



Single-cell RNA sequencing reveals extensive fibrotic remodeling and pathogenic chondrocyte subpopulations in developmental dysplasia of the hip

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Background: Developmental dysplasia of the hip (DDH) is a common pediatric orthopedic disorder that predisposes affected children to early-onset osteoarthritis (OA), yet the cellular heterogeneity and fibrotic remodeling of acetabular cartilage are poorly understood.

Methods: We performed single-cell RNA sequencing (scRNA-seq) on acetabular cartilage obtained from three children with DDH and two age-matched controls, thereby generating a transcriptomic atlas of 10,550 chondrocytes.

Results: Nine chondrocyte subpopulations were identified, including four previously unrecognized subsets (C1–C4). Compared with controls, DDH cartilage exhibited a marked shift toward fibrocartilage-like phenotypes, characterized by upregulation of fibrotic and matrix-remodeling genes, such as *COL1A1*, *COL1A2*, *COL3A1*, *POSTN*, *MMP13*, and *MMP14*, together with downregulation of key cartilage matrix genes, including *COL2A1*, *ACAN*, *CHAD*, and *CNMD*. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses revealed enrichment of extracellular matrix (ECM) organization and related pathways. Pseudotime analysis demonstrated a trajectory toward fibrotic and proliferative states, and CellChat analysis identified enhanced collagen and TGF- β signaling among fibrotic subsets. These transcriptional findings were validated by polymerase chain reaction (PCR), immunohistochemistry (IHC), and histological staining.

Conclusions: These findings reveal molecular remodeling of cartilage obtained from dislocated hips in DDH and identify fibrosis-associated chondrocyte subpopulations that may contribute to early degenerative changes.

Keywords: Developmental dysplasia of the hip (DDH); single-cell RNA sequencing (scRNA-seq); chondrocyte heterogeneity; fibrotic remodeling; extracellular matrix (ECM)

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Introduction

Developmental dysplasia of the hip (DDH) is one of the most common pediatric skeletal disorders, affecting approximately 0.56–3.8% of newborns worldwide (1,2). DDH encompasses a spectrum of anatomical abnormalities involving the femoral head and acetabulum, including instability, subluxation, and complete dislocation (3). If left unrecognized or inadequately treated, DDH disrupts hip joint biomechanics and markedly increases the risk of early-onset osteoarthritis (OA), leading to long-term functional disability (4).

The etiology of DDH remains incompletely understood. It is generally regarded as a multifactorial condition, influenced by both environmental and genetic factors. Reported risk factors include female sex, breech presentation, primiparity, oligohydramnios, and macrosomia (5). Several genes—such as *GDF5* (6), *ASPN* (7), *COL1A1* (8), and *DKK1* (9)—have been implicated in DDH susceptibility; however, findings across studies remain inconsistent, underscoring the complexity of DDH pathogenesis. Notably, little is known about the cellular composition of acetabular cartilage in DDH, the molecular signatures of specific chondrocyte populations, or the extent to which cellular heterogeneity contributes to cartilage degeneration and disease progression.

Single-cell RNA sequencing (scRNA-seq) has recently transformed the characterization of cartilage biology by enabling the identification of previously unrecognized

chondrocyte subpopulations and disease-associated transcriptional programs. This technology has revealed substantial chondrocyte heterogeneity in disorders such as OA (10), Kashin-Beck disease (KBD) (11), and intervertebral disc degeneration (IVDD) (12), offering new mechanistic insights into tissue remodeling and degeneration. However, to date, no single-cell transcriptomic studies have systematically investigated human DDH cartilage, leaving a critical gap in understanding the cellular landscape and pathological remodeling associated with this pediatric condition.

In this study, we performed scRNA-seq on acetabular cartilage obtained from children with DDH and age-matched controls to comprehensively characterize chondrocyte heterogeneity in DDH. By integrating differential gene expression, pathway enrichment, trajectory analysis, and cell-cell communication profiling, we constructed the first single-cell atlas of DDH cartilage. Our findings identify distinct pathogenic chondrocyte subpopulations and widespread fibrotic remodeling, providing new insights into DDH pathogenesis and highlighting potential translational targets for preserving joint integrity in affected children.

Methods

Ethical approval and informed consent

This study was approved by the Institutional Ethics Committee of the Children's Hospital of Fudan University [Approval No. (2022) 205]. Written informed consent was obtained from the legal guardians of all pediatric participants. This study was conducted in accordance with the approved study protocol and the Declaration of Helsinki and its subsequent amendments.

Sample acquisition

Acetabular cartilage samples from children diagnosed with DDH were collected intraoperatively during corrective hip surgery, following parental consent. Control cartilage was obtained from pediatric patients without DDH who underwent lower-limb amputation due to traumatic injury. Macroscopically intact acetabular cartilage distant from the injury site was collected during surgery, minimizing the potential influence of acute trauma-related tissue responses. Discarded acetabular cartilage tissue was harvested with guardian permission. All specimens were immediately processed for downstream scRNA-seq and histological

Highlight box

Key findings

- Single-cell RNA sequencing revealed distinct chondrocyte subpopulations in cartilage obtained from dislocated hips in developmental dysplasia of the hip (DDH), including fibrosis-associated subsets.

What is known and what is new?

- It is known that abnormal mechanical loading in DDH affects cartilage development.
- This study provides the first single-cell transcriptomic atlas of acetabular cartilage in DDH and identifies fibrotic chondrocyte subpopulations associated with early degeneration.

What is the implication, and what should change now?

- These findings suggest that cartilage alterations in DDH represent secondary molecular adaptations to abnormal joint mechanics and may inform future strategies for preserving cartilage integrity following early intervention.

validation. Clinical information for the DDH and control donors, including age, sex, diagnosis, and sample site, is summarized in [Table S1](#).

Representative radiographic materials of the DDH cases are shown in [Figures S1-S3](#).

ScRNA-seq and Seurat processing

Raw sequencing data were processed using Seurat (v4.2) in R. Cells with fewer than 200 detected genes, cells within the top 1% of total gene counts, or exhibiting >10% mitochondrial gene expression were excluded. To correct potential batch effects between samples, data from different donors were integrated using the Harmony algorithm. After integration, principal component analysis (PCA) was performed, and the top principal components were used for downstream analysis. Graph-based clustering was conducted using the FindNeighbors and FindClusters functions in Seurat with a clustering resolution of 0.5. Cluster visualization was generated using t-distributed stochastic neighbor embedding (t-SNE). t-SNE was used for visualization as it provided clear separation of the identified chondrocyte populations in this dataset. Additional quality control steps were applied to minimize potential doublets and ambient RNA contamination. Filtering of cells with extremely high gene counts and low-quality transcript profiles helped reduce the likelihood of doublets and background RNA signals.

