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Mini review

Genesis and morphogenesis of limb synovial joints and articular cartilage

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ABSTRACT

Limb synovial joints are intricate structures composed of articular cartilage, synovial membranes, ligaments and an articular capsule. Together, these tissues give each joint its unique shape, organization and biomechanical function. Articular cartilage itself is rather complex and organized in distinct zones, including the superficial zone that produces lubricants and contains stem/progenitor cells. For many years there has been great interest in deciphering the mechanisms by which the joints form and come to acquire such unique structural features and diversity. Decades ago, classic embryologists discovered that the first overt sign of joint formation at each prescribed limb site was the appearance of a dense and compact population of mesenchymal cells collectively called the interzone. Work carried out since then by several groups has provided evidence that the interzone cells actively participate in joint tissue formation over developmental time. This minireview provides a succinct but comprehensive description of the many important recent advances in this field of research. These include studies using various conditional reporter mice to genetically trace and track the origin, fate and possible function of joint progenitor cells; studies on the involvement and roles in signaling pathways and transcription factors in joint cell determination and functioning; and studies using advanced methods of gene expression analyses to uncover novel genetic determinants of joint formation and diversity. The overall advances are impressive, and the findings are not only of obvious interest and importance but also have major implications in the conception of future translational medicine tools to repair and regenerate defective, overused or aging joints.

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1. Introduction

The synovial joints in the limbs—be it the elbow, the hip or interphalangeal joints—are intricate and diverse organs. They are composed of reciprocally shaped surfaces covered by articular cartilage, stabilized mechanically by intrajoint and peri-joint ligaments, and insulated from the body environment by the synovial lining and a thick surrounding synovial capsule (Archer et al., 1999). Articular cartilage itself is rather complex and is organized in histologically and phenotypically distinct zones (Hunziker et al., 2007). The superficial zone contains elongated and flat-shaped cells oriented parallel to the articular surface, held together by a largely collagenous matrix, and producing lubricin, hyaluronate and other anti-adhesive macromolecules essential for frictionless joint movement (Jay et al., 2001). Articular chondrocytes in the middle zone are round in shape, usually organized in small vertical rows, and produce and maintain important extracellular components—particularly collagen II and aggrecan—that confer to the tissue its key biomechanical feature: resilience. The chondrocytes in the bottom zone tend to be larger in size, are also active in matrix production and

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maintenance, and face the critical tissue boundary—referred to as the tidemark- between articular cartilage and underlying subchondral bone (Broom and Poole, 1982). While the structure of the articular cartilage is similar throughout all synovial joints, the location and function of each joint defines its distinct architecture. For example, in the hip, the nearly spherical and concave acetabulum articulates with the nearly spherical and convex femoral head. In the knee, the dual distal femoral condyles articulate with the relatively flat proximal tibial plateau, but make no direct contact with the fibula. The knee also contains unique fibrocartilaginous structures such as the meniscus and intrajoint ligaments, while the hip displays the centrally located teres and the interphalangeal joints have externally positioned collateral ligaments. This extraordinary variety of anatomical, histological and biomechanical features is well documented and fairly well understood in terms of its multiple essential roles in joint function, maintenance, endurance, motion, and dissipation of mechanical loads (Li et al., 2013a). In comparison, what remains unclear is how the joints form and come to acquire such a variety of structural, organizational, and biomechanical features during embryogenesis and early postnatal life, each adept and adapted to specific anatomical locations and distinct function (Archer et al., 2003; Pacifici et al., 2005; Pitsillides and Ashhurst, 2008). For instance, what are the progenitor cells that give rise to the joints and their specialized tissues? How does articular cartilage acquire its stratified organization? How are the opposing sides of a joint molded in a reciprocal lock-and-key manner? Advances in these areas would be of obvious basic research value, but could also have critical biomedical implications, possibly one day leading to treatments for congenital disorders such as developmental dysplasia of the hip or acquired age-related disorders such as osteoarthritis. This mini-review then provides a concise description of important advances in joint development research obtained in the last few years.

2. Interzone origin and cell lineage tracing-tracking

Classic studies carried out decades ago in mammalian and avian embryos showed that the cartilaginous skeleton forming in the early limb is continuous and uninterrupted, as exemplified by the Y-shaped anlagen corresponding to the stylopod element (humerus or femur), the two zeugopod elements (radius–ulna and tibia–fibula), and the autopod rays corresponding to the phalangeal elements (Hinchliffe and Johnson, 1980; Hamrick, 2001). These and other studies led to the realization that the first overt histological sign of joint formation was the emergence of a compact avascular mesenchymal tissue layer at each prospective joint site, interrupting the adjacent cartilaginous elements, and thus originally named the “interzone” (Holder, 1977; Mitrovic, 1978). The interzone is composed of flat-shaped cells oriented perpendicularly to the limb's main axis, tightly bound via gap junctions and requiring the hypoxia regulator Hif-1α to function (Archer et al., 2003; Provot et al., 2007). Because the interzone cells emerge at sites previously occupied by chondrocytes, this led to the suggestion that the interzone cells originate from, and are the direct descendants of, de-differentiated chondrocytes (Craig et al., 1987; Nalin et al., 1995). When Holder microsurgically removed the interzone at prospective elbow sites of early chick embryos *in ovo*, no joint formed at later stages (Holder, 1977). These data revealed for the first time that the interzone is needed for joint formation, but did not clarify whether the interzone represents a mere physical signpost demarcating the joint site or has additional and more direct roles.

