The importance of proteoglycans in articular cartilage

Mini-project

Polina Deenichina

MSc: Regenerative Medicine & Technology, Utrecht University
Project supervisor & examiner: Dr. ir. Debby Gawlitta

Abbreviations

AC = Articular cartilage
ADAMTS-5 = A disintegrin and metalloproteinase with thrombospondin motifs 5
BMP = Bone morphogenic protein
CS = Chondroitin sulphate
DS = Dermatan sulphate
ECM = Extracellular matrix
FGF = Fibroblast growth factor
GAG = Glycosaminoglycan
HA = Hyaluronic acid
HS = Heparan sulphate
HSPG = Heparan sulphate proteoglycan
IHH = Indian hedgehog
IL = Interleukin
KS = Keratan sulphate
LP = Link protein
LRR = Leucine-rich repeat
MAPK = Mitogen-activated protein kinase
MMP = Matrix metalloproteinase
NF-kb = Nuclear factor kappa B
OA = Osteoarthritis
PG = Proteoglycan
SLRP = Small leucine-rich proteoglycan
TGF = Transforming growth factor
TLR = Toll-like receptor
TNF = Tumor necrosis factor
WISP1 = Wnt1-inducible signalling pathway protein 1
WNT = Wingless-related integration site

Articular cartilage

Hyaline cartilage is the most abundant type of cartilage in the human body. Hyaline is responsible for embryonic bone formation (endochondral ossification), and can be found in the respiratory system, in costal cartilages, and covering the tips of long bones (articular cartilage) in adults. The principal functions of articular cartilage are to provide lubrication for low friction articulation and to disperse compressive loads to the underlying subchondral bone. The biomechanical role of AC is underlied by its biphasic characteristics. AC consists of 2 phases: a fluid phase made up of water and sodium, calcium, chloride and potassium ions, and a solid phase made up of the extracellular matrix (ECM) components. The interplay between the ECM and the fluid phase allows resilience to compressive stress through electrostatic repulsion forces. The AC is composed of 4 zones: the superficial (tangential) zone, middle (transitional) zone, deep

(radial) zone, and calcified zone (Fig. 1). Each zone has a distinct cellular and extracellular composition that is dictated by the function of that zone within the cartilage tissue. Depending in which zone, cartilage cells or chondrocytes are at a different stage of chondrogenic differentiation and therefore they secrete different types of fibers (collagens and elastins), proteoglycans (PGs) and glycoproteins, that make up the ECM [1].

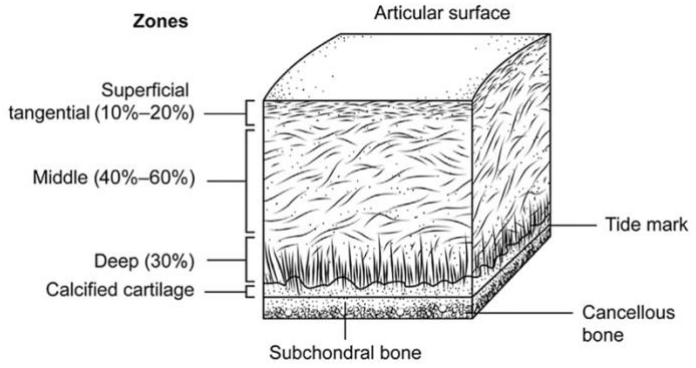

Figure 1. Layers of articular cartilage.

Superficial tangential zone is made up of flattened ellipsoid chondrocytes and parallelly distributed to the articular surface collagen fibrils. Has the highest collagen and lowest proteoglycan content. In the middle transitional zone, the cell density is lower and cells are spheroid. Collagen fibers are arranged obliquely, and proteoglycan concentration is higher. The cell density in the deep zone is the lowest, and cells are spheroidal in shape arranged perpendicularly to the subchondral bone surface. This zone contains the largest diameter of collagen fibers and has the highest concentration of proteoglycans. The tidemark represents the boundary between the uncalcified and calcified cartilage. The calcified cartilage zone is anchored to the subchondral bone by hydroxyapatite crystals and has the lowest number of cells. Figure and figure legend were adapted from Killen et al [2].

Glycosaminoglycans

Hyaline cartilage, and AC in particular, are characterised by having a high content of PG aggregates that are responsible for the turgid nature of cartilage and osmotic forces required to resist compressive stresses [3,4]. PGs are proteins that have one or more attached glycosaminoglycan (GAG) side chains, and some PGs have a non-GAG soluble version. GAGs are unbranched polysaccharide chains consisting of repeating disaccharide units. These disaccharides consist of one amino sugar and one hexuronic acid linked by glycosidic bonds [5]. Variations in disaccharide composition determine the major classes of GAGs: Hyaluronic Acid (HA), Chondroitin/Dermatan Sulphate (CS/DS), Keratan Sulphate (KS), and Heparin/Heparan Sulphate (HS) [6].

