EXTENDED REPORT

Forced exercise-induced osteoarthritis is attenuated in mice lacking the small leucine-rich proteoglycan decorin

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ABSTRACT

Objective Interterritorial regions of articular cartilage matrix are rich in decorin, a small leucine-rich proteoglycan and important structural protein, also involved in many signalling events. Decorin sequesters transforming growth factor β (TGFβ), thereby regulating its activity. Here, we analysed whether increased bioavailability of TGFβ in decorin-deficient ($Dcn^{-/-}$) cartilage leads to changes in biomechanical properties and resistance to osteoarthritis (OA).

Methods Unchallenged knee cartilage was analysed by atomic force microscopy (AFM) and immunohistochemistry. Active transforming growth factor β-1 (TGFβ1) content within cultured chondrocyte supernatants was measured by ELISA. Quantitative real-time (RT)-PCR was used to analyse mRNA expression of glycosaminoglycan (GAG)-modifying enzymes in C28/I2 cells following TGFβ1 treatment. In addition, OA was induced in $Dcn^{-/-}$ and wild-type (WT) mice via forced exercise on a treadmill.

Results AFM analysis revealed a strikingly higher compressive stiffness in $Dcn^{-/-}$ than in WT cartilage. This was accompanied by increased negative charge and enhanced sulfation of GAG chains, but not by alterations in the levels of collagens or proteoglycan core proteins. In addition, decorin-deficient chondrocytes were shown to release more active TGFβ1. Increased TGFβ signalling led to enhanced *Chst11* sulfotransferase expression inducing an increased negative charge density of cartilage matrix. These negative charges might attract more water resulting in augmented compressive stiffness of the tissue. Therefore, decorin-deficient mice developed significantly less OA after forced exercise than WT mice.

Conclusions Our study demonstrates that the disruption of decorin-restricted TGFβ signalling leads to higher stiffness of articular cartilage matrix, rendering joints more resistant to OA. Therefore, the loss of an important structural component can improve cartilage homeostasis.

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INTRODUCTION

Healthy articular cartilage forms a smooth coating on articulating bones and enables a frictionless movement of joints. The hyaline cartilage tissue contains chondrocytes, sparsely distributed within a dense extracellular matrix (ECM).[1]

Articular cartilage matrix comprises collagen fibrils and extrafibrillar matrix, rich in proteoglycans and glycoproteins. While the collagen fibrils are the main tensile tissue elements, proteoglycans and glycoproteins have multiple functions: they interconnect various ECM components, bind growth factors and cytokines, thereby influencing viability, differentiation and metabolic activity of chondrocytes. In addition, they attract large amounts of water and constitute a swelling pressure that counteracts compression under mechanical load.[2 3]

Decorin (*Dcn*) is a member of the class I small leucine-rich proteoglycans (SLRPs). It is widely expressed in connective tissues, including skin, cornea, tendon, bone as well as cartilage.[4] Its protein core contains 10 leucine-rich repeats between flanking cysteine-rich domains and is substituted at the N-terminus by a single dermatan sulfate (DS) or chondroitin sulfate (CS) chain.[5] Both the protein core and the glycosaminoglycan (GAG) chain of decorin bind various ECM proteins and signalling factors. The protein core of decorin interacts with collagens I, II, III and VI; fibronectin and matrilin-1, which is thought to be important for ECM assembly and physical ECM properties, such as permeability, compressibility or stretch resistance.[6–10] In addition, the protein core acts as a ligand for the epidermal growth factor receptor[11] and the insulin-like growth factor receptor[12] and sequesters tumour necrosis factor α (TNFα)[13] or transforming growth factor β-1 (TGFβ1),[14 15] while the GAG chain binds other signalling factors such as members of the fibroblast growth factor (FGF) family.[16] As a functional consequence of this large binding repertoire, decorin decisively affects the bioactivity of growth and differentiation factors under physiological or pathological conditions.

For this study, the decorin-controlled function of transforming growth factor β (TGFβ) seems important. TGFβ is synthesised as a dimeric proprotein and is converted in the trans-Golgi network into a complex of the mature TGFβ dimer and the latency-associated peptide (LAP). After secretion into the ECM, the TGFβ–LAP complex attaches to the latent TGFβ-binding protein (LTBP), giving rise to the large latent complex containing TGFβ in an inactive form. TGFβ activity requires the dissociation from the latent complex, but can be further restrained by sequestration of TGFβ by decorin.[17] Binding of TGFβ to decorin prevents activation of TGFβ receptors, their recruitment of Smad proteins

and the regulation of target genes.[18] Like other members of the TGFβ superfamily, TGFβ1 is crucially involved in cartilage homeostasis. For example, stimulation[18 19] and suppression[20] of collagen II and aggrecan syntheses by TGFβ have been reported. In addition, TGFβ can modulate post-translational modifications in chondroitin sulfate and dermatan sulfate chains.[21] TGFβ-induced alterations in the expression and activity of chondroitin sulfotransferases or dermatan sulfotransferases result in specific sulfation patterns within GAG chains, which control the immobilisation of signalling factors in the ECM and restrain their activity.[3]

