Cruciate ligament, patellar tendon, and patella formation involves differential cellular sources and dynamics as joint cavitation proceeds

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Abstract
Background: Cruciate ligament (CL) and patellar tendon (PT) are important elements of the knee joint, uniting femur, patella, and tibia into a single functional unit. So far, knowledge on the developmental mechanism of CL, PT, and patella falls far behind other skeletal tissues.

Results: Here, employing various lineage tracing strategies we investigate the cellular sources and dynamics that drive CL, PT, and patella formation during mouse embryonic development. We show that Gdf5 and Gli1 are generally expressed in the same cell population that only contributes to CL, but not PT or patella development. In addition, Col2 is expressed in two independent cell populations before and after joint cavitation, where the former contributes to the CL and the dorsal part of the PT and the latter contributes to the patella. Moreover, Prrx1 is always expressed in CL and PT progenitors, but not patella progenitors where it is switched off after joint cavitation. Finally, we reveal that patella development employs different cellular dynamics before and after joint cavitation.

Conclusions: Our findings delineate the expression changes of several skeletogenesis-related genes before and after joint cavitation, and provide an indication on the cellular dynamics underlying ligament, tendon, and sesamoid bone formation during embryogenesis.

Keywords
cellular dynamics, cruciate ligament, embryonic development, lineage tracing, patella, patellar tendon
1 | INTRODUCTION

Ligaments connect bone to bone and tendons connect muscle to bone, together uniting all musculoskeletal elements into an all-in-one system. They are closely related tissues that are made of tenocytes with similar gene expression profile. However, genetic deletion of certain key transcription factors critical for ligament and tendon development led to completely different phenotypes in these two structures, indicating that they might employ different developmental mechanisms. Cruciate ligament (CL) is one of the most common ligaments that are present in the knee joint. It is critical for the biomechanical stability and function of the knee joint and its rupture is often associated with a higher susceptibility to meniscal injury and early onset of degenerative changes. Patellar tendon (PT) is the distal part of the quadriceps tendon that connects the patella to the tibia. It is also known as patellar ligament since it involves bone-to-bone connection. So far, autograft-based surgical reconstruction is the most common treatment for CL injury, where bone (patella)-PT-bone (tibia) autograft is considered as a golden standard by some orthopedic surgeons, despite of certain controversies. Patella is the largest sesamoid bone in the human body and is part of the patellofemoral joint that maintains the mechanic and stability of the entire knee joint to facilitate locomotion. During mouse embryonic development, patella initially develops as a part of the femur and later separates from the femur and becomes superficially embedded in the quadriceps tendon.

Limb skeletogenesis initiates with the formation of undifferentiated and uninterrupted mesenchymal condensations that express Paired related homeobox 1 (Prx1) and Collagen type 2 (Col2). Soon after, a group of compact and closely associated mesenchymal cells appears at the future joint site within the original anlagen, known as the interzone that expresses Growth differentiation factor 5 (Gdf5). These Gdf5-positive cells serve as joint progenitors that generate a continuous influx of newly produced cells into many components of the developing knee joint, including the CL. In addition, Col2-expressing cells from the original anlagen have also been shown to be a major contributor for CL formation during mouse embryonic development.

Hedgehog (Hh) signaling is an evolutionarily conserved pathway that regulates multiple aspects of vertebrate development, including skeletal development. Binding of the Hh ligands to their receptors at the cell surface eventually leads to translocation of the Gli transcription factors from the cytoplasm into the nucleus, where they activate the transcription of Hh target genes, including Gli1 that is often used as a readout for Hh activity. Both Sonic hedgehog (Shh) and Indian hedgehog (Ihh) ligands of the Hh protein family have been shown to be essential for limb patterning and growth plate chondrocyte proliferation and differentiation, respectively. Recently, it has been shown that the Shh and Ihh-responsive Gli1 lineage cells contribute to the establishment and maturation of multiple tendon-bone attachments, that is, entheses.

In this study, we show that the expression of several skeletogenesis-related genes, including Gdf5, Gli1, Col2, and Prx1 changes in the progenitors of CL, PT, and patella as joint cavitation proceeds, leading to their spatially and temporally differential contribution to these structures during embryogenesis.

