# Identification of Gli1 as a Progenitor Cell Marker for Meniscus Injury Repair

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## Introduction

Meniscal tears are one of the most common injuries of the knee. They are likely to be an important early event in the initiation and later propagation of osteoarthritis (OA) and have been accepted as an important risk factor for OA clinically. As a treatment, meniscal resection has been demonstrated to accelerate degenerative disease and the most commonly performed surgery of partial meniscectomy is not restorative and only delays degeneration. Surgical repair remains a viable treatment for only a small portion of individuals. Various approaches, including stem cell transplantation, have been proposed to repair injured meniscus. However, meniscus-specific progenitors are still largely unknown. Gli1 was recently recognized as a marker for bone marrow and periosteal mesenchymal progenitor.[1,2] In this study, we constructed *Gli1-CreER Tomato (Gli1ER/Td)* mice and analyzed the progenitor properties of Gli1-labeled meniscus cells in development, homeostasis, and injury repair.

## Methods

### Animals

*Gli1-CreER* mice were crossed with *Rosa-tdTomato* mice to obtain *Gli1ER/Td*. Mice at various ages received Tamoxifen (Tam) injections (50 mg/kg $\times$ 2 days in pups and 75 mg/kg $\times$ 5 days in adults).

### Surgery

Male mice at 3 mo of age received Tam followed by surgical transection of the medial meniscus in right knees and sham operation in left knees a week later. During the surgery, the joint capsule was opened and the anterior horn of the medial meniscus was cut into two parts. For cell treatment, 5000 FACS sorted meniscus cells (Td$^+$ or Td$^-$ cells) were injected into the knee joint space right after meniscus surgery. For activator treatment, 2 $\mu$l Purmorphamine (1 mM) were injected into the knee joint space right after surgery. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania.

### Cell culture

Primary meniscus cells were enzymatic digested from the meniscus of 4-wk-old *Gli1ER/Td* mice. FACS sorted Td$^+$ and Td$^-$ cells were used for CFU, migration, proliferation, differentiation, and qRT-PCR assays.

### Histology

Knee joints were fixed in 4% PFA, decalcified in 10% EDTA, and processed for cryosections or paraffin sections.

### Human meniscus samples

They were prepared from de-identified specimens obtained at the total arthroplasty of the knee joints. Paraffin sections were stained by Safranin O/Fast green to evaluate degenerative stages and neighboring sections were used for Gli1 staining.

### Statistics

Data are expressed as means$\pm$SEM and analyzed by paired, two-tailed Student's t-test.

## Results

In *Gli1ER/Td* mice, Td did not label meniscus cells in newborn pups (Tam at P5-6). In 2-wk-old mice (Tam at P9-14), Td initially labeled the entire anterior meniscus and gradually concentrated at the superficial cells by 8 wk of age. Td started to label the posterior meniscus in 4-wk-old mice and also later focused on superficial cells only. In adult animals, Td$^+$ cells only occurred in the superficial layer of meniscus right after Tam injections and long term tracing did not detect their expansion (Fig. 1). In culture, Td$^+$ cells generated much more CFU-Fs (2.55-fold) and grew much faster than Td$^-$ cells (Fig. 2A). Using an activator (Purmorphamine) and an inhibitor (GANT-61) of hedgehog (Hh) pathway, we found that primary meniscus cells proliferate and migrate in an Hh-dependent manner (Fig. 2B, **C**). Td$^+$ meniscus cells also had the abilities to differentiate into osteoblasts and adipocytes (Fig. 2D). During meniscal differentiation, Td$^+$ cells expressed 2.9-fold more *Col1a1* and 61.2% less *Col2a1* than Td$^-$ cells. After meniscus injury, Td$^+$ cells quickly emerged at the injury ends and proliferated (Fig. 3). Without treatment, two ends remained separated 3 mo later. However, injection of Td$^+$ cells, but not Td$^-$ cells, from *GliER/Td* meniscus into the joint capsule of *WT* mice right after injury resulted in the
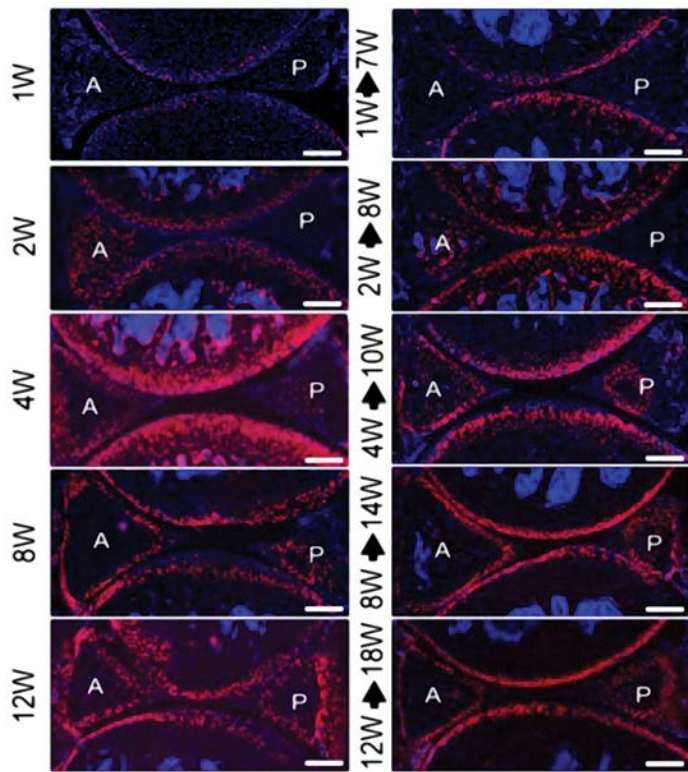
**Figure 1.** The distribution of Gli1+ cells and their progenies in mouse meniscus during development and homeostasis. *Gli1ER/Td* mice received Tam injections at various ages and their joints were harvested right after injections (left panel) or 6 weeks later (right panel) for sagittal cryosections. A and P represent the anterior and posterior side of meniscus, respectively. n = 5/age.

