresponding author:** Maria Vallet-Regí, Departamento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense de Madrid. Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12, Spain; Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain, Tel: +34913941861/ Fax: +34913941786; E-mail: [vallet@ucm.es](mailto:vallet@ucm.es)

## Abstract

Cancer-associated muscle dysfunction represents a deadly clinical problem, with ca. 80% mortality together with an increased toxicity from cancer treatment. The normal bone remodeling might 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 that muscle weakness might be attributed to the reduction on muscle mass or the reduction of muscle function. In fact, it has been demonstrated that in advanced cancers, it is probably caused by a combination of reductions, quantity and quality of muscle. This review focuses on the mechanisms that bone metastases promote skeletal muscle weakness.

**Received Date: October 28, 2016**

**Accepted Date: January 19, 2017**

**Published Date: January 27, 2017**

**Citation:** Vallet-Regí, M., et al. Mechanisms of Muscle Weakness Associated with Bone Metastases. (2017) *Int J Cancer Oncol* 4(1): 1- 5.

DOI: 10.15436/2377-0902.17.1188



## Introduction

Cancer-associated muscle weakness represents a research challenge and a serious clinical problem. In fact, there is an 80% of mortality and an increased toxicity from cancer treatment<sup>[1-3]</sup>. 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<sup>[4]</sup>. 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, 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

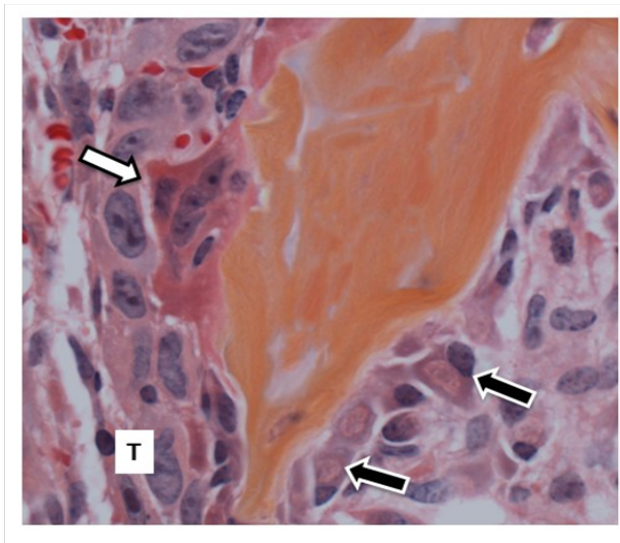
types of cancer like breast and prostate have particular affinity to grow in bone. The bone microenvironment is a fertile soil for tumor growth as it houses abundant growth factors which act on tumors to fuel growth and other invasive behavior.

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 result in loss of the normal structural integrity of the skeleton<sup>[5]</sup>. 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<sup>[6]</sup>, 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<sup>[4-7]</sup>.



**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- $\beta$ 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- $\beta$  (TGF- $\beta$ ) is the most abundant of these factors. TGF- $\beta$  is part of a large family of polypeptide growth factors that includes activins, inhibins, and bone morphogenetic proteins (BMPs). TGF- $\beta$  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- $\beta$ . High local levels of liberated TGF- $\beta$  result in increased tumor invasion, angiogenesis as well as immune suppression. In addition, TGF- $\beta$  stimulates tumor production of osteolytic factors that stimulate further bone resorption<sup>[8,9]</sup>. This categorizes TGF- $\beta$  as a crucial factor responsible for driving the feed-forward vicious cycle of tumor growth in bone. Therefore blocking TGF- $\beta$  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- $\beta$  including TGF- $\beta$  receptor I kinase inhibitors, TGF- $\beta$  neutralizing antibodies, soluble receptor decoy-Fc fusions and TGF- $\beta$  antisense oligonucleotides<sup>[10]</sup> 

### 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<sup>[11]</sup>. 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<sup>[12,13]</sup>. Cancer cachexia is associated with high levels of interleukin (IL)-6, which is regulated by HIF-1 $\alpha$ , NF $\kappa$ B and TGF- $\beta$ <sup>[11]</sup>. 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 role of RYR2 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<sup>[14]</sup>. The action potential depolarization is generated primarily by 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<sup>2+</sup> channel, also known as the dihydropyridine receptor (DHPR). In skeletal muscle, the DHPR is mechanically coupled to the ryanodine receptor Ca<sup>2+</sup> release channel type 1 (RyR1), which in contrast to the DHPR, rests in the sarcoplasmic reticulum<sup>[14]</sup>.

