Elevated FGF-21 Modulates Skeletal Muscle Fibrosis and Regeneration via Intercellular Interactions in Duchenne Muscular Dystrophy

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INTRODUCTION: Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy seen in children and is characterized by progressive degeneration of skeletal muscle and bone [1]. We previously identified FGF-21, a novel stress-induced hormone, as ectopically upregulated in dystrophic muscle in both DMD mouse models and patients [2], and demonstrated that elevated FGF-21 negatively affects bone homeostasis [3]. We further showed that satellite cells (SCs) are the major source of FGF-21 production in dystrophic muscle (2023 ORS Meeting). However, whether elevated FGF-21 contributes directly to skeletal muscle pathology remains unclear. To address this gap, we investigated the effects of FGF-21 on skeletal muscle using two complementary approaches: (1) an mdx FGF-21 overexpression (OE) model, (2) a global FGF-21 knockout mdx model. We hypothesized that FGF-21 modulates intercellular communication within dystrophic skeletal muscle, leading to impaired regeneration and increased fibrosis.

MATERIALS & METHODS: FGF-21 OE mdx mice (FGF21-Tg-mdx) were generated by crossbreeding mdx mice (Dys -/-) with ApoE promoter-driven FGF-21 transgenic mice. Global FGF-21 KO mdx mice were generated by crossbreeding mdx mice with FGF-21 loxP and Rosa-CreER mice, followed by tamoxifen induction at week 4. Male FGF21-Tg-mdx, global FGF-21 KO-mdx and their corresponding littermate controls were sacrificed at 16 weeks ($n \geq 6$ in each group). Serum and tissue FGF-21 levels were quantified. Skeletal muscle functions were assessed by forelimb grip strength and ex vivo isometric contraction of extensor digitorum longus (EDL) muscle. TA and diaphragm muscle tissues were harvested and evaluated for fibrosis via picrosirius red (PSR) staining and for regeneration via Pax7/Ki-67 co-immunostaining, central nucleation, and myofiber size distribution. To identify FGF-21 responding cell populations, KLB reporter mice (KLB-Cre; Ai14-tdtomato) were used to localize beta-Klotho (KLB)-expression in WT and mdx muscle and co-stained with PDGFRα (FAPs), F4/80 (macrophages), and perilipin-2 (adipocytes). FAPs were isolated from dystrophic muscles and treated with recombinant FGF-21 to assess transcriptional responses via RNA-seq. Statistical analyses used unpaired t-tests or ANOVA with appropriate post-hoc testing (α=0.05). All animal experiments were approved by IACUC.

RESULTS: FGF-21 OE mdx mice exhibited significantly increased fibrosis in TA and diaphragm, while global FGF-21 KO mdx mice demonstrated reduced fibrosis in both muscles, indicating a dose-dependent relationship between FGF-21 signaling and fibrotic remodeling (Fig. 1). FGF-21 OE mdx mice demonstrated reduced regenerative capacity, with decreased Pax7+/Ki-67+ satellite cell (SC) proliferation and lower central nucleation percentages compared with mdx controls (Fig. 2). KLB reporter analysis revealed robust KLB expression in dystrophic, but not WT, muscle. Expression localized primarily to PDGFRα+ fibro-adipogenic progenitors (FAPs), with additional signal in F4/80+ infiltrating macrophages and occasional perilipin-2+ adipocytes (Fig. 3A). These results suggest that multiple stromal populations are FGF-21-responsive targets. FGF-21-treated FAPs showed transcriptional changes associated with extracellular matrix remodeling and cytokine signaling, consistent with enhanced fibrosis (Fig. 3B). Collectively, these data support FGF-21 as a key modulator of stromal-myogenic interactions driving dystrophic muscle deterioration.

DISCUSSION: Elevated FGF-21 exacerbates dystrophic muscle pathology by promoting fibrosis and impairing regeneration, likely through SC-FAP-macrophage crosstalk. Conversely, loss of FGF-21 mitigates fibrosis, supporting its role as a potential regulator of extracellular matrix remodeling in DMD. These findings broaden our understanding of FGF-21 as a pathological mediator across musculoskeletal tissues and highlight its potential as a therapeutic target to improve bone health and to attenuate fibrosis and improve muscle regeneration in DMD. Future work will focus on defining the molecular mechanisms of FGF-21's action on FAPs and advancing FGF-21 targeting strategies.

SIGNIFICANCE: This work identifies FGF-21 as a key regulator of pathological intercellular signaling in dystrophic muscle. Targeting FGF-21 may represent a promising therapeutic strategy to reduce fibrosis, enhance regeneration, and preserve muscle function in DMD.

REFERENCES: [1] Frontiers in Physiology. 2021; PMID: 33762965. [2] Muscle & Nerve. 2018; PMID:30028902. [3]. Journal of Bone and Mineral Research. 2020; PMID: 31800971.

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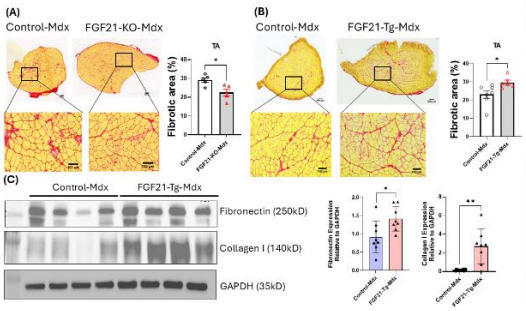

Fig.1: FGF-21 modulates skeletal muscle fibrosis in DMD. (A) PSR staining and fibrotic area quantification for Control-Mdx TA versus FGF-21-KO-Mdx TA. **(B)** PSR staining and fibrotic area quantification for Mdx-control TA versus FGF-21-Tg-Mdx TA. **(C)** Fibrotic marker western blot and quantification for Control-Mdx TA versus FGF-21-Tg-Mdx TA, normalized to GAPDH.

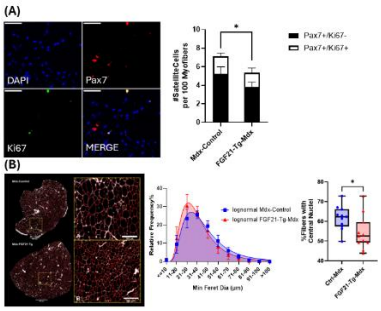

Fig.2: FGF-21 modulates skeletal muscle regeneration in DMD. (A) Pax7/Ki67 co-immunostaining and quantification of the number of quiescent and activated satellite cells per 100 myofibers in Control-Mdx TA versus FGF21-Tg-Mdx TA. **(B)** WGA lectin staining for myofiber size distribution and central nucleation analysis of Control-Mdx TA versus FGF21-Tg-Mdx TA.

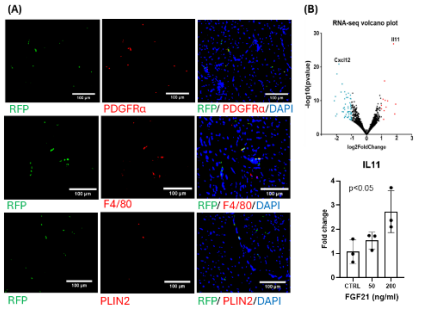

Fig. 3: FGF-21 may signal through multiple stromal cell populations in skeletal muscle in DMD. (A) Mdx-KLB reporter TA co-staining for RFP (green), PDGFRα, F4/80, and PLIN2 (red), and DAPI (blue). **(B)** RNA-seq volcano plot of FAP cells treated with FGF-21 & qRT-PCR validation of IL11 upregulation.