HIF2 Deficiency Reprograms FAP Fate and Limits Fibro-Adipogenic Differentiation During Rotator Cuff Muscle Injury

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INTRODUCTION: Rotator cuff (RC) tears represent one of the most common and debilitating musculoskeletal injuries and are frequently accompanied by progressive muscle atrophy and fatty degeneration [1]. Although these degenerations critically influence tendon repair outcomes, the underlying mechanisms driving rotator cuff muscle fatty infiltration remain poorly defined. Recent evidence has identified muscle fibro/adipogenic progenitors (FAPs) as the cellular source of intramuscular fatty infiltration [2]. Our previous work revealed localized hypoxia and altered expression of hypoxia-inducible factors (HIFs) in RC muscles following massive tendon tears [3], implicating hypoxic signaling in the role of RC muscle degeneration. Among HIF family members, HIF2 has been reported to regulate stem cell fate decisions; however, its role in muscle FAP differentiation has not been established. Thus, in this study, we aimed to define the role of HIF2 in regulating FAP differentiation and fatty infiltration after RC tears. We hypothesize that HIF2 functions as a central regulator of FAP lineage commitment, such that its loss suppresses fibro- and adipogenic differentiation while promoting regenerative capacity in rotator cuff muscle.

METHODS: FAP-specific HIF-2 knockout (HIF2 -/-) mice were generated by crossing Prrx1-Cre (JAX #005584) mice with HIF-2α floxed (JAX #008407) mice. For in vivo studies, 10-month-old Prrx1-Cre^{-}/HIF2a$^{lox/lox}$ (WT) and Prrx1-Cre^{+}/HIF2a$^{lox/lox}$ (HIF2 -/-) mice underwent combined unilateral supraspinatus (SS) tendon transection and suprascapular nerve transection (TT + DN) (n=4 per group). Six weeks post-injury, mice were sacrificed, and bilateral SS muscles were harvested, fresh-frozen, and cryosectioned at 10 µm for histological and immunohistochemical analysis. Fibrosis was quantified by Masson's Trichrome staining and collagen type Iα1 immunostaining, while fat infiltration was assessed using BODIPY and Perilipin-1 staining. All animal procedures were approved by our Institutional Animal Care and Use Committee (IACUC). For in vitro differentiation experiment, FAPs were isolated from additional WT and HIF 2 -/- mice (n=3 per group) by fluorescence-activated cell sorting (FACS) using the markers CD45^{-}/CD31^{-}/Sca1^{+}/PDGFRα^{+}. Cells were plated on ECM-coated dishes and maintained in growth medium [Ham's F-10 supplemented with 20% FBS and 5 ng/mL FGF2]. For fibrogenic differentiation, FAPs were cultured in DMEM containing 2% FBS and 10 ng/mL TGF-β1 for 4 days. Adipogenic differentiation was induced using Gibco's StemPro Adipogenesis Differentiation Kit in DMEM for 5 days. For transcriptome profiling, single-cell RNA sequencing (scRNA-seq) was performed using the 10x Chromium platform and sequenced on an Illumina NovaSeq. (10x Chromium platform). To improve yield, FAPs from three mice were pooled per strain for sequencing. All experiments were performed using both male and female mice.

RESULTS: FAP differentiation analysis showed a significant decrease in α-SMA intensity per nucleus under fibrogenic conditions (Fig. 1A) and a lower percentage of Perilipin-1+ cells under adipogenic conditions (Fig. 1B) in HIF2 -/- FAPs compared to WT FAPs (Fig. 1C), suggesting that HIF-2 promotes both fibrogenic and adipogenic lineage commitment of FAPs. In in vivo experiment, histological and immunofluorescence analyses revealed that the supraspinatus muscle from HIF2 -/- mice exhibited smaller fibrotic regions on Masson's Trichrome staining and reduced collagen I staining compared to WT mice at 6 weeks after rotator cuff tears (Fig. 2A, B). Similarly, BODIPY+ lipid area and Perilipin-1+ area were lower in HIF2 -/- mice (Fig. 2A, C) compared to WT mice, indicating attenuated fat infiltration in HIF2 -/- mice. To investigate transcriptome alteration in FAP with the loss of HIF2, single-cell RNA sequencing was performed on FAPs isolated from WT and HIF2-/- mice. UMAP clustering identified multiple transcriptionally distinct FAP subtypes, including canonical/quiescent, homeostatic-niche, stress-responsive, tendon-like, perivascular-like, adipogenic-primed, and myofibroblast populations (Fig. 3A, C). HIF2-/- mice showed a clear shift in FAP composition, with a significant reduction of canonical/quiescent FAPs and an increased proportion of homeostatic-niche, stress-responsive, immune-modulatory, and perivascular-like subsets compared with WT (Fig. 3B, D). Interestingly, although HIF HIF2 -/- FAPs contained a higher proportion of these active subsets, the expression levels of differentiation markers, Acta2 and Col1a1 (myofibroblast markers) and Apod and Fabp4 (adipogenic markers) were lower compared to WT (Fig. 3D). These findings indicate that HIF2 loss compromises the stemness of FAPs while constraining their terminal fibro- and adipogenic differentiation.

