Cigarettes Make You Weak: RANKL/RANK Link Changes in Muscle and Bone

It is increasingly appreciated that the chronic use of tobacco cigarettes leads to systemic inflammation that results in cellular and epigenetic changes throughout the whole organism. Signs of pathology can be detected in many organs even before there is evidence of lung damage. Patients with chronic obstructive pulmonary disease (COPD) often exhibit frailty owing to both a decrease in limb bone mass density (BMD) and muscle atrophy and weakness, referred to as sarcopenia (1, 2). Up to 70% of patients with COPD who exhibit osteoporosis also show signs of muscle wasting (3).

High levels of the RANKL (receptor activator of nuclear factor κ-B ligand) early on were detected in the saliva and teeth of heavy cigarette smokers and were associated with periodontal disease and tooth loss (4, 5). In bones, RANKL activates the differentiation of osteoclasts by binding and activating its receptor, RANK, thereby increasing bone resorption (6). In the extracellular space, OPG (osteoprotegerin) acts as a decoy receptor to downregulate RANKL–RANK interactions (7). Together, RANKL, RANK, and OPG expression regulate bone density. It is well known that muscle mass and force are strong indicators of bone health, and low BMD is usually detected in individuals with sarcopenia (8). More recently, it has been demonstrated that the use of tobacco cigarettes activates the RANKL/RANK/OPG pathway in osteoclasts and contributes to a low BMD and eventually to osteoporosis (9). Interestingly, postmenopausal women given a RANKL inhibitor for several years to treat their osteoporosis showed improvements in hand grip strength (10). In mice, overexpression of RANKL also leads to osteoporosis and decreases running speed, muscle mass, and oxidative myofibers, which can be restored by treating with a truncated OPG or with the RANK monoclonal antibody denosumab (10). Furthermore, in denervated muscle, RANK regulates myofiber Ca^{2+} storage through Stim1 (stromal interaction molecule 1), an important Ca^{2+} sensor that also regulates the activity of SERCA (sarco[endo]plasmic reticulum Ca^{2+}-ATPase) and muscle force (11, 12). Thus, there is precedent for the RANKL/RANK/OPG system in regulating muscle atrophy, fiber type transition, and contractile function.

In this issue of the *Journal*, Xiong and colleagues (pp. 617–628) show that chronic cigarette smoke (CS) exposure in mice activates the RANKL/RANK/OPG pathway in skeletal myofibers and contributes to smoke-induced hind limb muscle dysfunction (13). The authors had previously found that patients with COPD with low BMD (14) have higher levels of plasma RANKL compared with smokers without COPD or patients with COPD with normal BMD. The present study investigates the mechanisms by which RANKL/RANK/OPG contributes to muscle dysfunction brought about by long-term smoking. Xiong and colleagues (13) found that in mice exposed to daily periods of CS for 6 months, RANKL and RANK in myofibers increased and were localized to the sarcolemma. Surprisingly, when mice were treated with a RANKL antibody, CS-induced muscle dysfunction was prevented, and muscle mass, grip strength, and exercise endurance were maintained. Importantly, treatment with RANKL antibody attenuated muscle CS-induced inflammatory signaling (TNF-α, IL-6, and NFκB-p65) and atrophy-associated genes (myostatin, atrogin-1, MuRF1) (Figure 1). Therefore, the authors concluded that RANKL/RANK/OPG activation during CS exposure triggers hind limb muscle atrophy and weakness through inflammatory and atrophy signaling pathways (13). The direct effects of smoke components were also tested in differentiated C2C12 myotubes. Myotubes incubated with the water-soluble components of CS (CS extract) showed 2- to 3-fold higher RANK and RANKL expression together with stimulated atrophy and TNF-α–related inflammatory signaling. These changes were inhibited by RANKL siRNA (13). Their data suggest that tobacco CS components enter the bloodstream of smokers and directly stimulate myofiber RANKL expression to alter the inflammatory and atrophy pathways that lead to muscle atrophy and weakness.

RANKL/RANK alters three processes known to contribute to muscle atrophy: *1*) cytokine signaling, *2*) upregulation of atrophy pathways, and *3*) intracellular Ca^{2+} regulation. In the study by Xiong and colleagues (13), grip strength was used as an indicator of decreased muscle function and was accompanied by changes in cytokine signaling and atrophy pathways in CS-exposed mice. CS extract treatment *in vivo* has been previously shown to dysregulate myofiber Ca^{2+} handling through a slowing of Ca^{2+} pumping and SERCA function during contractions, which, in turn, reduces fatigue resistance (15). Future studies are necessary to more fully explore the role of the RANKL/RANK/OPG pathway in intracellular Ca^{2+} handling (e.g., Ca^{2+} stores and flux during contractions) and myofibrillar contractility (e.g., force development/kinetics and fatigue resistance) during smoke exposure.

