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Articular cartilage and joint development from embryogenesis to adulthood

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Abstract

Within each synovial joint, the articular cartilage is uniquely adapted to bear dynamic compressive loads and shear forces throughout the joint's range of motion. Injury and age-related degeneration of the articular cartilage often lead to significant pain and disability, as the intrinsic repair capability of the tissue is extremely limited. Current surgical and biological treatment options have been unable to restore cartilage *de novo*. Before successful clinical cartilage restoration strategies can be developed, a better understanding of how the cartilage forms during normal development is essential. This review focuses on recent progress made towards addressing key questions about articular cartilage morphogenesis, including the origin of synovial joint progenitor cells and postnatal development and growth of the tissue. These advances have provided novel insight into fundamental questions about the developmental biology of articular cartilage, as well as potential cell sources that may participate in joint response to injury.

Keywords

articular cartilage; joint formation; lineage tracing; progenitor cells; cartilage development

1. Introduction

During postnatal growth, the articular cartilage undergoes a series of tremendous structural and functional changes. While the tissue is highly cellular and isotropic at birth, unique zones develop as the tissue matures. This unique zonal architecture allows the articular cartilage to withstand significant shear and compressive forces throughout a joint's range of motion (Gannon et al., 2014; Helminen et al., 2000; Mienaltowski et al., 2008). At the surface adjacent to the joint cavity, the superficial zone is composed of elongated, flattened cells oriented parallel to the articular surface. These superficial cells play a key role in maintaining frictionless joint motion through production of hyaluronate, phospholipids and *Prg4*/lubricin (Jay et al., 2001). The adjacent underlying intermediate/transitional zone is made of slightly larger and rounder chondrocytes oriented more randomly and separated by

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appreciable matrix. The largest of the cartilage zones, the deep zone, consists of very large, round chondrocytes often found aligned in vertical stacks oriented perpendicularly to the articular surface. At the base of the articular cartilage, the subchondral junction provides physical stability and link to underlying bone (Broom and Poole, 1982). Throughout the articular cartilage, an abundant extracellular matrix composed primarily of collagen II organized in fibrils, and aggrecan organized into multimeric superstructures, provides the tissue with its key tensile strength and elasticity.

During normal aging and in response to injury, some or all of these vital components are often compromised. The intrinsic repair capacity of the articular cartilage is notoriously poor, and lost cartilage is often replaced by a structurally and functionally inferior fibrous scar tissue (Mienaltowski et al., 2009). While common surgical and biological treatment techniques are often able to temporarily improve joint function and reduce pain, they fail to reproduce the native characteristics of articular cartilage and are only partially effective long-term (Huey et al., 2012). In order for more successful reparative strategies to be developed, a better understanding of normal articular cartilage development is essential. Interestingly, there are indications that immature articular cartilage has at least partial innate regeneration capacity, although this ability appears to be lost with increasing age (Calandruccio and Gilmer, 1962; Ikegawa et al., 2015; Matsuoka et al., 2015; Namba et al., 1998). Key questions regarding the origin, fate, and role of synovial joint progenitor cells which may contribute to repair have been recently addressed, although not yet fully resolved. Such knowledge could be leveraged to create novel biological and pharmacological treatments designed to exploit normal articular cartilage biology. Such strategies have been widely used in other fields, although not yet fully realized in cartilage repair (Nicholas and Kriegstein, 2010; Szabo et al., 2010; Zhou and Melton, 2008). This review focuses on recent advances in knowledge of embryonic and postnatal articular cartilage development, growth and morphogenesis, providing essential insight into not only the developmental biology of the articular cartilage, but also into potential biomedical strategies for repair.

2. Origin of synovial joint progenitor cells

Within the uninterrupted cartilaginous anlagen of developing limbs, the first explicit sign of joint development is marked by the appearance of a region of flattened, condensed cells at putative joint sites. This compact region of mesenchymal cells has been classically defined as the interzone, and early studies found that its removal from chick embryos prevented formation of limb joints over time (Holder, 1977). The histological appearance of the interzone varies by developmental stage, joint location and species. Mitrovic described the interzone in the chick as having three distinct layers, including an intermediate zone consisting of dense, flattened cells in between layers of “chondrogenic” cells (Mitrovic, 1977). The putative mouse knee has also been described as consisting of a dense intermediate compartment and two flanking outer compartments with more loosely arranged cells (Hyde et al., 2007; Jenner et al., 2014). As the joint site forms, cells within the interzone region cease expression of early cartilage markers *Col2a1* and *Matn1,* and may be identified by increasingly restricted expression of *Wnt4*, *Wnt9a*, *Dcx*, *Gdf5* and *Erg* (Guo et al., 2004; Hartmann and Tabin, 2001; Hyde et al., 2008; Hyde et al., 2007; Spater et al.,

2006; Storm et al., 1994b; Iwamoto et al., 2007). Exploiting these unique gene expression patterns, several groups have developed transgenic mouse lines to gain further insight into the origin and eventual fate of these early cell populations. At early stages, *Gdf5* mRNA is highly expressed in regions flanking future joint sites, within the flattened intermediate interzone, and also, although less abundantly, in the outer interzone and adjacent regions of the cartilaginous anlagen (Storm and Kingsley, 1996). We and others have utilized compound *Gdf5*^{Cre};*ROSA*-reporter mice to investigate the lineage of early *Gdf5*-expressing cell populations at future joint sites (Decker et al., 2015; Dyment et al., 2015; Koyama et al., 2008; Rountree et al., 2004). While *Gdf5*mRNA expression in joint tissues is highly diminished or absent by the time of birth, *Gdf5*^{Cre};*R26R*^{LacZ} (*Gdf5*^{Cre}+) labeled cells are found within most mouse joint tissues into maturity - including the articular cartilage, synovial lining, meniscus and intrajoint ligaments (Fig. 1 A, C, E). This suggests that cells with a *Gdf5*-expressing lineage are not transient, actively take part in joint tissue formation, and constitute a progenitor cell cohort endowed with joint-formation capacity. After these initial experiments, it remained unclear if the broad cell population labeled by *Gdf5* was made of progenitors with multiple tissue differentiation capacity or included specific subsets of cells with unique roles in joint development.

