RESEARCH HIGHLIGHT

Physiological role of receptor activator nuclear factor-κB (RANK) in denervation-induced muscle atrophy and dysfunction

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Received: April 21, 2016
Published online: May 30, 2016

The bone remodeling and homeostasis are mainly controlled by the receptor-activator of nuclear factor κB (RANK), its ligand RANKL, and the soluble decoy receptor osteoprotegerin (OPG) pathway. While there is a strong association between osteoporosis and skeletal muscle dysfunction, the functional relevance of a particular biological pathway that synchronously regulates bone and skeletal muscle physiopathology remains elusive. Our recent article published in the American Journal of Physiology (Cell Physiology) showed that RANK is also expressed in fully differentiated C2C12 myotubes and skeletal muscles. We used the Cre-Lox approach to inactivate muscle RANK ($RANK^{mko}$) and showed that RANK deletion preserves the force of denervated fast-twitch EDL muscles. However, RANK deletion had no positive impact on slow-twitch Sol muscles. In addition, denervating $RANK^{mko}$ EDL muscles induced an increase in the total calcium concentration ($[Ca_T]$), which was associated with a surprising decrease in SERCA activity. Interestingly, the levels of STIM-1, which mediates Ca^{2+} influx following the depletion of SR Ca^{2+} stores, were markedly higher in denervated $RANK^{mko}$ EDL muscles. We speculated that extracellular Ca^{2+} influx mediated by STIM-1 may be important for the increase in $[Ca_T]$ and the gain of force in denervated $RANK^{mko}$ EDL muscles. Overall, these findings showed for the first time that the RANKL/RANK interaction plays a role in denervation-induced muscle atrophy and dysfunction.

Keywords: RANK; skeletal muscle; calcium; SERCA

To cite this article: Sébastien S. Dufresne, *et al*. Physiological role of receptor activator nuclear factor-κB (RANK) in denervation-induced muscle atrophy and dysfunction. Receptor Clin Invest 2016; 3: e1323. doi: 10.14800/rci.1323.

Introduction

Bone remodeling is an essential process for maintaining bone homeostasis throughout the life of an individual. It is under the control of local and systemic factors that orchestrate osteoblast/osteoclast activation [1-4]. RANK, the receptor-activator of RANKL, and the OPG triad (RANK/RANKL/OPG) play important roles in fine-tuning the activity of these two bone cell types. RANKL is mainly expressed by bone marrow mesenchymal stromal cells and

