Sulfation of Glycosaminoglycans and Its Implications in Human Health and Disorders

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Abstract

Sulfation is a dynamic and complex posttranslational modification process. It can occur at various positions within the glycosaminoglycan (GAG) backbone and modulates extracellular signals such as cell–cell and cell–matrix interactions; different sulfation patterns have been identified for the same organs and cells during their development. Because of their high specificity in relation to function, GAG sulfation patterns are referred to as the sulfation code. This review explores the role of GAG sulfation in different biological processes at the cell, tissue, and organism levels. We address the connection between the sulfation patterns of GAGs and several physiological processes and discuss the misregulation of GAG sulfation and its involvement in several genetic and metabolic disorders. Finally, we present the therapeutic potential of GAGs and their synthetic mimics in the biomedical field.

Contents

1. INTRODUCTION

PG: proteoglycan

ECM: extracellular matrix

GAG: glycosaminoglycan

Glycosylation is the most common of all known protein posttranslational modifications. Virtually all animal cells produce proteoglycans (PGs) and secrete them into the extracellular matrix (ECM), insert them into the plasma membrane, or store them in secretory granules. Thus, glycans, particularly glycosaminoglycans (GAGs), are key elements of the pericellular space, where they can either determine the physical characteristics of tissues or modulate the biological functions of cells. The biological activity of GAGs depends on several properties, such as molecular weight, monosaccharide constituents, and bonds between the disaccharide repeating units. Among these properties, negative charge, which is intrinsic to all GAGs, is paramount (1, 2). This negative charge is generally associated with the presence of sulfate groups, with only one exception: hyaluronic acid, or hyaluronan (HA), in which the charge is due to glucuronic acid

(GlcA). Curiously, HA is also the only GAG that does not covalently bind proteins to form PGs; instead, it is secreted by cells directly into the ECM.

Sulfation is a dynamic and complex posttranslational modification process that is orchestrated by a class of enzymes known as sulfotransferases (STs). It can occur at various positions within the glycan backbone. In contrast to phosphorylation, which is involved in intracellular signal transduction, sulfation modulates extracellular signals such as cell–cell and cell–ECM interactions. Changes in the degree of sulfation of GAGs are often associated with different disorders. Because of their high specificity in relation to the function, GAG sulfation patterns are often referred to as the sulfation code (2, 3).

GlcA: glucuronic acid

ST: sulfotransferase

PAPS: 3′-phosphoadenosine 5′-phosphosulfate

CS: chondroitin sulfate

GalNAc: *N*-acetylgalactosamine

IdoA: L-iduronic acid

DS: dermatan sulfate

2. SULFATION OF GLYCOSAMINOGLYCANS

In a mammalian cell, the sulfation process begins with uptake of inorganic sulfate from the extracellular milieu (**Figure 1**). The sulfate must be activated prior to reacting with the acceptor glycan molecule. In mammals, this activated form of the sulfate is the sulfonucleotide 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (**Figure 1**), which is the universal sulfonate donor for all ST reactions (4, 5).

3. FAMILIES OF SULFATED GLYCOSAMINOGLYCANS

STs contribute significantly to the diversity of GAGs: A simple octasaccharide may have thousands (or more) of different sequences because the sulfation can occur at various positions (e.g., C4, C6, and/or on the nonacetylated nitrogen). On the basis of their monosaccharide constituents and pattern/degree of sulfation, four families of sulfated GAGs have been established.

3.1. Chondroitin Sulfates

Chondroitin sulfates (CSs) consist of repeating *N*-acetylgalactosamine (GalNAc)-GlcA disaccharide units joined by β1,4 and β1,3 linkages, respectively. Several STs involved in 4-*O*- and 6-*O*-sulfation of GalNAc units are responsible for obtaining CSs with different degrees of sulfation (0.1–1.3 per disaccharide unit) and patterns (**Figure 2**). CSs may contain sulfate groups in both the C4 and C6 positions of the GalNAc unit (CS-E) but may also be exclusively 4-sulfated (CS-A) or 6-sulfated (CS-C). Three STs (C4ST1, C4ST2, and C2ST3) that catalyze the 4-*O*-sulfation of GalNAc in CS have been identified (9). The STs involved in 6-*O*-sulfation of GalNAc in CSs include C6ST1 and GlcNAc6ST4. The GlcA unit can also be sulfated at the C2 position, giving rise to CS-B (4-sulfated GalNAc and 2-sulfated GlcA) and CS-D (6-sulfated GalNAc and 2-sulfated GlcA). So far, only one 2-*O*-ST is known: CS/DS2ST, which catalyzes the sulfation of both GlcA and L-iduronic acid (IdoA) (10).

3.2. Dermatan Sulfates

Dermatan sulfates (DSs) are stereoisomers of CSs. DSs derive from CSs by enzymatically driven C5 inversion of varying amounts of GlcA to IdoA. The proportion of IdoA in DSs can vary from a few percent to almost 100% in a single chain. Two epimerases, DS epimerases 1 and 2 (DSepi1 and DSepi2), are involved in this process (**Figure 3**). These two enzymes share a common N-terminal epimerase domain with a 51%-similar amino acid sequence (11). DSepi1 has a C-terminal domain of unknown function, whereas the C-terminal domain in DSepi2 shares 16% of its amino acid identity with C4ST1, suggesting that this enzyme may have dual activity (epimerase and *O*-ST). The degree of sulfation of DSs can vary between one and three sulfates per disaccharide unit (12).

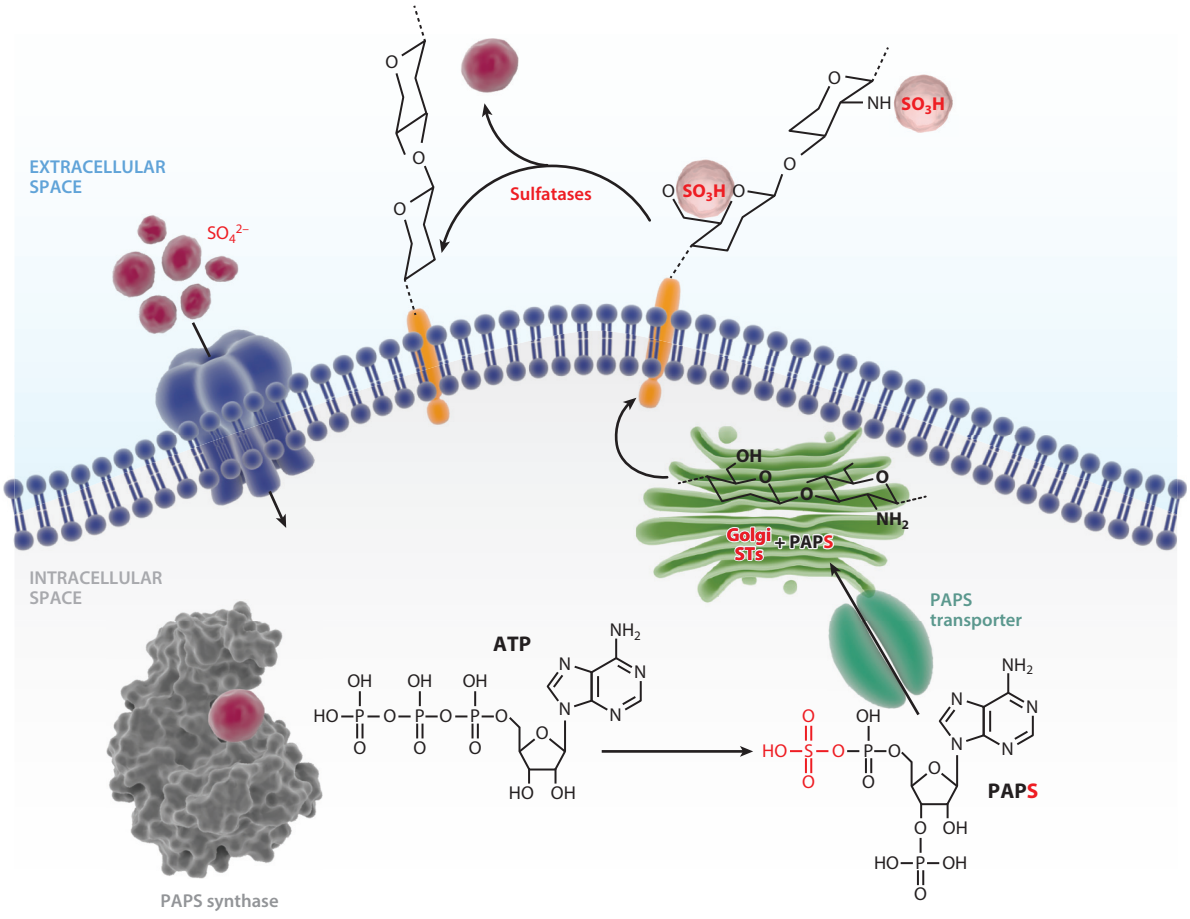


Figure 1

The sulfation cycle in mammalian cells. 3′-Phosphoadenosine 5′-phosphosulfate (PAPS) is synthesized in the cytosol and then transported into the Golgi apparatus by a specific transporter known as PAPS translocase (6). In this cellular compartment, the carbohydrate sulfotransferases (STs) catalyze the transfer of a PAPS sulfonyl group onto a hydroxyl or amino group(s) of the nascent glycoconjugates, which are then secreted into the extracellular matrix or inserted into the plasma membrane. So far, more than 30 Golgi-associated STs have been identified (see **Supplemental Table 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) (7, 8).