More recent work from several groups has addressed these key questions, and there is increasing evidence that synovial joint tissues may arise from cell populations originally contained within, as well as those flanking the primordial cartilaginous anlagen. Notably, populations of cells within each of these regions display *Gdf5* expression during early stages of joint development (Koyama et al., 2007; Rountree et al., 2004). As the interzone appears at sites previously occupied by chondrocytes, it was originally proposed that cells within the interzone were direct descendants of de-differentiated chondrocytes (Craig et al., 1987; Nalin et al., 1995). Using *Col2a1*^{Cre};*R26R*^{LacZ} and *Matn1*^{Cre};*R26R* ^{LacZ} reporter mice, Hyde and collaborators demonstrated that cells within the cartilaginous anlagen ceased *Col2a1* expression as the histological interzone was formed, later giving rise to portions of the articular cartilage, cruciate ligament and inner medial meniscus of the knee (Hyde et al., 2008; Hyde et al., 2007). Interestingly, these authors also noted that a band of chondrocytes adjacent to, but not within, the region of flattened cells constituting the intermediate histological interzone lacked *Matn1* expression and gave rise to articular chondrocytes. While *Col2a1* expression ceases within the intermediate interzone as the joint forms, expression of doublecortin (*Dcx*) is maintained. Studies on *Dcx*-reporter mice found that *Dcx* is initially expressed throughout the limb mesenchyme, and is maintained within the interzone but lost in the adjacent regions of the cartilaginous anlagen (Zhang et al., 2011). *Dcx* expression also overlaps that of the transcription factor *Sox9*, which is expressed by osteo - and chondro- progenitors in the developing limb mesenchyme (Akiyama et al., 2005; Zhang et al., 2011). Soeda and collaborators used *Sox9*$^{LacZ/+}$ mice as well as an inducible *Sox9*^{CreERT2}/+;R26R system to investigate stage dependent expression and lineage of *Sox9*+ cells (Soeda et al., 2010). *Sox9*$^{LacZ/+}$ expression was found in the knee interzone prior to embryonic day 13.5 (E13.5), and was thereafter limited exclusively to the outer regions of the interzone and flanking chondrocytes. When Sox9^{CreERT2}/+;R26R mice were injected with tamoxifen prior to E13.5, *Sox9+* cells were found within the cruciate ligaments, and injection after E14.5 resulted in a marked reduction of labeled cells. Thus, the authors

concluded that cells within the intermediate region of the interzone likely give rise to the cruciate ligaments. Hyde and collaborators (2008) also noted that at later stages during joint formation cells without a *Col2a1* lineage appear to invade the joint to form the ligaments, indicating that invading cell populations may combine with those in the original anlagen during morphogenesis of unique joint tissues. Indeed, earlier studies had also found that DiI labeled cells flanking the putative joint sites later migrated into developing chick joints *in ovo* (Pacifici et al., 2006). To determine if these flanking cell populations had a separate ancestry from those within the interzone, Koyama and collaborators crossed $Gdf5^{Cre};R26R^{LacZ}$ and Indian hedgehog null ($Ihh^{-/-}$) mice (Koyama et al., 2007). In the absence of *Ihh*, synovial joints failed to form (St-Jacques et al., 1999). Interestingly, we found that populations of $Gdf5^{Cre}$+ cells did form in regions flanking, but not within, prospective joint sites and expressed joint site-associated marker genes including *Erg* and *Tnc* (Decker et al., 2014; Koyama et al., 2007). Taken together, these data suggest that populations of joint progenitor cells broadly labeled by *Gdf5* are indeed of heterogeneous origin, consisting of de-differentiated chondrocytes from within the cartilaginous anlagen as well as from regions surrounding future joint sites. Further characterization of these flanking cell populations has been provided by Li and collaborators, who identified populations of *Tgfbr2*-expressing cells flanking the dorsal and ventral regions of digit joints (Li et al., 2012). Over time, these distinct cell populations were maintained in these local niches, eventually giving rise to cells in the groove of Ranvier, meniscal surface, synovial lining, and outer ligaments.

More recently, additional novel *Cre* mouse lines have been developed to investigate cell niches within developing synovial joints. In constitutively active $Gdf5^{Cre}$ mice, broad labeling of *R26R*-reporter cells is seen throughout multiple joint tissues (Fig. 1 A, C, E, Koyama et al., 2008). We recently developed a novel inducible BAC transgenic $Gdf5^{CreERT2}$ mouse line, and when the mice were crossed with $R26R^{zsGreen}$ mice, we were able to more selectively, although less abundantly, label specific cell populations (Decker et al., 2016). For example, when tamoxifen was administered at late embryonic time points, $Gdf5^{CreERT2}$+ cell labeling was more restricted to cells within the eventual articular cartilage. (Fig. 1 B, Decker et al., 2016). These positively labeled cells and/or their progeny remained in the articular cartilage through at least 6 months of age (Fig. 1 D, F).

The histological interzone, as well as immediately flanking regions within the cartilaginous anlagen, are broadly labeled by *Gdf5* but may have distinct destinies. Jenner and collaborators utilized laser capture microdissection to investigate gene expression in multiple regions of the interzone (Jenner et al., 2014). These authors found that genes associated with joint formation were more evident in cells from the intermediate compartment of the histological interzone, while genes associated with cartilage maturation and hypertrophy were over-represented in outer compartment/flanking cells. They concluded that the intermediate compartment of the histological interzone gave rise to articular cartilage, while cells in outer compartments were destined for endochondral ossification and thus part of the putative secondary ossification center formation. A very recent study has provided further insight into the origin and fate of *Gdf5* expressing cells surrounding the cartilaginous anlagen, as well as within the intermediate and flanking regions of the histological interzone. Shwartz and collaborators utilized a novel knock-in *Gdf5*CreER mouse line to perform a detailed analysis of $Gdf5^{CreER}$+ cell labeling and fate during various embryonic stages

(Shwartz et al., 2016). Through this approach, they were able to uniquely label sub-populations of cells by E18.5. These authors proposed that a continuous influx of cells which are *Gdf5* negative, *Sox9* positive and *Col2a1* negative migrate into the interzone of developing joints, where they later begin to express *Gdf5*. Notably, the onset, timing and localization of *Gdf5* expression may play a key role in lineage divergence to specific joint tissues. For example, active *Gdf5* expression is maintained for longer periods of time in cells that will contribute to the meniscus and articular cartilage, but is lost early in cells that will eventually contribute to the epiphysis. Together, these exciting studies have demonstrated that the articular cartilage and other synovial joint tissues arise from a broad, heterogeneous interzone progenitor cell population, which is found within the developing anlagen and is supplemented by progenitors flanking the prospective joint sites.

3. Mechanisms Regulating Synovial Joint Formation

Although initially mesenchymal in character, joint progenitors clearly undergo time and tissue specific phenotypic changes as the joint matures. Many recent studies have provided insight into mechanisms regulating joint progenitor cell phenotype and gene expression over time. While it has long been associated as a marker for developing joints, the control, and role of, *Gdf5* at putative joint sites remains under investigation. Absent or aberrant *Gdf5* expression results in malformations of the developing limb, including shortening of long bones and absence of cruciate ligament development (Harada et al., 2007; Storm et al., 1994; Shwartz et al., 2016). During early stages of limb development, *Gdf5* promotes cell adhesion, while likely increasing chondrocyte proliferation at later stages (Francis-West et al., 1999). Kan and collaborators found that *Gdf5* expression might be at least partially regulated by *Sox11,* which transitions from broad expression patterns in prechondrogenic limb condensations to become increasingly restricted to putative joint sites as development progresses (Kan et al., 2013). However, Bhattaram and collaborators did not observe *Sox11* expression at presumptive joint sites, but did find strong expression of *Sox4* (Bhattaram et al., 2014). These authors found an increase in *Gdf5* expression in $Sox4^{fl/fl}Prx1^{Cre}$ and $Sox11^{fl/fl}Prx1^{Cre}$ mutant mouse embryos, while Kan and collaborators found that overexpression of Sox11 stimulated *Gdf5* expression in vitro. Gao and coworkers showed that the zinc finger transcription factors *Osr1* and *Osr2* were not needed for onset of *Gdf5*, *Wnt4* and *Wnt9a* expression in incipient limb interzones, but in their absence the expression of those interzone genes was not sustained and was followed by fusion of limb joints over embryonic time (Gao et al., 2011). Ablation of Wnt/β-catenin in $Gdf5^{Cre}$/β-$catn^{fl/fl}$ mice was previously shown to result in defective joint formation, highlighting its importance in regulation of embryonic joint morphogenesis. At later stages, Wnt signaling is also required to sustain the function and regulate the thickness of the joint's superficial zone (Yasuhara et al., 2011; Yuasa et al., 2009). Searching for upstream regulators of *Wnt9a* expression, Kan and Tabin identified *c-Jun* as a pivotal regulator acting at the enhancer level that when ablated, deranged both Wnt signaling and the initiation and progression of joint formation (Kan and Tabin, 2013).