TGFβ can also be chondroprotective, as it restricts hypertrophy of articular chondrocytes by inhibiting proliferation. Indeed, during the development of osteoarthritis (OA), a deficit in TGFβ signalling results in chondrocyte proliferation[22] and differentiation, accompanied by marker expression of the hypertrophic differentiation stage, such as alkaline phosphatase,[23] type X collagen[24 25] and matrix metalloproteinase 13 (MMP-13),[26] with subsequent apoptotic death[27] and mineralisation of the diseased cartilage.[28] TGFβ1 can mediate its chondroprotective effects by binding to the activin-like kinase 5 (ALK-5) TGFβ type I/ TGFβ type II receptor heterodimer and activation of Smad2/3 signalling.[29] Van der Kraan and colleagues elegantly showed that TGFβ1 could also initiate signalling via the activin-like kinase 1 (ALK-1)-route (Smad1/5/8). During OA, a shift in TGFβ1 signalling pathways occurs. Increased signalling via ALK-1, and decreased ALK-5 signalling, stimulates MMP-13 expression and, thereby, collagen degradation. For this reason, the ALK-1/ALK-5 ratio, shown to be shifted during ageing and under pathophysiological conditions, seems crucial for the induction of cartilage loss during OA.[30–32] Further, diverse TGFβ actions on other joint tissues such as ligaments and/or subchondral bone can affect OA pathogenesis in a variable manner.[33]

MATERIALS AND METHODS

See online supplementary text.

RESULTS

Decorin deficiency alters ECM composition and articular cartilage stiffness

The histology of articular knee cartilage of 3.5-month-old *Dcn*$^{-/-}$ and wild-type (WT) mice was first studied by toluidine blue/Fast Green FCF (fast green for coloring food) staining. A normal cartilage structure, a comparable intensity of toluidine blue staining, a regular cell distribution and tidemark integrity were observed in femur and tibia without obvious differences between the genotypes (figure 1A). Indentation-type atomic force microscopy (IT-AFM) was applied on native tissue sections to analyse the compressive stiffness of the articular cartilage at the nanoscale. In both genotypes, histograms with bimodal stiffness distributions were observed. As previously reported, the lower stiffness peak can be attributed to the proteoglycan moiety, while the peak at higher stiffness relates to collagen fibrils.[34 35] As shown in figure 1B, stiffness peaks were observed in *Dcn*$^{-/-}$ cartilage for the proteoglycan network at 284 kPa and for the collagen network at 399 kPa. In WT cartilage, the average proteoglycan stiffness was 117 kPa and collagen stiffness was 324 kPa. Therefore, the extrafibrillar cartilage matrix mainly containing proteoglycans was clearly stiffer in *Dcn*$^{-/-}$ than in WT animals in spite of the similar overall appearance in the light microscope. This suggested that the osmotic swelling pressure exerted by the proteoglycan matrix is higher in decorin-deficient animals. In contrast, the compressibility of the collagen-containing fibrillar networks differed much less between the genotypes.

To evaluate changes in the proteoglycan networks, highly sulfated GAG chains were stained in cartilage sections (figure 1C) or micromass cultures (figure 1D) with Alcian blue at pH 1.0. Alcian blue staining was more intense around chondrocytes of *Dcn*$^{-/-}$ than of WT specimens indicating that the negative charge density of the territorial matrix was higher in *Dcn*$^{-/-}$ animals. Aggrecan is the most abundant proteoglycan in cartilage, whereas biglycan is the most closely related member of decorin within the SLRP family. In contrast to the protein core of decorin, undetectable in *Dcn*$^{-/-}$ cartilage, immunohistochemical staining of the protein cores of aggrecan and of biglycan was unaffected by the decorin knock-out (figure 1E). Likewise, staining for versican and perlecan did not vary (data not shown). However, detection of chondroitin-4 sulfate stubs after chondroitinase ABC digestion with an anti-ΔC4S antibody revealed higher levels in *Dcn*$^{-/-}$ cartilage (figure 1F). Therefore, the increased signal of Alcian blue staining was likely due to an increase in the charge density in proteoglycans rather than an increase in the overall content of proteoglycans. Immunohistochemical staining of the cartilage collagens II, VI and IX also revealed no obvious differences (figure 1G). We conclude that neither the density nor the amount of collagen networks was affected by decorin deficiency.

Increased levels of active TGFβ1 modulate GAG sulfation in *Dcn*$^{-/-}$ cartilage