Supplemental Material

The sulfation is catalyzed by the same STs that are involved in CS biosynthesis. There is only one specific DS ST (D4ST1) that catalyzes 4-*O*-sulfation of GalNAc (13). It acts immediately after C5 epimerization; therefore, it prevents reversible epimerization of the newly formed IdoA. Thus, D4ST1 together with DSepi1 and DSepi2 is absolutely required for DS biosynthesis (11). Many of the IdoA units thus obtained undergo 2-*O*-sulfation that is catalyzed by CS/DS2ST as in CSs. This ST has greater activity toward IdoA, and as a result, the 2-*O*-sulfated IdoA in DSs is more abundant than the 2-*O*-sulfated GlcA in CSs. Because of the different orientation of their –COOH group, DSs have more flexible chains than CSs, allowing specific interactions with several proteins and polysaccharides.

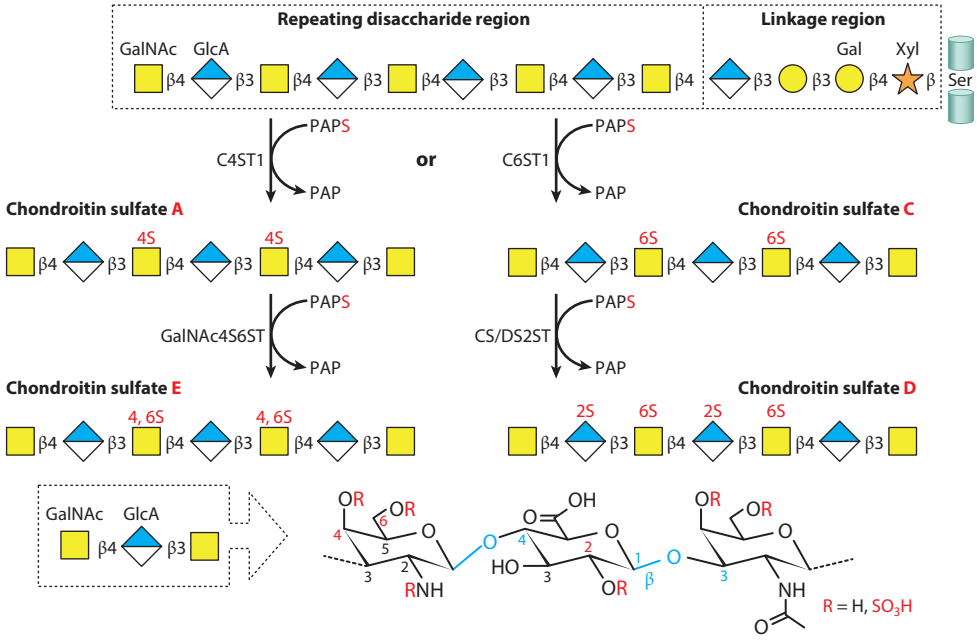


Figure 2

Biosynthetic pathways for chondroitin sulfate (CS) sulfation. The degree of sulfation and pattern of CSs are defined by the substrate specificity of the involved sulfotransferases (STs) (see **Supplemental Table 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). Abbreviations: GlcA, glucuronic acid; GalNAc, *N*-acetylgalactosamine; PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

Supplemental Material

3.3. Keratan Sulfates

Keratan sulfate (KS) chains contain repeating disaccharide units of galactose (Gal) and *N*-acetylglucosamine (GlcNAc) joined by β1,4 and β1,3 bonds, respectively. There are three types of KSs that differ in the linkage between the oligosaccharide and the core protein in the PGs (14): (*a*) KS I, which has been identified primarily in cornea; (*b*) KS II, which is found in cartilage; and (*c*) KS III, which has been isolated from brain tissue (**Figure 4**).

KSs vary in length and degree of sulfation. The sulfation pattern in different KSs is determined by the same STs. GlcNAc6ST5 catalyzes the 6-*O*-sulfation of GlcNAc, and it is essential for the elongation of the KS chain—in other words, sulfation and elongation occur concomitantly. GST1 is a KS-specific ST (15) that has 37% homology with C6ST1 and transfers sulfate at the C6 position of Gal. In contrast to GlcNAc6ST5, GST1 can act after chain elongation, that is, postsynthetically.

KS: keratan sulfate
Gal: galactose
GlcNAc: *N*-acetylglucosamine
Hep: heparin
HS: heparan sulfate

3.4. Heparins and Heparan Sulfates

Heparins (Heps) and heparan sulfates (HSs) are composed of alternating α1,4 GlcNAc and β1,4 GlcA units. During their assembly in the Golgi apparatus, they undergo extensive modification that includes concomitant or independent and sequential actions of a series of enzymes. So far, 26 enzymes that participate in HS biosynthesis have been identified. They catalyze processes such as

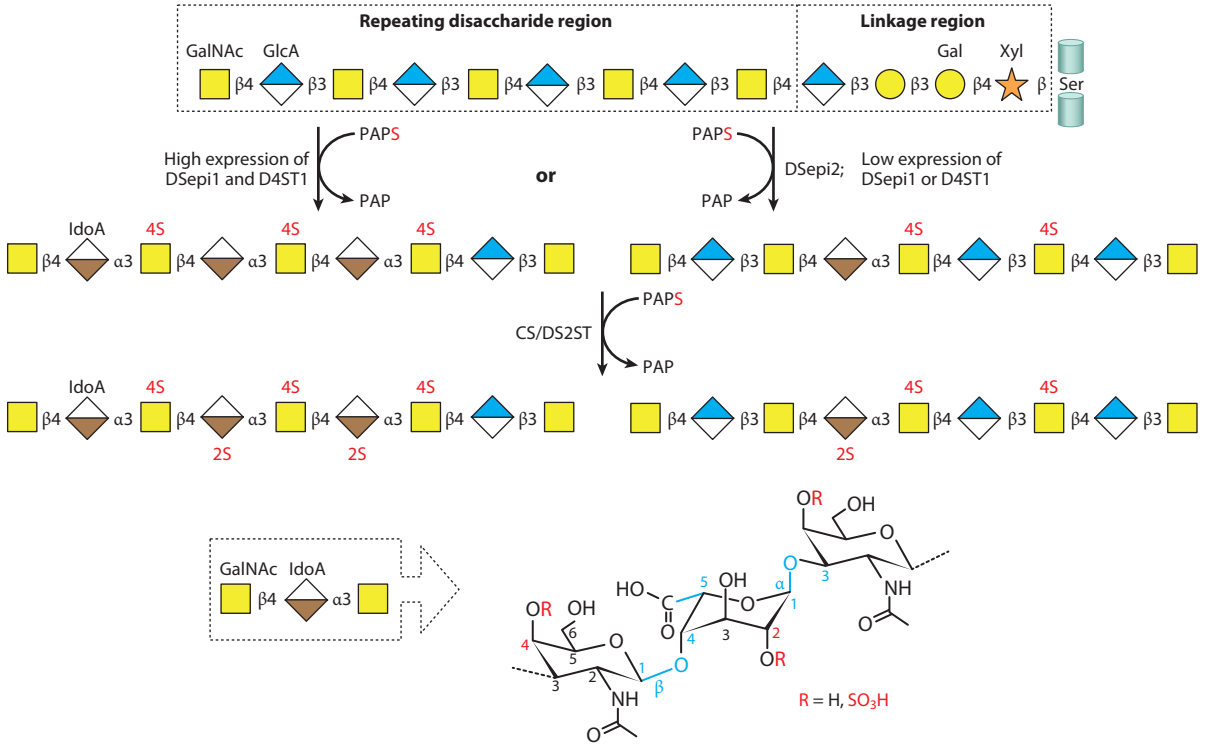


Figure 3

Dermatan sulfate (DS) is derived from chondroitin sulfate (CS) by C5 epimerization of glucuronic acid (GlcA) and is concomitant with 4-sulfation. Epimerization can occur during or after the formation of CS. Abbreviations: DSepi, DS epimerase; GalNAc, *N*-acetylgalactosamine; IdoA, L-iduronic acid; PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

N-deacetylation, *N*- and *O*-sulfation at different positions, and C5 epimerization of GlcA to IdoA (**Figure 5**) (12, 16). These modifications result in glycan chains that are highly heterogeneous in terms of chain length and size, space between the modified units, and extent of sulfation and epimerization. Note that although CS chains have long regions of fully modified disaccharides, in Hep/HS biosynthesis the modifications take place in clusters along the chain, with segments of sulfated sugars separating the unmodified regions. This structure generates a specific arrangement of sulfated residues and, thus, creates binding sequences for ligands (1, 17, 18).

The predominant disaccharide unit in Hep is *N*-sulfoglucosamine/2-*O*-sulfated IdoA (–GlcNS-IdoA2S–), but sulfation can also occur at the C6 position and, more rarely, at the C3 position of GlcNS, making Hep the biomacromolecule with the highest negative charge (2.7 sulfates per disaccharide unit on average) (1).

Supplemental Material

Although Hep and HS are structurally related, there are some differences between them (**Supplemental Table 2**). HS has a lower degree of modification (both sulfation and epimerization): Generally, it contains approximately one $-OSO_3H$ group per disaccharide unit, and GlcA is the predominant uronic acid component. Its polymer chains are more complex and longer than those of Hep macromolecules. HS always remains connected to its core protein. It is ubiquitously distributed on cell surfaces and is also a common component of the ECM.

