Loss of Type V Collagen Disrupts Mechanosensitive Signaling of TMJ Condylar Cartilage Progenitors

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INTRODUCTION: Temporomandibular joint (TMJ) condylar cartilage is a unique hybrid structure (integrating a fibrocartilage layer atop a hyaline layer), with each possessing distinct matrix and cellular characteristics [1]. The fibrous layer harbors progenitors that undergo postnatal chondrogenesis, forming hyaline cartilage and modulating endochondral ossification. [2]. Our previous studies showed that loss of collagen V, a minor fibrillogenesis regulatory collagen, leads to disrupted condylar growth and subchondral bone structure [3,4]. However, it remains unclear whether or how collagen V directly impacts the progenitor cell fate and function during TMJ postnatal growth. To address this gap in knowledge, we used a cartilage-specific collagen V knockout model (*Col5a1$^{f/f}$/AcanCre^{ER}*, or *Col5a1^{cKO}*) to test how loss of collagen V impacts condylar cartilage matrix integrity and cell fate.

METHODS: *Murine model:* Ablation of *Col5a1* was induced in *Col5a1^{cKO}* mice at 4 weeks of age via daily tamoxifen injections (4.5 mg/40 g) for 3 days. Phenotypes were evaluated at 5 and 8 weeks, with littermate *Col5a1$^{f/f}$* controls (approved by Drexel IACUC). Sagittal Sections were stained with Safranin-O/Fast Green, collagen V (ab7046, Abcam). EdU (5-ethynyl-2′-deoxyuridine, 100 mg/kg) was injected 24 h before euthanasia (Invitrogen). We applied TEM to assess collagen fibril structure [1], and AFM-nanoindentation for tissue modulus [5]. *Single-cell RNA sequencing (scRNA-seq)*: Single-cell suspensions from TMJ condylar cartilage (pooled from $n = 4$ animals) were processed with the 10x Genomics Chromium platform, sequenced on Illumina NovaSeq, and aligned to mm10 using Cell Ranger. Data were analyzed in Seurat (R) for quality control, clustering, cell cycle, and pathway enrichment.

RESULTS: In *Col5a1^{cKO}* TMJs at 5 weeks of age, we noted an aberrant increase in cell clustering and proliferation in the fibrous layer (Fig. 1a). By 8 weeks, condylar cartilage showed depletion of fibrous layer cells, and our previous μCT studies found an overgrowth of subchondral bone [4] (Fig. 1b), suggesting a crucial role for collagen V in regulating overall TMJ growth. We also found significant collagen fibril thickening (Fig. 1c,d) and reduced tissue modulus (Fig. 1e), evidencing impaired matrix elaboration. scRNA-seq identified superficial zone cells, progenitors, fibrogenic cells and chondrocytes (Fig. 2a). While there were no notable genotype-associated changes in cell types or proportions, loss of collagen V resulted in altered mechanosensitive signaling pathways both globally and in specifically progenitors (Fig. 2b,c). A higher proportion of progenitors were in the G1 phase and lower proportions in G2/M and S phases, supporting altered cell cycle and proliferation (Fig. 2d). Also, *Col5a1^{cKO}* cells exhibited differential expression of key matrix and mechanosensitive genes (Fig. 2e). This included decreased *Timp3* in cluster 1 progenitors, and increased *Mmp13* and decreased *Itga9* in cluster 2 progenitors, suggesting altered cell-matrix interactions and increased remodeling. In addition, cluster 0 fibrogenic cells showed lower *Fmod*, *Bgn*, *Acan* and *Fn1*, while cluster 5 chondrocytes showed increased *Col2a1* and *Col11a1* but decreased *Acan*, suggesting altered chondrogenesis.

DISCUSSION: Our results highlight the pivotal role of collagen V in regulating condylar cartilage matrix integrity and progenitor mechanosensitive signaling. In postnatal growth, the progenitor cells give rise to fibrogenic and chondrogenic cells that establish the hybrid matrix, which further develop into the subchondral bone [6]. Here, the altered expression of genes related to matrix content, cell-matrix interactions and remodeling observed in *Col5a1^{cKO}* TMJs suggest that collagen V not only directly regulates the elaboration of fibrous layer matrix, but also may alter the matrix-mediated mechanosensitive signaling of progenitors. It is possible that either collagen V directly regulates cell-matrix adhesion, or mediates the transmission of mechanical cues by modulating matrix integrity. The loss of collagen V impacts not only the progenitors, but also fibrogenic cells and chondrocytes (Fig. 2e), highlighting its crucial role in regulating the overall TMJ establishment and remodeling. These results are in contrast to our findings in the knee joint, which showed that embryonic loss of collagen V did not directly affect cellular function, despite pronounced matrix defects [7], underscoring the unique mechanosensitive nature of these TMJ progenitors.

