Knockout of DNA Repair Endonuclease ERCC1-XPF in Murine Pancreatic β-cells Affects Skeletal Muscle Regeneration

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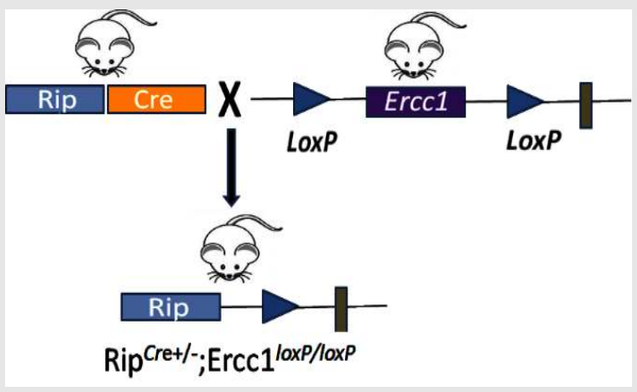
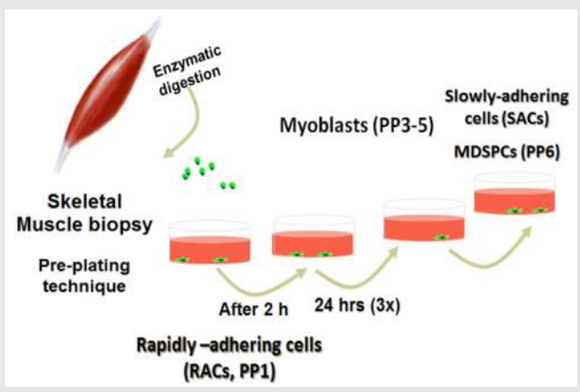
Objectives

Muscle aging is characterized by loss of mass and function, sarcopenia, and a decline in regenerative capacity due to functional impairment and reduced numbers of muscle progenitor cells [1-3]. Previously, we demonstrated that muscle-derived stem/progenitor cells (MDSPCs) isolated from the skeletal muscle of naturally-aged, wild-type (WT) mice and mice from a murine model of a human progeroid syndrome (called XFE progeroid syndrome caused by reduced expression of the DNA repair endonuclease, ERCC1-XPF, excision repair cross-complementing group 1) have a reduced ability to proliferate and differentiate, and impaired regenerative capacity compared to MDSPCs isolated from adult WT mice [4]. Here, we investigated whether age-related loss of MDSPC function is caused by cell non-autonomous mechanisms. Type 2 diabetes is an age-related disease and is associated with muscle wasting [**5**]. The goal of this study is to determine if deletion of *Ercc1* in pancreatic β-cells will affect the function of MDSPCs and muscle regeneration after injury.

Methods

Animals. Rip-KO mice were generated by crossing Rip-Cre$^{+/-}$;*Ercc1*$^{+/-}$ C57BL/6 mice with FVB/n *Ercc1*$^{+/flox}$ mice to create f1 Rip-Cre$^{+/-}$;*Ercc1*$^{-/flox}$. All mice were genotyped by TransnetYX (Cordova, TN) from an ear punch. ***Cell Isolation.*** MDSPCs were isolated from Rip-KO mice and littermate controls at 31-47 weeks of age via a modified pre-plate technique, as previously described [6].

