The RANK-RANK-L-OPG pathway: *trait d'union* between bone and muscle

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ABSTRACT

From an operational point of view, the musculoskeletal system can be considered a single organ that, displaying great plastic capacity, continuously remodels itself in response to various functional demands, which change over the course of human life. Many factors, mechanical and biochemical, underlie the constant interaction between bone and muscle. Among the main biochemical signals mediating this crosstalk, the receptor activator of the nuclear factor kappa-B (RANK)-RANK ligand (RANKL)-osteoprotegerin (OPG) system regulates not only bone remodeling but also muscle mass and performance, as well as its response to physiological or pathological stressing conditions. Administration of OPG or monoclonal antibodies (denosumab) against RANK-L improves skeletal mass and strength, and also plays an important role in reducing the risk of falls and improving the outcome of diseases involving skeletal and myocardial muscles.

KEYWORDS

Bone, muscle, RANK, RANKL, OPG, denosumab.

Introduction

The musculoskeletal system is a complex network of organs (bones, muscles, tendons, ligaments, cartilage, joints and other connective tissues) that work together to allow effective interaction with the outside world for the survival of the individual [1].

Bones and muscles together make up over 50% of human body weight, and striated muscle is responsible for over 30% of energy expenditure [2].

From an operational point of view, the musculoskeletal system can be considered a single organ that, a "cost-effective" product of evolution, performs several functions that go far beyond locomotion; furthermore, displaying great plastic capacity, it manages to continuously remodel itself in response to various functional demands, which change over the course of life [3]. This interaction between bone and skeletal muscle occurs throughout the individual's life and can be divided temporally into three major ontogenetic periods: patterning in embryonic life, allometric growth in the postnatal period, and the homeostatic relationship in adult life [4].

According to this concept, bone and muscle act as functional units, interacting from the period of the individual's body growth until old age [4]. Many factors underlie the constant interaction between bone and muscle. The concerted action of specific genes, along with environmental demands and stochastic processes, exerts a pleiotropic effect on bone/muscle crosstalk [5].

This concise review aims to examine and describe the mechanisms underpinning muscle-bone crosstalk, in particular the role of a biochemical system, the receptor activator of nuclear factor kappa-B (RANK)-RANK ligand (RANKL)-osteoprotegerin (OPG) pathway.

Article history

Received 21 Feb 2023 – Accepted 15 May 2023

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Muscle-bone crosstalk

Many hypotheses based on biomechanical and humoral mechanisms have been formulated over the past few decades to clarify the biological basis of muscle-bone interaction [6-8]. The "mechanostat theory" explained the longitudinal growth, bone modeling, and remodeling activities as results of the adaptation of bone to muscle forces [6]. High strains in response to resistance exercise not only initiate muscle protein synthesis but also induce bone formation, inhibiting remodeling, while low strains lead to bone resorption and subsequently its redistribution in space [6]. In addition to the role played by mechanical loading related to body weight, physical activity as well as disuse or age-related diseases, such as osteoporosis and sarcopenia, may contribute, through the biomechanical crosstalk between bone and muscle, to the maintenance of the efficiency of the musculoskeletal functional unit or to its deterioration (i.e., osteosarcopenia) [7].

However, in recent years, the concept of endocrine crosstalk between muscle and bone has also been described.

Muscle and bone both receive and secrete biochemical signals, such as myokines and osteokines, with bidirectional autocrine, paracrine, and endocrine effects [8]. For example, muscle secretes some interleukins (ILs) and cytokines that influence bone metabolism. Muscle-derived IL-6, for example, that is increased during exercise and muscle contraction, drives os-

teoclastogenesis via RANKL stimulation of osteoclasts, which in turn is induced by the release of RANK by osteoblasts, osteocytes, and leukocytes [9]. Myostatin, a myokine derived from the transforming growth factor β (TGFβ) family of cytokines, acts as a potent inhibitor of both skeletal muscle cell proliferation/growth and bone formation: similarly to IL-6, it acts by enhancing RANKL expression leading to bone resorption [10].

Irisin is a proteolytic product of the fibronectin type III domain containing 5 proteins. It is released from skeletal muscle to regulate metabolism, and is considered an exercise-induced myokine. Moreover, irisin promotes thermogenesis by "browning" white adipose tissues and it is involved in glucolipid metabolism, improving insulin resistance [11].

