A GUIDE TO...

A biological guide to glycosaminoglycans: current perspectives and pending questions

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Mammalian glycosaminoglycans (GAGs), except hyaluronan (HA), are sulfated polysaccharides that are covalently attached to core proteins to form proteoglycans (PGs). This article summarizes key biological findings for

Abbreviations
4-MU, 4-methylumbelliferone; ADAM, a disintegrin and metalloprotease; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AKT, RAC-alpha, beta, and gamma serine/threonine protein kinases; AMPK, AMP activated protein kinase; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; B3GALT6, β1,3-galactosyltransferase 6; B3GAT3, β1,3 glucuronosyltransferase 3; B4GALT7, β1,4-galactosyltransferase 7; C6ST, CS sulfotransferase; CAT, catalytic domain; CD, cluster of differentiation; CS, chondroitin sulfate; CSGALNACT, chondroitin sulfate *N*-acetylgalactosaminyltransferase; CSPG, chondroitin sulfate proteoglycan; D4ST, dermatan 4-sulfotransferase; D4ST1, dermatan 4-*O*-sulfotransferase 1; DS, dermatan sulfate; DSE, dermatan sulfate epimerase; DSPG, dermatan sulfate proteoglycan; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2; ERM, ezrin, radixin, and moesin; EXT, exostosin; EXTL, exostosin-like; FAK, focal adhesion kinase; FGly, *N*-formylglycine; GAG, glycosaminoglycan; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GalNAcT-II, *N*-acetylgalactosaminyltransferase II; GlcA, glucuronic acid; GlcAT-II, glucuronyltransferase II; GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; GlcNAc6ST, *N*-acetylglucosamine-6-*O*-sulfotransferase; GlcNS, *N*-sulfated glucosamine; HA, hyaluronan; HARE, hyaluronan receptor for endocytosis; HAS, hyaluronan synthase; HD, hydrophilic domain; HIF, hypoxia-inducible factor; HMGA2, high mobility group AT-hook protein 2; HMW, high molecular weight; HP, heparin; Hpa, heparanase; HS, heparan sulfate; HS2ST1, heparan sulfate 2-*O*-sulfotransferase; HS6ST, heparan sulfate 6-*O*-sulfotransferase; HSPG, heparan sulfate proteoglycan; HYAL, hyaluronidase; iASPP, inhibitor of ASPP protein; IdoA, iduronic acid; IL, interleukin; KO, knockout; KS, keratan sulfate; KSGalST, KS galactosyl sulfotransferase; KSPG, keratan sulfate proteoglycan; LMW, low molecular weight; LYVE, lymphatic vessel endothelial receptor; MAP-kinases, mitogen-activated protein kinases; Met, mesenchymal–epithelial transition factor; miR, microRNA; MMP, matrix metalloproteinase; MPS, mucopolysaccharidosis; MyD88, myeloid differentiation primary response 88; NDST, *N*-heparan sulfate sulfotransferase; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing 3; NOX, NADPH oxidase; p38, p38 mitogen-activated protein kinase; p42/44, p42/p44 mitogen-activated protein kinases; PD1, programmed cell death protein 1; PDCD4, programmed cell death protein 4; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PDL1, programmed cell death ligand 1; PG, proteoglycan; PI3K, phosphoinositide 3-kinase; RHAMM, receptor for hyaluronan-mediated motility; RHO-ROCK, rho-associated protein kinase; sHA, sulfated HA; SLRP, small leucine-rich proteoglycan; Src, src-family kinase; TGFβ, transforming growth factor β; TLR, toll-like receptor; TRIF, TIR domain-containing adaptor-inducing interferon β; TβR, transforming growth factor β receptor; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; Xyl, xylose; XylT, xylosyltransferase; β3GnT, β-1,3-*N*-acetylglucosaminyltransferase; β4GalT, β1,4-galactosyl transferase.

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the most widespread GAGs, namely HA, chondroitin sulfate/dermatan sulfate (CS/DS), keratan sulfate (KS), and heparan sulfate (HS). It focuses on the major processes that remain to be deciphered to get a comprehensive view of the mechanisms mediating GAG biological functions. They include the regulation of GAG biosynthesis and postsynthetic modifications in heparin (HP) and HS, the composition, heterogeneity, and function of the tetrasaccharide linkage region and its role in disease, the functional characterization of the new PGs recently identified by glycoproteomics, the selectivity of interactions mediated by GAG chains, the display of GAG chains and PGs at the cell surface and their impact on the availability and activity of soluble ligands, and on their move through the glycocalyx layer to reach their receptors, the human GAG profile in health and disease, the roles of GAGs and particular PGs (syndecans, decorin, and biglycan) involved in cancer, inflammation, and fibrosis, the possible use of GAGs and PGs as disease biomarkers, and the design of inhibitors targeting GAG biosynthetic enzymes and GAG–protein interactions to develop novel therapeutic approaches.

Introduction

Mammalian glycosaminoglycans (GAGs), except hyaluronan (HA), are sulfated polysaccharides covalently attached to core proteins to form proteoglycans (PGs). This article aims to provide an overview of the GAG biosynthetic pathways and of GAG biological significance in health and disease, with a focus on cancer, inflammation, and fibrosis, and to highlight the perspectives and pending questions in addition to the recently identified challenges [1]. This article complements the recently published roadmap article on GAG chemistry, analyses, structures, interactions, biophysics, and glycobioinformatics tools [2]. The chemical structures of GAGs are shown in Fig. 1.

The data on GAGs presented here mostly focus on HA, which is not sulfated, heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS), because they are found in numerous tissues and/or at the cell surface and are involved in extracellular matrix (ECM) organization, cell signaling, synaptic signaling, and development [3–7]. The challenges to be addressed include the fine details of the regulation of GAG biosynthesis and degradation, which are altered in diseases such as cancer, fibrosis, inflammation, and mucopolysaccharidoses, the design of inhibitors targeting GAG biosynthetic enzymes for therapeutic purposes, the molecular basis of the specificity of GAG–protein interactions, which is required to develop inhibitors of these interactions. The characterization of the human GAG profile (GAGome) in diseased tissues or cells is needed to get new insights into the GAG-mediated biological processes impacted by diseases. The heterogeneity of the tetrasaccharide linkage region and its role in determining the synthesis of HS or CS/DS chains and in linkeropathies are discussed. The interplay between GAGs and the PG core proteins being crucial to fully understand their functions and PG functions, pending questions include the organization of GAGs attached to PG core proteins at the cell surface and the mechanisms used by the ligands (e.g., growth factors) of cell surface receptors to move through the pericellular matrix to reach their receptors. These points are critical to decipher GAG-mediated signaling pathways. The roles of PGs bearing HS and/or CS chains (e.g., syndecans, biglycan, and decorin) and their possible use as disease biomarkers are also discussed here.

GAG biosynthetic pathways

HA biosynthesis

Hyaluronan does not possess sulfate groups and consists of the disaccharide unit [-β(1,4)-glucuronic acid (GlcA)-β(1,3)-*N*-acetylglucosamine (GlcNAc)-β1-]. It is synthesized at the plasma membrane as a free polysaccharide and is not covalently attached to any PG core protein. HA is primarily located in the ECM but is also found intracellularly in the endoplasmic reticulum (ER) and the nucleus under certain developmental and pathological conditions. The main sources of

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Fig. 1. The chemical structures of mammalian glycosaminoglycans. Hyaluronan (HA): -4)-β-D-GlcpA-(1-3)-β-D-GlcpNAc-(1-, Heparin/Heparan sulfate (HP/HS): -4)-α-D-GlcpNS(6S)-(1-4) α-L-IdopA(2S)-(1-, Chondroitin 4/6 Sulfate (CS): -4)-β-D-GlcpA-(1-3)-β-D-GalpNAc(4S/6S)-(1-, Dermatan Sulfate (DS): -4)-α-L-IdopA-(1-3)-β-D-GalpNAc(4S)-(1-, Keratan Sulfate (KS): -4)-β-D-GlcpNAc(6S)-(1-3) β-D-Galp -(1-.

intracellular HA are the endocytosis of extracellular HA by its cellular receptors and noncanonical synthesis within cells. Intracellular HA plays critical regulatory roles during mitosis, cell cycle progression, RNA processing, autophagy, and inflammatory processes [8].

Hyaluronan size is usually larger than the sulfated GAGs. Its molecular weight (Mw) ranges from 500 kDa to a few million Da, up to 10 million Da in the naked mole rat [9]. HA can be cleaved into bioactive fragments by hyaluronidases (HYALs), and free radicals such as oxygen and nitrogen reactive species [10]. HA size is critical for its viscoelastic and hydration properties and for its effects on cell migration and proliferation, the inflammatory response [11], and cell signaling triggered by HA receptors such as CD44 and RHAMM (receptor for HA-mediated motility). Outside the cells, HA can bind noncovalently to proteins called hyaladherins to spatially organize

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matrix proteins and PGs. HA can form covalent bonds with heavy chains from the glycoprotein inter-α-inhibitor (IαI), mediated by tumor necrosis factor-stimulated gene 6, which can use the CS chain from bikunin and link them to HA, producing large aggregates especially in tissues undergoing inflammatory remodeling [12]. HA can also be synthesized within cellular structures during autophagy. Although these mechanisms are still not completely understood, they shed light on a possible role of HA in cellular nutrition processes in the context of cellular stress [13,14].

There are three HA synthases (HAS1, HAS2, and HAS3) in humans, which raises the question of why three enzymes are needed to synthesize the simplest GAG. The classification of HASs has been recently updated [15]. Class I HASs are membrane-integrated enzymes that synthesize HA via a processive elongation mechanism and secrete it across the cell membrane. They comprise class I-R and class I-NR enzymes that catalyze HA elongation at the reducing or the nonreducing end, respectively. Class II HASs are membrane-associated peripheral enzymes with a nonprocessive, nonreducing end elongation mechanism and a separate secretion system for HA export [15]. HAS1 and HAS2 synthesize large HA polymers (about 2×10^6 Da) [9], whereas HAS3 produces lower Mw HA ranging from 1×10^5 to 1×10^6 Da. The mammalian HAS enzymes are differently expressed during development. HAS1 and HAS2 are the most abundant at the early stage, where HA is abundant and large, which enables it to promote cell migration and proliferation, whereas HAS3 is expressed in later stages and in pathological tissues such as tumors. HAS3 is associated with processes that modulate immune response [16,17], and with age-related ovary stiffness and fibrosis [18]. HAS2, the key HA-synthesizing enzyme, is involved in longevity [9], carcinogenesis [19,20], cardiovascular diseases including atherosclerosis and restenosis [21], and fibrosis [22]. The amount of HAS2 on the cell surface is regulated at several levels. In humans several transcription factors sustain a basal HAS2 mRNA transcription. In the nucleus, epigenetics controls *Has2* gene transcription via the long noncoding RNA (lncRNA) HAS2 antisense RNA 1, HAS2-AS1. HAS2-AS1 modulates the chromatin structure around the HAS2 promoter, controlling the accessibility of transcription factors in case of nutrient abundance, deprivation, or NF-κB activation [23]. Sirtuin 1 activation prevents nuclear translocation of NF-κB, which decreases the levels of HAS2-AS1 [21]. Moreover, HAS2-AS1 interacts with, and stabilizes, the HAS2 transcript, favoring HAS2 translation in some cancers [23]. HAS2 mRNA stability is also influenced in various tumor cells by several miRNAs that can interact with 3′ UTR influencing cell behavior [24,25]. HAS2 protein trafficking in the secretory pathway is regulated by post-translational modifications such as ubiquitination, O-GlcNAcylation, and phosphorylation [26,27], which can alter its enzymatic activity and stability (for a detailed review see [14]).

Hyaluronan synthesis is also regulated by the availability of UDP-GlcA, subjected to the activity of transporters (e.g., the nucleotide sugar transporter SLC35D1) [28,29], and of UDP-GlcNAc, which is modulated by glutamine fructose-6-phosphate amidotransferase, an enzyme of the hexosamine pathway [30]. This pathway integrates metabolites issued from amino acid, nucleotide, carbohydrate, and fatty acid metabolism, and the cytosolic concentration of UDP-GlcNAc reflects the cell metabolic status. When UDP-GlcNAc increases, *O*-GlcNAc transferase catalyzes the addition of a single GlcNAc sugar to the hydroxyl group of serine and threonine residues, which triggers several cellular responses. The regulation of HA synthesis is thus a multistep process, mainly mediated via HAS2 and epigenetic control (Fig. 2). HAS2 degradation depends on proteasome activity, lysosome, and autophagy [26,33].

Biosynthesis of CS/DS

The disaccharide unit of CS is made of uronic acid and *N*-acetyl hexosamine [(-4GlcAβ1-3GalNAcβ1-)n], whereas DS contains IdoA. CS and DS domains usually coexist in one chain. The CS/DS chains typically consist of 40–100 disaccharide units, and they are found in at least 32 PGs [34]. CSPGs and DSPGs regulate numerous physiological processes depending on their spatiotemporal distribution and the sulfation pattern of their GAG chains (e.g., in the mature central nervous system [35]).

The polymerization of CS and DS chains starts after the synthesis of a tetrasaccharide linker attached to a serine residue (GlcAβ1,3Galβ1,3Galβ1,4-Xylβ-*O*-serine). The importance of the linker region is discussed in the corresponding section. The amino acid sequence close to the serine residues bearing the GAG chains may affect the level of sulfation of the nascent GAG chain [36]. The next steps of CS/DS biosynthesis are catalyzed by glycosyltransferases, epimerases, and sulfotransferases (Fig. 3). Chondroitin synthases have dual glucuronyltransferase II (GlcAT-II) and *N*-acetylgalactosaminyltransferase II (GalNAcT-II) activities, but they cannot independently polymerize CS chains. The combination of two of them is required to achieve CS polymerization [39]. CS-synthesizing

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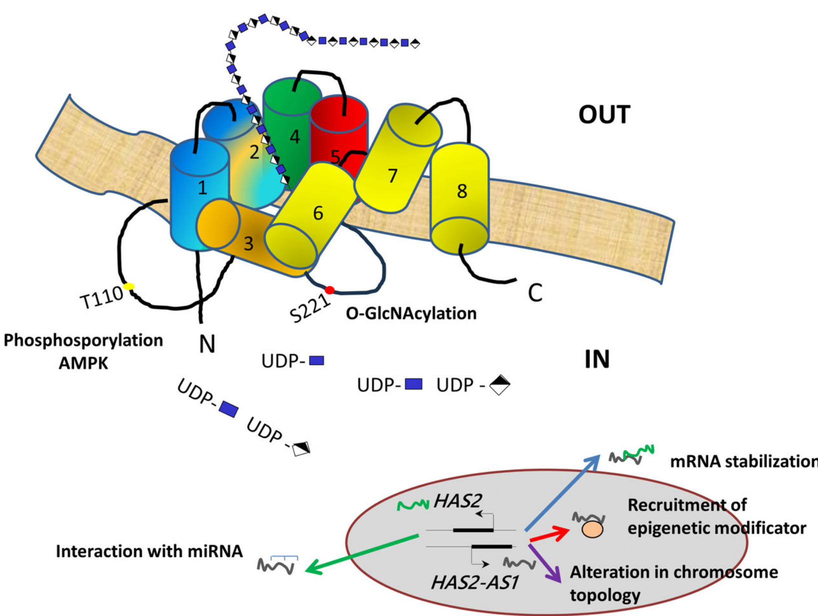

Fig. 2. Metabolic conditions regulating hyaluronan (HA) synthesis, a multistep process, mainly mediated via HA synthase 2 (HAS2), which undergoes transcriptional and post-transcriptional modifications, as well as via epigenetic control. HAS2 comprised five transmembrane helices and three amphipatic interface helices [31]. HAS2 can be phosphorylated on threonine 110 (T110) and O-GlcNAcylated on serine 221 (S221). UDP-glucose-6-dehydrogenase converts UDP-glucose to UDP-GlcA, releasing two molecules of NADH. The NAD/NADH ratio regulates sirtuins (NAD-dependent deacetylases) that in turn regulate HA synthesis [21]. HAS2-AS1 modulates the chromatin structure around the HAS2 promoter, controlling the accessibility of transcription factors in case of nutrient abundance, deprivation, or NF-κB activation [23] (AMPK, adenosine monophosphate-activated protein kinase; HAS2-AS1, long noncoding RNA [lncRNA] HAS2 antisense RNA 1; UDP, uridine diphosphate). The symbols used to represent GAG monosaccharides are those from the Symbol Nomenclature for Glycans (SNFG) [32].

enzymes utilize UDP-GlcA and UDP-GalNAc, originating from the cytosol [38], and UDP-glucose dehydrogenase regulates CS/DS biosynthesis [40]. The transport of UDP sugars from the cytosol to the Golgi and the ER is a critical step in GAG synthesis [41]. Some GlcA residues of CS are epimerized into IdoA by chondroitin GlcA-C5-epimerase/DS epimerases (DSE1 and 2), leading to the synthesis of the DS disaccharide [-4IdoUAα1-3GalNAcβ1-]n (Fig. 3).

The position of sulfate groups determines the synthesis of monosulfated (CS-A and CS-C) and disulfated (CS-B, CS-D, CS-E, and CS-K) CS subtypes. Dermatan 4-sulfotransferase (D4ST) catalyzes the sulfation of C4 in GalNAc residues leading to the formation of IdoA-GalNAc4S-rich clusters in DS chains. DSE-1, in contrast with DSE-2, can form a complex with D4ST, which is required to synthesize longer IdoA-containing chains [37]. The importance of the position of sulfate groups and the epimerization of GlcA residues is supported by the findings that the structure, conformation, and dynamics of CS4, CS6, and DS analyzed by molecular dynamics differ [42]. The 4-sulfation of GlcA residue by dermatan 4-*O*-sulfotransferase 1 (D4ST1) is required to stabilize epimerization [37,43]. The CS/DS ratio is critical for the development of many tissues [44].

***What remains to be solved**: The regulation and cooperation of the enzymes catalyzing CS and DS polymerization and sulfation should be studied more in depth to unravel their complex interplay and the influence of the metabolic status on the CS/DS precursor synthesis and transport. The molecular mechanisms leading to the synthesis of CS/DS rather than to the synthesis of HP/HS (e.g., the modifications of the tetrasaccharide linker and the amino acid sequences close to GAG attachment sites) warrant further investigation as detailed in the section describing the linkage region of GAGs to proteins.*

Biosynthesis of KS

KS is a complex GAG found in cornea, cartilage, and the nervous system where it plays a electrosensory and neuro-instructive role [45]. KS comprised three types (KS-I, KS-II, and KS-III), with different attachment sites to the core proteins of KSPGs [e.g., aggrecan, phosphacan, and small leucine-rich PGs (SLRPs), such as keratocan, fibromodulin, and lumican]. KS-I is N

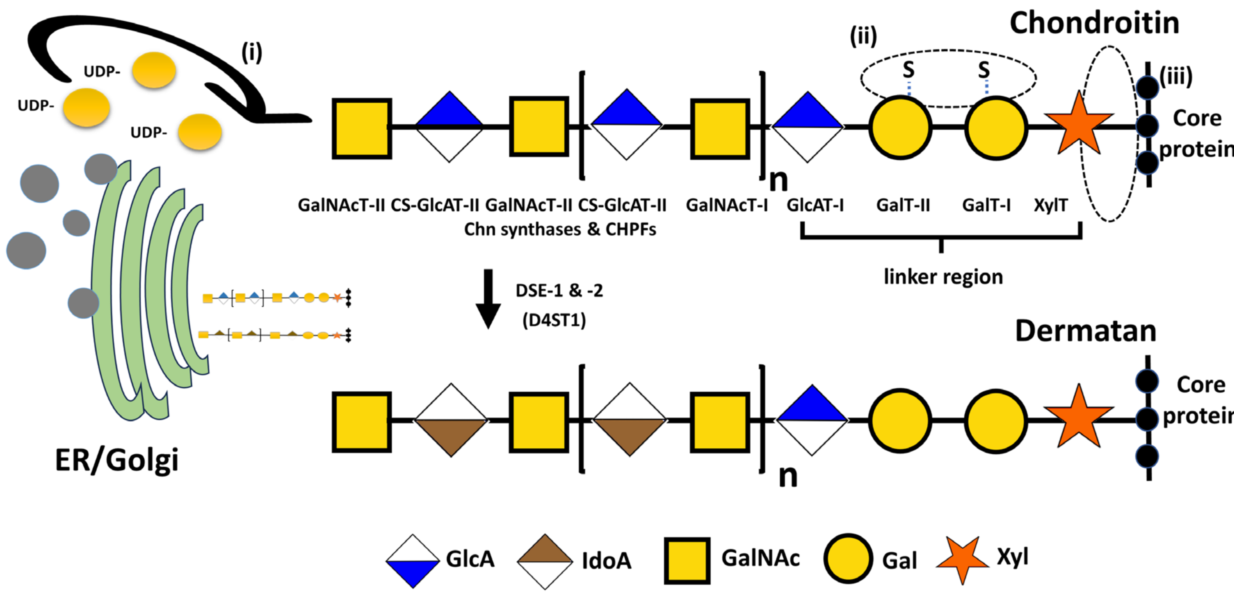

Fig. 3. Polymerization of the CS backbone in the endoplasmic reticulum (ER)/Golgi complex. The transfer of GalNAc to the nonreducing terminal GlcA residue of the tetrasaccharide linker is catalyzed by β1,4-*N*-acetylgalactosaminyltransferase-I (GalNAcT-I) encoded by the chondroitin sulfate *N*-acetylgalactosaminyltransferase CSGALNACT1 and CSGALNACT2 genes [37]. The addition of this residue triggers chondroitin sulfate CS polymerization [38]. The disaccharide backbone is synthesized by the alternate addition of GlcA and GalNAc residues performed by CS-β1,3glucuronyltransferase-II (CS-GlcAT-II) and β1,4*N*-acetylgalactosaminyltransferase-II (GalNAcT-II) respectively. Chondroitin synthase-1 and -2 and the chondroitin polymerizing factors I and II (CHPF-I and -II) have also been involved in CS/DS synthesis. The epimerization of GlcA residue into IdoA are catalyzed by dermatan sulfate epimerases (DSE1 and 2). CS/DS biosynthesis can be affected by (i) the availability of the UDP–sugar precursors, (ii) the modifications of the tetrasaccharide linker (obligatory sulfation for chondroitin synthesis), and (iii) the amino acid residue sequence in the vicinity of the GAG attachment site on the protein core. The analysis of the human urinary CS glycoproteome has shown that the CS sulfation level correlates with the acidity of the amino acid attachment motif [36]. The symbols used to represent GAG monosaccharides are those from the Symbol Nomenclature for Glycans (SNFG) [32].

linked to asparagine residues via a high mannose oligosaccharide, KS-II is O linked to threonine or serine residues, and KS-III is O linked to serine residues via a 2-*O*-mannose residue in PGs of the peripheral and central nervous system [45,46]. KS-II is subdivided into KS-IIA, which contains α(1-3)-linked fucose attached to sulfated GlcNAc residues and α(2-6)-linked *N*-acetylneuraminic acid, and KS-IIB, which lacks them [45]. The disaccharide unit of KS chains is composed of GlcNAc and Gal instead of a uronic acid. The biosynthesis of KS chains involves β-1,3-*N*-acetylglucosaminyltransferase (β3GnT), *N*-acetyl glucosaminyl-6-sulfotransferase (GlcNAc6ST), and β1,4-galactosyl transferase (β4GalT) (reviewed in [46]). The sulfation of KS chains is catalyzed by the KS gal actosyl sulfotransferase (KSGalST) and the GlcNAc-6-sulfotransferase (GlcNAc6ST) that transfer sulfate groups to C6 of Gal and GlcNAc, respectively. CS sul fotransferase (C6ST) also contributes to the sulfation of Gal in cartilage and corneal KS.

