Decorin Provides Molecular Linkages to Regulate the Integrity of Aggrecan Networks in Cartilage

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Objective

- **To determine the role of decorin, the most abundant small leucine-rich proteoglycan (SLRP) in cartilage, in mediating the content and structural integrity of aggrecan in cartilage extracellular matrix (ECM).**

Background

- The structural integrity of aggrecan, and its sulfated GAG side chains (sGAGs), are the key determinant of cartilage ECM mechanical functions [1].
- Our recent study showed that decorin knockout (***Dcn*$^{-/-}$**) murine cartilage develops substantially reduced aggrecan and sGAGs contents, resulting in impared elastic and poroelastic mechanical properties (Fig. 1) [2].

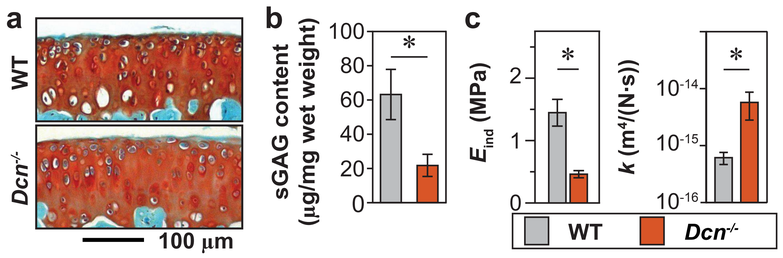

Fig. 1 Structure and mechanical phenotype of ***Dcn*$^{-/-}$** mice, including **a)** reduced Safranin-O staining, **b)** substantial reduction in sGAG content, and **c)** lower indentation modulus and higher hydraulic permeability.

- Decorin interacts with a wide array of matrix molecules [3,4], growth factors and surface receptors [5-7] (Fig. 2a). This indicates that the aggrecan content phenotype could arise from factors including decrease in synthesis, increase in degradation and /or decrease in retention.
- In developing ***Dcn*$^{-/-}$** cartilage, we did not detect degradation neo-epitopes of aggrecan by aggrecanases ($TEGE_{373}$) [8] or MMPs ($VDIPEN_{341}$) [9] (Fig. 2b), suggesting that decorin does not affect aggrecan degradation in vivo.
- Canonical functions of decorin include interaction with collagen [4] and aggrecan [3], and binding with TGF-β to sequester TGF-β signaling [10], indicating possible roles in mediating aggrecan synthesis and/or retention.

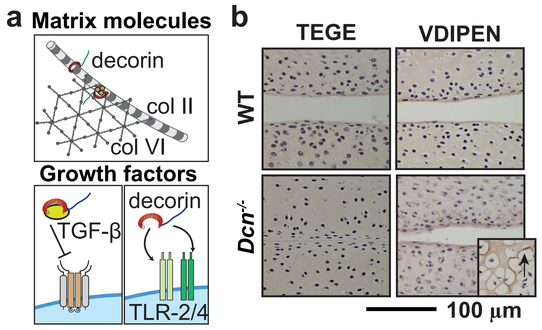

Fig. 2 a) Schematics of decorin interactions with matrix molecules and growth factors. **b)** IHC shows no differences in the degradation neo-epitopes of aggrecan (Inset: positive control by black arrow).

