# Multiomic approach to dissect cell-cell crosstalk between chondrocyte subtypes during embryonic knee development

Victoria L. Hansen[1], Yanshi Chen[2], Gulzada Kulzhanova[1], Chia-Lung Wu[1]
[1]Department of Orthopedics and Rehabilitation, [2]Department of Biology, Center for Musculoskeletal Research, University of Rochester, NY
Victoria_Hansen@URMC.Rochester.edu

**Disclosures:** All authors have nothing to disclose.

**INTRODUCTION:** The medical burden of osteoarthritis (OA) and associated cartilage damage on the US population is increasing[1], partially due to the absence of disease modifying drugs for OA. Tissue engineered cartilage is a promising OA treatment for eventual implantation in patients. While current differentiation protocols are capable of inducing chondrogenesis in stem cells, off-target differentiation continues to be a problem for efficiency. We propose that examining the transcriptional and epigenic profiles of developing embryonic cartilage will illuminate novel cell signals for enhancing chondrogenesis in stem cells. Here, we used a model system of chondrogenesis of murine knee cartilage during embryonic development where we performed scRNA-seq, scATAC-seq, and spatial transcriptomics (Spatial-seq) of the knee joints at key time points throughout gestation. By integrating these omics approaches and interrogating cell-cell crosstalk between chondrocyte subtypes, we may identify novel signaling pathways to achieve more effective *in vitro* chondrogenesis.

**METHODS:** Knee joints of murine hind limbs from embryonic day (E) 15.5 and 18.5 were harvested (IACUC approved). Samples were submitted for Spatial-seq (Visium, 10X Genomics) and multiomic scRNA-seq and scATAC-seq (Chromium, 10X Genomics) to the Genomics Research Center at the University of Rochester. Distinct cell populations were identified in multiomic data and then mapped to Spatial-seq sections to localize cell types. Bioinformatic tools including Seurat[2] and NicheNet[3] R packages were utilized to perform cell clustering and predict ligand-receptor interactions, respectively.

**RESULTS:** We identified four chondrocyte cell subtypes: early/mature (EM), hypertrophic (H), perichondrial (P), and interzone (I) in both E15.5 and E18.5 samples (**Fig. 1A**). Between E15.5 and E18.5 the percent of EM cells decreased from 47% to 45%, H cells decreased from 37% to 31%, P cells increased from 10% to 19%, and I cells decreased from 6% to 5% (**Fig. 1B**). Single cell clusters mapped to Spatial-seq sections revealed chondrocyte subpopulation locations (**Fig. 1C-F**). For P sender to EM receiver chondrocyte populations (**Fig. 2A**), we identified that Fgf2, Pdgfc, Postn, and Gpc3 are putative essential ligands between these two chondrocyte subtypes in E15.5 and E18.5 (**Fig. 2B**). These three ligands also exhibited higher expression at E18.5 than E15.5 (**Fig. 2C**). Importantly, Ccl7 and Dusp1 were detected to be possible downstream targets of the Postn and Pdgfc, respectively, with distinct chromatin accessibility in P vs. EM chondrocytes (**Fig. 3**).

**DISCUSSION:** We used a multiomic approach to identify cell-cell crosstalk between chondrocyte subtypes that regulate chondrogenesis during knee development. In the current work, we focus on the interactions between P and EM chondrocytes as the signaling pathways involved in lineage changes from EM to H chondrocytes are more well-studied, particularly via the endochondral ossification process. By examining the instances where P cells act as the sender and EM cells act as the receiver, we predict potential molecular mechanism(s) by which perichondrium regulates developing EM cartilage. Here, we identified that Pdgfc, Postn, and Gpc3 (glypican 3) were more highly expressed in P cells for the more developed state of E18.5 compared to E15.5. The time-dependent expression pattern of these genes may be essential in regulating maturation or proliferation of EM chondrocytes. CCL7 is a chemoattractant for immune cells *in vivo* but also acts as a recruiter of stem and progenitor cells to sites of injury and may have similar functionality during embryogeneis[4]. Nevertheless, the function role of CCL7 in mature chondrocytes remains to be determined. DUSP1, an inhibitor of the MAPK signaling pathway, may work to regulate chondrogenesis during embryogenesis by dephosphorylating MAPKs and transcription factors in a similar manner to other DUSP family members[5]. DUSP1 has also been implicated as an inhibitor of OA-associated inflammation (i.e., MMP13 and COX-2)[6]. We are currently using gene editing approaches to activate/suppress CCL7 and DUSP1 expression to elucidate their regulatory role in modulating chondrogenesis.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Effective long-term treatments for OA remain elusive though tissue engineering for implants shows promise. Here we use a murine model of chondrogenesis during embryonic cartilage development to identify novel gene targets, potentially leading to more efficient cartilage tissue engineering for OA therapeutic applications.