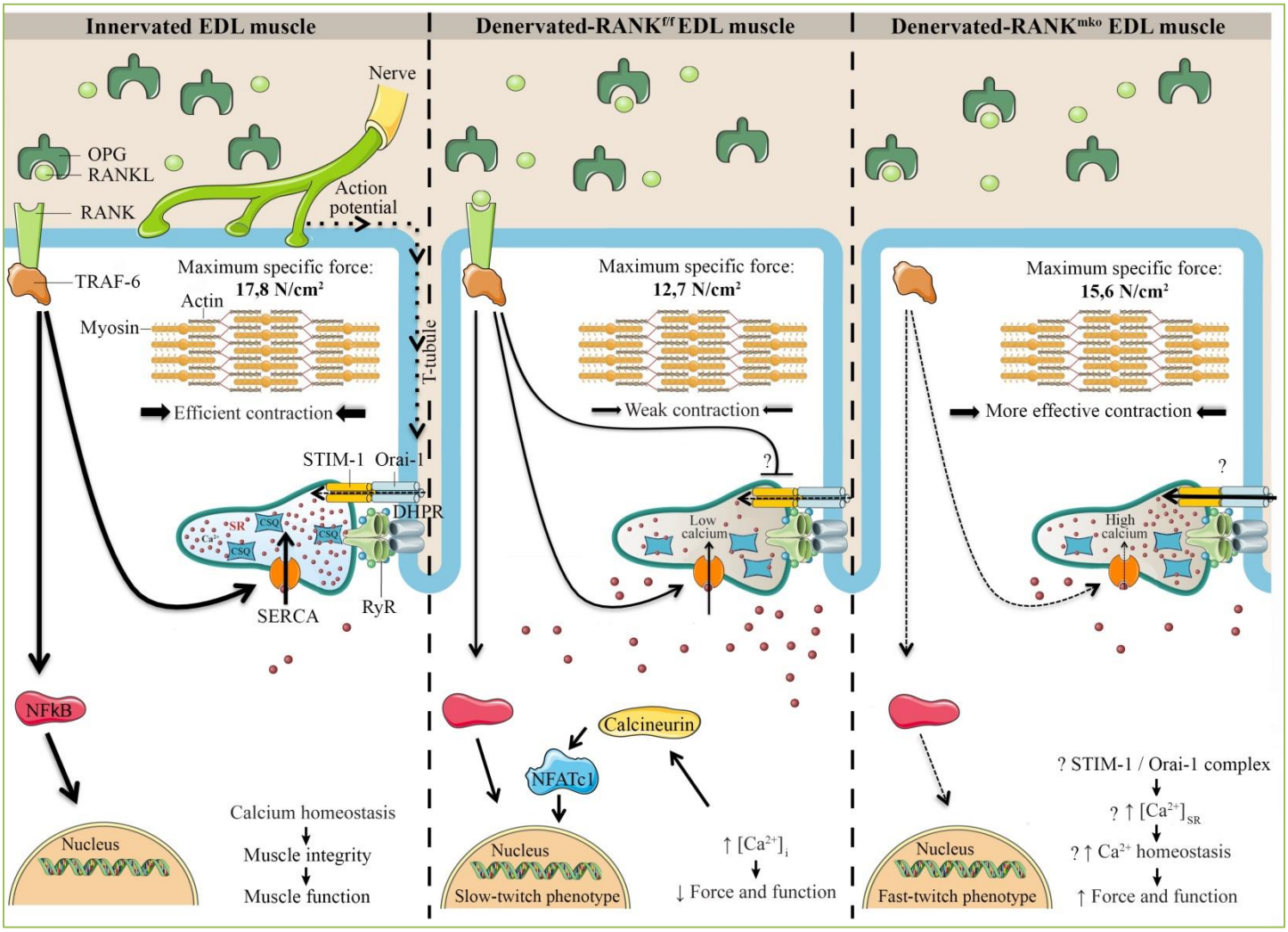

Figure 1. Schematic and hypothetical representation depicting the function of RANK in skeletal muscle. The action potential is conducted into the interior of the muscle cell along the T-tubules where DHPR, a voltage sensor protein, triggers Ca^{2+} release from the SR through RyR and muscle contraction. Once released, SERCA pumps back the Ca^{2+} into the SR allowing muscle relaxation and preparing muscle for the next contraction. In bone cells, RANKL/RANK interaction is important for SERCA activation, $[Ca^{2+}]_i$ oscillations and osteoclastogenesis. In skeletal muscle, a rise in $[Ca^{2+}]_i$ would stimulate calcineurin, a calcium dependent protein phosphatase that subsequently dephosphorylates NFATc1 and promotes a slower muscle phenotype. Muscle specific RANK deletion (RANK^{mko}) reduces SERCA activity but protects against sciatic denervation-induced muscle dysfunction and favors a fast-twitch phenotype. We speculate that STIM-1/Orai-1 complex would compensate for the lack of SERCA activity, refilling Ca^{2+} stores and improving muscle function in atrophied and denervated RANK^{mko} EDL muscles. Although the complete and precise role of muscle RANK remains poorly understood, our article in American Journal of Physiology (Cell physiology) shows that muscle RANK is an important regulator of Ca^{2+} storage, muscle phenotype, and muscle function in normal and pathological conditions.

osteoblasts [5-6]. The binding of RANKL to RANK on osteoclast precursors trimerizes its receptor and induces osteoclast differentiation and activation, resulting in bone resorption. RANK knockout impairs osteoclastogenesis and induces osteopetrosis, while the overproduction of RANKL induces osteoporosis [7, 8]. OPG, the third protagonist, is mainly produced by bone marrow mesenchymal stromal cells and osteoblasts and exerts an inhibitory effect on the osteoclastic process. OPG has a high affinity for RANKL and inhibits the RANKL/RANK interaction and subsequent bone degradation. In addition to RANKL, OPG serves as a low affinity decoy receptor for TRAIL. Pre-clinical studies have suggested that this interaction increases cell survival by blocking the apoptotic effects of the RANKL/RANK interaction [9, 10]. The fact that the overexpression of OPG or an exogenous OPG treatment [11] in mice results in osteopetrosis and that OPG-null mice are osteoporotic are testimony to the physiological importance of OPG [11].