Materials and Methods

- **Cell extraction and culture:** Primary chondrocytes were isolated from 1-month-old WT and *Dcn*$^{-/-}$ murine femoral head cartilage, and cultured in alginate beads. DMMB assay [11] was performed at 2, 4, 6 and 8 days of culture, and qPCR was performed on day 8.
- **Decorin-aggrecan and decorin-collagen molecular interactions via AFM:** Gold-coated silicon substrates and microspherical AFM tips ($R \approx 2.25$ μm) were end-functionalized with aggrecan [12]. Trypsin-treated bovine cartilage surfaces were used as the 2D collagen II network [13]. In PBS, with or without recombinant murine decorin protein, the tip was programmed to compress the substrate, held for 30 seconds, and then retracted at the same rate. The maximum adhesion force, F_{ad}, and total adhesion energy, E_{ad}, were calculated from each approach-retract force curve (e.g., Fig. 5a).
- **Decorin inducible knockout mice:** Using *Dcn*$^{fl/fl}$/*Rosa26CreER* (***Dcn*^{iKO}**) mice [14], we ablated the expression of decorin via tamoxifen injection (3 mg/40 g body weight) at 1-month age *Dcn*^{iKO} (Fig. 3), and tested at 3-month age with Safranin-O/Fast Green histology and DMMB to assess joint morphology and sGAG content, and AFM-nanorheometic test [15] to quantify poroelastic mechanical properties. Control mice include the same iKO mice treated with vehicle, and WT mice injected with the same dose of tamoxifen.

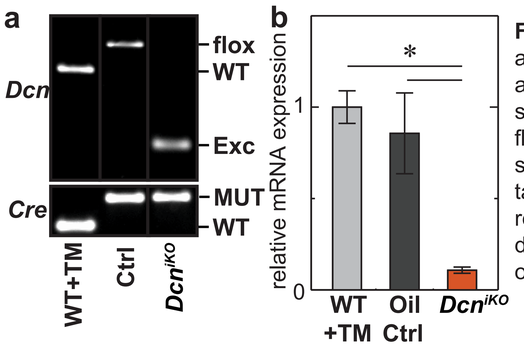

Fig. 3 a) Genotyping of WT and *Dcn*^{iKO} mice before and after tamoxifen injection showed the excision of floxed *Dcn* allele. **b)** qPCR showed daily injection of tamoxifen significantly reduced the expression of decorin to the baseline level on day 5 in cartilage.

Results and Discussion

1) Decorin mediates the assembly and stability of aggrecan in neo-matrix, but does not affect its synthesis by chondrocytes.

- *Dcn*$^{-/-}$ chondrocytes exhibited similar expressions of matrix genes other than decorin, with or without TGF-β (Fig. 4a). As expected, TGF-β treatment enhances anabolism for both genotypes (Fig. 4a).
- In accordance with qPCR results, ***Dcn*$^{-/-}$** chondrocyts synthesize similar total amount of sGAGs as WT, with or without the stimuli by TGF-β (Fig. 4b).
- A significantly reduced proportion of sGAGs was retained in the neo-matrix of ***Dcn*$^{-/-}$** chondrocytes, suggesting a key role of decorin in mediating aggrecan assembly, rather than synthesis (Fig. 4b).