Decorin can regulate TGFβ signalling in various tissues. Therefore, articular cartilage from *Dcn*$^{-/-}$ and WT animals was examined by immunohistochemical staining for TGFβ. The antibody employed primarily detects the precursor and the mature TGFβ1 and, to a lesser extent, transforming growth factor β-2 (TGFβ2). As shown in figure 2A, knee sections of *Dcn*$^{-/-}$ animals reveal a more intense TGFβ signal than WT cartilage. In addition, active TGFβ1 was determined by ELISA in cell culture supernatants of serum-free agarose cultures of epiphysial chondrocytes from newborn WT and *Dcn*$^{-/-}$ mice. About 2.5 pg/mL of active TGFβ1 was found in supernatants of WT chondrocyte cultures, which was well below the limit of reliability of the detection (7 pg/mL). However, culture media of *Dcn*$^{-/-}$ cells contained well detectable levels of active TGFβ1 (14.24±2.42 pg/mL, $p<0.05$, figure 2B). This suggests that under physiological conditions decorin binds and inactivates TGFβ1 in articular cartilage. To evaluate whether TGFβ signalling is increased in *Dcn*$^{-/-}$ cartilage, we analysed the TGFβ receptor subunits Alk-1 and Alk-5 as well as the phosphorylation of Smad2/3. While we observed no differences for ALK-1 and ALK-5 (see supplementary figure 1B), we detected a more intense phospho-Smad2/3 staining, suggesting an enhanced TGFβ signalling in *Dcn*$^{-/-}$ cartilage (figure 2A). Moreover, it was of interest whether active TGFβ1 influences GAG sulfation. To this end, C28/I2 micromass cultures were stimulated with TGFβ1 and subsequently analysed by Alcian blue staining (figure 2C). Stimulation with TGFβ1 significantly increased Alcian blue staining, suggesting a higher GAG sulfation in the presence of active TGFβ1. To determine which of the specific enzymes involved in GAG sulfation were induced by TGFβ1, quantitative RT-PCR expression analysis for *Chst11* and *Chst12* as well as for *Papss1* and *Papss2* was performed on RNA extracted from the micromass cultures.

Expression of the enzyme that transfers sulfate groups to the carbon-4 of *N*-acetylgalactosamine in the repetitive disaccharide units of CS chains, that is, *Chst11* was increased in *Dcn*$^{-/-}$ cartilage about fourfold ($p<0.005$, figure 2D). *Chst12* and the synthases of the sulfate donor 3′-phosphoadenosine-5′-phosphate, *Papss1*

