Seminars in Cell & Developmental Biology 62 (2017) 50–56

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Articular cartilage and joint development from embryogenesis to adulthood

Rebekah S. Decker*

Translational Research Program in Pediatric Orthopaedics, Division of Orthopaedic Surgery, The Children's Hospital of Philadelphia, PA 19104, United States

ARTICLE INFO

Article history:
Received 25 July 2016
Accepted 18 October 2016
Available online 20 October 2016

Keywords:
Articular cartilage
Joint formation
Lineage tracing
Progenitor cells
Cartilage development

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, 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.

Contents

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, distinct 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 [20,29,52]. 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 [38]. The adjacent underlying intermediate/transitional zone is made of slightly larger and rounder chondrocytes oriented more randomly and separated by 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 [5]. 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 [51]. While common surgical and bio-

* Current address: Genomics Institute of the Novartis Research Foundation, San Diego, CA, 92121, United States.
E-mail address: RDecker@gnf.org

http://dx.doi.org/10.1016/j.semcdb.2016.10.005
1084-9521/

logical 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 [31]. 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 [7,36,50,57]. 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 [58,74,81]. 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 [30]. 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 [53]. 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 [35,39]. 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* [24,26,34,35,70,72,37]. 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 [73]. 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 [14,17,43,65]. 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. 1A,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 [42,65]. 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 [12,56]. 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 [34,35]. 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 [80]. *Dcx* expression also overlaps that of the transcription factor *Sox9*, which is expressed by osteo – and chondro- progenitors in the developing limb mesenchyme [1,80]. 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 [68]. $Sox9^{LacZ/+}$ 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* [60]. 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 [42]. In the absence of *Ihh*, synovial joints failed to form [71]. 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* [13,42]. 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 [47]. 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. 1A,C,E, [43]. 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 [15]. For example, when tamoxifen was administered at late embryonic time