GlcNS: *N*-sulfoglucosamine

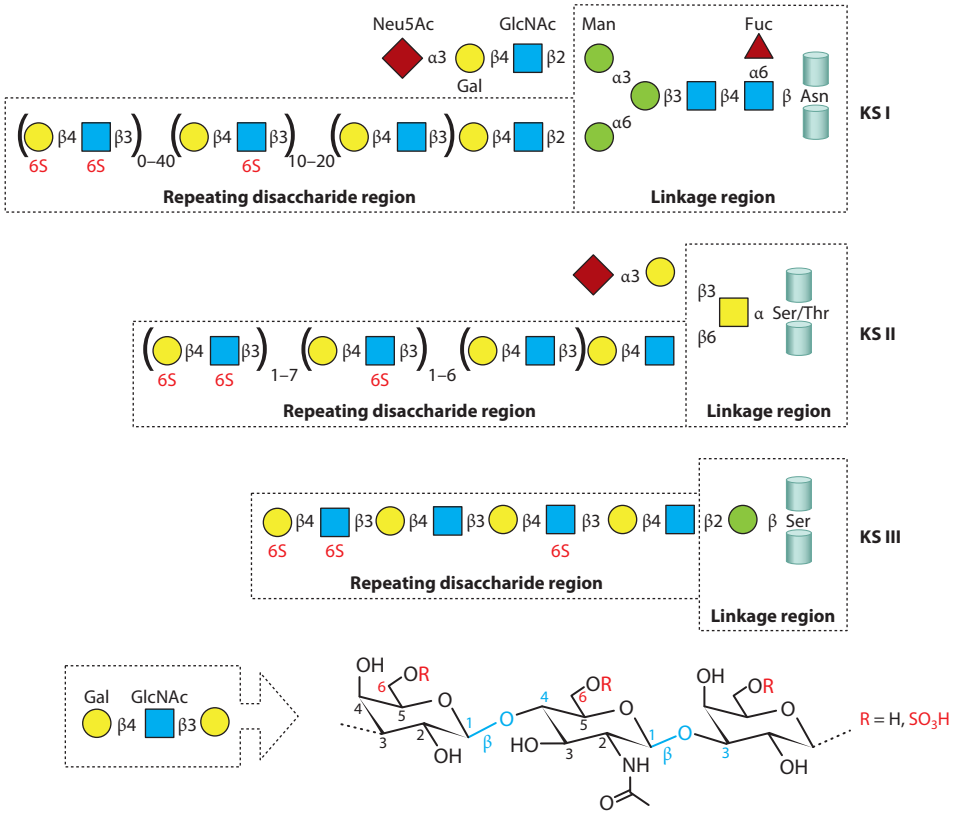


Figure 4

Protein linkage regions determine three classes of keratan sulfates (KSs). Two sulfotransferases, GlcNAc6ST5 and GST1, are involved in the 6-*O*-sulfation of KS. GlcNAc6ST5 is also essential for chain growth, and GST1 acts after elongation. Abbreviations: GlcNAc, *N*-acetylglucosamine; Neu5Ac, *N*-acetylneuraminic acid.

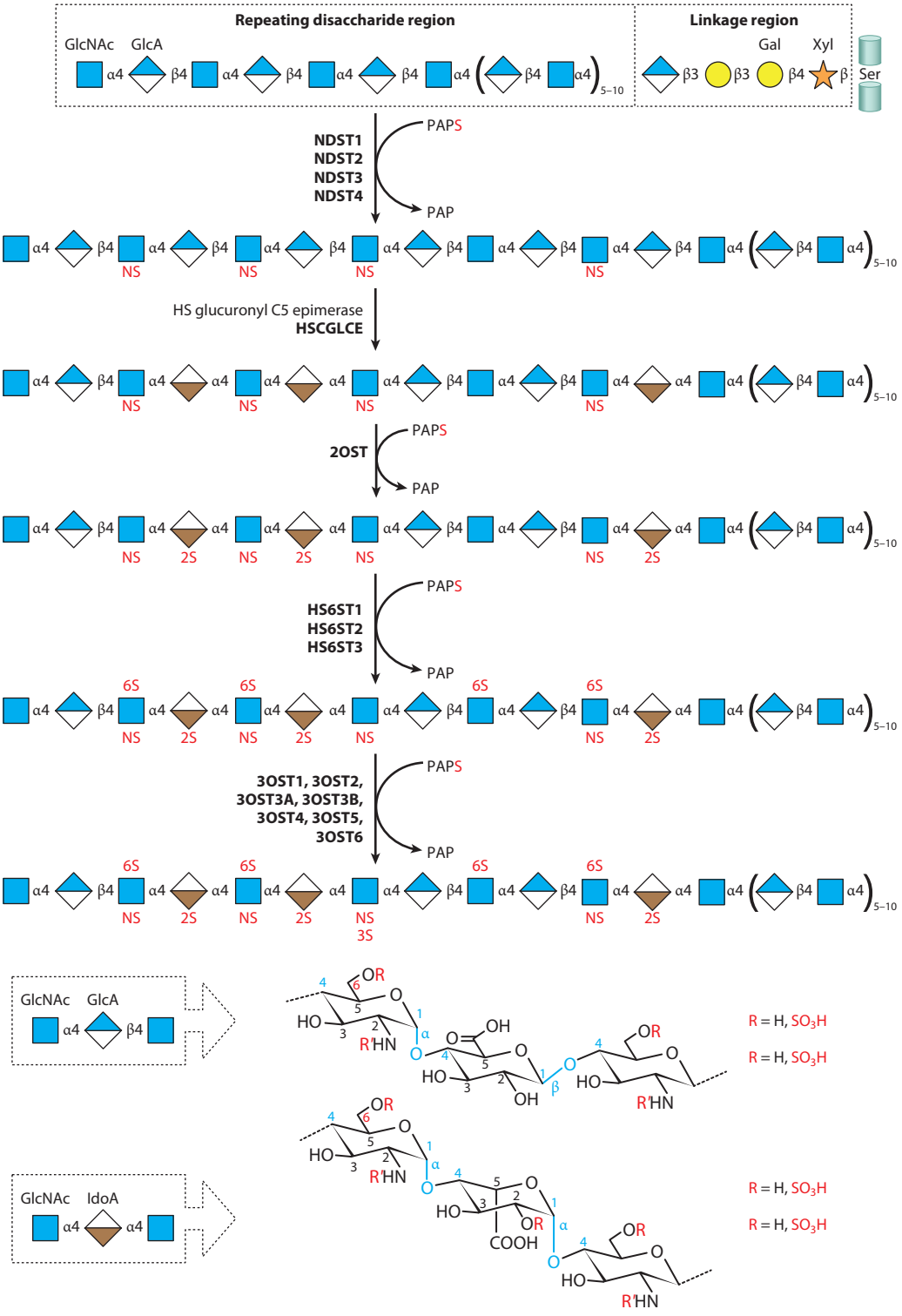
4. MECHANISM OF ACTION/BIOACTIVITY OF SULFATED GLYCOSAMINOGLYCANS

Sulfated GAGs are part of the pericellular space, where they are involved in a plethora of extracellular signaling events influencing cell-, tissue-, and organism-level development (**Supplemental Figure 1**) (16, 19–23). A significant body of evidence shows that sulfated GAGs exert their influence by interacting with other ECM components, mainly proteins, and that the degree of GAG sulfation—specifically, their negative charge—is the main driver of these interactions (24–26). Sulfation can influence GAG cross talk with other bioentities in the physiological environment either indirectly, such as by regulating protein folding via steric hindrance, exclusion, or recruitment (27), or directly, through electrostatic interactions that are often sequence specific (2, 28–30). Examples are presented in the following subsections.

Supplemental Material

4.1. Implications at the Cellular Level

Sulfated GAGs are usually part of PGs. The protein core determines whether the PGs are localized on the cell surface, in secretory granules, or in the ECM, while the GAG components


Repeating disaccharide region
Linkage region
GlcNAc
GlcA
Gal
Xyl
Ser
NDST1
NDST2
NDST3
NDST4
PAPS
PAP
HS glucuronyl C5 epimerase
HSCGLCE
2OST
HS6ST1
HS6ST2
HS6ST3
3OST1, 3OST2,
3OST3A, 3OST3B,
3OST4, 3OST5,
3OST6
IdoA
R = H, SO3H
R = H, SO3H
R = H, SO3H
R = H, SO3H

mediate interactions with a variety of extracellular ligands and adhesion molecules (**Supplemental Figure 1**).

GF: growth factor

PN: perineuronal net

CSPG: chondroitin sulfate proteoglycan

Supplemental Material

4.1.1. Glycosaminoglycans create specific microenvironments. GAGs can act as a physical and biochemical barrier, creating specific microenvironments around cells (**Supplemental Figure 1*a***). They build size-selective barriers that are permeable only by small entities, such as Ca^{2+} and Na^{+}, that can freely diffuse and promote extracellular cation homeostasis. GAGs also play a crucial role in the buildup of stem cell niches—specific microenvironments that save stem cells from depletion and protect the host from overexuberant stem cell proliferation. Stem cell niches are distinguished by the presence of low-sulfated GAGs, whose role is to avoid exposure of stem cells to growth factors (GFs) and receptor binding; thus, they help maintain the cells in an undifferentiated state (31, 32). When daughter cells are translocated outside the niche, they are no longer protected by this shield and are exposed to proteins that activate different signaling pathways (**Supplemental Figure 1*c–f***) and, hence, compelling processes such as proliferation and differentiation. Loss of pluripotency and differentiation are accompanied by changes in the sulfation pattern of GAGs in the ECM; a decrease in the level of nonsulfated disaccharides and an increase in sulfation are observed upon differentiation of human stem cells in different lineages (32). Note that although overall sulfation increases during differentiation, the patterns found within specific lineages are different; in other words, they are cell specific.

