Metabolic Labeling Reveals a Critical Role for Decorin in the Assembly and Turnover of Cartilage Matrix

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INTRODUCTION: The development of effective cartilage regeneration is challenged by our incomplete understanding of molecular activities that regulate the assembly, turnover and degradation of its extracellular matrix (ECM) [1], a hydrated composite of collagen II/IX/XI fibrils and aggrecan macromolecules [2]. Our recent study identified a critical role for the small leucine-rich (SLRP) proteoglycan, decorin, in regulating the ECM integrity in both health and osteoarthritis (OA). In decorin-null (*Dcn*$^{-/-}$) mice, articular cartilage exhibits reduced aggrecan levels in both the pericellular matrix (PCM) and territorial/interterritorial (T/IT)-ECM, and perturbed chondrocyte mechanotransduction [3]. This role was attributed to a novel "physical linkage" function of decorin, acting to enhance aggrecan-aggrecan and aggrecan-collagen fibril molecular adhesion [4]. In cartilage ECM, aggrecan undergoes active turnover [5], with a higher turnover rate in the PCM [6]. Given the role of decorin in increasing aggrecan retention, we investigated how decorin mediates the turnover of newly synthesized proteins and glycosaminoglycans (GAGs) using a bio-orthogonal click-labeling method [7] and elucidated how decorin regulates the collagen-aggrecan integration by AFM-nanoindentation to cartilage after enzymatic GAG removal and infiltration of exogenous decorin.

METHODS: Femoral head cartilage explants were harvested from 3-week-old decorin-null (*Dcn*$^{-/-}$) and wild-type (WT) C57BL/6 mice. Explants were pre-cultured for 2 days, and then cultured for another 3 days with either 30 mM N-azidoacetylgalactosamine-tetraacylated (GAL) (1086, ClickChemistryTools) or L-azidohomoalanine (AHA) (1066, ClickChemistryTools) in chondrogenic DMEM [8] to metabolically label newly synthesized GAGs and proteins [7], respectively. Explants were then tagged with 30 mM AZDye 488 DBCO (1278, ClickChemistryTools) and then cultured for an additional 6 days in chondrogenic DMEM, with or without stimulation by the inflammatory cytokine IL-1β (10 ng/mL) to elicit chondrocyte catabolism [9]. GAL-labeled nascent GAGs and AHA-labeled nascent proteins released into the supernatant were collected every other day for quantification. At day 6, tissues were digested with 2% papain to quantify the nascent protein/GAG retained in the explant. Confocal images were taken at day 0 and 6 following click labeling to assess the spatial distribution of nascent proteins and GAGs within the explant, using a Zeiss LSM700 confocal microscope. Following our established procedure [4], AFM-nanoindentation was applied to quantify the indentation modulus, E_{ind}, of 3-week-old *Dcn*$^{-/-}$ and WT femoral condyle cartilage using a microspherical tip ($R \approx 5$ μm, $k \approx 5.4$ N/m) in PBS. The test was applied to untreated cartilage, samples in which sulfated GAG was depleted by 0.5 U/ml chondroitinase ABC and 500 U/ml hyaluronidase digestion for 24 hrs, and sGAG-depleted tissues infiltrated with exogenous bovine decorin (D8428, Sigma, 20 μg/mL for 24 hours). Repeated measure two-way ANOVA was applied to study the effects of genotype and IL-1β on GAG/protein release, and two-way ANOVA was applied to study the effects of genotype and treatment on E_{ind}, followed by Tukey-Kramer multiple comparison at $\alpha = 0.05$.

RESULTS: Fluorescence images showed clear differences in the distributions of nascent GAGs (Fig. 1a) and proteins (Fig. 1b). From the GAL-labeling, we observed a higher concentration of nascent GAGs surrounding the cells, illustrating the preferred localization of nascent proteoglycans in the PCM, with or without IL-1β stimulation. From the AHA-labeling, we observed nascent proteins distributed nearly homogeneously throughout the intercellular space. At both day 0 and 6, we did not observe appreciable differences between the WT and *Dcn*$^{-/-}$ explants. When stimulated with IL-1β, an increasing percentage of nascent GAGs were released from the explant for both genotypes (Fig. 2), concurrent with a substantial decrease in GAL-signal in the PCM (Fig. 1a). With and without IL-1β, a higher proportion of GAGs were released from *Dcn*$^{-/-}$ cartilage, with no differences noted for protein release (Fig. 1b). Under AFM-nanoindentation, for WT cartilage, sGAG removal significantly reduced tissue modulus; this could be partially restored by decorin infiltration. For *Dcn*$^{-/-}$ cartilage, the modulus of untreated tissue was lower than that of WT, and neither sGAG removal nor decorin infiltration had a significant impact (Fig. 3).