SIGNIFICANCE/CLINICAL RELEVANCE: This work identifies collagen V as a crucial regulator of condylar cartilage matrix elaboration and progenitor cell function, establishing the basis for modulating TMJ growth and disease by leveraging collagen V-mediated cell-matrix interactions.

REFERENCES: [1] Chandrasekaran+ 2021. [2] Embree+ 2016. [3] Chandrasekaran+ 2024. [4] Alanazi+ 2023. [5] Chandrasekaran+ 2017. [6] Shen+ 2005. [7] Kwok+ 2025.

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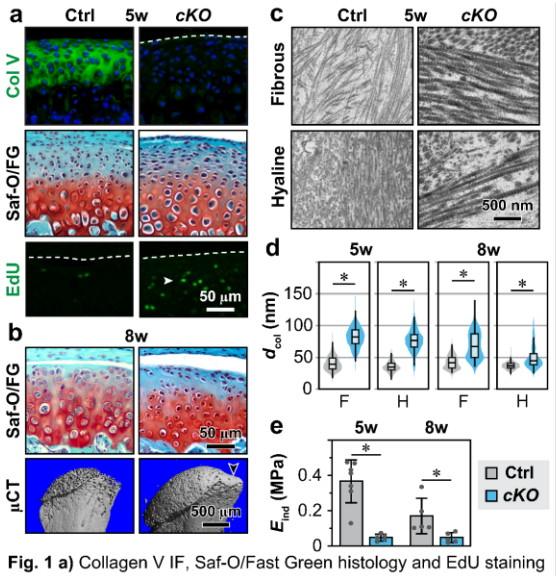

Fig. 1 a) Collagen V IF, Saf-O/Fast Green histology and EdU staining for condylar cartilage at 5 weeks of age, **b)** Histology and μCT (adapted from [4]) at 8 weeks for control and *Col5a1^{cKO}* (***cKO***) TMJs ($n \geq 5$), **c)** TEM images for both fibrous and hyaline layers at 5 weeks, **d)** Collagen fibril diameter at 5 and 8 weeks (> 350 fibrils from $n \geq 3$ animals, *: $p < 0.0001$), **e)** AFM-nanoindentation modulus of condylar cartilage at 5 and 8 weeks (mean ± 95% CI, $n \geq 5$, *: $p < 0.01$).

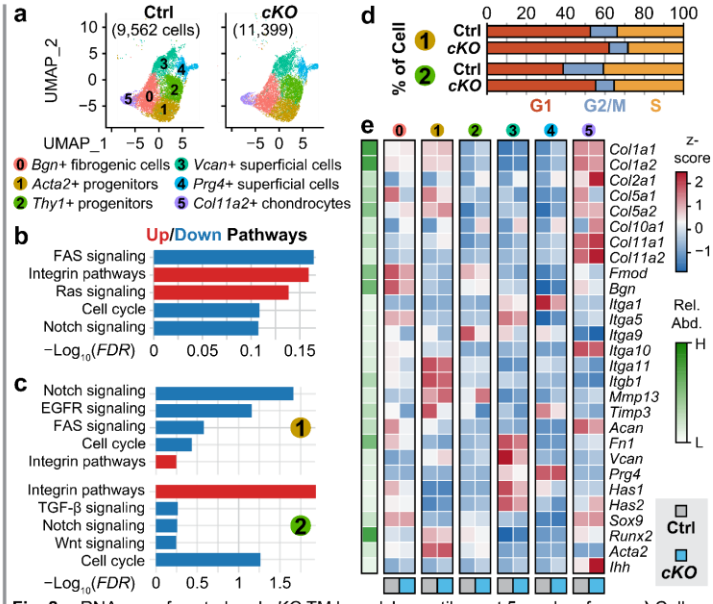

Fig. 2 scRNA-seq of control and *cKO* TMJ condylar cartilage at 5 weeks of age. **a)** Cell population UMAP after excluding irrelevant cells, **b,c)** Enrichment analysis highlighting altered selective mechanosensitive pathways: b) global changes, c) progenitor clusters 1 and 2. **d)** Progenitor cell proportion in each cell cycle phase, **e)** Heatmap of major matrix and mechanosensitive genes for each cluster (green: relative abundance).