TACSM Abstract

The RANKL and Nox2 Signaling in the Duchenne Muscular Dystrophy Models of Skeletal Muscle

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ABSTRACT

There is a severe sort of muscle disorder and dysfunction caused by a mutation of the dystrophin gene which is called Duchenne muscular dystrophy (DMD). The establishment of pathology in the limbs, respiratory systems, and hearts of DMD patients is the awareness of the main point for the inflammation and elevation of oxidative stress. NADPH oxidase-2 (Nox2) produces reactive oxygen species in response to skeletal muscle contractions. Recent studies show that various myopathy indicated the key stress response proteins which can modulate Nox2. The TRAF 6 and RANKL lead to Nox2 complex migration and construction. An increase of pro-inflammatory proteins in dystrophic muscle can lead to Nox2 assembly and disruption of Ca2+ homeostasis, including the Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL). The contribution of RANKL is involved in weakness, atrophy, inhibition of sarcoplasmic reticulum Ca2+-ATPase pump (SERCA), and insulin resistance in skeletal muscle. However, the mechanisms involved in the redox regulation of RANKL and their combined role in DMD pathology are not well understood. **PURPOSE**: We propose inhibiting RANKL and Nox2 will mitigate damage, loss of sarcolemmal DGD proteins, Ca2+ overload, and inflammation in the DMD pathology. **METHODS**: We set up experiments (a) Nox2 peptidyl inhibition and (b) RANKL IK22/55 knockdown. (a) In this initial study, 4 weeks old C57BL10 (WT and mdx mice) are partitioned into three groups (n = 6/ group): (1) wild-type, (2) mdx mice, and (3) mdx mice + Nox2 inhibitor gp91ds-tat (10 mg/kg/day i.p.); while second experiment (1) wild-type, (2) mdx mice, and (3) mdx mice + Ik22 antibody (10 mg/kg/3 days). After 8 days, Mice were euthanized, and the gastrocnemius muscles were dissected. Then muscles have frozen and snap-frozen for further analysis. **RESULTS**: We have observed that RANKL levels are elevated in dystrophic muscle, and the Nox2 inhibition attenuates the upregulation of RANKL, as confirmed with the RANKL inhibition treatment, suggesting potential feedback of Nox2 on RANKL. Protein abundance by western blot data reveals that inhibition of Nox2 and RANKL significantly reduces the tissue damage (IgG markers) and inflammation markers (CD68, P65, TRAF6 levels), and markers of Ca2+ overload (SRECA1, TRPC1, SRECA2, Sarcolipin, Calpain activity) in the mdx muscle compared to the mdx and WT groups. **CONCLUSION**: This is the first direct evidence of positive feedback signaling between RANKL and Nox2 in a model of DMD. Specifically, RANKL/Nox2 trigger damage and inflammation through exacerbation of the loss of sarcolemmal nNOSμ and Ca2+ overload by elevating Ca2+ channel TRPC1 and downregulating Ca2+ ATPase pumps (SERCA1, SERCA2a).