Loss of Decorin Accelerates Cartilage Surface Damage and Aberrant Fibrous Remodeling during Aging

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INTRODUCTION: Aging is the leading risk factor for osteoarthritis (OA), with more than 10% of the population > 60 years of age presenting with symptomatic disease [1]. While considerable efforts have been made to understanding the cellular hallmarks of aging [2], there is limited knowledge on how changes in cartilage extracellular matrix (ECM) contribute to the onset of aging-related spontaneous OA [3]. Our recent studies found that decorin, a small proteoglycan, plays a crucial role in regulating the retention of aggrecan during post-natal growth [4] and in injury-induced OA [5]. In aged constitutive decorin-null mice, we also noted accelerated loss of aggrecan and its sulfated glycosaminoglycans (sGAGs) relative to wild-type (WT) controls, indicating a potential role of decorin in cartilage maintenance during aging [6]. This study sought to investigate how decorin regulates cartilage matrix integrity and associated cell phenotypic changes via targeted, cartilage-specific decorin deletion in an aging murine model.

METHODS: We induced cartilage-specific knockout of *Dcn* by i.p. injection of tamoxifen to *Dcn*$^{f/f}$/*AcanCre*^{ER} (*Dcn*^{cKO}) mice [7] at 3 months of age, and evaluated joint phenotype at 9 and 18 months (9M and 18M). PicroSirius Red (PSR) staining and RNAscope were applied to sagittal paraffin sections to assess joint morphology, collagen fiber alignment and ECM gene expression. AFM-nanoindentation was applied to quantify femoral and tibial cartilage tissue modulus ($R \approx 5$ µm, $k \approx 8.9$ N/m) [8] and IF-AFM nanomechanical mapping was applied to delineate the micromodulus of the pericellular (PCM) and territorial/interterritorial (T/IT)-ECM on tibial cartilage cryo-sections ($R \approx 2.25$ µm, $k \approx 0.6$ N/m) [9], followed by Mann-Whitney U test at $\alpha = 0.05$. Single-cell RNA-sequencing (10X) was applied to 18M femoral cartilage (high-quality cells: 6,654 for the control, 5,165 for *Dcn*^{cKO} from 2 mice each). Seurat was used to identify cellular subsets and the PANTHER database to identify biological pathways impacted by *Dcn* deletion via statistical enrichment [10].

RESULTS: In 9M mice, we validated the reduction of *Dcn* expression (Fig. 1a), and found a decrease of sGAG staining, corroborating with our previously reported phenotype of decreased aggrecan content with the loss of decorin [4]. At this same time point, we did not notice appreciable *Col1a1* expression in either genotype (Fig. 1a), suggesting cartilage ECM retained its hyaline nature at 9M. By 18M, however, marked differences were observed between genotypes. Specifically, *Dcn*^{cKO} femoral cartilage surface formed a layer of highly aligned, thick collagen fibers, which were devoid of sGAGs. This new tissue layer harbored cells actively expressing *Col1a1*, indicative of fibrous tissue formation (Fig. 1b). This fibrous layer also showed aberrantly higher modulus compared the control, underscoring the loss of hyaline cartilage phenotype (Fig. 1c). Notably, cells in this layer actively expressed *Dcn* (Fig. 1b), suggesting that these cells may have migrated from adjacent tissues that did not express *Acan* at 3M. In support, scRNA-seq identified a new cell cluster in *Dcn*^{cKO} mice (cluster 2, Fig. 2a,b). These cells were highly metabolic, with high expression of collagens, proteoglycans, lysyl oxidases and MMPs, as well as altered biological pathways (Fig. 2c,d). In contrast, in other cell clusters, including articular chondrocytes (cluster 4), loss of decorin resulted in only mild changes in matrix organization genes. For the 18M tibial cartilage, however, we did not observe the formation of this fibrous layer. Instead, we found decreased sGAG staining (Fig. 1b) and reduced cartilage modulus both at the tissue-level (Fig. 1c) and within the PCM and T/IT-ECM (Fig. 1d) [4].

