Regulation of Cartilage Matrix Stability via Decorin Molecular Interactions with Collagen II and Aggrecan

Thomas Li[1], Gabriela Canziani[1], Biao Han[1], Neil Patel[1], X. Lucas Lu[2], David E. Birk[3], Renato V. Iozzo[4], Irwin M. Chaiken[1], Robert L. Mauck[5], Lin Han[1]
[1]Drexel University, Philadelphia, PA; [2]University of Delaware, Newark, DE; [3]University of South Florida, Tampa FL;
[4]Thomas Jefferson University, Philadelphia, PA; [5]University of Pennsylvania, Philadelphia, PA. Contact: Thomas Li: tl545@drexel.edu.

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INTRODUCTION: Current biomaterials therapeutic for osteoarthritis (OA) are hindered by our limited understanding of the molecular interaction governing assembly and stability of cartilage extracellular matrix (ECM) [1], a hydrated composite of collagen II/IX/XI fibrils and aggrecan [2]. Our recent study showed that decorin is essential for the proper establishment of cartilage ECM integrity. In decorin-null (*Dcn*$^{-/-}$) mice, cartilage exhibited reduced aggrecan content, contributing to impaired tissue-level biomechanics [3], pericellular matrix (PCM) micromechanics [4] and accelerated OA progression in the destabilization of the medial meniscus (DMM) model. In vivo, decorin exhibited a wide interactome with matrix molecules, cell surface receptors, growth factors and cytokines [5]. Although our previous studies showed that decorin could stabilize the ECM by increasing aggrecan-aggrecan and aggrecan-collagen II molecular adhesion [3], it remains unclear whether the primary function of decorin in cartilage is to regulate matrix integrity through its biophysical activities or to regulate chondrocyte biosynthesis through regulating major signaling pathways such as TGF-β [6]. This study aims to delineate the biophysical versus biological functions of decorin in cartilage.

METHODS: *Bulk RNA-sequencing* was performed on femoral head cartilages harvested from 1-month-old wild-type (WT) and *Dcn*$^{-/-}$ mice (Fig. 1) using commercial Eukaryotic Transcriptome resequencing (BGI America) (approved by Drexel IACUC). Gene clustering heatmaps were generated to assess changes in major chondrocyte signaling pathways (e.g., TGF-β). *Surface plasmon resonance* (SPR) was performed (BIAcore S200) to assess binding kinetics using ligand molecules (decorin or collagen II) immobilized on a CM5-PEG gold surface sensor with EDC/NHS coupling (Fig. 2a). In a direct-interaction "bi-element" setup, decorin (D8428, Sigma), collagen II (C3667, Sigma), aggrecan (A1960, Sigma) and aggrecan core protein were injected as the analyte in a series of decreasing concentrations (1,200-0.549 nM) (Fig. 2a) (n = 3 independent replicates). Aggrecan core protein was prepared by digestion of aggrecan with 500 U/ml hyaluronidase and 0.5 U/ml chondroitinase ABC overnight at 37ºC. A Monte Carlo simulation analysis was performed with three independent experiments to estimate the kinetic association/dissociation rates (k_a, k_d) and equilibrium dissociation constant (K_D) based on the classic Langmuir 1:1 model [9]. Additionally, a three-component "tri-element" setup was applied to assess a potential stabilizing role of decorin between aggrecan and collagen II. Concurrent bi-element ligand-analyte interactions were estimated with or without the pre-injection of 400 nM decorin as a coupler to the ligand, and the Langmuir 1:1 model was applied to estimate the decorin-mediated fold changes in K_D (n = 3 independent replicates).

RESULTS: Despite the pronounced aggrecan reduction in *Dcn*$^{-/-}$ cartilage [3], RNA-seq did not show notable changes in transcriptomes for *Dcn*$^{-/-}$ chondrocytes. Out of the > 15,000 genes analyzed, except for the expected reduction of *Dcn*, we only identified seven mildly altered genes with adjusted p < 0.05 (Fig. 1a). In turn, we found no evidence of altered signaling pathways, including canonical TGF-β (Fig. 1b) and other mechano-sensitive pathways. Meanwhile, SPR (Fig. 2a) showed direct molecular interactions of decorin with collagen II and aggrecan. In the bi-element setup, decorin exhibited similar orders of association rates k_a with decorin, aggrecan and its core protein, but much faster dissociation rates k_d with the core protein. Conversely, decorin showed slower association and dissociation with collagen II. In turn, decorin-decorin and decorin-aggrecan had comparable K_D values, which were lower than decorin-aggrecan core protein and decorin-collagen II (Table 1). Also, when applied as a coupler in the tri-element set-up, decorin increased the binding affinities of aggrecan and collagen II, as marked by significant decreases in K_D (Fig. 2c).

