Secreted ADAMTS-like 2 Promotes Muscle Regeneration After Injury

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Disclosures: None.

INTRODUCTION: Skeletal muscle (micro-) injuries due to daily activities or physical exercise regenerate efficiently. Endogenous muscle regeneration involves multiple cell types, including muscle stem cells (satellite cells), which differentiate into myoblasts that fuse to form contractile myofibers, and fibroadipogenic progenitors (FAPs), which regenerate the extracellular matrix (ECM) and restore muscle architecture. Endogenous muscle regeneration, however, is unable to restore or maintain muscle function after traumatic or surgical volumetric muscle loss or in muscular dystrophies, respectively. Therefore, there is a clear need to identify novel players that regulate muscle regeneration and that can potentially be harnessed as therapeutics to promote muscle regeneration after traumatic muscle loss or prevent muscle regeneration in muscular dystrophies. ADAMTS-like 2 (ADAMTS-L2) is a secreted regulatory ECM protein that negatively regulates TGFβ signaling in fibroblasts. In skeletal muscle, we recently identified a surprising pro-myogenic role for ADAMTS-L2, where it promoted myoblast differentiation and myotube/myofiber formation in a WNT-dependent manner. Based on its pro-myogenic role, we hypothesize that ADAMTS-L2 is required for muscle regeneration after injury. The objectives of this study are to determine if ADAMTS-L2 deficiency impairs muscle regeneration, and to demonstrate that ADAMTS-L2 can promote muscle regeneration after injury, paving the way for potential therapeutic development of ADAMTS-L2 peptides for clinical applications.

METHODS: Acute muscle injury was induced by barium chloride injection in the tibialis anterior (TA) muscle of wild type mice or mice, where ADAMTS-L2 was deleted in myogenic progenitor cells using MyoD-Cre. Barium chloride injection results in the rupture of myofibers due to hypercontraction while leaving muscle resident cell types largely intact. We followed muscle regeneration for 21 days by histomorphometry and quantified ADAMTS-L2 expression by real-time PCR. We also analyzed the cellular composition of regenerated wild type and ADAMTS-L2-deficient TA muscle by co-immunostaining for muscle stem cells (PAX7), myoblasts (MYOD), and proliferating cells (Ki67). This approach allows to distinguish different activation, differentiation, and commitment states of muscle stem cells and myoblasts. To test, if ADAMTS-L2 promotes muscle regeneration, we injected a myogenic ADAMTS-L2 peptide or vehicle in the TA after barium chloride injury and assessed regeneration at 7 days post injury (7 dpi) by histomorphometry. For statistical analyses, we compared independent samples with a Student’s t-test and 3 or more samples with a one-way ANOVA followed by a posthoc Tukey test. A p-value of <0.05 was considered statistically significant. Mouse experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Icahn School of Medicine at Mount Sinai.

RESULTS SECTION: ADAMTS-L2 mRNA was significantly induced at 4 and 14 dpi in the TA of wild-type mice, suggesting a role for ADAMTS-L2 during muscle regeneration (n=3). ADAMTS-L2 was also upregulated in muscles from 8-week old Duchenne muscular dystrophy mice (D2-mdx) (n=4, p<0.05). In regenerating ADAMTS-L2-deficient TA, we observed persistent embryonic myosin heavy chain (eMyHC) immunostaining, a transient maker of regenerating myofibers, and decreased myofiber cross-sectional area (CSA) at 7 and 14 dpi, respectively (n=5, p<0.05). Both parameters indicate delayed TA regeneration in the absence of ADAMTS-L2. Delayed regeneration was attributed to impaired differentiation of primary myogenic ADAMTS-L2-deficient myoblasts isolated from control and ADAMTS-L2-deficient extensor digitorum longus (EDL) muscles (n=3). Delayed differentiation also correlated with reduced canonical Wnt signaling indicated by reduced total β-catenin levels at day 2 after initiation of differentiation. When comparing myogenic cell populations, we noted a significantly lower number of proliferating cells (MyoD+/Ki67+) and committed myoblasts (MyoD+/Ki67-) in regenerating ADAMTS-L2-deficient TA muscle at 7 and 14 dpi (n=3 sections per condition). In addition, the number of proliferative and committed myoblasts (MyoD+/Ki67+) was significantly higher in the mutant animals, consistent with delayed muscle regeneration in the absence of ADAMTS-L2. ADAMTS-L2 may be required for myoblasts to exit the cell cycle and initiate differentiation. To test if ADAMTS-L2 promotes muscle regeneration after injury, we first injected 20 mg/kg of a recombinant C-terminally Myc-tagged ADAMTS-L2 peptide (TSR2-7), which contains the pro-myogenic ADAMTS-L2 domains, into wild type TA and determined its turnover (Fig. A, B). 2 days after injection, a robust signal for recombinant TSR2-7 (α-Myc immunostaining) was detected in TA cross-sections (Fig. C). 3 days after injection, signal intensity for TSR2-7 was substantially reduced (not shown). Based on this pilot study, we injected 20 mg/kg of the TSR2-7 or PBS in ADAMTS-L2-deficient muscle starting at 1 dpi followed by additional injections every other day for a total of three injections. TA muscles were harvested and analyzed at 7 dpi. In the ADAMTS-L2 peptide-injected injured TA muscles, we observed increased myofiber CSA, increased eMyHC fluorescence intensity and improved myofiber boundaries indicated by the integrity of the laminin-positive basal lamina (Fig. D-F, n=4, p<0.01). These changes are consistent with accelerated muscle regeneration in the presence of the ADAMTS-L2 peptide.

DISCUSSION: Collectively, our data support the hypothesis that ADAMTS-L2 promotes skeletal muscle regeneration after acute injury. We previously showed that ADAMTS-L2 promoted myoblast differentiation, which requires cell cycle exit. ADAMTS-L2 induction after injury, delayed regeneration in ADAMTS-L2-deficient TA muscle, and changes in the activation and differentiation state of myogenic progenitor cells are consistent with a role for ADAMTS-L2 in promoting differentiation of myoblasts. Our data also suggest that a myogenic ADAMTS-L2 peptide can promote muscle regeneration after injury and improve key indicators of efficient muscle regeneration. Hence, ADAMTS-L2 could become a part of skeletal muscle engineering approaches to improve the efficiency of myoblast to myotube differentiation. Since ADAMTS-L2 is also expressed in FAPs, an open question currently under investigation is, if ADAMTS-L2 from FAPs contributes to muscle regeneration and if there is a cross-talk between the two pools of ADAMTS-L2 in muscle.

SIGNIFICANCE/CLINICAL RELEVANCE: Inefficient regeneration of muscle function after traumatic muscle loss or in muscular dystrophies is a significant clinical problems with no satisfying therapies available. Identifying ECM proteins, such as ADAMTS-L2, as potential therapeutics to promote muscle regeneration after injury or to augment muscle regeneration in muscular dystrophies after the primary defect had been corrected, has tremendous translational potential for clinical and skeletal muscle engineering applications.

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ACKNOWLEDGEMENTS: Funding sources: NIH/NIAMS, Leni & Peter W. May Department of Orthopedics, Icahn school of Medicine at Mount Sinai, Ines Mandl Research Foundation.

ORS 2024 Annual Meeting Paper No. 1187