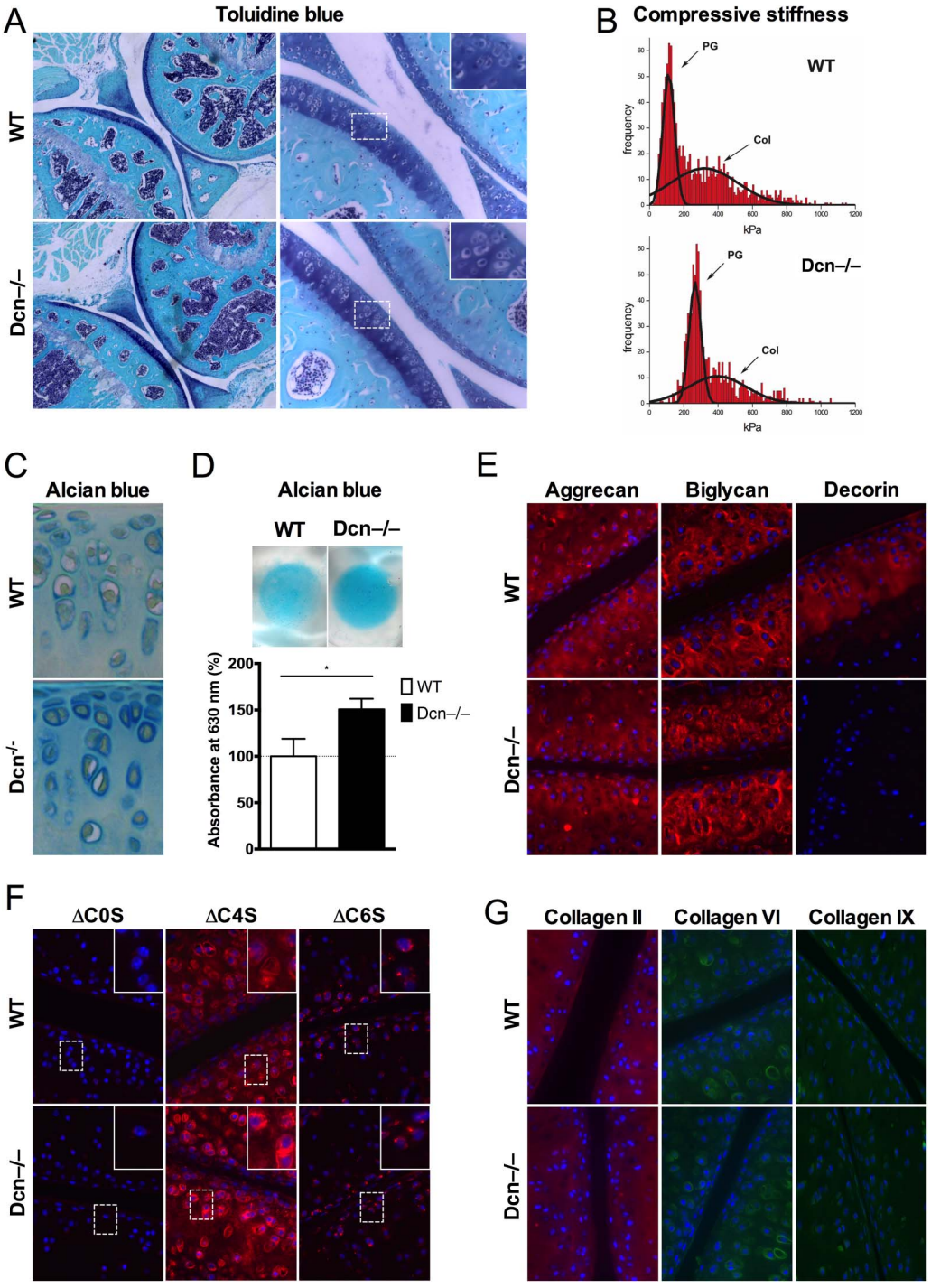

Figure 1 *Dcn* deficiency leads to altered extracellular matrix (ECM) composition and mechanical properties in 3.5-month-old murine articular cartilage. (A) Toluidine blue/Fast Green FCF staining of articular cartilage reveals no differences between unchallenged wild-type (WT) and $Dcn^{-/-}$ mice. A normal age-based cartilage structure with regular cell distribution, toluidine blue staining and tidemark integrity in femur and tibia of both genotypes is observed. Original magnification 25-fold (left panel), 100-fold (right panel). n≥9. (B) Histograms of elastic moduli obtained by indentation-type atomic force microscopy (IT-AFM) reveal a bimodal frequency distribution. The low modulus peak depicts the proteoglycan moiety, whereas the second peak represents the collagen fibril network (indicated by arrows, PG,proteoglycan, Col,collagen). The shift of the proteoglycan peak in $Dcn^{-/-}$ animals towards higher values characterises a stiffer ECM compared with WT animals. Combined histograms of three animals per genotype. (C) Alcian blue (pH 1.0) staining for sulfated glycosaminoglycan (GAG) chains of proteoglycans illustrates a higher negative charge in the $Dcn^{-/-}$ articular cartilage compared with WT controls. Original magnification 400-fold. n≥3. (D) Chondrocyte micromasses of $Dcn^{-/-}$ mice display an increased Alcian blue (pH 1.0) staining compared with WT controls, indicating a higher level of GAG sulfation. The optical density (OD) of extracted Alcian blue dye measured with a microplate ELISA reader at 630 nm is approximately 50% higher in $Dcn^{-/-}$ cells than in WT controls. *p≤0.05; n=3. (E) Distribution and staining intensity of the protein cores of aggrecan and biglycan do not differ between $Dcn^{-/-}$ and WT articular cartilage. Decorin protein core is present in WT but not in $Dcn^{-/-}$ cartilage. Original magnification 400-fold. n≥5. (F) Immunohistochemical staining with antibodies against specific chondroitin sulfate stub epitopes (Δ) displays increased ΔC4S, but similar ΔC6S and ΔC0S levels in $Dcn^{-/-}$ cartilage compared with WT tissue. Original magnification 400-fold. n≥5. (G) Immunohistochemical staining reveals no alterations in the distribution or staining intensity of collagens type II, type VI or type IX in $Dcn^{-/-}$ cartilage compared with WT tissue. Original magnification 400-fold. n≥5.