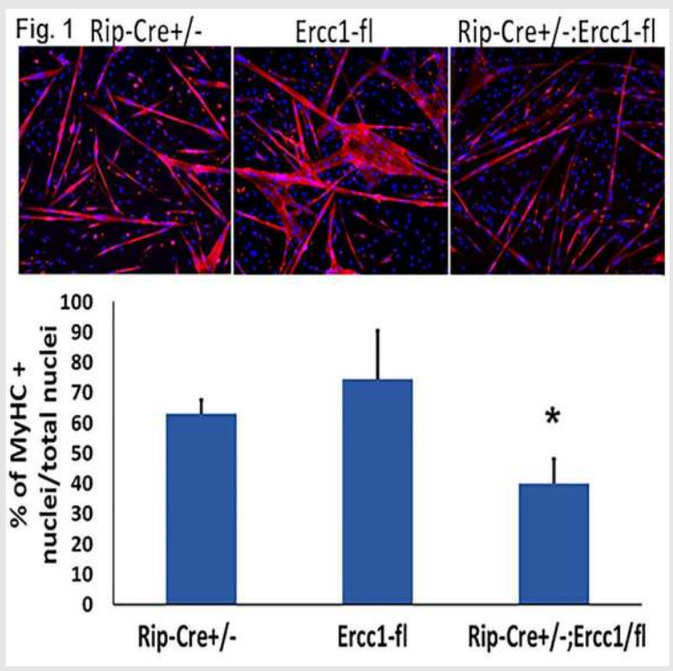




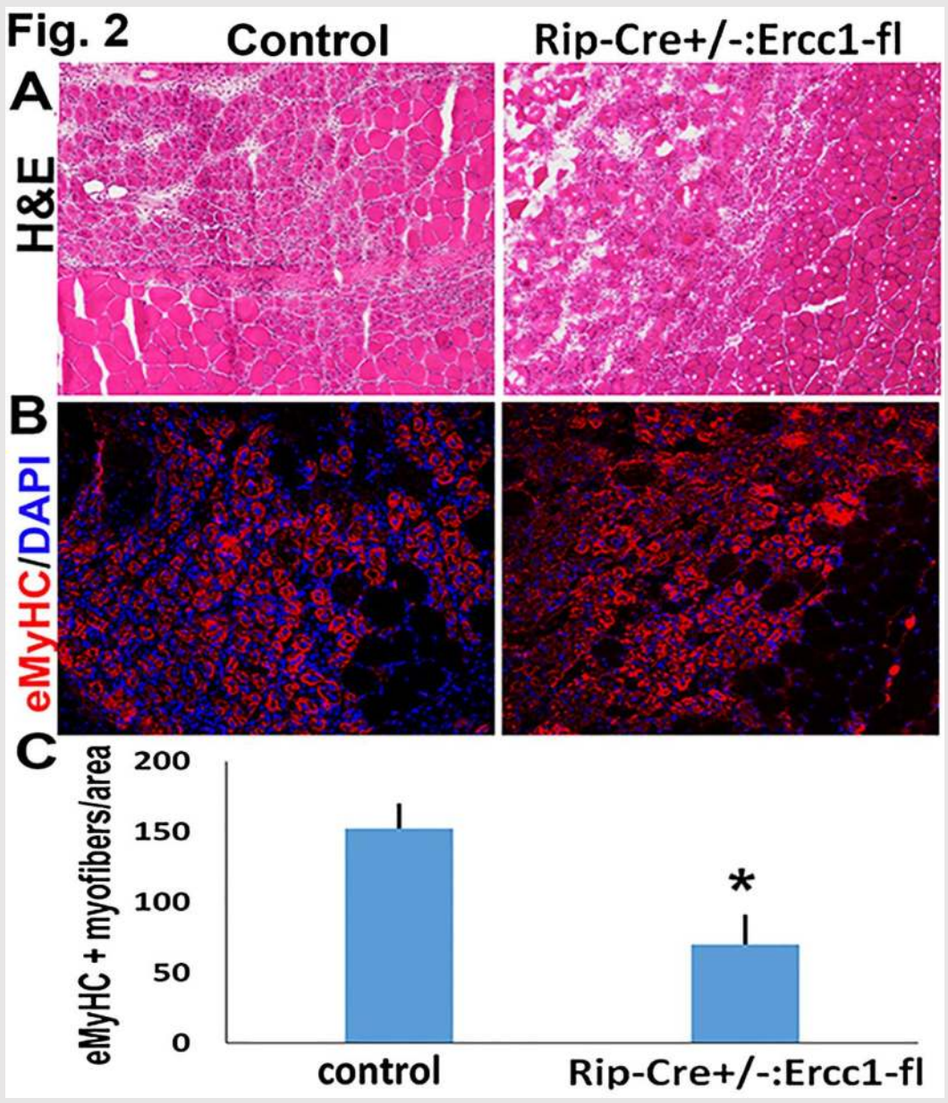
Myogenic differentiation assay. The myogenic differentiation capacity of the MDSPCs was assessed by switching the proliferation medium to fusion medium. After 3 days of culture, the cells were stained for fast myosin heavy chain (MyHCf), which is a marker of terminal myogenic differentiation. Myogenic differentiation levels were quantified as the percentage of nuclei in MyHC-positive myotubes relative to the total number of nuclei. ***Cardiotoxin (CTX) injury.*** 30ul of CTX (4 μM) was injected into one gastrocnemius muscle (GM) of each of 4 mice per group. Five days post-injury, the mice were sacrificed and the GMs were harvested, sectioned, and processed for staining with hematoxylin and eosin (H&E) stain, as well as antibodies specific for embryonic myosin heavy chain (eMyHC, newly generated myofibers). ***Staining for eMyHC.*** CTX-injured GM sections were stained for eMyHC using Mouse On Mouse (MOM) kits according to the manufacturer's protocol. Stained sections were visualized using a Nikon Eclipse Ni-E fluorescence microscope. Ten random images per slide were captured and the number of eMyHC+ myofibers were counted manually. ***Statistics.*** The Student's t-test was used to compare values between the groups, and $P<0.05$ was considered significant.

