Type V Collagen is Essential for the Initial Matrix Templating of Articular Cartilage and the Meniscus

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Disclosures: RL Mauck (4, *Mechano-Therapeutics*; 5, *4Web Medical*; 8, *JOR Spine*), no other disclosures.

INTRODUCTION: Development of effective regeneration strategies for articular cartilage and meniscus is challenged by our poor understanding of how these tissues first establish their extracellular matrices (ECMs) in vivo [1]. We recently showed that the primitive ECMs of both tissues undergo rapid regional specialization, with daily exponential stiffening during embryonic development. This establishes the template from which resident cells, guided by signals from their mechanical microenvironment, direct the growth of mature tissues [2]. At this early developmental stage, type V collagen is highly concentrated in both collagen II-rich articular cartilage and collagen I/II-hybrid meniscus [3], despite their distinct molecular composition and structure [4, 5]. This study queried the impact of collagen V depletion on the initial primitive matrix establishment and cell phenotype prior to further matrix elaboration.

METHODS: Knee joints were harvested from joint-specific *Col5a1*-knockout (*Col5a1$^{f/f}$/Gdf5Cre* [6, 7], or *Col5a1^{cKO}*) and control *Col5a1$^{f/f}$* mice at newborn (post-natal day 0, P0) and young adult (P90) ages. We applied *histology* and *immunofluorescence (IF)* to assess joint morphology, sGAG staining and collagen V distribution. *SEM* was used to quantify collagen fibril diameter, d_{col}. We applied *AFM-nanoindentation* using microspherical tips to quantify the micromodulus, E_{ind}, of different tissues and regions of cryo-sections from P0 samples ($R \approx 12.5$ µm, $k \approx 0.6$ N/m) [2] and intact tissues for P90 ($R \approx 5$ µm, $k \approx 8.9$ N/m) [8], followed by Mann-Whitney U test on E_{ind} at $\alpha = 0.05$. Joints from two P0 mice for each genotype were digested for single-cell RNA-sequencing (10X, final cell count: 7,281 for the control, 6,061 for *Col5a1^{cKO}*), followed by raw data filtering, dimension reduction and unsupervised cell subset clustering in Serat [9]. The PANTHER database was used to determine if specific biological pathways were impacted by the loss of collagen V [10].

RESULTS: In P0 *Col5a1^{cKO}* mice, ablation of *Col5a1* (Fig. 1a) resulted in moderate changes in the meniscus morphology, including a reduction in medial meniscus area (Fig. 1a, b) but did not change meniscus cell number. For articular cartilage, *Col5a1* depletion did not impact tissue area (Fig. 1b) or cell number (data not shown). However, we noted significant thickening of collagen fibrils (Fig. 1c) and reduction of micromodulus in both tissues (Fig. 1d), suggesting impaired matrix templates at this early time. In contrast, no micromechanical changes were noted in the epiphyseal cartilage (Fig. 1d), consistent with the absence of collagen V observed in this tissue region (Fig. 1a). In P90 mice, *Col5a1^{cKO}* joints developed much-reduced meniscus size, loss of sGAGs in articular cartilage (Fig. 1e), as well as significantly reduced modulus for both tissues (Fig. 1f), underscoring substantially disrupted post-natal growth.

Despite these marked changes in the primitive matrix at P0, scRNA-sequencing did not detect major changes in cell populations, cluster identities (Fig. 2a) or cell cycle phases (data not shown) in *Col5a1^{cKO}* joints. Differentially expressed gene (DEG) analysis revealed only modest changes in *Col5a1^{cKO}* cells, with limited gene fold changes in cartilage (clusters 2, 5), meniscus (cluster 6) and other fibrous tissues (clusters 7, 8), with most regulated pathways related to ER-stress-associated *Ergic* and ribosomes (Fig. 2b). Within these clusters, gene ontology (GO) analysis only detected mild changes in pathways related to translation (Fig. 2c). We did not find notable changes in the expression in any of the major collagens, proteoglycans or other matrix elements, aside from the expected reduction of *Col5a1*, and mildly changed DEGs related to β-actin (*Actb*), adherens junctions (*Mpp7*) and cell homeostasis (*Prdx1*) (Fig. 2d).

DISCUSSION: This study highlights a crucial role of collagen V in the initial matrix templating of both articular cartilage and the meniscus. At P0, before the start of weight bearing, loss of collagen V leads to thickened collagen fibrils (Fig. 1c), reduced modulus (Fig. 1d) and other morphometric changes (Fig. 1a, b). Meanwhile, the lack of changes in cell identity or major signaling pathways (Fig. 2) suggests that, at this early stage, collagen V does not drastically alter cell phenotype, and that resident cells have some degree of resistance to perturbations in their matrix microniche. On the other hand, the moderate changes in ribosome genes (Fig. 2b), cell-matrix and cell-cell interaction genes (e.g., *Actb*, *Mpp7*, Fig. 2d), may indicate that these cells are under some level of stress as a result of collagen V loss by P0. As a result, this collagen V-deficient matrix template leads to markedly disrupted maturation (Fig. 1e,f), despite the low presence of collagen V in adult WT joint [11]. Therefore, our finding points to a novel role of collagen V in regulating the initial establishment of hyaline and fibrocartilage matrices beyond its canonical function of mediating collagen I fibrillogenesis [12]. Modulating collagen V activities could provide a new path to direct the formation of both cartilage and meniscus matrices, without disrupting major signaling pathways, to promote functional regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: This study reveals an essential role of collagen V in the initial matrix templating for both collagen II-rich articular cartilage and collagen I/II-hybrid meniscus. Results support that collagen V is a potential target for improving the regeneration of both tissues.