Further analysis of gene expression at putative joint sites was performed by Longobardi and collaborators using laser capture-assisted gene arrays of tissue samples from E14.5 mouse embryo digits. They found that joint-forming interzone cells were characterized by low

expression of chemokines, and in particular *Mcp5,* compared to adjacent chondrocytes constituting the putative growth plate regions (Longobardi et al., 2012). In absence of *Tgfbr2,* limb synovial joints did not form (Seo and Serra, 2007; Spagnoli et al., 2007). Longobardi and colleagues found that joint formation could be rescued in *Tgfbr2*-deficient mouse embryos by concurrent blockade of the *Mcp5* receptor *Ccr2*. These authors concluded that joint formation is closely linked to, and coordinately regulated with, shaft development and that the *TgfβRII/Mcp5* axis is an essential crossroad for this process. In their study described above, Jenner and colleagues also performed transcriptional profiling of specific regions within putative joints (Jenner et al., 2014). In the mouse knee, they similarly found that genes related to inflammation and actin cytoskeletal organization were differentially regulated in the intermediate interzone. Clearly, many distinct effectors and molecular regulators act at the local and long-range levels and participate in the regulation of interzone gene expression patterns, fate and function (Decker et al., 2014; 2015).

4. Postnatal Articular Cartilage Morphogenesis

At birth, the articular cartilage exists as a dense tissue consisting of small cells within scant matrix. As described above, the tissue undergoes tremendous structural changes over postnatal life, increasing in thickness and acquiring a distinct zonal organization. How the articular cartilage grows and acquires its important zonal organization, and whether joint progenitors persist postnatally and participate in tissue morphogenesis have remained long standing questions. Early studies suggested that a region of proliferating cells “subjacent to the gliding surface of the joint” was responsible for interstitial growth of articular cartilage and increasing thickness of the articular surface (Mankin, 1962). In this same study, Mankin and collaborators found that proliferation continued within deeper regions of the tissue and adjacent to the calcified cartilage, but ceased within the sub-superficial zone at later stages of postnatal growth. The presence of these two proliferative cell regions was confirmed by tritiated thymidine incorporation in the articular cartilage of immature rabbits (Mankin, 1962; Mankin, 1963). Later, Archer and collaborators confirmed the presence of a proliferative cell region in the superficial zone, suggesting that these cells were primarily responsible for the appositional growth and thickening of the articular cartilage postnatally (Archer et al., 1994; Hayes et al., 2001). Unlike the previous studies, however, these authors did not find regions of proliferative cells within the deeper regions of the articular cartilage. Later, Hunziker and collaborators identified a population of bromodeoxyuridine (BrdU) labeled slow-cycling cells in the superficial zone, as well as more rapidly proliferating regions of chondrocytes within the deeper zones as detected by concurrent ^{3}H-thymidine labeling (Hunziker et al., 2007). These authors hypothesized that lateral expansion of the articular surface could be attributed to proliferation of cells within the superficial zone that would also give rise to daughter cells in a more rapidly proliferating cell population in the deeper zones leading presumably to vertical tissue growth.

Recently developed transgenic mouse models have permitted more detailed cell tracing and tracking during postnatal development. Kozhemyakina and collaborators created a novel inducible *Prg4*^{CreER} knock-in mouse line. The *Prg4* gene encodes diverse products, including lubricin/superficial zone protein (SZP), which is secreted by articular cartilage surface zone cells and synovial cells and is thought to play an essential role in joint

lubrication (Rhee et al., 2005). In contrast with the earlier studies described above, which found proliferating cells throughout specific zones or within multiple zones, these authors found that tamoxifen administration to *Prg4*^{CreER} mice at E17.5 resulted in *R26R*^{LacZ} labeling in only a *single* layer of cells at the articular surface by birth (Kozhemyakina et al., 2015). By one month of age, the labeled cells and/or their progeny were found throughout the entire thickness of the articular cartilage, leading the authors to conclude that the early *Prg4*-expressing superficial cell population served as progenitors which later gave rise to cells throughout the entire tissue by appositional growth. The initial, selective labeling of a single layer of cells *Prg4*^{CreER} ; *R26R*^{LacZ} mice was intriguing but also perplexing, as previous studies demonstrated that *Prg4* mRNA expression characterizes the entire incipient articular cartilage at late embryonic and neonatal stages (Iwamoto et al., 2007; Rhee et al., 2005). Indeed, when we repeated these experiments using these now commercially available knock-in *Prg4*^{CreER} mice, *R26R*^{LacZ} labeled cells were found throughout the entire thickness of the articular cartilage at birth after single tamoxifen administration at E17.5 (Decker et al., 2016). Thus, it is plausible that the patterns of reporter activation and cell progeny behavior observed and reported by Kozhemyakina may not accurately reflect those of endogenous *Prg4* expressing cell populations in either wild-type or *Prg4*^{CreER} ; *R26R*^{LacZ} mice. To mitigate concerns over haploinsufficiency in the knock-in *Prg4*^{CreER} mice, we recently developed novel BAC transgenic *Prg4*$^{\text{CreERT2}}$ mice, which maintain both functional copies of the *Prg4* gene. After tamoxifen administration at E17.5, *Prg4*$^{\text{CreERT2}/}$*R26R*^{tdTomato} labeled cells were found throughout the entire thickness of the articular cartilage at birth, recapitulating endogenous *Prg4* mRNA expression patterns at the stage of injection (Decker et al., 2016). After tamoxifen administration at later stages, we found populations of *Prg4*$^{CreERT2/}$*R26R*^{tdTomato} labeled cells were increasingly restricted to the superficial zone, accurately reflecting changes in *Prg4* mRNA over increasing postnatal age. While these *Prg4*$^{CreERT2/}$*R26R*^{tdTomato} labeling patterns did reflect endogenous gene expression patterns, they did not permit selective labeling and tracing of unique cell populations within the articular cartilage.