Among the bone-secreted factors with effects on muscle tissue, the undercarboxylated form of osteocalcin (OCN), released during bone resorption, controls several physiological processes, such as energy metabolism, by increasing insulin secretion and sensitivity, and muscle growth. This factor promotes nutrient uptake by muscle cells and the secretion of IL-6, thus further promoting bone resorption and increasing muscle mass in response to exercise [9].

OCN signaling is involved in myofiber adaptation to exercise and has been shown to directly promote protein synthesis in myotubes [12]. Moreover, in response to shear stress, osteocytes secrete prostaglandin (PG) E2 and Wnt 3a, which support myogenesis and muscle function [13]. Moreover, bone is a reservoir of several growth factors such as insulin-like growth factors, TGFβ and bone morphogenetic proteins (BMPs), which affect osteoblast proliferation and differentiation [5].

Finally, adipose tissue also seems to play a role in the regulation of muscle and bone metabolism through the secretion of adipokines [14]. During fat mobilization promoted by exercise, adipokines in concert with myokines have lipolytic effects improving body metabolism; moreover, they regulate bone turnover and bone mineral density (BMD) as well as catabolism of skeletal muscle in aging [15].

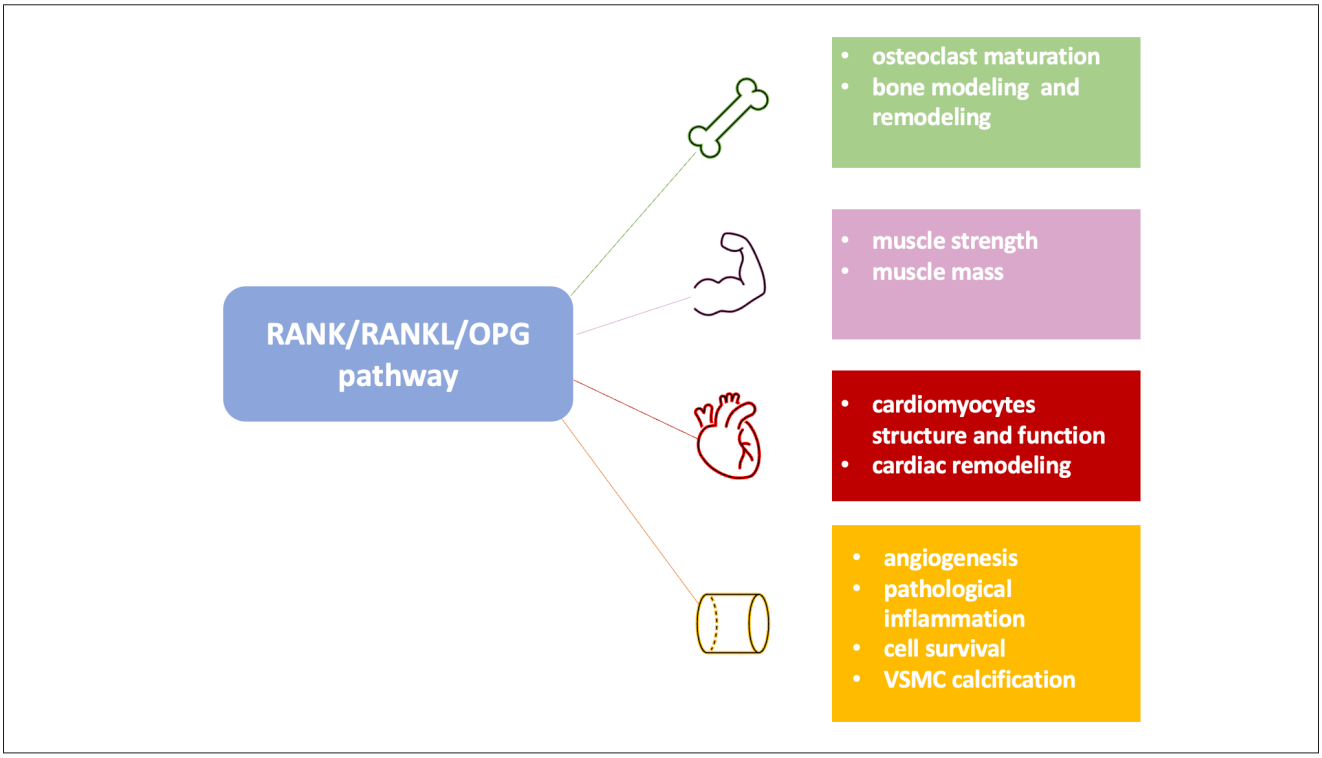
The RANK/RANKL/OPG pathway and its action on skeletal and cardiac muscles