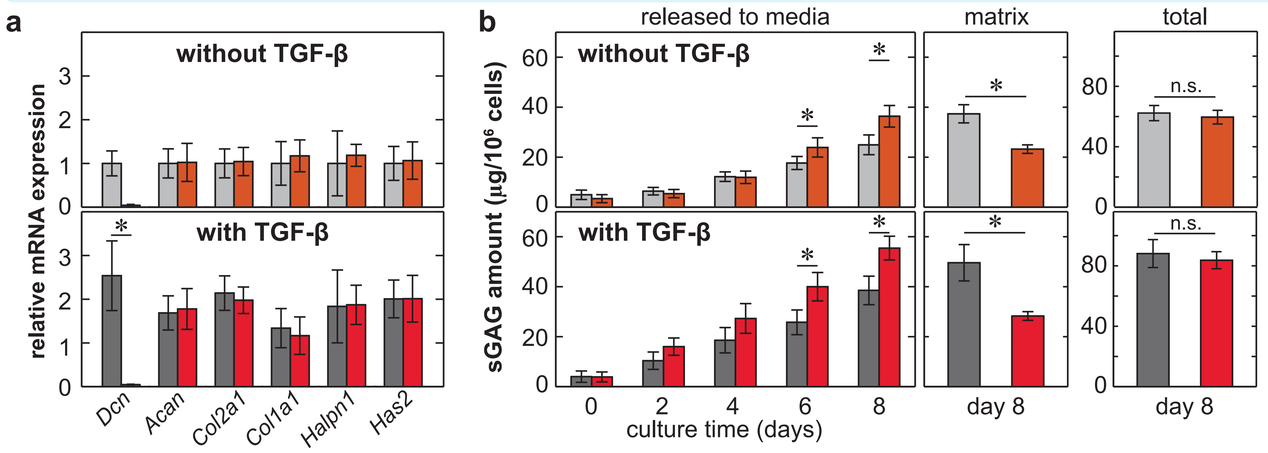

Fig. 4 a) qPCR detects similar expression of major anabolic genes between *Dcn*$^{-/-}$ and WT chondrocytes, and their stimulation by TGF-β. Col2a1/Col1a1 ratio are measured to be greater than 10 in all groups. **b)** DMMB shows more sGAGs are released to the media from *Dcn*$^{-/-}$ chondrocytes, while total amount synthesized are similar (mean ± 95% CI, $n = 7$, *: $p < 0.01$ via two sample *t*-test).

2) Decorin increases aggrecan-aggrecan and aggrecan-collagen fibrils adhesion.

- Under AFM colloidal force spectroscopy (Fig. 5a) [12], adding free decorin significantly increased both the maximum adhesion force, F_{ad}, and adhesion energy, E_{ad}, both between aggrecan and aggrecan (Fig. 5b), and between aggrecan and collagen fibrils (Fig. 5c).
- When purified bovine aggrecan was reconstituted on atomically flat mica surface, adding free decorin transforms aggrecan monomers into interconnected supramoleculer networks, shown by AFM imaging (Fig. 5d).

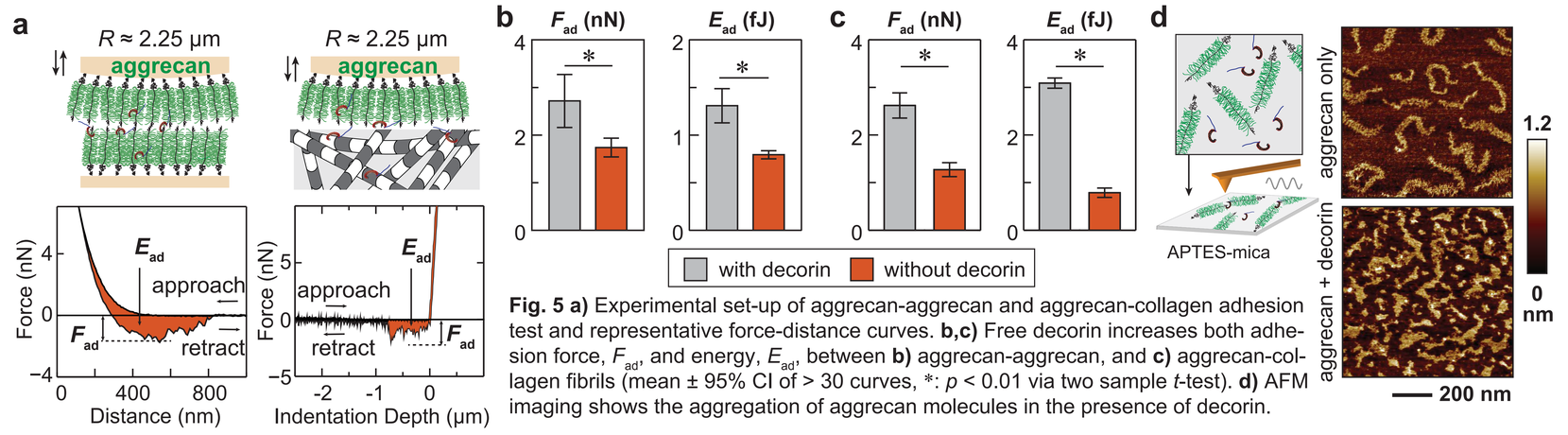

Fig. 5 a) Experimental set-up of aggrecan-aggrecan and aggrecan-collagen adhesion test and representative force-distance curves. **b,c)** Free decorin increases both adhesion force, F_{ad}, and energy, E_{ad}, between **b)** aggrecan-aggrecan, and **c)** aggrecan-collagen fibrils (mean ± 95% CI of > 30 curves, *: $p < 0.01$ via two sample *t*-test). **d)** AFM imaging shows the aggregation of aggrecan molecules in the presence of decorin.