Patterns of chondrocyte proliferation during articular cartilage maturation are not well understood. We recently utilized *Prg4*^{CreERT2}/*R26R*^{Confetti} mice to examine spatial expansion of *Prg4*-labeled cell populations postnatally. In *R26R*^{Confetti} mice, individual cells are traced by one of four color reporters (GFP, YFP, RFP and CFP), thus permitting simultaneous tracking of distinct cell progenies and their developmental roles. As with the single-color *R26R*^{tdTomato} reporter, *Prg4*^{CreERT2}/ *R26R*^{Confetti} labeled cells were found throughout the entire thickness of the articular cartilage after tamoxifen administration at E17.5 and collection at P0 (Decker et al., 2016). Over time, we found that clusters of uniquely-colored cells appeared to proliferate locally during postnatal growth, later aligning to form characteristic chondrocyte “stacks” with a mosaic color pattern. These experiments were repeated using the more broadly expressed *Gdf5*$^{\text{Cre}}$ as well as ubiquitous, non-biased *ROSA*^{CreER} mice, confirming that embryonically-labeled chondrocyte progenitors produced only small non-migratory progenies and that non-daughter lineage cells produced the vertical chondrocyte stacks, a result at variance with previous appositional models.

In the growth plate, tremendous increases in chondrocyte volume contribute greatly to lengthening of long bones (Farnum and Wilsman, 1998). To investigate changes in articular

chondrocyte volume during growth, we used multiphoton microscopy to evaluate chondrocyte size within the intact articular cartilage of mice harvested at various stages of postnatal development (Decker et al., 2016). We found that chondrocyte volume in the middle and deep layers increased by over 8 fold from birth to 2 months of age, while overall decreases in cell density reflecting an increase in extracellular matrix production occurred during this same period. Strikingly, we also observed that proliferation of uniquely labeled $ROSA^{CreER}/R26R^{Confetti}$ articular chondrocytes was limited after birth. Taken together, this suggests that, rather than being driven by proliferation and apposition of daughter cells from the surface to the deep zone to create cell columns (Dowthwaite et al., 2004; Hunziker et al., 2007; Kozhemyakina et al., 2015), thickening of the articular cartilage occurs primarily through an increase in the volume of articular chondrocytes and is aided by accumulation of extracellular matrix and formation of chondrocyte stacks. However, important questions remain unanswered regarding the mechanisms responsible for regulation of articular chondrocyte volume. This phenomenon has been more extensively studied in the growth plate, where changes in volume may be attributed partially to cell swelling and largely to intrinsic cell hypertrophy (Bush et al., 2008). Chondrocyte hypertrophy in the growth plate is a complex, directional event, in which volume progressively increases with distancing from the proliferating zone (Smits et al., 2004). Interestingly, the maximum size of articular chondrocytes is only about 60% of that found in the largest chondrocytes of the growth plate, indicating that location and function may uniquely dictate maximum chondrocyte volume. (Decker et al., 2016; Ginzberg et al., 2015; Wilsman et al., 1996; Youn et al., 2006). For example, articular chondrocytes are distinctively adapted to respond to inherent biomechanical stress. Many cell types display significantly negative resting membrane potentials, permitting rapid changes in membrane permeability in response to changes in osmotic pressure (Lewis et al., 2011). However, articular chondrocytes have less negative resting membrane potential, maintained by gadolinium-sensitive cation channels and permitting response to osmotic changes induced by biomechanical forces with only minimal changes in cell volume (Lewis et al., 2011).

Structurally, the cellular stacks of cells in mature articular cartilage likely form through alignment and repositioning of neighboring cells. This is in contrast to the distinct columns of chondrocytes in the growth plate, in which each individual column arises from a common ancestor (Decker et al., 2016). At birth, collagen fibrils within the extracellular matrix of the articular cartilage display an anisotropic distribution, later undergoing changes in deposition and orientation to form highly organized structures in the mature tissue (Clark et al., 1997; Hughes et al., 2005; Youn et al., 2006). It remains unclear if such changes within the extracellular matrix directly influence chondrocyte alignment, or if additional mechanisms may play a role. In embryonic structures, cell intercalation and convergent extension have been shown to directly influence cell migration (Tada and Heisenberg, 2012). During convergent extension, tissue growth and elongation occur while cells undergo very little proliferation, as is seen in the articular cartilage (Decker et al., 2016). The planar cell polarity pathway components *Vangl2* and *Ror2* regulate convergent extension and alignment of newly-formed chondrocytes along the proximo-distal axis of long bone cartilaginous rods during early limb skeletogenesis and patterning, and may play a role in postnatal tissue morphogenesis as well (Gao et al., 2011; Randall et al., 2012). Clearly, much work remains

to be done in investigating mechanisms directing postnatal articular cartilage morphogenesis.

5. Mature Articular Cartilage

The limited intrinsic repair capacity of mature articular cartilage is widely appreciated, and major efforts have been directed towards surgical and biological restoration of the tissue in arthritic patients (Caldwell and Wang, 2015; Johnstone et al., 2013; Mollon et al., 2013). However, substantial challenges are posed by the low cell density and avascular and aneural characteristics of mature articular cartilage. The limited ability of chondrocytes to proliferate and the essentially permanent nature of the extensive collagen matrix explain the failure of the tissue to turnover once it has reached maturity (Heinemeier et al., 2016). Several studies have demonstrated the presence of cells with progenitor potential in adult joint tissues (Candela et al., 2014a; Candela et al., 2014b; Dowthwaite et al., 2003; Grogan et al., 2009; Williams et al., 2010), and there is growing interest in further characterization and exploitation of these cell populations for repair strategies. Many key questions remain, including the origin, fate, and role of these populations in intrinsic repair.

There is increasing evidence that embryonically-derived cell populations with a *Gdf5, Dkk3*, and/or *Prg4* lineage remain present in joint tissues at maturity (Decker et al 2016; Koyama et al., 2008; Kozhemyakina et al., 2015). Sub-populations of slow-cycling cells have been identified in several tissues, including the articular cartilage and synovium. Early studies using tritiated thymidine demonstrated that embryonically labeled cells remained in the articular surface long-term (Ohlsson et al., 1992). More recent studies have confirmed the presence of cells with a progenitor or stem character in the superficial zone of adult articular cartilage (Alsalameh et al., 2004; Candela et al., 2014a; Dowthwaite et al., 2004; Williams et al., 2010b; Yasuhara et al., 2011). At the onset of osteoarthritis, cells in the superficial zone show increased proliferation and rearrangement (Rolauffs et al., 2010), but it is unknown how these cells may respond to acute injury. In the synovium, slow cycling cells expressing characteristic mesenchymal stem cell markers undergo proliferation and chondrogenic differentiation in response to injury (Kurth et al., 2011). Using novel cell lineage tracing strategies, we have recently demonstrated that embryonically labeled cells with a *Prg4*-expressing lineage respond massively to acute injury (Decker et al., 2016). After creation of a focal, full-thickness chondral defect, *Prg4*+ cells labeled at E17.5 were found within the defect site by 7d after injury (Fig. 2 D). EdU labeling indicated that cell proliferation within the synovium was much greater than within the articular cartilage adjacent to the defect site, suggesting that *Prg4*+cells within the synovium may play a primary role in the early injury response. Previous studies have also suggested that the synovium may be an essential source of cells contributing to articular cartilage injury, and our new data provide additional insight into the lineage and character of these cells (Kurth et al., 2011; Miyamoto et al., 2007; Rothwell, 1990). Future techniques designed to target synovium-associated *Prg4* + cells or inclusion of these cells in bioengineered constructs may provide promise for repair of damaged articular cartilage.