During osteoclastic resorption, various molecules are produced and released from the mineralized bone matrix, with autocrine, paracrine, and endocrine effects [16]. They include IGF-1 and -2, platelet-derived growth factor, BMPs, RANKL, OCN, fibroblast growth factors (FGFs), and others that have regulatory functions on bone cell activation sequences, maintaining normal bone remodeling [17]. Many of these substances also have a modulatory effect on skeletal muscle [TGFβ, sclerostin, RANKL, Dickkopf-1 (DKK-1), OCN, PGs, BMPs, FGF23], while others can influence cognitive impairment (osteocalcin, lipocalin-2, FGF23, RANKL) [18].

The main pathway regulating osteoclast formation and activation is the RANK-RANKL-OPG system [19,20]. In metabolic bone diseases, including osteoporosis, excess bone resorption leads to significantly increased release of many factors stored in the mineralized bone matrix via bone-derived exosomes that serve as carriers of biologically active molecules [21].

RANKL is produced by osteoblasts (OBs) in membrane form and is cleaved by metalloproteinases to its soluble form, which is neutralized by circulating OPG or is bound to RANK at the osteoclast membrane, inducing a signaling cascade involving tumor necrosis factor receptor-associated factors 2/5/6, phosphatidylinositol 3-kinase, and mitogen-activated protein

Figure 1 The role of the RANK/RANKL/OPG pathway in bone and skeletal, cardiac and smooth muscle (* VSMC: vascular smooth muscle cell).



kinase, leading to the activation of the transcription factors nuclear factor of activated T cells 1 (NFAT/Nfat) and nuclear factor kappa-B (NF-κB), for bone resorption [19]. Mature osteoclasts can also produce small extracellular RANK vesicles that bind to the membrane form of RANKL on OBs, inducing reverse signaling and promoting OB differentiation [19].

Recently, it has been well established that the RANK/RANKL/OPG (RRO) triad acts not only on bone tissue but also on skeletal, cardiac, and smooth muscles. RANK is expressed in skeletal muscle and activation of the NF-κB pathway mainly inhibits myogenic differentiation, which leads to skeletal muscle dysfunction and loss [22]. Figure 1 summarizes the roles of the RRO pathway.

The RRO pathway is also thought to be involved in cardiac remodeling following immunoinflammatory myocardial diseases or during chronic heart failure. It has been hypothesized that this pathway could play an active role in angiogenesis, pathological inflammation, cell survival, and vascular smooth muscle cell calcification [23].

RRO pathway modulation in muscle diseases

Modulation of the RRO pathway could exert beneficial effects on bone and muscle tissues. Dufresne et al. demonstrated that muscle cells can produce and secrete OPG; they also showed that in mice with muscular dystrophy treated with the RANKL inhibitor OPG-Fc (immunoglobulin Fc segment complex), progressive loss of muscle strength was reduced in a dose-dependent way and muscle integrity was even preserved, thereby strengthening the hypothesis that OPG could represent a potential new therapy not only for osteoporosis but also for muscle diseases [24].

Full-length OPG-Fc binds to RANKL and tumor necrosis factor-related apoptosis-inducing ligand, limiting inflammation and apoptotic pathways, and it also binds to a muscle receptor stimulating sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity and Ca^{2+} entry (from the cytosol into the ER lumen), thereby favoring Ca^{2+} cycling and homeostasis and ultimately muscle performance [24].

Also, anti-RANKL treatment protected against skeletal muscle dysfunctions while enhancing bone mechanical properties, thereby "filling two needs with one deed" in the context of muscular dystrophy [25].

The RANKL inhibitors could exert a positive influence on muscle mass and strength, particularly in conditions of osteoporosis and/or sarcopenia; their effects could be mediated by glucose regulation, since another key function of the RRO pathway, investigated in murine liver, is regulation of glucose homeostasis [26].