Cell-type identification and marker gene analysis

Marker genes for each cell cluster were identified using Seurat's FindAllMarkers function with the Wilcoxon rank-sum test. Only genes expressed in $\geq 10\%$ of cells within a cluster and displaying significant differential expression were retained. Cell identities were manually annotated based on canonical marker gene expression and prior published literature. Heatmaps, dot plots, and violin plots were generated using the DoHeatmap, DotPlot, and VlnPlot functions to visualize marker expression patterns.

Enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the clusterProfiler R package. The top 100 marker genes per cluster were used for enrichment analysis. Pathways with an adjusted P value <0.05 were considered

significantly enriched. Results were visualized using built-in R functions.

Differentially expressed gene (DEG) analysis

DEGs between DDH and control groups within each chondrocyte subpopulation were identified using Seurat FindMarkers with the Wilcoxon likelihood-ratio test and default parameters. Criteria for DEG significance included expression in $\geq 10\%$ of cells within a cluster and an average log fold-change >0.25. Given the limited number of donors in this pediatric cohort, results were interpreted with caution, and key transcriptional patterns were examined across individual samples to minimize potential donor-specific bias.

Pseudotime trajectory analysis

Chondrocyte developmental trajectories were reconstructed using the Monocle2 package to infer differentiation dynamics among chondrocyte populations. Genes were filtered based on the following criteria: expression in >10 cells, mean expression >0.1, and q value <0.01. Pseudotime ordering was performed to characterize cellular differentiation trajectories and lineage relationships among chondrocyte subsets.

Cell-cell communication analysis

Intercellular communication networks were inferred using the CellChat R package based on curated ligand-receptor interaction databases. Communication probabilities and signaling strengths were quantified using aggregateNet, while computeNetSimilarity assessed structural similarities among signaling pathways. Outgoing and incoming signaling roles were visualized using netAnalysis_signalingRole_heatmap, and aggregated ligand-receptor interactions were plotted using netVisual_aggregate.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from chondrocytes using the TRIzol reagent. Reverse transcription was performed using FastKing gDNA Dispelling RT SuperMix (TIANGEN, Beijing, China; KR118). RT-qPCR assays were conducted on an Applied Biosystems QuantStudio 3 system using Ultra-Rapid HotStart PCR Master Mix (Yeasen Biotechnology, Shanghai, China; 10157ES08).

Gene expression was normalized to β -actin, and relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

Clinical information for samples used in qPCR validation is summarized in [Table S2](#).

Hematoxylin-eosin (H&E) staining

DDH and control cartilage samples were fixed in formaldehyde at 4 °C for 24 hours and decalcified in 15% ethylenediaminetetraacetic acid (EDTA). Paraffin-embedded specimens were sectioned at 4 μ m, deparaffinized, and stained with hematoxylin for 10 minutes and eosin for 3 minutes. Images were captured using a Leica DM750 microscope equipped with a Leica ICC50 HD camera.

Safranin O-fast green staining

Cartilage tissues were fixed in 4% paraformaldehyde and decalcified in 15% EDTA for 4–5 weeks. After dehydration in graded ethanol and xylene, 4- μ m paraffin sections were stained with 0.2% Safranin O for 5 minutes and 2% fast green for 15 seconds, followed by a brief rinse in 1% acetic acid. Stained sections were imaged using a Leica DM750 microscope.

Immunohistochemistry (IHC)

Sections were deparaffinized, rehydrated, and subjected to antigen retrieval using 10 mM citrate buffer (pH 6.0) at 60 °C overnight. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 15 minutes, followed by blocking with 1% sheep serum for 1 hour. Sections were incubated overnight at 4 °C with primary antibodies: anti-*COL1A1* (Proteintech, Wuhan, China; 14695-1-AP, 1:1,000) and anti-*COL3A1* (Abcam, Cambridge, UK; A11691, 1:500). After incubation with HRP-conjugated secondary antibodies and hematoxylin counterstaining, slides were visualized using a Leica DM750 microscope equipped with an ICC50 HD camera.

Statistical analysis

Statistical analyses were performed using R software. Differential expression analysis was conducted using the Wilcoxon rank-sum test in Seurat. For RT-qPCR validation, data are presented as mean $\pm$ standard deviation (SD), and comparisons between groups were performed using Student's t-test. A P value <0.05 was considered

statistically significant.

Results

Identification of nine transcriptionally distinct chondrocyte subpopulations in DDH cartilage

ScRNA-seq of acetabular cartilage from three children with DDH and two controls yielded 10,550 high-quality chondrocytes, all expressing canonical cartilage markers, including *COL2A1*, *ACAN*, and *SOX9*, confirming their chondrocyte identity despite transcriptional heterogeneity. Unsupervised clustering analysis revealed nine transcriptionally distinct chondrocyte subpopulations, four of which (C1–C4) represent previously uncharacterized cell states in pediatric cartilage ([Figure 1A,1B](#)).

The C1 subset expressed *CD55* and *PRG4*, suggesting a lubricating or anti-inflammatory phenotype, whereas C2 was enriched for *CYTL1* and *CNMD*, genes associated with cartilage maintenance. C3 displayed elevated expression of *MMP2* and *HTRA1*, consistent with matrix-remodeling activity. C4, a subset detected predominantly in DDH samples, expressed *SPP1*, *TUBB2A*, and *TUBB2B*, indicating a potential DDH-specific chondrocyte phenotype. Marker genes and corresponding literature used for chondrocyte subset annotation are summarized in [Table S3](#).

Among previously reported populations, regulatory chondrocytes (RegCs) exhibited homeostatic markers (*CHI3L1*, *CFH*, and *PLA2G2A*), dividing chondrocytes (DivCs) represented proliferative chondrocytes (*TOP2A*, *CENPF*, and *CDK1*), fibrotic chondrocyte 1 (FC1) showed fibrosis-related markers (*ASPN* and *POSTN*), FC2 expressed fibrocartilage-associated collagens (*COL1A1* and *COL1A2*). Despite the upregulation of fibrosis-associated genes, these subsets retained expression of core chondrocyte markers, supporting their classification as fibrocartilage-like chondrocyte populations rather than contaminating fibroblasts. Hypertrophic chondrocytes (HTCs) demonstrated hypertrophic markers (*IBSP*, *COL10A1*, and *PTH1R*) ([Figure 1C](#)).