2 | RESULTS

2.1 | Gdf5-expressing cells only contribute to CL development, but not PT or patella

Given that Gdf5-positive cells are a major source for joint formation, we first employed the recently described tamoxifen-inducible Gdf5-CreERT2;R26R-Tomato mouse strain to label the interzone cells and investigated their contribution to the development of CL, PT and patella during embryogenesis. For the CL, both caudal and cranial ligaments were included in the analysis. We induced Cre recombination either before (embryonic day (E) 11.5 or E12.5) or after (E13.5 or E14.5) joint cavitation, and collected the knee joints at E17.5 for analysis (see Figure 1 for an overview). All samples were sectioned at the sagittal plane in the entire study. We found that the Gdf5-expressing cells labeled at E11.5 or E12.5 covered almost the entire CL at E17.5 (Figure 2A,B), while the density of these cells dropped by three- to four-folds when tracing was initiated at E13.5 or E14.5 (Figure 2C,D,M). This suggests that the Gdf5-expressing cells specified before joint cavitation are the major constituents of the CL and the Gdf5 expression is gradually reduced in the later-recruited progenitors as joint cavitation proceeds.

In contrast, almost no Gdf5-positive cells were observed in the PT during any of the tracing periods (Figure 2E-H,M). Similarly, Gdf5-positive cells were also found at an extremely low density in the patella during all tracing periods (Figure 2I-M). These results suggest that neither the PT nor the patella is originated from this cell population. Interestingly, most of the Gdf5-positive cells in the patella were found at the surface layer adjacent to the femur (highlighted by the yellow arrows in Figure 2I-L), hinting that these cells might be part of the femur cartilage before patella separation. Alternatively, since the Gdf5-positive cells were observed on both sides of the
patella/femur cavity (Figure 2J-L), the Gdf5 expression might be acquired during the onset of patella-femur cavitation.\(^\text{10}\)

Taken together, these results show that the Gdf5-expressing cells specified before joint cavitation mainly construct the CL in the inner knee joint; however, they make very little or no contribution to the PT or patella in the outer knee joint (Figure 2M). Considering the role of the Gdf5-expressing cells in the formation of other knee joint components,\(^\text{16}\) it seems that these interzone cells only contribute to the tissues in the inner knee joint, but not the ones in the outer joint.

2.2  |  Gli1 marks generally the same population of progenitor cells as Gdf5

Hh acts as a major morphogen in limb bud patterning\(^\text{24,25}\) and growth plate cartilage differentiation\(^\text{22}\) during murine embryonic development. In addition, the Hh-responsive Gli1 lineage cells are important contributors to the establishment and maturation of the entheses.\(^\text{23}\) Therefore, we then employed the Gli1-CreERT2;R26R-Tomato mouse strain\(^\text{26}\) to check their contribution to the development of CL, PT and patella using the four above-mentioned tracing periods. For CL, similar to the Gdf5-tracings, the Gli1-expressing cells specified at E11.5 marked almost the entire structure at E17.5 (Figure 3A); while the contribution dropped dramatically when tracings were initiated at later stages (Figure 3B-D,M). This suggests that the CL progenitors express both Gdf5 and Gli1 before joint cavitation, while the expression of both genes dramatically decreases as joint cavitation proceeds. In line with the previous study,\(^\text{23}\) the Gli1-expressing cells specified after joint cavitation, that is, at E13.5 or E14.5 were mainly found at the entheses, that is, the attaching points between the CL and femur/tibia (highlighted by the yellow arrows in Figure 3C,D).

For PT and patella, similar to the Gdf5 tracings, Gli1-positive cells were also found at an extremely low density in both structures during all tracing periods (Figure 3E-M), where most of them were observed at the entheses between PT and tibia and the surface layer of the patella adjacent to the femur, respectively (highlighted by the yellow arrows in Figure 3E-H and Figure 3L, respectively).

Taken together, these results show that the Gli1-positive cells behave similarly as the Gdf5-positive cells during CL, PT, and patella development (Figure 3M vs. Figure 2M), except their minor contribution to the PT entheses; suggesting that the two genes generally mark the same population of progenitor cells that mainly give rise to the CL.