6. Conclusion

Current clinical strategies for articular cartilage repair have failed to replicate the structure and function of innate articular cartilage. In recent years, novel genetic cell lineage tracing techniques have provided tremendous insight into the origin and morphogenesis of the articular cartilage during normal development, and this knowledge may be extremely useful for developing novel biological repair strategies. Taken together, these studies have revealed that the articular cartilage and other joint tissues have a heterogeneous origin, arising from de-differentiated chondrocytes as well as cells surrounding the cartilaginous anlagen. Postnatally, chondrocyte volume increase, matrix production, and realignment drive the growth and morphogenesis of the articular cartilage into a multifaceted tissue. While we now have a greater understanding of cell origin and fate, there is much work left to be done to decipher the molecular mechanisms responsible for guiding these processes and to find appropriate ways to manipulate them for therapeutic strategies.

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References

Akiyama H, Kim J-E, Nakashima K, Balmes G, Iwai N, Deng JM, Zhang Z, Martin JF, Behringer RR, Nakamura T, et al. Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. Proc. Natl. Acad. Sci. U.S.A. 2005; 102:14665–14670. [PubMed: 16203988]

Alsalameh S, Amin R, Gemba T, Lotz M. Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. Arthritis Rheum. 2004; 50:1522–1532. [PubMed: 15146422]

Archer CW, Morrison H, Pitsillides AA. Cellular aspects of the development of diarthrodial joints and articular cartilage. J. Anat. 1994; 184:447. [PubMed: 7928634]

Bhattaram P, Penzo-Méndez A, Kato K, Bandyopadhyay K, Gadi A, Taketo MM, Lefebvre V. SOXC proteins amplify canonical WNT signaling to secure nonchondrocytic fates in skeletogenesis. J. Cell. Biol. 2014; 207:657–671. [PubMed: 25452386]

Broom ND, Poole C. A functional-morphological study of the tidemark region of articular cartilage maintained in a non-viable physiological condition. J. Anat. 1982; 135:65. [PubMed: 7130057]

Bush PG, Parisinos CA, Hall AC. The osmotic sensitivity of rat growth plate chondrocytes in situ; clarifying the mechanisms of hypertrophy. J. Cell. Physiol. 2008; 214:621–629. [PubMed: 17786946]

Calandruccio RA, Gilmer WSJR. Proliferation, Regeneration, and Repair of Articular Cartilage of Immature Animals. J. Bone Joint Surg. Am. 1962; 44:431–455.

Caldwell KL, Wang J. Cell-based articular cartilage repair: the link between development and regeneration. Osteoarthr. Cart. 2015; 23:351–362.

Candela ME, Cantley L, Yasuaha R, Iwamoto M, Pacifici M, Enomoto-Iwamoto M. Distribution of Slow-Cycling Cells in Epiphyseal Cartilage and Requirement of β-Catenin Signaling for Their Maintenance in Growth Plate. J. Orthop. Res. 2014a; 32:661–668. [PubMed: 24415663]

Candela ME, Yasuhara R, Iwamoto M, Enomoto-Iwamoto M. Resident mesenchymal progenitors of articular cartilage. Matrix Biol. 2014b; 39:44–49. [PubMed: 25179676]

Clark JM, Norman A, Nötzli H. Postnatal development of the collagen matrix in rabbit tibial plateau articular cartilage. J. Anat. 1997; 191:215–227. [PubMed: 9306198]

Craig FM, Bentley G, Archer CW. The spatial and temporal pattern of collagens I and II and keratan sulphate in the developing chick metatarsophalangeal joint. Development. 1987; 99:383–391. [PubMed: 2958266]

Decker RS, Koyama E, Pacifici M. Genesis and morphogenesis of limb synovial joints and articular cartilage. Matrix Biol. 2014; 39:5–10. [PubMed: 25172830]

Decker RS, Koyama E, Pacifici M. Articular Cartilage: Structural and Developmental Intricacies and Questions. Current Osteoporosis Reports. 2015; 13:407–414. [PubMed: 26408155]

Decker RS, Um H, Dyment N, Cottingham N, Usami Y, Enotomi-Iwamoto M, Kronenburg M, Maye P, Koyama E, Pacifici M. Cell reposition, volume and local proliferation drive articular cartilage development, growth and response to injury. 2016 Manuscript submitted for publication.

Dowthwaite GP, Bishop JC, Redman SN, Khan IM, Rooney P, Evans DJ, Haughton L, Bayram Z, Boyer S, Thomson B. The surface of articular cartilage contains a progenitor cell population. J. Cell Sci. 2004; 117:889–897. [PubMed: 14762107]

Dyment NA, Breidenbach AP, Schwartz AG, Russell RP, Aschbacher-Smith L, Liu H, Hagiwara Y, Jiang R, Thomopoulos S, Butler DL. Gdf5 progenitors give rise to fibrocartilage cells that mineralize via hedgehog signaling to form the zonal enthesis. Dev. Biol. 2015; 405:96–107. [PubMed: 26141957]

Farnum CE, , Wilsman NJ. Skeletal Growth and Development AAOS; Rosemont: 1998 Effects of distraction and compression on growth plate function.; 517530

Francis-West PH, Abdelfattah A, Chen P, Allen C, Parish J, Ladher R, Allen S, MacPherson S, Luyten FP, Archer CW. Mechanisms of GDF5 action during skeletal development. Development. 1999; 126:1305–1315. [PubMed: 10021348]

Gannon A, Nagel T, Bell A, Avery N, Kelly D. Postnatal changes to the mechanical properties of articular cartilage are driven by the evolution of its collagen network. Eur. Cel.l Mater. 2014; 29:105–123.

Gao B, Song H, Bishop K, Elliot G, Garrett L, English MA, Andre P, Robinson J, Sood R, Minami Y. Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. Dev. Cell. 2011; 20:163–176. [PubMed: 21316585]

Ginzberg MB, Kafri R, Kirschner M. On being the right (cell) size. Science. 2015; 348:1245075. [PubMed: 25977557]

Grogan SP, Miyaki S, Asahara H, D'Lima DD, Lotz M. Mesenchymal progenitor cell markers in human articular cartilage: normal distribution and changes in osteoarthritis. Arthr. Res. Ther. 2009; 11:R85. [PubMed: 19500336]

Guo X, Day TF, Jiang X, Garrett-Beal L, Topol L, Yang Y. Wnt/beta-catenin signaling is sufficient and necessary for synovial joint formation. Genes Dev. 2004; 18:2404–2417. [PubMed: 15371327]

Harada M, Takahara M, Zhe P, Otsuji M, Iuchi Y, Takagi M, Ogino T. Developmental failure of the intra-articular ligaments in mice with absence of growth differentiation factor 5. Osteoarthr. Cart. 2007; 15:468–474.