Proteoglycans

In high concentrations, PGs generate large osmotic swelling pressure that draws water into the cartilage. This occurs because the GAG side chains of PGs carry negatively charged sulphate and carboxylate groups that create a large difference in ion concentration between the cartilage and the surrounding areas. Because of this osmotic imbalance, water and cations are drawn into the cartilage which causes it to swell and build up hydrostatic pressure that serves to resist mechanical loads. In addition, the ECM network of fibrils and PGs is stiff and resistant to deformation. Due to those two properties of cartilage, it behaves as a biphasic visco-elastic tissue. That is why the composition of GAGs and PGs determines how much water cartilage can retain and how much it can deform defining its biomechanical functions. For example, collagen content is highest at the superficial zone and decreasing in the direction of the deep zone, and inversely PGs are lowest at the superficial zone increasing as much as 50% into the middle and deep zones. That is because the superficial layer functions to disperse mechanical load and proteoglycan-rich layers build up pressure in water to resist the stress relayed from the superficial collagenous zone [3,4]. The chondro-osseous junctional region, CCL, is a mineralised cartilage that separates hyaline cartilage from subchondral bone [7]. In contrast to hyaline, CCL is made up of dispersed hypertrophic chondrocytes and primarily collagen type I and not type II, and nanohydroxyapatite crystals [8]. Chondrogenic collagen type II fibers transition into cemented collagen I osteoid that is deposited by osteoblasts. It has been shown that from cartilage to bone, there is an incremental increase in collagen content, but PGs are limited to the CCL, that is highlighting the importance of PGs for the function of cartilage [9,10].

Along with maintaining hydration, PGs play a role in regulating mineralisation in cartilage. The general consensus is that PGs in their native state prevent calcifications and stiffening of the ECM [11]. However, in hypertrophic and degenerate cartilage, PGs are modified in association with calcifications via e.g., enzymatic fragmentation. In turn, the modified PGs can no longer inhibit the mineralisation processes, but rather become a functioning component of the

hypertrophic matrix promoting further cartilage remodelling. Such cartilage transformations have been extensively studied in AC of osteoarthritic joints and in the degeneration of intervertebral discs.

Regardless of the initial trigger (e.g., mechanical load, inflammation) that disrupts cartilage homeostasis, the outcome is loss of structural integrity and substitution with stiffer ECM components (e.g., collagen I, hydroxyapatite, osteophytes) characteristic of bone, and not of cartilage. That is why it is important to identify factors that are differentially regulated in hyaline and in hypertrophic cartilage. In this mini-review we are going to compare PGs in different stages of cartilage differentiation.

Types of proteoglycans

PG aggregation relates to the ability of individual PGs to interact with HA, to form large PG aggregates. In AC, aggrecan is the aggregating PG that exists in association with HA and link protein (LP). Cartilage also contains small leucine-rich proteoglycans (SLRPs), namely decorin, biglycan, fibromodulin and lumican which maintain tissue integrity and metabolism. Additionally, there are transmembrane family of heparan sulphate proteoglycans (HSPGs), syndecans, glypicans and perlecan. A summary of proteoglycan characteristics and interactions in AC are shown in Table 1.

Aggrecan

Aggrecan is a large (>2500 kDa) modular PG with several functional domains capable of binding CS and KS. Aggrecan exists as an aggregate containing a central HA filament with up to 100 aggrecan molecules radiating from it, connected via LP at the N-terminal domain of aggrecan. Normal function of AC is determined by aggrecan content and size and GAG side chains. Large aggregates are able to attract more water and swell to resist mechanical stress. Moreover, aggrecan connects with collagen to resist deformation. In degenerating or hypertrophic cartilage aggrecan aggregates are not intact, but rather are found in fragments. Aggrecan fragmentation could be a result of the activity of matrix-degrading enzymes, proteolysis and lysosomal digestion. Following proteolytic cleavage of the core protein, two fragments are generated: one remains bound to HA and one is no longer able to interact with HA. The HA-free fragment is able to diffuse through the ECM and be lost into the synovial fluid. The remaining HA-fragment is estimated to have a half-life of 20 years. This has been considered an inhibition to repair processes as the fragment has an impaired load-bearing ability and yet occupies space on the HA that could be utilised by newly synthesised aggrecan. Aggrecan size polymorphisms would affect the network integrity and anionic charge density and decrease the ability to attract water and positrons. It has been well-established that dysregulation of osmosis in cartilage leads to the structural collapse of the tissue and remodelling associated with mineralisation, ultimately impairing the normal biomechanical function of cartilage [12,13].