2.3  |  Col2 marks two independent cell populations before and after joint cavitation that give rise to CL and PT, and patella, respectively

Given that the Col2-expressing cells label the cartilaginous anlagen before joint formation,\(^\text{12}\) as well as the chondrogenic and cartilaginous cells within the skeletal elements;\(^\text{27}\) we next employed the Col2-CreERT2 mouse strain and coupled it to the R26R-Confetti strain\(^\text{28}\) to investigate their cellular distribution during CL, PT and patella development using the four above-mentioned tracing periods. Following similar trend as the Gdf5- and Gli1-traced cells, Col2-positive cells labeled almost the entire CL during E11.5 to E17.5 tracing (Figure 4A), while the cellular density sharply decreased when tracings were initiated at later stages and dropped to zero when traced from E14.5 (Figure 4B-D,I). Given that the Col2-positive cells labeled at E11.5 are likely to be cells of the cartilaginous anlagen prior to joint formation, it is likely that the CL is mainly originated from this cell population. In addition, these results also suggest that Col2, Gdf5 and Gli1 likely mark a single pool of CL progenitors.
For PT development, a decent amount of Col2-positive cells were observed at E17.5 when traced from E11.5 or E12.5, where most of them were found in the dorsal part of the PT (Figure 4E,F,J,K); and the contribution dropped to almost zero when traced from E13.5 or E14.5 (Figure 4G-I). Considering the negligible roles of the Gdf5
and Gli1-expressing cells in PT formation, it seems that the dorsal part of PT are formed by an independent population of progenitor cells specified before joint cavitation that only express Col2, but not Gdf5 or Gli1. In addition, the orientation of PT proliferation might be along the ventral-dorsal axis. The overall contribution of the Col2-expressing cells to the PT was significantly lower than CL (Figure 4I), suggesting a potentially higher Col2

**FIGURE 3** Gli1 and Gdf5 are expressed in generally the same population of progenitors. A-L, Example images showing the distribution of Gli1-traced cells in crucial ligament (CL), patellar tendon (PT), and patella (Pa) of the knee joint during E11.5, E12.5, E13.5 or E14.5 to E17.5 tracing. M, Comparison of the Gli1-traced cellular density among CL, PT and Pa during the four tracing periods. One-way ANOVA was used to calculate the P values. Cellular density represents the number of tomato-positive cells over tissue area (mm²). Data represent mean ± SEM, where at least 5 embryos were analyzed. Ti, tibia, Fe, femur
content in the CL than the PT during their initial formation. Given that Col2 is a marker for the cartilaginous anlagen,\textsuperscript{12} as well as the chondrogenic and cartilaginous cells,\textsuperscript{27} a regulatory mechanism might exist in the initially Col2-expressing CL and PT progenitors to switch off the Col2 expression after joint cavitation so that these cells do not commit to the fate of becoming cartilaginous cells.

**FIGURE 4** Col2-expressing cells specified before and after joint cavitation make differential contribution to the CL, PT, and patella. A-H, Example images showing the distribution of Col2-traced cells in crucial ligament (CL), patellar tendon (PT), and patella (Pa) of the knee joint during E11.5, E12.5, E13.5 or E14.5 to E17.5 tracing. The PT was divided into ventral and dorsal parts with similar area in E and F. I, Comparison of the Col2-traced cellular density among CL, PT, and Pa during the four tracing periods. J and K, Comparison of cellular density between the dorsal and ventral parts of the PT in E11.5 (M) or E12.5 (N) to E17.5 tracings. One-way ANOVA was used to calculate the $P$ values. Cellular density represents the number of RFP, YFP, or CFP-positive cells over tissue area (mm$^2$). Data represent mean ± SEM, where at least 5 embryos were analyzed. Ti, tibia, Fe, femur.

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For patella development, density of Col2-positive cells followed a completely opposite trend as in the CL and PT, where the Col2-expressing cells specified at E14.5 marked almost the entire structure (Figure 4H); whereas the ones labeled at earlier stages, especially before joint cavitation, made very little contribution (Figure 4E-G,I). Increased contribution of Col2-expressing cells to patella at E13.5 and E14.5 likely reflects differentiation of the progenitors into chondrocytes in this skeletal element. However, contribution of Col2−, but not Gdf5− or Gli1-expressing cells to the dorsal part of PT suggests the existence of Col2+, Gdf5−, and Gli1− population of progenitors specifically enrolled in PT development.

