A loss of Cu/Zn Superoxide Dismutase (SOD1) impairs muscle stem cell function and regeneration

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Disclosures:

INTRODUCTION: The oxidative stress theory of aging predicts that accumulative damages resulting from excessive production of reactive nitrogen and oxygen species (RNOS), which target key cellular components, such as DNA/RNA, lipids, and proteins, leads to accelerated aging and age-related diseases, including sarcopenia. A lack of Cu/Zn-superoxide dismutase (SOD1), an essential antioxidant enzyme, accelerates the age-dependent loss of muscle mass and function. Despite recent advances in understanding oxidative stress and redox regulation in the musculoskeletal system, how RNOS and concomitant oxidative and nitrative stress contribute to muscle stem (satellite) cell function and muscle regeneration have not been thoroughly tested. To this end, we generated inducible muscle stem cell-specific *Sod1* knockout mice (*Pax7 Cre*^{ERT}; *Sod1*$^{flox/flox}$ mice) to establish a cause-and-effect relationship between oxidative damage in muscle stem cell function and muscle repair. We hypothesize that a deficiency in the antioxidant enzyme SOD1 in muscle stem cells will lead to an increase in RNOS production, ultimately resulting in the loss of myogenic function and deficits in muscle repair and regeneration.

METHODS: Age (4-6 months) and gender-matched Wildtype (WT) and *Pax7*^{CreERT}*Sod1flox* mice (referred to as *MuSC Sod1KO*) were used in this study. For *in vivo* muscle regeneration assessments, cryo-injury and $BaCl_2$ injections were performed on hindlimb muscles. Following 7-, 14-, and 28-day post-injury, immunofluorescence, histological staining, and the cross-sectional area of regenerating myofibers were analyzed for regenerative potential. A TUNEL assay was performed to investigate the cell death. For *in vitro* studies, primary myoblasts from WT and *MuSC Sod1KO* mice were cultured for proliferation, differentiation, and myogenic fusion. The automated live cell imaging was performed using Celloger Plus (Curiosis Inc., Seoul, South Korea), and fluorescent images were captured using Confocal microscopes (Leica Microsystems Mannheim, Germany).

RESULTS: Stains at the specified time points showed a significant deficiency in muscle regeneration in MuSC *Sod1KO* as measured by centrally nucleated fibers and the presence of embryonic myosin heavy chains. In addition, TUNEL+ and increased cleaved caspase-3 staining indicated that a loss of *Sod1* in satellite cells induced apoptosis following 7- and 14-day post-injury. In concurrence with *in vivo* experiments, primary myoblasts isolated from *Pax7*^{CreERT}*Sod1flox* mice exhibited significantly lower cellular confluency compared to WT myoblasts. Furthermore, the percentages of EdU+ cells in *mSod1KO* myoblasts were lower than WT ones. The fusion indices, reflecting the percentage of myotubes having <5; 5-10; >10 nuclei, showed a substantial decrease in *mSod1KO* myotubes with more than 10 nuclei compared to WT myotubes. Accordingly, the total number of myotubes formed from *mSod1KO* myoblasts was significantly lower than that from WT myoblasts.

DISCUSSION: These findings collectively demonstrate that SOD1 plays a pivotal role in myogenesis and subsequent tissue regeneration. Given that SOD1 functions as an antioxidant enzyme, scavenging superoxide radicals, its deficiency leads to an elevation in RNOS levels, thereby affecting cellular functions and potentially inducing cellular apoptosis of myoblasts, limiting myogenic fusion events. We postulate that increased oxidative stress prevalent in aged skeletal muscle accounts for the decline in muscle regenerative capacity. Notably, among various RNOS, the superoxide anion radical, nitric oxide, and hydrogen peroxide are key redox signaling agents, playing a pleiotropic role in regulating various biological activities, including angiogenesis, cell proliferation, cell differentiation, cell migration, and stress responses or adaptation. Consequently, the most effective intervention does not entail completely scavenging all RONS but rather maintaining RNOS at physiological concentrations to facilitate muscle regeneration and functional recovery following injury.

SIGNIFICANCE/CLINICAL RELEVANCE: This study underscores the essential role of SOD1 and emphasizes the significance of maintaining redox balance in muscle regeneration. These findings pave the way for therapeutic strategies exploiting redox-sensitive hydrogels, which can effectively control the RNOS levels.

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IMAGES AND TABLES:

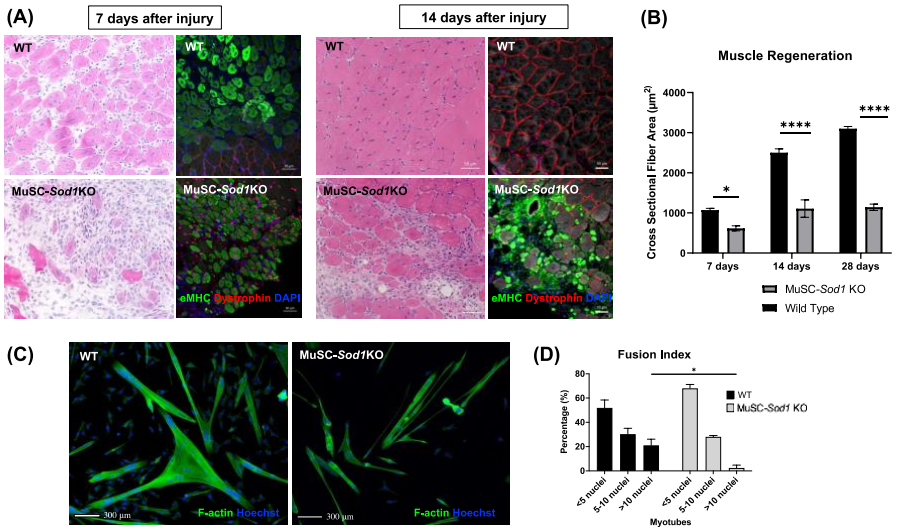

Figure 1: Hematoxylin and eosin (H&E) and immunofluorescent staining of the muscle tissue sections (A); Quantifying fiber cross-sectional area (B); Myotubes formed from WT and MuSC-*Sod1*KO myoblasts (C); Fusion index of WT and MuSC-*Sod1*KO primary myoblasts cultured *in vitro* (C). Data are shown as mean ± SEM (*t*-Test: *p < 0.05)