Keratan sulfate sulfation levels vary depending on tissues in brain containing highly sulfated KS chains [46]. Highly sulfated KS chains are also present in the developing notochord and otic vesicles of *Xenopus* embryos, and their biosynthesis requires the carbohydrate sulfotransferases Chst1, Chst3, and Chst5 [47]. The length of KS chains also depends on the tissue and the KS type. KS-I chains are usually longer than cartilage KS-II [45]. KS biosynthesis is altered in diseases such as macular dystrophy and amyotrophic lateral sclerosis due to mutations in the CHST6 gene encoding corneal-GlcNAc6ST, which result in altered KS sulfation [46]. CHST6 expression is upregulated in pancreatic metastatic tissue compared to primary tumors, which is consistent with the association of increased KS sulfation with tumorigenesis [48].

Biosynthesis of HS

Heparan sulfate disaccharide unit comprises a GlcNAc and a GlcA residue. However, in specific regions of the polysaccharide termed S domains, this disaccharide motif can undergo N-deacetylation/N-sulfation of GlcNAc into GlcNS (or more rarely into

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N-unsubstituted glucosamine), epimerization of GlcA into IdoA, and O sulfations on C2 of the uronic acid, C6 (and more rarely at C3) of glucosamine. HS elicits its numerous activities through the binding and modulation of a wide array of proteins mostly mediated by its S domains. HS biosynthesis primarily takes place in the Golgi apparatus and can be divided into three main phases: the synthesis of a tetrasaccharide linker, common to all GAGs covalently linked to PG core proteins and described in the sections on CS and DS biosynthesis and on the linkage region, the elongation of a nonmodified polysaccharide chain of proheparan; and the modifications of the polysaccharide chain leading to mature sulfated HS chains (Fig. 4, [50,51]). At least 26 enzymes participate in HS biosynthesis [50,51].

The elongation of the HS chain from the tetrasaccharide linker starts with the addition of a glucosamine. Concomitantly to chain elongation, the polysaccharide chain undergoes extensive modifications (Fig. 4). GlcNAc residues are deacetylated and N-sulfated by *N*-deacetylase/*N*-sulfotransferases (NDST1-4). This step is critical because it promotes subsequent modification reactions, and therefore dictates the size and distribution of S domain within the HS chain. The next steps involve the reversible C5 epimerization of GlcA residues next to GlcNS residues into IdoA. The addition of a 2-*O*-sulfate group that preferentially occurs on IdoA is irreversible and favors epimerization reaction equilibrium toward the generation of IdoA. The sulfation of HS chains at C6 of GlcNAc or GlcNS and C3 of GlcN is catalyzed by HS 6-*O*- and 3-*O*-sulfotransferases (Fig. 4). Although the occurrence of HS 3-O-sulfation is very low, it is catalyzed by seven HS3ST isoforms with distinct spatio-temporal expression and substrate specificities. This diversity likely indicates a functional relevance and the need for tight regulation of HS 3-O-sulfation in many biological processes.

Interactions between HS biosynthesis enzymes have been reported (see [51] for review), and a model in which the enzymes of HS biosynthesis form a complex, a GAGosome, has been proposed 15 years ago [52]. The structures of the EXT1/EXT2 heterodimer [53,54] and of the NDST1 homodimer [55] have been recently determined by cryoelectron electron microscopy, providing new insights into the catalytic mechanisms and substrate specificities of these enzymes. A physical interaction has been demonstrated so far between D-glucuronyl C5-epimerase and 2-*O*-sulfotransferase (HS2ST1), the formation of this complex influencing enzyme activities [56], and between D-glucuronyl C5 epimerase and HS 6-*O*-sulfotransferase 1 (HS6ST1). Additional interactions have been predicted by computational studies [57].

What remains to be solved? *Although HS biosynthesis enzymes have been identified and studied for more than 30 years, there are many pending issues. Most functional studies have been performed* in vitro *with isolated enzymes in solution and a limited number of substrates, but discrepancies between* in vitro *and* in vivo *data have underlined the importance of the physiological context on the activity of these enzymes. The generation of cell-based libraries engineered to knock-in/knockout genes encoding HS biosynthesis enzymes combined with HS structural analysis open new avenues for unraveling the complex regulation of HS biosynthesis* [58,59]. *The epigenetic and transcriptional mechanisms regulating the expression of HS biosynthesis enzymes and of other proteins (e.g., precursor synthases and transporters) contribute to this process. The role of the Golgi organization, the effects of Golgi pH gradients, the vesicle retrograde trafficking between ER and Golgi cisternae, and the concentration/bioavailability of precursors such as UDP sugars and 3′ phosphoadenosine-5′ phosphosulfate remain to be determined. Although most HS biosynthesis enzymes are type II transmembrane proteins, the role of their transmembrane and cytoplasmic domains and the consequences of their anchoring into the Golgi membrane have been poorly studied. The relevance and the topology of the GAGosome model remain to be established. Some complexes may be too transient to be experimentally identified, and/or may involve additional scaffold molecules such as substrate polysaccharides or linker proteins. Further investigations on the interaction network of HS biosynthesis enzymes are therefore needed. They will benefit from the development of highly sensitive binding assays, curated interaction data provided by databases and molecular dynamics simulations/artificial intelligence technologies for modeling these complexes.*

Postsynthetic mechanisms regulating HS structure and function

Heparan sulfate broad functions arise from its ability to bind, and to modulate the activity of, a vast array of proteins, including growth factors, cytokines, morphogens, matrix structural proteins, enzymes or enzyme inhibitors, and pathogen surface proteins [60,61]. HS-protein interactions involve highly sulfated motifs. Their size, disaccharide composition, and sulfation pattern determine the binding affinity and selectivity. HS biological properties are therefore governed by structure/function relationships, and HS structure is, in turn, tightly and dynamically controlled in the cells by the highly regulated HS biosynthesis machinery. Another level of regulation occurs at the cell surface

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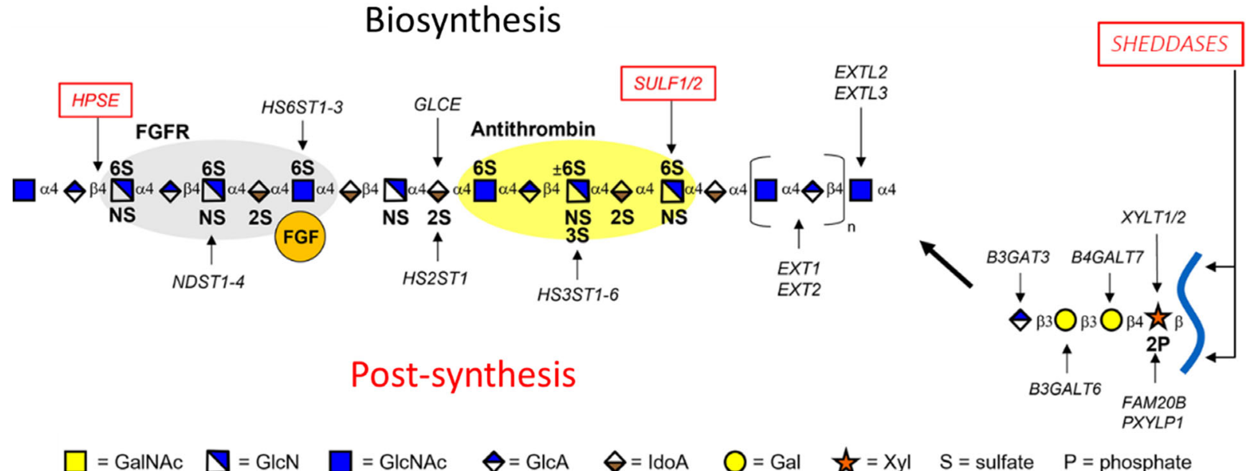

Fig. 4. Biosynthesis and postsynthesis modifications (in red) of heparin/heparan sulfate chains. Gray- and yellow-shaded areas show specific binding motifs for fibroblast growth factor (FGF) and antithrombin, respectively. The transfer of a xylose residue on a serine residue of the core protein is catalyzed by two *O*-xylosyltransferases (XylT-1 or XylT-2). Subsequent addition of two galactose (Gal) and a GlcA residue is achieved by two galactosyltransferases, β1,4-galactosyltransferase 7 (B4GALT7) and β1,3-galactosyltransferase 6 (B3GALT6), and a β1,3 glucuronosyltransferase 3 (B3GAT3). HS elongation starts with the addition of a GlcNAc residue to the linker catalyzed by exostosin-like 3 (EXTL3). Another exostosin family member, EXTL-2, acts as a negative regulator of heparan sulfate (HS) biosynthesis through a mechanism that remains to be clarified [49]. Following the addition of this first GlcNAc, the polymerization of the proheparan chain occurs through the alternative addition of GlcA and GLcNAc residues by exostosins EXT1 and EXT2. Concomitantly, the polysaccharide chain undergoes extensive modifications. GlcNAc residues are deacetylated and N-sulfated by *N*-deacetylase/*N*-sulfotransferases (NDST1-4). The C5 epimerization of GlcA into IdoA, and the 2-O-sulfation of IdoA are catalyzed by D-glucuronyl C5 epimerase (GLCE) and heparan sulfate 2-*O*-sulfotransferase (HS2ST1), respectively. The heparan sulfate 6-*O*-sulfotransferases (HS6ST1-3) and 3-*O*-sulfotransferases (HS3ST1-7) catalyze the transfer of a sulfate group at C6 of GlcNAc or GlcNS, and 3-O-sulfation of glucosamine (HPSE, heparinase).

and in the ECM through postsynthesis modifying enzymes catalyzing the cleavage of HS chains by the endoglycosidase heparanase, the 6-O-desulfation of HS by the Sulf endosulfatases, and the release of the soluble ectodomains of HSPGs by sheddases (Fig. 4).

The role of heparanase

Heparanase-1 (Hpa-1) is a multifaceted enzyme involved in a plethora of biological processes, primarily through, but not only, its ability to degrade cell surface and ECM HS. Hpa-1 discovery, structure, and functions have been extensively reviewed elsewhere [62,63]. Hpa-1 is the only HS-degrading endoglycosidase identified in mammals. While its physiological expression is low and restricted, it is strongly upregulated in various physiological conditions and diseases such as embryonic development, wound healing, inflammation, and cancer [64]. Activated Hpa-1 exhibits an endo-β-D-glucuronidase activity that catalyzes the hydrolysis of internal GlcA–GlcNS linkages, releasing sulfated fragments of 10–20 monosaccharides. The crystal structure of Hpa-1 in complex with HS oligosaccharides has shown that the enzyme catalytic cleft is exposed upon Hpa-1 proteolytic maturation and could accommodate a trisaccharide motif featuring N-sulfate at position -2 and a 6-O-sulfate at position +1 [65].

Lysosomal Hpa-1 contributes to HSPG degradation by cleaving HS chains into shorter fragments for subsequent degradation by HS exoglycosidases [66]. It can be translocated into the nucleus, where it affects the transcription of many tumor-related genes, or exported outside the cell to target and degrade cell surface and ECM HS. The extracellular activities of Hpa-1 are very versatile. Cleavage of HS by Hpa-1 strongly affects the structure and integrity of basement membranes and ECM and promotes tumor metastasis and angiogenesis by facilitating endothelial/tumor cell migration. Hpa-1 also catalyzes the release of HS bioactive fragments and HS ligands stored in the ECM, including growth factors such as fibroblast growth factor-2, vascular endothelial growth factor (VEGF), and cytokines [64,67]. Furthermore, Hpa-1 influences the secretion and composition of tumor cell-derived exosomes, which participate in the tumor/host cross-talk by acting on the syndecan–syntenin–ALIX exosome biogenesis pathway [64]. Hpa-1 also promotes cell autophagy, and both mechanisms contribute to the chemoresistance of cancer cells [64,68]. Hpa-1 may

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also regulate HS biosynthesis and promote HS sulfation and assembly of saccharide motifs activating proangiogenic and protumoral growth factors [64]. Interestingly, Hpa-1 elicits some of its biological functions through nonenzymatic driven mechanisms. Secreted latent Hpa-1 has been associated with the induction of signaling cascades, involving activation of Akt, ERK, protein tyrosine kinase 2, p38, and steroid receptor coactivator, and leading to enhanced cell adhesion, migration, survival, and VEGF upregulation. Although the underlying mechanisms remain unclear, the C-terminal domain of Hpa-1 mediates some of these nonenzymatic activities (see [64] for references). Aside from cancer and inflammation, Hpa-1 is associated with a wide range of other pathologies, including diabetes and diabetes-related disorders [69], fibrosis [70], neurodegenerative disease [71], kidney disease [72], atherosclerosis [73], or infections [74,75].

What remains to be solved? *Besides the well-documented role of Hpa-1 in cancer, accumulating evidence underlines the need to further explore the repertoire of Hpa-1 functions in both intracellular and extracellular compartments, related or not to its enzymatic activity* [76]. *Another pending issue is the study of the complex interplay between Hpa-1 and heparanase 2 (Hpa-2)* [66], *a homolog of Hpa-1, which lacks heparanase activity but retains its ability to bind to HS without triggering HSPG internalization. Hpa-2 may exert antitumoral properties through competition with Hpa-1 or an HS-independent mechanism. An important area of investigation is the development of Hpa-1 inhibitors. Hpa-1 has been extensively studied as a therapeutic target, notably in cancer, where it can be inhibited by monoclonal antibodies and sulfated polysaccharides including heparin derivatives* [77], *but these compounds may only target extracellular Hpa-1. New inhibitors and new design strategies are thus needed to target lysosomal and nuclear Hpa-1. Future developments will be sped up by the knowledge of the 3D structure of Hpa-1, which was recently used for the rational design of low-Mw Hpa-1 inhibitors* [78]. *The lack of heparinase enzyme assays that could be easily implemented for high-throughput screening also remains a technical challenge to be addressed* [79].

The endosulfatases Sulf-1 and Sulf-2

Sulfs are extracellular endosulfatases that catalyze the selective hydrolysis of HS 6-*O*-sulfate groups, thereby providing a unique postsynthesis mechanism to regulate HS sulfation patterns. The two human forms of Sulfs, HSulf-1 and HSulf-2, comprise an N-terminal catalytic domain (CAT), homologous to other eukaryotic sulfatases and including the active site, a central hydrophilic, highly basic, domain (HD) that is a unique feature of Sulfs, and is responsible for high-affinity binding to HS substrates, and a C-terminal region that may fold over the CAT domain to form the functional catalytic unit [80]. The maturation of Sulfs involves a furin cleavage within the HD domain, yielding two subunits linked by disulfide bridges. HSulfs undergo extensive post-translational modifications relevant to their structure and function. The cysteine residue strictly conserved in eukaryotic sulfatases and located within the CAT domain is converted into the catalytic *N*-formylglycine (FGly) residue essential for their enzymatic activity. Sulfs contain 10–11 putative N-glycosylation sites, mainly located within their N terminus [81], and HSulf-2 harbors a CS/DS GAG chain, which modulates substrate binding and its endosulfatase activity [82]. This GAG chain could affect Sulf-2 distribution and diffusion in tissues, and account for its functional differences compared to Sulf-1.

Sulfs essentially exert their endo-6-*O*-sulfatase activity on [UA2S-GlcNS6S] trisulfated disaccharide motifs where UA stands for glucuronic acid or iduronic acid, though limited desulfation was also reported on [UA-GlcNS6S] disulfated disaccharides [80]. N-sulfation is required for substrate recognition as GlcNAc,6S-containing disaccharides are not targeted by the enzymes. However, Sulfs process indiscriminately GlcA, IdoA, or heparinase-generated Δ4,5 uronic acid. Finally, the presence of 3-*O*-sulfate groups does not seem to prevent digestion by the Sulfs, and the antithrombin-III binding pentasaccharide can be efficiently 6-*O*-desulfated by the enzymes (see [51] for references). The mechanism underlying Sulf 6-O-desulfation of HS is orientated and processive.

High-affinity primary binding is governed by the HD domain, while low-affinity HS binding sites within the CAT domain ensure proper alignment of the HS chain with the enzyme active site [83]. 6-O-Desulfation of HS eventually leads to the dissociation of the Sulf–HS complex. Because of the strong preference for highly sulfated saccharide motifs, the main targets of the Sulfs are HS-sulfated domains, which comprise most of the binding sites for HS protein partners. Hence, although Sulfs impact on HS overall sulfation is minimal (typically leading to a ~ 4–5% sulfate loss), these changes dramatically affect HS binding properties for a large number of growth factors, morphogens, and chemokines, and modulate critical cellular functions such as cell growth, migration, and differentiation [80]. Sulfs have thus been associated with physiological processes and pathological conditions, including development, tissue repair, inflammation, and cancer [84,85].

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Sulf-1 and Sulf-2 exhibit nearly identical enzyme activity *in vitro*, but they can show very distinct functions *in vivo*. In developmental studies, single Sulf knockouts (KOs) showed little phenotypic defects, while the Sulf-1/Sulf-2 double KO features marked skeletal, renal, and lung abnormalities and high neonatal mortality. Analysis of HS from Sulf-1- and Sulf-2-deficient mice revealed enzyme-dependent specificities (see [6] for references). Sulf-1 KO mice featured specific neuronal development defects such as a reduced spine density, suggesting nonredundant functions of the Sulfs in the development of the mouse nervous system [80]. Similarly, although overlapping expression patterns of Sulfs were reported during bone development, only Sulf-2 expression was induced during bone fracture healing [86], suggesting that Sulfs play different roles in this process. Both Sulf-1 and Sulf-2 promote angiogenesis following postmyocardial infarction, but they act through distinct autocrine and paracrine mechanisms [87]. In contrast, functional divergences between Sulf-1 and Sulf-2 have been reported in cancer. Sulf-2 is strongly induced in many tumors and is a target for anticancer therapies, while Sulf-1 is associated with antioncogenic activities, and its role seems highly dependent on the tumor type [85]. These functional divergences underline the complexity, the context dependency, and the lack of understanding of Sulf regulation of HS biological functions.

What remains to be solved? *The study of Sulfs has essentially focused on their role in cancer and embryonic development, but they may play a role in tissue homeostasis, and their functional repertoire remains to be discovered. Another major unsolved issue is the molecular basis of the functional differences between Sulf-1 and Sulf-2* in vivo*, particularly in cancer. Differential spatiotemporal expression or restricted access to specific HS subsets may provide some rationale for these discrepancies as reported in head and neck squamous cell carcinoma where Sulf-2 is essentially expressed by cancer cells and Sulf-1 by cancer-associated fibroblasts of the tumor microenvironment* [85]. *The first discriminating molecular feature is the presence of a CS/DS chain attached to HSulf-2 but not to HSulf-1* [82]. *The interplay between Sulf-1 and heparanase deserves further studies to identify their possible cooperation or competition toward their common HS substrates and their contribution to HS postsynthesis regulatory mechanisms. Another major challenge is the determination of the structure of Sulfs, which will be difficult due to their numerous post-translational modifications, the presence of poorly structured regions in the HD domain, and their propensity to dimerize* [82]. *Structural insights would be crucial for understanding the molecular details governing their functions and designing specific inhibitors. This is a critical issue because Sulfs share arylsulfatase activity with many other sulfatases. The first attempts to increase inhibitor selectivity toward the Sulfs involved modifying at position C6 of glucosamine residues with an inhibitory sulfamate motif. This strategy was recently refined with the design of a synthetic HS sulfonamide-modified trisaccharide, an efficient inhibitor of HSulf-1* [88]. *However, as HSulf-1 and Hsulf-2 exhibit divergent activities in cancer, inhibitors targeting specifically Hsulf-2 should be sought. The CAT domain of the Sulfs and other sulfatases being homologous, targeting the poorly conserved HD domain may be the way forward to design Sulf selective inhibitors.*

The shedding of PGs

Although not directly targeting GAG chains, the proteolytic release of the extracellular domain of membrane-anchored HSPGs, termed shedding, has been associated with major biological processes in health and disease [89,90]. Shedding allows cells to dynamically regulate the expression, distribution, and function of cell-surface HSPGs by converting membrane-anchored HSPGs into soluble forms, which diffuse toward distant sites and exert paracrine activities. It may also affect cellular functions by modifying the balance of HPSGs present at the cell surface. The proteolytic release of HSPG ectodomains is catalyzed by sheddases, including matrix metalloproteinases (MMPs), elastase, thrombin, granzyme B, ADAMs (A disintegrin and metalloprotease) and ADAMTSs (A disintegrin and metalloproteinase with thrombospondin motifs) [90]. Heparanase, the degradation or the lack of HS chains, enhances the shedding of syndecan-1 [64,91].