Decorin

Decorin is a highly conserved class I small leucine-rich proteoglycan (SLRP) that has a ~36 kDa protein core that harbours either chondroitin sulphate (CS) or dermatan sulphate (DS) GAG side chine at its N-terminal [14–17]. The inner concave surface of decorin consists of 14 b-sheets and the outer convex surface of a-helices [17]. Most decorin binding occurs at the LRR 4-6 of the core protein. There is evidence to suggest that decorin interacts with other proteins as a monomer and dimerisation serves as decorin sequestration rather than protein stabilisation [18]. Although decorin concenrtrations in cartilage are comparable to aggrecan and decorin is actively expressed throughout life, the structural function of decorin in cartilage is unknown [19,20]. Apart from the canonical role of decorin in collagen fibrillogenesis and cell biology in other tissues, decorin was found to be an indispensable matrix constituent in cartilage in mice [14,21,22]. Specifically, decorin enhanced aggrecan-aggrecan and aggrecan-collagen molecular adhesion thereby maintaining the structural integrity of cartilage ECM [21]. It is well-accepted that the primary mechanism of aggrecan assembly in cartilage is through link protein-assisted aggrecan-HA aggregation [23,24]. It is known that in adult cartilage there is increased aggrecan fragmentation and dissociation from HA, even in healthy tissue [25]. However, there is a high degree of aggrecan retention despite fragmentation [26]. It has been hypothesised that decorin acts as a physical linker to maintain the integrity of the aggrecan network in older cartilage. This decorin-mediated aggrecan assembly was shown to be necessary for the biomechanical functions of cartilage in mice and for the retention of newly synthesised aggrecan in chondrocyte neo-matric *in vitro* [21]. Despite having a diverse interactome with other proteins, decorin did not have a direct effect on anabolism or chondrocyte proliferation. There are contradictory results

for decorin-TGF-*b* interactions. Gronau at al, claims that the removal of decorin helps resist the progression of OA due to increased negative charges through interactions with TGF-*b* leading to aberrant water retention and higher stiffness of AC matrix [27]. Those results are in disagreement with previously discussed findings, therefore more scientific effort is required to unravel the role of decorin in AC [21,27]. In contrast to direct aggrecan binding in mature cartilage, in soft chondrosarcoma cartilage, decorin and also biglycan were found to form complexes with matrilin-1. It was demonstrated that major structural entities collagen II and aggrecan were joined to collagen VI via a complex of matrilins and LRR-PGs [28]. Altogether, there is strong evidence that decorin is an important structural component of the cartilage ECM as it serves as a connector either alone or in combination with other proteins to form macromolecular assemblies. Non-glycosylated or soluble decorin has been associated with ageing cartilage and OA.

Biglycan

Biglycan, similar to decorin, is a class I SLRP that has a ~42 kDa protein core [29,30]. One or two GAG side chains of CS or DS can bind at the N-terminus of the LRR core protein [31,32]. Biglycan was discovered in the developing bone and is known to be involved in osteogenesis, immune processes, synaptic stability and muscle integrity through a multitude of interactions (e.g., collagen, BMPs, TGF-*b*, WISP1) [33]. However, the biglycan interactome in AC is elusive. It is known that biglycan is expressed in bones and tendons, and mice deficient in biglycan developed ectopic joint ossifications and gait impairment leading to unstable joints and OA development [34]. OA was a result of the structural collapse of tendons rather than the joint cartilage. In ageing AC however, similar to decorin, a non-GAG form of bigycan has been identified [35]. Both decorin and biglycan appear to have similar abundance in newborn AC, but biglycan was challenging to detect in the adult tissue [36]. Furthermore, soluble biglycan has been found in a greater abundance in adult human patellar cartilage and meniscus tear synovial fluid, compared to GAG-free decorin [37,38]. When added cartilage explants, the soluble biglycan significantly increased gene and protein expression of matrix-degrading metalloproteinases (MMP-1, -9, -13) and of pro-inflammatory cytokines (IL-6 and IL-8), whereas expression of anabolic markers aggrecan and collagen II was decreased [38]. The mechanism through which GAG-free biglycan induced these changes in chondrocytes was mainly through the TLR4-NF-kb pathway. High concentrations of soluble biglycan are associated with cartilage degradation that is characteristic of OA.

There are two possible mechanisms of how those non-glycosylated proteins arise: 1) they are synthesised as such or 2) they are a result of proteolytic processes. There is strong evidence in support of 2) whereby proteolytically modified forms of matrix components are a result of increased inflammation or tissue damage with age [35]. Due to fragmentation, the charge of molecules changes that affects interaction with other GAGs (preferentially bind to HA, rather than aggrecan) which results in aberrant water retention and consequently changes in the biomechanical properties of cartilage. That is why, non-glycosylated end products and matrix digested fragments are markers of collapsing cartilage homeostasis and predictive of OA development.

Syndecans

Syndecans-1, -2, -3 and -4 are a family of transmembrane HSPGs, 31, 20, 38 and 20 kDa in size, however, they migrate differently on polyacrylamide gels presumably due to extended structural configurations. The syndecan core protein is comprised of extracellular, N-terminal, transmembrane highly conserved domain and a cytoplasmic tail. The cytoplasmic tail contains two C-terminal domains and a variable domain specific to each syndecan member. Syndecan-1 and -4 can have both HS and CS on their core protein, while syndecan-2 and -3 can have only HS. Each domain interacts with a wide array of ECM components, mitogens, chemokines to relay signals from the external environment into the cell [39,40].