2.4 | Prrx1 is always expressed in CL and PT progenitors, but is switched off in patella progenitors after joint cavitation

Next, we employed the Prrx1-CreERT2 mouse strain to label the mesenchymal progenitor cells in the limb bud29 and coupled it with the R26R-Confetti strain to trace their contribution to the CL, PT, and patella using the four above-mentioned tracing periods. For CL development, unlike the Gdf5, Gli1, and Col2-tracings where there was a clear decrease in the number of labeled cells as joint cavitation proceeds (Figure 2M, 3M, 4I), Prrx1-positive cells marked almost the entire CL during all four tracing periods (Figure 5A-D,I), indicating that Prrx1 expression is maintained in the CL progenitors.

Similarly, PT was also mostly labeled with the Prrx1-positive cells during all tracing periods, despite an overall slightly lower density as compared to the CL, especially at later developmental stages (Figure 5E-I). No difference in Prrx1-positive cellular density was observed between the ventral and dorsal parts of PT during all tracing periods (Figure 5J). Considering the distribution pattern of the Col2-positive cells in the PT where they were mainly found in the dorsal part only when traced before joint cavitation (Figure 4E,F,J,K), these results might suggest that the PT is formed from the ventral side to the dorsal side, where the progenitor cells are initially Col2 and Prrx1 double positive and later only express Prrx1 after joint cavitation.

For patella development, contribution of Prrx1-expressing cells was particularly vivid when specified at E11.5 and dramatically dropped when specified at E12.5, E13.5, and E14.5 (Figure 5E-I). This is opposite to the Col2-tracings where the Col2-positive cells were mainly observed in the patella after joint cavitation (Figure 4G-I). These results suggest that Prrx1 and Col2 expression is mutually exclusive before and after joint cavitation in the patella. A regulatory mechanism might exist to activate the switch to facilitate these cells to commit their chondrocyte fate.

2.5 | Patella development involves different clonal dynamics before and after joint cavitation

Tracing of individual clones in the R26R-Confetti reporter mice allows us to quantify the number of cells in each Col2- and Prrx1-traced clone and analyze the clonal dynamics based on their size distribution during all tracing periods. Individual clones are difficult to be reliably identified in the CL and PT due to their extremely high clonal density, therefore only patella was analyzed. The Col2-traced clones showed similar size distribution pattern among all tracing periods, where over 60% of them contained only 2 to 3 cells (Figure 6A,B). This suggests that most of the Col2-expressing cells specified either before or after joint cavitation have only divided once or twice during patella formation, regardless of the length of the tracing periods. Therefore, cell influx, but not cell division may be the major cellular mechanism that drives the formation of patella after joint cavitation, considering that the Col2-expressing cells specified at E14.5 formed most of the patella structure (Figure 4H,I).

For the Prrx1-traced clones, we observed different clonal size distribution patterns between the E11.5 to E17.5 tracing and the other three tracing periods. There was no significant difference among the percentage of differently sized clones during E11.5 to E17.5 tracing (Figure 6C,D), whereas over 80% of the clones were composed of only 2 to 3 cells in the other three tracings (Figure 6D). Considering that the Prrx1-expressing cells specified at E11.5 made the most contribution to the patella (Figure 5E,I), these results suggest that the early stage patella progenitors that express Prrx1 employs cell division as the major proliferation mechanism; whereas when the progenitors switch off Prrx1 expression and start to express Col2, cell influx becomes the dominant mechanism of growth.

3 | DISCUSSION

Despite the unique and important roles of ligament, tendon and sesamoid bones in musculoskeletal function, understanding of their developmental mechanism falls far behind other skeletal tissues. In this study, using various lineage tracing strategies, we demonstrate that CL, PT, and patella are formed by Gdf5, Gli1, Col2, and Prrx1-expressing cells specified at various embryonic time points (before and/or after joint cavitation) in a temporally, spatially and quantitatively different manner (Figure 7). For the CL progenitors, they initially express all four markers at E11.5, while as the limb develops and joint cavitation proceeds most of the newly recruited progenitor cells turn off the expression of Gdf5, Gli1, and Col2,
whereas the Prx1 expression persists (Figure 7A,B). Interestingly, PT develops in a different fashion as compared to CL with almost no contribution from the Gdf5- or Gli1-positive progenitors and only partial contribution from the Col2-positive progenitors (Figure 7C). At the same time, Prx1-positive progenitors are the main contributors to the development of this structure (Figure 7C).