DISCUSSION: This study identifies HIF2 as a key regulator of FAP fate during muscle regeneration. Loss of HIF2 reduced both fibrogenic and adipogenic differentiation of FAPs and dampened fibro-fatty remodeling after rotator cuff injury. Transcriptomic profiling further revealed that HIF2 profoundly influences FAP differentiation, suggesting its essential role in maintaining the dynamic equilibrium between quiescent and activated FAP states. Collectively, these results identify HIF2 as a key transcriptional regulator coupling FAP state heterogeneity to fibro-fatty remodeling after rotator cuff injury, and suggest that targeting HIF2 may offer a potential strategy to limit fibrosis and fatty infiltration while promoting more effective muscle repair.

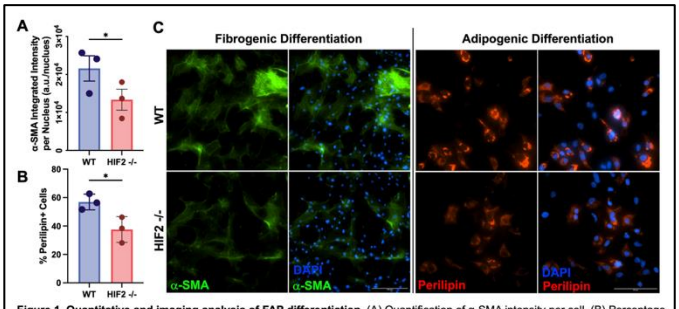

Figure 1. Quantitative and imaging analysis of FAP differentiation. (A) Quantification of α-SMA intensity per cell. (B) Percentage of perilipin-positive cells. (C) Confocal images showing fibrogenic and adipogenic differentiation of primary FAPs. α-SMA (green), perilipin-1 (red; lipid droplets), and nuclei (blue). Data are presented as mean ± SEM (n = 3 per group). Statistical significance was determined by unpaired two-tailed Student's t-test. P < 0.05. Scale bars: 200 µm (fibrogenic assay) and 50 µm (adipogenic assay).

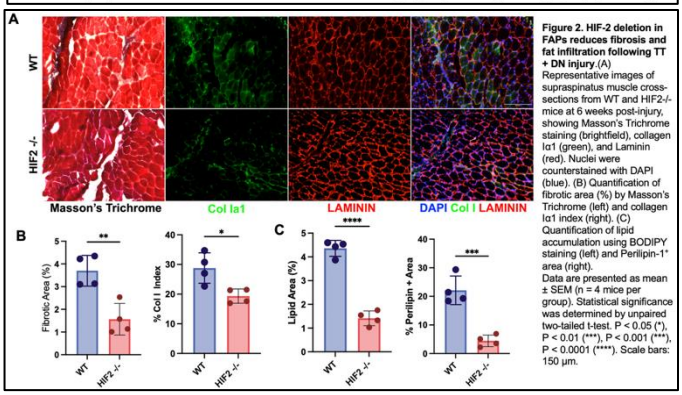

Figure 2. HIF-2 deletion in FAPs reduces fibrosis and fat infiltration following TT + DN injury. (A) Representative images of supraspinatus muscle cross-sections from WT and HIF2-/- mice at 6 weeks post-injury, showing Masson's Trichrome staining (brightfield), collagen Iα1 (green), and Laminin (red). Nuclei were counterstained with DAPI (blue). (B) Quantification of fibrotic area (%) by Masson's Trichrome (left) and collagen Iα1 index (right). (C) Quantification of lipid accumulation using BODIPY staining (left) and Perilipin-1+ area (right). Data are presented as mean ± SEM (n = 4 mice per group). Statistical significance was determined by unpaired two-tailed t-test. P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), P < 0.0001 (****). Scale bars: 150 µm.

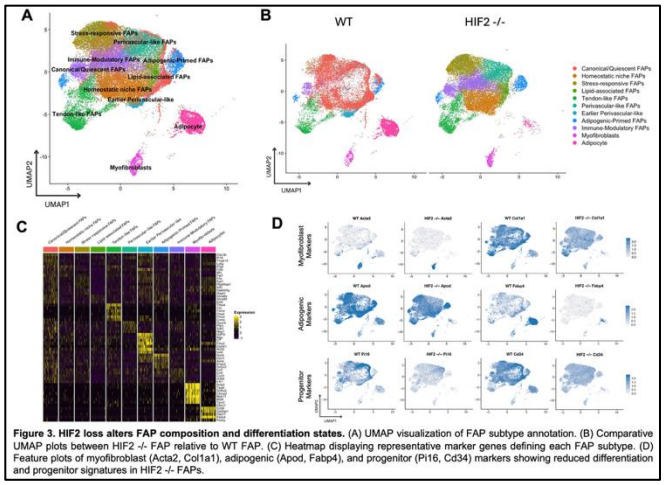

Figure 3. HIF2 loss alters FAP composition and differentiation states. (A) UMAP visualization of FAP subtype annotation. (B) Comparative UMAP plots between HIF2 -/- FAP relative to WT FAP. (C) Heatmap displaying representative marker genes defining each FAP subtype. (D) Feature plots of myofibroblast (Acta2, Col1a1), adipogenic (Apod, Fabp4), and progenitor (Pl16, Cd34) markers showing reduced differentiation and progenitor signatures in HIF2 -/- FAPs.

SIGNIFICANCE/CLINICAL RELEVANCE: HIF2 is a critical regulator of FAPs dynamics, highlighting its potential as a therapeutic target to preserve muscle integrity and promote regeneration after rotator cuff tears.

REFERENCES: [1] Bedi, et, al. Nat Rev Dis Primers. 2024 Feb 8;10(1). [2] Liu, et, al.Muscles Ligaments Tendons J. 2016 May 19;6(1):6-15. [3] Zhang, et. al., ORS 2021. Paper#1340.

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