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Cartilage proteoglycans

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The predominant proteoglycan present in cartilage is the large chondroitin sulfate proteoglycan 'aggrecan'. Following its secretion, aggrecan self-assembles into a supramolecular structure with as many as 50 monomers bound to a filament of hyaluronan. Aggrecan serves a direct, primary role providing the osmotic resistance necessary for cartilage to resist compressive loads. Other proteoglycans expressed during chondrogenesis and in cartilage include the cell surface syndecans and glypican, the small leucine-rich proteoglycans decorin, biglycan, fibromodulin, lumican and epiphycan and the basement membrane proteoglycan, perlecan. The emerging functions of these proteoglycans in cartilage will enhance our understanding of chondrogenesis and cartilage degeneration.

Key words: aggrecan / cartilage / CD44 / chondrocytes / hyaluronan

Introduction

Why is there a need for a separate discussion of cartilage proteoglycans? Part of the answer is historical and part relates to the unique proteoglycans of cartilage and other load-bearing tissues. Cartilage is particularly rich in extracellular matrix, with matrix making up 90% of the dry weight of the tissue.[1] As proteoglycans were originally considered as mere structural components of the extracellular matrix, cartilage proved a useful system for study especially in the early days of biochemical isolation and structural analysis. The predominate glycosaminoglycan present in cartilage has long been known to be chondroitin sulfate.[2] However, extraction of the chondroitin sulfate in a more native form, as a proteoglycan, proved to be a daunting task. The revolution in the field came about through the work of Hascall and Sajdera.[3] With the use of the strong chaotropic agent guanidinium hydrochloride, the proteoglycans of cartilage could now be readily extracted and separated into relatively pure monomers through the use of CsCl density gradient centrifugation. This provided the means to identify and characterize the major chondroitin sulfate proteoglycan of cartilage, later to be termed 'aggrecan' following the cloning and sequencing of its core protein.[4] From this start, aggrecan has gone on to serve as the paradigm for much of proteoglycan research.

Aggrecan was found to exhibit a unique feature in that the core protein had the capacity to interact with another glycosaminoglycan, hyaluronan.[5] As such, this defined one of the first protein–glycosaminoglycan interactions—a strong interaction with a binding affinity on the order of 10^{-7}–10^{-8} M.[6] Another singular feature of cartilage was the presence of a protein, highly homologous to the globular region of aggrecan core protein, that exhibited a capacity to bind both the aggrecan core protein as well as hyaluronan. This protein was given the simple name 'link protein'.[7] The tripartite linkage of aggrecan, link protein and hyaluronan is essentially non-dissociating and non-displaceable under physiological conditions. This interaction represents the primary reason why chaotropic agents are necessary to extract aggrecan from cartilage. Although originally thought to be a cartilage specific proteoglycan, aggrecan was subsequently found in other tissues such as aorta, disc and tendon. Nevertheless, deposition of aggrecan is considered a hallmark of chondrogenesis[8] and is a key marker in studies to elucidate molecular mechanisms of chondrogenesis. Conversely, proteoglycans common to other tissues

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such as decorin, biglycan and perlecan have also been identified in cartilage. Therefore, it is not that a unique proteoglycan provides cartilage with its intrinsic properties as a tissue. Rather, it is the unique blending of several proteoglycans, together with their organization within the extracellular matrix (Figure 1) that give cartilage its capacity to resist excessive loads and provide for the frictionless movement of joints.

Cartilage

Cartilage is a specialized connective in which the chondrocytes occupy only 5% of the volume (Figure 2, top panel). Chondrocytes have cell processes that extend a short distance into the matrix, but do not touch other cells. Thus in cartilage, cell–matrix interactions are essential for the maintenance of the extracellular matrix. The network of collagen fibers provides both tensile strength to the tissue and the capacity to contain the swelling pressure of the embedded proteoglycans. The heterotypic collagen fibrils of cartilage consist of collagens type II, IX and XI. The regulation of collagen fibril formation by both decorin and type IX collagen illustrates another functional role of proteoglycans in cartilage matrix assembly. The articular cartilage surface provides compressive stiffness and frictionless movement in the synovial joint. During embryogenesis, the cartilaginous anlagen pattern the rudiments of the skeleton. During growth of long bones, the growth plate (epiphyseal cartilage) undergoes an organization into columns and a process of hypertrophy.

