Anti-RANKL treatment attenuates sarcopenia via modulating mitochondria and macrophage infiltration

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INTRODUCTION: Sarcopenia is characterized by progressive loss of skeletal muscle mass, strength, and muscle function associated with typical aging, which increases the risk of a vast array of adverse health outcomes, including falls, morbidity, loss of independence, disability, and mortality. People with sarcopenia are associated with poor balancing abilities and substantial increase in fall risks that may result in increased fragility fracture rate to as high as 1.87 times in Hong Kong. The increase in chronic low-grade systemic inflammation during aging is associated with sarcopenia and frailty, and involves an increase in resident macrophage populations within aging muscles. M2 macrophages were reported to be elevated in aged mouse skeletal muscle and associated with increased fibrosis, while M1 macrophages declined with aging, making the total number of macrophages invariant with older age. The receptor activator of nuclear factor NF-κB ligand (RANKL) is expressed in skeletal muscle and its activation mainly inhibits myogenic differentiation, which leads to skeletal muscle dysfunction. Previous study showed that RANKL inhibition could improve muscle strength, insulin sensitivity and restore bone mass in adult osteoporotic mice, indicating a great potential of RANKL inhibition for treating sarcopenia, whereas no studies have yet investigated into this area in deep. The study aims to investigate the effects of anti-RANKL treatment on sarcopenic skeletal muscle and explore the related mechanisms in terms of mitochondria modulation and the polarization status of macrophages.

METHODS: Sarcopenic senescence-accelerated mouse P8 (SAMP8) mice at month 6 were treated intraperitoneally with 10mg/kg anti-RANKL (Bio X Cell) every 2 weeks and harvested at month 10 (Fig A). Senescence accelerated mouse resistant-1 (SAMR1) were collected at month 10 as age-matched non-sarcopenic group. Ex-vivo functional assessment, grip strength, oil red O and immunostaining of CD45, F4/80, CD206, iNOS, C/EBPa, and Pax7 were performed. Mitochondria morphology was examined with a transmission electron microscope (Hitachi H7700, Tokyo, Japan). One-way ANOVA analysis was done, and the significant level was set at $p \leq 0.05$.

RESULTS SECTION: After anti-RANKL treatment, tetanic, twitch force and grip strength were significantly higher than CTL group ($p<0.01$, $p<0.01$ and $p<0.05$, Fig B). The SAMP8 mice at month 10 expressed significantly more C/EBPα (intramuscular adipose marker), CD206 (M2 marker) and LYVE1 (macrophage marker) positive area than in SAMR1 (Fig C). Anti-RANKL treatment could significantly decrease CD45 (general leukocyte marker), F4/80 (M1 marker), iNOS (M1 Marker), C/EBPα and CD206 positive area and oil red O area, and significantly increase PAX7 (MDSC marker) positive cell numbers (Fig C and E). There was an increase in the number of intermyofibrillar mitochondria and restoration of mitochondria morphology in anti-RANKL group. The structure of mitochondria and their cristae were more compact, compared with swollen mitochondria in the isotype group (Fig. D).

DISCUSSION: These results indicated an excessive inflammation and the promoted shift from M1 to M2 in macrophage phenotype during sarcopenia. The anti-RANKL treatment protected against sarcopenic skeletal muscle through suppressing muscle inflammation and modulating mitochondria which may represent a novel therapeutic approach for sarcopenia.

SIGNIFICANCE/CLINICAL RELEVANCE: This study investigates molecular mechanism how RANKL inhibitor modulates macrophage infiltration and mitochondrial functions, indicating a great potential of RANKL inhibitor as an effective medication for treating sarcopenia.

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IMAGES AND TABLES:

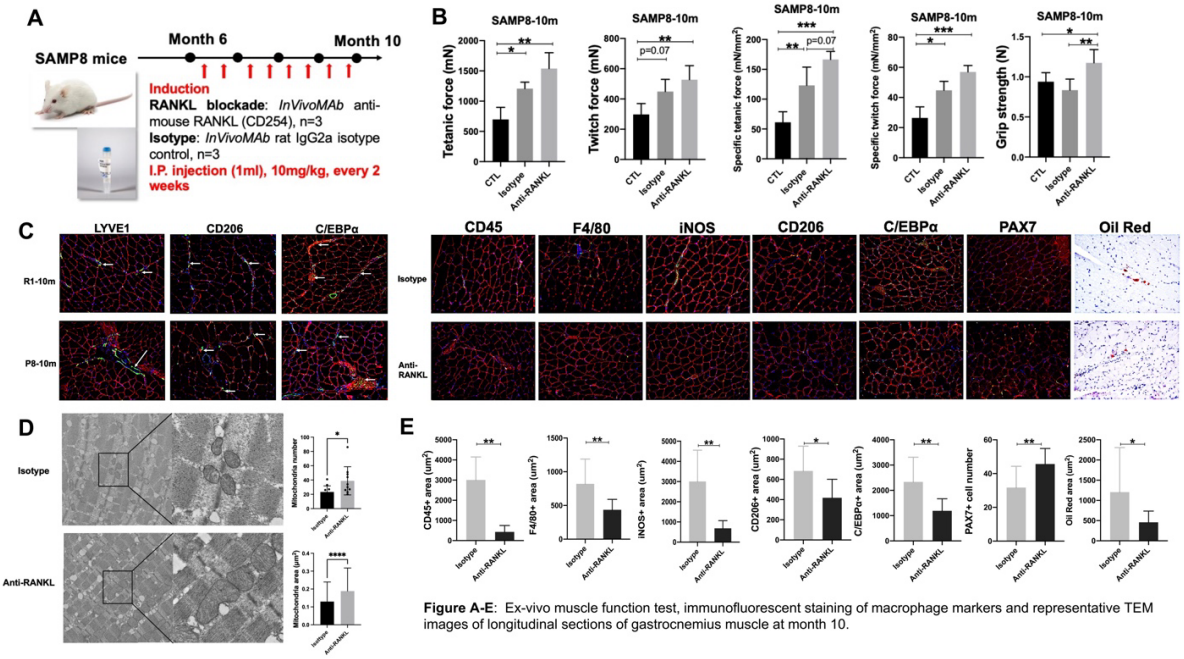

Figure A-E: Ex-vivo muscle function test, immunofluorescent staining of macrophage markers and representative TEM images of longitudinal sections of gastrocnemius muscle at month 10.