RANK-RANKL signaling in skeletal muscle mitochondrial dynamics

Paulo Henrique Cavalcanti de Araújo[1], Flávia Sayuri Matsuo[1], Anderson Vulczak[2], Maria Eduarda Ramos Cezine[1], Júlia Maranghetti Remoto[1], Luciane Carla Alberici[2], Mariana Kiomy Osako[1]

[1]Laboratory of Cell and Tissue Biology, Department of Cell and Molecular Biology and Pathogenic Bioagents, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto/SP, Brazil.

[2]Department of Biomolecular Sciences, Faculty of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto/SP, Brazil.

Introduction: The operational unit bone-muscle refers to the mechanical interaction between bone and muscle and the communication via soluble factors that reciprocally regulate their metabolism. RANKL (receptor activator of NFκB ligand) and OPG (osteoprotegerin) are examples of osteokynes that, together with RANK (receptor activator of NFκB), are essential for osteoclast activation. Our group has recently shown that RANK-RANKL signaling induces beige adipocyte differentiation (*browning* process) and increases extensive mitochondrial reticulum and energy expenditure. Mitochondrial dynamics, such as fusion, fission, and biogenesis, are regulated by PGC1-alpha expression. The skeletal muscle shows high levels of RANK expression, which has a fundamental role in regulating SERCA activity. Therefore, we hypothesized that RANK-RANKL signaling might impact skeletal muscle mitochondrial dynamic and contribute to muscle fiber switch to oxidative profile. **Methods**: Experiments with male C57BL/6J mice were approved by Ethical Principles in Animal Research (CONCEA nº 0069/2020). RANKL infusion was performed for 28 days using a mini-osmotic pump. Soleus and gastrocnemius were analyzed by transmission electron microscopy (TEM), HE staining, SDH staining, and immunofluorescence. Respiratory rates of soleus muscle were evaluated at high-resolution Oxygraph-2 k® (Oroboros, Innsbruk, Austria). Murine C2C12 myoblasts were differentiated for 5 days. Mitochondrial content was detected with Mitotracker® Red CMXRos (Invitrogen). Mitochondrial gene expression was quantified by RT-qPCR using QuantiFast SYBR® Green kit (Qiagen). Phosphorylation of CREB and p38 pathways was detected by western blot. Luciferase assays were performed with PPAR responsive elements (PPRE) in the promoter region of X3-TK-Luc plasmid

(Addgene). The oxygen consumption rate (OCR) of C2C12 myotubes was measured using a SeaHorse XFe96 Extracellular Flux Analyzer. **Results**: The soleus of RANKL treated mice showed elevated respiratory rates, cross-section area analysis also revealed muscle fiber with smaller diameter, increased SDH staining, an increased proportion of type IIa fibers, and TEM analysis revealed an increase of mitochondria number with decreased roundness morphology compared to PBS infused group. C2C12 myotubes stimulated with different doses of RANKL showed a rise in mitochondrial markers such as ATP synthase, Citrate synthase, NRF-2, MFN-2, OPA-1, and higher activity of PPRE in the luciferase assay. OCR analysis of C2C12 myotubes revealed an increase of spare respiratory capacity in cells stimulated with RANKL, demonstrating the ability of these cells to respond to the rise of energy demand. **Conclusion**: These data indicate that RANKL modulates the mitochondrial dynamics fundamental for skeletal muscle metabolism and fiber type conversion, provides new insights in the communication between bone-muscle and clarifies the still unreported role of RANKL in the healthy muscle fiber.