Adult muscle stem cells are morphologically heterogeneous in vivo with dynamically regulated cellular extensions

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Abstract

Muscle stem cells (MuSCs) are essential for tissue homeostasis and regeneration, but the potential contribution of MuSCs morphology to *in vivo* function remains unknown. Pax7 is a marker of adult MuSCs and we recently generated a Pax7EGFP reporter mouse to track MuSCs. Utilizing this model, we demonstrate that quiescent MuSCs in unperturbed muscles exhibit long protrusions that are morphologically heterogeneous. Adult MuSCs could be classified into three morphologically and functionally distinct subtypes, responsive, intermediate, and sensory. Upon injury, protrusion length was dynamically re-adjusted, with responsive cells responding first, followed by responses from sensory cells. As regeneration progressed and stem cell self-renewal returned, MuSC protrusions reappeared coincident with quiescence. The functional significance of our findings was reinforced by the aberrant regulation of MuSC protrusions in response to aging and dystrophy. Given the emerging interest of stem cell protrusions in other systems, our findings on the fundamental regulatory aspects of MuSC morphology may greatly inform the field.

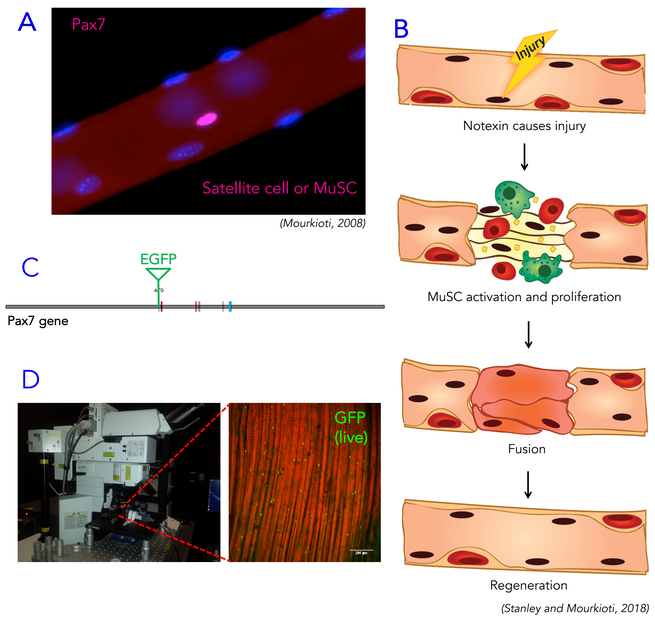
Introduction

FIGURE A: Muscle fibers consist of 2 different types of nuclei (stained by DAPI: blue) : myonuclei and muscle stem cells or satellite cells (stained by Pax7: pink). In an uninjured muscle, MuSCs typically are found on the sides of fibers between the basal lamina and the the sarcolemma and remain quiescent until injury of the muscle.

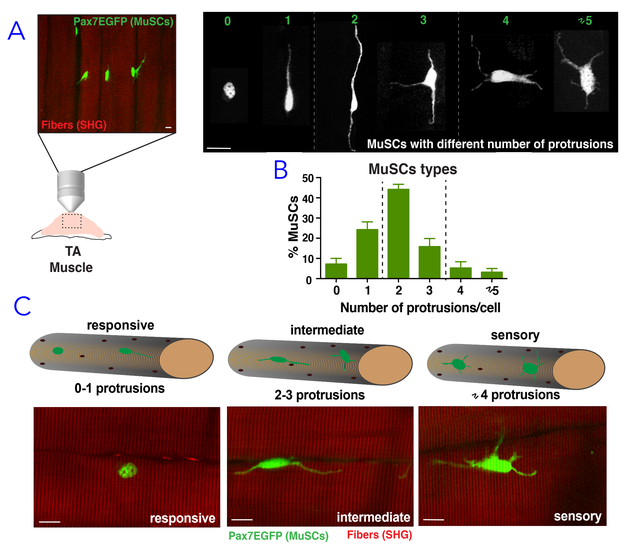
FIGURE B: Muscle stem cells are important in muscle regeneration following skeletal muscle injury. In experimental situations, the injury is usually introduced by notexin. This activates the muscle stem cells (red) and recruits macrophages (green) to clean up the tissue. The muscle stem cells proliferate and then fuse with the surrounding tissue, and the muscle stem cell is fully regenerated after a few days.

