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Critical Review

Decorin and its Galactosaminoglycan Chain: Extracellular Regulator of Cellular Function?

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Summary

A molecular network of extracellular matrix molecules determines the tissue architecture and accounts for mechanical properties like compressibility or stretch resistance. It is widely accepted that the elements of the cellular microenvironment are important regulators of the cellular behavior *in vitro* and *in vivo*. One large group comprising these molecules is the family of proteoglycans. Both, the core proteins and, in particular, the attached galactosaminoglycans, contribute to the regulation network as they bind a variety of signaling molecules, *e.g.* cytokines, chemokines, growth, and differentiation factors. We would like to emphasize specific patterns of epimerization and sulfation within the galactosaminoglycans chains, because these result in "motifs" that are responsible for the modulation of signal factor binding, release and activity. This property is crucial in physiological and pathological conditions, for example development and wound healing.

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Keywords extracellular matrix; decorin; galactosaminoglycan chain; sulfation patterns.

INTRODUCTION

Multicellular organisms are comprised of different tissues that consist of cells enclosed in a specialized extracellular matrix (ECM) of secreted proteins and carbohydrates. In some of these tissues, like the epithelium or muscle, the amount of ECM is low and the neighboring cells are in contact via cell-cell junctions. In contrast, in the connective tissues, like skin or cartilage, the interspace between the cells is wider and the sparsely distributed cells are surrounded by large amounts of different matrix molecules. On one hand this molecular network of ECM molecules determines the tissue histoarchitecture and accounts for mechanical properties like compressibility or stretch resistance. On the other hand, the cells interact with different components of the matrix via cell-matrix receptors. It is known that the cellular behavior in vitro and in vivo is influenced by elements of the microenvironment. Cell-matrix interactions participate in the control of metabolic activities, morphogenesis, proliferation, differentiation, cell fate specification, gain or loss of tissue-specific functions, cell migration, tissue repair, and cell death. Therefore, the composition of the direct cellular microenvironment is crucial to maintain or regulate cell function (*1–4*). In contrast, changes in the matrix composition or pericellular proteolysis inducing dysfunctions in cell-matrix interactions lead to certain diseases such as cancer and arthritis (*5–7*).

The extracellular matrix is built from a complex mixture of proteins and carbohydrates. These proteins belong to the families of collagens, proteoglycans, laminins, vitronectins, fibronectins, and elastins. Most carbohydrates of the ECM are found as long unbranched polysaccharide chains, called glycosaminoglycans (GAGs). With exception of hyaluronan, the GAGs are covalently linked to protein backbones (cores) of the proteoglycans. One can distinguish between chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate proteoglycans. Unlike other GAGs, hyaluronan is not sulfated, contains no core protein, and is synthesized in the plasma membrane rather than in the Golgi (*8*). The GAGs of keratan sulfate proteoglyans can either be *N*- or *O*-linked and are based on repeating disaccharide units: -4GlcNAc-β1,3-Gal-β1-. Sulfation occurs generally at the C-6 of the *N*-acetylglucosamine. However, sulfation is rarely found at the C-6 of the galactose (*9*). Heparan sulfates are glycosaminoglycans linked via a linkage-region to the core proteins and composed of *N*-acetylglucosamine (GlcNAc) and D-glucuronic acid (D-GlcA) or L-iduronic acid (L-IdoA). The heparan sulfate chain is further modified by sequential enzymatic modifications to replace the *N*-acetyl group of GlcNAc with a sulfate group resulting in clusters of modified and

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unmodified regions. Furthermore, the D-GlcA adjacent to $GlcNSO_3$ is epimerized to L-IdoA. The function of heparan sulfate is extensively studied (*10*); however, chondroitin/dermatan sulfate, on which we focus in this review, is receiving more attention. The proteoglycans can also be subclassified based on their core proteins into the lectican family which contain large (»200 kDa) core proteins and none or a low amount of L-IdoA in the sugar chains (*11, 12*) and the small leucine-rich repeat proteoglycan family (SLRPs), which will be described in detail in the following section.