Clinical studies have clearly shown that there is an association between osteoporosis and muscle atrophy and that the worsening of these conditions happens in tandem [12, 13]. While bones and skeletal muscles are closely related and are mechanically linked, the possibility of dynamic

molecular cross-talk between these tissues and a common signaling pathway that can efficiently control them has been underappreciated to date. Since the RANK/RANKL/OPG pathway is the most important cytokine network involved in bone biology and diseases, we postulated that this bone pathway is also involved in muscle atrophy and dysfunction. We first showed by Western blotting and confocal microscopy that C2C12 myotubes and fully differentiated skeletal muscles express RANK on the membranes of fast and slow-twitch myofibers [14]. Based on the protective effect of RANK deletion on bone mass [15], we next postulated that selective muscle RANK deletion preserves muscle mass and function and favors a fast-twitch phenotype following denervation.

Muscle phenotype and RANK

Skeletal muscles are primarily composed of four muscle fiber types: type I fibers (slow and oxidative), type IIa fibers (fast and oxidative), and type IIx and IIb fibers (fast and glycolytic). The function largely dictates the phenotype and composition of each skeletal muscle. For example, the Sol muscle, a postural muscle, is largely composed of slow oxidative type I fibers while the EDL muscle, a phasic muscle, is almost exclusively composed of fast-twitch fibers [16]. These two muscles encompass the two extremes of the phenotypic spectrum of the skeletal muscle contractile apparatus. Type I fibers play an important role in maintaining the body in an upright position, while type IIb and IIx fibers are responsive during movement and physical activity. Type IIa fibers are a hybrid of type I and type IIb fibers and can perform short or prolonged exercises. Specific exercises, immobilization, unloading, denervation, muscle diseases, or glucocorticoid treatments affect all four muscle fiber phenotypes to different degrees. For instance, decreases in mechanical loading and neuromuscular activity favor muscle atrophy and a conversion of muscle fiber phenotypes from slow to fast [17]. Functional muscle overload causes a gain in muscle mass while prolonged exercise leads to the transformation of pre-existing fast-twitch muscle fibers to a slow-twitch oxidative phenotype. Type IIb fibers, the most powerful fibers, are converted to oxidative phenotype fibers (type I or IIa) and disappear through a necrotic process during myopathies, aging, and glucocorticoid treatments [18, 19]. Intracellular/cytosolic Ca^{2+} ($[Ca^{2+}]_i$) fluctuations play a crucial role in the maintenance and adaptation of muscle phenotypes. The increase in $[Ca^{2+}]_i$ is caused by a Ca^{2+} influx from the extracellular space and/or Ca^{2+} release from $[Ca^{2+}]_i$ stores. The SR is the major releasable $[Ca^{2+}]_i$ store. Ca^{2+} channels and pumps maintain a steep Ca^{2+} concentration gradient between different cell compartments. Generally speaking, the spatial pattern of increased free $[Ca^{2+}]_i$ at rest induces the activation of calmodulin and downstream Ca^{2+} decoders, namely calcineurin and CaMKII [20]. These two decoders modify gene transcription by activating transcription factors such as NFATc1 and MEF2 and transcription modulators such as HDAC and PGC-1□, promoting the slow myofiber phenotype [21] (Figure 1). Interestingly, the increase in $[Ca^{2+}]_{SR}$ is essential to activate NFATc1, while extracellular Ca^{2+} is required to maintain its activation [22] and to replenish $[Ca^{2+}]_i$ stores [23]. Since over 97% of $[Ca^{2+}]_i$ is stored in the SR at rest, the measurement of total Ca^{2+} ($[Ca_T]$) is an estimate of $[Ca^{2+}]_{SR}$ content [24]. We used Cre-Lox technology to inactivate muscle RANK and found that the selective deletion of muscle RANK ($RANK^{mko}$) decreases $[Ca_T]$ in fast-twitch EDL muscles but not in Sol muscles, when compared to $RANK^{flox/flox}$ ($RANK^{f/f}$) muscles. However, sciatic denervation markedly increased $[Ca_T]$ in $RANK^{mko}$ EDL muscles. In addition, we discovered that denervated $RANK^{mko}$ EDL muscles are smaller, more fatigable, and surprisingly stronger than $RANK^{f/f}$ EDL muscles. Since force production is usually proportional to muscle size, we hypothesized that the increase in $[Ca_T]$, i.e., $[Ca^{2+}]_{SR}$ at rest, would favor Ca^{2+} release, increasing free $[Ca^{2+}]_i$ and thereby the specific force of denervated $RANK^{mko}$ EDL muscles. The phenotypic profiles, which are consistent with these observations, showed a lower proportion of slow-twitch type I and fast-twitch type IIx fibers and a higher proportion of fast-twitch type IIa/IIb fibers in $RANK^{mko}$ EDL muscles relative to $RANK^{f/f}$ EDL muscles. However, muscle fatigue was significant and could not be explained by the modest changes in myofiber type in sham and denervated $RANK^{mko}$ EDL muscles. In this context, it would be highly relevant to determine whether muscle RANK deletion influences mitochondria Ca^{2+} uptake, another important regulator of $[Ca^{2+}]_i$ and energy production. Our results showed for the first time that muscle RANK is an important regulator of Ca^{2+} storage, muscle phenotype, and muscle fatigue in normal and pathological conditions.