Hartmann C, Tabin CJ. Wnt-14 plays a pivotal role in inducing synovial joint formation in the developing appendicular skeleton. Cell. 2001; 104

Hayes AJ, MacPherson S, Morrison H, Dowthwaite G, Archer CW. The development of articular cartilage: evidence for an appositional growth mechanism. Anat. Embryol. (Berl). 2001; 203:469–479. [PubMed: 11453164]

Heinemeier KM, Schjerling P, Heinemeier J, Møller MB, Krogsgaard MR, Grum-Schwensen T, Petersen MM, Kjaer M. Radiocarbon dating reveals minimal collagen turnover in both healthy and osteoarthritic human cartilage. Science Trans. Med. 2016; 8:346ra390–346ra390.

Helminen HJ, Hyttinen MM, Lammi MJ, Arokoski JP, Lapvetelainen T, Jurvelin J, Kiviranta I, Tammi MI. Regular joint loading in youth assists in the establishment and strengthening of the collagen network of articular cartilage and contributes to the prevention of osteoarthrosis later in life: a hypothesis. J. Bone Miner. Metab. 2000; 18:245–257. [PubMed: 10959613]

Holder N. An experimental investigation into the early development of the chick elbow joint. J. Embryology Experimen. Morphol. 1977; 39

Huey DJ, Hu JC, Athanasiou KA. Unlike bone, cartilage regeneration remains elusive. Science. 2012; 338:917–921. [PubMed: 23161992]

Hughes L, Archer C, Ap Gwynn I. The ultrastructure of mouse articular cartilage: collagen orientation and implications for tissue functionality. A polarised light and scanning electron microscope study and review. Eur. Cell Mate.r. 2005; 9:e84.

Hunziker EB, Kapfinger E, Geiss J. The structural architecture of adult mammalian articular cartilage evolves by a synchronized process of tissue resorption and neoformation during postnatal development. Osteoarthr. Cartilage. 2007; 15:403–413.

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. [PubMed: 19014360]

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. [PubMed: 17313942]

Ikegawa N, Sasho T, Yamaguchi S, Saito M, Akagi R, Muramatsu Y, Akatsu Y, Fukawa T, Nakagawa K, Nakajima A. Identification of genes required for the spontaneous repair of partial-thickness cartilage defects in immature rats. Connect. Tissue Res. 2016; 57(3):190–9. [PubMed: 26719950]

Iwamoto M, Tamamura Y, Koyama E, Komori T, Takeshita N, Williams JA, Nakamura T, Enomoto-Iwamoto M, Pacifici M. Transcription factor ERG and joint and articular cartilage formation during mouse limb and spine skeletogenesis. Dev. Biol. 2007; 305:40–51. [PubMed: 17336282]

Jay GD, Tantravahi U, Britt DE, Barrach HJ, Cha CJ. Homology of lubricin and superficial zone protein (SZP): products of megakaryocyte stimulating factor (MSF) gene expression by human synovial fibroblasts and articular chondrocytes localized to chromosome 1q25. J. Orthop. Res. 2001; 19:677–687. [PubMed: 11518279]

Jenner F, IJpma A, Cleary M, Heijsman D, Narcisi R, van der Spek PJ, Kremer A, van Weeren R, Brama P, van Osch GJ. Differential Gene Expression of the Intermediate and Outer Interzone layers of developing articular cartilage in murine embryos. Stem Cells Dev. 2014; 23(16):1883–98. [PubMed: 24738827]

Johnstone B, Alini M, Cucchiarini M, Dodge GR, Eglin D, Guilak F, Madry H, Mata A, Mauck RL, Semino CE, et al. Tissue engineering for articular cartilage repair. The state of the art. Eur. Cells Mater. 2013; 25:248–267.

Kan A, Ikeda T, Fukai A, Nakagawa T, Nakamura K, Chung U.-i. Kawaguchi H, Tabin CJ. SOX11 contributes to the regulation of GDF5 in joint maintenance. BMC Dev. Biol. 2013; 13:4. [PubMed: 23356643]

Koyama E, Ochiai T, Rountree RB, Kingsley DM, Enomoto-Iwamoto M, Iwamoto M, Pacifici M. Synovial joint formation during mouse limb skeletogenesis: roles of Indian hedgehog signaling. Ann. N. Y. Acad. Sci. 2007; 1116:100–112. [PubMed: 18083924]

Koyama E, Shibukawa Y, Nagayama M, Sugito H, Young B, Yuasa T, Okabe T, Ochiai T, Kamiya N, Rountree RB. A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletogenesis. Dev. Biol. 2008; 316:62–73. [PubMed: 18295755]

Kozhemyakina E, Zhang M, Ionescu A, Ayturk UM, Ono N, Kobayashi A, Kronenberg H, Warman ML, Lassar AB. Identification of a Prg4- expressing articular cartilage progenitor cell population in mice. Arthritis Rheum. 2015; 67:1261–1273.

Kurth TB, Dell'Accio F, Crouch V, Augello A, Sharpe PT, De Bari C. Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo. Arthritis Rheum. 2011; 63:1289–1300. [PubMed: 21538315]

Lewis R, Asplin KE, Bruce G, Dart C, Mobasheri A, Barrett-Jolley R. The Role of the Membrane Potential in Chondrocyte Volume Regulation. J. Cell. Physiol. 2011; 226:2979–2986. [PubMed: 21328349]

Li T, Longobardi L, Myers TJ, Temple JD, Chandler RL, Ozkan H, Contaldo C, Spagnoli A. Joint TGF-β type II receptor-expressing cells: ontogeny and characterization as joint progenitors. Stem cells and development. 2012; 22:1342–1359.

Mankin HJ. Localization of tritiated thymidine in articular cartilage of rabbits. J. Bone Joint Surg. 1962; 44:688–698.

Mankin HJ. Localization of tritiated thymidine in articular cartilage of rabbits. J. Bone Joint Surg. 1963; 45:529–540.

Matsuoka M, Onodera T, Sasazawa F, Momma D, Baba R, Hontani K, Iwasaki N. An articular cartilage repair model in common C57Bl/6 mice. Tissue Eng. Part C Methods: Methods. 2015; 21:767–772.

Mienaltowski MJ, Huang L, Frisbie DD, McIlwraith CW, Stromberg AJ, Bathke AC, Macleod JN. Transcriptional profiling differences for articular cartilage and repair tissue in equine joint surface lesions. BMC Med. Genomics. 2009; 2:60. [PubMed: 19751507]

Mienaltowski MJ, Huang L, Stromberg AJ, MacLeod JN. Differential gene expression associated with postnatal equine articular cartilage maturation. BMC Musculoskelet. Disord. 2008; 9:149. [PubMed: 18986532]

Mitrovic DR. Development of the metatarsophalangeal joint of the chick embryo: Morphological, ultrastructural and histochemical studies. Am. J. Anat. 1977; 150:333–347. [PubMed: 920633]

Miyamoto A, Deie M, Yamasaki T, Nakamae A, Shinomiya R, Adachi N, Ochi M. The role of the synovium in repairing cartilage defects. Knee Surg. Sports Traumatol. Arthrosc. 2007; 15:1083–1093. [PubMed: 17279425]

Mollon B, Kandel RA, Chahal J, Theodoropoulos J. The clinical status of cartilage tissue regeneration in humans. Osteoarthr. Cart. 2013; 21:1824–1833.