In mineralised tissues, syndecans are expressed in a spatiotemporal manner according to the developmental stage and tissue type. In early skeletal development, syndecan-1 is transiently expressed, whereas syndecan-4 is ubiquitous [41–44]. Importantly, syndecan-1 was shown to be upregulated in the AC of a mouse model of OA [45–47]. Combined with the known role in skeletogenesis and wound healing, the authors suggest that syndecan-1 is overexpressed in the early stages of cartilage degeneration in an attempt to repair cartilage fibrillations. And although sydnecan-4 is hardly expressed in normal AC, it is strongly upregulated in human and animal models of OA [46,47]. Interestingly, the OA mice deficient in syndecan-4 had a significant reduce of severity and cartilage damage. One mechanism of syndecan-4 action could be that during inflammation TNF and IL-1b regulate the expression of syndecan-4 which promotes aggrecan degradation by the aggrecanase ADAMTS-5 thereby inducing damage to the cartilage tissue [48,49]. Syndecan-4 has been specifically implicated in the OA of knee cartilage and in a ‘shedded’ or proteolytically processed version of syndecan-4 [50].

In mice it was revealed that syndecan-2 was restricted to mesenchymal-derived tissues. Syndecan-2 is also expressed at the onset of periosteum osteogenesis, in the condensing pre-chondrogenic core and in the periochondrium, but is decreased during chondrocyte differentiation, heavily implicating syndecan-2 in bone formation. It has been established that syndecan-2 is a negative regulator of Wnt signalling in osteosarcoma cells, however, it is known that the syndecan-4-driven Wnt/PCP axis is important for head cartilage morphogenesis [44,51]. There is convincing evidence that syndecan-2 and 4 have redundant functions in bone fracture repair. In a syndecan-4 knockout, syndecan-2 was upregulated, however, expression was dependent on the presence of inflammatory TNF-a and less on Wnt-3a [52]. Although syndecan-2 and -4 display some redundancy, there is evidence that they have distinct roles in skeletal and chondrogenic patterning, however, not fully understood [41].

Syndecan-3 is involved in chondrocyte differentiation during growth plate formation and is restricted to the proliferative and hypertrophic zones, indicating a role in chondrocyte proliferation [53–55]. Chondrocyte proliferation is thought to be mediated through syndecan-3 interaction with FGF-2 and IHH [56–58]. In healthy AC, syndecan-3 had a basal expression, however, in OA cartilage syndecan-3 was highly upregulated, along with other chondrogenic differentiation and proliferation markers [55]. This evidence suggests that during OA, chondrocytes in the AC undergo a syndican-3-mediated differentiation process similar to growth plate chondrocytes. If that is the case, hypertrophic or terminally differentiated chondrocytes would synthesise matrix components important for mineralisation and overall ECM stiffening, that are typically not found in AC.

Despite these data, however, the data on the specific function of syndecans during cartilage damage is elusive. There is a growing body of evidence that implicates syndecans in the inflammatory response not only in cartilage degradation, but also in other systems [59,60]. It is likely that syndecans are more strongly associated with the skeletal system under normal conditions, but during stress, they could be misregulated and contribute to pathological processes in cartilage via inflammation.

Glypicans

Glypicans are another class of PG that contain HS chains but instead of a transmembrane domain, they are anchored to the cell membrane with a glycosylphosphatidylinositol (GPI). Out of 6 glypicans, glypican-3 was found to be predominantly expressed in foetal AC and limited in adult tissues [61,62]. In bone biology, glypican-3 has been shown to inhibit osteogenesis through BMP-2 and to regulate osteoclast differentiation that correlated with the persistence of hypertrophic chondrocytes in embryos [63,64]. Because it was implicated in bone remodelling, it was speculated that glypican-3 might also contribute to the development of OA. Udomsinprasert et al, discovered that glypican-3 levels were remarkably increased in the synovial fluid of patients with OA, although the mechanism was not revealed [65]. Given the evidence, we can speculate that the appearance of glypican-3 in OA could be an early attempt for the inhibition of cartilage ossifications.

Perlecan

Perlecan is a PG that bares HS or CS GAG side chains. There is a large body of literature that implicates perlecan in cartilage development. Genetic abrogation of perlecan is either embryonic lethal or leads to severe skeletal and cartilage disorganisation, highlighting the importance of this gene during development [66,67]. Importantly, perlecan is pronounced in the tissues undergoing endochondral ossification and in the hypertrophic zone of the growth at all developmental stages [68,69]. Perlecan was shown to be a chondrogenic promoter *in vitro*, however, insufficient to drive subsequent development alone [70]. In a ‘domain mapping’ investigation of perlecan it was revealed that the abolishment of GAG attachment sites inhibits that chondrogenic activity [71]. In contrast, there is evidence that the ablation of perlecan HS domain I provides chondroprotection through the complex interplay of FGF signalling [72]. It was demonstrated that perlecan was upregulated in late-stage OA of the knee at the site of injury and particularly associated with fine collagen fibers [73]. In normal cartilage, perlecan interacts with adhesive macromolecules to provide structural support of the chondrocyte pericellular matrix and promotes chondrocyte adhesion to the substratum [74–76]. The increased amount of perlecan at the main defect that coinsides with collagen distribution could be a compensatory effect for the loss of other ECM components such as aggrecan. Furthermore, perlecan was shown to be necessary for osteophyte development in knee OA, likely because perlecan regulates the chondrogenic differentiation of synovial mesenchymal cells through TGF-*b* [77,78].

