ERRγ Overexpression in Skeletal Muscle Promotes the Muscle Regeneration in Aged Mice After CTX Injury

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INTRODUCTION

The ability to repair skeletal muscle injury and maintain skeletal muscle health declines with age (1). Potential physiological explanations for why these age-related changes occur may be related to metabolic loss, vascular deficits, or neurological degeneration (1,2). To offer a novel approach in combating the musculoskeletal decline related to aging our team has performed extensive ontology work on the estrogen related receptor gamma gene (ERR-γ) which has exposed multiple encoded programs that are actively involved in lipid metabolism, mitochondrial functions, and angiogenesis (3). Promoting oxidative myofibril remodeling in skeletal muscle by overexpressing ERR-γ in the transgenic mouse model (TG) has shown promising potential for overcoming challenges related to musculoskeletal injury rehabilitation (2,3). To determine if overexpressing ERR-γ maintains skeletal muscle health throughout the aging process we induced an acute muscle cardiotoxin (CTX) injury in both the TG and age-matched wild-type (WT) control at three different age-phases (6-8wk, 12mo, 22mo). We hypothesize that the overexpression of ERR-γ will promote muscle regeneration and alleviate skeletal muscle deficits associated with aging. If these results are indicative, overexpressing ERR-γ may be a novel approach to preventing age-imposed defects on the skeletal muscle system.

METHODS

Animals: The TG colony was obtained from Dr. Narkar's laboratory. The animal protocol used for these experiments was approved by Colorado State University's Animal Care and Use Committee. **Muscle injury**: 4μM of cardiotoxin (CTX) were injected into the gastrocnemius muscles (GM) of TG and WT mice. Five days after the CTX injury the mice were euthanized. The GMs were then harvested and flash-frozen in liquid nitrogen-cooled 2-methylbutane for cry-sectioning (10μm sections). **H&E staining**: HE was performed according to the manufacturer's instructions. **Immunohistochemistry**: The GM cryosections were fixed with 4% PFA, blocked with M.O.M blocking solution and then incubated with an antibody against mouse IgG (Biotinylated) to determine the extent of muscle fiber necrosis. An antibody against embryonic myosin heavy chain (eMyHC) was used to evaluate myogenic regeneration and an antibody against CD68 (macrophage marker) was used to analyze the extent of inflammation/macrophage infiltration in the muscle tissues. The biotinylated goat anti-mouse IgG, Alexafluor 594 conjugated anti-mouse IgG and Alexafluor 488 conjugated anti-rat IgG were used as secondary antibodies, and Streptavidin Cy3 conjugate was added to act as the tertiary antibody. The nuclei were stained with DAPI. The number of necrotic fibers, percent of CD68+ area, and cross-sectional area (CSA) of eMyHC expressing regenerative fibers were analyzed using ImageJ software. **Statistical analysis:** All results are presented as mean ± standard deviation (SD). Means from the CTX injured GM of WT and TG mice were compared using a Two-way ANOVA with Tukey's multiple comparisons test; significance is indicated by a p value < 0.05.

RESULTS

Muscle healed faster and had less necrosis in the injured muscles of aged TG mice compared to age-matched WT mice. From the HE and mouse IgG staining, we observed that the TG mice in both the 12mo and 22mo age group had significantly less muscle damage and necrosis when compared to the age-matched WT mice. Interestingly, the degree of muscle damage in the TG mice did not increase with age (**Fig. 1A-B**). Conversely, CTX muscle damage progressively worsened in the older WT age groups (**Fig. 1A-B**). In quantifying the number of IgG+ fibers (necrotic marker) only the WT mouse had a significant correlation between increased necrosis and age (**Fig. 1C, p < 0.0001**). Note that the number of necrotic fibers in the 12mo and 22mo TG groups had no significant difference when compared to the baseline 6-8wk TG mice group (**Fig. 1C, p > 0.9435**). ***There was reduced macrophage infiltration in the injured muscles of the aged TG mice.*** The results from CD68 staining showed significantly decreased macrophage infiltration in both the 12mo and 22mo aged TG mice compared to the age-matched WT mice (**Fig. 2A and B, p > 0.0001**). ***Muscle regeneration was accelerated in the injured muscles of aged TG mice compared to age-matched WT mice***. Muscle regeneration declines during aging. eMyHC staining and quantification data demonstrated that the muscle regeneration potential of WT mice declined as expected, showing a decrease in CSA of eMyHC+ fibers with age (**Fig. 3A and B, p < 0.0029**). In comparison, the newly regenerated myofibers of aged TG mice did not lose regenerative potential (**Fig. 3A-B, p < 0.0001).** Together, these results suggest overexpressing ERR-γ in the skeletal muscle can enhance overall muscle healing by combating age-related defects and promoting muscle regeneration.