Interestingly, the work of Xiong and colleagues (13) and others show that RANKL/RANK/OPG are biomarkers that can be used to monitor muscle and bone health in smokers, current and former, and patients with COPD. It should be noted that the RANKL/RANK/OPG pathway is not limited to muscle and bone but also plays a role in the immune system in bone marrow development, lymph node and thymus development, gastrointestinal tract, and central nervous system. RANKL also plays a role in the regulation of mammary glands, the thermoregulatory center, blood vessels, hair follicles, and liver (16). Interestingly, expression levels are roughly twice as high in the brain than bone (10). Thus, there is a potential for future therapies that regulate the RANKL/RANK/OPG pathway (e.g., RANK receptor antagonists, RANKL monoclonal antibodies, or OPG treatment) to treat patients with COPD. Care should be taken as it is not known

Supported by Tobacco-Related Disease Program (TRDRP) 26IP-0033S and T29KT0397.

Originally Published in Press as DOI: 10.1165/rcmb.2021-0098ED on March 12, 2021

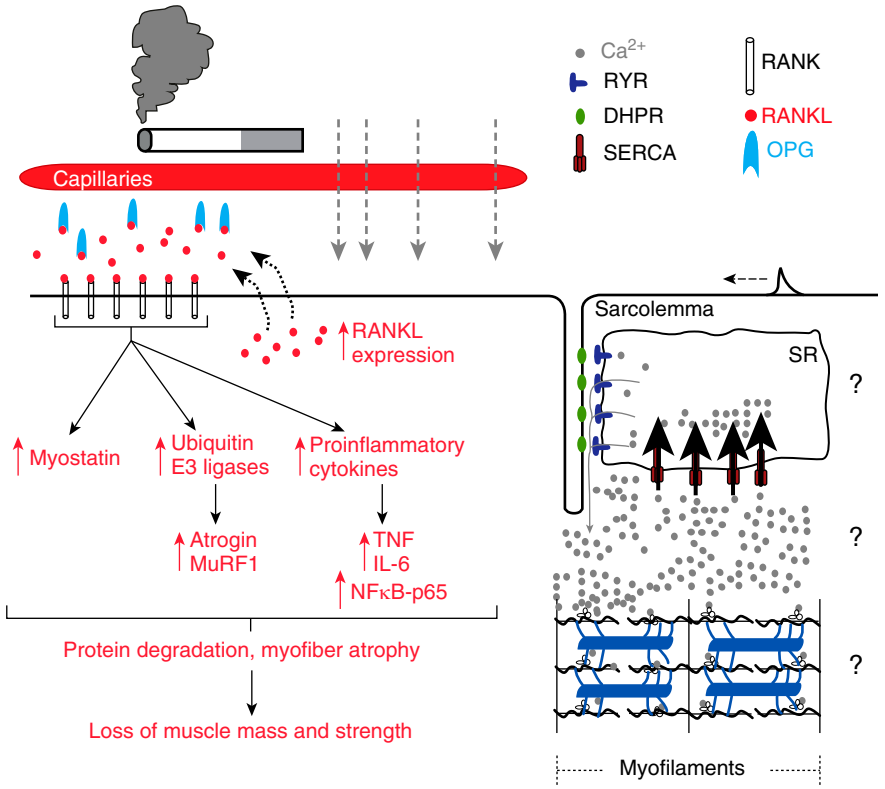

Figure 1. Mechanism for muscle atrophy owing to chronic cigarette smoke exposure in mice triggered by the expression of RANKL. Three processes may be implicated in RANKL-dependent cigarette smoke–induced atrophy: *1*) atrophy-associated gene activation (myostatin and Ubiquitin E3 ligases), *2*) activation of proinflammatory cytokines, and *3*) myofibrillar contractility controlled by intracellular Ca^{2+} handling and myofilament function. DHRP = dihydropyridine receptor; OPG = osteoprotegerin; MuRF1 = muscle ring-finger protein 1; RANK = receptor activator of NFκB; RANKL = RANK ligand; RYR = ryanodine receptors; SERCA = SR Ca^{2+}-ATPase; SR = sarcoplasmic reticulum.

whether RANKL therapies will have beneficial or harmful effects on other tissues that rely on this pathway for their normal function.