Ryanodine receptors (RyRs) are intracellular Ca<sup>2+</sup> 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<sup>[15]</sup>. It is a Ca<sup>2+</sup> permeable non-selective cation channel that releases Ca<sup>2+</sup> stored in the sarco-endoplasmic reticulum of excitable and non-excitable cells. This ion channel is a tetramer and each sub unit contains approximately 5000aa, so one single ion channel weights around 2MDa<sup>[16]</sup>. 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<sup>2+</sup> channels (VGCCs) on the plasma membrane<sup>[15]</sup>. Pathological oxidation of RyR1 results in leaky channels that contribute to muscle weakness<sup>[17,18]</sup>.

### 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<sup>[19]</sup>. Reactive oxygen species (ROS) is one of the most important species of free radicals. It is produced by various metabolic pathways, including mitochondrial aerobic metabolism. It plays 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<sup>[20]</sup>.

In the case of chronic inflammation, the secretion of reactive oxygen/nitrogen species (ROS/RNS) might lead to the amplification of dysregulated processes and eventually to a de-

velopment of preneoplastic condition. If the amount of cellular ROS/RNS produced is high enough to overcome endogenous antioxidant response, an irreversible oxidative damage to nucleic acids, lipids, and proteins may cause 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 could result in genetic/epigenetic changes that drive the initiation of carcinogenesis<sup>[21]</sup>. 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<sup>[22]</sup>.

### 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-7 and ZR-75-1), lung cancer (A549 and RWGT2), prostate cancer (PC-3) and multiple myeloma (JJN3). We compared the results obtained from each group of mice with those from non-tumor bearing mice. The tumor-bearing mice developed muscle weakness when compared to the non-tumor control mice. This is presented as a reduction in forelimb grip strength and the 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<sup>[23]</sup>. This observation was extended in murine models of several other cancer that caused osteolytic lesion including lung cancer, prostate cancer and multiple myeloma as well as in murine models with osteoblastic bone metastases including the ZR-75-1 and MCF-7 breast cancer model.

Since our data indicate that muscle weakness strongly correlated with bone destruction and remodeling, we further investigated whether the interaction between tumor-bone microenvironment plays a role in cancer-associated muscle weakness. Using a mammary fat pad breast cancer mouse model, we inoculated breast cancer cell MDA-MB-231 cells (106) into the mammary fat pad. In this model tumor grow locally and do 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 had 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 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<sup>[17,24]</sup>. To determine whether the RyR1 modifications observed in the mouse 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 and 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-benzothiazepine 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<sup>[25]</sup>. 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<sup>[26]</sup>. 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 ( $P_o$ ), 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<sup>[27]</sup> 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 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- $\beta$ inhibition improves muscle strength

As mentioned earlier, TGF- $\beta$  stimulates tumor production of osteolytic factors that stimulate further bone resorption<sup>[8,9]</sup>. Our data showed that TGF- $\beta$ -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- $\beta$  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- $\beta$  released from the sites of bone destruction and circulate in the blood, measurements of serum TGF- $\beta$  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- $\beta$  released as a consequence of osteolytic bone destruction was determined to play a role in cancer-associated muscle weakness when TGF- $\beta$  release from bone was inhibited using various pharmacological agents. Using TGF- $\beta$  receptor I kinase inhibitor SD-208, anti-resorptive bisphosphonate therapy or combination of the two drugs, in mouse model of breast cancer bone metastases, to reduce TGF- $\beta$  release from the bone matrix had a significant reduction in SMAD3 phosphorylation, indicating a block to TGF- $\beta$  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- $\beta$ . These data suggest that bone-derived TGF- $\beta$  plays a critical role in cancer-associated muscle weakness.

