A Subset of FAP Cells Expressing Gli1 Promote Muscle Regeneration With Less Fat Accumulation

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Disclosures

None

Introduction

Skeletal muscle has a remarkable capacity for regeneration after injury. Recently, a new type of muscle-resident progenitor cell, referred to as fibro-adipogenic progenitors (FAPs), was identified to be critical in supporting the process of injured muscle regeneration. To date, FAPs remain a poorly defined, heterogeneous population without any specific genetic markers. Using a lineage tracing mouse model (*Gli1-CreER Tomato, Gli1ER/Td*), we recently discovered that Gli1 marks a small subset of muscle-resident FAPs (17%) that preferentially expand upon muscle injury (40% of FAPs at day 3 after injury). Here, we performed cell ablation, pharmacologic manipulation, and single cell transcriptomics to further investigate the role of Gli1$^+$ FAPs in muscle regeneration and fat deposition after injury.

Methods

Animals

All animal work was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. *Gli1-CreER Rosa-tdTomato* (*Gli1ER/Td*) mice and *Gli1-CreER Rosa-tdTomato DTA* (*Gli1ER/Td/DTA*) mice were generated. To induce *CreER* activity, 2-month-old male mice received tamoxifen (Tam) injections (75 mg/kg/day) for 5 days. Acute muscle injury was induced by injection of 10 μl Notexin (NTX, 10 μg/mL) or 50 μl glycerol (50% vol/vol) into the Tibialis Anterior (TA) muscle.

Histology

TA muscles were processed for cryosections followed by H&E, WGA, or Perilipin antibodies staining.

Single cell RNA sequencing (scRNA-seq) analysis

Pre-aligned and filtered single-cell RNA-seq matrix files of mouse muscle cells were acquired from GEO: GSE110878. Unsupervised clustering was conducted by Seurat and GO/KEGG term enrichment were analyzed by Clusterprofiler.

Statistics

Data are expressed as means ± standard error of the mean and analyzed by t-tests or one-way ANOVA.

Results

In the TA muscle of *Gli1ER/Td* mice, Td$^+$ cells were exclusively FAPs located in the interstitial area of myofibers (Figure 1A). Though initially presented at a low level in freshly digested muscle cells, Td$^+$ cells constituted 40% of confluent cells after culturing. Sorted Td$^+$ FAPs formed 6.2-

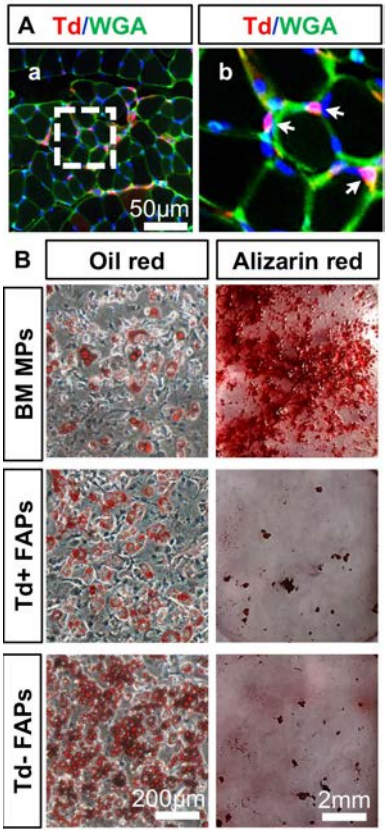

Figure 1. Td in the muscle of *Gli1ER/Td* mice labels a subpopulation of FAPs with less adipogenicity. (A) Td$^+$ cells located in the interstitial area of TA muscle in *Gli1ER/Td* mice. **(B)** Representative images of osteogenic and adipogenic differentiation of BM MPs (Bone marrow mesenchymal progenitors), Td$^+$ FAPs and Td$^-$ FAPs.

