Regenerative Index Quantitation Reveals a Reduced Regenerative Potential in Duchenne Muscular Dystrophy From 7-11 Years of Age

POSTER 282-T

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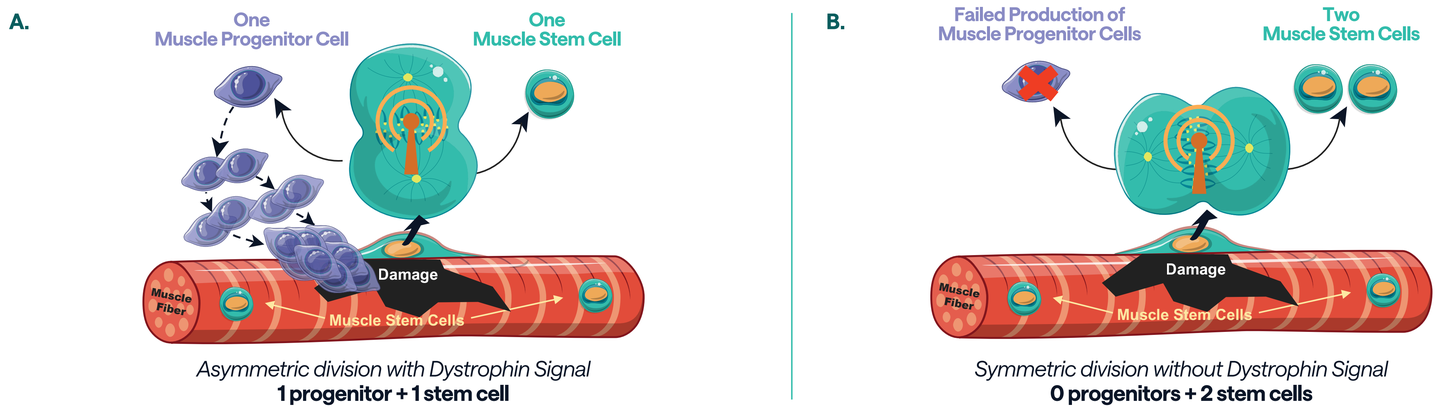
BACKGROUND

- In healthy skeletal muscle with normal dystrophin expression, stem cells respond to damage by dividing in a balanced way – some become new muscle progenitor cells to repair damaged tissue and regenerate muscle fibers, while other remain as stem cells to support future repair (**Figure 1a**; Kuang et al. Cell 2007, 129(5): 999-1010).
- Previous studies characterizing the trajectory of DMD myopathology highlight a lack of muscle regeneration (Cardone et al. Acta Neuropathol Commun 2023, 11(1): 167; Abdel-Salam et al. Acta Myol 2009, 28(3): 94-100).
- Intrinsic deficits from the lack of dystrophin in muscle stem cells has been shown to be the cause, leading to reduced polarity, loss of asymmetric divisions, and failure to maintain a pool of progenitor cells adequate to facilitate muscle regeneration (**Figure 1b**; Dumont et al. Nat Med 2015, 21(12): 1455-1463).
- An orally-administered small molecule (SAT-3247) to rescue stem cell polarity and restore muscle regeneration is currently under evaluation in two clinical trials in non-ambulatory adults (NCT06867107) and ambulatory children (NCT07287189) living with DMD, and is being explored in other neuromuscular disorders (MDA Presentation #399-O).
- Because the regenerative process is closely linked to the cumulative severity of muscle damage, which is variably distributed within and between muscle groups, accurately quantifying muscle regeneration has remained a significant challenge.
- Here, we describe development and validation of a Regeneration Index (RI), which is a methodology to objectively assess and quantitate muscle regenerative capacity, and to monitor the effectiveness of potential muscle regenerating therapies in DMD and other disorders.