Chondrogenesis

Condensation is a principal mechanism by which the morphological units of the vertebrate skeleton develop. Cells within these condensations express elevated levels of cell surface adhesion molecules, including N-cadherin, N-CAM, CD44 and syndecan-3 as well as matrix components including fibronectin, tenascin, versican and heparan sulfate proteoglycans. Temporal and cooperative function of these molecules mediate the requisite cell–cell and cell–matrix interactions that initiate the condensation event. The progression from condensation and growth to chondrocyte differentiation also involves members of the FGF and BMP families and the activation of homeobox genes including *Msx-1* and *Msx-2*.[9] Thus, the temporal and spatial patterning of the heparan sulfate proteoglycans may alter the presentation of the heparin-binding growth factors to their signaling receptors to regulate anlagen growth and differentiation.

Prior to the condensation process, cells are surrounded by an extensive extracellular matrix, in which hyaluronan is the predominant glycosaminoglycan. Depletion of the hyaluronan content of the matrix is likely via receptor-mediated uptake of hyaluronan for its lysosomal degradation by hyaluronidases. Chondrogenic cells use some of the residual hyaluronan to form essential cell-to-cell cross-bridging prior to chondrogenesis. Disruption of native hyaluronan-cell interactions with hyaluronan hexasaccharides resulted in a delay in the formation of condensations and the expression of cell surface PNA binding during *in vitro* chondrogenesis of embryonic limb bud mesenchyme.[10] Reducing the extracellular space allows the progression of condensation and signaling events mediated by ligation of specific receptors.

Other proteoglycans participate in cartilage matrix organization during chondrogenesis. Versican (also referred to as PG-M) is a large chondroitin sulfate proteoglycan expressed in limb buds during prechondrogenic condensation. The use of alternative exon splicing of the versican gene can generate four core proteins, with the largest one present in embryonic limb buds.[11] PG-M/versican may function as an anti-adhesion molecule during the initiation of matrix assembly and is no longer expressed after the deposition of aggrecan in the developing cartilage anlagen. The G3 domain of versican containing the EGF-like motifs (see below) inhibited mesenchymal chondrogenesis, while an antisense strategy to eliminate versican synthesis resulted in a stimulation of chondrogenesis.[12] The interaction of the chondrogenic cells with matrix components may establish the edges of the cartilage anlagen, for example the binding of syndecan-3 with tenascin-C.[9] The ability of tenascin to bind to the lectin-like C-terminal domain of lecticans,[13] as well as its temporal expression, suggests its role in proteoglycan remodeling within the embryonic matrix. The restricted expression pattern of the small proteoglycan epiphycan during cartilage maturation suggests its role in matrix organization in the growth plate.[14] Progress from condensation to the differentiation of chondrocytes involves the replacement of the initial matrix of fibronectin and versican with the deposition of the cartilage matrix, principally of type II col-