DISCUSSION

Throughout the aging process, the loss of normal biological functions impairs muscle healing. These losses result in increased myofiber necrosis (myonecrosis) and prolonged inflammation within the skeletal muscle niche. Myofiber regeneration (myogenesis), essential for skeletal muscle repair, also declines during aging. Both the TG and WT gastrocnemius muscles were successfully injured in all three age groups, but while the WT mice demonstrated classic age-related decline in skeletal muscle healing substantial deviations were noted in the ERR-γ TG mice. We observed that ERR-γ overexpression effectively decreases myonecrosis and macrophage infiltration (indicated by the IgG/CD68 staining) while promoting myogenesis by accelerating the maturation of newly regenerated fibers in aged mice. A potential explanation for why TG mice have enhanced muscle healing may be related to their upregulation of vascular endothelial growth factor (VEGF) which has also been found to play a significant role in enhancing muscle regeneration of aged mice (3, 4). Although, the mechanism for how the overexpression of ERR-γ combats these age-related declines is not currently understood, further investigation is needed so these imperishable benefits can be clinically implemented.

SIGNIFICANCE. ERR-γ is a nuclear receptor that can be targeted by current drug applications to alleviate age-related muscle decline.

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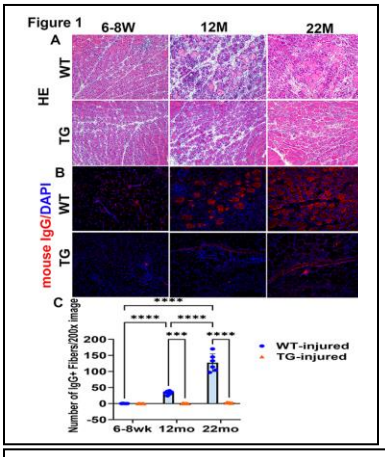

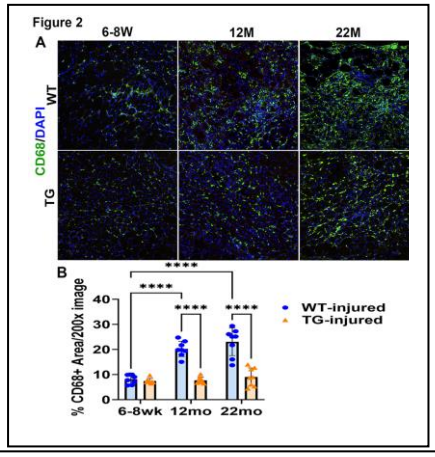

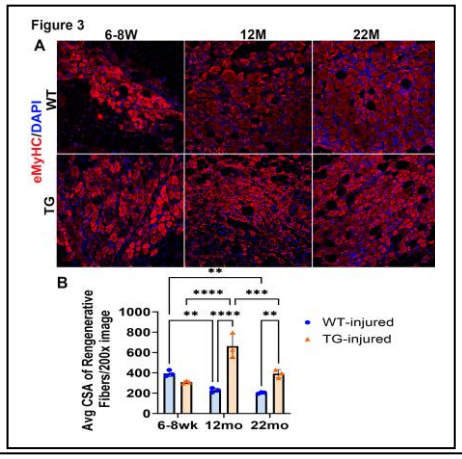

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