Exploiting the fact that incipient interzone cells express growth and differentiation factor 5 (*Gdf5*; Storm and Kingsley, 1996), we and our collaborators carried out genetic cell lineage tracing-and-tracking experiments using compound *Gdf5-Cre*;*ROSA R26R* (*LacZ*) reporter mice (Rountree et al., 2004; Koyama et al., 2008). We found that *LacZ*-positive interzone cells and their progeny gave rise to many joint tissues over developmental time—including articular cartilage, synovial lining, and intrajoint ligaments—that persisted into adulthood (Fig. 1A–B). Thus, the data showed that interzone cells are not transient, actively take part in joint tissue formation, and constitute a progenitor cell cohort endowed with joint-formation capacity. In concurrent studies, Hyde and collaborators carried out similar cell lineage tracing-and-tracking experiments in the developing knee using the compound *matrillin 1-Cre*;*R26R* mice; matrillin 1 is normally expressed by all chondrocytes except articular chondrocytes (Hyde et al., 2007). They found that nascent articular chondrocytes emerging in the incipient knee joint at E13.5 were *LacZ*-negative, while the adjacent shaft chondrocytes were positive, and that this pattern persisted over time. In related experiments, the same group used *Col2a1-Cre*;*R26R* mice to track cells in developing knees and found that reporter-positive cells initially gave rise to articular cartilage, cruciate ligament, and medial meniscus (Hyde et al., 2008). Reporter-negative cells appeared in the developing joint at E14.5 (termed intermediate zone cells) and were also present at later stages in the lateral portions of the meniscus. The authors concluded that knee development involves cells present in the original anlagen with a *Col2a1* history, as well as invading *Col2a1*-negative cells recruited from the surroundings. Similarly, we had previously used *in ovo* fluorescent DiI cellular labeling-tracing in chick embryo limbs to provide evidence that surrounding cells migrate into developing joints (Pacifici et al., 2006). Likewise, we studied *Indian hedgehog*-null ($Ihh^{-/-}$) mouse embryos in which the limb skeletal elements remain wholly cartilaginous and lack joints (Fig. 1E–F) that are well appreciable in age-matched control littermates (Fig. 1C–D; Koyama et al., 2007). Using compound $Ihh^{-/-}$;*Gdf5-Cre*;*R26R* embryos, we found that *LacZ*-positive cells did form at prospective joint sites in the mutants (Fig. 1E, arrowheads), but flanked and surrounded the uninterrupted joint sites (Fig. 1F, arrowhead). These reporter positive cells expressed interzone marker genes including *Gdf5* and *Erg* (Fig. 1J–L, arrowheads), and phenotypically similar cells were present in prescribed patterns within the joints of control littermates as expected (Fig. 1C–D and G–I). We interpreted the data to indicate that prospective joint progenitor cells had emerged and were topographically specified in the *Ihh*-null mutants, but could not penetrate the fused joint sites. Recruitment and immigration of flanking cells into the interzone has also been suggested by Mundlos and coworkers in their study of joint development defects in the *Short digits* mouse mutant (Niedermaier et al., 2005).

Recent cell lineage-tracking experiments with *Sox9CreERT2*;*R26R* mouse embryos receiving tamoxifen at E11.5 and examined by E17.5 have indicated that *Sox9*-expressing cells are the precursors of both articular and growth plate chondrocytes, as well as ligaments and tendons (Soeda et al., 2010). In experiments using endogenous *doublecortin* (*Dcx*) to drive *LacZ* or *eGFP* expression in mouse embryos, Zhang et al. found that *Dcx*-expressing cells initially constitute much of the limb mesenchyme, but later are restricted to the interzone and articular chondrocytes (Zhang et al., 2010). In line with the *Sox9* study above, they concluded that articular and growth plate chondrocytes derive from common mesenchymal precursors originally expressing *Dcx*, which then bifurcate into articular and growth plate chondrocytes. The TGFβ type II receptor (Tgfbr2) is essential for joint formation, particularly in the autopod (Spagnoli et al., 2007). Novel transgenic reporter mice bearing a *Tgfbr2-βGal-GFP-BAC* construct and expressing both β-galactosidase (βGal) and green fluorescence protein (GFP) as reporters were used to monitor the spatiotemporal distribution of *Tgfbr2*-expressing cells in developing digit joints over time (Li et al., 2013b). *Tgfbr2-βGal*-positive cells were first limited to dorsal and ventral regions of E13.5 joints and were undetectable in the central region of the interzone. By E16.5 and postnatally, positive cells were observed in the synovial lining, meniscal surface, ligaments, and groove of Ranvier, and BrdU-labeling showed that *Tgfbr2*-expressing cells also constitute slow cycling stem/progenitor cells. The authors reached the interesting conclusion that during interzone development, *Tgfbr2*-expressing cells would act as progenitors that orchestrate joint development within specified cell niches.