What remains to be solved? *Despite increasing evidence on the role of the shed HSPG ectodomains in a number of biological processes, the mechanisms of its regulation remain largely unknown at the molecular level. The regulation of sheddase expression, activation, and specificity warrants further investigation. Increased serum levels of shed syndecan have been detected in several diseases (e.g., cancer, sepsis, cardiovascular, bowel, and kidney diseases), and future studies should help clarify if shed syndecans and/or glypicans could be diagnostic or prognostic biomarkers to monitor disease progression.*

The linkage region of GAGs to core proteins

Glycosaminoglycan chains are covalently attached to serine residues of core proteins via a tetrasaccharide linker (GlcA-Gal-Gal-Xyl-O), which can be sulfated

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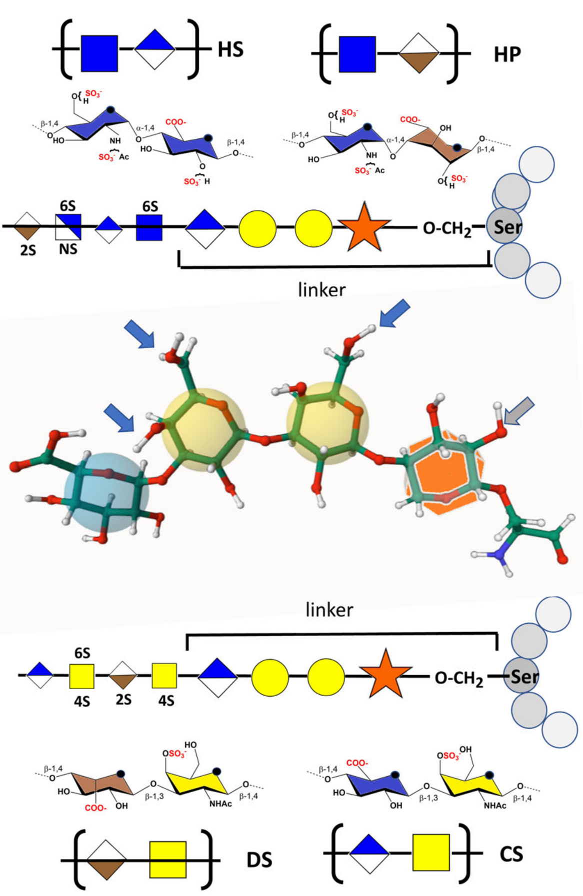

Fig. 5. The linker region of proteoglycans. The three-dimensional structure of the tetrasaccharide linker connecting serine residues of the core protein of proteoglycans (PGs) and the glycosaminoglycan (GAG) chains is displayed in the middle panel. Blue arrows: sulfation, gray arrow: phosphorylation. The symbols used to represent GAG monosaccharides are those from the Symbol Nomenclature for Glycans (SNFG) [32].

and sialylated on the galactose residues and phosphorylated and fucosylated on the xylose residue [92] (Fig. 5). The transient phosphorylation of the xylose residue by the kinase FAM20B enhances the efficiency of glycosyltransferases, and the sulfation of both Gal residues on C4 or C6 favors the synthesis of CS/DS chains [38]. Molecular dynamics simulations have shown that the tetrasaccharide acts as a relatively rigid unit extended over 20 Å and that significant conformational heterogeneity exists for rotation about bonds connecting the first monosaccharide (xylose) to the serine residue [93]. The elongation of the polysaccharide chain from the tetrasaccharide linker starts with the addition of a first hexosamine residue, the nature of which (galactosamine or glucosamine) will direct the biosynthesis pathway toward the assembly of either CS/DS or HS chains. The molecular mechanism leading to the synthesis of CS or HS has been recently elucidated. The CS *N*-acetylgalactosaminyltransferase 2 (CSGALNACT2) initiating CS biosynthesis modifies all glycopeptide substrates, whereas the *N*-acetylglucosaminyltransferase EXTL3, initiating HS biosynthesis, is selective. Acidic amino acid residues in the glycopeptides and a basic exosite in EXTL3 are important for HS biosynthesis [94].

The GAG attachment sites of the known human CSPGs, HSPGs, and KSPGs have been recently reviewed [95,96]. There is no canonical consensus sequence for GAG attachment, but the serine residues bearing GAG chains are generally followed by a glycine residue. Acidic residues are usually found close to GAG attachment sites, and hydrophobic residues could play a role in the selective attachment of HS chains [95,97]. The location of CS/DS and HS chains on hybrid PGs such as syndecans 1 and 3 are conserved, suggesting interactions between the PG core protein and the enzymes involved in the regulation of the tetrasaccharide linker modifications, or in GAG chain polymerization. In contrast, as mentioned in the section on KS biosynthesis, there are three types of linkage of KS to the core proteins of KSPGs via asparagine (KS-I), serine (KS-II, KS-III), or threonine residues (KS-II) [46].

A glycoproteomics approach, based on enrichment by ion-exchange chromatography, enzymatic digestion, and liquid chromatography–tandem mass spectrometry (LC–MS/MS), has been developed to analyze glycopeptides and hence GAG linkage regions and attachment sites on core proteins [98]. This approach allows the analysis of both the GAG and protein parts of PGs, which have often been investigated separately. New CSPGs have been identified in human urine and cerebrospinal fluid, including five prohormones (cholecystokinin, chromogranin A, neuropeptide W, secretogranins 1 and 3) [99]. Additional CSPGs and HSPGs were identified in mammalian insulin-secreted cells (e.g., the HSPGs and prohormones chromogranin A and secretogranin-1) [100], in *Caenorhabditis elegans* (15 novel CSPGs) [101], and in *Drosophila* (Windpipe, a novel membrane CSPG) [102]).

A noncanonical CS linkage region, comprised three sugar residues (GlcA-Gal-Xyl) instead of four, has been identified in urinary bikunin of healthy subjects [103], confirming the heterogeneity of this CS linkage region [104]. Defective synthesis of the canonical tetrasaccharide linker has been reported in rare heritable

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connective tissue diseases known as linkeropathies [105,106]. A few rare Ehlers–Danlos syndromes (spondylodysplastic Ehlers–Danlos) are linked to a defective formation of the tetrasaccharide linker due to β4GalT7 or β3GalT6 deficiency [43]. The urinary bikunin of the three affected siblings has both the tetrasaccharide and the noncanonical trisaccharide linkers. In contrast, only the tetrasaccharide was found in the urinary bikunin of their unaffected mother. The linkage region of urinary bikunin could thus be a marker for the β-3GalT6-deficient syndrome [107]. In a b3galt6 knockout zebrafish model, associated with a decrease in GAG content and disorganized collagen fibrils, a small amount of PGs contain a trisaccharide linker [108]. These findings highlight the importance of the linker region.

What remains to be solved? *A first challenge would be to determine how the serine residues serving as GAG attachment sites to the PG protein core are selected. The heterogeneity of the linkage region, its influence on the conformation of the GAG chains attached to the core proteins, its association with linkeropathies, and its potential use as disease biomarkers also warrant further investigations. The functions of newly identified CSPGs and HSPGs remain to be determined.*

GAGs and their protein moieties in disease

Glycosaminoglycans contribute to cell homeostasis and human disease. Their spatiotemporal diversity and regulation in physiopathological situations [109–111] and the global impact of PG science on human diseases have been recently reviewed [112]. Recent advances in GAG analysis and sequencing (e.g., ion mobility–mass spectrometry, gas-phase infrared spectroscopy, and nanopores) have been detailed in a companion article [2], and are not discussed here. We mostly focus below on the role of CS, HS, syndecans, and SLRPs in cancer and inflammation. Table 1 summarizes the roles of PGs in disease progression and the signaling pathways involved, whereas the potential applications of PGs as biomarkers of disease diagnosis and monitoring are listed in Table 2.

Disorders associated with an impaired degradation of GAGs

Lysosomal storage of undegraded or partially degraded GAGs are a hallmark of mucopolysaccharidoses (MPS) that are associated with skeletal, cardiac, eye, and hearing abnormalities. They result from the deficiency of one of the 12 enzymes required for the catabolism of GAGs [134], namely sulfatases (iduronate-2-sulfatase, heparan *N*-sulfatase, *N*-acetylglucosamine-sulfate-6-sulfatase, *N*-acetylgalactosamine-6-sulfate sulfatase, *N*-acetylgalactosamine-4-sulfatase, and arylsulfatase K), α-L-iduronidase, α-*N*-acetylglucosaminidase, β-galactosidase, α-glucuronidase, hyaluronidase, and heparan-alpha-glucosaminide *N*-acetyltransferase [134]. Current therapeutic approaches for MPSs include enzyme replacement therapy and hematopoietic stem cell transplantation [134–136]. Despite promising preliminary results, substrate reduction therapy has not been demonstrated to be effective in randomized and controlled clinical trials [136].

What remains to be solved? *There are ongoing clinical trials for gene therapy of MPSs targeting α-L--iduronidase, α-iduronate-2-sulfatase, and heparan N-sulfatase [134,136]. However, it remains to be determined what would be the best strategy (i.e.,* ex vivo *or* in vivo *gene therapy) depending on the patients [137]. Targeted delivery of recombinant lysosomal enzymes using fusion with peptide/protein, nanoscaled delivery systems, or encapsulation in liposomes might improve their efficacy [138]. Other strategies such as gene editing to correct the defective genes [135] and the use of cell homeostatic modulators to restore affected pathways and organelles in MPSs together with enzyme replacement therapy [134] are promising therapeutic approaches for mucopolysaccharidoses but they require further development for clinical applications.*

Hyaluronan signaling in health and disease

Elucidation of the mechanisms driving activation of HA–CD44 signaling

The discovery of CD44 as a receptor for HA made possible to understand why one of the HA physiological functions was to capture circulating cells such as lymphocytes and lead them to inflamed sites. Growth factors, such as platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β, induce HA production in normal fibroblasts and mammary epithelial cells. HAS2 is strongly upregulated in both mesenchymal and epithelial cells, and knockout of the Has2 isoform in mice led to embryonic lethality [139,140]. HAS2 is important for TGFβ-induced epithelial-to-mesenchymal transition (EMT). TGFβ-induction of Smad and non-Smad pathways results in the transcriptional expression of high-mobility group AT-hook protein 2 (HMGA2) and the natural antisense *HAS2-AS1*, which cooperatively promote HAS2 expression. HAS2-synthesized HA activates CD44, triggering downstream signaling and contributing to

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Table 1. Summary of the roles of syndecans and two small leucine-rich proteoglycans, decorin and biglycan, in disease with the signaling pathways involved.

Proteoglycans	Disease	Cell/Tissue function	Signaling pathways
Syndecan-1	Breast cancer	Promotes breast cancer stem cell phenotype	IL-6/STAT3, Notch and EGFR [113]
Syndecan-1	Colon cancer	Decreases cancer cell stemness, availability, and resistance to chemotherapy	FAK/Wnt [114]
Syndecan-1	Triple-negative breast cancer	Promotes angiogenesis	VEGF-A and tissue factor (TF) [115]
Syndecans	Cancer	Regulates cell proliferation, invasion and survival	Receptor tyrosine kinases [116]
Syndecan-4	Gastric cancer	Promotes cancer cell invasion and communication	HS-containing SDC4 defines extracellular vesicle organ distribution, uptake, and oncogenic functional effects on recipient cell populations [117]
Biglycan	Hepatorenal inflammation	Acts as a switch between inflammation and autophagy	TLR2 and 4, CD14, CD44 [118]
Biglycan	Ischemia/reperfusion injury	Promotes autophagy	CD44/Toll-like receptor 4 [119]
Biglycan	Secondary polycythemia	Promotes erythropoiesis	TLR2 [120]
Biglycan	Acute pancreatitis	Enhances immune response	Advanced glycosylation end-product-specific receptor (AGER) [121]
Decorin	Epidural fibrosis	Inhibits fibrosis	TGFβ [122]
Decorin	Peritoneal dialysis-associated peritonitis	Inhibits fibrosis	Decreased TGF-βRI, p38 MAPK, and AKT/PI3K phosphorylation and increased glycogen synthase kinase-3β phosphorylation [122]

Table 2. The putative roles of proteoglycans (PGs) as biomarkers for fibrosis and cancer.

PG	Disease	Source	Biomarker	Analysis method
Oncofetal chondroitin sulfate (ofCS) PG	Pan-cancer	Serum	Cancer detection	Immunohistochemistry assay based on the binding of the recombinant *Plasmodium falciparum* protein rVAR2 to ofCS [123]
Biglycan	Chronic hepatitis B	Serum	Fibrosis marker	ELISA [124]
Biglycan	Nonalcoholic steatohepatitis and liver fibrosis	Serum	Disease detection	ELISA [125]
Biglycan	Lung cancer	Cancer tissue/preoperative serum	Prognosis marker	Immunohistochemistry/ELISA [126]
Biglycan	Stomach adenocarcinoma	Databases	Prognosis marker	Gene expression profiling interactive analysis (Gepia) database (http://gepia.cancer-pku.cn/) and Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) [127]
Biglycan	Gastric cancer	Databases/cancer tissues	Prognostic marker	TCGA and GTEx databases analysis – real-time PCR and immunohistochemistry [128]
Degraded biglycan	Systemic sclerosis	Serum	Prognostic marker	ELISA [129]
Decorin	Malignant peripheral nerve sheath tumors	Serum	Prognostic marker	Label-free quantitative proteomics and mass spectrometry [130]
Decorin	Preeclampsia	First-trimester serum	Prognostic marker	ELISA [131]
Decorin	Idiopathic pulmonary fibrosis	Serum	Prognostic marker	ELISA [132]
Cathepsin-S degraded decorin	Fibrotic lung disorders	Serum	Prognostic marker	ELISA [133]

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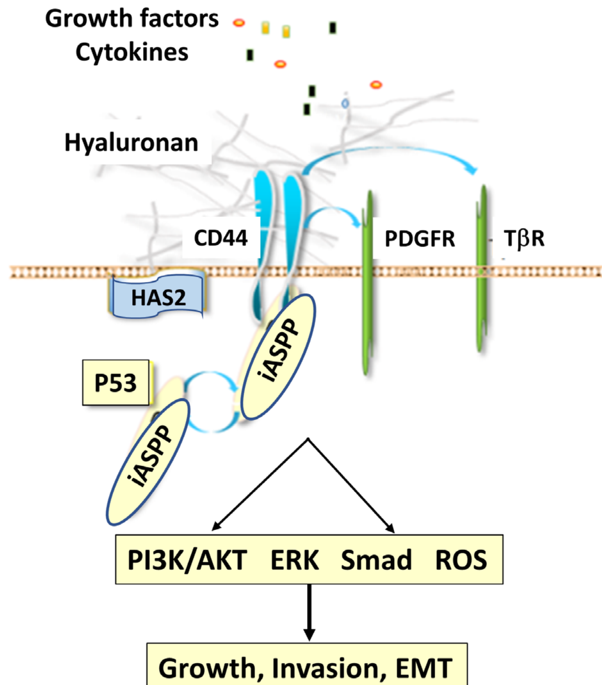

Fig. 6. Hyaluronan-mediated CD44 activation regulates cell fate. Growth factors and cytokines activate hyaluronan synthase 2 (HAS2), leading to the production of hyaluronan (HA). Then, HA binds to CD44, activating direct signaling and affecting signaling as a coreceptor for other receptors such as PDGF and transforming growth factor receptors (AKT, RAC-alpha, beta, and gamma serine/threonine–protein kinases; EMT, epithelial–mesenchymal transition; iASPP, inhibitor of ASPP protein; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; TβR, transforming growth factor receptor).

EMT and tumor cell invasion [141] (Fig. 6). Furthermore, the activation of protein kinase C by phorbol 12-myristate 13-acetate and the energy stress marker AMP-activated protein kinase (AMPK) regulates HAS2 activity by phosphorylation.

CD44 is a transmembrane glycoprotein involved in a myriad of physiological functions. CD44 is expressed at cell–cell contact sites during endothelial cell differentiation, and perturbation of the HA/hyaluronidase/CD44 axis impairs the formation of a regular vessel-like network. The perturbation of HA signaling contributes to the progression of diseases such as aggressive breast cancer [142], lung cancer [143], and dengue virus infection [144]. CD44 genetic depletion in glioblastoma cells decreases proliferation and self-renewal ability [145]. Furthermore, CD44 ablation impairs autocrine PDGF signaling and suppresses HAS2 expression with concomitant suppression of HA synthesis. These data support that a HA/CD44 feedback circuit contributes to glioblastoma progression in cooperation with PDGF signaling [145]. Beyond this, CD44 is a marker of cancer stem cells in various malignancies and a target for p53 tumor-suppressive activity [146], which is inhibited by the inhibitor of ASPP protein (iASPP). CD44 physically interacts with iASPP, and the balance between iASPP–CD44 and iASPP–p53 complexes affects cell survival [147] (Fig. 6).

What remains to be solved? *Because HA plays a central role in tissue homeostasis and elevated amounts correlate to the progression of certain diseases, a better understanding of the molecular mechanisms regulating its synthesis and turnover and its receptor-mediated signaling is required.* O-*GlcNAcylation and ubiquitination events are interconnected, and most likely, the former regulates the latter, suggesting* O-*GlcNAcylation of HAS2 may modulate the recruitment of the HAS2 deubiquitinase USP17 and/or USP4. CD44 acts as a coreceptor affecting several receptor signaling. Therefore, it may be interesting to inhibit HA–CD44 signaling by developing small molecule inhibitors targeting it and/or specific HAS inhibitors.*

Mechanisms driving activation of HA receptor for HA-mediated motility (RHAMM) interplay

RHAMM has been identified as a soluble protein binding to HA, and RHAMM isoforms have been detected both inside the cell and at the cell surface, even though RHAMM does not have a signal peptide. RHAMM is a supercoiled coil protein including an N-terminal domain, a central rod-like made up of five coils domain, and a short C-terminal domain, but lacks a transmembrane domain. Extracellular RHAMM must thus interact with other transmembrane receptors such as CD44 and PDGF receptor to initiate downstream intracellular signaling [148]. Extracellular RHAMM and RHAMM/HA interactions modulate response to growth factors, cell growth, and migration [149,150]. Intracellular RHAMM regulates the cell cycle, mitotic spindle, and microtubule formation. RHAMM is overexpressed in cancer and supports tumor growth and dissemination [148,151]. Its overexpression correlates with aggressive disease and poor disease outcomes in various cancers, including breast cancer, where RHAMM can be both a therapeutic target and a disease marker [152]. RHAMM defines an invasive niche associated with tumor progression and predicts poor outcomes in breast cancer patients [153].

RHAMM binds to HA via B(X7)B sequences, B being lysine or arginine residues and X being a nonacidic amino acid, located in its C-terminal domain,

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LMW-HA preferentially binds to RHAMM, facilitating cell growth and motility of tumor or injured cells, suggesting that the interaction is size dependent [154,155]. Upon interacting with HA, RHAMM modulates key intracellular pathways, including ERK1/2, RHO-ROCK, and Wnt/β-catenin [156,157]. Cells overexpressing RHAMM have increased basal ERK1/2 activation correlated to enhanced cancer cell motility. HA/RHAMM-induced ERK1/2 activation upregulates the level of FAK tyrosine phosphorylation, increasing the turnover of focal adhesion sites and cell migration (Fig. 7). This interaction also affects the cytoskeletal organization by triggering the RHO-ROCK pathway, which regulates the polymerization of actin and promotes cell unidirectional movement [158]. In this model, RHAMM is suggested to act as a scaffold protein binding β-catenin and Axin-2 at different cellular compartments to enhance β-catenin transcriptional activity [159].

In addition to affecting RHAMM downstream signaling, HA regulates its expression. Exposure of fibroblasts, breast cancer cells, and human umbilical vein endothelial cells to HA activates the CD44/protein kinase Cδ pathway, inducing the nuclear translocation of c-fos and c-jun to facilitate RHAMM expression. HA affects the ability of RHAMM to interact with transmembrane coreceptors (Fig. 7). The formation of the CD44/RHAMM complex is stimulated when cells are immobilized on HA, but not when treated with soluble HA. Furthermore, both RHAMM and CD44 expressions are regulated by HA, suggesting the existence of putative feedback loops [160], and RHAMM expression positively correlates with HA deposition [161].

Tubulin-derived peptides generated to target HA are endocytosed by prostate and breast cancer cells, blocking intracellular HA-dependent RHAMM activities [162]. The inhibition of HA synthesis by 4-methylumbelliferone (4-MU) decreases RHAMM expression and downstream signaling pathways involved in cancer progression. Targeting HA in the glycocalyx of the peritoneal cavity could inhibit cancer cell adhesion to peritoneum distant sites by blocking interactions of HA-binding proteins [163].

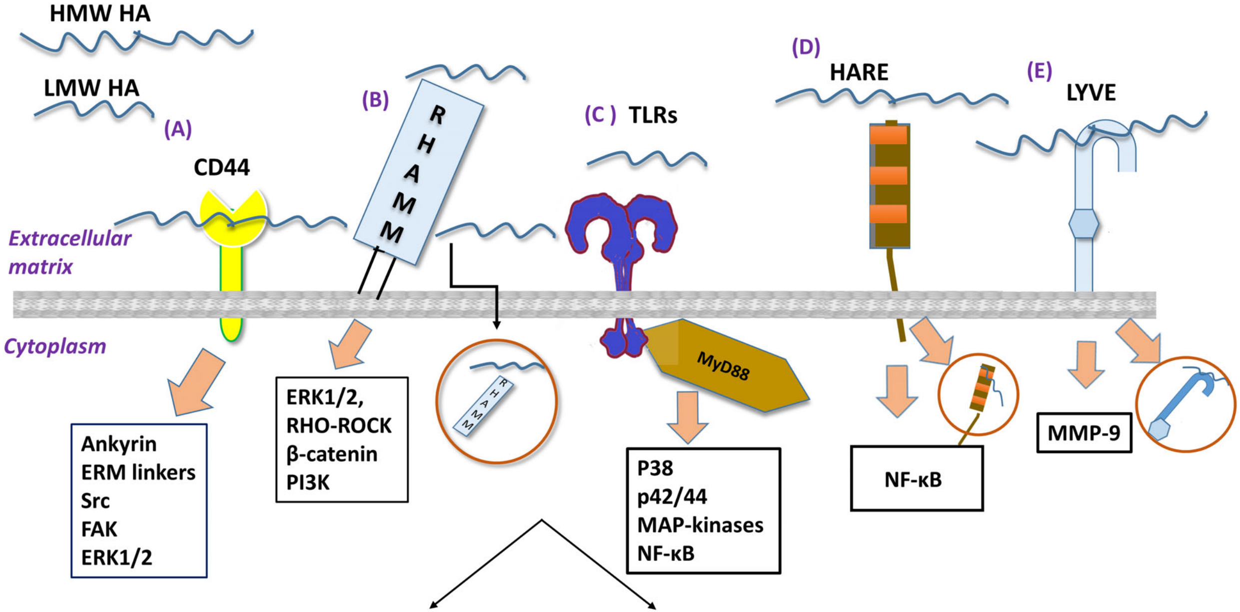

Fig. 7. Signaling pathways mediating RHAMM/HA effects. (A) HA-RHAMM binding increases RHAMM expression and regulates its cellular deposition. (B) HA/RHAMM-induced ERK1/2 activation upregulates FAK phosphorylation, increasing the turnover of focal adhesion sites and cell migration. (C) HA enhances RHAMM intracellular deposition and RHAMM-ERK1/2 complex formation, mediating adhesion and migration. (D) HA-RHAMM interactions facilitate RHAMM/β-catenin complex formation, β-catenin stabilization, nuclear translocation, and increased cell growth. (E, F) HA-RHAMM interaction may facilitate RHAMM colocalization with transmembrane receptors/proteins (PDGFR/CD44) to activate intracellular signaling pathways resulting in ERK1/2 phosphorylation, nuclear translocation, and transcription of mitogenic and motogenic genes (ERK1/2, extracellular signal-regulated kinase 1/2; ERM, ezrin, radixin, and moesin; FAK, focal adhesion kinase; HARE, hyaluronan receptor for endocytosis; LYVE, lymphatic vessel endothelial receptor; MAP kinases, mitogen-activated protein kinases; MMP-9, matrix metalloproteinase-9; NF-κB, nuclear factor kappa B; p38, p38 mitogen-activated protein kinase; p42/44, p42/p44 mitogen-activated protein kinases; PI3K, phosphoinositide 3-kinase; RHO-ROCK, Rho-associated protein kinase; Src, src-family kinase; TLR, toll-like receptor).