Comparative analysis of cell-type proportions showed that C1, FC1, and DivC were expanded in DDH cartilage, whereas C2, RegC, and HTC were more abundant in controls, indicating a shift toward proliferative and fibrotic phenotypes in DDH ([Figure 1D](#)). Expression patterns of representative marker genes across clusters are shown in [Figure 1E](#). Cells from different donors were broadly distributed across the identified clusters, indicating that the

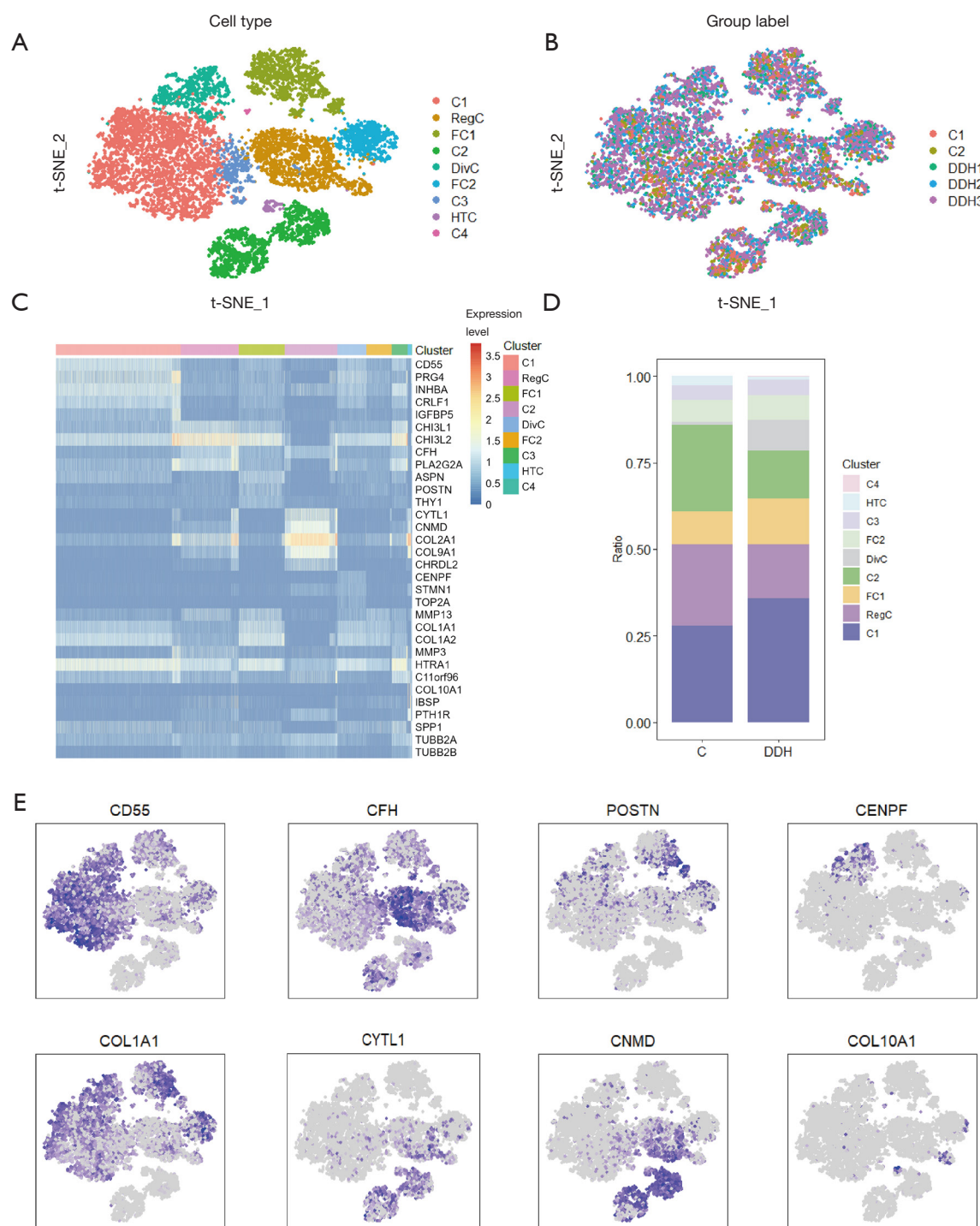


Figure 1 Identification of chondrocyte subsets and transcriptional signatures in human DDH cartilage. (A) t-SNE visualization of single-cell transcriptomes showing nine chondrocyte subsets identified in human acetabular cartilage. (B) t-SNE plots colored by sample origin, distinguishing DDH and control cells. (C) Heatmap showing scaled expression of representative marker genes defining each chondrocyte subset. (D) Proportional distribution of the nine chondrocyte populations in DDH and control samples. (E) Dot plot illustrating the expression patterns of canonical marker genes across the identified chondrocyte clusters. DDH, developmental dysplasia of the hip; t-SNE, t-distributed stochastic neighbor embedding.