Name	Type	Size (kDa)	Structure	Interaction	Role in caerilage
Aggrecan		>2500	- core protein of G1, G2, G3 domains and IGD - N-terminal site for CS and KS - Exists in aggregated form connected to HA via LP	HA, Collagen, FGFs, IHH, BMPs	Structural support and tissue swelling to resist mechanical stress. A target of proteolytic fragmentation that is correlated with the ageing cartilage and also with deterioration during OA.
Decorin	SLRP, class I	36	- 12 LRR core - N-terminal site for a single CS or DS - LRR 4-6 is a high affinity binding site	Interacts with aggrecan and collagen as a monomer.	Indispensable for structural integrity and biomechanical functions of cartilage through interacting with the aggrecan network. Does not directly influence chondrocyte biology.
Biglycan	SLRP, class I	42		TLR4-NF-kb pathway	Important structure and ossification of collagen-I-rich tissues (tendons), indirectly regulating joint homeostasis. Non-GAG biglycan is highly associated with inflammation and cartilage degradation in OA.
Fibromodulin [79–81]	SLRP, class II	42	- 12 LRR core, flanked by cysteine-rich N- and C-terminals and a signal peptide - N-terminal site for KS	Collagen I, Complement system, TGF-*b*, BMP	Its expression in cartilage is spatiotemporally determined by the developmental stage and prevents chondrocyte apoptosis. Canonical function is collagen fibrillogenesis. Proteolytic or inflammation-related fragmentation of fibromodulin are highly correlated with OA. Deletion of fibromodulin promote OA.
Lumican [81–83]	SLRP, class II	40	- 11 LRR core, flanked by N- and C- terminals and a signal peptide - N-terminal site for KS	Collagen I, TLR4	Controls fibril formation and maturation in normal AC. Lumican promotes inflammation, cartilage degradation and macrophage activation in OA.
Syndecan-1	HSPG	31	Extracellular, transmembrane, N-terminal domain and a cytoplasmic tail true for all syndecans. - N-terminal site for HS or CS		Transiently expressed in early skeletogenesis. Overexpressed in OA, but not in AC. Might have a protective effect on chondrocytes in early OA.
Syndecan-2	HSPG	20	- N-terminal site for HS	TNF-a, Wnt-3a	Important for bone formation and repair. Could be a negative prognostic factor for osteogenesis and cartilage calcifications.
Syndecan-3	HSPG	38	- N-terminal site for HS	FGF-2, IHH	Chondrocyte differentiation in normal AC and unfavourable chondrocyte hypertrophy in OA.
Syndecan-4	HSPG	20	- N-terminal site for HS or CS	TNF, IL-1b, ADAMTS-5, Wnt	Not expressed in normal AC. Fragmented syndecan-4 is correlated with cartilage degradation and inflammation in OA.
Glypican-3	HSPG		- Linked to the cell surface through a GPI anchor - N-terminus, globular domain of 14 cysteine residues, GAG attachment domain, C-terminus containing the GPI anchor - Bares HS		Normally expressed in foetal AC and inhibits osteogenesis through BMP-2. In OA appears in synovial fluid of the knee.
Perlecan	HSPG	400-470	- 5 domains (I, II, III, IV, V) - N-terminal site for mostly HS but can also bind CS	Smad, MAPK, TGF-*b,* collagen, FGFs	Essential for cartilage development, but not required for chondrogenesis in later stages. Regulates chondrogenic differentiation of synovial mesenchymal cells. Colocalises with collagen at the site of injury in OA.

Table 1. Summary of proteoglycan properties and interactome in articular cartilage