On the other hand, as demonstrated by Carbone et al. in a murine model of myocardial infarction, RANKL plays a role in neutrophil degranulation and migration and, consequently, in infarct size and post-lesional cardiac dysfunction [27]. The authors proved that RANKL inhibition before reperfusion ameliorated infarct size and improved cardiac function in these mice. Also in myocardial muscle, SERCA2a, expressed in the heart, mediates reuptake, by the sarcoplasmic reticulum, of Ca^{2+} from the cytoplasm, thus determining the rate of myocardial relaxation and affecting cardiac function [27]. The expression and activity of SERCA2a are reduced in failing hearts. Anti-RANKL antibody, enhancing Ca^{2+} cycling and homeostasis also in myocardial muscle, could favor muscle performance [27].

Denosumab is a fully human monoclonal anti-RANKL antibody that decreases bone resorption and increases BMD, reducing the risk of fracture [28]. Furthermore, the RRO system also plays a key role in muscle metabolism and the development of sarcopenia. In mice, overexpression of RANKL decreased muscle mass, force, and glucose uptake associated with an upregulation of anti-myogenic and inflammatory genes [26]. RANKL inhibitors seem to correct these findings in *huRANKL* mice and in *Pparb*$^{-/-}$ osteo-sarcopenic mice, thus demonstrating that the RANKL-RANK system is involved in the development of muscle weakness, regardless of the triggering mechanism [26].

In the FREEDOM trial, denosumab was demonstrated to be effective in reducing the risk of falls (4.5% of subjects in the denosumab group and 5.7% in the placebo group (p= 0.02) [29].

These findings support the use of denosumab in synergy with a proper musculoskeletal rehabilitation plan for the adequate management of patients with increased fracture risk.

In community-dwelling older adults, too, denosumab was demonstrated to be effective in decreasing the risk of falling [30].

Conclusion

Skeletal bones and muscles, as well as the myocardium, have the same embryonic origin and interact with each other to enable functions essential for survival such as breathing, movement, and postural maintenance.

Just as they grow in perfect synchrony, they degenerate at the same time and in similar ways under various physiological conditions such as aging, or in pathological scenarios such as tumors, prolonged bed rest, neuromuscular diseases, or other pathologies that reduce spontaneous movement.

The functional coupling of bone and muscle tissue relies on their sharing of signaling pathways such as the RANK/RANKL/OPG pathway. This pathway is essential for bone homeostasis and fundamental in the maintenance of skeletal muscle health, but also as a role in the onset of sarcopenia, atherosclerosis, and cardiovascular disease.

Denosumab, a potent RANKL inhibitor, appears to represent an efficient agent that could reduce both fall risk and fragility fractures in osteoporotic patients, by acting on both skeletal and muscle tissue.

References

1. Kerkman JN, Daffertshofer A, Gollo LL, Breakspear M, Boonstra TW. Network structure of the human musculoskeletal system shapes neural interactions on multiple time scales. Sci Adv. 2018;4(6):eaat0497.
2. Periasamy M, Herrera JL, Reis FCG. Skeletal muscle thermogenesis and its role in whole body energy metabolism. Diabetes Metab J. 2017;41(5):327-36.