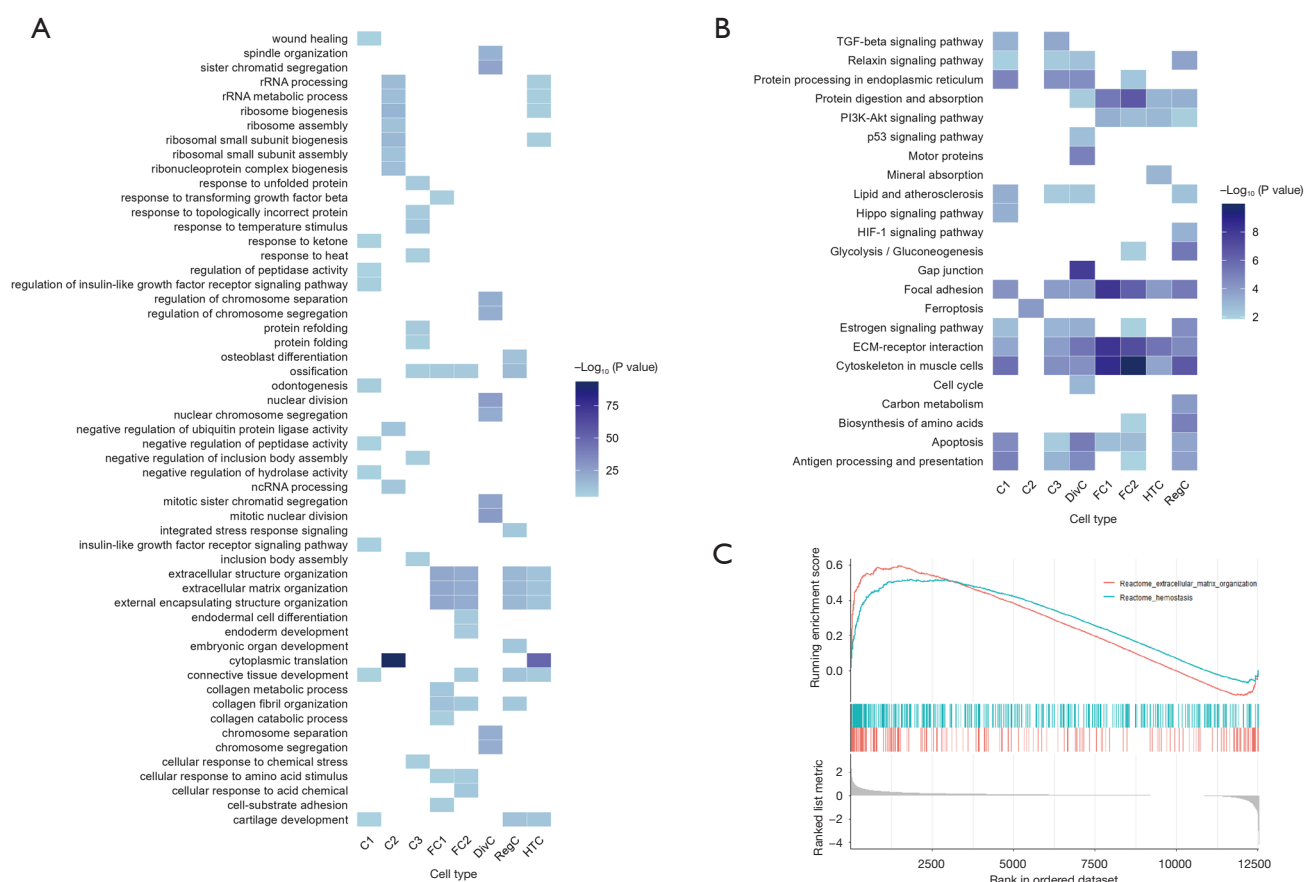


Figure 2 GO and KEGG pathway enrichment across chondrocyte subsets. (A,B) Bubble plot depicting the top 10 enriched GO biological process terms within seven major chondrocyte subsets, based on the top 100 highly variable genes identified in each subset. (C) KEGG pathway enrichment analysis showing significantly upregulated pathways in DDH-associated chondrocyte subsets compared with controls, based on DEGs identified using the FindMarkers algorithm. DEG, differentially expressed gene; DivC, dividing chondrocyte; FC, fibrotic chondrocyte; GO, Gene Ontology; HTC, hypertrophic chondrocyte; KEGG, Kyoto Encyclopedia of Genes and Genomes; RegC, regulatory chondrocyte.

clustering structure was not driven by donor-specific batch effects.

GO and KEGG analyses reveal enrichment of extracellular matrix (ECM) remodeling, ossification, and adhesion pathways in DDH-associated subsets

GO analysis demonstrated that C1 cells were enriched in insulin-like growth factor (IGF) signaling, with prominent expression of *IGFBP5* and *BMP2*, whereas RegC, FC1, and FC2 were enriched in ECM organization and ossification, consistent with their putative roles in cartilage remodeling (Figure 2A). FC1 and FC2 displayed strong enrichment of matrix-remodeling genes, with *MMP2* predominating in

FC1 and *MMP13* in FC2, suggesting enhanced cartilage degradation in DDH.

KEGG pathway analysis further revealed activation of ECM-receptor interaction and focal adhesion pathways across multiple DDH-associated subsets, including C1, C3, FC1, FC2, and HTC (Figure 2B). RegC uniquely exhibited enrichment in glycolysis/gluconeogenesis pathways, suggesting a metabolic shift in response to an altered cartilage microenvironment. In addition, apoptosis-related pathways were significantly enriched across several DDH subpopulations, implicating programmed cell death in DDH pathogenesis. Gene set enrichment analysis further supported enrichment of extracellular matrix organization and hemostasis-related pathways (Figure 2C).

DDH cartilage exhibits a fibrotic and degenerative transcriptional program

Gene expression comparisons between DDH and control samples revealed marked transcriptional remodeling across multiple chondrocyte subpopulations. While C2 and HTC subsets showed largely similar expression patterns between groups, other clusters demonstrated substantial disease-associated differences.

In DDH cartilage, FC1 and FC2 subsets exhibited strong induction of fibrosis-related genes, including *IGFBP4*, *THY1*, and *POSTN*, as well as matrix-remodeling enzymes such as *MMP14* and *MMP13*. Collagen genes *COL1A1* and *COL1A2* were also significantly elevated in these subsets (Figure 3A). These fibrotic markers were minimally expressed in control samples, indicating that fibrocartilage-like phenotypes are specifically activated in DDH cartilage.

The DivC subset showed selective upregulation of proliferative genes (*CENPF*, *STMN1*, *CDK1*, and *TOP2A*) in DDH, suggesting aberrant chondrocyte proliferation (Figure 3B). In contrast, key structural cartilage genes—*COL2A1*, *ACAN*, *CHAD*, and *CNMD*—were consistently downregulated in several DDH subpopulations, reflecting impaired ECM integrity.

These transcriptional findings were further supported by molecular and histological validation. RT-qPCR demonstrated decreased expression of *COL2A1* and *CYTL1*, accompanied by increased expression of *MMP13*, *COL3A1*, and *COL1A1* in DDH tissues (Figure 4A). Safranin O-fast green and H&E staining revealed reduced proteoglycan content, disrupted ECM organization, and decreased chondrocyte density. Immunohistochemistry further confirmed elevated deposition of *COL1A1* and *COL3A1* in DDH cartilage (Figure 4B), and Safranin O-fast green and H&E staining demonstrated reduced proteoglycan content and disrupted cartilage architecture (Figure 4C).