Perineuronal nets (PNs) represent another specific cellular environment. PNs are highly condensed ECM that surrounds the cell bodies and proximal dendrites of some types of neurons found in the human entorhinal cortex, amygdala, hippocampus, motor and somatosensory cortex, visual cortex, and prefrontal cortex (33). Once considered to be simple structural supports, PNs are now attracting a great deal of attention in neuroscience research because of their crucial role in the regulation of synaptic function and plasticity (the ability of neurons to adapt their responses to a changing environment) during postnatal development and in adulthood (34). Moreover, their malfunction is associated with such pathologies as Alzheimer disease (35, 36) and epilepsy (37), and their deficit is a hallmark of schizophrenia (38, 39). Contrary to stem cell niches, PNs are rich in sulfated GAGs, with a particular abundance of CSs presented as PGs (CSPGs) such as aggrecan; versican; and brevican and neurocan, which are specific to neuronal tissues (**Supplemental Figure 2**) (40–42).

The sulfation pattern of CSPGs determines their function(s) and contributes greatly to the molecular heterogeneity of PNs. In the early postnatal period of brain development, largely soluble complexes of proteins are the main constituents of PNs. During this period, CSPGs are expressed in different brain regions, where they guide neurite outgrowth by providing signals that promote or inhibit neurite outgrowth or modulate neuronal polarity in vitro (43, 44). In the late postnatal period, PN composition changes to create a defined and stable microenvironment around the neurons: Highly charged, insoluble complexes of CSPGs are responsible for synaptic stabilization, ion homeostasis, and neuroprotection (41, 44). The end of this period coincides with the end of the so-called critical period. In the mature neural circuit, CSPGs are associated with constraining neural plasticity; they act as a physical barrier that inhibits the formation of new synaptic contacts, but they also bind other molecules that constrain activity-dependent synaptic modification (41).

Figure 5

Heparin/heparan sulfate (Hep/HS) biosynthesis involves a series of modification reactions, including sulfation and epimerization, that are catalyzed by as many as 26 enzymes. Abbreviations: GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; IdoA, L-iduronic acid; PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

FGF-2: fibroblast growth factor 2

Supplemental Material

4.1.2. Glycosaminoglycans serve as storage depots for proteins. GAGs are linear negatively charged macromolecules, which act as molecular wires that present multiple binding contact points to positively charged regions/amino acids in proteins. These molecular wires restrict the movement of bound proteins (e.g., cytokines) to one dimension in three-dimensional space. As a result, protein gradients are created next to the site of secretion, where they are stored and protected against degradation until further use. This protective role of GAGs is illustrated by the interaction between fibroblast growth factor 2 (FGF-2) and Hep in the ECM (1, 27, 45). FGF-2 interacts with Hep via a pentasaccharide (**Supplemental Figure 3**) as soon as it is secreted by cells. An active form of the resulting complex can be isolated upon proteolytic degradation of the ECM, demonstrating that FGF-2 is protected from such degradation (27). The interaction of FGF-2 with soluble Hep can also be used to increase the activity of the GF. In this case, the FGF-2–Hep complex does not bind to the Hep immobilized in the ECM (because its Hep binding sites are unavailable), and it diffuses further than the FGF-2 molecule alone (27). As a result, the complex stimulates morphological changes in a significantly larger area than FGF-2 alone when released from a defined source on a cellular monolayer. Other examples of GFs that are stored in the ECM by their specific interaction with GAGs are presented in **Supplemental Table 3**.

4.1.3. Signaling via sulfated glycosaminoglycans. GAGs on the cell surface localize and/or immobilize various ligands for receptor binding and signal transduction (**Supplemental Figure 1*c–f***). The restriction of protein movement next to the cells that express the proteins facilitates cell–ECM and intercellular communication via both autocrine and paracrine signaling.

Among the families of sulfated GAGs, the Hep/HS family is the best-studied one in terms of the biointeractions that affect cell behavior via the striking diversity of the specific Hep/HS cell ligands (**Supplemental Table 4**). Most of these interactions are complex, often involving the formation of highly organized complexes of more than two macromolecules. This multivalency, together with the complex environment in which the interactions occur, makes investigations of such interactions difficult and elucidation of their mechanisms quite challenging. The ionic interactions between the carboxyl and sulfate groups from the GAGs and the positively charged amino acid (e.g., lysine and arginine) residues in the proteins, which are usually ordered in Cardin–Weintraub sequences, are the main contributors to the formation of GAG–protein complexes (46). However, in some cases there are significant contributions by nonionic interactions, such as hydrogen bonding, van der Waals forces, and hydrophobic forces. The results of such specific multivalent interactions can be (*a*) protection of proteins from degradation, as discussed above; (*b*) conformational change of the protein that can evoke its activation or deactivation (e.g., antithrombin; **Supplemental Figure 3*b***); and/or (*c*) clustering of binding complexes at the cell surface (**Supplemental Figure 2*c***). In all cases, the resulting GAG–protein complexes have profound physiological effects on processes such as cell growth and migration; therefore, they affect the development of tissues, organs, and organisms.

Protein conformational changes induced following GAG–protein interactions are at the root of the oldest known GAG bioactivity—the anticoagulant activity of Hep (47, 48). This activity is based on an interaction between the protein antithrombin and a specific pentasaccharide from Hep (**Supplemental Figure 3*a***). As a result, the conformation of antithrombin changes (**Supplemental Figure 3*b***), and it can bind thrombin and Factor Xa more efficiently: Hep enhances the rate of inactivation of thrombin and Factor Xa by 1,000-fold. After thrombin is inactivated, the complex loses affinity for Hep, dissociates, and is ready to interact with another antithrombin molecule.

A very similar mechanism triggers the activation of different ECM components. Conformational changes of ECM proteins resulting from their complexation with cell surface GAGs modulate the interaction between these proteins and their respective ligands (e.g., integrins, GFs) at the

cell surface. An example is the Hep/HS–fibronectin (Fn) complex. Hep/HS binds reversibly to Fn, thereby inducing a conformational change that is retained even after Hep/HS unbinding. This new conformation exposes more binding sites and thus dramatically increases Fn's affinity for GFs (49, 50). The specific interaction between Fn and Hep/HS also forms the basis of cell attachment. In this process, syndecans (transmembrane PGs that carry three to five HS and CS chains) and integrins are the main players. HSs from the syndecan bind Fn, thereby localizing it next to the cell surface and the integrins. The proximity between the integrins and the Fn promotes Fn's specific binding (51).

Fn: fibronectin

HSPG: heparan sulfate proteoglycan

Sulfated GAGs (as either ECM components or transmembrane PGs) can also act as coreceptors regulating ligand function via different mechanisms (52, 53). In the simplest scenario, they enhance receptor complex formation by facilitating ligand binding to their canonical signaling receptors. This is the case for the activation of FGF-2 (**Supplemental Figure 3*c***). As mentioned above, FGF-2 is stored and protected in the ECM through its specific interactions with Hep. The stored FGF-2 is also activated by Hep, but a longer (10-mer) sequence is required to trigger the formation of a tight ternary complex between FGF-2, Hep, and the FGF-2 receptor (45).

Supplemental Material

4.2. Engagement of Sulfated Glycosaminoglycans in Development of Organs and Tissues

The role of GAG sulfation patterns in maintaining organs and tissues in a healthy state can be understood via two distinct processes: development and regeneration. Structural changes in GAGs that have no effect at the cellular level often have a huge impact at the organ/tissue or organism level. Thus, knockout animals have been used as models in such studies. However, evidence for the importance of GAG sulfation in human health and development is mostly based on genetic disorders involving mutations in genes encoding GAG sulfation. The fact that many of these mutations cause developmental abnormalities provides compelling evidence for the importance of sulfation in many stages of development in multicellular organisms. Regeneration processes, such as recovery of tissue after injury, have also contributed significantly to our understanding of the importance of sulfation in human health.

4.2.1. Development of the central nervous system. Whereas only nonsulfated GAGs are abundant in bacteria, sulfated GAGs are found throughout the animal kingdom (vertebrates and invertebrates). Interestingly, this discrepancy coincides with the appearance of tissues that are organized into germ layers that produce neurons (54). Among the sulfated GAGs, CSs are the most abundant in the central nervous system (CNS), where they can be found as 16 different CSPGs that play a critical role in the development and pathophysiology of the brain and spinal cord (55–57). Histological analyses of prenatal and early postnatal human cerebrum have revealed that the quantity of CSs is high in early embryos [26–31 postconceptional weeks (PCW)], gradually decreases until birth (32–34 PCW), and disappears in newborns (58).

The structure of CSs also changes during this period. CS-C (6-sulfated), which is predominant in the embryonic period, gradually decreases and is replaced by CS-A (4-sulfated), which is predominant at the end of embryogenesis (59, 60). This change is consistent with the observation that CS-A, but not CS-C, exhibits a strong negative guidance cue to cerebellar neurons (61). Heparan sulfate proteoglycans (HSPGs) are also present in the CNS, although in a smaller quantity. Their role(s) in the CNS development is less well studied than the involvement of CSPGs, but they are known to act mainly by recruitment and activation of different GFs and morphogens (60).