3. Murphy AC, Muldoon SF, Baker D, et al. Structure, function, and control of the human musculoskeletal network. PLoS Biol. 2018;16(1):e2002811.
4. Karasik D, Kiel DP. Genetics of the musculoskeletal system: a pleiotropic approach. J Bone Miner Res. 2008;23(6):788-802.
5. Maurel DB, Jähn K, Lara-Castillo N. Muscle-bone crosstalk: emerging opportunities for novel therapeutic approaches to treat musculoskeletal pathologies. Biomedicines. 2017;5(4):62.
6. Frost HM. Bone "mass" and the "mechanostat": a proposal. Anat Rec. 1987;219(1):1-9.
7. Li G, Zhang L, Wang D, et al. Muscle-bone crosstalk and potential therapies for sarco-osteoporosis. J Cell Biochem. 2019;120(9):14262-73.
8. Lee JH, Jun HS. Role of myokines in regulating skeletal muscle mass and function. Front Physiol. 2019;10:42.
9. Battafarano G, Rossi M, Marampon F, Minisola S, Del Fattore A. Bone control of muscle function. Int J Mol Sci. 2020;21(4):1178.
10. Elkasrawy MN, Hamrick MW. Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. J Musculoskelet Neuronal Interact. 2010;10(1):56-63.
11. He C, He W, Hou J, et al. Bone and muscle crosstalk in aging. Front Cell Dev Biol. 2020;8:585644.
12. Mera P, Laue K, Wei J, Berger JM, Karsenty G. Osteocalcin is necessary and sufficient to maintain muscle mass in older mice. Mol Metab. 2016;5(10):1042-7.
13. Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. Gene. 2012;492(1):1-18.
14. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548-56.
15. Kirk B, Feehan J, Lombardi G, Duque G. Muscle, bone, and fat crosstalk: the biological role of myokines, osteokines, and adipokines. Curr Osteoporos Rep. 2020;18(4):388-400.
16. Han Y, You X, Xing W, Zhang Z, Zou W. Paracrine and endocrine actions of bone-the functions of secretory proteins from osteoblasts, osteocytes, and osteoclasts. Bone Res. 2018;6:16.
17. Siddiqui JA, Partridge NC. Physiological bone remodeling: systemic regulation and growth factor involvement. Physiology (Bethesda). 2016;31(3):233-45.
18. Oury F, Khrimian L, Denny CA, et al. Maternal and offspring pools of osteocalcin influence brain development and functions. Cell. 2013;155(1):228-41.
19. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys. 2008;473(2):139-46.
20. Kohli SS, Kohli VS. Role of RANKL-RANK/osteoprotegerin molecular complex in bone remodeling and its immunopathologic implications. Indian J Endocrinol Metab. 2011;15(3):175-81.
21. Zhou X, Cao H, Guo J, Yuan Y, Ni G. Effects of BMSC-derived EVs on bone metabolism. Pharmaceutics. 2022;14(5):1012.
22. Marcadet L, Bouredji Z, Argaw A, Frenette J. The roles of RANK/RANKL/OPG in cardiac, skeletal, and smooth muscles in health and disease. Front Cell Dev Biol. 2022;10:903657.
23. Rochette L, Meloux A, Rigal E, Zeller M, Cottin Y, Vergely C. The role of osteoprotegerin and its ligands in vascular function. Int J Mol Sci. 2019;20(3):705.
24. Dufresne SS, Boulanger-Piette A, Bossé S, et al. Genetic deletion of muscle RANK or selective inhibition of RANKL is not as effective as full-length OPG-fc in mitigating muscular dystrophy. Acta Neuropathol Commun. 2018;6(1):31.
25. Boulanger Piette A, Hamoudi D, Marcadet L, et al. Targeting the muscle-bone unit: filling two needs with one deed in the treatment of Duchenne muscular dystrophy. Curr Osteoporos Rep. 2018;16(5):541-53.
26. 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(8):3214-23 [published correction appears in J Clin Invest. 2020;130(6):3329].
27. Carbone F, Crowe LA, Roth A, et al. Treatment with anti-RANKL antibody reduces infarct size and attenuates dysfunction impacting on neutrophil-mediated injury. J Mol Cell Cardiol. 2016;94:82-94.
28. Ominsky MS, Stouch B, Schroeder J, et al. Denosumab, a fully human RANKL antibody, reduced bone turnover markers and increased trabecular and cortical bone mass, density, and strength in ovariectomized cynomolgus monkeys. Bone. 2011;49(2):162-73.
29. Cummings SR, San Martin J, McClung MR, et al; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009;361(8):756-65 [published correction appears in N Engl J Med. 2009;361(19):1914].
30. Wu XD, Huang W, Qiu GX. Denosumab for the prevention of falls in older people: we need more evidence. J Bone Miner Res. 2020;35(8):1609-10.

Author Contributions: *Each author contributed equally and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.*
Funding: *This research received no external funding.*
Institutional Review Board Statement: *Not applicable.*
Informed Consent Statement: *Not applicable.*
Data Availability Statement: *Not applicable.*
Acknowledgments: *None.*
Conflicts of Interest: *The authors declare no conflict of interest.*