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Targeting HA metabolism in cancer

The functions of HA highly depend on its size and interactions with its cellular receptors, mainly CD44 [140,164]. The antioncogenic and anti-inflammatory functions of high-molecular weight (HMW) HA have been demonstrated in naked mole rats, which produce larger HA polymers and exhibit less detectable HA fragmentation than humans or mice and have an increased life span and an unusual resistance to cancer [165,166]. Silencing HAS genes or overexpressing HYAL2 in naked mole rat cells increases their susceptibility to malignant transformation. HMW-HA/CD44 interactions activate the tumor-suppressive Hippo signaling pathway, while low-molecular weight (LMW) HA produced by the action of HYALs inhibits Hippo signaling by competing with HMW-HA for CD44 binding, thus promoting its pro-oncogenic functions [166]. Moreover, LMW-HA exerts proinflammatory actions by stimulating Toll-like receptors (TLR2/TLR4), resulting in the enhanced synthesis of cytokines and chemokines [140].

The increased deposition of HA in tumors results in the abundance of LMW-HA fragments in the tumor microenvironment (TME) due to the elevated expression and activity of hyaluronidases and the sustained action of reactive oxygen species. HA degradation in the TME combines the actions of both tumor and tumor-associated cells. Breast tumors, particularly the aggressive HER-2-positive and triple-negative breast cancer subtypes, are solid tumors containing immune cell-infiltrated stroma [167]. The cross-talk between tumor-recruited HYAL2-expressing myeloid cells and HA-producing tumor and stromal cells triggers its enhanced degradation and the accumulation of proinflammatory and proangiogenic LMW-HA [168]. In addition, HA-enriched tumor stroma drives the formation of programmed cell death ligand 1 (PD-L1)-expressing macrophages, thus contributing to the establishment of an immunosuppressive, tolerogenic microenvironment by creating a PD-L1 shield preventing immune responses mediated by T cells via programmed cell death protein 1 (PD1)/PD-L1 pathway [169,170]. Peritumoral HA-dependent matrices are important for the adhesion of metastatic breast cancer cells to CD44 expressed by microvascular endothelial cells, promoting their metastatic potential.

Targeting the HA network metabolic enzymes and interacting proteins/receptors, which affect multiple cellular processes, may provide new therapeutic approaches for human pathologies, such as inflammation and cancer. Several strategies have been used to target either HA synthesis or degradation or its interactions with cellular receptors such as CD44 [164]. Silencing HAS genes, suppression of HA synthesis by inhibitors and modification (i.e., sulfation) of HA significantly impair tumor growth in several *in vitro* and *in vivo* models. 4-MU, the only currently established inhibitor of HA biosynthesis that mainly targets HAS2, inhibits the proliferation, migration, and invasion of multiple cancer types *in vitro* and *in vivo* by mechanisms mainly involving the depletion of UDP–sugar precursors as well as downregulation of HAS2 mRNA [171]. The effect of HA inhibition in main breast cancer subtypes has highlighted the impact of 4-MU on mammary carcinoma cells of distinct estrogen receptor expression profiles, a major criterion in the prognosis and therapeutic management of breast cancers. 4-MU induces cell apoptosis, possibly anoikis, and suppresses cell invasiveness in low metastatic estrogen receptor-positive cells, whereas it induces cell growth arrest and attenuates cell migration and invasion in highly metastatic estrogen receptor-negative cells.

Salicylate, an *in vivo* breakdown product of the common low-cost nonsteroidal anti-inflammatory drug aspirin, inhibits HA biosynthesis and deposition in metastatic estrogen receptor-negative breast cancer cells through phosphorylation and activation of AMPK that inactivates HAS2. These changes are associated with decreased cancer cell growth and motility, and suppression of the metastatic potential of breast cancer cells [172]. Moreover, sulfated HA (sHA) attenuates breast cancer cell proliferation, migration, and invasion and increases cell adhesion on collagen I. It also modulates the expression of EMT markers and downregulates matrix remodeling enzymes such as matrix metalloproteinases. This suggests that sHA has an antioncogenic role and likely competes with endogenous HA for binding to cellular receptors [173].

What remains to be solved*? Overall, these studies may offer a direction for future matrix-based targeted treatments of specific breast cancer subtypes through inhibition of HA, a normal constituent of human tissues that can be converted into a proangiogenic, proinflammatory, and tumor-promoting effector promoting persistent inflammation and cancer progression. There is an urgent need to develop more specific inhibitors of HA metabolic enzymes (e.g., HAS2) that would bring to light novel therapeutic approaches to combat inflammation and cancer.*

HA in inflammation

During inflammation and after tissue injury, HA and its $< 5 \times 10^5$ Da degradation products accumulate

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[174]. As previously mentioned, the large (> 500 kDa) native HA chains support homeostasis, whereas the HA fragments in the 10–500 kDa range primarily exert proinflammatory functions [175]. The presence of myriad-size HA fragments confirms that cells utilize efficient and specific enzymatic mechanisms to regulate HA turnover and metabolism [11]. The generation of reactive oxygen or nitrogen species due to inflammation, ischemia–reperfusion injury, sepsis, and cancer results in specific HA cleavage [10]. A complex size-dependent relationship has been established as HA fragments in the 40–400 kDa size range trigger NF-κβ-modulated downstream inflammatory effects through the HA receptor for endocytosis (HARE). HA fragments of approximately 2.5×10^5 Da trigger inflammation in renal tubular epithelial cells by inducing the expression of monocyte chemoattractant protein-1. LMW-HA fragments of 15–40 kDa act as danger-associated molecular patterns (DAMPs), initiate and propagate contact allergen-dependent sensitization of keratinocytes utilizing a TLR4/ NF-κB (nuclear factor kappa light chain enhancer of activated B cells) signaling axis [176]. HA fragments attenuate the expression of proinflammatory TNF-α, IL-6, IL-1, and IFN-β in an *in vivo* model of lipopolysaccharide (LPS)-induced inflammation and upregulate the production of the anti-inflammatory cytokine IL-10 in LPS-treated macrophages, suggesting that HA fragments abrogate the triggering of the TLR4 signaling pathway [177]. Both LMW-HA and HMW-HA fragments can be proinflammatory under pathological conditions, activating multiple receptors and triggering proinflammatory signaling in response to various stimuli.

Hyaluronan interaction with RHAMM contributes to the modulation of inflammatory processes [177]. RHAMM is suggested to facilitate the process of wound repair through inflammatory cues. Blocking RHAMM/HA binding with an RHAMM-mimetic peptide attenuates inflammation and fibrogenesis in excisional skin wounds. RHAMM expression is strongly upregulated in immune atopic diseases and correlated with the severity of inflammation, suggesting that it can be classified as a DAMP. Mice overexpressing RHAMM exhibit upregulated macrophage accumulation in their lungs, a 30-fold increase in HA level in bronchoalveolar lavage, and exaggerated lung fibrosis in response to bleomycin-mediated injury compared to wild-type injured mice [178].

What remains to be solved? *RHAMM ability to interact with a number of proteins, associated with its complex pattern of cellular localization, suggests it has a key role in regulating biological functions. It has been proposed to act as a scaffold protein regulating interactions mediated by other biomolecules. However, the structure, interactions, and functions of its different isoforms remain to be determined and correlated to cellular behavior in physiological and pathological conditions.*

Sulfated GAGs and PGs in cancer

Changes in GAG synthesis in cancer

Glycosaminoglycans and PGs are involved in cancer [179–181]. The fine structure and expression level of GAGs can profoundly impact the function of these molecules. Changes in the expression of GAG biosynthetic enzymes have been reported in a variety of diseases. GAG biosynthetic enzymes and PGs are dysregulated in malignant tumors [182]. For example, the HS 3-*O*-sulfotransferase HS3ST2 is epigenetically silenced in numerous tumor entities, including breast and ovarian carcinoma [183]. These changes occur not only at the gene expression level but also at the GAG level. A 33% reduction in 2-*O*-sulfation on iduronic acid and a 20% reduction in total N sulfation have been observed during tumor progression in colon cancer cells compared with adenoma cells. The dysregulation of specific biosynthetic enzymes has been correlated to either a better or worse prognosis for affected patients, as exemplified by the prognostic role of HS3ST2 in ovarian [183] and breast cancer [184], of HS 2-*O*-sulfotransferase HS2ST1 in breast cancer [184], and of HAS2 in ovarian cancer [185]. These studies highlight the clinicopathological relevance of altered GAG amounts in malignant and nonmalignant diseases and suggest a possible mechanistic involvement as outlined next.

HS and CSPG in cancer

The dysregulated expression of GAGs and PGs in cancer has been functionally linked to key mechanisms that drive tumor progression. The "Hallmarks of Cancer" originally coined by Hanahan and Weinberg have defined a set of cell biological processes frequently dysregulated in tumors, contributing to their progression. Taking this influential concept as a template, it is noteworthy that changes in PGs and GAGs modulate virtually all the hallmarks of cancer, highlighting their relevance to malignant disease.

Numerous PGs and GAGs play a role in sustaining proliferative signaling, the first hallmark of cancer [186]. A textbook example is the role of cell surface HSPGs that act as coreceptors for growth factor-mediated receptor tyrosine kinase signaling, where the

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HS chain aids in the formation of a ternary complex of growth factor, receptor tyrosine kinase, and the GAG chain, which substantially promotes and enhances mitogenic signaling promoting tumor growth [187]. Furthermore, the GAG chain's fine structure can profoundly influence tumor cell proliferation. Upregulation of HS3ST2 in MDA-MB-231 breast cancer cells increases cell viability and p44/42 MAPK signaling, whereas upregulation of HS2ST1 reduces viability and MAPK signaling in these cells [188].

The second hallmark of cancer, evading growth suppressors, is associated with a modulation of the molecular pathways linked to growth inhibition. In this context, the modulation of the TGFβ pathway by the cell surface HSPGs syndecans 1 and 4 is noteworthy, as is the well-known regulation of TGFβ function by the CS/DSPG decorin [189,190]. Moreover, decorin is an efficient tumor suppressor as it acts as a potent inhibitor of multiple receptor tyrosine kinase pathways in a variety of cancer [190,191], and induces upregulation of the cell cycle regulator p21.

The third hallmark of cancer, resisting cell death, is linked to a well-documented role of PGs and GAGs in regulating apoptosis. Enzymatic degradation of cell surface HS in MDA-MB-231 and HCC1806 breast cancer cells increases apoptosis [192]. In addition, the interaction of HA with its receptor CD44 induces anti-apoptotic signaling in concert with receptor tyrosine kinases [164]. Likewise, depletion of the HSPG syndecan-1 in MDA-MB-231 cells protects them from apoptosis, but this effect cannot be modulated by the exogenous addition of HA [193].

The fourth hallmark, enabling replicative immortality, is closely linked to the concept of cancer stem cells, or tumor-initiating cells, which are characterized by an unlimited proliferative potential and properties such as more efficient DNA repair and multidrug resistance protein expression, which ultimately lead to increased therapeutic resistance [194]. A well-investigated PG in this context is syndecan-1, which promotes breast cancer stem cell phenotype as an essential modulator of the Wnt and Notch signaling pathways [113]. In colon cancer cells, syndecan-1 depletion is associated with an enhanced cancer stem cell phenotype, partially influencing similar signaling pathways [114]. This demonstrates that PG/GAG-mediated functions in cancer are often context dependent. Likewise, overexpression of HS 2-*O*- and 3-*O*-sulfotransferases influences the breast cancer stem cell phenotype in a complex and context-dependent manner [195]. Besides PGs, HA is a prominent modulator of cancer stem cell properties via its interaction with CD44. This interaction affects the maintenance of cancer stem cells and their niche, drug resistance, and EMT and involves the activation of GSK3β and the stromal cell-derived factor 1/CXCR4 axis [194].

There is ample evidence for the involvement of PGs and GAGs in the fifth hallmark, inducing neoangiogenesis. Cell surface PGs, such as syndecans, can promote tumor angiogenesis as coreceptors for VEGF signaling and ensure the proper expression of proangiogenic cytokines, including VEGF and constituents of the tissue factor pathway [115]. Moreover, syndecan-1 can promote tumor angiogenesis via the association of its ectodomain with IGF-like family receptor (IGFR) and proangiogenic αϖβ3 integrin as a mechanistic basis for the action of the antiangiogenic peptide synstatin [116]. The proangiogenic properties of the basement membrane HSPG perlecan are converted into antiangiogenic effects when it is proteolytically degraded, resulting in the release of endorepellin, which exerts its antiangiogenic effects via a dual receptor antagonism involving VEGFR2 and integrin α2β1 [196]. The DSPG decorin exhibits antiangiogenic effects through AMPK-dependent induction of endothelial autophagy and the inhibition of multiple proangiogenic signaling pathways [196]. HMW-HA has proangiogenic properties concerning GAG-mediated effects, including the TSG-6-dependent priming of breast cancer-associated monocytes/macrophages in the tumor microenvironment [197].

Finally, the sixth of the original hallmarks of cancer, activating invasion and metastasis, is modulated by numerous GAGs and PGs. Degradation of basement membrane HSPGs by heparanase promotes local invasion and the metastatic spreading of tumors by removing steric constraints. Moreover, the downregulation of syndecan-1 in breast cancer cells promotes cell motility and invasiveness in a focal-adhesion kinase- and Rho-GTPase-dependent manner, suggesting a cross-talk with integrin-associated pathways. Considering GAG-dependent effects, overexpression of the HS 2-*O*-sulfotransferase HS2ST1 reduces invasion and cell motility of MDA-MB-231 breast cancer cells in an HS-dependent manner. In contrast, overexpression of the HS3-*O*-sulfotransferase HS3ST2 increases invasiveness, suggesting a differential impact of specific HS sulfation patterns on cell behavior [188]. Another critical mechanism promoting the invasive behavior of cancer cells is the EMT process by which epithelial cells loosen cell–cell contacts and acquire a migratory mesenchymal-like phenotype more prone to metastasis. PGs and GAGs contribute to this proinvasive behavior. Reduced expression of syndecan-1 during development and in breast cancer cells correlates with a reduction in the membrane expression of the epithelial

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marker and cell–cell adhesion molecule E-cadherin and acquisition of a migratory phenotype [198]. Treatment of prostate cancer cells with heparanase results in a downregulation of syndecan-1, E-cadherin, and multiple mesenchymal markers, demonstrating an impact of HS on the process of EMT [199]. The upregulation of the HS 6-*O*-sulfate editing enzyme Sulf-2 in hepatocellular carcinoma cells results in the activation of the TGFβ pathway and induces EMT in cocultured cancer-associated fibroblasts [200]. Overall, these examples provide ample evidence for the role of PGs and GAGs as modulators of the hallmarks of cancer as key steps of tumor progression.

Glycosaminoglycans and PGs are also involved in gastrointestinal cancers induced by the gastric pathogen *Helicobacter pylori*, which is considered a carcinogenic agent, as chronic infection with *H. pylori* is associated with a higher risk of developing gastric cancer. *Helicobacter pylori* colonizes the gastric epithelium by binding to glycan epitopes, mostly Lewis antigens but also HS chains. Highly pathogenic strains of *H. pylori*, harboring the cagPAI pathogenicity island, induce syndecan-4 expression, which can contribute toward the higher aggressiveness of these strains. The increased expression of syndecan-4 in gastric tumors of the intestinal subtype is associated with poor patient prognosis and with gastric cancer cell aggressive features such as higher migration and invasion [117]. Syndecan-4 bearing HS chains is present in the extracellular vesicles secreted by gastric cancer cells and impacts their uptake by recipient cells, therefore affecting their organ distribution. Moreover, syndecan-4 knockout disrupts the tropism of extracellular vesicles for the common gastric cancer metastatic sites [117]. Several glycosylation modifications have been reported in gastric cancer, including aberrant synthesis of GAGs by the tumor cells. Human gastric carcinoma tissue generally contains more GAGs than normal tissues and is enriched in CS chains. Nonsulfated CS/DS levels are significantly increased in gastric cancer compared to normal tissues, whereas HA level is significantly decreased in advanced gastric cancer [201]. Regarding HS biosynthesis, microRNA (miR-191)-mediated downregulation of NDST1 results in apoptosis resistance in a human gastric carcinoma cell line, demonstrating an impact of HS structural modification on this process. The deregulation of HS biosynthetic machinery has been described as an important event underlying HS abnormal abundance in cancer [202]. The lack of EXTL2 enzymatic activity results in the remodeling of GAGs on gastric cancer cell glycocalyx, promoting syndecan-4 expression and overproduction of HS with an altered sulfation profile. These changes promote a more aggressive and invasive phenotype [49].

What remains to be solved? *Considering the variety of functions of cell-surface HSPGs, the contribution of the individual PGs, and their potential compensatory effects warrant further investigations. As a given cell displays multiple HSPGs on its surface, the question arises why a process like a coreceptor function for growth factor-mediated signaling cannot be taken over to a large extent by another PG when one of them is downregulated. Another question refers to our understanding of how GAG chains organize and associate with other constituents in the ECM or at the cell surface. Difficulties in analyzing the structure of these highly complex molecules* in vivo *have hampered our conceptual understanding of this structural integration. A last open question concerns interpreting context-dependent effects of PGs and GAGs in a cancer setting. For example, upregulation of syndecan-1 is associated with a poor prognosis in breast cancer but with a good prognosis in colon cancer. This feature is also reflected by* in vitro *findings showing that experimental knockdown of syndecan-1 in breast cancer cells weakens the cancer stem cell phenotype, which is enhanced in colon cancer cells* [113,114]. *Surprisingly, there is even an overlap of molecular pathways affected by syndecan-1 depletion in both models (e.g., IL-6 signaling, FAK signaling), though the consequences on the cell phenotype are quite different* [114]. *The multifunctional role of syndecan-1 and differential expression of multiple HS-dependent signaling pathways in various tumor entities may explain such differences, but more research is needed to solve this point. In the era of personalized cancer treatment, there is an urgent need for highly specific biomarkers for early diagnosis, improving patient stratification and enlarging the repertoire of therapeutic targets. Other relevant challenges include (1) identification of key enzymatic regulatory events that underlie GAG aberrant synthesis in tumors; (2) understanding the molecular mechanisms through which aberrant GAGosylation regulates tumor cell signaling and disease progression; (3) how to distinguish between the contribution of the core protein and GAG chains to observed functions; (4) how to obtain detailed structural information about cancer-associated site-specific GAG features, particularly regarding sulfation profiles; and (5) identification of cancer cell GAGosylation profiles that would allow specific targeting and avoid therapy side effects.*

The human GAGome in cancer

Glycosaminoglycans have been investigated as potential biomarkers for MPSs and other disorders,

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including encephalopathy, epilepsy, fatty acid metabolism, respiratory, renal, and liver disorders. Their serum levels were increased in patients with encephalopathy, but further studies are needed to draw firm conclusions [203]. Since GAGs are important players in molecular events underlying cancer progression, and since their abundance and structural features, such as length and sulfation, are altered in tumors as discussed in the section on HA signaling in health and disease, they are good biomarker candidates. CS, HS, and HA has been investigated in blood, tissues, or urine of patients with various cancer types to determine their potential clinical applications [204,205]. A GAG score based on the concentrations and sulfation of CS and HS in plasma and urine with a high sensitivity and specificity for the occurrence of metastatic clear cell renal cell carcinoma has been developed [205].

Oncofetal CS (ofCS), which is highly sulfated on C4 of the vast majority of GalNAc residues [123], is covalently attached to CD44, CSPG4, and syndecan-1 in cancer cells or secreted into the cell microenvironment and body fluids. The plasma levels of ofCS/ofCSPGs are significantly higher in cancer patients and could serve as potential plasma biomarkers in cancer [206]. The detection of GAGs in body fluids is a promising strategy for noninvasive cancer diagnosis [207].

Glycosaminoglycan biosynthesis can be altered in diseases, as discussed in the section on GAGs and their protein moieties in disease, due to changes in the expression of their biosynthetic enzymes, but the quantitation of GAGs in biological samples is not straightforward. However, a method has been recently developed to characterize human-free GAGomes (the protein-free fraction of GAGomes) in body fluids based on the quantification of 15 CS, HS, and HA disaccharides. The GAGome profiling is performed by the enzymatic release of GAG disaccharides, which are labeled, separated by ultra-high-performance liquid chromatography, and detected by electrospray ionization triple-quadrupole mass spectrometry through multiple reaction monitoring [208]. The levels of free GAGs have been measured in the urine and plasma of healthy adults using this approach to determine their reference intervals, which is a prerequisite for using GAGomes as disease biomarkers. There are no significant variations in human GAGomes related to age, but the concentration of several CS and HS disaccharides is higher in males [209]. Urine and plasma GAGomes are altered in cancer, and they can be used as tumor metabolism biomarkers for multicancer early detection, as shown in a study including 14 cancer types and about 2000 samples from 1260 cancer or healthy subjects [207]. GAGome profiling could perform better than genomic biomarkers in detecting early-stage cancers.

An ultra-high sensitivity method combining reverse-phase HPLC and laser-induced fluorescence detection of BODIPY-FL-labeled disaccharides enable detection of HS disaccharides in the zeptomolar range, and HS disaccharide compositional analysis from minute tissue samples [210].

