Gli1 Labels a Subpopulation of Fap Cells that Respond to Muscle Injury

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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.[1] To date, FAPs remains a poorly defined, heterogeneous population without any specific genetic markers. Gli1 was recently recognized as a marker for bone marrow and periosteal mesenchymal progenitor.[2, 3] In this study, we used *Gli1-CreER* to label FAPs and characterized their changes in healthy, aged, and diseased muscle.

Methods

Animals

All animal work performed in this report was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. Gli1-CreER Rosa-tdTomato (Gli1ER/Td) mice were generated by breeding Rosa-tdTomato mice with Gli1-CreER. Gli1ER/Td/mdx mice were generated by breeding Gli1ER/Td mice with mdx^{4cv} mice.[4] To induce CreER activity, mice received tamoxifen (Tam) injections (75 mg/kg/day) at 2 months of age for 5 days. Acute muscle injury was induced by injection of 10 µl Notexin (10 µg/mL) into Tibialis Anterior (TA) muscle.

Histology

TA muscle samples were fixed in 4% PFA for 1 day, and then immersed into 30% sucrose at 4°C overnight. They were processed for cryosections followed by H&E, WGA, Biodipy, Sca1, PDGFRα, or αSMA antibodies staining.

FAP cell isolation

Hindlimb muscles (quadriceps, gastrocnemius, and tibialis anterior) were dissected and enzymatically dissociated with 0.1% collagenase and 4.8 units/mL dispase in DMEM using the gentleMACs system. The cell slurry was pulled through a 21-gauge needle until all remaining muscle tissue was broken apart, after which the cell solution was filtered through a 40 µm cell strainer. After red blood cell lysis, cells were stained with lineage cell markers (CD45, CD31, CD11b), Sca1, α7-integrin (Itga7) and CD34 antibodies for flow analysis.

Statistics

Data are expressed as means±SEM and analyzed by unpaired, two-tailed Student's t-test.

Results

In the TA muscle of *Gli1ER/Td* mice, Td$^+$ cells were exclusively located in the interstitial area of myofibers after Tam induction (Fig. 1A). The majority of them were co-stained with FAP markers, PDGFRα and Sca1 (Fig. 1B). Quantification revealed that Gli1$^+$ cells constitute a small portion of PDGFRα$^+$Sca1$^+$ FAPs (Fig. 1C). In flow analysis, FAPs are defined by Lin$^-$Sca1$^+$CD34$^+$Itga7$^-$. In line with the staining data, Td$^+$ cells were mostly FAPs (97.4% Lin-, 78.5% Sca1$^+$, 78.2% CD34$^+$/Itga7$^-$, Fig. 1D) and they labeled 3.2 ± 0.1% and 7.6 ± 0.5% of digested muscle cells at P66 and P72, respectively (Tam at P61-65). Meanwhile, 10.9 ± 0.7% and of 17.3 ± 0.3% FAPs at P66 and P72, respectively, were Td$^+$ cells (Fig. 1E). In 1-year-old mice, the percentage of FAP cells in digested muscle cells decreased to 7.94 ± 0.6% and *Gli1-CreER* labeled cells (95.15 ± 4.45 cells/mm^2) reduced drastically compared to adult mice (147.09 ± 14.98 cells/mm^2, n = 3/age, $p < 0.05$). Though initially presented at a low level in freshly digested muscle cells, Td$^+$ cells constituted 40% of confluent cells after culturing (Fig. 2A). Sorted Td$^+$ cells exhibited fibroblastic and adipogenic differentiation abilities, but not osteogenic differentiation ability (Fig. 2B). To investigate their in vivo function, *Gli1ER/Td* mice received Notexin intramuscularly at P72 (Tam at P61-65) to create acute muscle injury. Td$^+$ cells peaked at day 3 post injury, gradually decreased at day 6, and almost receded to normal levels by day 9 (Fig. 3A, B). To further validate the role of Gli1-labeled cells in chronic injury, we crossed *Gli1ER/Td* mice with dystrophic *mdx^{4cv}* mice. After Tam at P61-65, Td$^+$ cells were significantly increased in the interstitial area of myofibers of *Gli1ER/Td/mdx* muscle compared to *Gli1ER/Td* control muscle (P72, control: 198.81 ± 22.66 cells/mm^2, mdx: 360.33 ± 53.70 cells/mm^2, n = 6/group, $P < 0.05$, Fig. 3C).