reconnection of two ends within a month (Fig. 4). Injection of Purmorphamine also exhibited a strong repair effect. After meniscus injury, OA generally developed in the cartilage 2 mo later. Strikingly, injection of Td+ cell or Hh activator right after surgery significantly delayed OA initiation (Fig. 5). Analyzing human meniscus samples from OA patients confirmed an increase of Gli+ cells in meniscus during degeneration (Fig. 6).

## Discussion

By using a lineage tracing line, cell culture, and a meniscus injury model, we demonstrated that Gli1 is a mesenchymal progenitor marker in mouse meniscus. Gli1-labeled cells contribute to meniscus development and injury response. Activation of Gli1/hedgehog signaling in adult meniscus leads to accelerated meniscus healing process in response to surgically induced meniscus degeneration, indicating a protective role of hedgehog signaling on meniscus against degeneration. Analyzing Gli1 expression profile in human meniscus samples with different meniscus degenerative stages strongly implicates the clinical relevance of our study

## Significance

Our studies uncover the critical role of Gli1 in adult knee meniscus and provide proof-of-principle evidence for targeting this novel pathway as meniscus injury therapy for preventing OA development.
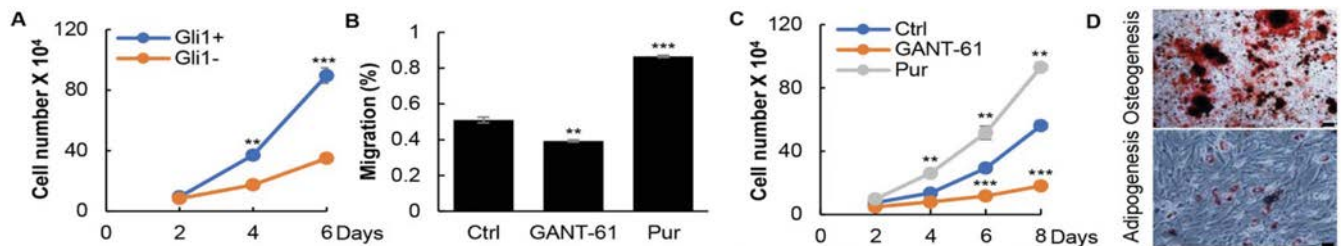


**Figure 2.** Gli1+ cells exhibit mesenchymal progenitor properties in vitro. **(A)** The growth curve of sorted Gli1+ and Gli1− meniscus cells. **(B, C)** The growth curve **(B)** and migration ability **(C)** of primary meniscus cells treated with GANT-61 (1 mM) or Purmorphamine (0.1 mM). **P $<$ 0.01, ***P $<$ 0.001 vs Control. **(D)** Osteogenic (Alizarin Red staining) and adipogenic (Oil Red O staining) differentiation of sorted Gli1+ primary meniscus cells. n = 3/group.
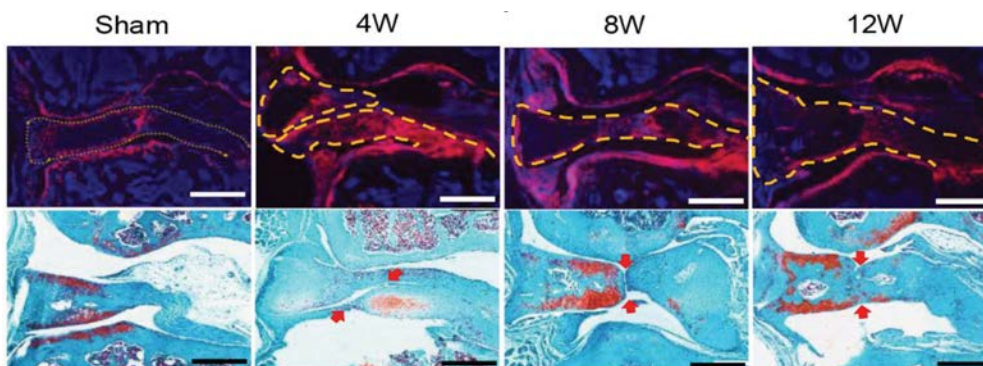