4.2.2. Development of cartilage and bone. CSPGs, KSPGs, and (to a lesser extent) DSPGs are the major components of the cartilage ECM. They have two main roles in this tissue: (*a*) to generate

TGF-β: transforming growth factor β

BMP: bone morphogenetic protein

Supplemental Material

an osmotic swelling pressure, which enables the cartilage to withstand a wide range of compressive loads, and (*b*) to directly influence chondrocytes' activity, either through cell–ECM interactions or through the binding of specific GFs in the ECM. Among the different PGs, aggrecan is the most abundant component of human articular cartilage (**Supplemental Figure 4**). It consists of a protein core with attached KSs and CSs. The structure of aggrecan varies with anatomical site and tissue depth, but the age of the individual seems to be the most important factor associated with the composition of the cartilage (62, 63). The sulfation pattern undergoes major changes until age 20 years—an age that is associated with maturation of the cartilage and until which the chondrocytes are most active. The content of 6-sulfated CSs increases during this period, whereas that of 4-sulfated CSs decreases immediately after birth, and this diminution persists up to 20 years of age (**Supplemental Figure 5**). Thereafter, the content of both 6- and 4-sulfated CSs remains the same until age 85 years (62). Importantly, these changes are not related to mechanical loading, as demonstrated by quantification of CSs in different areas of cartilage. Although poorly understood, these changes suggest that regulation of GAG synthesis may be useful in the treatment of cartilage disorders. For example, treatment of mature cartilage with transforming growth factor β (TGF-β) modifies matrix synthesis by decreasing the ratio of 6- to 4-sulfated CSs; in other words, the resulting composition resembles less "mature" articular cartilage (63).

CSs also play a key role in skeletal development; they represent 67–97% of the total GAG content of bone. The CSPGs decorin, biglycan, and aggrecan have been identified in this tissue (64, 65). As in cartilage, the GAG composition in normal bone is age dependent—it decreases in elderly persons but also in individuals with hormonal imbalances. The CS composition (the ratio of 4- to 6-sulfated CSs) depends on the location of the tissue: Whereas 4-sulfation is predominant in alveolar bone (65), the 6 isomer is prevalent in femoral head bone (64).

Changes in sulfation patterns can greatly influence bone formation and development, processes that are tightly regulated by molecules including systemic and local soluble factors [Indian hedgehog, parathyroid hormone–related peptide, FGFs, members of the TGF-β family, bone morphogenetic proteins (BMPs)]. GAGs, especially CSs, usually act as coreceptors for these factors and are involved in numerous signaling cascades. Therefore, any imbalance in GAG sulfation or composition modifies these signaling pathways, thereby affecting skeletal development. In the following section, we discuss deficiencies in GAG sulfation mechanisms that can cause tissue abnormalities.

5. GENETIC DEFECTS IN GLYCOSAMINOGLYCAN SULFATION

Approximately 2% of the human genome encodes enzymes involved in glycan biosynthesis (66). However, inherited disorders related to defects in these genes were not discovered until very recently. In general, these are rare and very heterogeneous (both clinically and biochemically) disorders that usually affect multiple organ systems. Their mechanisms are far from being understood, and so far no effective treatments have been proposed for them.

5.1. *CHST3*-Related Skeletal Dysplasia

CHST3-related skeletal dysplasia is also known as autosomal recessive Larsen syndrome; spondyloepiphyseal dysplasia, Omani type; humero-spinal dysostosis; and chondrodysplasia with multiple dislocations. This recessively inherited disorder is clinically characterized by bone and joint abnormalities that worsen over time. Affected individuals have short stature throughout life and severely reduced adult height (110–130 cm) (67). Joint dislocations, most often affecting the knees, hips, and elbows, are present at birth (congenital) (68). Other bone and joint abnormalities can include an inward- and upward-turning foot (clubfoot), a limited range of motion in large joints, and abnormal curvature of the spine (67, 68). As its name suggests, the disorder is related to the

CHST3 gene, which encodes the ST C6ST1. Although C6ST1 is expressed in various human tissues and organs (**Supplemental Table 1**), the disorder affects only cartilage and bone, and the reason for this selectivity is unknown. An analysis of the disaccharide composition of CS chains produced from affected patients (either in fibroblasts or in urine) revealed that the proportion of 6-*O*-sulfated disaccharide units was markedly decreased but not zero, which may be attributable to GlcNAc6ST4. An increase in nonsulfated units has also been detected in sufferers (67).

Supplemental Material

5.2. Macular Corneal Dystrophy

The transparency of the corneas is essential for vision. This transparency depends on the characteristic spatial arrangement of collagen fibrils achieved by electrostatic interactions between collagen I and KS. Macular corneal dystrophy (MCD) is caused by mutations in the *CHST6* gene, which encodes the ST GlcNAc6ST5. Defects in the KS sulfation pattern resulting from this mutation cause distortions in fibril organization and corneal opacity (69, 70).

The first symptoms of MCD are corneal clouding and periodic photophobia, which appear in the second decade of life (i.e., in patients 10–12 years of age) (69). MCD is progressive and results in bilateral loss of vision (69–71). When patients reach the age of 20–30 years, corneal transplantation is usually required. There are two types of MCD: MCD type I is characterized by the absence of sulfated KS in serum, and MCD type II is characterized by its presence. These two types have clinically indistinguishable phenotypes.

5.3. Noninflammatory Peeling Skin Syndrome

Noninflammatory peeling skin syndrome, type A, is manifested at birth or during infancy with generalized white scaling associated with painless and spontaneous peeling of the skin (72). Direct contact with water, dust, or sand may cause skin irritation. Patients are in good general health. The cause is largely unknown, but recent evidence suggests a connection to mutations in the *CHST8* gene, which change the hydrophilicity of the amino acids in the coded Golgi transmembrane ST GalNAc4ST1 and reduce the ST's molecular weight. Moreover, decreased levels of total sulfated GAGs are observed in cells expressing mutant GalNAc4ST1 compared with wild type, suggesting a loss of function in mutant GalNAc4ST1 proteins.

5.4. Ehlers–Danlos Syndrome, Musculocontractural Type 1

Ehlers–Danlos syndrome, musculocontractural type 1 (EDS I), is also known as adducted thumb–clubfoot syndrome and Dundar syndrome. The classical EDS is associated with defects in connective tissue, and the symptoms can vary from mildly loose joints to life-threatening complications. Patients with EDS have soft, velvety skin that is highly elastic (stretchy) and fragile (73, 74). Sufferers have mutations in several genes that code collagen assembly. Recently, EDS was also associated with mutations in the *CHST14* gene, which encodes the ST D4ST1 (73–75). These mutations result in a deficiency of DSs in affected tissues (75). D4ST1 is involved in the synthesis (epimerization and sulfation) of DSs (**Figure 3**). Patients with EDS I are able to epimerize GlcA-GalNAc to IdoA-GalNAc, but they are not able to sulfate the IdoA-GalNAc thus obtained. As a result, the levels of IdoA-GalNAc, GlcA-GalNAc, and GlcA-GalNAc4S increase significantly; in other words, a large fraction of DSs is replaced by CSs. DSPGs are involved in the generation of morphogen gradients in epithelia; they are also involved in several specific interactions with TGF-β1, tenascin-X, and heparin cofactor II, among others. In EDS I, the CSs that are generated fail to replace DSs in these bioactivities, and this failure causes the disorder.

5.5. Hypogonadotropic Hypogonadism 15 with or Without Anosmia

Supplemental Material

Hypogonadotropic hypogonadism 15 (HH15) is manifested by absent or incomplete sexual maturation in conjunction with low levels of circulating gonadotropins (76). Patients with HH15 may also have an impaired sense of smell. Mutations of the *HS6ST1* gene, which encodes an ST with the same abbreviation (**Supplemental Table 1**), were recently connected with this disorder. It is not yet clear how the generated defects in HS sulfation patterns (missing or reduced 6-*O*-sulfation of GlcNS) influence the course of the disorder. The genetic mutations alone may not be sufficient to cause the disease; rather, they may contribute to a complex setup involving other HH15 genes that regulate neuronal branching (e.g., *kal-1*, *FGFR*, *FGF*) (76).

6. DISEASES ASSOCIATED WITH MISREGULATION OF GLYCOSAMINOGLYCAN SULFATION

Misregulation of GAG sulfation (either under- or oversulfation) is associated with several diseases (**Supplemental Table 5**). Importantly, the role of sulfated GAGs in these disorders is not trivial and straightforward; in cancer, for example, HSPGs may act as either inhibitors or promoters of tumor progression, depending on the type and stage of the disease.

6.1. Alzheimer Disease

Amyloidopathies, including Alzheimer disease, are characterized by extracellular plaques with fibrillated amyloid-β (Aβ) protein. Sulfated GAGs are key participants in this fibrillation process: Both CSPGs and HSPGs accumulate around the fibrillar cores, stabilize them, and protect them from proteolytic degradation, thereby aiding amyloidogenesis (77–79). Interestingly, sulfated GAGs alone reduce the aggregation and toxicity of Aβ, as well as the secretion of amyloid precursor protein; in other words, they inhibit Aβ fibrillogenesis (80). GAGs, therefore, play a dual role in amyloidosis both as a "good" protein partner, aiding nontoxic fibrillar conformation, and as a "bad" pathological chaperone, inducing protein aggregation. Initially, investigators speculated that changes in the degree of sulfation of GAGs were at the root of this dual role. However, patients with Alzheimer disease and healthy subjects show a very similar degree of sulfation of GAGs in the cerebral cortex (81). The only difference is in the GAGs' *N*-sulfation pattern. Curiously, the specific recognition sequence of HS for Aβ is exactly within the regions of consecutive *N*-sulfated disaccharide units and coincides with the FGF-2 binding site (82). The common binding site can explain the dual role of GAGs: Neurotoxic and neuroprotective signals may converge by competing for the same binding sites. Consequently, these shared HS domains have been employed as a therapeutic target for the inhibition of amyloid formation (82, 83).