Trajectory and cell-cell communication analyses reveal progressive differentiation toward fibrotic states in DDH

Pseudotime trajectory analysis positioned C2 and HTC subsets at the earliest developmental stages, consistent with their roles in cartilage homeostasis and matrix maturation (Figure 5A–5E).

In contrast, DDH chondrocytes were preferentially distributed at later pseudotime states corresponding to C1, DivC, FC1, and FC2, indicating a progression toward proliferative and fibrotic phenotypes (Figure 5D). Along the

trajectory, expression of cartilage matrix genes (*COL2A1* and *ACAN*) gradually decreased, whereas fibrosis- and degradation-associated markers (*MMP13*) increased, revealing a shift toward degenerative remodeling (Figure 5E).

CellChat analysis further showed altered outgoing and incoming signaling patterns among chondrocyte subsets (Figure 6A). Representative ligand-receptor interactions supporting the highlighted pathways, including collagen- and TGF- β -related signaling, are shown in Figure 6B. Representative ligand-receptor pairs supporting these signaling pathways included interactions such as *COL1A1*-*ITGA1* and *TGFB1*-*TGFB1*.

Discussion

In this study, we constructed the first single-cell transcriptomic atlas of acetabular cartilage from children with DDH. Nine transcriptionally distinct chondrocyte populations were identified, including four previously unrecognized subsets (C1–C4). Compared with age-matched controls, DDH cartilage exhibited extensive alterations in cellular composition, ECM remodeling, and intercellular communication. Collectively, these findings uncover fundamental changes in chondrocyte states in cartilage obtained from dislocated hips in DDH. Importantly, these molecular alterations likely represent secondary adaptations to abnormal mechanical loading and joint instability associated with hip dislocation, rather than intrinsic defects of cartilage itself.

Cartilage obtained from dislocated hips demonstrated marked fibrotic remodeling and loss of chondrogenic identity.

One of the most prominent findings of this study is the widespread activation of fibrotic and matrix-degrading programs in DDH cartilage. FC1 and FC2 subsets in DDH exhibited strong induction of *COL1A1*, *COL1A2*, *POSTN*, *MMP13*, and *MMP14*, accompanied by reduced expression of cartilage-specific ECM genes such as *COL2A1*, *ACAN*, *CHAD*, and *CNMD*. These signatures were validated by qPCR, immunohistochemistry, and histological staining, which consistently demonstrated increased collagen I/III deposition and decreased proteoglycan content (13,14).

Similar fibrotic remodeling has been described in end-stage OA, where collagen I replacement and upregulation of matrix proteases mark irreversible degeneration (15,16). Our data suggest that DDH cartilage undergoes fibrotic remodeling much earlier in life, potentially driven by abnormal mechanical loading resulting from acetabular

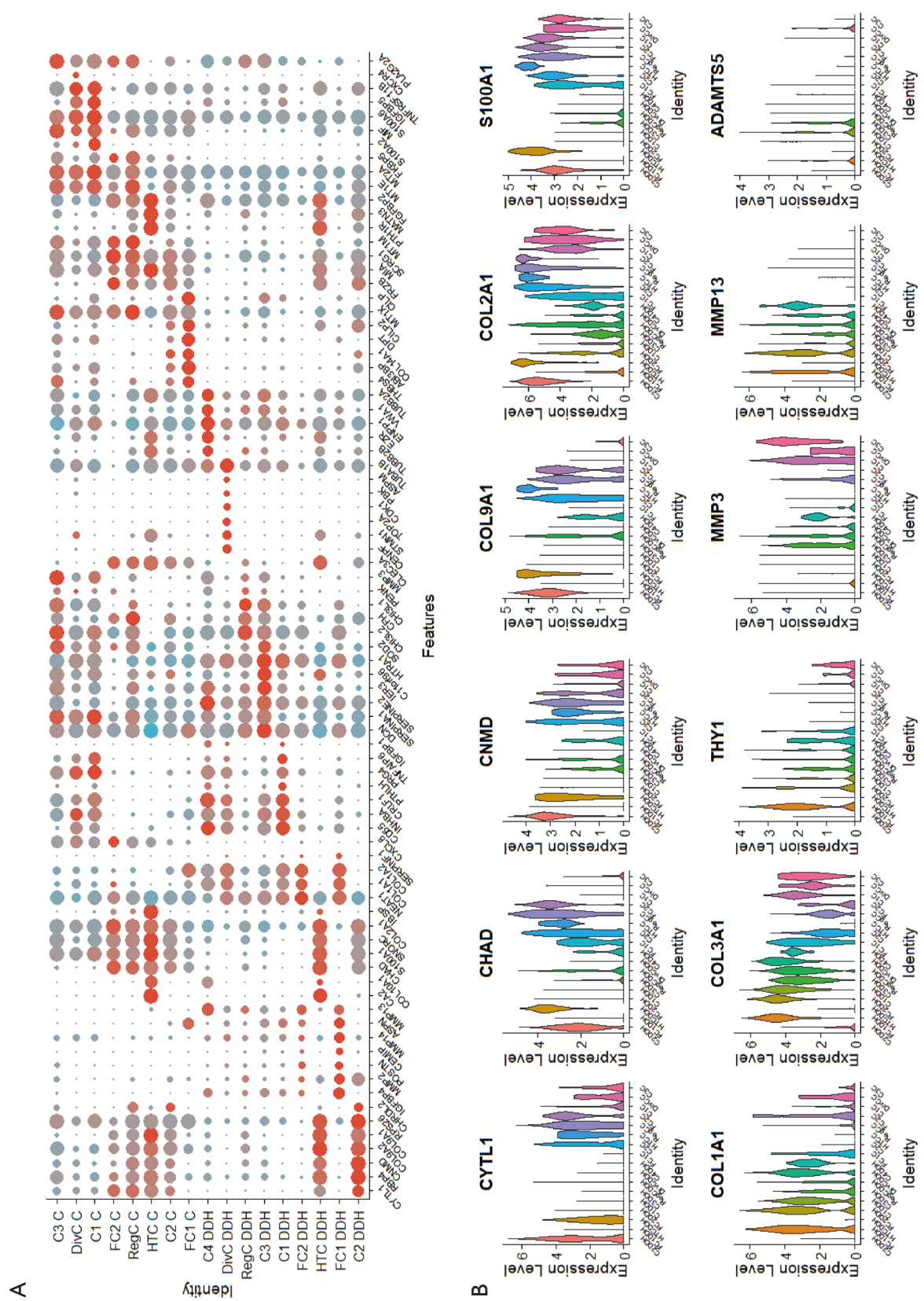


Figure 3 Differential gene expression between DDH and control chondrocytes. (A) Dot plot showing the expression of top marker genes across chondrocyte subsets in DDH versus control samples, based on aggregated FindAllMarkers analysis. (B) Violin plots illustrating differential expression of representative fibrosis-, proliferation-, and cartilage matrix-related genes between DDH and control groups. DDH, developmental dysplasia of the hip.