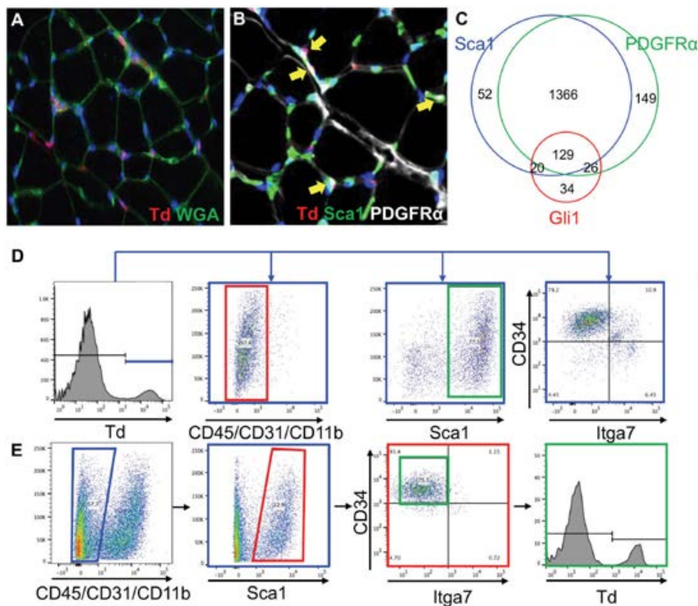

Figure 1. Td in the muscle of *Gli1ER/Td* mice labels a subpopulation of FAPs. **(A)** Td^+ cells located in the interstitial area of TA muscle in *Gli1ER/Td* mice. **(B)** Td^+ cells co-express FAP staining markers, Sca1 and PDGFRα (yellow arrows). **(C)** Venn diagram of $Sca1^+$, $PDGFR\alpha^+$, and Td^+ cells in TA muscles. **(D)** Examination of FAP flow markers on Td^+ cells. **(E)** A portion of FAPs identified by flow are Td^+.

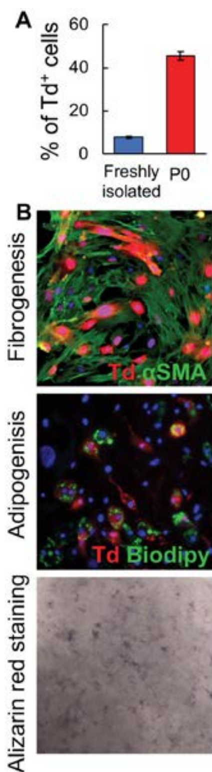

Figure 2. Td^+ cells in the muscle of *Gli1ER/Td* mice are FAPs in vitro. **(A)** The percentage of Td^+ cells in freshly digested muscle cells and in cultured P0 cells. **(B)** Sorted Td^+ cells can differentiate into fibroblasts (αSMA^+) and adipocytes ($biodipy^+$) but not osteoblasts (Alizarin red staining).

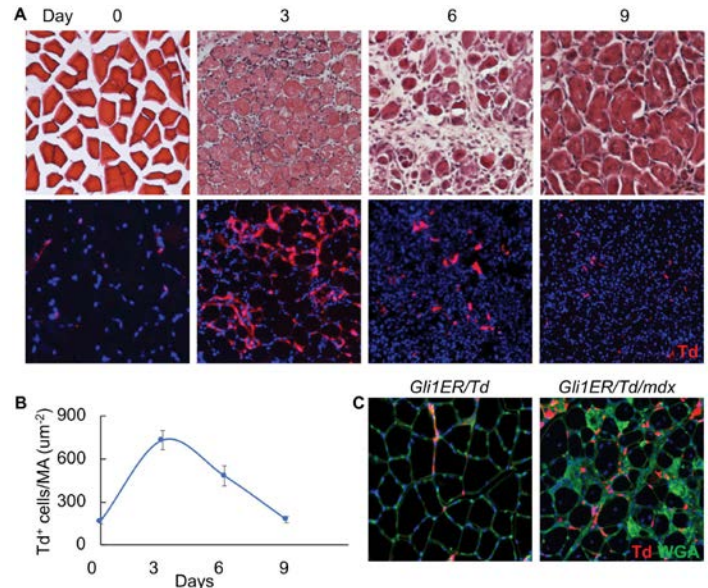

Figure 3. Td^+ cells in the muscle of *Gli1ER/Td* mice rapidly response to muscle injury. **(A)** HE staining (top panel) and immunofluorescence of Td^+ cells (bottom panel) in TA muscles at day 0, 3, 6 and 9 post Notexin-induced muscle injury. **(B)** The time course of Td^+ cells after injury. MA: muscle area. **(C)** Immunofluorescence of Td^+ cells in TA muscles of control and dystrophic mdx^{4cv} mice.

Discussion

In our study, we demonstrated that *Gli1-CreER* labels a subpopulation of FAP cells that undergo age-dependent reduction. Interestingly, they show the same response kinetics of FAPs after acute and chronic muscle injury, suggesting that Gli1-labeled subpopulation of FAP cells play a predominant role in the regeneration process of injured skeletal muscles. Since Gli1 is an effector of Hedgehog (Hh) signaling, our data also implied a possible role of Hh signaling in regulating FAP action.

Significance

We identified *Gli1-CreER* as a suitable model to genetically target a subpopulation of FAPs that respond to muscle injury. Understanding the cellular and molecular mechanism of FAPs is crucial for designing new treatments to promote muscle regeneration under aging and diseased conditions.

References

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