A completely different mechanism has been elucidated for PNs, which have a protective role in Alzheimer disease. CSPGs from the PNs neutralize oxidative stress—they act as scavengers for iron ions, which are involved in the transformation of hydrogen peroxide (HO) to reactive oxygen species (84). Moreover, CSs also increase HO-1 activity (HO is an antioxidant enzyme with two isoforms: an inducible isoform, HO-1, and a constitutive isozyme, HO-2), providing efficient antioxidant protection without compromising cell viability (85).

6.2. Cancer

Cancer is characterized by abnormal cell growth that involves a range of unique alterations in intracellular and intercellular space. Because GAGs are the main mediators of communication (cell–cell and cell–ECM communication) in the intracellular space, they play a role in malignant transformation and tumor metastasis, as expected, and they can act as either promoters or inhibitors

of the disease. Indeed, changes in the degree of sulfation and/or the pattern of CSs and HSs are associated with breast (86, 87), ovarian (88, 89), colorectal (90), prostate (91–94), and gastric (95) cancers, among others. In most cases, these changes have been proposed as cancer biomarkers (86, 91, 93, 94). However, person-to-person variations in GAG composition must be considered when using these markers (96). Moreover, the same cancers at different stages are associated with different sulfation changes, which is expected but difficult to predict (90, 91).

SAR: structure–activity relationship

Most of the cancers that have been studied in this context showed altered 6-*O*-sulfation of CSs and/or HSs, which can be either decreased or increased and are usually accompanied by another change (or changes) in GAG sulfation (e.g., 2-*O*-sulfation) or expression (**Supplemental Table 5**) (97). The physiological significance of these modifications is not fully understood, but it is clear that the modified GAGs affect tumor progression by regulating proliferation, invasion, angiogenesis, and metastasis (98, 99). There is evidence that during tumor growth and proliferation, GAGs mainly act as coreceptors for GFs (100–103). They can also aid metastasis, either by facilitating tumor adhesion via participation in selectin binding (87) or by altering the expression of heparanase (99, 104, 105): High heparanase expression and activity result in higher metastatic potentials. HSs with a low degree of sulfation, typical in some cancers, do not effectively inhibit heparanase. As a result, this enzyme degrades the ECM and facilitates metastasis (96). The altered GAG sulfation patterns influence tumor invasiveness as well: Destabilized focal cell adhesions between cancer cells and the surrounding environment occur as a consequence of altered GAG structure (93, 94, 104).

Supplemental Material

7. THERAPIES AND REGENERATION APPROACHES INVOLVING NATURAL SULFATED GLYCOSAMINOGLYCANS AND SYNTHETIC MIMICS

The diverse bioactivities of sulfated GAGs make them an attractive class of therapeutics. Due to its anticoagulant activity, Hep is the best-studied carbohydrate therapeutic to date. CSs and KSs are also widely used for the treatment of osteoarthritis (OA) and corneal dystrophy, respectively. Other than these examples, there are few current applications of natural sulfated GAGs, for several reasons: (*a*) Isolating GAGs from natural sources is costly and labor intensive, resulting in low yields; (*b*) there is significant microheterogeneity because the biosynthesis and diversification of GAGs involve several complex steps regulated by multiple factors, including metabolic levels of sugar nucleotides, expression and localization of glycosylation enzymes, and protein-trafficking mechanisms; and (*c*) their structure–activity relationship (SAR) is largely unknown (106, 107). GAGs also present challenges to synthetic chemists because of the many functional groups that have to be protected in order to get one specific group to react (106, 108, 109). The introduction of sulfate groups in specific positions adds another level of complexity to the already-challenging synthesis process. Consequently, the existing methods for the assembly of sulfated GAGs are costly, time consuming, and limited to short oligosaccharides (generally up to 10 units). Thus, alternative, simpler molecules that mimic the targeted features of individual GAGs have been developed for tissue engineering, regenerative medicine, and pharmaceutics (110).

7.1. Artificial Two- and Three-Dimensional Cellular Environments Created by Glycosaminoglycans

Generally, GAG mimics can be divided into three groups: (*a*) two-dimensional supports from GAGs analogs, (*b*) bulky three-dimensional models from GAG mimics, and (*c*) exogenous soluble GAG mimics. The two-dimensional substrates usually imitate the glycans from the cell surface

(106, 111). Such functional platforms are designed to be compatible with different surface characterization techniques, which are very sensitive and can be used to characterize the weak multivalent interactions in which GAGs participate along with other bioentities (24, 112, 113). Thus, these platforms are very useful for elucidating the mechanisms by which GAGs code and transfer information and for identifying new specific biomolecules with which GAGs interact. GAGs are also a crucial component of the ECM. Mimicking the ECM requires different bulky models that more closely resemble the natural three-dimensional cellular environment. As in the two-dimensional models, the bulky substrates are useful in fundamental studies but can also be employed as substitutes of GAGs in different regenerative approaches (114). Finally, soluble GAGs mimics are supplemented either to compensate for pathological GAG deficits or to interfere with specific signaling pathways causing different diseases (115).

7.1.1. Immobilization of sulfated glycosaminoglycans. The study of GAGs' biointeractions is inherently difficult and requires the design of suitable analytical platforms. Recently, our group proposed simple two-dimensional substrates that are based on single-component or mixed self-assembled monolayers and mimic GAGs with a defined degree of sulfation (24, 112, 113). Despite their minimalism, these substrates are useful in studies elucidating the influence of the degree of sulfation of GAGs on their interactions with proteins and cells. However, they do not represent certain important features of GAGs, such as sulfation pattern or chain mobility. Entire GAG molecules can be immobilized to create closer mimics. The main challenge in this approach is to preserve the GAGs' bioactivity while immobilizing them irreversibly. Covalent immobilization via conventional protocols such as *N*-hydroxysuccinimide activation followed by carbodiimide coupling, biotinylation, hydrazide functionalization, and more recently, oxime click chemistry is often used (116–120). The functionalization can occur either at the reductive end of the GAGs (end-on manner) or through the whole GAG chain (side-on manner). Because end-on modification only minimally alters the GAG's structure, it is more suitable for the development of platforms targeting the elucidation of GAGs' bioactivity or SAR studies (116, 121). The density of the immobilized molecules is another important issue to consider in the design of analytical platforms: If the immobilized GAGs are very dense, they can be inaccessible to or hindered by the ligand. By contrast, if the GAGs are at a very low density, multivalent interactions between them and their ligands are not possible, and determination of their bioactivity is also compromised.

Layer-by-layer constructs that incorporate sulfated GAGs can be also employed as ECM mimics. These provide a feasible platform for the study of specific cell–ECM interactions and the influence of ECM mechanical properties on cellular behavior (122–124).

The development of different GAG platforms has potentiated the rapid, sensitive, and high-throughput screening of GAGs' interactions with cells and proteins (cytokines, GFs, and antibodies), thereby helping to elucidate various signaling pathways. These screening platforms are also the basis for valuable diagnostic tools (for, e.g., serum screening of specific GFs, cytokines, and/or chemokines) and for drug discovery involving GAGs in a variety of pathologies.

7.1.2. Glycosaminoglycan-based hydrogels as effective three-dimensional mimics of extracellular matrix. Hydrogels are an appealing scaffold material due to their structural similarity to natural ECM, mild processing conditions, and minimally invasive delivery. Artificial ECM can be created by mixing native ECM components, such as GAGs and proteins or their derivatives, such as functional peptides and crosslinkable GAGs (125, 126). Tissue engineering, culture and expansion of therapeutically relevant cells, and drug/protein delivery are among the common applications of these gels (114, 127). The application determines the choice of GAG—for example, CSs support chondrogenesis, and CS-based hydrogels have been proposed for cartilage tissue

engineering (128, 129) and as fillers for damaged articular cartilage promoting normal metabolic function and ECM remodeling (130). CS hydrogels can also improve nerve growth by mimicking neural ECM and delivering neurotrophic signals to cells (131). Hep/HS has been used in the design of hydrogels mimicking ECM (132). Hep covalently bound to different polymer cores has been used as an HSPG mimic, yielding a multivalent biomaterial that is capable of controlled release of GFs, such as FGF-2 (119, 132). Furthermore, Hep-based hydrogels are a promising matrix for the encapsulation and maintenance of different cells, such as difficult-to-culture primary hepatocytes (133).

SCI: spinal cord injury

7.2. Examples of the Potential Uses of Sulfated Glycosaminoglycans in Regenerative Medicine and Disease Control

Our limited knowledge of GAGs' bioactivity and SAR significantly impedes the biomedical application of glycans in various pathological scenarios. Patient-to-patient variations in GAG composition and the dynamic structural changes that occur during the life cycle are other issues that are difficult to address both in practice and in terms of regulatory approval. As a result, there is a significant body of evidence demonstrating the enormous therapeutic potential of GAGs in different animal models, but the application of GAGs in human treatments is in its infancy, with very few clinical trials and approved products.