Calcium signaling, RANK, and skeletal muscle

Muscle contraction involves the depolarization of the transverse-tubular system and activation of DHPRs, which in turn open RYR1 receptors adjacent to the SR membrane [25]. This results in the rapid efflux of large amounts of Ca^{2+} into the cytoplasm and the binding of Ca^{2+} to troponin C, causing the formation of cross-bridges between actin and myosin, the shortening of the sarcomere, and force development [26]. To avoid permanent contraction and allow muscle relaxation, Ca^{2+} is in part pumped back into the SR by an ATP-dependent Ca^{2+} pump called SERCA. Ca^{2+} reuptake into the SR is mainly controlled by the fast-twitch SERCA-1a and slow-twitch SERCA-2a isoforms in myofibers. The movement of Ca^{2+} is reduced when PLN is associated with SERCA. Under

β-adrenergic stimulation, like the stress hormones adrenaline and noradrenaline, or sympathomimetic drugs, the phosphorylation-dependent dissociation of PLN by PKA increases SERCA activity and Ca^{2+} movement, enhancing skeletal muscle and heart functions (Figure 1). Alternatively, PLN deletion increases cardiac output by decreasing the time to peak pressure and the half-relaxation time [27], indicating that efficient Ca^{2+} mobilization and the disinhibition of SERCA are prerequisites for powerful contractions. Conversely, dysfunctional SERCA expression and activity impair $[Ca^{2+}]_i$ homeostasis, reducing the force production and power output of skeletal and cardiac muscles [28, 29]. This is particularly true for fast-twitch muscles, like in dystrophic EDL muscles where Ca^{2+} handling is altered with the expression of the slow isoform of SERCA-2a [30]. Increased Ca^{2+} influx thus activates proteolytic pathway, which in turn causes even greater Ca^{2+} influx, giving rise to a possible vicious pathological cycle in dystrophic muscles [31]. Interestingly, the overexpression of SERCA mitigates muscular dystrophy and rescues cardiac function in a model of pressure overload, highlighting the importance of SERCA in muscle performance and disease [32, 33]. In our paper, we report a number of intriguing and challenging observations, including the fact that RANK deletion in denervated EDL muscles increases $[Ca_T]$ and specific force and decreases SERCA activity. This apparent discrepancy between the increase in $[Ca_T]$ and the decrease in SERCA activity may be due to an alternative source of Ca^{2+} refilling the SR. Indeed, extracellular Ca^{2+} and SOCE are important for optimal muscle force production, while SOCE inhibition is associated with a decrease in contractile properties [34]. More specifically, Orai-1 Ca^{2+} channels are key sensors and major contributors to Ca^{2+} entry into the SR via an interplay with STIM-1. When SR Ca^{2+} stores are depleted, STIM-1 activates Orai-1 to refill the stores [35]. Additionally, Stiber *et al*. (2008) demonstrated that STIM-$1^{+/+}$ myotubes maintain full Ca^{2+} stores whereas STIM-$1^{-/-}$ myotubes fail to refill SR Ca^{2+} stores following chronic stimulations [36]. Interestingly, we showed that denervated $RANK^{mko}$ EDL muscles have a markedly higher STIM-1 content. Since the increase in $[Ca_T]$ and the decrease in SERCA activity in denervated $RANK^{mko}$ EDL muscles are somewhat irreconcilable, we proposed that STIM-1 and Orai-1 compensate for the lack of SERCA activity, refilling SR Ca^{2+} stores in the absence of muscle RANK.