FIGURE C: A Pax7EGFP mouse was generated by inserting a cytoplasmic EGFP cassette at the ATG of the Pax7 gene the gene.

FIGURE D: Using 2-photon microscopy, a muscle of an anesthetized mouse was imaged to show live muscle stem cells (green) among muscle fibers (red).



Results (steady state)



Adult Muscle Stem Cells are a morphologically heterogeneous population in vivo.

FIGURE A: Schematic representation of Tibialis Anterior (TA) muscle imaging using the 2-photon microscopy. Muscle stem cells are green (EGFP) and sarcomeres are marked by second harmonic generation (SHG) in red. Scale bar: 10μm. Representative images of quiescent MuSCs (white) with 0, 1, 2, 3, 4 and ≥5 protrusions.

FIGURE B: Quantification of MuSCs with different number of protrusions show that the majority of cells have 2 protrusions, followed by cells with 1 and 3. n=3 mice (both legs, total 6 TA adult muscles), N>600 cells/muscle were analyzed.

FIGURE C: Schematic representation of the 3 different types of quiescent MuSCs: responsive, intermediate and sensory cells.

Under steady state conditions, protrusions are not used to mobilize MuSCs along or across the fiber over a period of 8 days.

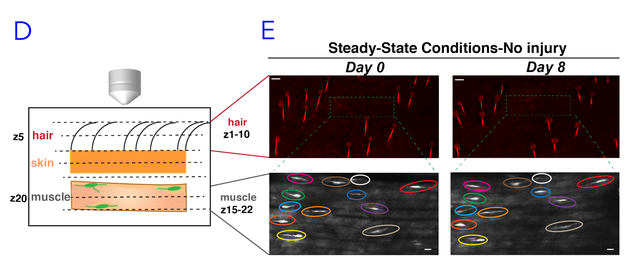
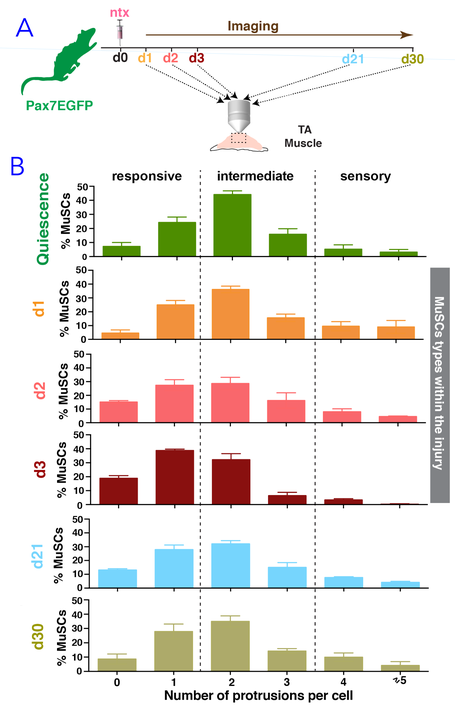


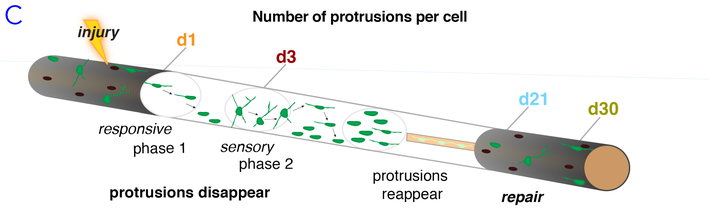
FIGURE D: Schematic representation of imaging from surface to deep tissue: hair follicles, then skin and below the skeletal muscle.