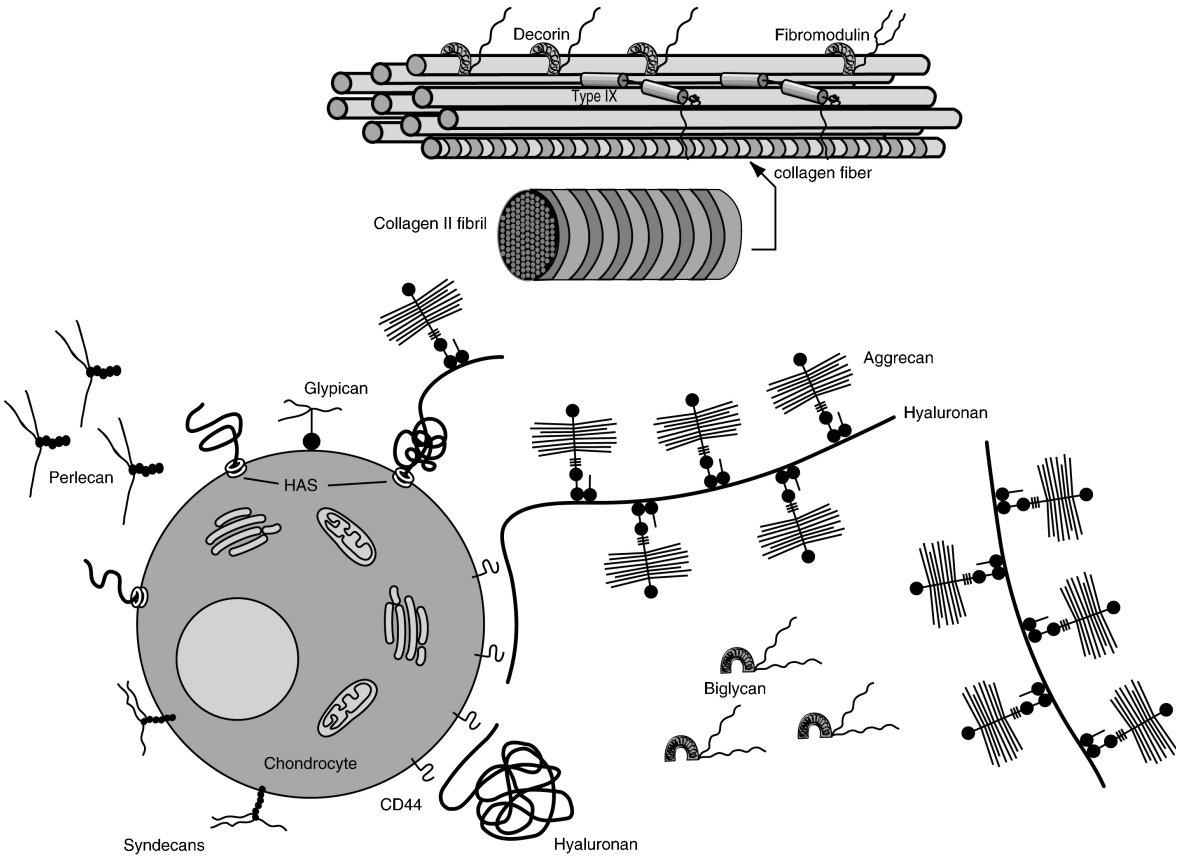

Figure 1. Overview of the proteoglycans present in cartilage. Depicted is a cartilage chondrocyte. Associated with the cell surface are the transmembrane spanning syndecan proteoglycans, the GPI-linked heparan sulfate proteoglycan, glypican, and two forms of hyaluronan, namely hyaluronan bound to the hyaluronan synthase and hyaluronan tethered to CD44. Aggrecan binds to cell surface-associated hyaluronan as well as hyaluronan within the further removed extracellular matrix. Several small proteoglycans namely, decorin, fibromodulin and type IX collagen have been shown to form strong associations with cartilage collagen fibrils (collagens types II, IX and XI). Other proteoglycans such as biglycan and perlecan are also present within cartilage but their localization and binding partners have not been firmly identified.

lagen and aggrecan. Cells undergoing chondrogenesis express occupied cell surface hyaluronan receptors.[15] Hyaluronan bound to the CD44 receptors is thus poised in the pericellular matrix for assembly of proteoglycan aggregates with newly synthesized aggrecan and proteoglycan.[16]

Aggrecan

The core protein of aggrecan (see reviews by Watanabe *et al.*[11] and Schwartz *et al.*[8]) has a molecular weight of ~230 kDa and consists of three globular domains, G1, G2 and G3, and three interglobular domains, the keratan sulfate and chondroitin sulfate glycosaminoglycan attachment domains located between G2 and G3 and a short interglobular domain between G1 and G2. The N-terminus comprises the G1 globular domain, which interacts with hyaluronan and link protein. Mild trypsin treatment generates a protein fragment containing the G1 and G2 domains, which retains hyaluronan binding capacity, and thus is termed the hyaluronan-binding region (HABR). The G1 domain contains three looped subdomains, A, B and B′. Both the B and B′ loops form disulfide bonding double loop structures called proteoglycan tandem repeat (PTR) units. The PTR loop contains the functional site of the binding of aggrecan to hyaluronan. The secondary structure is significant and the HABR no longer binds hyaluronan under reducing conditions. Oegema suggested that newly