DISCUSSION: This study highlights a crucial role for decorin in regulating the active turnover and retention of newly synthesized aggrecan in healthy and degenerative cartilage. In cartilage ECM, the majority of GAGs constitute the side chains of aggrecan [10]. Thus, accelerated release of nascent GAGs from *Dcn*$^{-/-}$ cartilage (Fig. 2a) supports the essential role of decorin in the retention of newly synthesized aggrecan. Conversely, the lack of difference in nascent protein release (Fig. 2b) suggests that decorin does not directly impact collagen retention. Such role is crucial not only for the retention of aggrecan in healthy cartilage, but under OA-like conditions, when the fragmentation of aggrecan is aggravated by increased catabolism (e.g., IL-1β treatment).

The observation that exogenous decorin increased the modulus of sGAG-depleted WT cartilage (Fig. 3) suggests that decorin strengthens the collagen fibrillar network, likely by providing physical linkages through its leucine-rich, hydrophobic core protein to connect adjacent collagen II fibrils. In doing so, the decorin-strengthened collagen network provides higher resistance to the diffusive loss of aggrecan entrapped therein, which is driven by the high swelling pressure from the negative charges on sGAG [11]. Since the collagen fibril network is a prominent feature of both PCM and T/IT-ECM [3], decorin likely regulates the aggrecan-collagen integration in both domains. Indeed, while localization of nascent GAGs in the PCM (Fig. 1a) evidences specific interactions between aggrecan and PCM-specific molecules, this is not regulated by decorin, as nascent GAGs showed similar localization for both genotypes (Fig. 1a). Notably, addition of exogenous decorin did not rescue properties in *Dcn*$^{-/-}$ cartilage (Fig. 3). One explanation may be that loss of decorin alters collagen fibril structure, i.e., higher fibril heterogeneity [4], and thus, exogenous decorin is unable to effectively establish physical linkages with this altered network. In conclusion, our results highlight a critical role for decorin in both normal maintenance and disease-related cartilage remodeling.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests a crucial role for decorin in increasing the retention of newly synthesized aggrecan by strengthening the collagen fibril network, enabling the development of decorin-based strategies for improving cartilage regeneration and OA intervention.

REFERENCES: [1] Huey+ 2012. [2] Han+ 2011. [3] Chery+ 2021. [4] Han+ 2019. [5] Lohmander+ 1999. [6] Quinn+ 1999. [7] Porter+ 2022. [8] Loebel+ 2020. [9] Li+ 2020. [10] Poole+ 1996. [11] Warren+ 2022.

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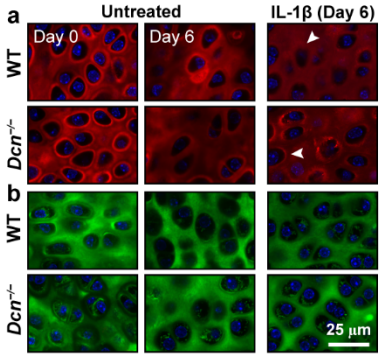

Fig. 1 Representative confocal images of click-labeled **a)** nascent GAGs by GAL and **b)** nascent proteins by AHA with (day 6) and without (day 0, 6) IL-1β stimulation.

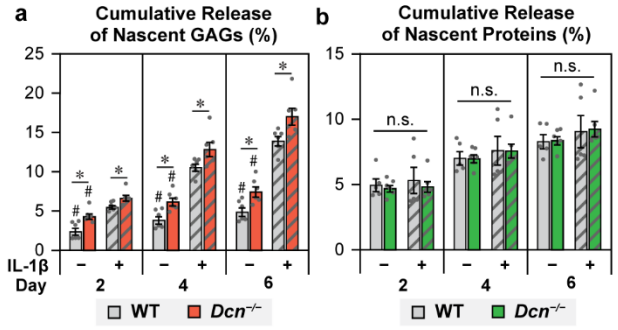

Fig. 2 Cumulative release of **a)** newly synthesized sGAGs measured by GAL and **b)** newly synthesized proteins by AHA click-labeling on WT and *Dcn*$^{-/-}$ P21 femoral head cartilage explants cultured with or without the stimulation of IL-1β at day 2, 4, and 6 of culture (mean ± S.E.M., $n \geq 5$, *: $p < 0.05$ between each genotypes; #: $p < 0.05$ between treatments).

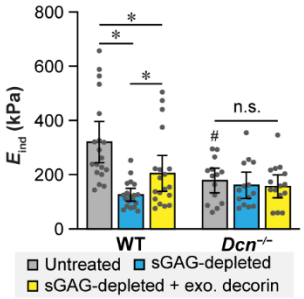

Fig. 3 AFM-nanoindentation modulus, E_{ind}, of WT and *Dcn*$^{-/-}$ cartilage for untreated, sGAG-depleted and decorin-infiltrated samples (mean ± 95% CI, $n \geq 14$, *: $p < 0.05$ between each treatment, #: $p < 0.05$ between genotypes).