REFERENCES: [1] Kwon+ 2019. [2] Kwok+ 2023a. [3] Kwok+ 2023b. [4] Han+ 2011. [5] Makris+ 2011. [6] Sun+ 2011. [7] Rountree+ 2004. [8] Wang+ 2020. [9] Stuart+ 2019. [10] Mi+ 2009. [11] Chandrasekaran 2021+. [12] Wenstrup+ 2004.

ACKNOWLEDGEMENTS: This work was supported by NSF CMMI-2047073, NIH R01AR075418 and UPenn PCMD NIH P30AR069619.

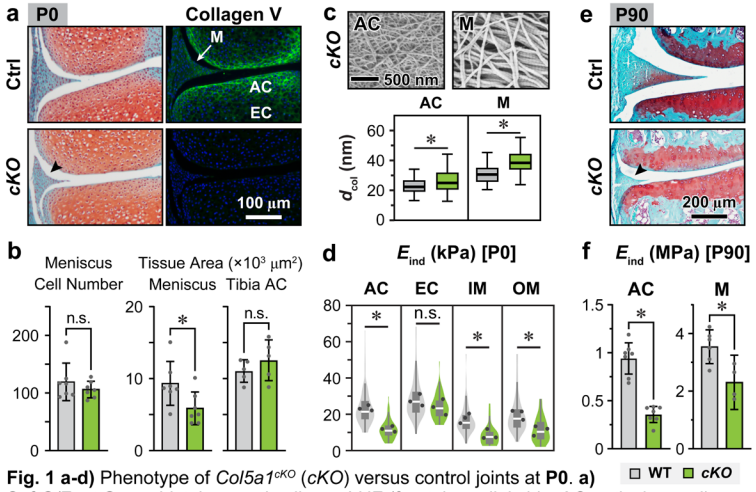

Fig. 1 a-d) Phenotype of *Col5a1^{cKO}* (*cKO*) versus control joints at **P0**. **a)** Saf-O/Fast Green histology and collagen V IF (frontal, medial side, AC: articular cartilage, EC: epiphyseal cartilage, M: meniscus). **b)** Morphometric analysis of medial M area, cell number and AC area ($n \geq 5$). **c)** SEM images of *cKO* joint cryo-sections, and comparison of fibril diameter, d_{col} (≥ 200 fibrils). **d)** Micromodulus of AC, EC, inner (IM) and outer meniscus (OM) by AFM on frontal cryo-sections. **e-f)** Phenotype at **P90**. **e)** Medial knee joint histology. **f)** Tissue modulus by AFM ($n \geq 5$). (mean ± 95% CI, each data represents average of one animal, *: $p < 0.05$, black arrowheads in a, e: reduced meniscus size)

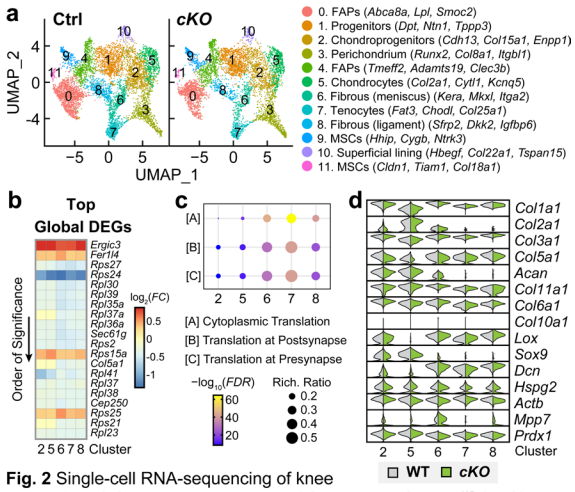

Fig. 2 Single-cell RNA-sequencing of knee joint at **P0**. **a)** Cell population UMAP of Control and *Col5a1^{cKO}* (*cKO*) mice after excluding irrelevant cells. **b)** Most significant differentially expressed genes (DEGs) mainly consist of ribosome related genes (*Rpls*). **c)** Gene ontology analysis reveals largest changes in protein translation pathways, strongly correlating to changes in ribosome gene expression. **d)** Matrix genes of interest show modest changes between control and *cKO* cells, except for moderate DEGs of *Actb*, *Mpp7* and *Prdx1*.