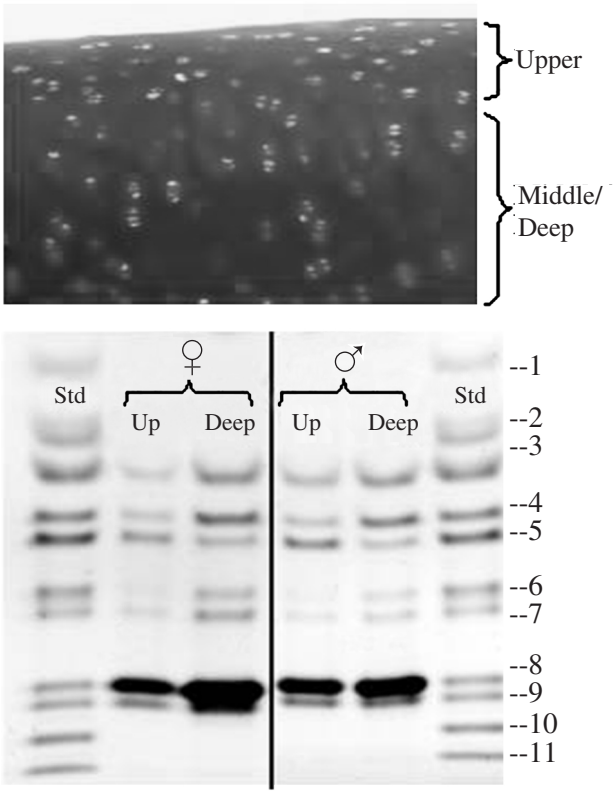

Figure 2. Fluorophore-assisted carbohydrate electrophoresis (FACE) analysis of two samples of normal donor human articular cartilage (talocrural joint cartilage). Two representative donor tissues were examined in this study; one from a 33 year old female (♀), Collins' grade 0 and another, from a 67 year old male (♂), Collins' grade 1. In this analysis the upper (Up) one-third of the cartilage was dissected from the bottom middle/deep layers (Deep) and processed separately. An example of safranin O stained human articular cartilage is shown at the top of the figure. Following proteinase K, hyaluronidase and chondroitinase ABC treatment, the 1,4 unsaturated disaccharides (ΔDi) were reacted with 2-aminoacridone and separated as distinct fluorescent bands following electrophoresis.[25,64] Numbered standards (Std) are represented as follows: N-acetylgalactosamine (1), mannose (2), glucose (3), ΔDi-hyaluronan (4), ΔDi-chondroitin (5), N-acetylgalactosamine-6-sulfate (6), N-acetylgalactosamine-4-sulfate (7), ΔDi-chondroitin-6-sulfate (8), ΔDi-chondroitin-4-sulfate (9), ΔDi-chondroitin-2-sulfate (10), ΔDi-chondroitin-4,6-disulfate (11) and, ΔDi-chondroitin-2,4,6-trisulfate (12). The well-characterized standards were obtained from Calabro *et al.*,[25] as described. As can be seen, little difference in overall glycosaminoglycan composition was found between these two different donors.

synthesized aggrecan is not immediately incorporated into aggregates and furthermore that this delayed aggregation is age dependent.[17] Recent studies by Bayliss *et al.* suggest that newly synthesized aggrecan is processed in pools with different capacities for aggregation with hyaluronan and stabilization by link protein and that tissue compartments, perhaps defined by extracellular pH, show differences with age and with disease state.[18] The C-terminus comprises the G3 domain that includes an EGF module, a C-type lectin module and a complement regulatory protein (CRP) module. With aging, there is an increase in the population of aggrecan monomers that lack the G3 domain, most likely due to extracellular proteolytic degradation.

Other hyaluronan-binding proteoglycans, namely, versican/PG-M, neurocan and brevican contain a G1 and a G3 domain similar to aggrecan.[11] Thus these four large proteoglycans can be grouped into a family alternatively named for their common G1 or G3 functional domains, respectively, as the hyaluronan-binding proteoglycans or the lecticans. Until quite recently, the retention of these proteoglycans was thought to be based solely on their interaction with hyaluronan. However, fibulin-1 as a ligand for the C-type lectin domain of aggrecan and versican[19] has the potential for modulating the assembly or organization of these proteoglycans within the extracellular matrix. Fibulin-1 expression is very low in adult cartilage, but there is significant fibulin-1 expression in the growth plate suggesting its function in remodeling of versican in embryonic cartilage and in the initial organization of proteoglycan aggregates during cartilage maturation.