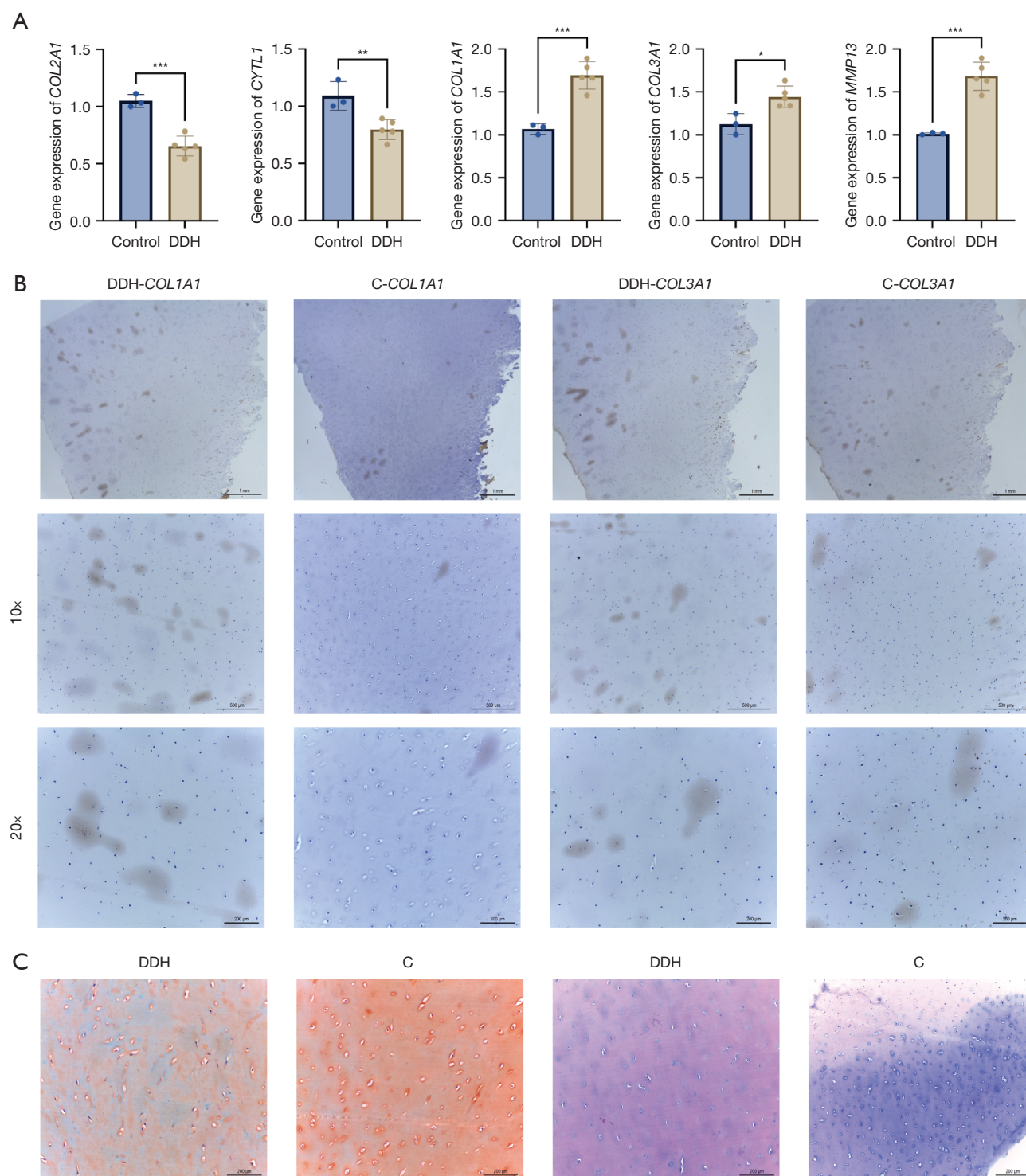


Figure 4 Molecular and histological validation of ECM alterations in DDH. (A) RT-qPCR analysis of *COL2A1*, *CYTL1*, *MMP13*, *COL3A1*, and *COL1A1* expression in DDH and control acetabular tissues. (B,C) Representative images of IHC staining for *COL1A1* and *COL3A1*, together with Safranin O-fast green and H&E staining, demonstrated ECM disorganization and altered collagen deposition in DDH cartilage. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. C, control; DDH, developmental dysplasia of the hip; ECM, extracellular matrix; H&E, hematoxylin-eosin; IHC, immunohistochemistry; RT-qPCR, real-time quantitative polymerase chain reaction.