**Figure 3.** Mouse meniscus does not heal at 3 mo post injury. *Gli1ER/Td* mice received Tam injections at 12 wk of age followed by meniscus injury. Joints harvested at various times were sectioned at a coronal plane for fluorescent image of Td signal (top panel) and Safranin O/Fast Green staining (bottom panel). n = 8/group. Red arrows point to injury sites.
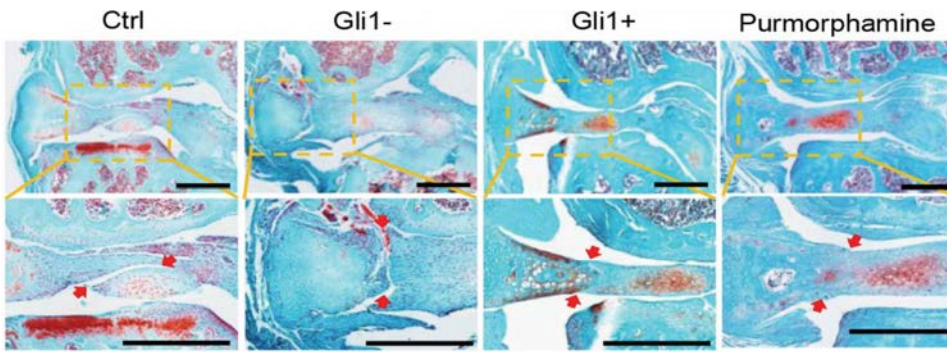
**Figure 4.** Activating Hh signaling promotes meniscus repair. At 4 wk post injury, mouse knees received Gli1-labeled meniscus cells or Purmorphamine injections right after surgery showed reconnection of two meniscus ends. Red arrows point to prior meniscus injury sites. n = 8/group. Bottom panels are magnified images of top panels.

**Figure 5.** Meniscus repair delays OA initiation. **(A)** Safranin O staining of mouse knee joints (sagittal sections) at 8 wk postsurgery. Mice received PBS, Gli1+ cell or Purmorphamine treatment right after surgery. **(B)** The OA severity was measured by Mankin score. n = 10/group. *P < 0.05, ***P < 0.001 vs PBS injury.
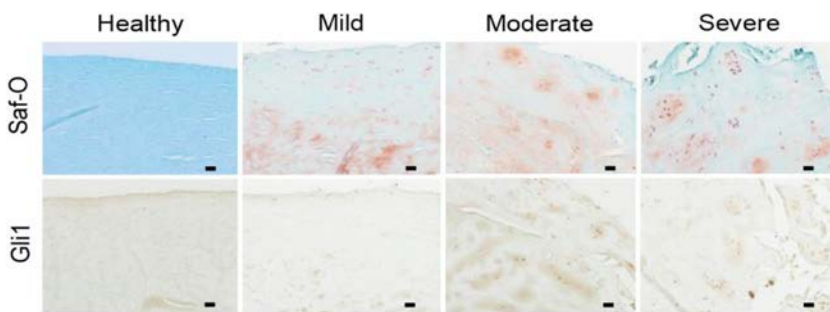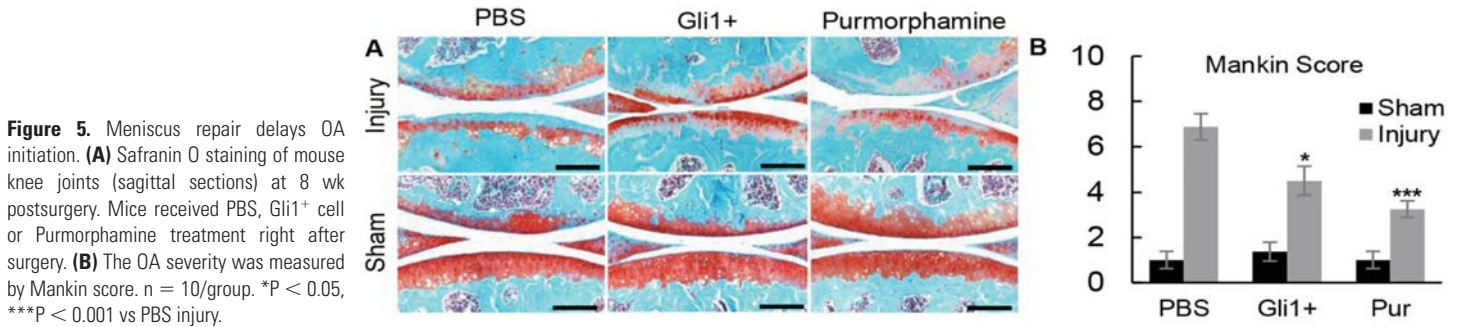




**Figure 6.** Human meniscus samples from OA patients show a positive correlation between meniscus degeneration severity and Gli1 expression. n = 5/group. Scale bar in all images, 200 mm.

# References

**1. Shi Y et al.** *Nat Commun.* 2017;8(1):2043
**2. Wang L et al.** *J Bone Miner Res.* 2019;34(3):520-532.