Results

MDSPCs isolated from Rip-KO mice exhibited a reduction in myogenic differentiation capacity compared to MDSPCs isolated from control mice. We observed that the MDSPC populations isolated from Rip-KO formed less multi-nucleated myotubes than control MDSPCs, which indicate that the degree of myogenic differentiation was significantly decreased in Rip-KO MDSPCs compared to the MDSPCs isolated from control mice ($P<0.05$, **Fig. 1**).



Knockout of Ercc1 in pancreatic β-cells affects skeletal muscle regeneration. CTX injury of GM was performed to examine the muscle regeneration potential of Rip-KO mice. H&E (**Fig. 2A**), and eMyHC (**Fig. 2B**) staining of GM sections revealed that there was significant decline in eMyHC+ newly generated myofibers in Rip-KO muscle ($P<0.05$, **Fig. 2C**), further validating that MDSPCs from Rip-KO mice were impaired in terms of muscle regeneration.



Discussion

Researchers are interested in identifying the causes of muscle progenitor cell dysfunction during the aging process, with the ultimate goal of rejuvenating old muscle progenitor cells and improving muscle function in elderly people. However, the mechanisms of age-associated stem cell loss and dysfunction have not been clearly defined. It has been reported that age-associated functional defects of muscle progenitor cells may reflect alterations in epigenetic and transcriptional programs [7]. Also, systemic circulating factors in serum of aged mice can suppress muscle stem cell proliferation and myogenic differentiation, as well as neurogenesis, as demonstrated by heterochronic parabiosis experiments [8]. The goal of this project was to determine if MDSPCs become dysfunctional with aging via a cell non-autonomous mechanism using a murine model of muscle-specific aging through targeted inactivation of *Ercc1,* a gene associated with human progeroid syndromes. We demonstrated that the loss of *Eccr1* specifically in pancreatic β-cells had an adverse effect on the function of MDSPCs *in vitro* and *in vivo*. In addition, the levels of blood glucose in the Rip-KO mice were elevated. Interestingly, it has been reported that muscle regeneration is impaired in diabetic mice [7]. It is not clear if impaired function of MDSPCs in Rip-KO mice is due to high levels of glucose or other circulating factors. Our future studies will continue to investigate the causes of muscle progenitor cell dysfunction in this Rip-KO model.

Significance

Identifying cell autonomous and non-autonomous mechanisms of aging-related stem cell decline will yield novel insight for developing strategies for maintaining stem cell function and tissue homeostasis during the aging process.

References

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