Figure 1: Asymmetric stem cell division and progenitor formation in healthy skeletal muscle (A) and symmetric division and impaired progenitor formation in DMD (B)



METHODS & RESULTS

Methods

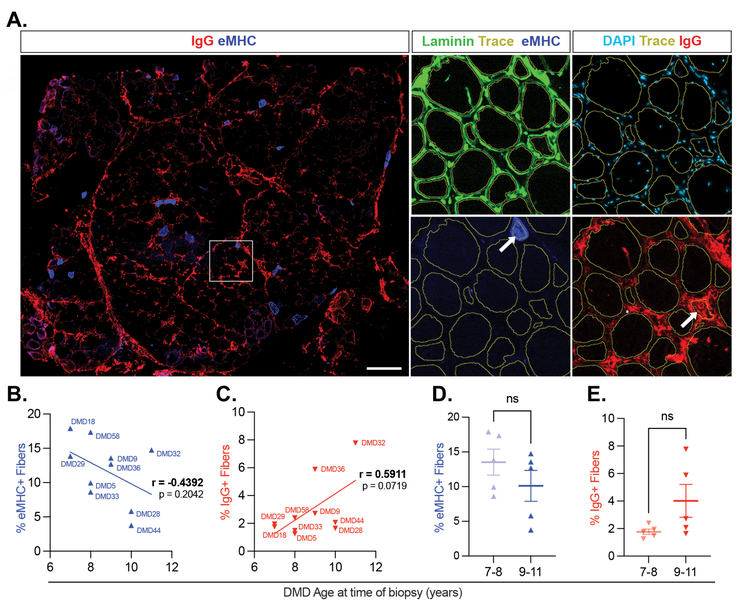
- The research described here represents a collaborative effort between Satellos Bioscience, the Ottawa Hospital Research Institute, and the University of Iowa Department of Pathology.
- Detailed methodology for provision of muscle biopsy samples, immunofluorescence protocols, morphometric analyses, and determination of RI in DMD and non-DMD biopsy samples are described elsewhere (bioRxiv 2026.01.05.697715; QR code for access provided below).

Determination of RI

- While detection of embryonic myosin heavy chains (eMHC) has long been used to identify regeneration of newly forming myofibers, evaluation of regeneration alone does not provide a complete representation as it comes in response to myonecrosis.
- Further, myonecrosis can be quantified by the immunodetection of immunoglobulin G (IgG) infiltration. However, variability within and between muscles limits the utility of any determination of the rate of regeneration.
- To address these issues, regeneration of myofibers was normalized to myonecrosis to reduce this variability and assess net regenerative potential of muscle:

Regenerative Index = newly forming eMHC⁺ fibers ÷ necrotic IgG⁺ fibers

Figure 2: Decreased regeneration and increased degeneration in DMD muscle. Representative immunofluorescence images of DMD muscle biopsies stained for immunoglobulin G (IgG) and embryonic myosin heavy chains (eMHC), laminin, DNA by DAPI, and automated outlining of individual fibers in yellow (A). The presence or absence of either eMHC or IgG is denoted by white arrows. Correlations between percent of eMHC⁺ (B) and IgG⁺ fibers (C) and age, and direct comparisons in percent eMHC⁺ and IgG⁺ in muscle biopsy samples from younger (7-8 years) and older (9-11 years) (D and E, respectively).