DISCUSSION: This study highlights an unexpected emergent phenotype marked by aberrant fibrous remodeling on the femoral surface of aged cartilage caused by the loss of decorin. The presence of *Col1a1* and *Dcn*-expressing cells indicates the fibrous character of this surface remodeling and suggests infiltration of cells from adjacent tissues. This fibrous layer is much stiffer (Fig. 1c), lacks the sGAG-rich hyaline traits (Fig. 1c), and thus, does not possess the poroelastic energy dissipation functions of cartilage. These changes represent accelerated, severe degradation of femoral condyle cartilage with the deletion of decorin. Given the observed degenerating of femoral cartilage at 9M (Fig. 1a), it is possible that the progressive aggrecan loss due to decorin deletion and continuous physiologic loading leads to cartilage erosion and infiltration of these metabolically active cells from adjacent tissues, possibly synovial lining and fat pad, forming this aberrant layer covering the damaged surface by 18M. As cartilage erosion and fibrillation are common symptoms in advanced OA patients [11], these results point to a crucial role of decorin in inhibiting cartilage damage and OA progression in aged individuals. Notably, such role is primarily manifested through decorin's direct impacts on ECM integrity, rather than chondrocyte signaling, as supported by the mild changes in *Dcn*^{cKO} chondrocyte signaling pathways (cluster 4, Fig. 2c,d). Meanwhile, the observation that the tibial cartilage retained its hyaline cartilage traits at 18M (Fig. 1b-d) indicates higher susceptibility of femoral cartilage to degeneration, possibly due to the thinner thickness of femoral cartilage in young adults [12].

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlights a crucial role of decorin in maintaining cartilage ECM integrity and inhibiting fibrous remodeling during aging, establishing decorin as a novel target for enhancing cartilage maintenance and ameliorating aging-related degeneration.

REFERENCES: [1] Zhang+ 2010. [2] Loeser+ 2016. [3] Fan+ 2022. [4] Han+ 2019. [5] Li+ 2020. [6] Fan+ 2023. [7] Henry+ 2009. [8] Doyran+ 2017. [9] Chery+ 2020. [10] Knights+ 2023. [11] Pritzker+ 2006. [12] Li+ 2005.

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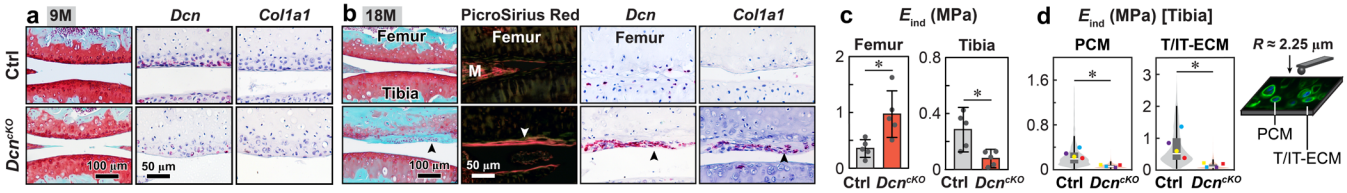

Fig. 1 a,b) Safranin-O/Fast Green, Picrosirius Red and RNAscope images of *Dcn*^{cKO} and control mice at **a)** 9 and **b)** 18 months (arrowhead: *Col1a1*-expressing fibrous layer on *Dcn*^{cKO} femoral condyle surface; M: meniscus). **c)** Tissue modulus of intact femoral and tibial cartilage at 18M (mean ± 95% CI, $n = 5$, *: $p < 0.05$). **d)** Micromodulus of tibial cartilage PCM and T/IT-ECM at 18M by IF-AFM ($n = 4$, *: $p < 0.05$; Each data point represents the average value from one animal).

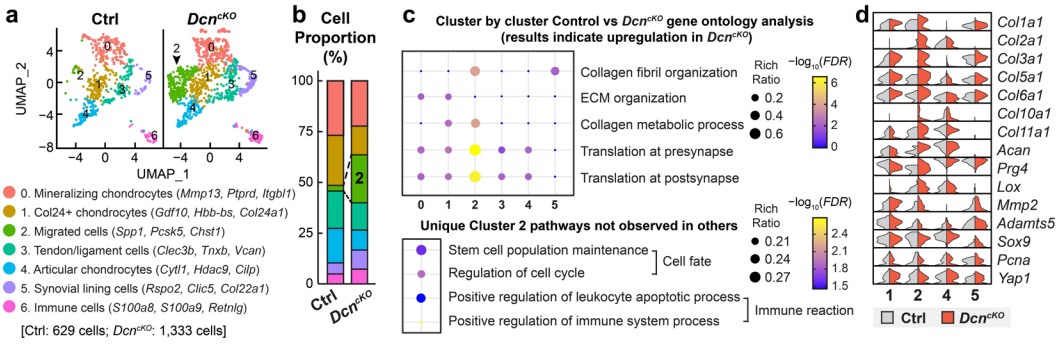

Fig. 2 ScRNA-seq of 18M femur cartilage. **a)** UMAP shows a new cell Cluster 2 for *Dcn*^{cKO} group (arrowhead) after excluding irrelevant cells. Top 3 gene markers are listed after filtering by pct.1 > 0.5 and sorted by average $\log_2$-fold comparision between *Dcn*^{cKO} and control. **b)** Comparison of cell proportions highlights the infiltration of Cluster 2 cells. **c)** Gene enrichment analysis of altered biological pathways. **d)** Violin plots of genes of interest related to ECM organization.