fold more CFU-Fs than Td$^-$ FAPs. They had fibroblastic and adipogenic differentiation abilities, but generated much fewer adipocytes than Td$^-$ FAPs (Figure 1B). Both Td$^+$ and Td$^-$ cells had no osteogenic differentiation ability (Figure 1B). To investigate their *in vivo* function, we generated *Gli1ER/Td/DTA* mice. At 2 months of age,Tam injections quickly reduced Td$^+$ cells by 80% and eliminated Td$^+$ CFU-F colonies from muscle (n = 3/group), indicating successful cell ablation. Interestingly, myofibers regenerated at day 7 and day 28 in *Gli1ER/Td/DTA* mice after Notexin injury were 34.1% and 22.7%, respectively. These were smaller than those in *Gli1ER/Td* mice (Figure 2A, n = 5/group), suggesting an impairment of myogenesis.

Gli1 is an effector of Hedgehog (Hh) signaling. Intramuscular injection of purmorphamine (PUR), an Hh agonist, expanded Td$^+$ cells at day 4 and increased myofiber size by 1.6-fold at day 30 after Notexin injury (Figure 2B, C, n = 4/group). Local glycerol injection causes muscle degeneration with fat infiltration. Strikingly, Td$^+$ FAPs were less likely to become adipocytes compared to Td$^-$ FAPs and ablation of Td$^+$ cells led to 39.9% more adipocytes in muscle (Figure 3A-D, n = 4/group). Hh signaling inhibitor, GANT61, induced 78.3% more adipocytes, while PUR almost completely depleted adipocyte infiltration (n = 5/group). ScRNA-seq analysis of mouse muscle mononucleated cells showed that FAPs can be subclustered into Gli1$^+$ and Gli1$^-$ FAPs (Figure 3E, F). Pathway analysis suggested that Gli1$^+$ FAPs are more metabolic active and more related to tissue regeneration than Gli1$^-$ FAPs. For example, they have higher Interleukin-6 (Il6) production and TGF-beta signaling, two known positive regulators of myoblast proliferation and tissue regeneration. Furthermore, Gli1$^+$ FAPs express higher levels of known muscle regulators, such as *Tgfb1, Wisp1, Malat1, Igf1*, *Il15* and *Il33* (Figure 3G), compared with Gli1$^-$ FAPs, which was further validated by qRT-PCR (Figure 3H). *Il15* and *Wisp1*, are also involved in inhibiting FAPs differentiation into adipocytes. Further analysis revealed increased expression of anti-adipogenic regulators, such as *Tsc22d3*, *Dlk1*, *Ddit3* and *Nr4a1* (Figure 3I), and reduced expression of pro-adipogenic regulator, such as *Zfp423* and *Ebf1* (Figure 3J).

Discussion

Our study found that *Gli1-CreER* labels a subpopulation of FAP cells. Compared to Gli1$^-$ FAPs, Gli1$^+$ FAPs are more metabolically active for muscle repair and less likely to contribute to muscle adiposity. Our studies demonstrated that Hh/Gli1 signaling pathway is critical for regulating muscle regeneration and fat accumulation, indicating that