In summary, the work of Xiong and colleagues (13) provides valuable information about a critical signaling pathway involved in the activation of muscle protein degradation leading to muscle atrophy. Additional studies are needed to investigate several important unanswered questions: *1*) How does smoke exposure or its components activate the expression of RANKL in myofibers? *2*) Does smoking also change the extracellular concentration of OPG? *3*) Are muscle calcium handling and contractile function regulated by RANKL signaling in smokers? And *4*) do the effects of RANKL expression on muscle mass occur before the development of pulmonary symptoms? Their study defines a new mechanistic link between muscle and bone that is likely to be present in cigarette smokers and those that go on to develop COPD. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Leonardo Nogueira, Ph.D.
Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine
University of California San Diego
La Jolla, California
and
Instituto de Bioquímica Médica Leopoldo de Meis
Universidade Federal do Rio de Janeiro
Rio de Janeiro, Brazil

Ellen C. Breen, Ph.D.
Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine
University of California San Diego
La Jolla, California

ORCID ID: 0000-0001-5194-9126 (L.N.).

References

1. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigaré R, *et al*.; ATS/ERS Ad Hoc Committee on Limb Muscle Dysfunction in COPD. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014;189:e15–e62.
2. Degens H, Gayan-Ramirez G, van Hees HW. Smoking-induced skeletal muscle dysfunction: from evidence to mechanisms. *Am J Respir Crit Care Med* 2015;191:620–625.
3. Chua JR, Tee ML. Association of sarcopenia with osteoporosis in patients with chronic obstructive pulmonary disease. *Osteoporos Sarcopenia* 2020;6:129–132.
4. Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. *J Clin Periodontol* 2012;39:239–248.
5. Tanaka H, Tanabe N, Shoji M, Suzuki N, Katono T, Sato S, *et al*. Nicotine and lipopolysaccharide stimulate the formation of osteoclast-like cells by increasing macrophage colony-stimulating factor and prostaglandin E2 production by osteoblasts. *Life Sci* 2006;78:1733–1740.
6. Teitelbaum SL. Bone resorption by osteoclasts. *Science* 2000;289:1504–1508.

7. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, *et al*. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997;89:309–319.
8. He C, He W, Hou J, Chen K, Huang M, Yang M, *et al*. Bone and muscle crosstalk in aging. *Front Cell Dev Biol* 2020;8:585644.
9. Xiong J, Tian J, Zhou L, Le Y, Sun Y. Interleukin-17A deficiency attenuated emphysema and bone loss in mice exposed to cigarette smoke. *Int J Chron Obstruct Pulmon Dis* 2020;15:301–310.
10. Bonnet N, Bourgoin L, Biver E, Douni E, Ferrari S. RANKL inhibition improves muscle strength and insulin sensitivity and restores bone mass. *J Clin Invest* 2019;129:3214–3223.
11. Lee KJ, Hyun C, Woo JS, Park CS, Kim DH, Lee EH. Stromal interaction molecule 1 (STIM1) regulates sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA1a) in skeletal muscle. *Pflugers Arch* 2014; 466:987–1001.
12. Dufresne SS, Dumont NA, Boulanger-Piette A, Fajardo VA, Gamu D, Kake-Guena SA, *et al*. Muscle RANK is a key regulator of Ca2+ storage, SERCA activity, and function of fast-twitch skeletal muscles. *Am J Physiol Cell Physiol* 2016;310:C663–C672.
13. Xiong J, Le Y, Rao Y, Zhou L, Hu Y, Guo S, *et al*. RANKL mediates muscle atrophy and dysfunction in a cigarette smoke–induced model of chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2021;64:617–628.
14. Bai P, Sun Y, Jin J, Hou J, Li R, Zhang Q, *et al*. Disturbance of the OPG/RANK/RANKL pathway and systemic inflammation in COPD patients with emphysema and osteoporosis. *Respir Res* 2011;12:157.
15. Nogueira L, Trisko BM, Lima-Rosa FL, Jackson J, Lund-Palau H, Yamaguchi M, *et al*. Cigarette smoke directly impairs skeletal muscle function through capillary regression and altered myofibre calcium kinetics in mice. *J Physiol* 2018;596:2901–2916.
16. Ono T, Hayashi M, Sasaki F, Nakashima T. RANKL biology: bone metabolism, the immune system, and beyond. *Inflamm Regen* 2020; 40:2.