**REFERENCES:** 1. Xu & Wu, *Scientific Reports*, 2021; 2. Hao+, *Cell*, 2021; 3. Browaeys+, *Nature Methods*, 2020; 4. Cheng+, *Cytokine*, 2014; 5. Bobick & Kulyk, *Birth Defects Research Part C: Embryo Today: Reviews*, 2008; 6. Peng+, *Molecular Medicine Reports*, 2017.

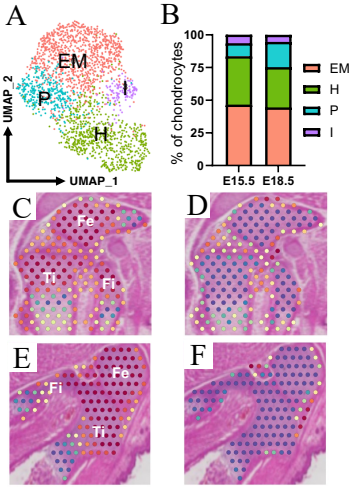


**Fig. 1:** (A) UMAP and (B) percents of early/mature (EM), hypertrophic (H), perichondrial (P), and interzonal (I) chondrocyte subclusters in single cell data. (C) E15.5 EM and (D) P locations. (E) E18.5 EM and (F) P locations. Warmer color is high likelihood and cooler is low. Fe = femur, Fi = fibula, T = tibia.

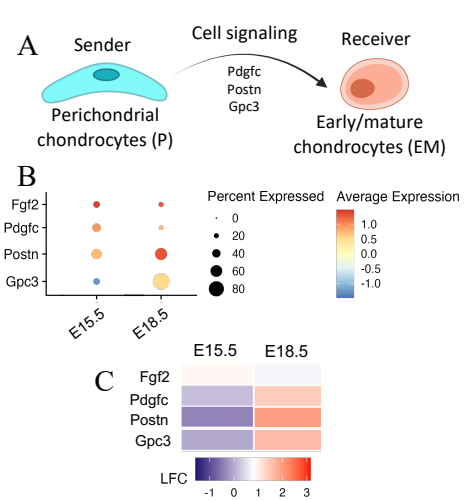


**Fig. 2:** (A) Diagram of cell communication between P and EM groups. (B) Average expression and percent of single cells expressing Fgf2, Pdgfc, Postn, and Gpc3 in P clusters at E15.5 and E18.5. (C) Log2 fold change of Fgf2, Pdgfc, Postn, and Gpc3 for P cluster in E15.5 versus E18.5 samples.

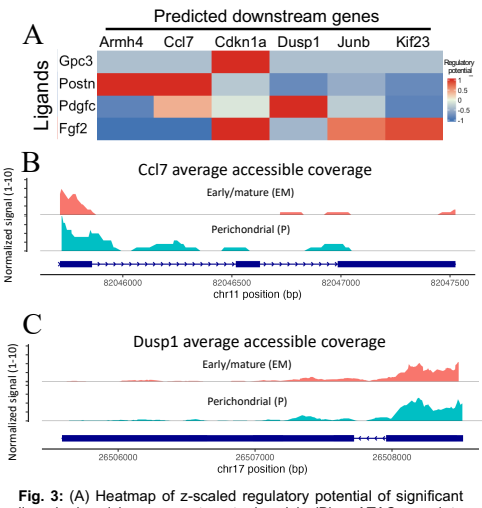


**Fig. 3:** (A) Heatmap of z-scaled regulatory potential of significant ligands (y-axis) on gene targets (x-axis). (B) scATAC-seq data demonstrates average accessible chromatin coverage of Ccl7 is greater in P than EM. (C) scATAC-seq data demonstrates average accessible chromatin coverage of Dusp1 is slightly elevated in P than EM.