Previous study has hinted some discrepancies in the origins of the CL and PT progenitors: CL is labeled in the newborn mice of the Sox9-CreERT2;R26R strain when
Tamoxifen was injected at either E12.5 or E13.5, whereas
PT is only labeled at E12.5 injection, but not E13.5.30 Our
data further suggest that PT likely develops from a popula-
tion of progenitors, which is different from the one that
forms the CL. In addition, specification of the
Prrx1-positive progenitors into PT does not require activa-
tion of Gdf5 or Gli1, suggesting a different mechanism of
patterning for the two structurally similar tissues. Given
that the expression of Prrx1 is restricted to the periosteum
of the limb skeleton at later embryonic stages,11 the robust
labeling of the CL and PT by the Prrx1-CreERT2 at late
embryonic stages may represent a tissue specific behavior
of the Prrx1 enhancer. Alternatively, the continuously-
high contribution of Prrx1-positive progenitors to both CL
and PT might suggest an influx model of their formation
with constant addition of new cells into these structures
during development. It is worth noting that no obvious dif-
fERENCE was observed between the caudal and cranial cru-
ciate ligaments in term of tracing patterns with any of the
examined strain.

CL injury is one of the most common knee liga-
ment injuries that affect many young and active indi-
viduals who participate in sports that involve jumping
and rapid change of direction.31 On the other hand,
PT tear requires a very strong force by direct impact
to the front of the knee. Our results might provide an
indication from the cell origin point of view on this
difference in injury susceptibility between the two
tissues.

It has been shown that the degenerating CL cells start
to exhibit chondrocyte-like characters, at both the histo-
logical and gene expression levels.32 In addition, induc-
tion of Col2 expression has been observed in the
fibrocartilaginous regions of ruptured or degenerating lig-
aments.33 Our observation that CL is mainly made by
Col2-expressing cells specified before joint cavitation sug-
gests that a regulatory mechanism might exist to suppress
Col2 expression in normal CL cells and prevent them
from differentiating into chondrocytes and the subse-
quent degeneration. It is also in line with the previous

Figure 6: Different developmental mechanism is employed during patella development before and after joint cavitation. A and C,
Example images illustrating the clonal distribution of E14.5 to E17.5 traced Col2-positive clones (A) and E11.5 to E17.5 traced Prrx1-positive
clones (C) in the patella. White dashed lines outline individual traced clones. B and D, Size distribution of the Col2- (B) and Prrx1-traced
(D) clones in patella. Clonal size represents the number of cells within a clone of the same color. One-way ANOVA was used to calculate the
P values. Data represent mean ± SEM, where at least 5 embryos were analyzed.
observation that the CL-derived cells have a high chondrogenic potential in vitro.\textsuperscript{34}

Patella comprises two independent cellular populations before and after joint cavitation, which are characterized by Prrx1 and Col2 expression, respectively (Figure 7E,F). It is likely that after joint cavitation the mesenchymal cells have differentiated into mature chondrocytes, where they switch off Prrx1 expression and start to express Col2. Previous study showed that patella initially develops as a cartilaginous process as part of the femur using cells from the anlagen and later it separates from the femur and starts to use the invading chondrogenic cells as the major source for further development.\textsuperscript{10} Therefore, the Prrx1-expressing population that we observed before joint cavitation is likely cells from the original anlagen and the later Col2-expressing population may represent the invading chondrocytes. These observations suggest that there might be a regulatory program guiding the specification of the fate of the progenitor cells by altering the expression level of several critical transcription factors.
Autograft-based surgical reconstruction is the major strategy to treat CL injury, where bone-PT-bone is one of the most popular option, thanks to its long-term stability after surgical reconstruction.\(^5,6\) Our study explored the differential gene expression and cellular dynamics underlying CL, PT, and patella development during embryogenesis, which might provide indications on the graft choice for CL reconstruction from the developmental biology point of view.