**RyR1 oxidation in response to TGF- $\beta$  is mediated by NADPH oxidase 4 (Nox4)**

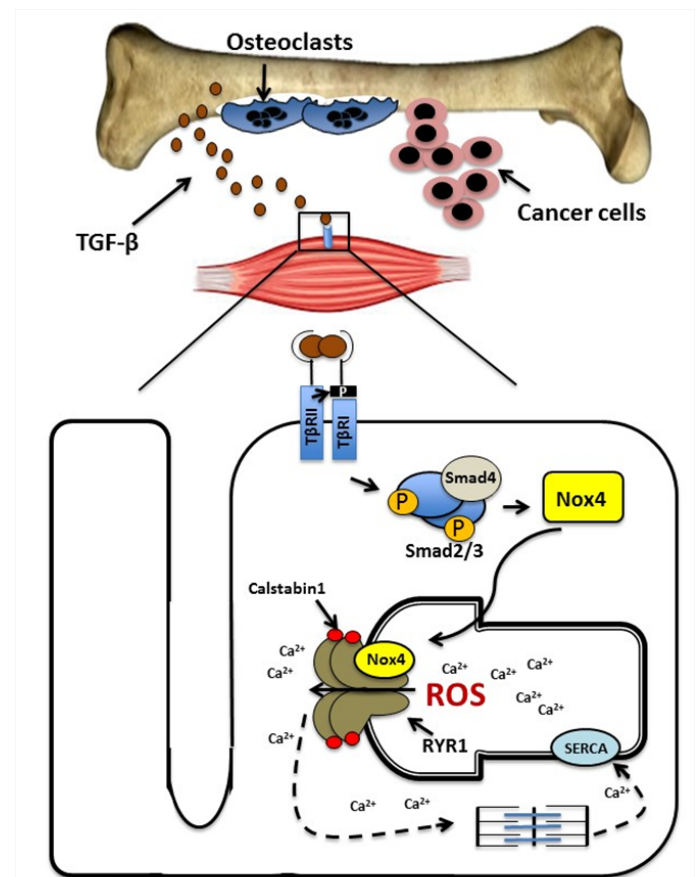
The mechanism by which TGF- $\beta$  mediates oxidation of RyR1 was shown to be through Nox4. Nox4 is expressed in skeletal muscle and contributes to oxidative stress in cardiomyocytes and Nox4 protein is increased in muscle in response to TGF- $\beta$  signaling<sup>[28,29]</sup>. Previously published data show that Nox4 protein interacts with RyR1 channels<sup>[30]</sup>. 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 mouse model 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- $\beta$  treatment increased Nox4 protein expression in myotubes. This observed increase in Nox4 protein expression was blocked using TGF- $\beta$

RI kinase inhibitor, SD208, but not S107. When myotubes were treated with TGF- $\beta$  Nox4 binding to RyR1 channels was increased. When we knocked down Nox4 in myotubes TGF- $\beta$  treatment did not induce RyR1 is remodeling or reduction of calstabin1 binding to RyR1 compared to scrambled control samples. This data definitely shows that Nox4 mediates TGF- $\beta$  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.



**Figure 2:** TGF- $\beta$  released from bone as a result of bone destruction by osteoclast that is stimulated by cancer cell. TGF- $\beta$  drives the expression of Nox4 in muscle through Smad2/3 signaling and Nox4 produce reactive oxygen species (ROS) which cause oxidation of the RYR1 on the Sarcoplasmic Reticulum (SR). Oxidation of RYR1 leads to binding of the RYR1 stabilizing unit (calstabin1) which leads to Ca<sup>2+</sup> leak from the SR. Thus depleting the Ca<sup>2+</sup> store and causes reduction in muscle force.

Different type 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- $\beta$  using TGFBR1 kinase inhibitor or by antiresorptive drugs (bisphosphonates or denosumab) or blocking RYR1 calcium leak 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).

Thus, targeting skeletal muscle weakness caused by the TGF- $\beta$ -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.

**Acknowledgment:** The authors thank the Ministerio de Economía y Competitividad (Spain) through the grant no. CSO2010-11384; the Ramón Areces Foundation that organized the International Workshop on Cancer and Aging entitled “Cancer as a result of aging: possible solutions”, that took place in Madrid (Spain) the 3rd November 2015; and the European Research Council through the Advanced Grant VERDI (ERC-2015-AdG proposal no. 694160).