1. Carballo, C. B. *et al*. Basic Science of Articular Cartilage. *Clin Sports Med* **36**, 413–425 (2017).
2. Killen, M. C. & Charalambous, C. P. Advances in cartilage restoration techniques. *Advances in Medical and Surgical Engineering* 71–83 (2020) doi:10.1016/B978-0-12-819712-7.00006-1.
3. Roughley, P. J. The structure and function of cartilage proteoglycans. *Eur Cell Mater* **12**, 92–101 (2006).
4. Roughley, P. J. & Lee, E. R. Cartilage proteoglycans: Structure and potential functions. *Microsc Res Tech* **28**, 385–397 (1994).
5. Blanco, A. & Blanco, G. Carbohydrates. *Med Biochem* 73–97 (2017) doi:10.1016/B978-0-12-803550-4.00004-5.
6. Sasisekharan, R. *et al.* Glycomics approach to structure-function relationships of glycosaminoglycans. *Annu Rev Biomed Eng* **8**, 181–231 (2006).
7. anatomy, J. C.-J. of & 1990, undefined. The structure of vascular channels in the subchondral plate. *ncbi.nlm.nih.gov* **171**, 105–115 (1990).
8. Hoemann, C. D. *et al*. The Cartilage-Bone Interface. *J Knee Surg* **25**, 085–098 (2012).
9. NT, K. *et al.* FTIR-I compositional mapping of the cartilage-to-bone interface as a function of tissue region and age. *J Bone Miner Res* **29**, 2643–2652 (2014).
10. Wang, W. *et al.* Roles of the calcified cartilage layer and its tissue engineering reconstruction in osteoarthritis treatment. *Front Bioeng Biotechnol* **0**, 1499 (2022).
11. Campo, R. D. & Romano, J. E. Calcified Tissue International Changes in Cartilage Proteoglycans Associated with Calcification. *Calcif Tissue Int* **39**, 175–184 (1986).
12. Kiani, C. *et al*. Structure and function of aggrecan. *Cell Research 2002 12:1* **12**, 19–32 (2002).
13. Roughley, P. J. & Mort, J. S. The role of aggrecan in normal and osteoarthritic cartilage. *Journal of Experimental Orthopaedics 2014 1:1* **1**, 1–11 (2014).
14. Gubbiotti, M. A. *et al.* Decorin interacting network: A comprehensive analysis of decorin-binding partners and their versatile functions. *Matrix Biol* **55**, 7–21 (2016).
15. Krusius, T. & Ruoslahti, E. Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. *Proc Natl Acad Sci U S A* **83**, 7683 (1986).
16. Mann, D. M. *et al*. Analysis of Glycosaminoglycan Substitution in Decorin by Site-directed Mutagenesis*. *THE JOURNAL. OF BIOLOGICAL CHEMLWRY* **265**, 199 (1990).
17. Weber, I. T. *et al.* Model structure of decorin and implications for collagen fibrillogenesis. *J Biol Chem* **271**, 31767–31770 (1996).
18. Gubbiotti, M. A. *et al*. Decorin interacting network: A comprehensive analysis of decorin-binding partners and their versatile functions. *Matrix Biology* **55**, 7–21 (2016).
19. Sampaio, L. de O. *et al.* Dermatan sulphate proteoglycan from human articular cartilage. Variation in its content with age and its structural comparison with a small chondroitin sulphate proteoglycan from pig laryngeal cartilage. *Biochemical Journal* **254**, 757 (1988).
20. Poole, A. R. *et al.* Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. *J Orthop Res* **14**, 681–689 (1996).
21. Han, B. *et al.* Decorin Regulates the Aggrecan Network Integrity and Biomechanical Functions of Cartilage Extracellular Matrix. *ACS Nano* **13**, 11320 (2019).
22. Birk, D. E. & Chen, S. The regulatory roles of small leucine-rich proteoglycans in extracellular matrix assembly. *FEBS J* **280**, 2120–2137 (2013).
23. Hardingham, T. E. & Muir, H. The specific interaction of hyaluronic acid with cartillage proteoglycans. *Biochim Biophys Acta* **279**, 401–405 (1972).
24. Buckwalter, J. A. *et al*. The effect of link protein on proteoglycan aggregate structure. An electron microscopic study of the molecular architecture and dimensions of proteoglycan aggregates reassembled from the proteoglycan monomers and link proteins of bovine fetal epiphyseal cartilage. *Journal of Biological Chemistry* **259**, 5361–5363 (1984).
25. Wells, T. *et al.* Age-related changes in the composition, the molecular stoichiometry and the stability of proteoglycan aggregates extracted from human articular cartilage. *Biochemical Journal* **370**, 69 (2003).
26. Lee, H. Y. *et al.* Age-related nanostructural and nanomechanical changes of individual human cartilage aggrecan monomers and their glycosaminoglycan side chains. *J Struct Biol* **181**, 264–273 (2013).