FIGURE E: At Day 0, the landmark of hair follicles marks the specific area (green rectangle) where the underlying muscle will be imaged. In the specific muscle area, different MuSCs are circled in different colors.

At Day 8, the same area as marked by the characteristic pattern of the hair follicles is located and imaged and the MuSCs are again circled in different colors. Upper panel scale bar: 10μm. Lower panel scale bar: 20μm

Results (Regeneration)





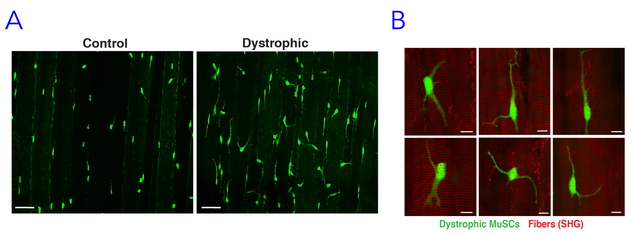
MuSC protrusions are dynamically regulated during muscle regeneration.

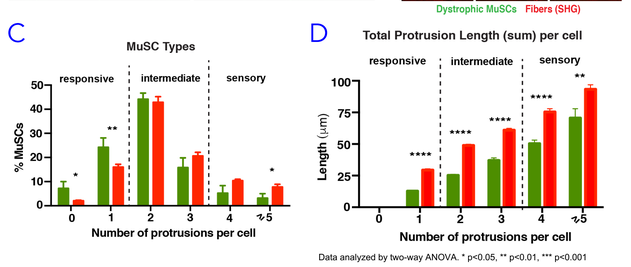
FIGURE A: Schematic representation of imaging the TA muscle at different time points (days) post-notexin injury using 2-photon microscopy.

FIGURE B: Distribution of different MuSC types pre-injury, and at different time-points post-injury. Distribution of MuSC types 2 days (d2) post-injury show a shift towards more responsive cells. Distribution of MuSC types 3 days (d3) post-injury show the disappearance of sensory cells and the increase shift towards more responsive cells. At 21 days post-injury (d21), there is a redistribution of MuSC types suggesting that protrusions reappear at later stages of regeneration. At day 30 (d30) the distribution of MuSC types have returned to pre-injury conditions.

FIGURE C: Proposed model of the behavior of sensory and responsive cells during regeneration. n=3 mice were analyzed and N>150 cells per animal for each time-point in B

Results (diseased)





MuSCs do not regulate properly their protrusions in muscular dystrophy.

FIGURE A: Representative maximum intensity projections of an uninjured Pax7EGFP (left) and dystrophic (mdx/Pax7EGFP) muscle (right) at 3 months old. Note the increased number and complexity of protrusions in the dystrophic compared to uninjured muscles. Pax7EGFP MuSCs are shown in green. Scale bar: 50μm.

FIGURE B: Representative images of dystrophic MuSCs show the complexity of the protrusions in these cells. Note the aberrant fibers shown by SHG imaging. Scale bars: 10μm.

FIGURE C: Reduced number of responsive cells and increased number of sensory cells in dystrophic muscles.

FIGURE D: Increased protrusion length in all types of dystrophic MuSCs.

Discussion

- Using the Pax7EGFP mouse we visualized for the first time adult quiescent MuSCs in their natural environment in skeletal muscles
- MuSCs are morphologically heterogeneous and exhibit axon-like protrusions
- In uninjured conditions, protrusions do not mobilize MuSCs but instead they extend along and across the fiber to "sense" their environment
- MuSCs' protrusions are extremely sensitive and their length alters in response to local injuries
- During regeneration, protrusions are dynamically regulated
  - Responsive cells are the first to be activated upon injury, while sensory cells are activated later on and transition towards responsive
- Dystrophic MuSCs fail to adequately regulate protrusions

Acknowledgments

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References

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