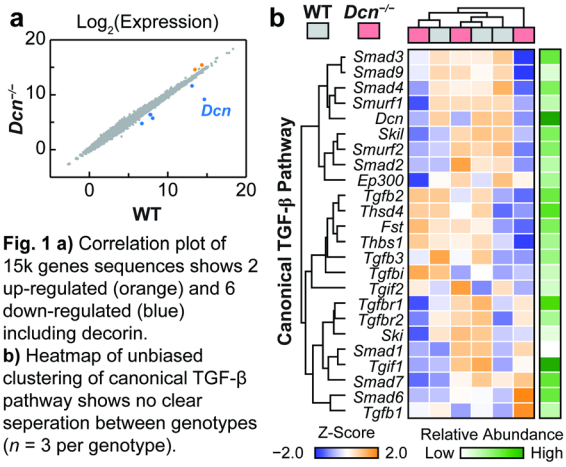

Fig. 1 a) Correlation plot of 15k genes sequences shows 2 up-regulated (orange) and 6 down-regulated (blue) including decorin. **b)** Heatmap of unbiased clustering of canonical TGF-β pathway shows no clear seperation between genotypes (n = 3 per genotype).

DISCUSSION: This study provides molecular insight into the biophysical roles of decorin in stabilizing the cartilage matrix assembly at the molecular level. Specifically, the lower K_D values of decorin-decorin and decorin-aggrecan interactions (Table 1) indicate that decorin plays an integral role in reinforcing the collagen II-aggrecan interactions and formation of the complex. This is further supported by our tri-element SPR outcomes, which confirms the ability of decorin to strengthen aggrecan-aggrecan and aggrecan-collagen II interactions (Fig. 2b), corroborating earlier findings showing that decorin increases the aggrecan-aggrecan and aggrecan-collagen II molecular adhesion [12]. Second, by connecting collagen II molecules, decorin could strengthen the collagen II fibrillar network, thereby enhancing the confinement effect of the collagen fibril network against the diffusive loss of aggrecan driven by osmotic pressure [13]. Therefore, these decorin-endowed interactions work in synergy to increase aggrecan-collagen II integration, contributing to the overall ECM integrity. On the other hand, the lack of changes from RNA-seq analysis indicates that decorin does not directly influence chondrocyte fate or mechanosensitive signaling in vivo. This reaffirms that the primary role of decorin in cartilage is structural, and suggests that chondrocytes retain a certain degree of innate resilience to microenvironmental perturbations. However, the disrupted matrix integrity and cell mechano-sensing may indicate higher susceptibility to disease onset, as supported by the accelerated OA in *Dcn*$^{-/-}$ mice after DMM [14].

Table 1. Estimated bi-element binding kinetics values via Monte Carlo simulation (mean ± 95% CI)

Ligand	Analyte	k_a ($\times 10^4$ $M^{-1}\cdot s^{-1}$)	k_d ($\times 10^{-3}$ s^{-1})	K_D ($\times 10^{-9}$ M)
Decorin	Decorin	3.09 ± 0.01	0.26 ± 0.01	8.39 ± 0.02
Decorin	Aggrecan	5.38 ± 0.01	0.20 ± 0.02	3.75 ± 0.02
Decorin	Aggrecan Core Protein	1.97 ± 0.01	1.95 ± 0.01	98.8 ± 0.1
Collagen II	Decorin	0.608 ± 0.001	0.455 ± 0.001	74.8 ± 0.8

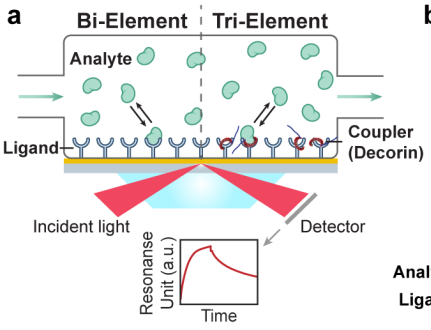

Fig. 2 a) Schematic of surface plasmon resonance (SPR) setup for bi-element and tri-element binding. **b)** Fold changes in the inverse of equilibrium dissociation constant, $1/K_D$, show increased binding affinities (lower K_D) for all tested collagen II-aggrecan bindings when decorin is applied as a coupler in tri-element setup (mean ± SEM, n = 3).

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides molecular-level insights into the biophysical role of decorin in regulating cartilage matrix stability, providing the foundation for using decorin-targeting strategies for OA intervention or cartilage repair.

REFERENCES: [1] Huey+ 2012. [2] Han+ 2011. [3] Han+ 2019. [4] Chery+ 2021. [5] Gubbiotti+ 2016. [6] Hildebrand+ 1994. [7] Myszka+ 1997. [8] Karlsson+ 1997. [9] Carroll+ 2016. [10] Müller+ 1998. [11] Tenni+ 2002. [12] Han+ 2019. [13] Wight+ 1991. [14] Li+ 2020.

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