27. Gronau, T. *et al.* Forced exercise-induced osteoarthritis is attenuated in mice lacking the small leucine-rich proteoglycan decorin. *Ann Rheum Dis* **76**, 442–449 (2017).
28. Wiberg, C. *et al.* Complexes of Matrilin-1 and Biglycan or Decorin Connect Collagen VI Microfibrils to Both Collagen II and Aggrecan *. *Journal of Biological Chemistry* **278**, 37698–37704 (2003).
29. Schaefer, L. & Iozzo, R. v. Biological Functions of the Small Leucine-rich Proteoglycans: From Genetics to Signal Transduction. *J Biol Chem* **283**, 21305 (2008).
30. Bianco, P. *et al.* Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. *J Histochem Cytochem* **38**, 1549–1563 (1990).
31. Choi, H. U. *et al.* Characterization of the dermatan sulfate proteoglycans, DS-PGI and DS-PGII, from bovine articular cartilage and skin isolated by octyl-sepharose chromatography. *Journal of Biological Chemistry* **264**, 2876–2884 (1989).
32. Roughley, P. J. & White, R. J. Dermatan sulphate proteoglycans of human articular cartilage. The properties of dermatan sulphate proteoglycans I and II. *Biochemical Journal* **262**, 823–827 (1989).
33. Nastase, M. *et al*. A Multivalent Proteoglycan Providing Structure and Signals. *Journal of Histochemistry and Cytochemistry* **60**, 963 (2012).
34. Ameye, L. *et al.* Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. *FASEB J* **16**, 673–680 (2002).
35. Roughley, P. J. *et al.* Non-proteoglycan forms of biglycan increase with age in human articular cartilage. *Biochemical Journal* **295**, 421–426 (1993).
36. Melching, L. I. & Roughley, P. J. The synthesis of dermatan sulphate proteoglycans by fetal and adult human articular cartilage. *Biochem. J* **261**, 501–508 (1989).
37. Witsch-prehm, P. *et al*. PRESENCE O F SMALL PROTEOGLYCAN FRAGMENTS IN NORMAL AND ARTHRITIC HUMAN CARTILAGE. doi:10.1002/art.1780350909.
38. Barreto, G. *et al.* Soluble biglycan: a potential mediator of cartilage degradation in osteoarthritis. *Arthritis Res Ther* **17**, (2015).
39. Agere, S. A. *et al*. Syndecans in chronic inflammatory and autoimmune diseases: Pathological insights and therapeutic opportunities. *J Cell Physiol* **233**, 6346 (2018).
40. Silbert, J. E. *et al*. Proteoglycans: a special class of glycoproteins. *New Comprehensive Biochemistry* **29**, 1–31 (1997).
41. Oh, E., Science, J. C.-M. & C. (Springer & 2004, undefined. Syndecans-2 and-4; close cousins, but not identical twins. *search.ebscohost.com*.
42. Molteni, A. *et al.* Differential expression of fibroblast growth factor receptor-1, -2, and -3 and syndecan-1, -2, and -4 in neonatal rat mandibular condyle and calvaria during osteogenic differentiation in vitro. *Bone* **24**, 337–347 (1999).
43. Bai, X. M. *et al.* expression of multiple cell-surface heparan sulfate proteoglycans during embryonic tooth development. *J Histochem Cytochem* **42**, 1043–1053 (1994).
44. Mansouri, R. *et al*. Role of syndecan-2 in osteoblast biology and pathology. *Bonekey Rep* **4**, 666 (2015).
45. Salminen-Mankonen, H. *et al.* Syndecan-1 expression is upregulated in degenerating articular cartilage in a transgenic mouse model for osteoarthritis. *Scand J Rheumatol* **34**, 469–474 (2005).
46. Barre, P. E. *et al.* Semiquantitative reverse transcription-polymerase chain reaction analysis of syndecan-1 and -4 messages in cartilage and cultured chondrocytes from osteoarthritic joints. *Osteoarthritis Cartilage* **8**, 34–43 (2000).
47. Echtermeyer, F. *et al.* Syndecan-4 regulates ADAMTS-5 activation and cartilage breakdown in osteoarthritis. *Nat Med* **15**, 1072–1076 (2009).
48. Wang, J. *et al.* TNF-α and IL-1β promote a disintegrin-like and metalloprotease with thrombospondin type I motif-5-mediated aggrecan degradation through syndecan-4 in intervertebral disc. *J Biol Chem* **286**, 39738–39749 (2011).
49. Echtermeyer, F. *et al.* Syndecan-4 regulates ADAMTS-5 activation and cartilage breakdown in osteoarthritis. *Nat Med* **15**, 1072–1076 (2009).
50. Sanchez, C. *et al.* Syndecan-4 Is Increased in Osteoarthritic Knee, but Not Hip or Shoulder, Articular Hypertrophic Chondrocytes. *https://doi.org/10.1177/1947603519870855* **13**, 862S-871S (2019).
51. Ohkawara, B. *et al.* Rspo3 binds syndecan 4 and induces Wnt/PCP signaling via clathrin-mediated endocytosis to promote morphogenesis. *Dev Cell* **20**, 303–314 (2011).