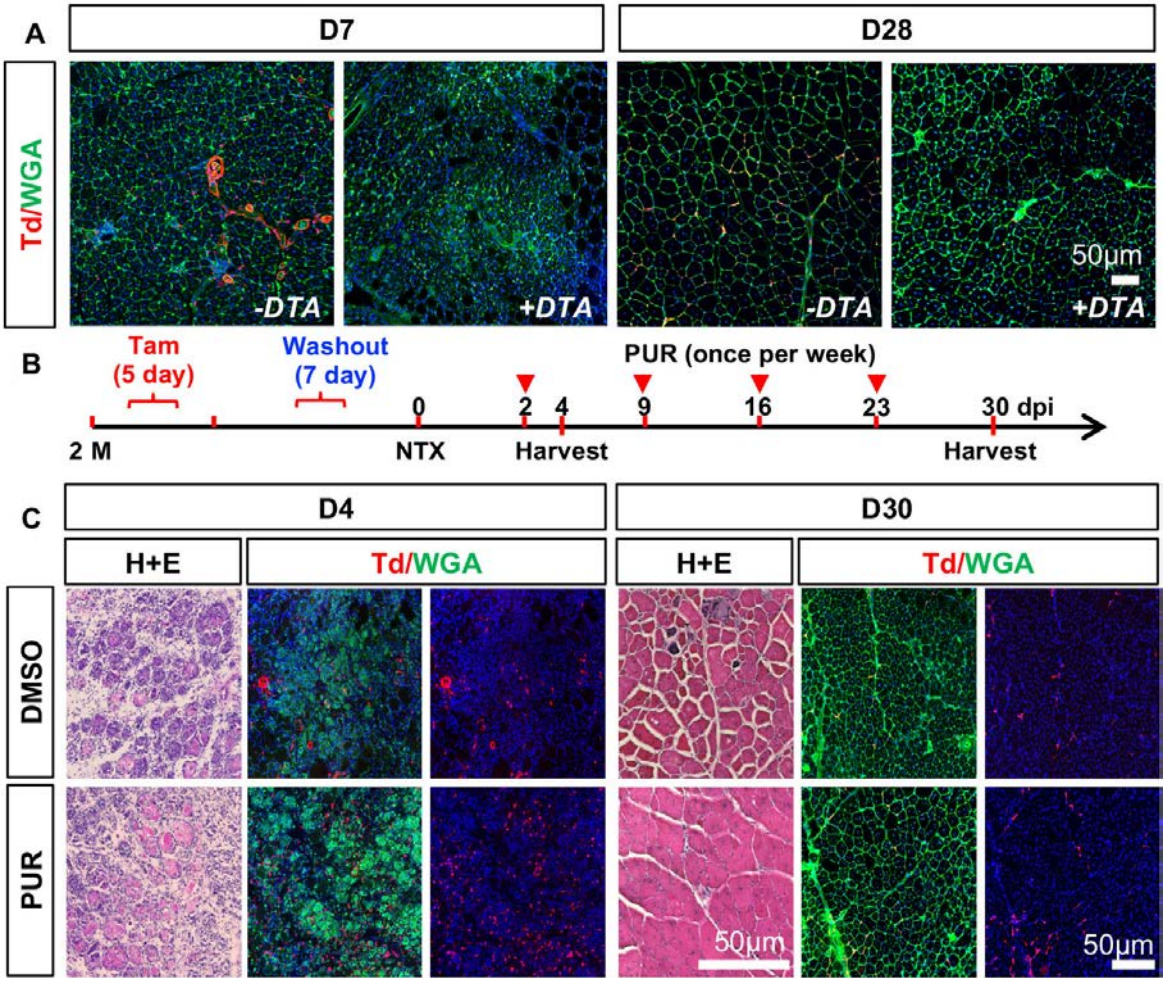

Figure 2. Ablation of Gli1$^+$ cells causes delayed muscle regeneration and activation of Hh signaling accelerates muscle healing. (A) Representative immunofluorescence imaging of *Gli1ER/Td* and *Gli1ER/Td/DTA* muscle at day 7 and 28 post NTX injury. **(B)** Schematic plot of mouse muscle NTX injury model with PUR treatment. **(C)** Representative H&E staining and immunofluorescence images of TA muscles with DMSO or PUR treatment group at day 4 and day 30 post NTX injury.