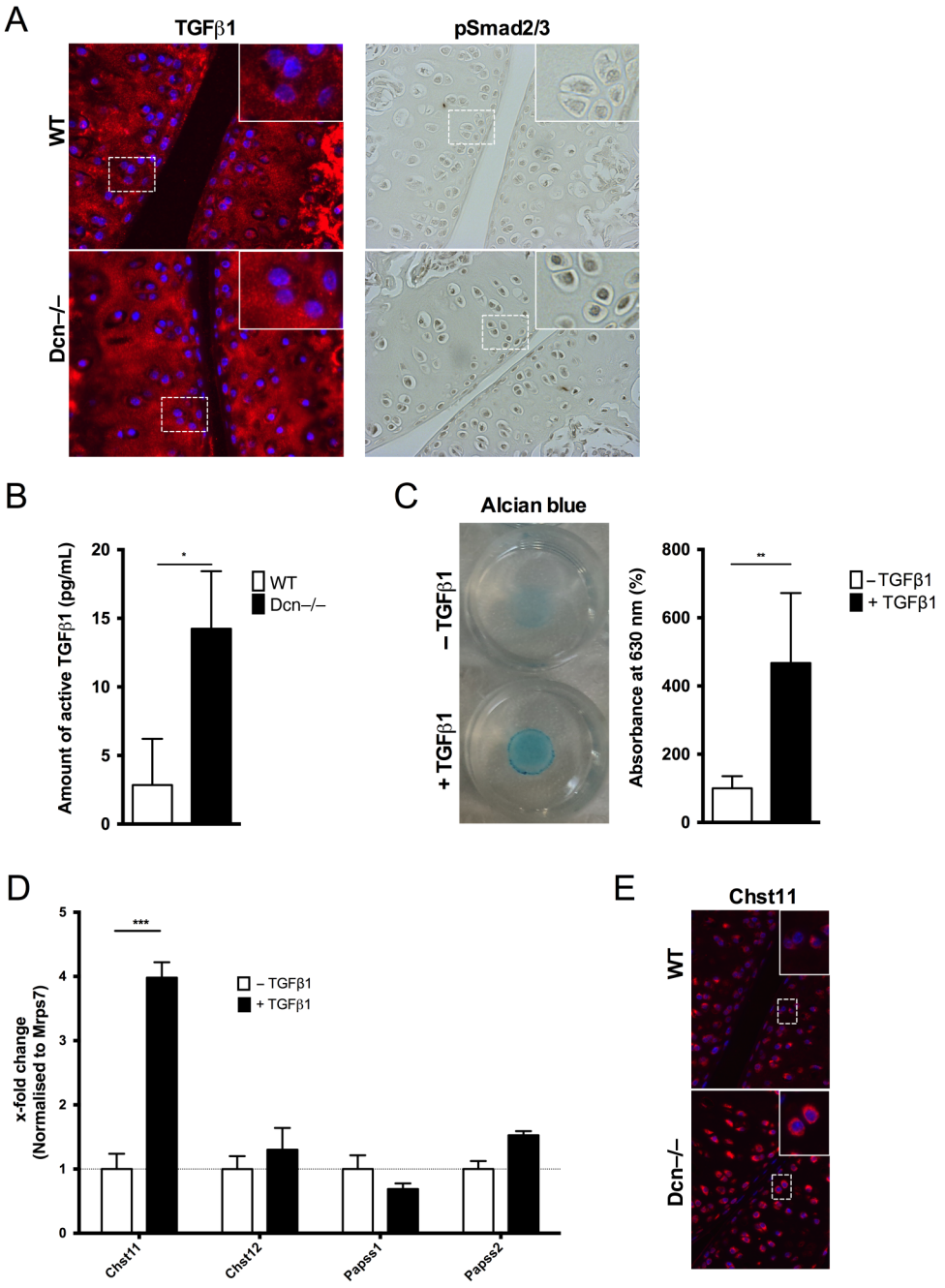

Figure 2 Higher levels of active transforming growth factor β-1 (TGFβ1) in *Dcn*$^{-/-}$ articular cartilage result in increased Smad 2/3 phosphorylation, enhanced Alcian blue staining and increased expression of *Chst11*. (A) Immunohistochemical staining of 3.5-month-old knee sections reveals higher TGFβ1 levels (left panel), inducing the phosphorylation of Smad 2/3 (right panel) in *Dcn*$^{-/-}$ cartilage compared with wild-type (WT) tissue. Original magnification 400-fold. *p≤0.05; n≥5. (B) ELISA analyses confirmed a more than fivefold increased level of active TGFβ1 in the supernatant of cultured *Dcn*$^{-/-}$ chondrocytes compared with WT cells. (C) TGFβ1 stimulation of C28/I2 chondrocyte micromasses increased Alcian blue staining, indicating a higher level of glycosaminoglycan (GAG) sulfation in the presence of this cytokine. **p≤0.01; n=3. The optical density (OD) of extracted Alcian blue dye measured with a microplate ELISA reader at 630 nm is approximately fivefold higher in TGFβ1-stimulated C28/I2 chondrocytes compared with unstimulated controls. n=3. (D) Quantitative RT-PCR expression analysis reveals a fourfold increased expression of *Chst11* in TGFβ1-stimulated C28/I2 chondrocytes compared with controls. In contrast, *Chst12*, *Papss1* and *Papss2* are equally expressed in the presence or absence of TGFβ1. ***p≤0.001; n=3. (E) Immunohistochemical staining of 3.5-month-old knee sections reveals a higher protein expression of *Chst11* in *Dcn*$^{-/-}$ cartilage compared with WT tissue. Original magnification 400-fold. n≥3.

and *Papss2*, were not affected by TGFβ1. Immunohistochemical staining confirmed enhanced *Chst11* expression in *Dcn*$^{-/-}$ cartilage on protein level (figure 2E).

Decorin deficiency attenuates exercise-induced OA

Given the TGFβ-evoked changes in proteoglycan sulfation increasing compressive stiffness in articular *Dcn*$^{-/-}$ cartilage, we hypothesised that decorin deficiency could mitigate the development of OA in vivo. To experimentally address this hypothesis, we subjected mice to forced exercise on a treadmill (40 min/day, 6 weeks, see online supplementary figure S1C). This regimen leads to cartilage degeneration and ultimately to OA without initial inflammation. The latter occurs in surgical OA animal models, which are more suitable to mimic traumatic induction

mechanism of OA. Figure 3A shows representative examples of toluidine blue/Fast Green FCF-stained knee cartilage sections of 5-month-old mice after 6 weeks of forced exercise. Both genotypes display osteoarthritic changes but, indeed, decorin deficiency attenuates the development of exercise-induced OA. Reduced proteoglycan staining is limited to the upper superficial layer in *Dcn*$^{-/-}$ cartilage. However, proteoglycan loss was apparent in all layers, including the deep zones, of WT cartilage. Furthermore, an obvious roughening of the joint surface as well as fissures strikingly developed in WT but not in *Dcn*$^{-/-}$ animals. The osteoarthritic changes were scored in both genotypes via a modified Mankin protocol in a double-blinded manner (figure 3B). The data were subjected to a statistical analysis by a two-tailed Mann-Whitney test revealing a significant difference between the genotypes with a median Mankin score of 6.5 for the WT animals and 4.5 for *Dcn*$^{-/-}$ mice ($p<0.005$).