RANKL/RANK interactions and intracellular signaling networks in bone and skeletal muscle

In bone, RANKL/RANK interactions activate TRAF-6, which then induces the activation of downstream signaling molecules and $[Ca^{2+}]_i$ oscillations [37]. SERCA is essential for $[Ca^{2+}]_i$ oscillations and plays a critical role in osteoclastogenesis [38]. It has recently been reported that SERCA-2a heterozygote mice (SERCA$2a^{+/-}$) present defects in osteoclast differentiation and the suppression of RANKL-induced Ca^{2+} oscillations [39]. Tmem64, a positive modulator of osteoclast differentiation, regulates RANKL-induced Ca^{2+} signaling via SERCA2a [40]. In addition to SERCA, Ca^{2+} oscillations may partly derive from extracellular Ca^{2+}. Gadolinium, a specific SOCE blocker, abolishes RANKL-induced Ca^{2+} oscillations [41] while the knockdown of STIM-1 significantly reduces the frequency of RANKL-induced Ca^{2+} oscillations, indicating that SOCE and RANK communicate. TRP channels are also candidates for the Ca^{2+} influx involved in RANKL-induced Ca^{2+} oscillations [42]. Chamoux *et al*. (2010) showed that RANKL induces a significant increase in $[Ca^{2+}]_i$ from outside, presumably through the TRPV-5 Ca^{2+} channel. While an elaborate approach has been proposed to elucidate how RANKL/RANK induces Ca^{2+} oscillations in bone cells, the importance of this pathway in skeletal muscle is extremely limited. Our work reported in the American Journal of Physiology (Cell Physiology) was, to our knowledge, the first attempt to assign a regulatory role for RANKL/RANK interactions on SERCA activity in bone and muscle cells [14]. The identification of downstream effectors of muscle RANK will be an important step in determining precisely how the RANKL/RANK interaction modulates SERCA activity. Bioinformatic approaches may eventually be required to improve our understanding of the cell signaling network and to determine how the RANKL/RANK interaction regulates SERCA activity and possibly other Ca^{2+} channels in skeletal muscles.

Future directions and perspectives

We convincingly show in another publication in the American Journal of Pathology (2015) [43] that OPG-Fc (OPG fused to immunoglobulin Fc) completely restores the function of dystrophic EDL muscles and significantly improves the function of Sol and diaphragm muscles. OPG treatments also provide significant protection against eccentric-contraction-induced muscle damage. As observed with OPG-treated dystrophic mice, muscle RANK deletion preferentially protects fast-twitch fibers. It is important to note that fast-twitch fibers are essential for brief and powerful contractions. They are predominantly affected directly or indirectly in aging and in several forms of muscle disease and chronic illness, including diabetes, congestive heart failure, renal failure, chronic heart disease, and chronic obstructive pulmonary disease. Our results clearly show that the role of the RANK/RANKL/OPG pathway extends well beyond bone remodeling and osteoporosis and that skeletal muscles and other tissues may share the

RANK/RANKL/OPG pathway as a common denominator.

Conflicting interests

The authors have declared that no competing interests exist. Jérôme Frenette was supported by the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC).

Author contributions

J.F. and S.S.D. conceived the project and its design; J.F., S.S.D., A.B.P. and S.B. wrote the manuscript; and all authors checked for scientific content and approved the final manuscript.

Abbreviations

RANK: receptor-activator of nuclear factor κB; RANKL: ligand of receptor-activator of nuclear factor κB; OPG: osteoprotegerin; TRAF-6: tumor necrosis factor associated factor-6; NFATc1: nuclear factor of activated T cells 1; MAPKs: mitogen activated protein kinases; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; Sol: soleus muscle; EDL: extensor digitorum longus muscle; $[Ca^{2+}]_i$, intracellular calcium; CSQ: calsequestrin; CaMKII: calmodulin-dependent protein kinase II; MEF2: myocyte enhancer factor; HDAC: histone deacetylase; PGC-1: peroxisome-proliferator-activated receptor gamma, co-activator 1; SR: sarcoplasmic reticulum; $[Ca_T]$: total calcium content; $RANK^{mko}$: specific RANK skeletal muscle deletion; $RANK^{f/f}$: $RANK^{flox/flox}$ skeletal muscle; DHPR: dihydropyridine receptor; RYR1: ryanodine receptor; SERCA: sarco(endo)plasmic reticulum Ca^{2+}ATPase; PLN: phospholamban; PKA: protein kinase A; SOCE: store-operated Ca^{2+} entry; STIM-1: stromal interaction molecule-1; ER: endoplasmic reticulum; ECC: excitation contraction coupling; ub: ubiquitination; TRP: Transient receptor potential; Tmem64: transmembrane protein 64.

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