Nalin AM, Greenlee TK Jr. Sandell LJ. Collagen gene expression during development of avian synovial joints: transient expression of types II and XI collagen genes in the joint capsule. Dev. Dyn. 1995; 203:352–362. [PubMed: 8589432]

Namba RS, Meuli M, Sullivan KM, Le AX, Adzick NS. Spontaneous repair of superficial defects in articular cartilage in a fetal lamb model. J. Bone Joint Surg. 1998; 80:4. [PubMed: 9469302]

Nicholas CR, Kriegstein AR. Regenerative medicine: Cell reprogramming gets direct. Nature. 2010; 463:1031–1032. [PubMed: 20182502]

Ohlsson C, Nilsson A, Isaksson O, Lindahl A. Growth hormone induces multiplication of the slowly cycling germinal cells of the rat tibial growth plate. Proc. Natl. Acad. Sci. U S A. 1992; 89:9826–9830. [PubMed: 1409707]

Pacifici M, Koyama E, Shibukawa Y, WU C, Tamamura Y, Enomoto-Iwamoto M, Iwamoto M. Cellular and Molecular Mechanisms of Synovial Joint and Articular Cartilage Formation. Ann. N. Y. Acad. Sci. 2006; 1068:74–86. [PubMed: 16831907]

Randall RM, Shao YY, Wang L, Ballock RT. Activation of Wnt Planar cell polarity (PCP) signaling promotes growth plate column formation in vitro. J. Orthop. Research. 2012; 30:1906–1914. [PubMed: 22674351]

Rhee DK, Marcelino J, Baker M, Gong Y, Smits P, Lefebvre V, Jay GD, Stewart M, Wang H, Warman ML. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. J. Clin. Invest. 2005; 115:622–631. [PubMed: 15719068]

Rolauffs B, Williams JM, Aurich M, Grodzinsky AJ, Kuettner KE, Cole AA. Proliferative remodeling of the spatial organization of human superficial chondrocytes distant from focal early osteoarthritis. Arthritis Rheum. 2010; 62:489–498. [PubMed: 20112377]

Rothwell A. Synovium transplantation onto the cartilage denuded patellar groove of the sheep knee joint. Orthopedics. 1990; 13:433–442. [PubMed: 2333257]

Rountree RB, Schoor M, Chen H, Marks ME, Harley V, Mishina Y, Kingsley DM. BMP receptor signaling is required for postnatal maintenance of articular cartilage. PLoS Biol. 2004; 2:e355. [PubMed: 15492776]

Seo H-S, Serra R. Deletion of< i> Tgfbr2</i> in Prx1-cre expressing mesenchyme results in defects in development of the long bones and joints. Dev. Biol. 2007; 310:304–316. [PubMed: 17822689]

Smits P, Dy P, Mitra S, Lefebvre V. Sox5 and Sox6 are needed to develop and maintain source, columnar, and hypertrophic chondrocytes in the cartilage growth plate. The Journal of Cell Biology. 2004; 164:747–758. [PubMed: 14993235]

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 (New York, N.Y. : 2000). 2010; 48:635–644.

Spagnoli A, O'Rear L, Chandler RL, Granero-Molto F, Mortlock DP, Gorska AE, Weis JA, Longobardi L, Chytil A, Shimer K. TGF- signaling is essential for joint morphogenesis. J. Cell Biol. 2007; 177:1105. [PubMed: 17576802]

Spater D, Hill TP, O'Sullivan R J, Gruber M, Conner DA, Hartmann C. Wnt9a signaling is required for joint integrity and regulation of Ihh during chondrogenesis. Development. 2006; 133:3039–3049. [PubMed: 16818445]

St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes & Develop. 1999; 13:2072–2086. [PubMed: 10465785]

Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee S-J. Limb alterations in brachypodism mice due to mutations in a new member of the TGF[beta]-superfamily. Nature. 1994a; 368:639–643. [PubMed: 8145850]

Storm EE, Kingsley DM. Joint patterning defects caused by single and double mutations in members of the bone morphogenetic protein (BMP) family. Development. 1996; 122:3969–3979. [PubMed: 9012517]

Szabo E, Rampalli S, Risueno RM, Schnerch A, Mitchell R, Fiebig-Comyn A, Levadoux-Martin M, Bhatia M. Direct conversion of human fibroblasts to multilineage blood progenitors. Nature. 2010; 468:521–526. [PubMed: 21057492]

Tada M, Heisenberg C-P. Convergent extension: using collective cell migration and cell intercalation to shape embryos. Development. 2012; 139:3897–3904. [PubMed: 23048180]

Williams R, Khan IM, Richardson K, Nelson I, McCarthy HE, Analbelsi T. Identification and clonal characterirization of a progenitor cell sub-population in normal human articular cartilage. PLoS One. 2010a; 5:e13246. [PubMed: 20976230]

Wilsman NJ, Leiferman EM, Fry M, Farnum CE, Barreto C. Differential growth by growth plates as a function of multiple parameters of chondrocytic kinetics. J. Orthop. Res. 1996; 14:927–936. [PubMed: 8982136]

Yasuhara R, Ohta Y, Yuasa T, Kondo N, Hoang T, Addya S, Fortina P, Pacifici M, Iwamoto M, Enomoto-Iwamoto M. Roles of β-catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells. Lab. Invest. 2011; 91:1739–1752. [PubMed: 21968810]

Youn I, Choi J, Cao L, Setton L, Guilak F. Zonal variations in the three-dimensional morphology of the chondron measured in situ using confocal microscopy. Osteoarthr. Cartilage. 2006; 14:889–897.

Zhang Q, Cigan AD, Marrero L, Lopreore C, Liu S, Ge D, Savoie FH, You Z. Expression of doublecortin reveals articular chondrocyte lineage in mouse embryonic limbs. Genesis. 2011; 49:75–82. [PubMed: 21162077]

Zhou Q, Melton DA. Extreme makeover: converting one cell into another. Cell Stem Cell. 2008; 3:382–388. [PubMed: 18940730]