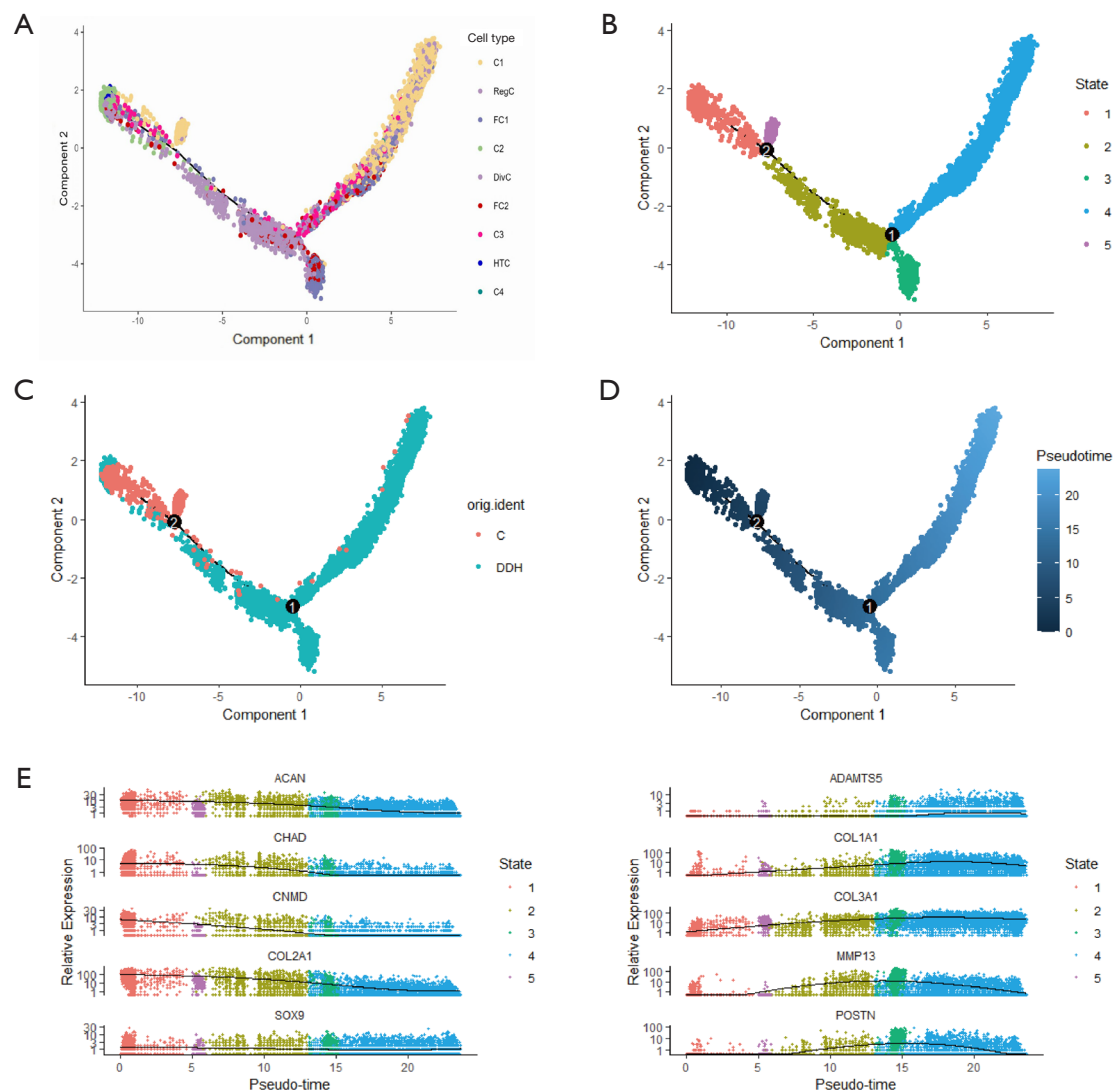


Figure 5 Pseudotime trajectory analysis of chondrocyte differentiation states. (A) Pseudotime trajectory illustrating the distribution of nine chondrocyte subsets along the inferred developmental path. (B) Visualization of pseudotime states assigned to individual chondrocytes. (C) Distribution of control and DDH cells along the trajectory. (D) Pseudotime progression highlighting developmental timing. (E) Expression dynamics of representative chondrocyte marker genes along pseudotime. C, control; DDH, developmental dysplasia of the hip; DivC, dividing chondrocyte; FC, fibrotic chondrocyte; HTC, hypertrophic chondrocyte; RegC, regulatory chondrocyte.

malformation (17,18). Such an early fibrotic transition may, at least in part, predispose DDH patients to the well-recognized risk of premature OA.

Disruption of key chondrogenic regulators may contribute to impaired cartilage maturation

Multiple genes critical for cartilage development were downregulated across DDH populations, including

CYTL1, *CHAD*, *S100A1* (15), and *CNMD* (16). *CYTL1* promotes early chondrogenesis and protects against cartilage degradation, and its deficiency accelerates OA progression in mouse models (19,20). Similarly, *CHAD* contributes to collagen fibril organization and early skeletal development (21,22). In addition, *S100A1* and *CNMD* play important roles in maintaining chondrocyte differentiation and cartilage homeostasis (23,24). The coordinated downregulation of these genes suggests a failure to maintain