Dcn$^{-/-}$ cartilage exhibited higher protein levels of TGFβ1, *Chst11* and ΔC4S under unchallenged conditions as well as after forced exercise (figure 3C). Additionally, osteoarthritic changes were monitored by immunohistochemical staining of type X collagen, matrix metalloproteinase 3 (MMP-3) and MMP-13 (figure 3D). Both markers were more intense in WT

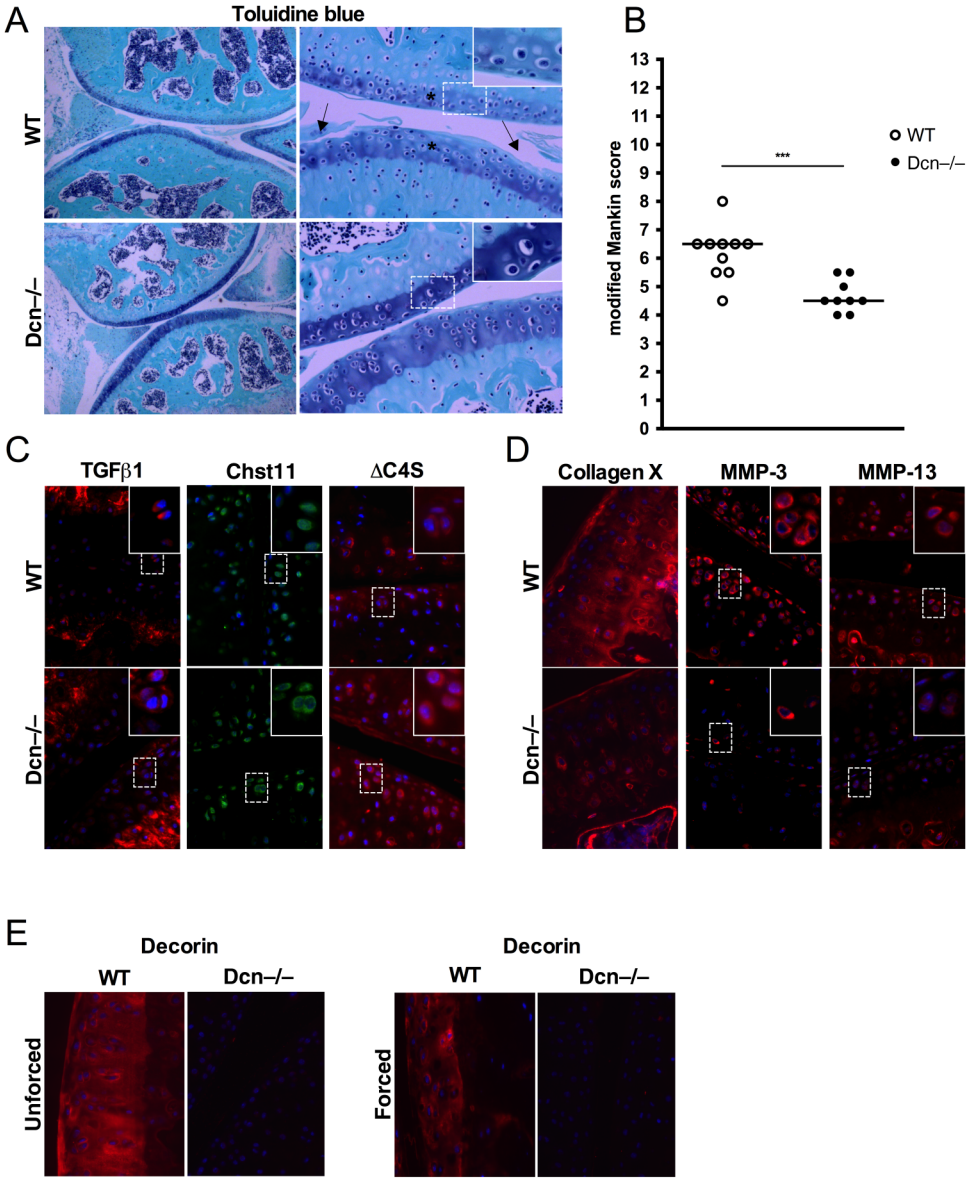

Figure 3 Five-month-old *Dcn*$^{-/-}$ mice exhibit less severe osteoarthritic changes after 6 weeks of forced exercise than wild-type (WT) mice. (A) Toluidine blue/Fast Green FCF staining of WT and *Dcn*$^{-/-}$ articular knee cartilage obtained after 6 weeks of forced exercise on a treadmill reveals fewer osteoarthritic changes in *Dcn*$^{-/-}$ mice. Note a less pronounced decrease in toluidine blue staining (*) in Dcn$^{-/-}$ animals. Additionally, fissuring and cleft formation (→) are observed in WT, but not in *Dcn*$^{-/-}$ knee cartilage. Original magnification 25-fold (left panel), 100-fold (right panel). n≥9. (B) Blind assessment of osteoarthritic changes via a modified Mankin score confirms less severe cartilage degradation in *Dcn*$^{-/-}$ animals (median Mankin score: 4.5, n=9) versus WT animals (median Mankin score: 6.5, ***$p\leq0.001$; n≥9). (C) Immunohistochemical staining of WT and *Dcn*$^{-/-}$ articular knee cartilage displays higher protein levels of transforming growth factor β-1 (TGFβ1), *Chst11* and ΔC4S in *Dcn*$^{-/-}$ mice compared with WT controls after forced exercise. Original magnification 400-fold. n≥3. (D) Immunohistochemical staining of collagen X, a chondrocyte hypertrophy marker and of the cartilage degrading proteases MMP-3 and MMP-13 reveals an elevated protein expression in WT mice during osteoarthritis (OA) development in comparison with *Dcn*$^{-/-}$ cartilage. Original magnification 400-fold. n≥3. (E) Immunohistochemical staining of decorin decreases during OA development in WT mice after 6 weeks of forced exercise on a treadmill compared with unforced controls. Original magnification 400-fold. n≥3.