4 | EXPERIMENTAL PROCEDURES

4.1 | Mice handling

All animal work was permitted by the Ethical Committee on Animal Experiments (Stockholm North Animal Ethics Committee or Linköping Animal Ethics Committee) and conducted according to The Swedish Animal Agency’s Provisions and Guidelines. Genetic recombination in pregnant female mice was induced by intraperitoneal (ip) injection of tamoxifen (Sigma #T5648). Tamoxifen was dissolved in corn oil at a concentration of 20 mg/mL. Three or five milligrams of tamoxifen was injected into the pregnant dam depending on the embryonic stages. A total of 3 mg dosage per animal was used for injections at E11.5 and E12.5 and 5 mg dosage per animal was used for injections at E13.5 and E14.5. For Gli1CreERT2;R26RTomato tracing, 2 mg dosage per animal was used for injection at all embronic stages. For plug checking, male and female mice were put together in the evening at around 5 PM and vaginal plug was checked early next morning at around 7 AM. The day when the plug was observed was considered as E0.5. To better identify individual clones in the patella at a lower density for E14.5 to E17.5 tracing of the Col2CreERT2-Confetti embryos and E11.5 to E17.5 tracing of the Prx1CreERT2-Confetti embryos, we also performed these tracings with a dosage of 1 mg tamoxifen per animal.

The Col2-CreERT2, Prmx1-CreERT2, Gdf5-CreERT2, Gli1-CreERT2, and R26RConfetti, R26RTomato mice have been previously described.\(^12,16,26,28,29\)

4.2 | Tissue preparation

Mice were sacrificed with isoflurane (Baxter KDG9623) followed by cervical dislocation. Embryos were dissected into ice-cold PBS and fixed in freshly prepared 4% paraformaldehyde (PFA) for 2-5 hours at 4°C. Samples were then washed in PBS at 4°C for 1 hour before being placed into 30% sucrose at 4°C overnight to eliminate the remaining PFA. Tissues were subsequently embedded in OCT (Tissue-Tek #25608-930) and sectioned into 30-50 \(\mu\)m-thick sections at \(-20°C\).

4.3 | Microscopy, image analysis, and data quantifications

Images were acquired with an LSM880 confocal microscope and processed with the Imaris software. Image analysis was performed with the Imaris and the Image J software.

4.4 | Statistical analysis

Data were presented as mean ± SEM. In each group, at least 5 embryos were analyzed. Unpaired Student’s t-test and one-way ANOVA were utilized to calculate \(P\)-values.

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REFERENCES

1. Asahara H, Inui M, Lotz MK. Tendons and ligaments: connecting developmental biology to musculoskeletal disease pathogenesis. J Bone Miner Res. 2017;32:1773-1782.

2. Ito Y, Toriuchi N, Yoshitaka T, et al. The Mohawk homeobox gene is a critical regulator of tendon differentiation. Proc Natl Acad Sci U S A. 2010;107:10538-10542.

3. Murchison ND, Price BA, Conner DA, et al. Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons. Development. 2007; 134:2697-2708.

4. Nakahara H, Hasegawa A, Otabe K, et al. Transcription factor Mohawk and the pathogenesis of human anterior cruciate ligament degradation. Arthritis Rheum. 2013;65:2081-2089.

5. Zoran Z, Ivan V, Egon B, Dubravka V, Vjekoslav W, Vjekoslav K. Knee stability after arthroscopic anterior cruciate ligament reconstruction using the middle third of the patellar ligament and quadrupled hamstring tendons grafts—a two-year follow-up. Injury. 2015;46(Suppl 6):S91-S95.

6. Carmichael JR, Cross MJ. Why bone-patella tendon-bone grafts should still be considered the gold standard for anterior cruciate ligament reconstruction. Br J Sports Med. 2009;43: 323-325.
7. Slone HS, Romine SE, Premkumar A, Xerogeanes JW. Quadriceps tendon autograft for anterior cruciate ligament reconstruction: a comprehensive review of current literature and systematic review of clinical results. *Art Ther.* 2015;31:541-554.

8. Pearson K, Davin AG. On the sesamoids of the knee-joint: part II. Evolution of the sesamoids. *Biometrika.* 1921;13:350-400.

9. Sutton FS Jr, Thompson CH, Lipke J, Kettelkamp DB. The effect of patellectomy on knee function. *J Bone Joint Surg Am.* 1976;58:537-540.

10. Eyal S, Blitz E, Shwartz Y, Akiyama H, Schweitzer R, Zelzer E. On the development of the patella. *Development.* 2015;142:1831-1839.