It is noteworthy that several other hyaluronan-binding proteins contain the G1 domain or a single PTR loop. These include the link protein of cartilage, the tumor necrosis factor stimulated gene-6 (TSG-6) which is also expressed in cartilage and the cell surface receptor for hyaluronan, CD44.[11] The ability of aggrecan to bind to hyaluronan, stabilized by the link protein, provides a mechanism for the fixation of aggrecan within the extracellular matrix. However, our studies have demonstrated that the binding of hyaluronan to the cell surface receptor CD44 allows the retention of a subpopulation of aggrecan in the pericellular matrix of the chondrocytes (Figure 1).[20–22] Thus CD44, hyaluronan and aggrecan comprise important elements for pericellular matrix assembly and maintenance of matrix homeostasis. Disruption of native hyaluronan-cell interactions with hyaluronan hexasaccharides resulted in a delay in

the formation of condensations as well as a delay in the deposition of type II collagen and aggrecan.[10]

Synthesis of aggrecan involves >30 enzymes performing post-translational modifications. The aggrecan core protein is modified co-translationally by the addition of N-linked mannose-rich oligosaccharides. Xylosylation, the initiation step for chondroitin sulfate chain modification of the core protein, begins in the late endoplasmic reticulum compartment and continues into the cis Golgi.[23] The keratan sulfate domain is located just C-terminal to the G2 domain. The potential consensus sequences for attachment of keratan sulfate are E(E,K)PFPS or EEP(S,F)PS.[8] The chondroitin sulfate domain contains approximately 120 ser-gly repeats that can serve as sites for chondroitin sulfate chain elongation and typically up to 100 chondroitin sulfate chains are attached to a single core protein. A consensus sequence, consisting of an acidic amino acid followed by a hydrophobic residue and then the ser-gly residues, is used for xylosylation; but other ser-gly sites are also recognized.[8]

The chondroitin sulfate side chains of aggrecan in mammals are predominantly sulfated at the 6-O-hydroxyl of the galactosamine residue. Plaas *et al.*,[24] have shown that the ratio of 6-O-sulfatation to 4-O-sulfation is <1 in fetal and newborn human articular cartilage, but is >20 in adolescent and adult tissues. The functional significance of these changes remains unclear. New methodologies have been developed to more readily characterize the glycosaminoglycan composition of tissues like cartilage. One new methodology termed fluorophore-assisted carbohydrate electrophoresis (FACE) can be used to quantify the glycosaminoglycan disaccharides generated by chondroitinase ABC digestion.[25] Example profiles of disaccharides derived from a total digest of normal adult articular cartilage of two donors are depicted in Figure 2. The predominant product is the 6-O-sulfated galactosamine-containing unsaturated disaccharide. The 4-O-sulfated and non-sulfated chondroitin disaccharides can also be observed as well as 4-O- and 6-O-sulfated galactosamine monosaccharides derived from the termini of the chains. Hyaluronan disaccharides are also present and represent 2–3% of the total disaccharide. Of particular interest is that the disaccharide profile derived from both donor cartilages are remarkably similar. Analyses of additional donor samples will determine if this represents a true high-fidelity property of 6- and 4-O-sulfotransferases. Nonetheless, this technique demonstrates that glycosaminoglycans can be profiled and quantified with the same ease and accuracy as cDNA fragments.

The aggrecan gene structure has been recently reviewed by Schwartz *et al.*[8] including the results of preliminary promoter analyses. As yet there has been little examination of the transcriptional regulation of the aggrecan gene in either its tissue-specific distribution or its temporal expression during chondrogenesis, although numerous potentially active cis elements have been found in the 1.8 kb 5′ flanking region.[26] SOX9 is a transactivating factor that stimulates the expression and maintenance of the chondrocyte phenotype. Recently it was reported that SOX9 enhanced the aggrecan gene promoter activity through a Sry/Sox consensus sequence.[27]

Other than aggrecan

Although the turnover of collagen type II is extremely low, with a half-life of >100 years,[28] aggrecan and other proteoglycans continue to be synthesized and deposited into the cartilage matrix. The role of the these minor proteoglycans in the initial assembly of the cartilage extracellular matrix, and their role in the maintenance of adult cartilage is an exciting area of study that combines the use of transgenic animals and the study of normal and diseased human cartilage.