What are the future applications? *The development of the above methods and their use in large-scale studies will allow GAG profiling in various cancers at different stages and the monitoring of GAGome variations in the course of cancer and other diseases such as inflammatory and fibrotic diseases, and hence the mapping of GAG alterations and their role(s) in diseases.*

Sulfated GAGs and PGs in inflammatory processes

Inflammation, a regulated immune response to exogenous or endogenous stimuli, is a hallmark associated with the pathology of various diseases. The role of sterile inflammation in disease ontology and progression has recently gained considerable interest [211,212]. The small leucine-rich PGs (SLRPs) structurally characterized into five distinct classes are the architectural regulators of the ECM composed of a central protein core with leucine-rich repeat (LRR) motifs covalently linked to GAG chains [213]. Secreted SLRPs in their soluble form function as DAMPs or signaling molecules capable of interacting with diverse receptors regulating immune response pathways and autophagy, resulting in either resolution or progression of inflammation, thereby playing a decisive role in the prognosis, pathogenesis, and therapeutic outcome of diverse inflammatory diseases [118,214,215]. Biglycan and decorin are the best-characterized members of the class I SLRP family with negatively charged CS or DS chains [213]. Both biglycan and decorin are DAMPs capable of inducing autophagy [119,214], playing a decisive role in regulating the delicate balance between inflammation and cell death, leading to either resolution or chronification of disease. The role of these two SLRPs in the pathophysiology of inflammation-associated diseases is briefly discussed next and summarized in Fig. 8.

Role of biglycan in the inflammatory milieu

In response to tissue stress and injury, both *de novo* synthesized and soluble biglycan released from the ECM act as an endogenous ligand of innate immunity triggering signaling via interaction with diverse

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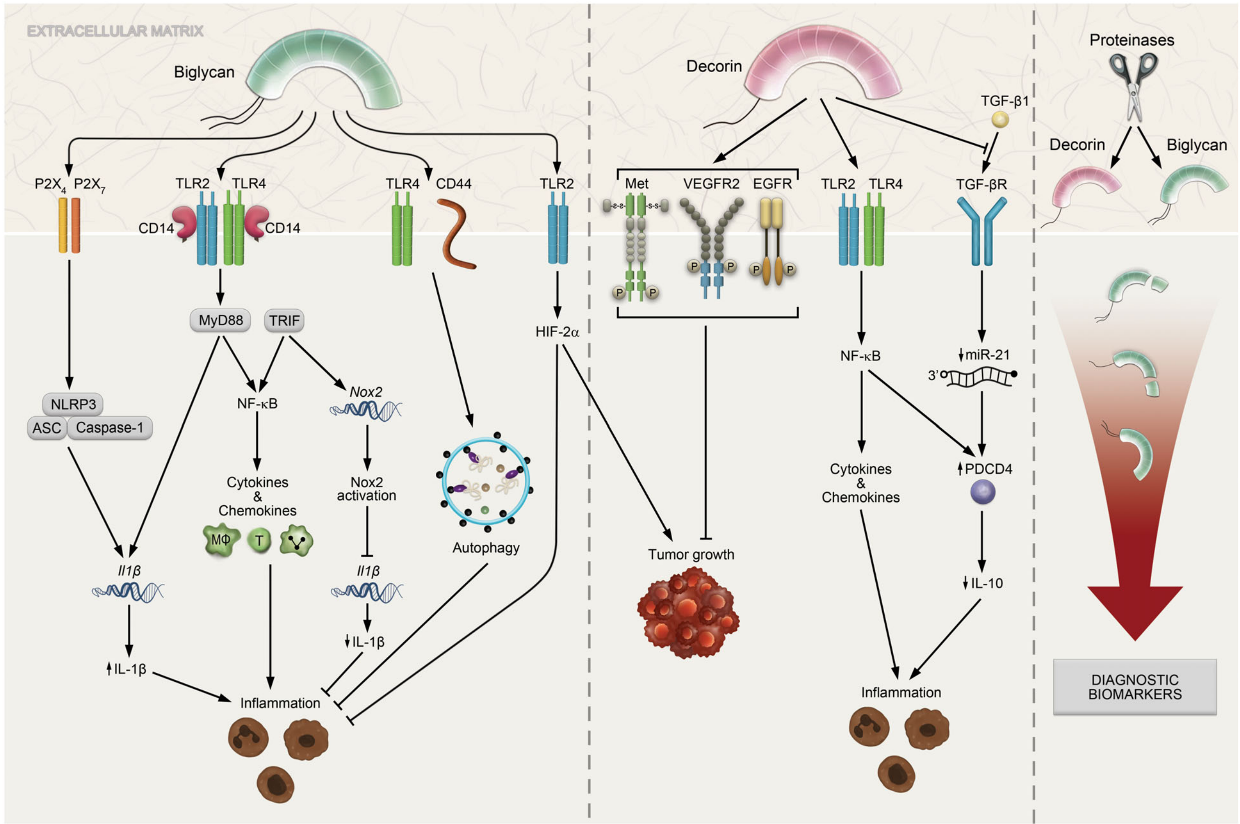

Fig. 8. Role of biglycan and decorin in the inflammatory milieu. (A) Biglycan determines pro- and anti-inflammatory signaling response by switching between TLR2/TLR4/CD14, $P2X_4/P2X_7$, and TLR4/CD44 axes. Biglycan induces proinflammatory signaling by clustering the purinergic receptors $P2X_4/P2X_7$ to trigger the NLRP3 inflammasome assembly leading to the turnover of pro-IL-1β, by activated caspase-1, to active IL-1β. Soluble biglycan via TLR2/TLR4/CD14 activates proinflammatory NF-κB signaling, leading to chemokine and cytokine production, immune cell recruitment, and pro-IL-1β production. Together, these responses augment inflammation, leading to the chronification of disease. Biglycan inhibits inflammation through the TLR4/CD44 signaling-induced autophagy and TLR2/TLR4/TRIF pathway-induced expression of NOX2, leading to the inhibition of biglycan-TLR2/TLR4/MyD88-mediated IL-1β production. These responses limit unmitigated inflammation, leading to the resolution of diseases. Soluble biglycan inhibits inflammation and induces tumor-associated angiogenesis by triggering TLR2-dependent HIF-2α production. (B) Decorin-mediated signaling in inflammation and tumorigenesis. Decorin inhibits tumorigenesis by binding and signaling via the Met, EGFR, and VEGFR2 receptors. Decorin promotes innate immunity and inflammation by a dual mechanism. By binding to TLR2/TLR4, decorin activates NF-κB signaling and induces the expression of proinflammatory cytokines. As an endogenous antagonist of TGFβ1, decorin blocks TGFβ1 binding and subsequent activation of the TGFβ receptor (TGFβR), thus inhibiting the maturation of microRNA-21, a post-transcriptional inhibitor of PDCD4. Increased PDCD4 abundance reduces levels of IL-10, resulting in augmented inflammation. (C) During disease progression, *de novo* synthesized and proteinases cleaved ECM-bound biglycan and decorin released into systemic circulation are promising prognostic markers for inflammatory diseases. ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; CD, cluster of differentiation; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; HIF, hypoxia-inducible factor; IL, interleukin; Met, mesenchymal–epithelial transition factor; miR, microRNA; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing 3; NOX, NADPH oxidase; PDCD4, programmed cell death protein 4; TGF, transforming growth factor; TLR, Toll-like receptor; TRIF, TIR domain-containing adaptor-inducing interferon-β; VEGFR2, vascular endothelial growth factor receptor 2.

receptors, including TLR2, TLR4, $P2X_4$, and $P2X_7$, coreceptor CD14, and adaptor molecules the myeloid differentiation primary response 88 (MyD88) or Toll/IL-1R domain-containing adapter inducing interferon (IFN)-β (TRIF). As a proinflammatory DAMP, biglycan activates NF-κB- and the (NOD-, LRR-, and pyrin domain-containing protein 3) NLRP3 inflammasome/caspase-1 pathways, and interleukin-1β (IL-1β) synthesis, triggering sterile inflammation [216]. On the contrary, as an anti-inflammatory signaling molecule, biglycan/TLR4/CD44-mediated autophagy leads to the resolution of inflammation and tissue

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repair [119]. Biglycan-induced expression of NADPH oxidase-2 (*Nox2*) via TLR4/TRIF and subsequent activation of NOX2 enzyme complex inhibits biglycan/TLR2/TLR4/MyD88-mediated IL-1β production and proinflammatory response [217]. The signaling ability of biglycan has significant clinical implications in inflammatory hepatic and renal diseases. As a chemical messenger, soluble biglycan can trigger inflammation in an autocrine and paracrine manner mediating cross-talk between organs as in hepatorenal dysfunction [118]. Liver injury/pathology triggers increased synthesis and release of biglycan in the systemic circulation, as evidenced in hepatitis B, suggesting a regulatory role in hepatic inflammation and autophagy, supporting its application as a diagnostic biomarker [124]. As an anti-inflammatory modulator, biglycan stabilizes the hypoxia-inducible factor 2α (HIF-2α), a master regulator of cellular hypoxia response in the liver and kidney [120]. Biglycan-TLR4-CD44-axis promotes anti-inflammatory M2 macrophage polarization, leading to the resolution of renal inflammation [119]. On the contrary, soluble biglycan/TLR/TRIF/MyD88-axis promotes proinflammatory signaling by triggering the synthesis of CXCL9, CXCL10, CCL20, and TLR2/4-dependent expression of CXCL13 to recruit neutrophils, macrophages, B and T cells to the diseased kidney [218]. Enhanced biglycan upregulates the expression of MCP-1, RANTES, MIP-1α, and TNFα cytokines in the kidney. Accordingly, deficiency of biglycan ameliorates pathogen-mediated and sterile inflammatory disease outcomes, including sepsis, lupus nephritis, insulin-dependent diabetes mellitus, and autoimmune perimyocarditis. Thus, based on the physiological context of the cellular microenvironment, biglycan regulates inflammation either as a proinflammatory or anti-inflammatory molecule. Soluble biglycan autonomously triggers sterile inflammation or potentiates inflammatory response in pathogen-mediated diseases. Understanding the distinct types of signaling initiated by biglycan in a pathophysiological scenario is crucial to help decide the appropriate therapeutic intervention and disease outcome.

Role of decorin in the inflammatory milieu

The therapeutic benefits of soluble decorin as an antifibrotic and antitumorigenic agent are attributed to regulating several biological processes, including innate immunity, inflammation, autophagy, wound healing, and angiogenesis. Decorin by binding and signaling via the receptor tyrosine kinases Met, epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor, vascular endothelial growth factor receptor 2 (VEGFR2), and a ligand to the innate immunity receptors TLR2 and TLR4, is a potent modulator of inflammation and angiogenesis in the pathophysiology of pathogen- and sterile-inflammatory diseases and tumorigenesis. Decorin-TLR2/4-signaling axis activates p38, MAPK, and NF-κB pathways, leading to enhanced expression of the proinflammatory cytokines TNF-α and IL-12 [120]. Decorin-mediated inactivation of TGFβ1 inhibits mature microRNA-21 (miR-21) to promote the transcription of the tumor suppressor programmed cell death protein 4 (PDCD4), a specific translational suppressor of IL-10, resulting in augmented levels of TNF-α and IL-12. Although decorin-PDCD4-microRNA-21-axis aggravates inflammation in sepsis, the same is beneficial in inhibiting tumor growth. The proinflammatory role of decorin is also evidenced in the pathology associated with chronic pancreatitis, delayed-type hypersensitivity, and allergic asthma.

Decorin is known to both promote and inhibit inflammation depending on the cellular microenvironment and distinct signaling pathways. In a pathophysiological inflammatory scenario, the potent anti-inflammatory effect of decorin was proven beneficial as a preclinical therapeutic agent for treating COVID-19-related complications [219]. Furthermore, pharmacological inhibition of the decorin-advanced glycosylation end-product-specific receptor axes in macrophages is protective against ferroptotic death-related acute pancreatitis, limiting the induction of tumor-protective immune response by ferroptotic cancer cells [121]. Thus, decorin promotes proinflammatory signaling as an endogenous ligand of TLR2/TLR4 and TGFβ1 inhibitor.

The antifibrotic effect of decorin mediated by its ability to bind and neutralize TGFβ signaling presents the therapeutic potential for the treatment of epidural fibrosis and adhesions after laminectomy [122], lung fibrosis, liver fibrosis [220], trabecular meshwork fibrosis in open-angle glaucoma, corneal scarring, diabetic cardiomyopathy, peritoneal fibrosis in patients on long-term peritoneal dialysis [221], recessive dystrophic epidermolysis bullosa [222], scar formation during wound healing [223], and tubulointerstitial fibrosis. The roles of PGs in pathogenesis and the involved signaling pathways are summarized in Table 1.

Role of biglycan and decorin as biomarkers and therapeutic targets

Currently, hepatological therapeutics restrict the use of soluble biglycan as a noninvasive fibrosis marker with a significant correlation to developing hepatic necroinflammation, fibrosis, and disease severity [125]. Serum/

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soluble biglycan is used as a diagnostic biomarker to detect inflammatory renal diseases and multiple human cancers [126–128,224], Crohn's disease [225], early changes in the equine osteoarthritic subchondral bone [226], and systemic sclerosis [129]. Like biglycan, decorin is used as a prognostic biomarker to detect malignant peripheral nerve sheath tumors [130], oocyte potential [227], preeclampsia [131], fibrotic lung disorders [132,133], and fetal growth restriction. Serum decorin and biglycan are potential biomarkers in predicting preterm premature rupture of fetal membranes in early gestation.

At the therapeutic front, circulating biglycan is a promising marker for targeting statin therapy in patients with heart failure. Decorin is a predictive immunomarker of the clinical response to neoadjuvant chemotherapy (NAC) using S-1 (S-1 NAC) and patient prognosis in oral cancer. Decorin-mediated oncosuppression holds potential as a future adjuvant therapy for epithelial cancers [228]. Decorin gene therapy alleviates fibrosis and the progression of renal failure [229]. Thus, visualization of biglycan and decorin beyond the conceptualized inert ECM derivate might provide possibilities for multiple therapeutic interventions to combat inflammatory and fibrotic diseases. The putative roles of PGs as markers of diagnosis and disease progression are summarized in Table 2.

What remains to be solved? *In their soluble form, the SLRPs biglycan and decorin act as signaling molecules and are potent activators of sterile inflammation and autophagy with implications in the pathophysiology of several diseases. The choice of pro-, anti-inflammatory signaling, and autophagy by biglycan and decorin in the cellular context is decisive for disease and therapeutic outcomes. Studies elucidating tissue/cell type-specific and selective interactions of biglycan and decorin with receptors/adaptor molecules and interorgan cross-talk would help develop novel and efficient anti-inflammatory drugs. During the early stages of the disease, the accumulation of copious amounts of SLRPs released into systemic circulation by de novo synthesis and action of proteases as representative end products of ECM remodeling makes them promising prognostic biomarkers for the early detection and progression of diseases. Further studies are warranted to elucidate the role of biglycan and decorin-evoked signaling that harbors the potential for developing prognostic biomarkers and therapeutics for inflammatory and fibrotic diseases.*

GAGs as biomaterials

The therapeutic use of GAGs from various origins, such as marine sources, has been investigated, though potential drawbacks of CS use in medicine are reported [230]. Another critical area for future investigation is using GAGs and PGs as biomaterials and hydrogels for tissue engineering [231–234]. GAG-based biomaterials can be used for growth factor and cytokine delivery [235]. Collagen/GAG-based matrices enhance skin wound healing [236]. GAGs can be chemically modified [237] and then serve as building blocks for biomaterial coatings and hydrogels in regenerative medicine [238] as shown for ReGeneraTing Agents (RGTA®) mimicking HS [239]. GAG chemical derivatives can also be used for their biological and binding properties. sHA, for example, is antiangiogenic [240], antioncogenic [173], exhibits pro-osteogenic properties [241], and alters the formation of the complex formed by the tissue inhibitor of metalloproteinase-3 with the low-density lipoprotein receptor-related protein LRP-1 [242]. GAGs and their derivatives can also be used as bioinks for bioprinting as reported for HA [243,244], and CS and DS in cartilage regeneration [245].

GAG and PG interactions and interactomes

Biological functions being mediated by interactions, identifying GAG-binding proteins is crucial to determine the functional repertoire of GAGs and to understand the role of GAG–protein interactions in diseases [246]. Interactions were first identified using low-throughput biochemical and biophysical techniques performed with purified GAGs and proteins. However, numerous GAG-binding proteins have been identified in the last 10 years via high-throughput techniques such as GAG or protein microarrays [247,248], and affinity proteomics using various biological samples [249]. Building and analyzing the GAG interaction network gives new insights into the molecular functions of GAG-binding proteins and the biological pathways and processes they are involved in [250]. GAG networks can be generated using the MatrixDB database, which focuses on interactions established by extracellular proteins and GAGs [251,252].

The most comprehensive GAG interactome generated so far comprises 4290 interactions and 3464 unique GAG-binding proteins [61,253]. In contrast to studies carried out with purified molecules, proteomic-based approaches identify both direct and indirect GAG–protein interactions (i.e., those which might be mediated *in vivo* via a third biomolecule), but they can be used to generate GAG interaction networks in subcellular compartments, cells, tissues, and biological fluids in health and diseases. HA, HS, and several PG core proteins

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such as glypican, biglycan, perlecan [254], and syndecan-1 [255], for example, translocate to the nucleus in various conditions. Identifying GAG-binding proteins in the nucleus using affinity–mass spectrometry could provide new insights into the biological functions of GAGs and PGs in this organelle. The analysis of the nuclear interactome of syndecan-1 in mesothelioma cells has identified the role of this syndecan in RNA biogenesis [256], and the analyses of cardiac interactomes of syndecans 2 and 4 have shown that syndecan-4 plays a role in the nuclear translocation of the muscle LIM protein [257,258]. The consensus interactome of the four syndecans gathering their common partners has been used to predict the canonical functions of the syndecan family mediated by these partners [259].

What remains to be solved? *GAGs regulate the formation of several functional complexes, including signaling complexes* [260]. *The structure and dynamics of GAG–protein and PG–protein complexes are needed to decipher the molecular mechanisms regulating GAG functions. This will be achieved by combining NMR spectroscopy, molecular modeling, and molecular dynamics as done for HA–protein complexes* [261] *and several GAG–protein complexes* [262–264]. *One challenge will be to simulate GAG chains with lengths similar to those found* in vivo *and to extend this approach to more GAG-binding proteins. Kinetics and affinity* [265], *specificity of GAG–protein interactions* [60,266], *and GAG sequences binding to proteins warrant further investigation to better understand their relationships with GAG molecular recognition and functions. This will require routine GAG sequencing techniques* [267]. *Solid-state nanopore sequencing of GAGs* [268–270] *will undoubtedly be helpful for this purpose. The discovery of bacterial exolytic heparinases that cleave heparin chains from their reducing ends should also ease HP/HS sequencing* [271]. *A deeper understanding of the selectivity of GAG–protein interactions will open new perspectives to design inhibitors of GAG–protein complexes targeted for therapeutic purposes and GAG-based drugs. Another mechanism worth exploring is to determine how the protein ligands of cell surface receptors move through the pericellular layer to reach their receptors and if GAGs displayed at the cell surface contribute to the mobility of cell surface receptors. The pericellular coat formed by HA and the picket fence formed by its receptor CD44 limit the mobility of phagocytic receptors* [272], *and it will be interesting to determine if this could happen for other GAGs and the receptors they bind to. The role of the cell membrane in the organization of cell surface PGs will be investigated* in situ *using nonlinear spectroscopic methods such as vibrational sum-frequency generation spectroscopy, which has been developed to analyze GAGs at biological interfaces* [273]. *Last, more cell-, tissue-, and disease-specific GAG interactomes should be generated by carrying out affinity proteomics with biological samples of specific cells, tissues, or diseases, and/or by integrating glycomics datasets in the GAG interactome 2.0* [61] *to identify GAG-specific interaction repertoires. The curation of the GAG interactions included in this comprehensive network and their integration in publicly available databases such as MatrixDB focused on ECM and GAGs* [251] *and the large global IntAct database* [274] *will result in a freely available curated GAG interaction dataset to be reused by the GAG and ECM communities.*

Conclusion

Glycosaminoglycans were first considered as structural components of the ECM and were then found to regulate numerous biological processes such as signaling, cell–matrix and host–pathogen interactions. This article focuses on the biological landscape of GAGs in complement to their structural and analytical characteristics discussed in a companion article [2]. It highlights recent findings on the biosynthesis of GAGs, their roles in health and disease, both as individual glycans and as part of PGs, and on the major challenges to overcome to fully understand their biological functions and the molecular mechanisms underlying them, to develop new therapeutic applications, new GAG mimetics or GAG-based therapeutic strategies, and to explore their use as biomarkers for diagnosing diseases and monitoring their progression.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All the authors, SR-B, RRV, LS, MG, RM, AP, PH, AM, CAR, SSS, NKK, SP, and DN, contributed to the manuscript writing, and designed figures, SP

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optimized the figures, SR-B drafted the manuscript, SR-B, SP, and DN revised the manuscript.