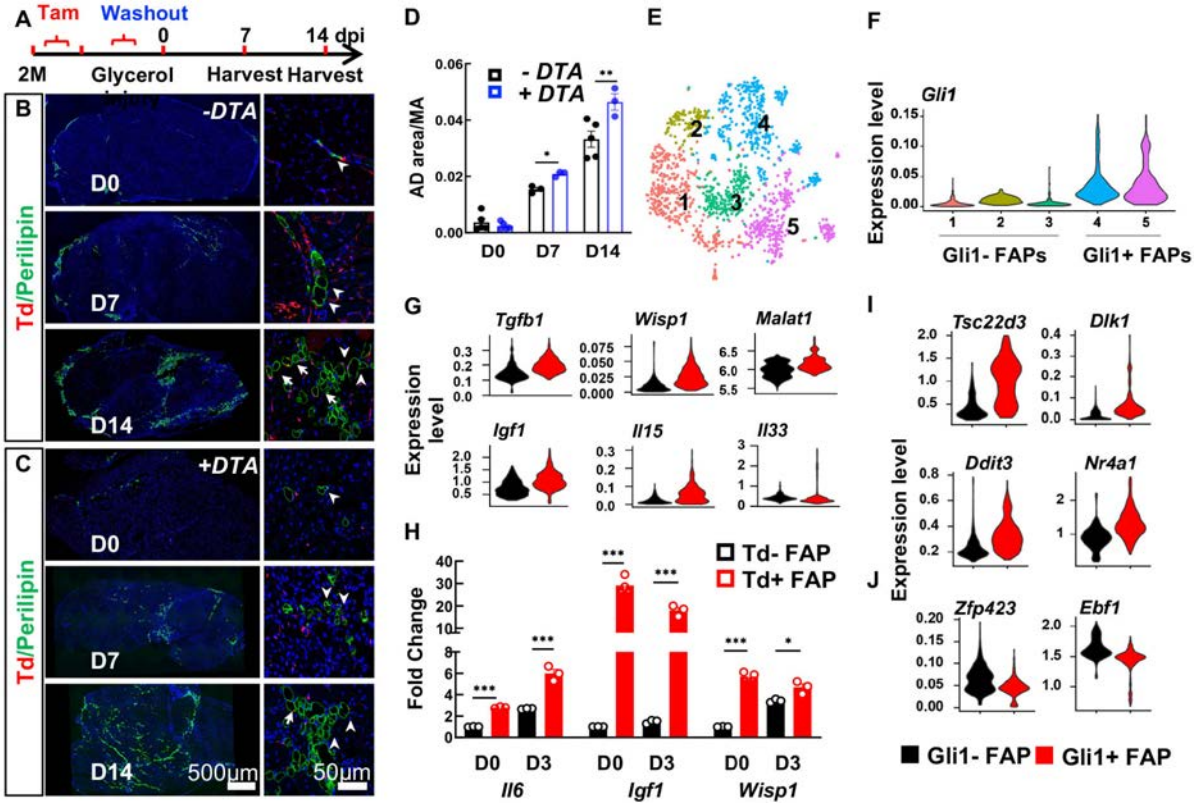

Figure 3. Gli1⁺ FAPs suppress intramuscular adipogenesis and Single cell RNA-seq analysis of FAPs. (A) Schematic plot of mouse muscle glycerol injury model. **(B, C)** Representative immunofluorescence images of TA muscle from *Gli1ER/Td* or *Gli1ER/Td/DTA* mice at day 0, 7, and 14 post glycerol injury with perilipin staining. Arrows point to Perilipin⁺Td⁺ cells and arrowheads point to Perilipin⁺Td⁻ cells. **(D)** Quantification of muscle adipocyte area revealed that muscle adiposity is increased in *Gli1ER/Td/DTA* mice. **(E)** Further subclustering of FAPs generates 5 subclusters. **(F)** Violin plots show clusters 4 and 5 express Gli1 at a higher level compared to clusters 1, 2 and 3. **(G)** Violin plot of myogenic factors expression. **(H)** qPCR of *Il6, Igf1* and *Wisp1* expression at D0 and D3 after injury of sorted Td⁻ and Td⁺ FAPs. **(I)** Violin plot of anti-adipogenic factors expression. **(J)** Violin plot of pro-adipogenic factors expression.

pharmacological activation of this pathway could be a therapeutic approach to boost muscle regeneration.

Significance

Our study revealed a subpopulation of FAPs that preferentially promotes muscle regeneration with less fat accumulation and implied a potential therapeutic effect of Hedgehog signaling in muscle diseases.