52. Bertrand, J. *et al.* Syndecan 4 Supports Bone Fracture Repair, but Not Fetal Skeletal Development, in Mice. *Arthritis Rheum* **65**, 743–752 (2013).
53. Kirsch, T. *et al.* Syndecan-3 Is a Selective Regulator of Chondrocyte Proliferation*. (2002) doi:10.1074/jbc.M207209200.
54. Shimazu, A. *et al.* Syndecan-3 and the control of chondrocyte proliferation during endochondral ossification. *Exp Cell Res* **229**, 126–136 (1996).
55. Pfander, D. *et al.* Expression of Early and Late Differentiation Markers (Proliferating Cell Nuclear Antigen, Syndecan-3, Annexin VI, and Alkaline Phosphatase) by Human Osteoarthritic Chondrocytes. *Am J Pathol* **159**, 1777–1783 (2001).
56. Pacifici, M. *et al.* Syndecan-3: a cell-surface heparan sulfate proteoglycan important for chondrocyte proliferation and function during limb skeletogenesis. *J Bone Miner Metab* **23**, 191–199 (2005).
57. Shimazu, A. *et al.* Syndecan-3 and the control of chondrocyte proliferation during endochondral ossification. *Exp Cell Res* **229**, 126–136 (1996).
58. Shimo, T. *et al.* Indian Hedgehog land Syndecans-3 Coregulate Chondrocyte Proliferation and Function during Chick Limb Skeletogenesis. *Developmental Dynamics* **229**, 607–617 (2004).
59. Pap, T. & Bertrand, J. Syndecans in cartilage breakdown and synovial inflammation. *Nature Reviews Rheumatology 2012 9:1* **9**, 43–55 (2012).
60. MGopal, S. Syndecans in inflammation. *FASEB J* **17**, 575–591 (2003).
61. Tretiakova, M. *et al.* Glypican 3 overexpression in primary and metastatic Wilms tumors. *Virchows Arch* **466**, 67–76 (2015).
62. Lee, C. R. *et al.* A phenotypic comparison of intervertebral disc and articular cartilage cells in the rat. *Eur Spine J* **16**, 2174–2185 (2007).
63. Viviano, B. L. *et al.* Altered hematopoiesis in glypican-3-deficient mice results in decreased osteoclast differentiation and a delay in endochondral ossification. *Dev Biol* **282**, 152–162 (2005).
64. Dwivedi, P. P. *et al.* Regulation of bone morphogenetic protein signalling and cranial osteogenesis by Gpc1 and Gpc3. *Bone* **55**, 367–376 (2013).
65. Udomsinprasert, W. *et al.* Plasma and Joint Fluid Glypican-3 Are Inversely Correlated with the Severity of Knee Osteoarthritis. *Cartilage* **12**, 505 (2021).
66. Bolton, M. Perlecan is essential for cartilage development. *Arthritis Research & Therapy 1999 3:1* **3**, 1–4 (1999).
67. Arikawa-Hirasawa, E. *et al.* Perlecan is essential for cartilage and cephalic development. *Nat Genet* **23**, 354–358 (1999).
68. French, M. M. *et al.* Expression of the heparan sulfate proteoglycan, perlecan, during mouse embryogenesis and perlecan chondrogenic activity in vitro. *J Cell Biol* **145**, 1103–1115 (1999).
69. Handler, M. *et al.* Developmental Expression of Perlecan During Murine Embryogenesis. *Dev. Dyn* **210**, 130–145 (1997).
70. Gomes, R. *et al.* Perlecan: an important component of the cartilage pericellular matrix. *J Musculoskelet Neuronal Interact* **2**, 511 (2002).
71. French, M. M. *et al.* Chondrogenic activity of the heparan sulfate proteoglycan perlecan maps to the N-terminal domain I. *J Bone Miner Res* **17**, 48–55 (2002).
72. Shu, C. C. *et al.* Ablation of Perlecan Domain 1 Heparan Sulfate Reduces Progressive Cartilage Degradation, Synovitis, and Osteophyte Size in a Preclinical Model of Posttraumatic Osteoarthritis. *Arthritis Rheumatol* **68**, 868–879 (2016).
73. Tesche, F. & Miosge, N. Perlecan in late stages of osteoarthritis of the human knee joint. *Osteoarthritis Cartilage* **12**, 852–862 (2004).
74. Costell, M. *et al.* Perlecan maintains the integrity of cartilage and some basement membranes. *J Cell Biol* **147**, 1109–1122 (1999).
75. Hassel, J. *et al.* Role of perlecan in skeletal development and diseases. *Glycoconj J* **19**, 263–267 (2002).
76. SundarRaj, N. *et al.* Perlecan is a component of cartilage matrix and promotes chondrocyte attachment. *J Cell Sci* **108 (Pt 7)**, 2663–2672 (1995).
77. Arita, H. *et al.* Role of synovial perlecan in osteophyte formation in early stage knee osteoarthritis. *Osteoarthritis Cartilage* **27**, S52 (2019).

78. Mayuko, K. *et al.* Perlecan regulates chondrogenic differentiation from synovial mesenchymal cells via Smad and MAPK signaling pathways. *Osteoarthritis Cartilage* **24**, S177 (2016).
79. Jan, A. T. *et al.* Fibromodulin: A regulatory molecule maintaining cellular architecture for normal cellular function. *International Journal of Biochemistry and Cell Biology* **80**, 66–70 (2016).
80. Li, C. *et al.* Fibromodulin – A New Target of Osteoarthritis Management? *Front Pharmacol* **10**, 1475 (2019).
81. Chakravarti, S. Functions of lumican and fibromodulin: Lessons from knockout mice. *Glycoconj J* **19**, 287–293 (2003).
82. Barreto, G. *et al.* Lumican is upregulated in osteoarthritis and contributes to TLR4-induced pro-inflammatory activation of cartilage degradation and macrophage polarization. (2020) doi:10.1016/j.joca.2019.10.011.
83. Kao, W. W.-Y. *et al*. Focus on Molecules: Lumican. *Exp Eye Res* **82**, 3 (2006).