References

1 Merry CLR (2021) Exciting new developments and emerging themes in glycosaminoglycan research. *J Histochem Cytochem* **69**, 9–11.
2 Perez S, Makshakova O, Angulo J, Bedini E, Bisio A, de Paz JL, Fadda E, Guerrini M, Hricovini M, Hricovini M *et al.* (2023) Glycosaminoglycans: what remains to be deciphered? *JACS Au* **3**, 628–656.
3 Mii Y & Takada S (2020) Heparan sulfate proteoglycan clustering in Wnt signaling and dispersal. *Front Cell Dev Biol* **8**, 631.
4 Rushton E, Kopke DL & Broadie K (2020) Extracellular heparan sulfate proteoglycans and glycan-binding lectins orchestrate trans-synaptic signaling. *J Cell Sci* **133**, jcs244186.
5 Merry CLR, Lindahl U, Couchman J & Esko JD (2022) Proteoglycans and sulfated Glycosaminoglycans. In Essentials of Glycobiology (Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Mohnen D, Kinoshita T, Packer NH, Prestegard JH, *et al.*, eds), 4th edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
6 Hayes AJ & Melrose J (2023) HS, an ancient molecular recognition and information storage glycosaminoglycan, equips HS-proteoglycans with diverse matrix and cell-interactive properties operative in tissue development and tissue function in health and disease. *Int J Mol Sci* **24**, 1148.
7 Mikami T & Kitagawa H (2023) Chondroitin sulfate glycosaminoglycans function as extra/pericellular ligands for cell surface receptors. *J Biochem* **173**, mvac110.
8 Skandalis SS, Karalis T & Heldin P (2020) Intracellular hyaluronan: importance for cellular functions. *Semin Cancer Biol* **62**, 20–30.
9 Tian X, Azpurua J, Hine C, Vaidya A, Myakishev-Rempel M, Ablaeva J, Mao Z, Nevo E, Gorbunova V & Seluanov A (2013) High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* **499**, 346–349.
10 Berdiaki A, Neagu M, Spyridaki I, Kuskov A, Perez S & Nikitovic D (2023) Hyaluronan and reactive oxygen species signaling-novel cues from the matrix? *Antioxidants (Basel)* **12**, 824.
11 Tavianatou AG, Caon I, Franchi M, Piperigkou Z, Galesso D & Karamanos NK (2019) Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer. *FEBS J* **286**, 2883–2908.
12 Day AJ & Milner CM (2019) TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. *Matrix Biol* **78–79**, 60–83.
13 Rahman AA, Soto-Avellaneda A, Yong Jin H, Stojkovska I, Lai NK, Albright JE, Webb AR, Oe E, Valarde JP, Oxford AE *et al.* (2020) Enhanced Hyaluronan signaling and autophagy dysfunction by VPS35 D620N. *Neuroscience* **441**, 33–45.
14 Caon I, Parnigoni A, Viola M, Karousou E, Passi A & Vigetti D (2021) Cell energy metabolism and Hyaluronan synthesis. *J Histochem Cytochem* **69**, 35–47.
15 DeAngelis PL & Zimmer J (2023) Hyaluronan synthases; mechanisms, myths, & mysteries of three types of unique bifunctional glycosyltransferases. *Glycobiology* **33**, cwad075.
16 Kessler SP, Obery DR & de la Motte C (2015) Hyaluronan synthase 3 null mice exhibit decreased intestinal inflammation and tissue damage in the DSS-induced colitis model. *Int J Cell Biol* **2015**, 745237.
17 Homann S, Grandoch M, Kiene LS, Podsvyadek Y, Feldmann K, Rabausch B, Nagy N, Lehr S, Kretschmer I, Oberhuber A *et al.* (2018) Hyaluronan synthase 3 promotes plaque inflammation and atheroprogression. *Matrix Biol* **66**, 67–80.
18 Amargant F, Manuel SL, Tu Q, Parkes WS, Rivas F, Zhou LT, Rowley JE, Villanueva CE, Hornick JE, Shekhawat GS *et al.* (2020) Ovarian stiffness increases with age in the mammalian ovary and depends on collagen and hyaluronan matrices. *Aging Cell* **19**, e13259.
19 Caon I, Bartolini B, Parnigoni A, Caravà E, Moretto P, Viola M, Karousou E, Vigetti D & Passi A (2020) Revisiting the hallmarks of cancer: the role of hyaluronan. *Semin Cancer Biol* **62**, 9–19.
20 Tavianatou A-G, Piperigkou Z, Barbera C, Beninatto R, Masola V, Caon I, Onisto M, Franchi M, Galesso D & Karamanos NK (2019) Molecular size-dependent specificity of hyaluronan on functional properties, morphology and matrix composition of mammary cancer cells. *Matrix Biol Plus* **3**, 100008.
21 Caon I, Bartolini B, Moretto P, Parnigoni A, Caravà E, Vitale DL, Alaniz L, Viola M, Karousou E, De Luca G *et al.* (2020) Sirtuin 1 reduces hyaluronan synthase 2 expression by inhibiting nuclear translocation of NF-κB and expression of the long-noncoding RNA HAS2-AS1. *J Biol Chem* **295**, 3485–3496.
22 Yang YM, Noureddin M, Liu C, Ohashi K, Kim SY, Ramnath D, Powell EE, Sweet MJ, Roh YS, Hsin I-F *et al.* (2019) Hyaluronan synthase 2-mediated hyaluronan production mediates Notch1 activation and liver fibrosis. *Sci Transl Med* **11**, eaat9284.
23 Parnigoni A, Caon I, Moretto P, Viola M, Karousou E, Passi A & Vigetti D (2021) The role of the multifaceted long non-coding RNAs: a nuclear-cytosolic interplay to regulate hyaluronan metabolism. *Matrix Biol Plus* **11**, 100060.

24 Röck K, Tigges J, Sass S, Schütze A, Florea A-M, Fender AC, Theis FJ, Krutmann J, Boege F, Fritsche E *et al*. (2015) miR-23a-3p causes cellular senescence by targeting hyaluronan synthase 2: possible implication for skin aging. *J Invest Dermatol* **135**, 369–377.
25 Pan B, Toms D & Li J (2018) MicroRNA-574 suppresses oocyte maturation via targeting hyaluronan synthase 2 in porcine cumulus cells. *Am J Physiol Cell Physiol* **314**, C268–C277.
26 Melero-Fernandez de Mera RM, Arasu UT, Kärnä R, Oikari S, Rilla K, Vigetti D, Passi A, Heldin P, Tammi MI & Deen AJ (2019) Effects of mutations in the post-translational modification sites on the trafficking of hyaluronan synthase 2 (HAS2). *Matrix Biol* **80**, 85–103.
27 Kasai K, Kuroda Y, Takabuchi Y, Nitta A, Kobayashi T, Nozaka H, Miura T & Nakamura T (2020) Phosphorylation of Thr328 in hyaluronan synthase 2 is essential for hyaluronan synthesis. *Biochem Biophys Res Commun* **533**, 732–738.
28 Vigetti D, Viola M, Karousou E, De Luca G & Passi A (2014) Metabolic control of hyaluronan synthases. *Matrix Biol* **35**, 8–13.
29 Viola M, Brüggemann K, Karousou E, Caon I, Caravà E, Vigetti D, Greve B, Stock C, De Luca G, Passi A *et al*. (2017) MDA-MB-231 breast cancer cell viability, motility and matrix adhesion are regulated by a complex interplay of heparan sulfate, chondroitin−/dermatan sulfate and hyaluronan biosynthesis. *Glycoconj J* **34**, 411–420.
30 Oikari S, Makkonen K, Deen AJ, Tyni I, Kärnä R, Tammi RH & Tammi MI (2016) Hexosamine biosynthesis in keratinocytes: roles of GFAT and GNPDA enzymes in the maintenance of UDP-GlcNAc content and hyaluronan synthesis. *Glycobiology* **26**, 710–722.
31 Maloney FP, Kuklewicz J, Corey RA, Bi Y, Ho R, Mateusiak L, Pardon E, Steyaert J, Stansfeld PJ & Zimmer J (2022) Structure, substrate recognition and initiation of hyaluronan synthase. *Nature* **604**, 195–201.
32 Neelamegham S, Aoki-Kinoshita K, Bolton E, Frank M, Lisacek F, Lütteke T, O'Boyle N, Packer NH, Stanley P, Toukach P *et al*. (2019) Updates to the symbol nomenclature for Glycans guidelines. *Glycobiology* **29**, 620–624.
33 Chen CG & Iozzo RV (2020) Angiostatic cues from the matrix: endothelial cell autophagy meets hyaluronan biology. *J Biol Chem* **295**, 16797–16812.
34 Thelin MA, Bartolini B, Axelsson J, Gustafsson R, Tykesson E, Pera E, Oldberg Å, Maccarana M & Malmstrom A (2013) Biological functions of iduronic acid in chondroitin/dermatan sulfate. *FEBS J* **280**, 2431–2446.
35 Fawcett JW & Kwok JCF (2022) Proteoglycan sulphation in the function of the mature central nervous system. *Front Integr Neurosci* **16**, 895493.
36 Noborn F, Nilsson J, Sihlbom C, Nikpour M, Kjellén L & Larson G (2023) Mapping the human chondroitin sulfate glycoproteome reveals an unexpected correlation between glycan sulfation and attachment site characteristics. *Mol Cell Proteomics* **22**, 100617.
37 Mizumoto S & Yamada S (2023) Histories of dermatan sulfate epimerase and dermatan 4-O-sulfotransferase from discovery of their enzymes and genes to Musculocontractural Ehlers-Danlos syndrome. *Genes (Basel)* **14**, 509.
38 Mizumoto S & Yamada S (2021) An overview of in vivo functions of chondroitin sulfate and dermatan sulfate revealed by their deficient mice. *Front Cell Dev Biol* **9**, 764781.
39 Izumikawa T, Koike T, Shiozawa S, Sugahara K, Tamura J & Kitagawa H (2008) Identification of chondroitin sulfate glucuronyltransferase as chondroitin synthase-3 involved in chondroitin polymerization: chondroitin polymerization is achieved by multiple enzyme complexes consisting of chondroitin synthase family members. *J Biol Chem* **283**, 11396–11406.
40 Zimmer BM, Barycki JJ & Simpson MA (2021) Integration of sugar metabolism and proteoglycan synthesis by UDP-glucose dehydrogenase. *J Histochem Cytochem* **69**, 13–23.
41 Parker JL & Newstead S (2019) Gateway to the Golgi: molecular mechanisms of nucleotide sugar transporters. *Curr Opin Struct Biol* **57**, 127–134.
42 Pągielska M & Samsonov SA (2023) Molecular dynamics-based comparative analysis of chondroitin and dermatan sulfates. *Biomolecules* **13**, 247.
43 Syx D, Delbaere S, Bui C, De Clercq A, Larson G, Mizumoto S, Kosho T, Fournel-Gigleux S & Malfait F (2022) Alterations in glycosaminoglycan biosynthesis associated with the Ehlers-Danlos syndromes. *Am J Physiol Cell Physiol* **323**, C1843–C1859.
44 Mizumoto S & Yamada S (2022) The specific role of dermatan sulfate as an instructive glycosaminoglycan in tissue development. *Int J Mol Sci* **23**, 7485.
45 Melrose J (2019) Functional consequences of Keratan sulfate Sulfation in Electrosensory tissues and in neuronal regulation. *Adv Biosyst* **3**, e1800327.
46 Caterson B & Melrose J (2018) Keratan sulfate, a complex glycosaminoglycan with unique functional capability. *Glycobiology* **28**, 182–206.
47 Yasuoka Y (2023) Tissue-specific expression of carbohydrate sulfotransferases drives keratan sulfate biosynthesis in the notochord and otic vesicles of *Xenopus* embryos. *Front Cell Dev Biol* **11**, 957805.
48 Leiphrakpam PD, Patil PP, Remmers N, Swanson B, Grandgenett PM, Qiu F, Yu F & Radhakrishnan P

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(2019) Role of keratan sulfate expression in human pancreatic cancer malignancy. *Sci Rep* **9**, 9665.
49 Marques C, Poças J, Gomes C, Faria-Ramos I, Reis CA, Vivès RR & Magalhães A (2022) Glycosyltransferases EXTL2 and EXTL3 cellular balance dictates heparan sulfate biosynthesis and shapes gastric cancer cell motility and invasion. *J Biol Chem* **298**, 102546.
50 Li J-P & Kusche-Gullberg M (2016) Heparan sulfate: biosynthesis, structure, and function. *Int Rev Cell Mol Biol* **325**, 215–273.
51 Annaval T, Wild R, Crétinon Y, Sadir R, Vivès RR & Lortat-Jacob H (2020) Heparan sulfate proteoglycans biosynthesis and post synthesis mechanisms combine few enzymes and few Core proteins to generate extensive structural and functional diversity. *Molecules* **25**, 4215.
52 Presto J, Thuveson M, Carlsson P, Busse M, Wilén M, Eriksson I, Kusche-Gullberg M & Kjellén L (2008) Heparan sulfate biosynthesis enzymes EXT1 and EXT2 affect NDST1 expression and heparan sulfate sulfation. *Proc Natl Acad Sci USA* **105**, 4751–4756.
53 Leisico F, Omeiri J, Le Narvor C, Beaudouin J, Hons M, Fenel D, Schoehn G, Couté Y, Bonnaffé D, Sadir R *et al*. (2022) Structure of the human heparan sulfate polymerase complex EXT1-EXT2. *Nat Commun* **13**, 7110.
54 Li H, Chapla D, Amos RA, Ramiah A, Moremen KW & Li H (2023) Structural basis for heparan sulfate co-polymerase action by the EXT1-2 complex. *Nat Chem Biol* **19**, 565–574.
55 Vallet SD, Annaval T, Vives RR, Richard E, Hénault J, Le Narvor C, Bonnaffé D, Priem B, Wild R & Lortat-Jacob H (2023) Functional and structural insights into human N-deacetylase/N-sulfotransferase activities. *Proteoglycan Res* **1**, e8.
56 Préchoux A, Halimi C, Simorre J-P, Lortat-Jacob H & Laguri C (2015) C5-epimerase and 2-O-sulfotransferase associate in vitro to generate contiguous epimerized and 2-O-sulfated heparan sulfate domains. *ACS Chem Biol* **10**, 1064–1071.
57 Zhang T, Yu M, Li H, Maccarana M, Zhang W, Shi D, Kan Y, Zhang X, Chi L, Lindahl U *et al*. (2023) Interacting polymer-modification enzymes in heparan sulfate biosynthesis. *Carbohydr Polym* **299**, 120191.
58 Chen Y-H, Narimatsu Y, Clausen TM, Gomes C, Karlsson R, Steentoft C, Spliid CB, Gustavsson T, Salanti A, Persson A *et al*. (2018) The GAGOme: a cell-based library of displayed glycosaminoglycans. *Nat Methods* **15**, 881–888.
59 Qiu H, Shi S, Yue J, Xin M, Nairn AV, Lin L, Liu X, Li G, Archer-Hartmann SA, Dela Rosa M *et al*. (2018) A mutant-cell library for systematic analysis of heparan sulfate structure-function relationships. *Nat Methods* **15**, 889–899.
60 Kjellén L & Lindahl U (2018) Specificity of glycosaminoglycan-protein interactions. *Curr Opin Struct Biol* **50**, 101–108.
61 Vallet SD, Berthollier C & Ricard-Blum S (2022) The glycosaminoglycan interactome 2.0. *Am J Physiol Cell Physiol* **322**, C1271–C1278.
62 Masola V, Bellin G, Gambaro G & Onisto M (2018) Heparanase: a multitasking protein involved in extracellular matrix (ECM) remodeling and intracellular events. *Cell* **7**, 236.
63 Vlodavsky I, Sanderson RD & Ilan N (2020) Heparanase: From Basic Research to Clinical Applications. Springer Nature, Cham, Switzerland.
64 Sanderson RD, Elkin M, Rapraeger AC, Ilan N & Vlodavsky I (2017) Heparanase regulation of cancer, autophagy and inflammation: new mechanisms and targets for therapy. *FEBS J* **284**, 42–55.
65 Wu L, Viola CM, Brzozowski AM & Davies GJ (2015) Structural characterization of human heparanase reveals insights into substrate recognition. *Nat Struct Mol Biol* **22**, 1016–1022.
66 Vlodavsky I, Gross-Cohen M, Weissmann M, Ilan N & Sanderson RD (2018) Opposing functions of Heparanase-1 and Heparanase-2 in cancer progression. *Trends Biochem Sci* **43**, 18–31.
67 Jayatilleke KM & Hulett MD (2020) Heparanase and the hallmarks of cancer. *J Transl Med* **18**, 453.
68 Si J, Li W, Li X, Cao L, Chen Z & Jiang Z (2021) Heparanase confers temozolomide resistance by regulation of exosome secretion and circular RNA composition in glioma. *Cancer Sci* **112**, 3491–3506.
69 Simeonovic CJ, Popp SK, Brown DJ, Li F-J, Lafferty ARA, Freeman C & Parish CR (2020) Heparanase and type 1 diabetes. *Adv Exp Med Biol* **1221**, 607–630.
70 Masola V, Gambaro G & Onisto M (2020) Impact of Heparanse on organ fibrosis. *Adv Exp Med Biol* **1221**, 669–684.
71 Li J-P & Zhang X (2020) Implications of Heparan sulfate and Heparanase in amyloid diseases. *Adv Exp Med Biol* **1221**, 631–645.
72 Rabelink TJ, van den Berg BM, Garsen M, Wang G, Elkin M & van der Vlag J (2017) Heparanase: roles in cell survival, extracellular matrix remodelling and the development of kidney disease. *Nat Rev Nephrol* **13**, 201–212.
73 Nguyen TK, Paone S, Chan E, Poon IKH, Baxter AA, Thomas SR & Hulett MD (2022) Heparanase: a novel therapeutic target for the treatment of atherosclerosis. *Cell* **11**, 3198.
74 Agelidis A & Shukla D (2020) Heparanase, Heparan sulfate and viral infection. *Adv Exp Med Biol* **1221**, 759–770.
75 Lebsir N, Zoulim F & Grigorov B (2023) Heparanase-1: from cancer biology to a future antiviral target. *Viruses* **15**, 237.

17424658, 0, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.17107 by Asst Dei 7 Laghi, Wiley Online Library on [13/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

76 Mayfosh AJ, Nguyen TK & Hulett MD (2021) The Heparanase regulatory network in health and disease. *Int J Mol Sci* **22**, 11096.
77 Coombe DR & Gandhi NS (2019) Heparanase: a challenging cancer drug target. *Front Oncol* **9**, 1316.
78 de Boer C, Armstrong Z, Lit VAJ, Barash U, Ruijgrok G, Boyango I, Weitzenberg MM, Schröder SP, Sarris AJC, Meeuwenoord NJ *et al.* (2022) Mechanism-based heparanase inhibitors reduce cancer metastasis in vivo. *Proc Natl Acad Sci USA* **119**, e2203167119.
79 Chhabra M & Ferro V (2018) The development of assays for Heparanase enzymatic activity: towards a gold standard. *Molecules* **23**, 2971.
80 El Masri R, Seffouh A, Lortat-Jacob H & Vivès RR (2017) The "in and out" of glucosamine 6-O-sulfation: the 6th sense of heparan sulfate. *Glycoconj J* **34**, 285–298.
81 Seffouh I, Przybylski C, Seffouh A, El Masri R, Vivès RR, Gonnet F & Daniel R (2019) Mass spectrometry analysis of the human endosulfatase Hsulf-2. *Biochem Biophys Rep* **18**, 100617.
82 El Masri R, Seffouh A, Roelants C, Seffouh I, Gout E, Pérard J, Dalonneau F, Nishitsuji K, Noborn F, Nikpour M *et al.* (2022) Extracellular endosulfatase Sulf-2 harbors a chondroitin/dermatan sulfate chain that modulates its enzyme activity. *Cell Rep* **38**, 110516.
83 Seffouh A, El Masri R, Makshakova O, Gout E, Hassoun ZEO, Andrieu J-P, Lortat-Jacob H & Vivès RR (2019) Expression and purification of recombinant extracellular sulfatase HSulf-2 allows deciphering of enzyme sub-domain coordinated role for the binding and 6-O-desulfation of heparan sulfate. *Cell Mol Life Sci* **76**, 1807–1819.
84 El Masri R, Crétinon Y, Gout E & Vivès RR (2020) HS and inflammation: a potential playground for the Sulfs? *Front Immunol* **11**, 570.
85 Yang Y, Ahn J, Edwards NJ, Benicky J, Rozeboom AM, Davidson B, Karamboulas C, Nixon KCJ, Ailles L & Goldman R (2022) Extracellular Heparan 6-O-Endosulfatases SULF1 and SULF2 in head and Neck squamous cell carcinoma and other malignancies. *Cancers (Basel)* **14**, 5553.
86 Zaman G, Staines KA, Farquharson C, Newton PT, Dudhia J, Chenu C, Pitsillides AA & Dhoot GK (2016) Expression of Sulf1 and Sulf2 in cartilage, bone and endochondral fracture healing. *Histochem Cell Biol* **145**, 67–79.
87 Korf-Klingebiel M, Reboll MR, Grote K, Schleiner H, Wang Y, Wu X, Klede S, Mikhed Y, Bauersachs J, Klintschar M *et al.* (2019) Heparan sulfate-editing extracellular sulfatases enhance VEGF bioavailability for ischemic heart repair. *Circ Res* **125**, 787–801.
88 Chiu L-T, Sabbavarapu NM, Lin W-C, Fan C-Y, Wu C-C, Cheng T-JR, Wong C-H & Hung S-C (2020) Trisaccharide sulfate and its sulfonamide as an effective substrate and inhibitor of human Endo-O-sulfatase-1. *J Am Chem Soc* **142**, 5282–5292.
89 Piperigkou Z, Mohr B, Karamanos N & Götte M (2016) Shed proteoglycans in tumor stroma. *Cell Tissue Res* **365**, 643–655.
90 Bertrand J & Bollmann M (2019) Soluble syndecans: biomarkers for diseases and therapeutic options. *Br J Pharmacol* **176**, 67–81.
91 Hadigal S, Koganti R, Yadavalli T, Agelidis A, Suryawanshi R & Shukla D (2020) Heparanase-regulated syndecan-1 shedding facilitates herpes simplex virus 1 egress. *J Virol* **94**, e01672-19.
92 Gomez Toledo A, Nilsson J, Noborn F, Sihlbom C & Larson G (2015) Positive mode LC-MS/MS analysis of chondroitin sulfate modified Glycopeptides derived from light and heavy chains of the human inter-α-trypsin inhibitor complex. *Mol Cell Proteomics* **14**, 3118–3131.
93 Ng C, Nandha Premnath P & Guvench O (2017) Rigidity and flexibility in the tetrasaccharide linker of proteoglycans from atomic-resolution molecular simulation. *J Comput Chem* **38**, 1438–1446.
94 Sammon D, Krueger A, Busse-Wicher M, Morgan RM, Haslam SM, Schumann B, Briggs DC & Hohenester E (2023) Molecular mechanism of decision-making in glycosaminoglycan biosynthesis. *Nat Commun* **14**, 6425.
95 Noborn F, Nilsson J & Larson G (2022) Site-specific glycosylation of proteoglycans: a revisited frontier in proteoglycan research. *Matrix Biol* **111**, 289–306.
96 Noborn F, Nikpour M, Persson A, Nilsson J & Larson G (2021) Expanding the chondroitin sulfate glycoproteome – but how far? *Front Cell Dev Biol* **9**, 695970.
97 Esko JD & Zhang L (1996) Influence of core protein sequence on glycosaminoglycan assembly. *Curr Opin Struct Biol* **6**, 663–670.
98 Noborn F, Nikpour M, Persson A, Sihlbom C, Nilsson J & Larson G (2022) A glycoproteomic approach to identify novel proteoglycans. *Methods Mol Biol* **2303**, 71–85.
99 Noborn F, Gomez Toledo A, Sihlbom C, Lengqvist J, Fries E, Kjellén L, Nilsson J & Larson G (2015) Identification of chondroitin sulfate linkage region glycopeptides reveals prohormones as a novel class of proteoglycans. *Mol Cell Proteomics* **14**, 41–49.
100 Nikpour M, Nilsson J, Persson A, Noborn F, Vorontsov E & Larson G (2021) Proteoglycan profiling of human, rat and mouse insulin-secreting cells. *Glycobiology* **31**, 916–930.
101 Noborn F & Larson G (2021) Characterization of *C. elegans* chondroitin proteoglycans and their large functional and structural heterogeneity; evolutionary aspects on structural differences between humans and the nematode. *Adv Exp Med Biol* **21**, 155–170.

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102 Takemura M, Noborn F, Nilsson J, Bowden N, Nakato E, Baker S, Su T-Y, Larson G & Nakato H (2020) Chondroitin sulfate proteoglycan windpipe modulates hedgehog signaling in drosophila. *Mol Biol Cell* **31**, 813–824.