The family of small leucine-rich proteoglycans, termed the SLRPs[29] can be divided into three classes; members of each class can be identified in cartilage. Class I includes decorin and biglycan both of which bind TGF-β and are found in cartilage. Epiphyseal cartilage stains prominently for decorin and only weakly for biglycan, whereas in presumptive articular cartilage biglycan is found in the pericellular matrix whereas this developing zone is free of decorin.[30] In adult articular cartilage decorin is present in the interterritorial matrix while biglycan is found in the pericellular matrix. Decorin is associated with collagen fibrils as a decorating proteoglycan and carries one chondroitin or dermatan sulfate side chain. The message level for decorin in cartilage is by far the most abundant of all the SLRP family members and shows increases with increasing age in human articular cartilage.[31] The horseshoe shape of decorin, and the overall dimensions of this arch, support a model for its interaction with a single triple helix of collagen. Decorin deficient mice exhibit reduced tensile strength of skin and tendons

and irregularities in the collagen fibril diameter.[32] Although decorin decorates type II collagen fibrils in cartilage (Figure 1), no effects on type II collagen fiber diameter in the decorin knockout mice have been reported as of yet. Since the concave surface of the decorin core protein is presumed to bind in the gap zone of the collagen fibril, the glycosaminoglycan side chain located near the N-terminus of decorin would be free to maintain the fibril-to-fibril spacing.

The tissue localization and the potential interaction with other cartilage matrix components have been less clearly defined for biglycan, which carries two chondroitin or dermatan sulfate side chains. Mice with a targeted disruption of the biglycan gene were apparently normal at birth, with no skeletal patterning defects. However, the biglycan null mice, that expressed unaffected levels of decorin, showed decreased postnatal skeletal growth, indicating that biglycan is a positive regulator of bone formation and bone mass.[33]

Class II SLRPs expressed in cartilage include fibromodulin, lumican and the protein known as PRELP (proline arginine-rich end leucine-rich repeat protein.) Fibromodulin carries up to four keratan sulfate side chains.[34] Fibromodulin has the capacity to decorate the surface of collagen fiber and therefore may regulate fibril diameter. The message levels for fibromodulin and lumican show increases with increasing age of human articular cartilage.[31] Lumican is the major keratan sulfate proteoglycan in the cornea, but also shows widespread distribution in connective tissues, including cartilage. The modification of lumican with keratan sulfates may contribute to corneal transparency,[35] and it is interesting that in young cartilage, lumican is found as a keratan sulfate proteoglycan while after IL-1 treatment, chondrocytes synthesize and secrete the lumican protein devoid of glycosaminoglycan substitution.[36] PRELP exhibits protein sequence similarity to both lumican and fibromodulin and has four potential N-linked glycosylation sites. Thus although it is a member of the SLR proteins, it apparently functions as a cartilage matrix protein with the capacity for matrix organization.[37]

The class III SLRP expressed in cartilage is epiphycan (also referred to as PG-Lb). It is a dermatan sulfate proteoglycan that can be separated from decorin and biglycan from epiphyseal cartilage, from which its name derives.[38] Two serine residues exhibit the typical glycosaminoglycan attachment consensus structure similar to aggrecan, decorin and biglycan. The expression of epiphycan during development of the growth plate lags behind that of aggrecan, and is excluded from both the layer of presumptive articular cartilage and the hypertrophic zone.[14] These investigators proposed that epiphycan could function to organize the matrix of the growth plate, especially the zone of flattened chondrocytes wherein it is abundant.

Type II collagen represents 80–90% of the collagen content of cartilage, while type IX collagen represents 10% of the total collagen in embryonic cartilage and only 1–2% of the total collagen in adult articular cartilage.[39,40] Type IX collagen can bridge camps of the collagen and proteoglycan research world as it is substituted with a single chondroitin sulfate on the $\alpha 2$ type IX chain (NC3 domain). Although the superficial layer of articular cartilage is enriched in both decorin and type IX collagen, throughout the remaining depth of the tissue decorin is present mainly in the interterritorial compartment while type IX collagen is in the territorial matrix surrounding the chondrocytes. Hagg *et al.*[41] showed by immunoelectron microscopy that the collagen fibrils of hyaline cartilage were heterogeneous, with the majority of the thinnest fibrils decorated with type IX collagen, while the fibrils of larger diameter were decorated with decorin. The thin collagen fibrils found in embryonic cartilage may thus depend on the surface presence of type IX collagen. It has been proposed that a function of the type IX collagen may be to limit fibril diameter; however, the collagen fibrils in cartilage from mice deficient in type IX collagen do not exhibit morphological differences from control.