So far, the original papers and reviews usually focus either on the GAG portion of proteoglycans (*13*) or on the protein core (*14, 15*); only few manuscripts cover the complete proteoglycan (*16*) and take into account that a proteoglycan core protein acts only in concurrence with its GAG chains. This fact is demonstrated by the phenotype of a patient with a progeroid Ehlers-Danlos syndrome described by Kresse et al. (*17*) who showed a defective decorin biosynthesis resulting in 30–70% decorin lacking a GAG chain. In addition, there are other patients with a mild form of Ehlers-Danlos syndrome in which the decorin biosynthesis is altered the same way (*18, 19*). These results demonstrate the importance of a single GAG chain on a proteoglycan. For this reason the focus in this review is on the interface between protein cores, chondroitin/dermatan sulfate chain, sulfation pattern, and their impact as a whole on cellular function.

SMALL LEUCINE-RICH REPEAT PROTEOGLYCANS

Proteoglycans of the SLRP family include keratocan, mimecan, decorin, biglycan, fibromodulin, epiphycan, osteoadherin, and lumican (*14*) and have reasonably small molecular sizes with core proteins of ~40 kD, containing 6–10 leucine-rich repeat units between flanking cystein-rich disulfide crosslinked domains. Despite their core protein homologies, the GAGs vary from keratan sulfate of fibromodulin and lumican or chondroitin/dermatan sulfate in decorin and biglycan. Decorin and biglycan comprise one subfamily because they share the highest homology on gene organization resulting in 57% identity on protein level (*15*). Here we will focus on decorin that is composed of ten leucine-rich repeats flanked by cysteine loops and contains N-linked di- or tri- antennary glycans. Furthermore, at the N-terminus decorin is covalently linked with a GAG chain of chondroitin/dermatan sulfate type depending on the tissue in which it is expressed.

The function of decorin can be defined by the role of the core protein and the role of the GAG chains. Overall the well studied functions of the core protein are quite diverse. At first, decorin was considered exclusively as a matrix proteoglycan. It binds to collagen fibrils at the "d" or "e" bands and "decorates" the fibrils (*20*). One consequence of this interaction is a delay in triple helix formation and a reduction in the final diameter of the collagen fibrils *in vitro* (*21*). However, *in vivo*, decorin was detected preferentially associated with a population of thicker fibrils in the interterritorial regions of articular cartilage, and was absent on the thinner fibrils in the territorial matrix around the chondrocytes, where collagen IX was detected in greater amounts (*22*). The role of decorin in fibrillogenesis was undermined by the phenotype of decorin deficient mice, which mainly show skin problems. In particular, the lack of decorin leads to abnormal fusion of collagen bundles and to increased skin fragility (*23*). The effect of irregular and heterogeneous diameter of collagen fibrils could be reproduced *in vitro* using decorin deficient fibroblasts. This phenotype could be rescued by exogenous decorin (*24*). Interestingly, the GAG chain of decorin had a reducing effect on collagen fibril diameter at early stages of fibrillogenesis (*25*).

Decorin not only regulates collagen fibril formation, but it also acts as a bridging molecule between type I and type VI collagen. Decorin interacts with both collagens via different binding sites (*26*). Although banded fibril-forming and filamentous beaded collagens form independent networks, they intermingle with each other *in vivo*, providing mechanical stabilization of tissues. Electron microscopy studies indicate that the banded fibril forming collagens are traversed specifically near their "d" bands, within the gap region of the collagen fibrils, by the filamentous beaded structures of the type VI collagen-containing network (*27, 28*). More recently, a complex formation between the globular domains of collagen type VI and a decorin/matrilin-1 complex has been described which can act as a bridge between type VI and type II collagen in cartilage, where decorin binds to the globular N-terminal domain of type VI collagen (*29*).

In addition to the structural functions, the decorin core protein was described to function as a signaling mediator. Decorin acts as a ligand for both the epidermal growth factor receptor (EGF-R) (*30*) and the insulin-like growth factor receptor (*31*). The fact that decorin is interacting with EGF-R and is blocking the signaling in cancer in *in vivo* models (*32*) makes this molecule useful as a possible therapeutic agent in cancer treatment. The different functions of the decorin core protein are extensively studied and described. However the galactosaminoglycan moiety of decorin has become a center of attention.