7.2.1. Recovery after spinal cord injury. Recovery from injuries to the CNS, including spinal cord injury (SCI), is extremely limited. Upon SCI, macrophages, microglia, oligodendrocyte precursors, meningeal cells, and astrocytes migrate to the lesion, where they secrete different inhibitory molecules such as CSPGs (42, 134). The microenvironment formed around the injury site is known as a glial scar—it is composed mainly of astrocytes and CSPGs, and its role is to limit the extent of tissue damage. In addition to its beneficial role immediately after injury, the CSPGs in a glial scar inhibit axonal sprouting and therefore limit the regeneration process in the subacute and chronic stages of the injury. Digestion of CSPGs with chondroitinase ABC enhances axon regeneration and functional recovery in different animal models. Although the mechanism by which chondroitinase acts is not completely understood, it has been suggested that the enzyme digests not only the CSPGs from the glial scar but also those from PNs, thereby enhancing plasticity (134). Despite promising results from animal experiments, this treatment has a drawback related to the broad spectrum of action of chondroitinase ABC, which can also digest other GAGs, such as HA, that are crucial for functional maintenance of the ECM. Therefore, we conclude that CSPGs are a good target for treatments of SCI but that a more selective enzyme or inhibitor(s) must be used at the lesion site.

7.2.2. Cartilage and bone defects. OA is a degenerative joint disease that affects mainly the articular cartilage but is often extended to the subchondral bone. It is characterized by pain, stiffness, and loss of function and affects the knees, fingers, and hips, usually in elderly patients. The disease is associated with unbalanced synthesis/degradation of ECM and abnormal secretion of PGs. Initial attempts at OA treatment relied on the assumption that supplementation with exogenous precursors of ECM components would help articular cartilage cells restore the damaged environment (115). Glucosamine (GlcN) is a biosynthetic precursor of GlcNAc, the main building block of GAGs; owing to its known safety and high abundance in nature (from chitin), GlcN oral administration was proposed as a treatment for damaged cartilage. These attempts failed, as the supplemented GlcN was rapidly metabolized and thus did not aid the synthesis of new GAGs. Following these failed treatments, GlcN sulfate was tested as an alternative (135, 136).

LMWH: low-molecular-weight heparin

Treatment of OA chondrocytes with GlcN sulfate caused a dose-dependent increase in cell-associated GAG content. However, chondrocytes failed to respond to treatment in 40% of the patients studied (136). Because these treatments with GAG precursors did not have the expected effect, investigators then tested the administration of CSs. Different clinical trials showed that oral administration of a combination of GlcN sulfate and low-molecular-weight CSs is successful (137, 138). However, the mechanism of action of orally administered CSs is unknown. CSs are poorly absorbed through the digestive system, which means that they are not systemically delivered to the cartilage; it is likely that they indirectly stimulate chondrocytes to synthesize ECM components (139).

GAGs are also involved in bone cell homeostasis: They promote osteogenesis and suppress the paracrine support of osteoclast functions, thereby promoting bone remodeling (120, 125). The inhibition of osteoclast differentiation and bone resorption depends mainly on the degree of sulfation of GAGs rather than on the monosaccharide composition (125). The ability of CSs to interact specifically with bone-regulating proteins (e.g., cytokines of the TGF-β superfamily) makes these GAGs promising biomaterials for bone tissue regeneration (140).

7.2.3. Treatment of Alzheimer disease. Sulfated GAGs or their synthetic mimics prevent the formation of amyloid fibrils and the binding of amyloidogenic proteins to the cell surface by direct competition with natural PGs (77, 141). Sulfation of GAGs is crucial in this process—fully desulfated GAGs are not active in Aβ fibrillogenesis, whereas selectively *O*- or *N*-desulfated GAGs are, with dramatically reduced activity (77).

The most-studied GAG mimic as an agent for antiamyloid aggregation is 3-aminopropylsulfonic acid (homotaurine, tramiprosate; trade name Alzhemed™), which emerged in a screen of different low-molecular-weight GAGs and their mimics (141, 142). Although a Phase I study demonstrated that 3-aminopropylsulfonic acid is a safe compound, a Phase II study was not conclusive, and Phase III failed to show any clinical benefit (143). The reasons for this failure are not clear, as some unexpected problems were detected among the control group. However, this failure may be associated with the other pathology behind Alzheimer disease—some studies have shown that tramiprosate promotes an abnormal aggregation of the tau protein in neuronal cells (144). Highly sulfated low-molecular-weight Heps (LMWHs) such as neuroparin (C3) are also potential therapeutics, but so far they have been tested only in animal models (145, 146).

7.2.4. Controlling tumor progression. As mentioned above, sulfated GAGs have important roles in oncogenesis; thus, they represent potential therapeutics for human cancers. Indeed, Heps and LMWHs are the most widely used anticancer therapeutics among different GAGs and are already on the market (98, 147). Their clinical application relies on (*a*) inhibition of the binding of P- and L-selectins to sialyl Lewis X ligand, which is usually overexpressed in cancer cells; (*b*) binding of heparanase; or (*c*) inhibition of the interactions between GF and Hep/HS through competition with natural HSPGs (148–151). P-, E-, and L-selectins mediate tumor interactions with blood cells such as platelets (P-selectins), leukocytes (L-selectins), and endothelium (E-selectins); thus, they are involved in tumor metastasis through the blood circulation (150). Once tumor cells access the vasculature, they can form large aggregates with platelets and leukocytes, which can then settle in the small vessels of distant organs (98).

Heparanases are directly related to the tumor's metastatic potential (105). They are required for tumor cells to invade the vascular basement membrane: The heparanases released by migrating tumor cells can liberate sequestered GFs by degrading the GAG component of the HSPGs from its healthy surroundings. Hep-binding GFs such as FGFs, vascular endothelial growth factor, TGF-β, and interleukin-8 are related to tumor growth and vascularization. In a tumor

environment, cancer cells produce these GFs and use them in both an autocrine and a paracrine (i.e., on the surrounding host cells) fashion (98). GAGs, particularly Hep, act as coreceptors for these GFs, facilitate their storage, and protect them from degradation.

8. OUTLOOK AND FUTURE DIRECTIONS

Sulfated GAGs bind many ligands, modulate numerous cellular processes, and are essential for tissue architecture and physiology. Although the mechanisms underlying several biological processes regulated in vivo by GAGs are known, the pathways of GAG action, and specifically of their sulfation patterns, generally remain to be elucidated, especially in humans. There is clear evidence that altered sulfation patterns affect protein binding. Subtle variations in the GAG sulfation pattern modulate interactions with different enzymes, inhibitors, cell surface receptors, and ECM proteins, resulting in developmental defects and misregulation of signaling pathways. However, we do not yet know whether findings obtained in different animal models can be applied to the action of GAGs in humans. Nevertheless, the GAG sulfation pattern in human health and pathology is undoubtedly important, as its misregulation causes several degenerative diseases and malignant neoplasms. A better understanding of the underlying mechanisms would open new opportunities for disease control and therapy. Translating new findings into therapies requires the development of technologies enabling precise control over GAGs sulfation in living cells and organisms. Thus, the design of specific enzymes and inhibitors that can modulate sulfation patterns is one of the most powerful and promising tools for the application of GAGs as therapeutic agents.

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LITERATURE CITED

1. Capila I, Linhardt RJ. 2002. Heparin–protein interactions. *Angew. Chem. Int. Ed.* 41:391–412
2. Gama CI, Tully SE, Sotogaku N, Clark PM, Rawat M, et al. 2006. Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. *Nat. Chem. Biol.* 2:467–73
3. Habuchi H, Habuchi O, Kimata K. 2004. Sulfation pattern in glycosaminoglycan: Does it have a code? *Glycoconj. J.* 21:47–52
4. Farooqui AA. 1980. 3′-Phosphoadenosine 5′-phosphosulfate metabolism in mammalian tissues. *Int. J. Biochem.* 12:529–36
5. Venkatachalam KV. 2003. Human 3′-phosphoadenosine 5′-phosphosulfate (PAPS) synthase: biochemistry, molecular biology and genetic deficiency. *IUBMB Life* 55:1–11
6. Ozeran JD, Westley J, Schwartz NB. 1996. Identification and partial purification of PAPS translocase. *Biochemistry* 35:3695–703
7. Kusche-Gullberg M, Kjellén L. 2003. Sulfotransferases in glycosaminoglycan biosynthesis. *Curr. Opin. Struct. Biol.* 13:605–11
8. Bowman KG, Bertozzi CR. 1999. Carbohydrate sulfotransferases: mediators of extracellular communication. *Chem. Biol.* 6:R9–22
9. Mikami T, Kitagawa H. 2013. Biosynthesis and function of chondroitin sulfate. *Biochim. Biophys. Acta* 1830:4719–33
10. Kobayashi M, Sugumaran G, Liu JA, Shworak NW, Silbert JE, Rosenberg RD. 1999. Molecular cloning and characterization of a human uronyl 2-sulfotransferase that sulfates iduronyl and glucuronyl residues in dermatan chondroitin sulfate. *J. Biol. Chem.* 274:10474–80
11. Thelin MA, Bartolini B, Axelsson J, Gustafsson R, Tykesson E, et al. 2013. Biological functions of iduronic acid in chondroitin/dermatan sulfate. *FEBS J.* 280:2431–46