Perlecan is a heparan sulfate proteoglycan, with five major protein modules.[42] The N-terminal domain contains the glycosaminoglycan attachment sites. Domain II is homologous to the LDL receptor, domain III exhibits homology with laminin, and domain IV contains 21 immunoglobulin repeats resembling N-CAM. The C-terminal domain V contains EGF-like motifs and two LRE tripeptides that could mediate laminin binding. As perlecan is the major proteoglycan in basement membranes it was surprising when it was demonstrated in cartilage,[43,44] a tissue organized without a basement membrane. Perlecan can undergo self-aggregation, and can interact with laminin, nidogen and fibronectin. Integrins have been proposed to function as cell surface receptors for perlecan. Perlecan in adult articular cartilage is enriched in the pericellular matrix and is a chondroitin sulfate/heparan sulfate hybrid proteoglycan.[43] Perlecan is found in cartilage

anlagen after the expression of type II collagen and aggrecan. Perlecan coated onto dishes promoted chondrogenesis and maintains chondrogenic differentiation, perhaps working in concert with SOX9.[45] The perlecan knock-out mouse[46,47] exhibits skeletal abnormalities, defective endochondral ossification, but no early defects in the formation of cartilage anlagen, so *in vivo* the early events of condensation and chondrocyte differentiation are apparently unaffected by the perlecan deficiency. However, in the knockout animals, there is disorganization of the growth plate, including a disruption of the chondrocyte columnar organization and a reduction of the fibrillar collagen network suggesting the role of perlecan in matrix structure. It has also been suggested that perlecan may influence chondrocyte metabolism via signaling receptors or by modulating the expression of FGFs.[44,45]

Cell surface proteoglycans

Chondrocytes also express cell surface proteoglycans; members of the transmembrane family of syndecans and the phosphatidylinositol linked heparan sulfate proteoglycan, glypican (Figure 1). The syndecans may carry two or more heparan sulfate chains, alone or in combination with chondroitin sulfate, and thus have the potential to interact with bFGF, and modulate its interaction with its signaling receptor. Analyses of mRNA from articular chondrocytes demonstrated that message for amphiglycan (syndecan-4) was of the highest abundance. Low level expression of fibroglycan (syndecan-2), glypican and the TGF-β type III receptor betaglycan was also detected.[48] Syndecan-3 is expressed briefly and specifically during early stages of chondrogenesis.[9] Syndecan-3 function as a co-receptor for FGF would promote proliferation and the inhibition of differentiation by this growth factor. Subsequently, syndecan-3 may promote the formation of precartilage condensations by facilitating required cell–cell and cell–matrix interactions.[49] During maturation in the growth plate, expression of syndecan-3 persists in the zone of proliferating chondrocytes, but is not detected in the layer of presumptive articular chondrocytes.[50]

Osteoarthritis

Any remodeling and turnover of cartilage components must be carefully regulated in order to maintain the biomechanical properties of the tissue. The interglobular domain of aggrecan, between G1 and G2 domains, is sensitive to cleavage by a variety of proteases. A major focus of studies on the elucidation of the pathogenesis of osteoarthritis is the identification of candidate 'aggrecanases' that would generate the primary aggrecan fragment containing the NITEGE neoepitope, including ADAMTS-1,[51] ADAMTS-4,[52] MMP-8[53] and MMP-13[54] as well as other enzymes that generate another neoepitope, the VDIPEN epitope, including MMP-3 (stromolysin) and MMP-8, and MMP-13.[55]

Other changes in aggrecan, biglycan, decorin and fibromodulin have been identified in samples of human osteoarthritic and rheumatoid cartilage.[56] Most of the osteoarthritic samples contain the proteoglycan species characteristic of normal articular cartilage, but the capacity for aggregate formation is much lower, and evidence of degradation or changes in charge density was observed. A significant proportion of the keratan sulfate and chondroitin-6-sulfate epitopes of aggrecan was lost from all diseased cartilages, with an elevation of the chondroitin-4-sulfate or fetal-type epitopes. There was an increase in the content of intact decorin, biglycan and fibromodulin as well as fragments of these proteoglycans in diseased cartilage. These results imply an attempt at matrix repair by proteoglycan synthesis by chondrocytes within diseased cartilage.