103 Persson A, Nilsson J, Vorontsov E, Noborn F & Larson G (2019) Identification of a non-canonical chondroitin sulfate linkage region trisaccharide. *Glycobiology* **29**, 366–371.

104 Nilsson J, Noborn F, Gomez Toledo A, Nasir W, Sihlbom C & Larson G (2017) Characterization of glycan structures of chondroitin sulfate-glycopeptides facilitated by sodium ion-pairing and positive mode LC-MS/MS. *J Am Soc Mass Spectrom* **28**, 229–241.

105 Ritelli M, Cinquina V, Giacopuzzi E, Venturini M, Chiarelli N & Colombi M (2019) Further defining the phenotypic Spectrum of B3GAT3 mutations and literature review on Linkeropathy syndromes. *Genes (Basel)* **10**, 631.

106 Haouari W, Dubail J, Poüs C, Cormier-Daire V & Bruneel A (2021) Inherited proteoglycan biosynthesis defects-current laboratory tools and Bikunin as a promising blood biomarker. *Genes (Basel)* **12**, 1654.

107 Nikpour M, Noborn F, Nilsson J, Van Damme T, Kaye O, Syx D, Malfait F & Larson G (2022) Glycosaminoglycan linkage region of urinary bikunin as a potentially useful biomarker for β3GalT6-deficient spondylodysplastic Ehlers-Danlos syndrome. *JIMD Rep* **63**, 462–467.

108 Delbaere S, De Clercq A, Mizumoto S, Noborn F, Bek JW, Alluyn L, Gistelinck C, Syx D, Salmon PL, Coucke PJ *et al.* (2020) b3galt6 Knock-out zebrafish recapitulate β3GalT6-deficiency disorders in human and reveal a trisaccharide proteoglycan linkage region. *Front Cell Dev Biol* **8**, 597857.

109 Basu A, Patel NG, Nicholson ED & Weiss RJ (2022) Spatiotemporal diversity and regulation of glycosaminoglycans in cell homeostasis and human disease. *Am J Physiol Cell Physiol* **322**, C849–C864.

110 Morla S (2019) Glycosaminoglycans and glycosaminoglycan Mimetics in cancer and inflammation. *Int J Mol Sci* **20**, E1963.

111 Song Y, Zhang F & Linhardt RJ (2021) Glycosaminoglycans. *Adv Exp Med Biol* **1325**, 103–116.

112 Xie C, Schaefer L & Iozzo RV (2023) Global impact of proteoglycan science on human diseases. *iScience* **26**, 108095.

113 Ibrahim SA, Gadalla R, El-Ghonaimy EA, Samir O, Mohamed HT, Hassan H, Greve B, El-Shinawi M, Mohamed MM & Götte M (2017) Syndecan-1 is a novel molecular marker for triple negative inflammatory breast cancer and modulates the cancer stem cell phenotype via the IL-6/STAT3, notch and EGFR signaling pathways. *Mol Cancer* **16**, 57.

114 Kumar Katakam S, Tria V, Sim W-C, Yip GW, Molgora S, Karnavas T, Elghonaimy EA, Pelucchi P, Piscitelli E, Ibrahim SA *et al.* (2021) The heparan sulfate proteoglycan syndecan-1 regulates colon cancer stem cell function via a focal adhesion kinase-Wnt signaling axis. *FEBS J* **288**, 486–506.

115 Nassar E, Hassan N, El-Ghonaimy EA, Hassan H, Abdullah MS, Rottke TV, Kiesel L, Greve B, Ibrahim SA & Götte M (2021) Syndecan-1 promotes angiogenesis in triple-negative breast cancer through the Prognostically relevant tissue factor pathway and additional angiogenic routes. *Cancers (Basel)* **13**, 2318.

116 Rapraeger AC (2021) Syndecans and their synstatins: targeting an organizer of receptor tyrosine kinase signaling at the cell-matrix Interface. *Front Oncol* **11**, 775349.

117 Poças J, Marques C, Gomes C, Otake AH, Pinto F, Ferreira M, Silva T, Faria-Ramos I, Matos R, Ribeiro AR *et al.* (2023) Syndecan-4 is a maestro of gastric cancer cell invasion and communication that underscores poor survival. *Proc Natl Acad Sci USA* **120**, e2214853120.

118 Schulz M, Diehl V, Trebicka J, Wygrecka M & Schaefer L (2021) Biglycan: a regulator of hepatorenal inflammation and autophagy. *Matrix Biol* **100–101**, 150–161.

119 Poluzzi C, Nastase M-V, Zeng-Brouwers J, Roedig H, Hsieh LT-H, Michaelis JB, Buhl EM, Rezende F, Manavski Y, Bleich A *et al.* (2019) Biglycan evokes autophagy in macrophages via a novel CD44/toll-like receptor 4 signaling axis in ischemia/reperfusion injury. *Kidney Int* **95**, 540–562.

120 Frey H, Moreth K, Hsieh LT-H, Zeng-Brouwers J, Rathkolb B, Fuchs H, Gailus-Durner V, Iozzo RV, de Angelis MH & Schaefer L (2017) A novel biological function of soluble biglycan: induction of erythropoietin production and polycythemia. *Glycoconj J* **34**, 393–404.

121 Liu J, Zhu S, Zeng L, Li J, Klionsky DJ, Kroemer G, Jiang J, Tang D & Kang R (2022) DCN released from ferroptotic cells ignites AGER-dependent immune responses. *Autophagy* **18**, 2036–2049.

122 Ding Q, Wei Q, Sheng G, Wang S, Jing S, Ma T, Zhang R, Wang T, Li W, Tang X *et al.* (2021) The preventive effect of decorin on epidural fibrosis and epidural adhesions after laminectomy. *Front Pharmacol* **12**, 774316.

123 Khazamipour N, Al-Nakouzi N, Oo HZ, Ørum-Madsen M, Steino A, Sorensen PH & Daugaard M (2020) Oncofetal chondroitin sulfate: a putative therapeutic target in adult and pediatric solid tumors. *Cell* **9**, 818.

124 Ciftciler R, Ozenirler S, Yucel AA, Cengiz M, Erkan G, Buyukdemirci E, Sönmez C & Esendaglı GY (2017) The importance of serum biglycan levels as a fibrosis marker in patients with chronic hepatitis B. *J Clin Lab Anal* **31**, e22109.

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125 Cengiz M, Yilmaz G & Ozenirler S (2021) Serum biglycan as a diagnostic marker for non-alcoholic steatohepatitis and liver fibrosis. *Clin Lab* **67**, doi: 10.7754/Clin.Lab.2020.200709
126 Morimoto H, Hida Y, Maishi N, Nishihara H, Hatanaka Y, Li C, Matsuno Y, Nakamura T, Hirano S & Hida K (2021) Biglycan, tumor endothelial cell secreting proteoglycan, as possible biomarker for lung cancer. *Thorac Cancer* **12**, 1347–1357.
127 Shao C, Cheng C, Shao Q & Chen B (2021) Identification and validation of Biglycan as prognosis and therapy markers for patients with stomach adenocarcinoma. *Int J Gen Med* **14**, 3497–3509.
128 Zhang S, Yang H, Xiang X, Liu L, Huang H & Tang G (2022) BGN may be a potential prognostic biomarker and associated with immune cell enrichment of gastric cancer. *Front Genet* **13**, 765569.
129 Siebuhr AS, Juhl P, Bay-Jensen A-C, Karsdal MA, Franchimont N & Chavez JC (2019) Citrullinated vimentin and biglycan protein fingerprints as candidate serological biomarkers for disease activity in systemic sclerosis: a pilot study. *Biomarkers* **24**, 249–254.
130 Jia X, Chen C, Chen L, Yu C & Kondo T (2019) Decorin as a prognostic biomarker in patients with malignant peripheral nerve sheath tumors. *Oncol Lett* **17**, 3517–3522.
131 Adanaş Aydın G, Ayvacı H & Özgen G (2020) The first-trimester serum decorin levels as a potential predictor of preeclampsia. *J Perinat Med* **48**, 779–785.
132 Nikaido T, Tanino Y, Wang X, Sato Y, Togawa R, Kikuchi M, Misa K, Saito K, Fukuhara N, Kawamata T *et al*. (2018) Serum decorin is a potential prognostic biomarker in patients with acute exacerbation of idiopathic pulmonary fibrosis. *J Thorac Dis* **10**, 5346–5358.
133 Kehlet SN, Bager CL, Willumsen N, Dasgupta B, Brodmerkel C, Curran M, Brix S, Leeming DJ & Karsdal MA (2017) Cathepsin-S degraded decorin are elevated in fibrotic lung disorders – development and biological validation of a new serum biomarker. *BMC Pulm Med* **17**, 110.
134 Leal AF, Benincore-Flórez E, Rintz E, Herreño-Pachón AM, Celik B, Ago Y, Alméciga-Díaz CJ & Tomatsu S (2022) Mucopolysaccharidoses: cellular consequences of Glycosaminoglycans accumulation and potential targets. *Int J Mol Sci* **24**, 477.
135 Fachel FNS, Frâncio L, Poletto É, Schuh RS, Teixeira HF, Giugliani R, Baldo G & Matte U (2022) Gene editing strategies to treat lysosomal disorders: the example of mucopolysaccharidoses. *Adv Drug Deliv Rev* **191**, 114616.
136 Penon-Portmann M, Blair DR & Harmatz P (2023) Current and new therapies for mucopolysaccharidoses. *Pediatr Neonatol* **64** (Suppl 1), S10–S17.
137 Rossi A & Brunetti-Pierri N (2023) Gene therapies for mucopolysaccharidoses. *J Inherit Metab Dis* **47**, 135–144.
138 Safary A, Akbarzadeh Khiavi M, Omidi Y & Rafi MA (2019) Targeted enzyme delivery systems in lysosomal disorders: an innovative form of therapy for mucopolysaccharidosis. *Cell Mol Life Sci* **76**, 3363–3381.
139 Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A, Kubalak S, Klewer SE & McDonald JA (2000) Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J Clin Invest* **106**, 349–360.
140 Heldin P, Lin C-Y, Kolliopoulos C, Chen Y-H & Skandalis SS (2019) Regulation of hyaluronan biosynthesis and clinical impact of excessive hyaluronan production. *Matrix Biol* **78–79**, 100–117.
141 Kolliopoulos C, Lin C-Y, Heldin C-H, Moustakas A & Heldin P (2019) Has2 natural antisense RNA and Hmga2 promote Has2 expression during TGFβ-induced EMT in breast cancer. *Matrix Biol* **80**, 29–45.
142 Bernert B, Porsch H & Heldin P (2011) Hyaluronan synthase 2 (HAS2) promotes breast cancer cell invasion by suppression of tissue metalloproteinase inhibitor 1 (TIMP-1). *J Biol Chem* **286**, 42349–42359.
143 Kolliopoulos C, Chatzopoulos A, Skandalis SS, Heldin C-H & Heldin P (2021) TRAF4/6 is needed for CD44 cleavage and migration via RAC1 activation. *Cancers (Basel)* **13**, 1021.
144 Lin C-Y, Kolliopoulos C, Huang C-H, Tenhunen J, Heldin C-H, Chen Y-H & Heldin P (2019) High levels of serum hyaluronan is an early predictor of dengue warning signs and perturbs vascular integrity. *EBioMedicine* **48**, 425–441.
145 Kolliopoulos C, Ali MM, Castillejo-Lopez C, Heldin C-H & Heldin P (2022) CD44 depletion in glioblastoma cells suppresses growth and Stemness and induces senescence. *Cancers (Basel)* **14**, 3747.
146 Dhar D, Antonucci L, Nakagawa H, Kim JY, Glitzner E, Caruso S, Shalapour S, Yang L, Valasek MA, Lee S *et al*. (2018) Liver cancer initiation requires p53 inhibition by CD44-enhanced growth factor signaling. *Cancer Cell* **33**, 1061–1077.e6.
147 Lin C-Y, Basu K, Ruusala A, Kozlova I, Li Y-S, Skandalis SS, Heldin C-H & Heldin P (2023) Hyaluronan-induced CD44-iASPP interaction affects fibroblast migration and survival. *Cancers (Basel)* **15**, 1082.
148 Hinneh JA, Gillis JL, Moore NL, Butler LM & Centenera MM (2022) The role of RHAMM in cancer: exposing novel therapeutic vulnerabilities. *Front Oncol* **12**, 982231.

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149 Garantziotis S & Savani RC (2019) Hyaluronan biology: a complex balancing act of structure, function, location and context. *Matrix Biol* **78–79**, 1–10.
150 Tolg C, Yuan H, Flynn SM, Basu K, Ma J, Tse KCK, Kowalska B, Vulkanesku D, Cowman MK, McCarthy JB *et al.* (2017) Hyaluronan modulates growth factor induced mammary gland branching in a size dependent manner. *Matrix Biol* **63**, 117–132.
151 Buttermore ST, Hoffman MS, Kumar A, Champeaux A, Nicosia SV & Kruk PA (2017) Increased RHAMM expression relates to ovarian cancer progression. *J Ovarian Res* **10**, 66.
152 Kahl I, Mense J, Finke C, Boller A-L, Lorber C, Győrffy B, Greve B, Götte M & Espinoza-Sánchez NA (2022) The cell cycle-related genes RHAMM, AURKA, TPX2, PLK1, and PLK4 are associated with the poor prognosis of breast cancer patients. *J Cell Biochem* **123**, 581–600.
153 Tarullo SE, He Y, Daughters C, Knutson TP, Henzler CM, Price MA, Shanley R, Witschen P, Tolg C, Kaspar RE *et al.* (2023) Receptor for hyaluronan-mediated motility (RHAMM) defines an invasive niche associated with tumor progression and predicts poor outcomes in breast cancer patients. *J Pathol* **260**, 289–303.
154 Liu M, Tolg C & Turley E (2019) Dissecting the dual nature of Hyaluronan in the tumor microenvironment. *Front Immunol* **10**, 947.
155 Tolg C, Messam BJ-A, McCarthy JB, Nelson AC & Turley EA (2021) Hyaluronan functions in wound repair that are captured to fuel breast cancer progression. *Biomolecules* **11**, 1551.
156 Parnigoni A, Viola M, Karousou E, Rovera S, Giaroni C, Passi A & Vigetti D (2022) Hyaluronan in pathophysiology of vascular diseases: specific roles in smooth muscle cells, endothelial cells, and macrophages. *Am J Physiol Cell Physiol* **323**, C505–C519.
157 Carvalho AM, Reis RL & Pashkuleva I (2023) Hyaluronan receptors as mediators and modulators of the tumor microenvironment. *Adv Healthc Mater* **12**, e2202118.
158 Thangavel C, Boopathi E, Liu Y, Haber A, Ertel A, Bhardwaj A, Addya S, Williams N, Ciment SJ, Cotzia P *et al.* (2017) RB loss promotes prostate cancer metastasis. *Cancer Res* **77**, 982–995.
159 Berdiaki A, Thrapsanioti L-N, Giatagana E-M, K Karamanos N, C Savani R, N Tzanakakis G & Nikitovic D (2023) RHAMM/hyaluronan inhibit β-catenin degradation, enhance downstream signaling, and facilitate fibrosarcoma cell growth. *Mol Biol Rep* **50**, 8937–8947.
160 Carvalho AM, Soares da Costa D, Paulo PMR, Reis RL & Pashkuleva I (2021) Co-localization and crosstalk between CD44 and RHAMM depend on hyaluronan presentation. *Acta Biomater* **119**, 114–124.
161 Carvalho AM, Soares da Costa D, Reis RL & Pashkuleva I (2022) RHAMM expression tunes the response of breast cancer cell lines to hyaluronan. *Acta Biomater* **146**, 187–196.
162 Esguerra KVN, Tolg C, Akentieva N, Price M, Cho C-F, Lewis JD, McCarthy JB, Turley EA & Luyt LG (2015) Identification, design and synthesis of tubulin-derived peptides as novel hyaluronan mimetic ligands for the receptor for hyaluronan-mediated motility (RHAMM/HMMR). *Integr Biol (Camb)* **7**, 1547–1560.
163 Soliman F, Ye L, Jiang W & Hargest R (2022) Targeting hyaluronic acid and peritoneal dissemination in colorectal cancer. *Clin Colorectal Cancer* **21**, e126–e134.
164 Karousou E, Misra S, Ghatak S, Dobra K, Götte M, Vigetti D, Passi A, Karamanos NK & Skandalis SS (2017) Roles and targeting of the HAS/hyaluronan/CD44 molecular system in cancer. *Matrix Biol* **59**, 3–22.
165 Takasugi M, Firsanov D, Tombline G, Ning H, Ablaeva J, Seluanov A & Gorbunova V (2020) Naked mole-rat very-high-molecular-mass hyaluronan exhibits superior cytoprotective properties. *Nat Commun* **11**, 2376.
166 Ooki T, Murata-Kamiya N, Takahashi-Kanemitsu A, Wu W & Hatakeyama M (2019) High-molecular-weight Hyaluronan is a hippo pathway ligand directing cell density-dependent growth inhibition via PAR1b. *Dev Cell* **49**, 590–604.e9.
167 Cha YJ & Koo JS (2020) Role of tumor-associated myeloid cells in breast cancer. *Cell* **9**, 1785.
168 Dominguez-Gutierrez PR, Kwenda EP, Donelan W, O'Malley P, Crispen PL & Kusmartsev S (2021) Hyal2 expression in tumor-associated myeloid cells mediates cancer-related inflammation in bladder cancer. *Cancer Res* **81**, 648–657.
169 Dominguez-Gutierrez PR, Kwenda EP, Donelan W, Miranda M, Doty A, O'Malley P, Crispen PL & Kusmartsev S (2022) Detection of PD-L1-expressing myeloid cell clusters in the Hyaluronan-enriched stroma in tumor tissue and tumor-draining lymph nodes. *J Immunol* **208**, 2829–2836.
170 Donelan W, Dominguez-Gutierrez PR & Kusmartsev S (2022) Deregulated hyaluronan metabolism in the tumor microenvironment drives cancer inflammation and tumor-associated immune suppression. *Front Immunol* **13**, 971278.
171 Vitale DL, Icardi A, Rosales P, Spinelli FM, Sevic I & Alaniz LD (2021) Targeting the tumor extracellular matrix by the natural molecule 4-Methylumbelliferone: a complementary and alternative cancer therapeutic strategy. *Front Oncol* **11**, 710061.

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172 Karalis TT, Chatzopoulos A, Kondyli A, Aletras AJ, Karamanos NK, Heldin P & Skandalis SS (2020) Salicylate suppresses the oncogenic hyaluronan network in metastatic breast cancer cells. *Matrix Biol Plus* **6–7**, 100031.

173 Koutsakis C, Tavianatou A-G, Kokoretsis D, Baroutas G & Karamanos NK (2021) Sulfated Hyaluronan modulates the functional properties and matrix effectors expression of breast cancer cells with different estrogen receptor status. *Biomolecules* **11**, 1916.

174 Misra S, Hascall VC, Markwald RR & Ghatak S (2015) Interactions between Hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. *Front Immunol* **6**, 201.

175 Hauser-Kawaguchi A, Luyt LG & Turley E (2019) Design of peptide mimetics to block pro-inflammatory functions of HA fragments. *Matrix Biol* **78–79**, 346–356.

176 Kavasi R-M, Berdiaki A, Spyridaki I, Papoutsidakis A, Corsini E, Tsatsakis A, Tzanakakis GN & Nikitovic D (2019) Contact allergen (PPD and DNCB)-induced keratinocyte sensitization is partly mediated through a low molecular weight hyaluronan (LMWHA)/TLR4/NF-κB signaling axis. *Toxicol Appl Pharmacol* **377**, 114632.

177 You N, Chu S, Cai B, Gao Y, Hui M, Zhu J & Wang M (2021) Bioactive hyaluronic acid fragments inhibit lipopolysaccharide-induced inflammatory responses via the toll-like receptor 4 signaling pathway. *Front Med* **15**, 292–301.

178 Cui Z, Liao J, Cheong N, Longoria C, Cao G, DeLisser HM & Savani RC (2019) The receptor for Hyaluronan-mediated motility (CD168) promotes inflammation and fibrosis after acute lung injury. *Matrix Biol* **78–79**, 255–271.

179 De Pasquale V & Pavone LM (2020) Heparan sulfate proteoglycan signaling in tumor microenvironment. *Int J Mol Sci* **21**, 6588.

180 Faria-Ramos I, Poças J, Marques C, Santos-Antunes J, Macedo G, Reis CA & Magalhães A (2021) Heparan sulfate Glycosaminoglycans: (un)expected allies in cancer clinical management. *Biomolecules* **11**, 136.

181 Dituri F, Gigante G, Scialpi R, Mancarella S, Fabregat I & Giannelli G (2022) Proteoglycans in cancer: friends or enemies? A special focus on hepatocellular carcinoma. *Cancers (Basel)* **14**, 1902.

182 Karamanos NK, Piperigkou Z, Passi A, Götte M, Rousselle P & Vlodavsky I (2021) Extracellular matrix-based cancer targeting. *Trends Mol Med* **27**, 1000–1013.

183 Huang R-L, Chen H-J, Chen L-Y, Chao T-K, Lin W-Y, Liew P-L, Su P-H, Weng Y-C, Wang Y-C, Liao C-C *et al*. (2018) Epigenetic loss of heparan sulfate 3-O-sulfation sensitizes ovarian carcinoma to oncogenic signals and predicts prognosis. *Int J Cancer* **143**, 1943–1953.

184 Kuehn J, Espinoza-Sanchez NA, Teixeira FCOB, Pavão MSG, Kiesel L, Győrffy B, Greve B & Götte M (2021) Prognostic significance of hedgehog signaling network-related gene expression in breast cancer patients. *J Cell Biochem* **122**, 577–597.

185 Riecks J, Parnigoni A, Győrffy B, Kiesel L, Passi A, Vigetti D & Götte M (2022) The hyaluronan-related genes HAS2, HYAL1-4, PH20 and HYALP1 are associated with prognosis, cell viability and spheroid formation capacity in ovarian cancer. *J Cancer Res Clin Oncol* **148**, 3399–3419.

186 Karamanos NK, Piperigkou Z, Theocharis AD, Watanabe H, Franchi M, Baud S, Brézillon S, Götte M, Passi A, Vigetti D *et al*. (2018) Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics. *Chem Rev* **118**, 9152–9232.

187 Hassan N, Greve B, Espinoza-Sánchez NA & Götte M (2021) Cell-surface heparan sulfate proteoglycans as multifunctional integrators of signaling in cancer. *Cell Signal* **77**, 109822.