GALACTOSAMINOGLYCANS

In decorin the attached sugar chains are of chondrotin/dermatan sulfate type. They are also called galactosaminoglycans due to the disaccharide units *N*-acetylgalactosamine (D-GalNAc) and uronic acid. Structural characterization of chondroitin/dermatan sulfate is challenging because of the heterogeneity of these polymers. Galactosaminoglycans are highly sulfated oligosaccharides. Depending on the tissue type, the D-GlcA is converted into the C5 epimer L-IdoA. The chondroitin C5-epimerase (EC 5.1.3.19), was the last missing enzyme, involved in the biosynthesis of dermatan sulfate (*33*). Interestingly, the chondroitin C5-epimerase was first described as SART2, a protein of unknown function which is over expressed in cancer cells (*34*).

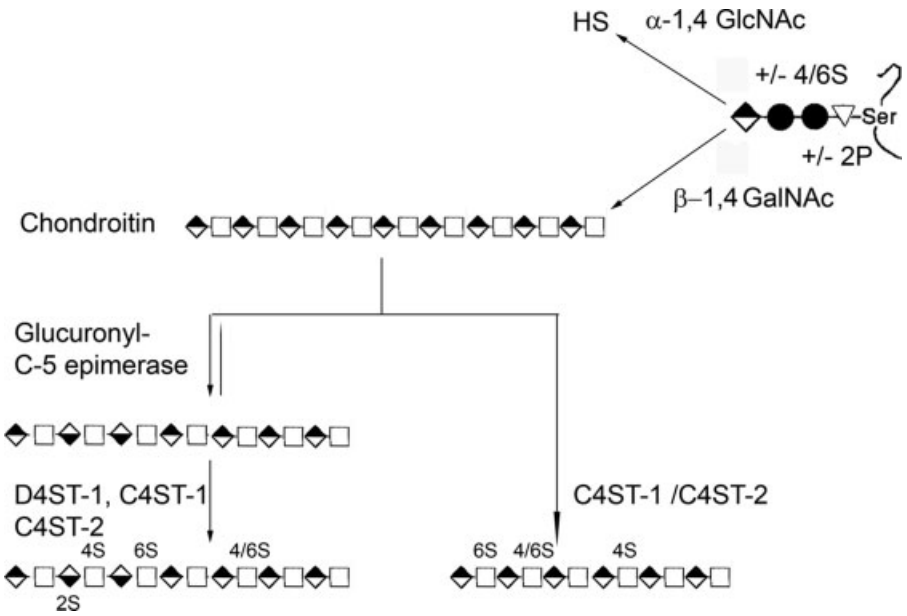

Figure 1. Scheme of chondroitin/dermatan sulfate (CS/DS) biosynthesis. The synthesis of galactosaminoglycans starts with the tetrasaccharide and the modifications determined by the transfer of GalNAc residue. The chondroitin synthase with β1,3-GlcA transferase and β1,4-GalNAc transferase activities polymerizes the chondroitin using the respective UDP-sugars (*37*). The glucuronyl C5-epimerase is able to epimerize GlcA into the C-5 stereoisomer IdoA. The conversion from GlcA to IdoA is a two-base mechanism and a freely reversible reaction with favored retention of the D-gluco-configuration. The sulfation event prevents back-epimerization (*33*). Symbols: ▽ Xyl; ●: Gal; ◆ GlcA; □: GalNAc; ◈ IdoA. Abbreviations: D4ST-1, dermatan 4-sulfotransferase-1; C4ST, chondroitin 4-sulfotransferase; heparan sulfate, HS; P, phosphate; S, sulfate.

To increase the complexity, the sugar moieties are further modified by sulfate esters. The D-GalNAc can be sulfated at C-4 and C-6 and the L-IdoA at C-2 (*11*). The dermatan sulfate specific *N*-acetylgalactosamine 4-*O* sulfotransferase (D4ST-1) transfers specifically activated sulfates to GalNAc residues adjacent to L-IdoA (*35*), whereas the chondroitin 4-*O* sulfotransferases transfers activated sulfates to D-GalNAc residues in chondroitin and dermatan sulfate chains adjacent to D-GlcA (*36*) (Fig. 1). In addition, Cheng et al. have reported O-6 sulfation in dermatan sulfate obtained from decorin and biglycan (*16*). Therefore, chondrotin/dermatan sulfate on the same core protein from different cells can show consistent structural differences such as variations in size, epimerization, and *O*-sulfation. Among the GAGs, dermatan sulfate and heparan sulfate show considerable conformational flexibility due to the presence of L-IdoA residues, which change easily between chair and skew boat conformation resulting in similar functions of heparan and dermatan sulfate (*38*).