Inherent changes in the metabolism of chondrocytes occur in osteoarthritis, but one consistent pathogenic feature of this arthropathy is the loss of matrix macromolecules from the cartilage, especially aggrecan.[57] This loss may be due to increased degradation via elevated MMP activity as discussed above, inhibition of biosynthesis, and/or an inhibition of proteoglycan retention due to deficits in hyaluronan. Studies in our laboratory have demonstrated that binding to CD44 is the primary means of retention and anchoring proteoglycan aggregates to the surface of chondrocytes.[20–22] In addition, CD44 also participates in the internalization and turnover of HA in articular chondrocytes as well as other cells.[58,59] Disruption of CD44 function may disturb cartilage homeostasis, leading to pathological changes and loss of extracellular matrix such as observed in degenerative diseases. Uncoupling hyaluronan-cell interactions with hyaluronan oligosaccharides initiated chondrocytic chondrolysis.[60] The down-regulation of CD44 expression in cartilage by the use of antisense oligonucleotides in explant cultures also resulted in a decrease in matrix proteoglycans.[61] Thus, in order to repair the extracellular matrix,

it will likely be necessary to increase not only the biosynthesis of aggrecan, but also, the molecules necessary for retention of the aggrecan within the cartilage.

BMP-7 (OP-1) stimulates type II collagen and aggrecan synthesis in bovine and human articular chondrocytes without inducing chondrocyte hypertrophy.[62] We have recently demonstrated that treatment of articular chondrocytes with OP-1 stimulates hyaluronan synthase-2 (HAS-2) and CD44 mRNA expression as well as aggrecan, resulting in an increase in functional pericellular matrix.[63,64] Hyaluronan binding studies indicate that the increase in CD44 mRNA is correlated with an increase in functional receptors present at the chondrocyte cell surface. Furthermore, OP-1 promotes proteoglycan and hyaluronan retention in cartilage explant cultures. Thus, OP-1 stimulates not only the synthesis of type II collagen and aggrecan but also hyaluronan and CD44, which play a pivotal role in pericellular matrix formation and cartilage matrix repair.

Mutations

The brachymorphic mouse has a sulfation deficiency and thus aggrecan is undersulfated, with a reduced negative charge and the cartilage tissue is reduced in volume as well.[65] Two autosomal recessive chondrodystrophies have been shown to be the result of mutations in the aggrecan gene. In nanomelia in chickens, the skeletal elements are severely shortened and the cartilage rudiments are highly cellular consisting of little extracellular matrix. A single G to T transversion generates a premature stop codon and a truncated aggrecan core protein that is not processed for secretion. The nanomelic aggrecan core protein is modified by N-linked oligosaccharides and accumulates in the ER of embryonic chondrocytes.[18] The cartilage matrix deficiency (*cmd*) locus in the mouse maps to the aggrecan gene, in which a truncated aggrecan core protein in the result. These mouse embryos also have shortened skeletal elements and no aggrecan in the highly cellular cartilage extracellular matrix. By electron microscopy, the collagen fibrils in *cmd* homozygotic embryos also show abnormal bundling and banding patterns, and an increase in diameter, suggesting a role of aggrecan in collagen fibril formation.[11] The *cmd* heterzygotic mice are viable, but exhibit axial and appendicular skeletal problems that develop with age. Both chondrodystrophies are embryonic lethal, exhibit reduced cartilage extracellular matrix and disorganization of the growth plates, reflecting the key function of aggrecan in these developmental processes. The correlation of a defect of the human aggrecan gene and a human genetic disease has not yet been reported.

In summary, cartilage displays a wide diversity of proteoglycans. The archetypal cartilage proteoglycan, aggrecan, is a hallmark of chondrogenesis and clearly participates in a direct, functional role supplying much of the osmotic resistance of the tissue. However, other proteoglycans are being found to be critical to the structure and function of cartilage. These proteoglycans may sequester growth factors, mediate critical events during prechondrogenic condensation, and organize the fine structure of the extracellular matrix thus regulating the expression of the chondrocyte phenotype.

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