Results

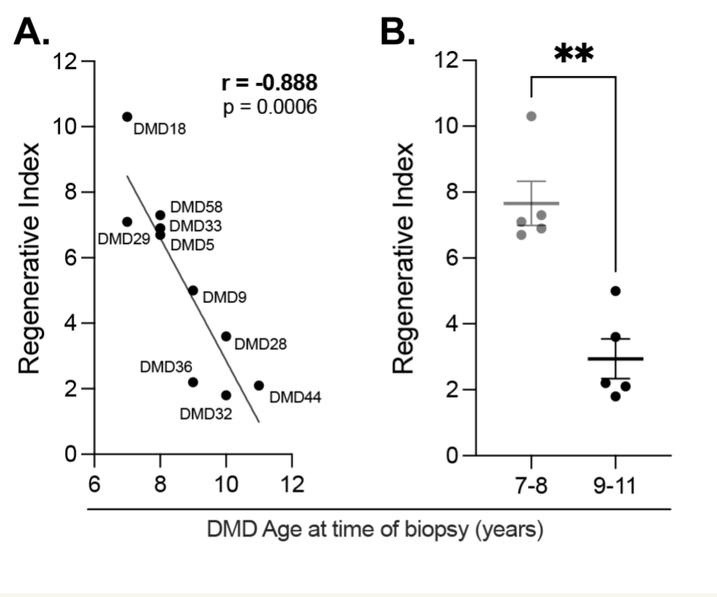
Histological Analysis of DMD Progression and Morphologic Differences vs Healthy Controls

- Standard markers of DMD disease progression in muscle biopsy samples, including fiber cross-sectional area (CSA), mean fiber Feret (MFF), and circularity were assessed, as well as the number of Pax7-expressing satellite cells.
- Muscle biopsies from DMD boys displayed significantly elevated average CSA and MFF compared to age-matched healthy controls.
- A trend toward increasing Feret's diameter, CSA, and circularity was observed with increasing age (7-8 vs. 9-11 years old) in DMD boys.
- A significant increase in the number of Pax7-expressing satellite cells was observed in DMD biopsies relative to healthy controls, along with a trend for an increased number of Pax7-expressing cells with age in DMD samples.

Determination and Analysis of RI

- Evaluation of regenerating (eMHC⁺) and necrotic (IgG⁺) myofibers via immunofluorescence revealed age-related trends in muscle regeneration and degeneration (**Figure 2**). The percent of eMHC⁺ fibers showed a significant negative correlation with age whereas the percent of IgG⁺ fibers showed a trend for a positive correlation with age. No significant differences in the percentages of eMHC⁺ and IgG⁺ myofibers were observed between biopsy samples from younger (7-8 years) and older (9-11 years) DMD boys.
- In contrast, determination of RI revealed a strong inverse relationship with age in DMD boys (**Figure 3a**), and direct comparison revealed that younger (7-8 years) boys had a markedly higher RI than older (9-11 years) boys (**Figure 3b**).

Figure 3: Regeneration Index is sharply decreased with age in DMD boys. A strong negative correlation between RI (ratio of newly-forming eMHC⁺ to necrotic IgG⁺ myofibers) and increasing age was observed (A). Direct comparison of RI in muscle biopsies obtained from younger (7-8 years) and older (9-11 years) DMD boys (B).



CONCLUSIONS & NEXT STEPS

- Because the regenerative process of muscle is closely linked to the cumulative severity of damage, which is variably distributed within and between muscle groups, accurately quantifying regeneration has remained a significant challenge. The currently described RI approach, which normalizes regeneration to myonecrosis, was developed to reduce this variability and quantitate the net regenerative potential of muscle.
- A sharp decline and significant inverse correlation in RI with increasing age in DMD boys aged 7-11 years, even in the presence of relatively stable myofiber morphology and satellite pool, underscores that muscle's ability to regenerate relative to myonecrosis deteriorates very rapidly in DMD, which is due in part to a previously described loss of satellite cell polarity in the absence of dystrophin.
- Further, the age-associated RI changes described here reinforce the potential of the RI as a quantitative measure that accurately reflects ongoing regenerative activity in DMD. Taken together with results previously shown in a canine DMD model (QR code provided for access), the RI provides a robust means of evaluating the effectiveness of novel therapies aimed at improving muscle regeneration.
- Future longitudinal studies that correlate RI with functional outcomes in DMD, examining RI in other muscle diseases and age groups, as well as integrating transcriptomic and metabolic profiling will be important to further validate and refine the use of RI in research and clinical settings.

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