188 Vijaya Kumar A, Brézillon S, Untereiner V, Sockalingum GD, Kumar Katakam S, Mohamed HT, Kemper B, Greve B, Mohr B, Ibrahim SA *et al*. (2020) HS2ST1-dependent signaling pathways determine breast cancer cell viability, matrix interactions, and invasive behavior. *Cancer Sci* **111**, 2907–2922.

189 Ponandai-Srinivasan S, Saare M, Boggavarapu NR, Frisendahl C, Ehrström S, Riethmüller C, García-Uribe PA, Rettkowski J, Iyengar A, Salumets A *et al*. (2020) Syndecan-1 modulates the invasive potential of endometrioma via TGF-β signalling in a subgroup of women with endometriosis. *Hum Reprod* **35**, 2280–2293.

190 Baghy K, Reszegi A, Tátrai P & Kovalszky I (2020) Decorin in the tumor microenvironment. *Adv Exp Med Biol* **1272**, 17–38.

191 Xie C, Mondal DK, Ulas M, Neill T & Iozzo RV (2022) Oncosuppressive roles of decorin through regulation of multiple receptors and diverse signaling pathways. *Am J Physiol Cell Physiol* **322**, C554–C566.

192 Krautschneider SL, Troschel FM, Vadillo E, Eich HT, Götte M, Espinoza-Sánchez NA & Greve B (2022) Enzymatic digestion of cell-surface Heparan sulfate alters the radiation response in triple-negative breast cancer cells. *Arch Med Res* **53**, 826–839.

193 Valla S, Hassan N, Vitale DL, Madanes D, Spinelli FM, Teixeira FCOB, Greve B, Espinoza-Sánchez NA, Cristina C, Alaniz L *et al*. (2021) Syndecan-1 depletion has a differential impact on hyaluronic acid metabolism and tumor cell behavior in luminal and triple-negative breast cancer cells. *Int J Mol Sci* **22**, 5874.

17424658, 0, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.17107 by Asst Dei 7 Laghi, Wiley Online Library on [13/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

194 Vitale D, Kumar Katakam S, Greve B, Jang B, Oh E-S, Alaniz L & Götte M (2019) Proteoglycans and glycosaminoglycans as regulators of cancer stem cell function and therapeutic resistance. *FEBS J* **286**, 2870–2882.

195 Teixeira FCOB, Vijaya Kumar A, Kumar Katakam S, Cocola C, Pelucchi P, Graf M, Kiesel L, Reinbold R, Pavão MSG, Greve B *et al.* (2020) The Heparan sulfate sulfotransferases HS2ST1 and HS3ST2 are novel regulators of breast cancer stem-cell properties. *Front Cell Dev Biol* **8**, 559554.

196 Gubbiotti MA, Buraschi S, Kapoor A & Iozzo RV (2020) Proteoglycan signaling in tumor angiogenesis and endothelial cell autophagy. *Semin Cancer Biol* **62**, 1–8.

197 Spinelli FM, Vitale DL, Icardi A, Caon I, Brandone A, Giannoni P, Saturno V, Passi A, García M, Sevic I *et al.* (2019) Hyaluronan preconditioning of monocytes/macrophages affects their angiogenic behavior and regulation of TSG-6 expression in a tumor type-specific manner. *FEBS J* **286**, 3433–3449.

198 Couchman JR (2021) Syndecan-1 (CD138), carcinomas and EMT. *Int J Mol Sci* **22**, 4227.

199 Masola V, Franchi M, Zaza G, Atsina FM, Gambaro G & Onisto M (2022) Heparanase regulates EMT and cancer stem cell properties in prostate tumors. *Front Oncol* **12**, 918419.

200 Wang C, Shang C, Gai X, Song T, Han S, Liu Q & Zheng X (2021) Sulfatase 2-induced cancer-associated fibroblasts promote hepatocellular carcinoma progression via inhibition of apoptosis and induction of epithelial-to-mesenchymal transition. *Front Cell Dev Biol* **9**, 631931.

201 Weyers A, Yang B, Park J-H, Kim Y-S, Kim S-M, Lee S-E, Zhang F, Lee KB & Linhardt RJ (2013) Microanalysis of stomach cancer glycosaminoglycans. *Glycoconj J* **30**, 701–707.

202 Marques C, Reis CA, Vivès RR & Magalhães A (2021) Heparan sulfate biosynthesis and Sulfation profiles as modulators of cancer signalling and progression. *Front Oncol* **11**, 778752.

203 Amendum PC, Khan S, Yamaguchi S, Kobayashi H, Ago Y, Suzuki Y, Celik B, Rintz E, Hossain J, Xiao W *et al.* (2021) Glycosaminoglycans as biomarkers for mucopolysaccharidoses and other disorders. *Diagnostics (Basel)* **11**, 1563.

204 Wei J, Hu M, Huang K, Lin S & Du H (2020) Roles of proteoglycans and glycosaminoglycans in cancer development and progression. *Int J Mol Sci* **21**, 5983.

205 Hua SH, Viera M, Yip GW & Bay BH (2022) Theranostic applications of Glycosaminoglycans in metastatic renal cell carcinoma. *Cancers (Basel)* **15**, 266.

206 Zhang P-F, Wu Z-Y, Zhang W-B, He Y-Q, Chen K, Wang T-M, Li H, Zheng H, Li D-H, Yang D-W *et al.* (2023) Establishment and validation of a plasma oncofetal chondroitin sulfated proteoglycan for pan-cancer detection. *Nat Commun* **14**, 645.

207 Bratulic S, Limeta A, Dabestani S, Birgisson H, Enblad G, Stålberg K, Hesselager G, Häggman M, Höglund M, Simonson OE *et al.* (2022) Noninvasive detection of any-stage cancer using free glycosaminoglycans. *Proc Natl Acad Sci USA* **119**, e2115328119.

208 Tamburro D, Bratulic S, Abou Shameh S, Soni NK, Bacconi A, Maccari F, Galeotti F, Mattsson K, Volpi N, Nielsen J *et al.* (2021) Analytical performance of a standardized kit for mass spectrometry-based measurements of human glycosaminoglycans. *J Chromatogr B Analyt Technol Biomed Life Sci* **1177**, 122761.

209 Bratulic S, Limeta A, Maccari F, Galeotti F, Volpi N, Levin M, Nielsen J & Gatto F (2022) Analysis of normal levels of free glycosaminoglycans in urine and plasma in adults. *J Biol Chem* **298**, 101575.

210 Maciej-Hulme ML, Leprince ACN, Lavin A, Guimond SE, Turnbull JE, Pelletier J, Yates EA, Powell AK & Skidmore MA (2023) High sensitivity (zeptomole) detection of BODIPY-labelled heparan sulfate (HS) disaccharides by ion-paired RP-HPLC and LIF detection enables analysis of HS from mosquito midguts. *Anal Methods* **15**, 1461–1469.

211 Otani K & Shichita T (2020) Cerebral sterile inflammation in neurodegenerative diseases. *Inflamm Regen* **40**, 28.

212 Zeng-Brouwers J, Pandey S, Trebicka J, Wygrecka M & Schaefer L (2020) Communications via the small leucine-rich proteoglycans: molecular specificity in inflammation and autoimmune diseases. *J Histochem Cytochem* **68**, 887–906.

213 Iozzo RV & Schaefer L (2015) Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. *Matrix Biol* **42**, 11–55.

214 Schaefer L & Dikic I (2021) Autophagy: instructions from the extracellular matrix. *Matrix Biol* **100–101**, 1–8.

215 Frevert CW, Felgenhauer J, Wygrecka M, Nastase MV & Schaefer L (2018) Danger-associated molecular patterns derived from the extracellular matrix provide temporal control of innate immunity. *J Histochem Cytochem* **66**, 213–227.

216 Roedig H, Nastase MV, Frey H, Moreth K, Zeng-Brouwers J, Poluzzi C, Hsieh LT-H, Brandts C, Fulda S, Wygrecka M *et al.* (2019) Biglycan is a new high-affinity ligand for CD14 in macrophages. *Matrix Biol* **77**, 4–22.

217 Hsieh LT-H, Frey H, Nastase M-V, Tredup C, Hoffmann A, Poluzzi C, Zeng-Brouwers J, Manon-Jensen T, Schröder K, Brandes RP *et al.* (2016) Bimodal role of NADPH oxidases in the regulation of

17424658, 0, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.17107 by Asst Dei 7 Laghi, Wiley Online Library on [13/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

biglycan-triggered IL-1β synthesis. *Matrix Biol* **49**, 61–81.

218 Nastase MV, Zeng-Brouwers J, Beckmann J, Tredup C, Christen U, Radeke HH, Wygrecka M & Schaefer L (2018) Biglycan, a novel trigger of Th1 and Th17 cell recruitment into the kidney. *Matrix Biol* **68–69**, 293–317.

219 Allawadhi P, Singh V, Khurana I, Rawat PS, Renushe AP, Khurana A, Navik U, Allwadhi S, Kumar Karlapudi S, Banothu AK *et al*. (2021) Decorin as a possible strategy for the amelioration of COVID-19. *Med Hypotheses* **152**, 110612.

220 Chen G, Zhu Y, Liang X, Wang X, Yu W, Guo J, Zhu L & Ma R (2020) The effect of lecithins coupled decorin nanoliposomes on treatment of carbon tetrachloride-induced liver fibrosis. *Biomed Res Int* **2020**, 8815904.

221 Jiang N, Zhang Q, Chau MK, Yip MS, Lui SL, Liu S, Chu KM, Ngan HY, Chan TM & Yung S (2020) Anti-fibrotic effect of decorin in peritoneal dialysis and PD-associated peritonitis. *EBioMedicine* **52**, 102661.

222 Cianfarani F, De Domenico E, Nyström A, Mastroeni S, Abeni D, Baldini E, Ulisse S, Uva P, Bruckner-Tuderman L, Zambruno G *et al.* (2019) Decorin counteracts disease progression in mice with recessive dystrophic epidermolysis bullosa. *Matrix Biol* **81**, 3–16.

223 Vijayan AN, Solaimuthu A, Murali P, Gopi J, Y MT, R AP & Korrapati PS (2022) Decorin mediated biomimetic PCL-gelatin nano-framework to impede scarring. *Int J Biol Macromol* **219**, 907–918.

224 Zhao X, Li M, Dai X, Yang Y, Peng Y, Xu C, Dai N & Wang D (2020) Downregulation of exosomal miR-1273a increases cisplatin resistance of non-small cell lung cancer by upregulating the expression of syndecan binding protein. *Oncol Rep* **44**, 2165–2173.

225 Mortensen JH, Manon-Jensen T, Jensen MD, Hägglund P, Klinge LG, Kjeldsen J, Krag A, Karsdal MA & Bay-Jensen A-C (2017) Ulcerative colitis, Crohn's disease, and irritable bowel syndrome have different profiles of extracellular matrix turnover, which also reflects disease activity in Crohn's disease. *PLoS One* **12**, e0185855.

226 Adepu S, Ekman S, Leth J, Johansson U, Lindahl A & Skiöldebrand E (2022) Biglycan neo-epitope (BGN262), a novel biomarker for screening early changes in equine osteoarthritic subchondral bone. *Osteoarthr Cartil* **30**, 1328–1336.

227 Sawada Y, Sato T, Saito C, Ozawa F, Ozaki Y & Sugiura-Ogasawara M (2018) Clinical utility of decorin in follicular fluid as a biomarker of oocyte potential. *Reprod Biol* **18**, 33–39.

228 Sainio AO & Järveläinen HT (2019) Decorin-mediated oncosuppression – a potential future adjuvant therapy for human epithelial cancers. *Br J Pharmacol* **176**, 5–15.

229 Li R, Wang Q-F, Li W-W & Niu X-C (2018) Effect of treating renal failure with decorin gene therapy. *Eur Rev Med Pharmacol Sci* **22**, 2483–2489.

230 Pomin VH, Vignovich WP, Gonzales AV, Vasconcelos AA & Mulloy B (2019) Galactosaminoglycans: medical applications and drawbacks. *Molecules* **24**, 2803.

231 Neves MI, Araújo M, Moroni L, da Silva RMP & Barrias CC (2020) Glycosaminoglycan-inspired biomaterials for the development of bioactive hydrogel networks. *Molecules* **25**, 978.

232 Sodhi H & Panitch A (2020) Glycosaminoglycans in tissue engineering: a review. *Biomolecules* **11**, E29.

233 Menezes R, Vincent R, Osorno L, Hu P & Arinzeh TL (2022) Biomaterials and tissue engineering approaches using Glycosaminoglycans for tissue repair: lessons learned from the native extracellular matrix. *Acta Biomater* **163**, 210–227.

234 Rnjak-Kovacina J, Tang F, Whitelock JM & Lord MS (2018) Glycosaminoglycan and proteoglycan-based biomaterials: current trends and future perspectives. *Adv Healthc Mater* **7**, e1701042.

235 Hachim D, Whittaker TE, Kim H & Stevens MM (2019) Glycosaminoglycan-based biomaterials for growth factor and cytokine delivery: making the right choices. *J Control Release* **313**, 131–147.

236 Anderegg U, Halfter N, Schnabelrauch M & Hintze V (2021) Collagen/glycosaminoglycan-based matrices for controlling skin cell responses. *Biol Chem* **402**, 1325–1335.

237 Palhares LCGF, London JA, Kozlowski AM, Esposito E, Chavante SF, Ni M & Yates EA (2021) Chemical modification of glycosaminoglycan polysaccharides. *Molecules* **26**, 5211.

238 Schnabelrauch M, Schiller J, Möller S, Scharnweber D & Hintze V (2021) Chemically modified glycosaminoglycan derivatives as building blocks for biomaterial coatings and hydrogels. *Biol Chem* **402**, 1385–1395.

239 Barritault D, Gilbert-Sirieix M, Rice KL, Siñeriz F, Papy-Garcia D, Baudouin C, Desgranges P, Zakine G, Saffar J-L & van Neck J (2017) RGTA® or ReGeneraTing agents mimic heparan sulfate in regenerative medicine: from concept to curing patients. *Glycoconj J* **34**, 325–338.

240 Koehler L, Ruiz-Gómez G, Balamurugan K, Rother S, Freyse J, Möller S, Schnabelrauch M, Köhling S, Djordjevic S, Scharnweber D *et al*. (2019) Dual action of sulfated Hyaluronan on angiogenic processes in relation to vascular endothelial growth factor-a. *Sci Rep* **9**, 18143.

241 Vogel S, Arnoldini S, Möller S, Schnabelrauch M & Hempel U (2016) Sulfated hyaluronan alters fibronectin matrix assembly and promotes osteogenic differentiation of human bone marrow stromal cells. *Sci Rep* **6**, 36418.

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242 Rother S, Samsonov SA, Hempel U, Vogel S, Moeller S, Blaszkiewicz J, Köhling S, Schnabelrauch M, Rademann J, Pisabarro MT *et al*. (2016) Sulfated Hyaluronan alters the interaction profile of TIMP-3 with the endocytic receptor LRP-1 clusters II and IV and increases the extracellular TIMP-3 level of human bone marrow stromal cells. *Biomacromolecules* **17**, 3252–3261.

243 Mörö A, Samanta S, Honkamäki L, Rangasami VK, Puistola P, Kauppila M, Narkilahti S, Miettinen S, Oommen O & Skottman H (2022) Hyaluronic acid based next generation bioink for 3D bioprinting of human stem cell derived corneal stromal model with innervation. *Biofabrication* **15**, doi: 10.1088/1758-5090/acab34

244 Schwab A, Hélary C, Richards RG, Alini M, Eglin D & D'Este M (2020) Tissue mimetic hyaluronan bioink containing collagen fibers with controlled orientation modulating cell migration and alignment. *Mater Today Bio* **7**, 100058.

245 Lafuente-Merchan M, Ruiz-Alonso S, Zabala A, Gálvez-Martín P, Marchal JA, Vázquez-Lasa B, Gallego I, Saenz-Del-Burgo L & Pedraz JL (2022) Chondroitin and dermatan sulfate bioinks for 3D bioprinting and cartilage regeneration. *Macromol Biosci* **22**, e2100435.

246 Shi D, Sheng A & Chi L (2021) Glycosaminoglycan-protein interactions and their roles in human disease. *Front Mol Biosci* **8**, 639666.

247 Pomin VH & Wang X (2018) Synthetic oligosaccharide libraries and microarray technology: a powerful combination for the success of current glycosaminoglycan interactomics. *ChemMedChem* **13**, 648–661.

248 Vallet SD, Miele AE, Uciechowska-Kaczmarzyk U, Liwo A, Duclos B, Samsonov SA & Ricard-Blum S (2018) Insights into the structure and dynamics of lysyl oxidase propeptide, a flexible protein with numerous partners. *Sci Rep* **8**, 11768.

249 Ricard-Blum S & Perez S (2022) Glycosaminoglycan interaction networks and databases. *Curr Opin Struct Biol* **74**, 102355.

250 Ricard-Blum S (2023) Building, visualizing, and analyzing glycosaminoglycan-protein interaction networks. *Methods Mol Biol* **2619**, 211–224.

251 Clerc O, Deniaud M, Vallet SD, Naba A, Rivet A, Perez S, Thierry-Mieg N & Ricard-Blum S (2019) MatrixDB: integration of new data with a focus on glycosaminoglycan interactions. *Nucleic Acids Res* **47**, D376–D381.

252 Berthollier C, Vallet SD, Deniaud M, Clerc O & Ricard-Blum S (2021) Building protein-protein and protein-glycosaminoglycan interaction networks using MatrixDB, the extracellular matrix interaction database. *Curr Protoc* **1**, e47.

253 Vallet SD, Clerc O & Ricard-Blum S (2021) Glycosaminoglycan-protein interactions: the first draft of the glycosaminoglycan Interactome. *J Histochem Cytochem* **69**, 93–104.

254 Hayes AJ & Melrose J (2021) What are the potential roles of nuclear perlecan and other heparan sulphate proteoglycans in the normal and malignant phenotype. *Int J Mol Sci* **22**, 4415.

255 Kumar-Singh A, Parniewska MM, Giotopoulou N, Javadi J, Sun W, Szatmári T, Dobra K, Hjerpe A & Fuxe J (2021) Nuclear syndecan-1 regulates epithelial-mesenchymal plasticity in tumor cells. *Biology (Basel)* **10**, 521.

256 Kumar-Singh A, Shrinet J, Parniewska MM, Fuxe J, Dobra K & Hjerpe A (2020) Mapping the interactome of the nuclear heparan sulfate proteoglycan syndecan-1 in mesothelioma cells. *Biomolecules* **10**, E1034.

257 Mathiesen SB, Lunde M, Aronsen JM, Romaine A, Kaupang A, Martinsen M, de Souza GA, Nyman TA, Sjaastad I, Christensen G *et al*. (2019) The cardiac syndecan-4 interactome reveals a role for syndecan-4 in nuclear translocation of muscle LIM protein (MLP). *J Biol Chem* **294**, 8717–8731.

258 Mathiesen SB, Lunde M, Stensland M, Martinsen M, Nyman TA, Christensen G & Carlson CR (2020) The cardiac syndecan-2 interactome. *Front Cell Dev Biol* **8**, 792.

259 Gondelaud F & Ricard-Blum S (2019) Structures and interactions of syndecans. *FEBS J* **286**, 2994–3007.

260 Smock RG & Meijers R (2018) Roles of glycosaminoglycans as regulators of ligand/receptor complexes. *Open Biol* **8**, 180026.

261 Guvench O (2022) Atomic-resolution experimental structural biology and molecular dynamics simulations of hyaluronan and its complexes. *Molecules* **27**, 7276.

262 Kogut MM, Marcisz M & Samsonov SA (2022) Modeling glycosaminoglycan–protein complexes. *Curr Opin Struct Biol* **73**, 102332.

263 Nagarajan B, Holmes SG, Sankaranarayanan NV & Desai UR (2022) Molecular dynamics simulations to understand glycosaminoglycan interactions in the free- and protein-bound states. *Curr Opin Struct Biol* **74**, 102356.

264 Künze G, Huster D & Samsonov SA (2021) Investigation of the structure of regulatory proteins interacting with glycosaminoglycans by combining NMR spectroscopy and molecular modeling – the beginning of a wonderful friendship. *Biol Chem* **402**, 1337–1355.

265 Peysselon F & Ricard-Blum S (2014) Heparin-protein interactions: from affinity and kinetics to biological roles. Application to an interaction network regulating angiogenesis. *Matrix Biol* **35**, 73–81.

266 Xu D & Esko JD (2014) Demystifying heparan sulfate-protein interactions. *Annu Rev Biochem* **83**, 129–157.

17424658, 0, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.17107 by Asst Dei 7 Laghi, Wiley Online Library on [13/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

267 Song Y, Zhang F & Linhardt RJ (2021) Analysis of the glycosaminoglycan chains of proteoglycans. *J Histochem Cytochem* **69**, 121–135.

268 Xia K, Hagan JT, Fu L, Sheetz BS, Bhattacharya S, Zhang F, Dwyer JR & Linhardt RJ (2021) Synthetic heparan sulfate standards and machine learning facilitate the development of solid-state nanopore analysis. *Proc Natl Acad Sci USA* **118**, e2022806118.

269 Im J, Lindsay S, Wang X & Zhang P (2019) Single molecule identification and quantification of glycosaminoglycans using solid-state nanopores. *ACS Nano* **13**, 6308–6318.

270 Bayat P, Rambaud C, Priem B, Bourderioux M, Bilong M, Poyer S, Pastoriza-Gallego M, Oukhaled A, Mathé J & Daniel R (2022) Comprehensive structural assignment of glycosaminoglycan oligo- and polysaccharides by protein nanopore. *Nat Commun* **13**, 5113.

271 Zhang Q, Cao H-Y, Wei L, Lu D, Du M, Yuan M, Shi D, Chen X, Wang P, Chen X-L *et al*. (2021) Discovery of exolytic heparinases and their catalytic mechanism and potential application. *Nat Commun* **12**, 1263.

272 Freeman SA, Vega A, Riedl M, Collins RF, Ostrowski PP, Woods EC, Bertozzi CR, Tammi MI, Lidke DS, Johnson P *et al*. (2018) Transmembrane pickets connect cyto- and pericellular skeletons forming barriers to receptor engagement. *Cell* **172**, 305–317.e10.

273 Szekeres GP, Pagel K & Heiner Z (2022) Analytical challenges of glycosaminoglycans at biological interfaces. *Anal Bioanal Chem* **414**, 85–93.

274 Del Toro N, Shrivastava A, Ragueneau E, Meldal B, Combe C, Barrera E, Perfetto L, How K, Ratan P, Shirodkar G *et al*. (2022) The IntAct database: efficient access to fine-grained molecular interaction data. *Nucleic Acids Res* **50**, D648–D653.

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