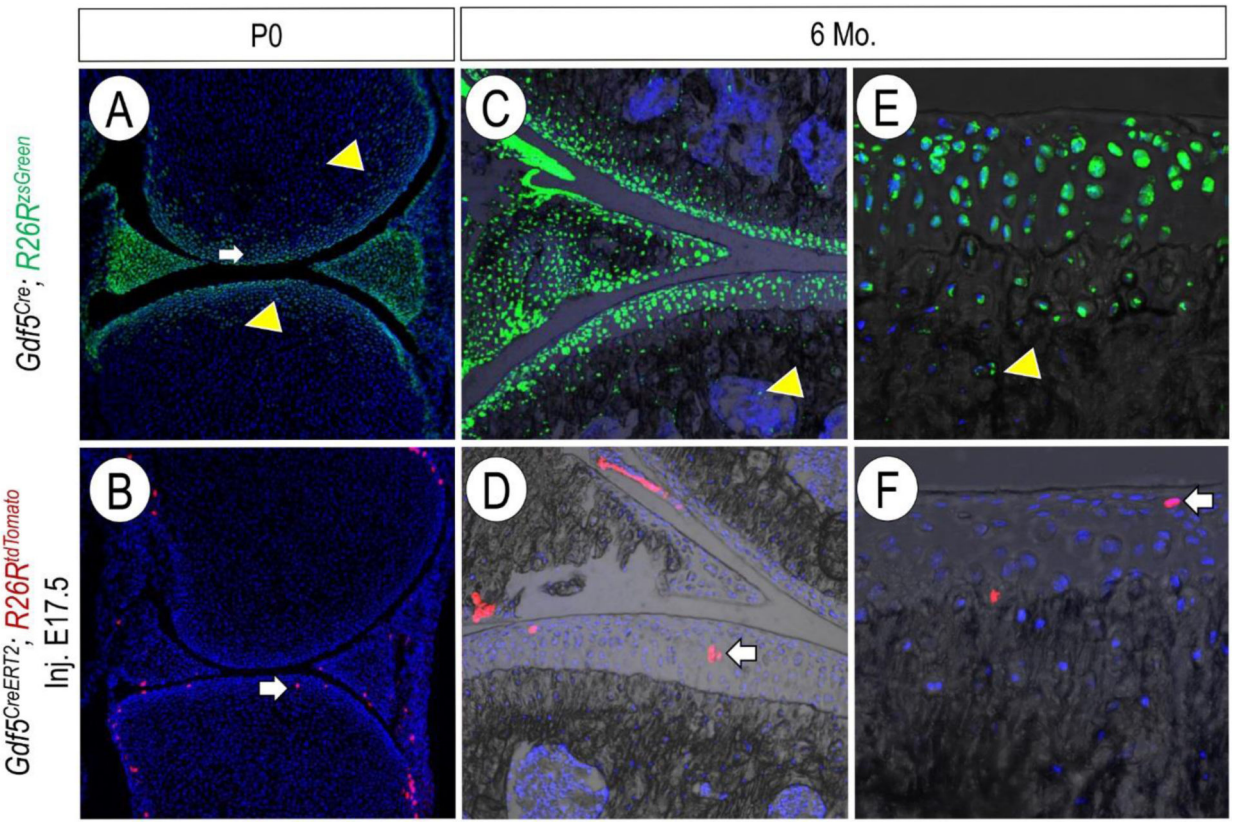

Fig. 1.

Selective labeling of *Gdf5*-expressing cells in *Gdf5*^{CreERT2} mice. At birth, *Gdf5*^{Cre};*R26R*^{zsGreen} labeled cells are found throughout the joint, including within the articular cartilage (white arrow) and the putative secondary ossification center (yellow arrowhead) (A). After tamoxifen administration at E17.5, *Gdf5*^{CreERT2}; *R26R*^{tdTomato} labeled cells are less numerous, and more restricted towards the articular cartilage (white arrow) (B). *Gdf5*^{Cre};*R26R*^{zsGreen} remain present in all joint tissues through 6 months of age, including within the secondary ossification center (yellow arrowhead, C) as well as the entire thickness of the articular cartilage (E). *Gdf5*^{CreERT2}; *R26R*^{tdTomato} labeled cells remain restricted primarily to the articular cartilage (white arrows, D-F).

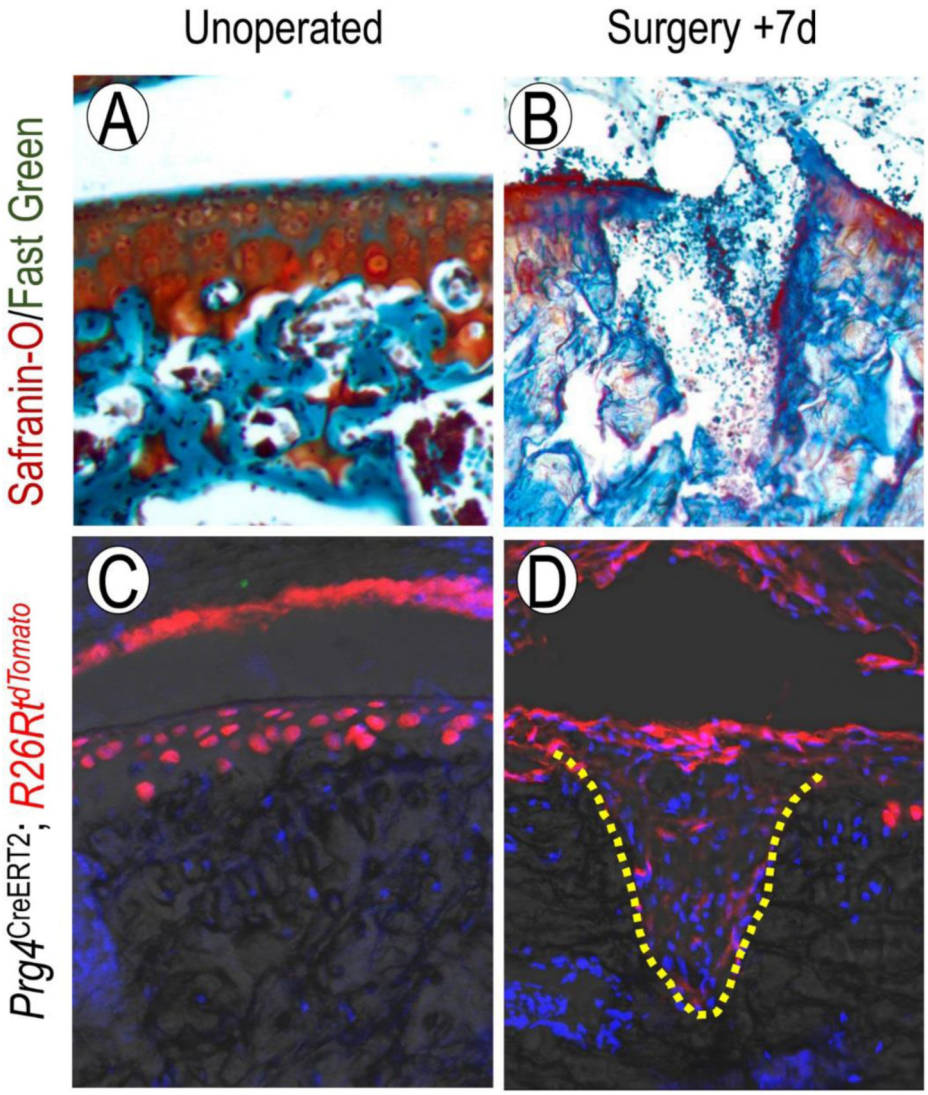

Fig. 2.

Cells with a *Prg4*+ lineage respond to articular cartilage injury. Safranin-O/Fast green staining of mature mouse femoral articular cartilage before (A) and 7 days (B) after creation of a full-thickness chondral defect. After tamoxifen administration to *Prg4*^{CreERT2}/*R26R*^{tdTomato} mice at 1month of age, labeled cells are found throughout several layers of the articular cartilage (C). One week after injury, cells within the defect site (dotted line) are *Prg4*+, indicating that cells with a *Prg4*-expressing lineage participate in the acute injury response (D).