## References

1. Fearon, K., Arends, J., Baracos, V. Understanding the mechanisms and treatment options in cancer cachexia. (2013) *Nat Rev Clin Oncol* 10(2): 90-99.
2. Giordano, A., Calvani, M., Petillo, O., et al. Skeletal muscle metabolism in physiology and in cancer disease. (2003) *J Cell Biochem* 90(1): 170-186.
3. von Haehling, S., Anker, S.D. Prevalence, incidence and clinical impact of cachexia: facts and numbers-update 2014. (2014) *J Cachexia Sarcopenia Muscle* 5(4): 261-263.
4. Roodman, G.D. Mechanisms of disease: Mechanisms of bone metastasis. (2004) *New Eng J Med* 350(16): 1655-1664.
5. Seeman, E., Delmas, P.D. Bone quality--the material and structural basis of bone strength and fragility. (2006) *N Engl J Med* 354(21): 2250-2261.
6. Coleman, R.E. Skeletal complications of malignancy. (1997) *Cancer* 80(8 Suppl): 1588-1594.
7. Mundy, G.R., Metastasis to bone: Causes, consequences and therapeutic opportunities. (2002) *Nat Rev Cancer* 2(8): 584-593.
8. Kang, Y., Siegel, P.M., Shu, W., et al. A multigenic program mediating breast cancer metastasis to bone. (2003) *Cancer Cell* 3(6): 537-549.
9. Yin, J.J., Selander, K., Chirgwin, J.M., et al. TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. (1999) *J Clin Invest* 103(2): 197-206.
10. Yingling, J.M., Blanchard, K.L., Sawyer, J.S. Development of TGF-beta signalling inhibitors for cancer therapy. (2004) *Nat Rev Drug Discov* 3(12): 1011-1022.
11. Fearon, K.C., Glass, D.J., Guttridge, D.C. Cancer cachexia: mediators, signaling, and metabolic pathways. (2012) *Cell Metab* 16(2): 153-166.
12. Anandavadivelan, P., Lagergren, P. Perioperative nutritional intervention: a way to improve long-term outcomes. (2016) *Nat Rev Clin Oncol* 13(3): 198.
13. Martinez-Outschoorn, U., Sotgia, F., Lisanti, M.P. Tumor microenvironment and metabolic synergy in breast cancers: critical importance of mitochondrial fuels and function. (2014) *Semin Oncol* 41(2): 195-216.
14. Hernandez-Ochoa, E.O., Stephen Pratt, J.P., Lovering R.M., et al., Critical Role of Intracellular RyR1 Calcium Release Channels in Skeletal Muscle Function and Disease. (2015) *Front Physiol* 6: 420.
15. Zalk, R., Clarke, O.B., des Georges, A., et al. Structure of a mammalian ryanodine receptor. (2015) *Nature* 517(7532): 44-49.
16. Guerrero-Hernandez, A., Avila, G., Rueda, A. Ryanodine receptors as leak channels. (2014) *Eur J Pharmacol* 739: 26-38.
17. Bellinger, A.M., Reiken, S., Carlsson, C., et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. (2009) *Nat Med* 15(3): 325-330.
18. Andersson, D.C. Marks, A.R. Fixing ryanodine receptor Ca leak - a novel therapeutic strategy for contractile failure in heart and skeletal muscle. (2010) *Drug Discov Today Dis Mech* 7(2): e151-e157.
19. Sosa, V., Moliné, T., Somoza, R., et al. Oxidative stress and cancer: an overview. (2013) *Ageing Res Rev* 12(1): 376-390.
20. Nourazarian, A.R., Kangari, P., Salmaninejad, A. Roles of oxidative stress in the development and progression of breast cancer. (2014) *Asian Pac J Cancer Prev* 15(12): 4745-4751.
21. Murata, M., Thanan, R., Ma, N., et al. Role of nitrate and oxidative DNA damage in inflammation-related carcinogenesis. (2012) *J Biomed Biotechnol* 2012: 623019.
22. Sharma, A., Rajappa, M., Saxena, A., et al. Antioxidant status in advanced cervical cancer patients undergoing neoadjuvant chemoradiation. (2007) *Br J Biomed Sci* 64(1): 23-27.
23. Waning, D.L., Mohammad, K.S., Reiken, S., et al. Excess TGF-beta mediates muscle weakness associated with bone metastases in mice. (2015) *Nat Med* 21(11): 1262-1271.
24. Wehrens, X.H., Lehnart, S.E., Reiken, S., et al. Enhancing calstabin binding to ryanodine receptors improves cardiac and skeletal muscle function in heart failure. (2005) *Proc Natl Acad Sci USA* 102(27): 9607-9612.
25. Bellinger, A.M., Reiken, S., Dura, M., et al. Remodeling of ryanodine receptor complex causes “leaky” channels: a molecular mechanism for decreased exercise capacity. (2008) *Proc Natl Acad Sci USA* 105(6): 2198-2202.
26. Andersson, D.C., Betzenhauser, M.J., Reiken, S., et al. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. (2011) *Cell Metab* 14(2): 196-207.
27. Guerrero-Hernandez, A., Dagnino-Acosta, A., Verkhatsky, A. An intelligent sarco-endoplasmic reticulum Ca<sup>2+</sup> store: release and leak channels have differential access to a concealed Ca<sup>2+</sup> pool. (2010) *Cell Calcium* 48(2-3): 143-149.
28. Kuroda, J., Ago, T., Matsushima, S., et al. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. (2010) *Proc Natl Acad Sci USA* 107(35): 15565-15570.
29. Hubackova, S., Krejcikova, K., Bartek, J., et al. IL1- and TGF-beta-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine ‘bystander senescence’. (2012) *Ageing (Albany NY)* 4(12): 932-951.
30. Sun, Q.A., Hess, D.T., Nogueira, L., et al. Oxygen-coupled redox regulation of the skeletal muscle ryanodine receptor-Ca<sup>2+</sup> release channel by NADPH oxidase 4. (2011) *Proc Natl Acad Sci USA* 108(38): 16098-16103.