animals than in *Dcn*$^{-/-}$ animals. This indicated a higher number of hypertrophic chondrocytes and an enhanced proteolytic activity in WT than in *Dcn*$^{-/-}$ mice. This is consistent with more osteoarthritic alterations in the WT and in contrast to normal, non-diseased cartilage, in which collagen X, MMP-3 and MMP-13 are absent (see online supplementary figure S1A). Moreover, degenerative conditions led to a loss of decorin in murine cartilage samples (figure 3E) as also known for other ECM proteins.

DISCUSSION

In this report, we show that the deficiency in the SLRP decorin ameliorates exercise-induced OA in mice. The fact that the deficiency in an important structural component of the ECM actually results in an improvement of the biomechanical performance of joint cartilage may be surprising at the first glance. However, a compromised TGFβ sequestration in joint cartilage of *Dcn*$^{-/-}$ mice entails an increased availability of active TGFβ, accompanied by enhanced TGFβ signalling via phospho-Smad2/3. As a result, GAG sulfation of proteoglycans, but not their protein core synthesis, is intensified resulting in elevated levels of immobilised negative charges. Larger amounts of water are attracted which, in turn, results in an augmented compressive stiffness of the tissue and a delayed susceptibility to load-induced cartilage degeneration (figure 4). Thus, the salient feature of the work presented here is the regulation of growth factor function and signalling by SLRPs. This is consistent with postulates proposed earlier that decorin can bind and functionally modulate members of the TGFβ superfamily,[14 15] which plays a pivotal role also in cartilage homeostasis.[36 37]

Enzymes involved in the post-translational modification of GAG chains are regulated in articular cartilage in a TGFβ-dependent manner.[19 38 39] Therefore, TGFβ could stimulate also the synthesis of the sulfate group donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS), catalysed by phosphoadenosine phosphosulfate synthase 2 (*Papss2*).[21] However, this was not observed in this study. By contrast, the expression of *Chst11*, the sulfotransferase catalysing the formation of *N*-acetylgalactosamine-4-sulfate in CS chains, was increased by higher levels of TGFβ1, whereas the sulfatase arylsulfatase B, which hydrolyses C4S groups remains unaltered (see online supplementary figure S1B). Taken together, our results presented here are entirely consistent with the established notion that the proteoglycans, carrying sulfated GAG chains, are responsible for most of the physicochemical properties of cartilage, including the generation of an osmotic swelling pressure to withstand compressive load.[40 41] Swelling is dependent on fixed charges, the stiffness of the matrix and the ion concentration in the interstitium.[42] In addition, electrostatic repulsion between highly charged GAG chains contributes to increased strength of the articular cartilage matrix.[43]

Missing SLRP interactions with collagens impair normal fibril formation.[44] In SLRP-deficient animals, structurally or mechanically defective collagen fibrils appear in tendons or ligaments. Therefore, the structures may lose their physiological function and cannot withstand tension. This results in unstable joints with partial overload conditions and subsequent articular cartilage degeneration. Examples for this OA phenotype are published for biglycan[45] deficient mice and fibromodulin deficient mice[46] and biglycan/fibromodulin double[45]-deficient mice. However, for decorin-deficient mice, such a correlation has

