Single-Cell Analysis of Articular Cartilage Reveals Diverse Cellular Populations and a Novel Chondrocyte Cluster Characterized by Increased Cholesterol Metabolism and Endoplasmic Reticulum Stress Response in Knee Osteoarthritis

Yu Kobori[1], Kohei Nishitani[1], Hiroyuki Yoshitomi[1], Akinori Murakami[1], Shinichi Kuriyama[1], Akio Sakamoto[1], Takashi Noguchi[1], Yugo Morita[1], Takashi Sono[1], Takayuki Fujii[1], Koichi Murata[1], Junya Toguchida[1], Hideki Ueno[1], Yasuhiro Murakawa[2], Chikashi Terao[2], Shuichi Matsuda[1]
[1] Kyoto University, Kyoto, Japan, [2]RIKEN, Yokohama, Japan
Email of Presenting Author: kobo1128@kuhp.kyoto-u.ac.jp

Disclosures: Yu Kobori(N), Kohei Nishitani(8-JOR), Hiroyuki Yoshitomi(N), Akinori Murakami(N), Shinichi Kuriyama(N), Akio Sakamoto(N), Takashi Noguchi(N), Yugo Morita(N), Takashi Sono(N), Takayuki Fujii(N), Koichi Murata(N), Junya Toguchida(N), Hideki Ueno(N), Yasuhiro Murakawa(N), Chikashi Terao(N), Shuichi Matsuda(N)

INTRODUCTION: Knee osteoarthritis (OA) is a common, disabling joint disease marked by progressive cartilage degeneration, yet the cellular programs driving its progression remain unclear. Histological grading describes structural changes, but the molecular definition of chondrocyte subpopulations and their spatial distribution in human cartilage is incomplete. Single-cell RNA sequencing (scRNA-seq) enables high-resolution mapping of cellular heterogeneity[1]. Here, we used scRNA-seq on OA and non-OA human cartilage. Gene expression profiles between OA and control cartilage were compared to evaluate the cellular and molecular mechanisms underlying OA. The overall cartilage cell population was examined. Subsequent analyses focused on associations between cluster composition and OA-related background characteristics, including aging and lifestyle-related factors. In addition, the spatial distribution of chondrocyte clusters between the superficial and deep zones was assessed, based on the hypothesis that phenotypic variation may arise in response to structural changes associated with cartilage thinning.

METHODS: Cartilage was obtained from nine OA and three non-OA patients (7 males, 5 females; age 16–91 years) with ethics approval and informed consent. Tissue was digested, dissociated, and processed using 10x Genomics Chromium Single Cell 5′ kits; sequencing was performed on an Illumina NovaSeq 6000. Trajectories were inferred with Slingshot and scVelo; cell–cell communication was analyzed with CellChat. OA–control comparisons used both single-cell and pseudobulk analyses. Functional validation used TC28a2 and primary chondrocytes, with CRISPR/Cas9 knockouts of candidate genes, cholesterol and ER-stress stimulation, and pellet culture. Immunostaining and Western blotting assessed protein expression. For spatial analysis, in five OA knees, ICRS 0–1 cartilage was divided into superficial and deep halves, and ICRS 2–3 regions were sampled as degenerated cartilage.

RESULTS: We obtained 104,951 cells across 11 clusters. Three non-chondrocytic clusters—hematopoietic (*HLA-DRA*+, *PTPRC*+), mesenchymal stromal (*THY1*+, *NT5E*+, *ENG*+), and *THY1*− fibroblast-like synoviocytes—were excluded as likely contamination. Remaining chondrocytes were annotated into eight subtypes: fibrocartilage chondrocytes (FCs), hypertrophic (HTCs), prefibrocartilage (preFCs), proliferative (ProCs), effector (ECs), homeostatic (HomCs), regulatory (RegCs), and stress-responsive metabolic chondrocytes (MetabCs) (Figure1). Pseudotime and RNA velocity revealed two main branches from HomCs via RegCs: one toward FCs, showing reduced anabolic and increased fibrous genes, and one toward MetabCs through EC, showing increased anabolic and catabolic genes. MetabCs were increased in OA compared to controls (median 1.8% vs. 0.9%; P = 0.009), while HomCs were reduced (11.6% vs. 14.9%; P = 0.04). The proportions of preFCs and FCs positively correlated with age (r = 0.61, 0.67, respectively), whereas ProCs negatively correlated with age (r = -0.90). MetabCs expressed cholesterol biosynthesis genes (*MSMO1*, *SQLE*) and showed enrichment of cholesterol metabolism and ER-stress pathways. MetabC proportion correlated with serum cholesterol level (Figure2). Tunicamycin induced cholesterol synthesis genes; cholesterol loading decreased *SOX9* and *COL2A1*, increased *IL1B* and *MMP13*gene expression, and upregulated phosphorylation of BiP, PERK, and CHOP. Pseudobulk analysis for differentially expressed genes highlighted *HILPDA* (higher in controls) and *AKR1C2* (higher in OA). *HILPDA* knockout reduced anabolic and increased catabolic genes; *AKR1C2* knockout had the opposite effect. In pellet culture, HILPDA-KO pellets were small and irregular, while AKR1C2-KO pellets were spherical with strong matrix staining. In OA, FC/preFC interactions decreased, while EC/HTC/MetabC interactions increased, with outgoing signals shifting from FC/preFCs toward ECs/MetabCs. Spatially, degenerated cartilage resembled the superficial zone in both cluster proportions and gene-expression correlations. Several clusters differed between deep and degenerated regions. Superficial zones were enriched for preFCs, RegCs, HomCs, and FCs; deep zones for HTCs and ECs (Figure3).

DISCUSSION: This single-cell atlas refines the cellular landscape of human articular chondrocytes in OA and revealed four key findings. First, the cartilage samples contained various cell types, including non-chondrocytic cells[2]. Thus, careful preselection is required to analyze chondrocyte populations. Second, two novel genes potentially associated with OA pathology were identified. Third, a novel chondrocyte phenotype related to cholesterol synthesis and ER stress was identified. Finally, stratification analysis of cluster proportions revealed that chondrocytes in degenerated cartilage had acquired superficial characteristics. Trajectory and proportional analyses suggested that the trajectory toward FCs representing an aging process, while the trajectory toward MetabCs is disease-related. A novel cluster (MetabC) exhibited enrichment gene sets associated with UPR and mTORC1 signaling, both involved in ER stress, suggesting that MetabCs may represent a stress-resistant cell population. Although the relationship between cholesterol levels and knee OA remains controversial, previous studies have reported elevated cholesterol levels in the synovial fluid of OA patients[3].These findings highlight the importance of ER stress mechanisms in OA pathology. Spatial analysis suggests that chondrocytes in OA cartilage alter their phenotype in response to spatial distribution changes caused by degeneration and destruction. These findings demonstrate that lipid–ER stress pathways and specific gene programs contribute to OA pathogenesis and that rigorous preselection and exclusion of contaminants enhance the accuracy of cartilage scRNA-seq analyses.

SIGNIFICANCE/CLINICAL RELEVANCE: This study identifies a novel OA-related chondrocyte phenotype characterized by cholesterol metabolism and ER stress, providing a potential cellular target for disease-modifying OA therapies and underscoring the importance of spatial context in cartilage biology.

REFERENCES: 1. Ji Q, et al. 2019. Ann. Rheum. Dis. 78(1):100; 2. Nishitani K, et al. 2025. Ann. Rheum. Dis. 84(3):e10–e11
3. Choi W-S, et al. 2019. Nature 566(7743):254–258.