11. Martin JF, Olson EN. Identification of a prx1 limb enhancer. *Genes Dev.* 2000;21:1531-1538.

12. Hyde G, Boot-Handford RP, Wallis GA. Col2a1 lineage tracing reveals that the meniscus of the knee joint has a complex cellular origin. *J Anat.* 2008;213:531-538.

13. Holder N. An experimental investigation into the early development of the chick elbow joint. *J Embryol Exp Morphol.* 1977;39:115-127.

14. Mitrovic D. Development of the diarthroial joints in the rat embryo. *Am J Anat.* 1978;151:475-485.

15. Koyama E, Shibukawa Y, Nagayama M, et al. A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletogenesis. *Dev Biol.* 2008;316:62-73.

16. Shwartz Y, Viukov S, Krief S, Zelzer E. Joint development involves a continuous influx of Gdf5-positive cells. *Cell Rep.* 2016;15:2577-2587.

17. Hyde G, Dover S, Aszodi A, Wallis GA, Boot-Handford RP. Lineage tracing using matrilin-1 gene expression reveals that articular chondrocytes exist as the joint interzone forms. *Dev Biol.* 2007;304:825-833.

18. Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate smoothened functions at the primary cilium. *Nature.* 2005;437:1018-1021.

19. Humke EW, Dorn KV, Milenkovic L, Scott MP, Rohatgi R. The output of hedgehog signaling is controlled by the dynamic association between suppressor of fused and the Gli proteins. *Genes Dev.* 2010;24:670-682.

20. Rohatgi R, Milenkovic L, Scott MP. Patched1 regulates hedgehog signaling at the primary cilium. *Science.* 2007;317:372-376.

21. Chiang C, Litingtung Y, Lee E, et al. Cyclopia and defective axial patterning in mice lacking sonic hedgehog gene function. *Nature.* 1996;383:407-413.

22. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev.* 1999;13:2072-2086.

23. Felsenthal N, Rubin S, Stern T, et al. Development of migrating tendon-bone attachments involves replacement of progenitor populations. *Development.* 2018;145:dev165381.

24. Kicheva A, Cohen M, Briscoe J. Developmental pattern formation: insights from physics and biology. *Science.* 2012;338:210-212.

25. Wang B, Fallon JF, Beachy PA. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell.* 2000;100:423-434.

26. Ahn S, Joyner AL. Dynamic changes in the response of cells to positive hedgehog signaling during mouse limb patterning. *Cell.* 2004;118:505-516.

27. Nakamura E, Nguyen MT, Mackem S. Kinetics of tamoxifen-regulated Cre activity in mice using a cartilage-specific CreER(T) to assay temporal activity windows along the proximodistal limb skeleton. *Dev Dyn.* 2006;235:2603-2612.

28. Snippert HJ, van der Flier LG, Sato T, et al. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell.* 2010;143:134-144.

29. Kawanami A, Matsushita T, Chan YY, Murakami S. Mice expressing GFP and CreER in osteochondro progenitor cells in the periosteum. *Biochem Biophys Res Commun.* 2009;386:477-482.

30. Soeda T, Deng JM, de Crombrugghe B, Behringer RR, Nakamura T, Akiyama H. Sox9-expressing precursors are the cellular origin of the cruciate ligament of the knee joint and the limb tendons. *Genesis.* 2010;48:635-644.

31. Boden BP, Dean GS, Feagin JA Jr, Garrett WE Jr. Mechanisms of anterior cruciate ligament injury. *Orthopedics.* 2000;23:573-578.

32. Mullaji AB, Marawar SV, Simha M, Jindal G. Cruciate ligaments in arthritic knees: a histologic study with radiologic correlation. *J Arthroplasty.* 2008;23:567-572.

33. Samiric T, Parkinson J, Ilic MZ, Cook J, Feller JA, Handley CJ. Changes in the composition of the extracellular matrix in patellar tendinopathy. *Matrix Biol.* 2009;28:230-236.

34. Furumatsu T, Hachioji M, Saiga K, Takata N, Yokoyama Y, Ozaki T. Anterior cruciate ligament-derived cells have high chondrogenic potential. *Biochem Biophys Res Commun.* 2010;391:1142-1147.

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