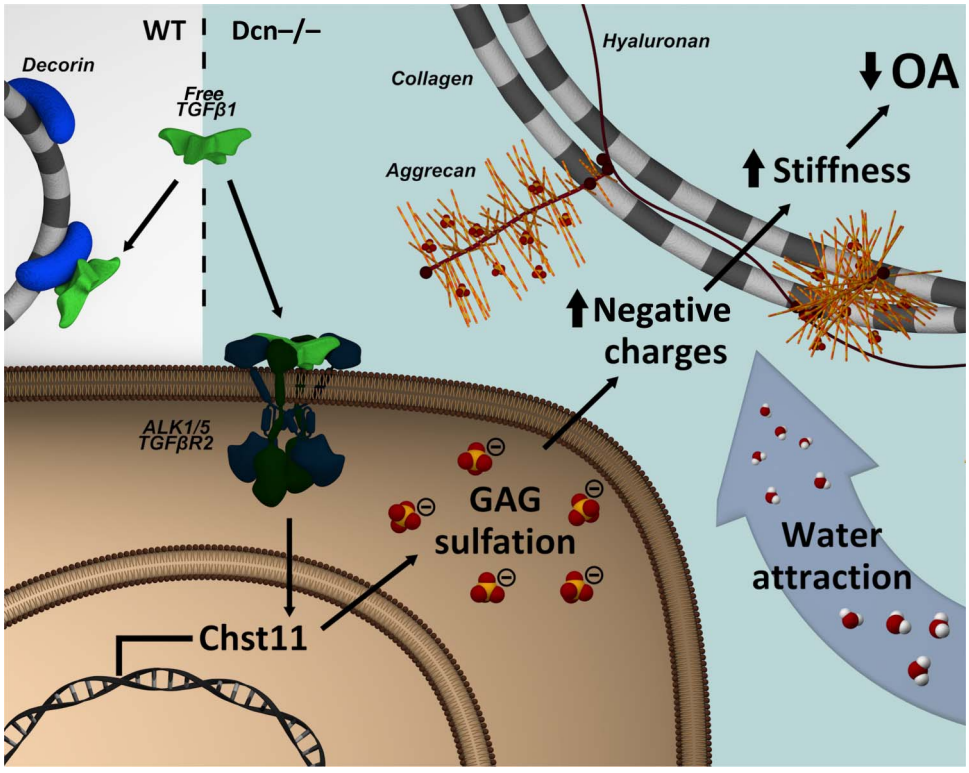

Figure 4 Schematic model of transforming growth factor β (TGFβ)-mediated stiffness increase in *Dcn*$^{-/-}$ mice. The absence of decorin-mediated sequestration of TGFβ in *Dcn*$^{-/-}$ mice necessitates that higher quantities of active TGFβ bind to TGFβ receptors. As a result, *Chst11* expression increases and glycosaminoglycan (GAG) sulfation of proteoglycans is intensified resulting in elevated levels of immobilised negative charges in the extracellular matrix (ECM). Larger amounts of water are attracted, which, in turn, results in an augmented compressive stiffness of the tissue and a reduced susceptibility to load-induced cartilage degeneration during osteoarthritis (OA). ALK-1, activin-like kinase 1; ALK-5, activin-like kinase 5; WT, wild-type.

been described for dermal stability but not for articular cartilage. SLRPs including decorin are, together with other extrafibrillar macromolecules, incorporated into the fibrillar suprastructures.[10 47 48] It is assumed that surface properties of the fibrils are modulated, including interactions with the extrafibrillar matrix and the susceptibility to collagenases, for example, by MMP-13, and other catabolic enzymes.[49]

During endochondral bone formation, TGFβ is involved in chondrogenesis and orchestrates chondrocyte proliferation and differentiation.[50] It has been shown in cultures of chicken chondrocytes that TGFβ2 restricts chondrogenic differentiation[20] and hypertrophic development.[51] These predictions were validated also in vivo in mice overexpressing a dominant negative TGFβ type II receptor[52] or in Smad3 knockout (KO) mice.[53] In agreement with the notion that TGFβ inhibits chondrocyte hypertrophy also in vivo, these animals exhibit reduced proliferative but enlarged hypertrophic zones in their growth plates. The same seems true for normal permanent cartilage, for example, in young and healthy joints. Under the influence of TGFβ chondrocytes step up synthesis of cartilage proteoglycans, remain in a quiescent state and refrain from proliferation and hypertrophic differentiation.[54] In aged joints however, TGFβ also can induce negative effects, for example, the stimulation of hypertrophic differentiation in articular chondrocytes with subsequent expression of matrix-degrading enzymes, such as MMP-13.[55]

The ambiguity of beneficial and deleterious effects of TGFβ in joint cartilage of young and older individuals, respectively, may be explained by an age-dependent variation in the expression of TGFβ receptors and their subsequent signalling pathways. In young and healthy cartilage, TGFβ seems to act via TGFβ receptor II/ ALK-5, phosphorylation of Smad2/3 and induction of SOX9, which stabilises the chondrocytic phenotype. In old or osteoarthritic cartilage, ALK-5 declines and ALK-1 is increased. Therefore, Smads1/5/8 are recruited favouring collagen X and MMP-13 expression.[56 57] Moreover, canonical Wnt signalling was described to be able to convert TGFβ signalling towards the ALK-1 (Smad 1/5/8) pathway, at least during experimental OA.[58]

The ambivalent importance of matrix sequestration of TGFβ has been recognised in the pathogenesis of several disorders. Selected examples include kidney fibrosis,[59] Marfan syndrome[60] or epidermolysis bullosa.[61] Decorin and other SLRPs are important in this context.[62] Unlike in fibrotic diseases, TGFβ sequestration by decorin, as shown here, may have detrimental consequences in the development OA. Therefore, therapeutic strategies aimed at local mobilisation of TGFβ from the decorin pool in joint tissues, for example, by specific anti-decorin antibodies, may be promising.

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Contributors TG, PB and RD incepted and designed the experiments, analysed the data and prepared the manuscript. Experimental work was performed by TG, KK, CP and IG with contributions from AA, FCM, HC-S, JB and TP. RVI provided *Dcn*$^{-/-}$ animals. The manuscript was approved by all authors.

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