In general, dermatan and chondroitin sulfate are widely distributed in the ECM, in the pericellular matrix as well as at the cell surface where they have several different structural functions. The dermatan sulfate GAGs of decorin are involved in the organization of the fibrils (*20*). In patients with a variant of Ehlers-Danlos Syndrome, about half of the secreted decorin (*17, 19*) and biglycan lack the GAG chain (*19*). These patients show a ß4 galactosyltransferase I (ß4GalT-7; E.C. 2.4.1.133) deficiency resulting in a reduced amount of L-IdoA in decorin and biglycan which are linked with a GAG chain (*19*). Notably, some of these patients have a skin fragility phenotype that resembles that of the decorin null mice.

GALACTOSAMINOGLYCANS AND CYTOKINES

It has been demonstrated that GAGs are required for the activity of growth factors, cytokines, and chemokines *in vivo*. In this section we will focus on the interaction of oversulfated IdoA-rich galactosaminoglycans. Over the years it was established that decorin and biglycan can interact with several cytokines such as transforming growth factor-ß (TGF-ß), tumor necrosis factor-β (TNF-β), and Wnt-1-induced secreted protein 1 (WISP-1) both via the protein cores and the dermatan sulfate chains (*39, 40, 41*), therefore, playing an important role in physiological and pathological conditions.

An additional group of cytokines binding to GAG chains includes the fibroblast growth factor (FGF) family. There are more than 20 known members of this family, most of which are predicted to have a conserved heparin-binding site (*42*). The binding of FGF-1 and FGF-2 to FGF receptors is well studied for heparin and heparan sulfates (*43, 44*). In addition, it has also been demonstrated that in wound healing dermatan sulfate can also function as a coreceptor for FGF-2 and induce cell signaling (*45*). Oversulfated octa- or longer saccharides of the

dermatan sulfate chain derived from decorin can bind to FGF-2 (*46*). The function of FGF-2 is closely associated with the binding to specific sulfation patterns within the oligosaccharides inducing modulation of growth factor receptor activity. It is possible to determine sulfated oligosaccharides up to eicosasaccharides without losing the liable sulfates (*47*) which enable the investigation of more complex signaling factor binding motifs. Another example is the FGF-7-induced proliferation in cells in presence of dermatan sulfate that plays a role in wound healing (*48*).

Additional to the role in wound healing, galactosaminoglycans, in general, play an important role on cellular levels for example in axon regeneration (*49*) and neural stem cell proliferation (*50*). The involvement of galactosaminoglycans in brain development received a particular interest. Different oversulfated dermatan sulfates from ascidians containing specific disaccharide units: like IdoA(2-O-sulfate)β1-3GalNAc(6-O-sulfate) or IdoAβ1-3GalNAc(4,6-O-sulfate) promote human neurite outgrowth *in vitro* (*51*). In particular, this neurite outgrowth was also seen with oligosaccharides from embryonic brain composed of GlcA(2-O-sulfate)β1-3GalNAc(6-O-sulfate) and IdoA(2-O-sulfate)β1-3GalNAc(6-O-sulfate) by interacting with pleiotropin (*52*). Only oversulfated disaccharides containing L-IdoA residues in the growth factor-binding and neuritogenic activities of these chains were examined but pattering of these disaccharide units into binding motifs was not investigated (*53*).

Aging or differentiation is another part where decorin and biglycan and their GAG chains play a role. It has been demonstrated that in bone cells epimerization of the GAG chains of these two SLRPs is altered. The epimerization of D-GlcA to L-IdoA is increasing with age. The comparison of epimerization pattern in aging and osteogenesis imperfecta revealed that osteoblast derived from osteogenesis imperfecta patients resemble a fetal-like phenotype rather than aging (*54*). A detailed analysis of sulfation pattern in cartilage development and repair by Hayes et al. shows distinct sulfation motifs in chondroitin sulfate galactosaminoglycans to be associated with cartilage progenitor cells. These specific GAGs were linked to aggrecan (*55*).

Therefore, the knowledge of sulfation pattern within a GAG chain on specific proteoglycans-like decorin or aggrecan is important to understand and elucidate physiological and pathological conditions and should be considered for future studies in order to improve therapeutic approaches. Nevertheless, both the core protein along with its GAG chains are responsible for the function of a given proteoglycan.

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