3) The role of decorin is manifested through late maturation, when collagen turnover is slow.

- After decorin was ablated in young adult mice (1-month age), similar structural and mechanical defects were observed in adult mice (3-month age) (Fig. 6).
- At the later stages of maturation, the turnover of collagen fibrils is slow [16], indicating the impact of decorin on aggrecan integrity is possibly independent of collagen fibrillogenesis in cartilage.

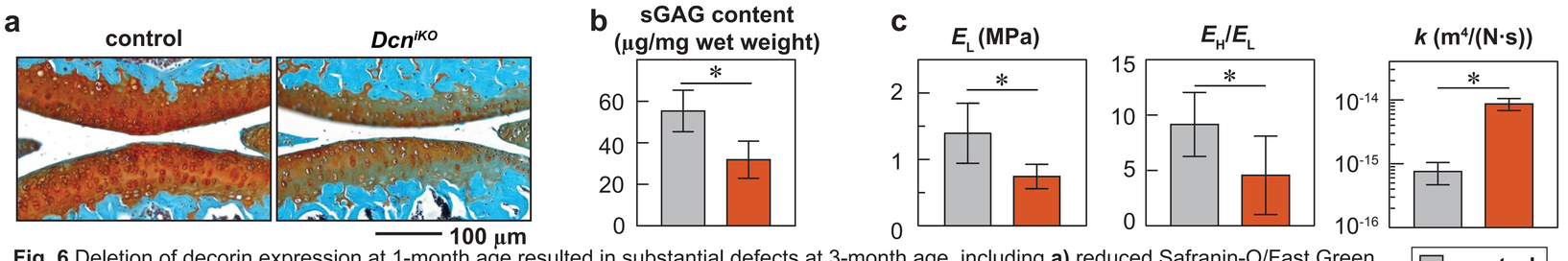

Fig. 6 Deletion of decorin expression at 1-month age resulted in substantial defects at 3-month age, including **a)** reduced Safranin-O/Fast Green histology staining, **b)** decreased sGAG content via DMMB, and **c)** decrease in low frequency modulus, E_L, self-stiffening ratio E_H/E_L and increase in hydraulic permeability, *k*, via AFM nanorheometric test (mean ± 95% CI, $n \geq 4$,*: $p < 0.05$ via Mann-Whitney U test).

Conclusion and Outlook

- **Our results suggest that decorin increases aggrecan stability and retention via increasing its molecular adhesion, and this role is critical to the integrity of cartilage aggrecan network during post-natal growth (Fig. 7).**
- This new role of decorin is a clear departure from its known functions in mediating collagen fibrillogenesis, and thus, extends our understanding of decorin's structural role in various tissues.
- Building on these results, our ongoing studies are testing the potential of using decorin to improve regeneration by modulating the assembly of neo-matrix and mechanotransduction of chondrocytes.

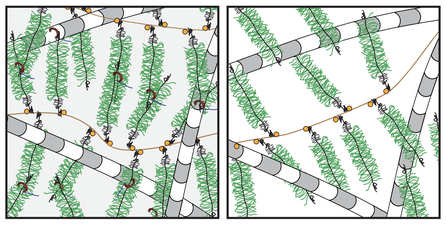

Fig. 7 Schematics show the hypothesized role of decorin in cartilage, and the impact of decorin loss on the reduction of aggrecan.

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