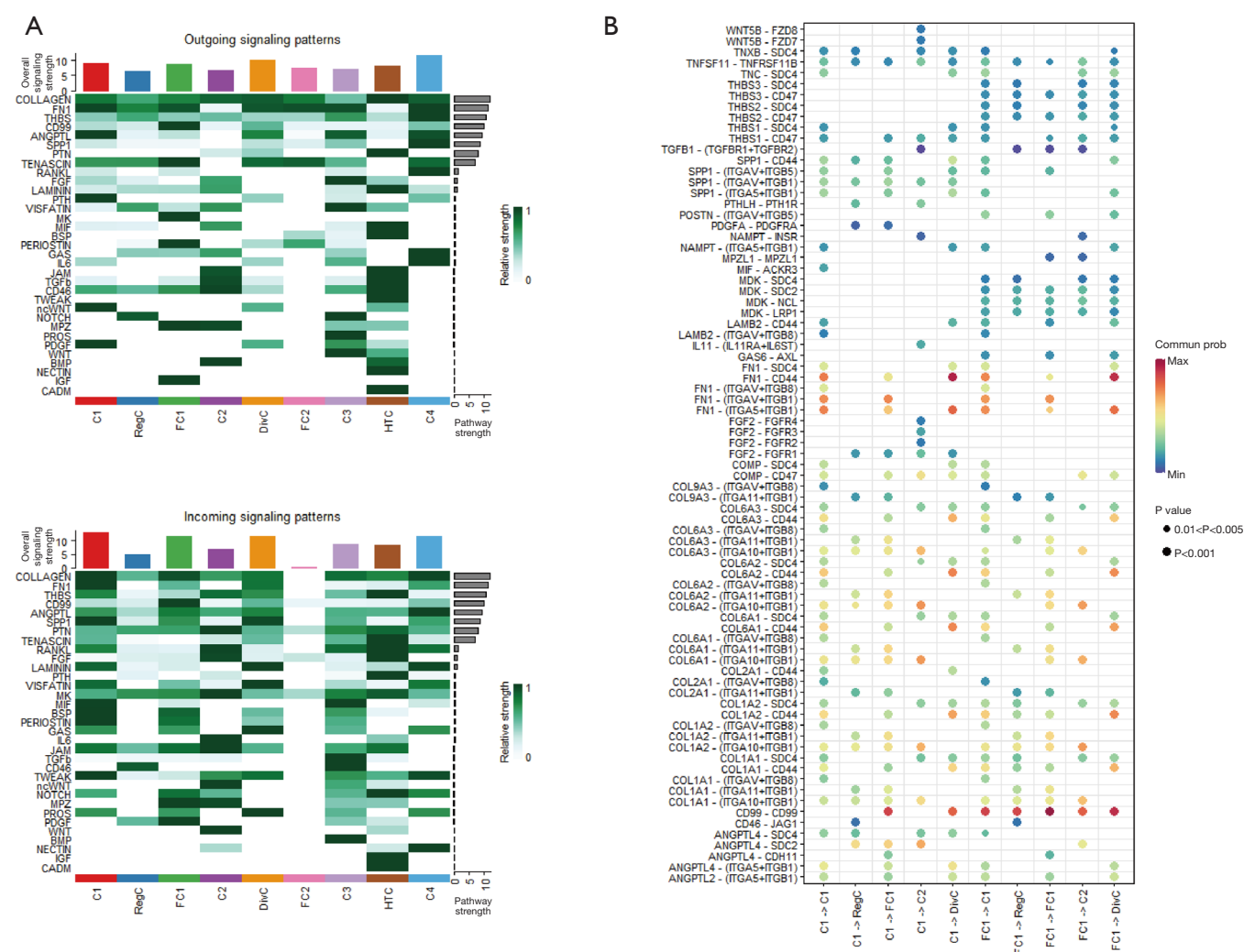


Figure 6 Cell-cell communication networks altered in DDH cartilage. (A) Heatmap showing outgoing and incoming signaling patterns among chondrocyte subsets. (B) Dot plot showing representative ligand-receptor interactions supporting the highlighted signaling pathways. DDH, developmental dysplasia of the hip; DivC, dividing chondrocyte; FC, fibrotic chondrocyte; HTC, hypertrophic chondrocyte; RegC, regulatory chondrocyte.

normal chondrocyte differentiation and ECM assembly, thereby contributing to the structural instability of DDH cartilage (25).

Single-cell trajectory analysis indicates a shift toward late, degenerative chondrocyte states

Pseudotime reconstruction positioned C2 and HTC cells at early developmental stages, whereas DDH chondrocytes were predominantly located in more advanced pseudotime states enriched for fibrotic and proliferative phenotypes (C1, FC1, FC2, and DivC). Along this trajectory, we observed

progressive loss of cartilage matrix genes and increased expression of degradative enzymes such as *MMP13*. These findings are consistent with a scenario in which abnormal joint mechanics and altered loading conditions associated with hip dislocation drive chondrocytes toward degenerative and fibrosis-prone states, rather than maintaining stable articular cartilage identity (26,27).

Altered intercellular communication highlights collagen and TGF- β signaling as key drivers of DDH pathology

CellChat analysis revealed strengthened collagen signaling

networks in nearly all DDH subsets, consistent with the widespread fibrosis observed histologically. In addition, the TGF- β pathway—known to promote ECM synthesis, fibrosis, and chondrocyte hypertrophy—was predominantly active in the C3 population. Dysregulated TGF- β signaling has been implicated in both OA and skeletal dysplasia (28–30), and our findings suggest that aberrant activation of this pathway may contribute to pathological ECM remodeling in DDH.

IGF axis dysregulation may underlie impaired cartilage growth in DDH

We observed altered expression of IGF-binding proteins (IGFBP1, IGFBP3, IGFBP5, and IGFBP6) within the C1 subset. Because IGFBPs modulate IGF availability and receptor signaling, disruptions in this axis could potentially impair normal cartilage growth and repair. Prior studies reporting reduced circulating IGFBP5 levels in infants with DDH further support this hypothesis and suggest that impaired IGF signaling may have diagnostic or therapeutic relevance. However, further mechanistic studies will be required to clarify the precise functional role of IGF signaling dysregulation in DDH (31,32).

Clinical implications

Together, our findings suggest that cartilage obtained from dislocated hips in DDH undergoes substantial molecular remodeling characterized by fibrosis, ECM disorganization, and altered chondrocyte differentiation. These transcriptional changes likely represent secondary responses to abnormal joint mechanics and loading conditions rather than primary intrinsic cartilage defects.

Understanding these secondary molecular adaptations may help explain why prolonged hip dislocation predisposes patients to early degenerative joint changes and may provide insights into potential strategies for preserving cartilage integrity once mechanical alignment of the hip is restored.

Limitations

There are several limitations in this study. First, the sample size and number of donors were limited due to the difficulty of obtaining pediatric acetabular cartilage samples. Although consistent transcriptional patterns were observed across DDH samples, donor-level variability cannot be completely excluded and should be addressed in future studies with larger cohorts.

Second, only cartilage tissue was analyzed; integration with synovium, ligament, or subchondral bone may reveal additional pathological interactions.

Third, functional experiments to validate the roles of candidate markers such as *IGFBP5* or *MMP13* were beyond the scope of this study. Future investigations using *in vitro* and *in vivo* models will be necessary to establish causal mechanisms.

Another limitation relates to the source of control cartilage, which was obtained from trauma-related surgical cases. Although macroscopically intact acetabular cartilage distant from the injury site was collected, potential stress- or inflammation-related transcriptional effects cannot be completely excluded. However, the primary molecular changes observed in this study mainly involved ECM remodeling rather than acute inflammatory signatures.

Conclusions

In summary, this study provides a comprehensive single-cell atlas of cartilage obtained from dislocated hips in DDH and reveals profound alterations in chondrocyte composition, ECM remodeling, and signaling pathways.

These findings offer new insights into the molecular adaptations associated with abnormal joint mechanics in DDH and may help inform future studies aimed at preserving cartilage health following correction of hip instability.

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None.

Footnote

Data Sharing Statement: Available at <https://tp.amegroups.com/article/view/10.21037/tp-2025-1-891/dss>

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Ethics Committee of the Children's Hospital of Fudan University [Approval No. (2022) 205]. Written informed consent was obtained from the legal guardians of all pediatric participants. This study was conducted in accordance with the approved study protocol and the Declaration of Helsinki and its subsequent amendments.

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