12. Lindahl U, Hook M. 1978. Glycosaminoglycans and their binding to biological macromolecules. *Annu. Rev. Biochem.* 47:385–417
13. Pacheco B, Maccarana M, Malmström A. 2009. Dermatan 4-*O*-sulfotransferase 1 is pivotal in the formation of iduronic acid blocks in dermatan sulfate. *Glycobiology* 19:1197–203
14. Funderburgh JL. 2002. Keratan sulfate biosynthesis. *IUBMB Life* 54:187–94
15. Fukuta M, Inazawa J, Torii T, Tsuzuki K, Shimada E, Habuchi O. 1997. Molecular cloning and characterization of human keratan sulfate Gal-6-sulfotransferase. *J. Biol. Chem.* 272:32321–28
16. Bishop JR, Schuksz M, Esko JD. 2007. Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature* 446:1030–37
17. Esko JD, Selleck SB. 2002. Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Annu. Rev. Biochem.* 71:435–71
18. Mulloy B, Linhardt RJ. 2001. Order out of complexity—protein structures that interact with heparin. *Curr. Opin. Struct. Biol.* 11:623–28
19. Linhardt RJ, Toida T. 2004. Role of glycosaminoglycans in cellular communication. *Acc. Chem. Res.* 37:431–38
20. Ganguly C, Thakurata GG, Mukherjee KL, Bhattacharya N. 2016. Mucopolysaccharides, water and electrolytes of human fetal organs. In *Human Fetal Growth and Development*, ed. N Bhattacharya, PG Stubblefield, pp. 99–120. Cham, Switz.: Springer
21. Lensen JFM, Rops ALWMM, Wijnhoven TJM, Hafmans T, Feitz WFJ, et al. 2005. Localization and functional characterization of glycosaminoglycan domains in the normal human kidney as revealed by phage display–derived single chain antibodies. *J. Am. Soc. Nephrol.* 16:1279–88
22. Mizumoto S, Yamada S, Sugahara K. 2015. Molecular interactions between chondroitin–dermatan sulfate and growth factors/receptors/matrix proteins. *Curr. Opin. Struct. Biol.* 34:35–42
23. Sasisekharan R, Raman R, Prabhakar V. 2006. Glycomics approach to structure–function relationships of glycosaminoglycans. *Annu. Rev. Biomed. Eng.* 8:181–231
24. Amorim S, Pires RA, Soares da Costa D, Reis RL, Pashkuleva I. 2013. Interactions between exogenous FGF-2 and sulfonic groups: in situ characterization and impact on the morphology of human adipose-derived stem cells. *Langmuir* 29:7983–92
25. Jemth P, Kreuger J, Kusche-Gullberg M, Sturiale L, Giménez-Gallego G, Lindahl U. 2002. Biosynthetic oligosaccharide libraries for identification of protein-binding heparan sulfate motifs—exploring the structural diversity by screening for fibroblast growth factor (FGF)1 and FGF2 binding. *J. Biol. Chem.* 277:30567–73
26. Xu D, Esko JD. 2014. Demystifying heparan sulfate–protein interactions. *Annu. Rev. Biochem.* 83:129–57
27. Flaumenhaft R, Moscatelli D, Rifkin DB. 1990. Heparin and heparan sulfate increase the radius of diffusion and action of basic fibroblast growth factor. *J. Cell Biol.* 111:1651–59
28. Faham S, Hileman RE, Fromm JR, Linhardt RJ, Rees DC. 1996. Heparin structure and interactions with basic fibroblast growth factor. *Science* 271:1116–20
29. Casu B, Oreste P, Torri G, Zoppetti G, Choay J, et al. 1981. The structure of heparin oligosaccharide fragments with high anti-(factor Xa) activity containing the minimal antithrombin III–binding sequence. Chemical and C-13 nuclear-magnetic-resonance studies. *Biochem. J.* 197:599–609
30. Atha DH, Stephens AW, Rosenberg RD. 1984. Evaluation of critical groups required for the binding of heparin to antithrombin. *PNAS Biol. Sci.* 81:1030–34
31. Smith RAA, Meade K, Pickford CE, Holley RJ, Merry CLR. 2011. Glycosaminoglycans as regulators of stem cell differentiation. *Biochem. Soc. Trans.* 39:383–87
32. Gasimli L, Hickey AM, Yang B, Li G, dela Rosa M, et al. 2014. Changes in glycosaminoglycan structure on differentiation of human embryonic stem cells towards mesoderm and endoderm lineages. *Biochim. Biophys. Acta* 1840:1993–2003
33. Pantazopoulos H, Murray EA, Berretta S. 2008. Total number, distribution, and phenotype of cells expressing chondroitin sulfate proteoglycans in the normal human amygdala. *Brain Res.* 1207:84–95
34. Soleman S, Filippov MA, Dityatev A, Fawcett JW. 2013. Targeting the neural extracellular matrix in neurological disorders. *Neuroscience* 253:194–213
35. Baig S, Wilcock GK, Love S. 2005. Loss of perineuronal net *N*-acetylgalactosamine in Alzheimer's disease. *Acta Neuropathol.* 110:393–401

36. Lendvai D, Morawski M, Negyessy L, Gati G, Jager C, et al. 2013. Neurochemical mapping of the human hippocampus reveals perisynaptic matrix around functional synapses in Alzheimer's disease. *Acta Neuropathol.* 125:215–29
37. McRae PA, Porter BE. 2012. The perineuronal net component of the extracellular matrix in plasticity and epilepsy. *Neurochem. Int.* 61:963–72
38. Berretta S, Pantazopoulos H, Markota M, Brown C, Batzianouli ET. 2015. Losing the sugar coating: potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophr. Res.* 167:18–27
39. Mauney SA, Athanas KM, Pantazopoulos H, Shaskan N, Passeri E, et al. 2013. Developmental pattern of perineuronal nets in the human prefrontal cortex and their deficit in schizophrenia. *Biol. Psychiatry* 74:427–35
40. Sugahara K, Mikami T. 2007. Chondroitin/dermatan sulfate in the central nervous system. *Curr. Opin. Struct. Biol.* 17:536–45
41. Miao Q-L, Ye Q, Zhang X-H. 2014. Perineuronal net, CSPG receptor and their regulation of neural plasticity. *Acta Physiol. Sin.* 66:387–97
42. Rhodes KE, Fawcett JW. 2004. Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? *J. Anat.* 204:33–48
43. Bovolenta P, Fernaud-Espinosa I. 2000. Nervous system proteoglycans as modulators of neurite outgrowth. *Prog. Neurobiol.* 61:113–32
44. Hartmann U, Maurer P. 2001. Proteoglycans in the nervous system—the quest for functional roles in vivo. *Matrix Biol.* 20:23–35
45. Zhang FM, Zhang ZQ, Lin XF, Beenken A, Eliseenkova AV, et al. 2009. Compositional analysis of heparin/heparan sulfate interacting with fibroblast growth factor · fibroblast growth factor receptor complexes. *Biochemistry* 48:8379–86
46. Cardin AD, Weintraub HJR. 1989. Molecular modeling of protein–glycosaminoglycan interactions. *Arteriosclerosis* 9:21–32
47. Björk I, Lindahl U. 1982. Mechanism of the anticoagulant action of heparin. *Mol. Cell. Biochem.* 48:161–82
48. Rosenber RD, Damus PS. 1973. Purification and mechanism of action of human antithrombin–heparin cofactor. *J. Biol. Chem.* 248:6490–505
49. Mitsi M, Hong ZN, Costello CE, Nugent MA. 2006. Heparin-mediated conformational changes in fibronectin expose vascular endothelial growth factor binding sites. *Biochemistry* 45:10319–28
50. Martino MM, Hubbell JA. 2010. The 12th–14th type III repeats of fibronectin function as a highly promiscuous growth factor–binding domain. *FASEB J.* 24:4711–21
51. Morgan MR, Humphries MJ, Bass MD. 2007. Synergistic control of cell adhesion by integrins and syndecans. *Nat. Rev. Mol. Cell Biol.* 8:957–69
52. Kirkbride KC, Ray BN, Blobe GC. 2005. Cell-surface co-receptors: emerging roles in signaling and human disease. *Trends Biochem. Sci.* 30:611–21
53. Mythreye K, Blobe GC. 2009. Proteoglycan signaling co-receptors: roles in cell adhesion, migration and invasion. *Cell. Signal.* 21:1548–58
54. Yamada S, Sugahara K, Ozbek S. 2011. Evolution of glycosaminoglycans: comparative biochemical study. *Commun. Integr. Biol.* 4:150–58
55. Dyck SM, Karimi-Abdolrezaee S. 2015. Chondroitin sulfate proteoglycans: key modulators in the developing and pathologic central nervous system. *Exp. Neurol.* 269:169–87
56. Rowlands D, Sugahara K, Kwok JCF. 2015. Glycosaminoglycans and glycomimetics in the central nervous system. *Molecules* 20:3527–48
57. Bandtlow CE, Zimmermann DR. 2000. Proteoglycans in the developing brain: new conceptual insights for old proteins. *Physiol. Rev.* 80:1267–90
58. Kostović I, Jovanov-Milošević N, Radoš M, Sedmak G, Benjak V, et al. 2014. Perinatal and early postnatal reorganization of the subplate and related cellular compartments in the human cerebral wall as revealed by histological and MRI approaches. *Brain Struct. Funct.* 219:231–53
59. Bertolotto A, Rocca G, Schiffer D. 1990. Chondroitin-4-sulfate proteoglycan forms an extracellular network in human and rat central nervous system. *J. Neurol. Sci.* 100:113–23

60. Smith PD, Coulson-Thomas VJ, Foscarin S, Kwok JCF, Fawcett JW. 2015. "GAG-ing with the neuron": the role of glycosaminoglycan patterning in the central nervous system. *Exp. Neurol.* 274:100–14
61. Wang H, Katagiri Y, McCann TE, Unsworth E, Goldsmith P, et al. 2008. Chondroitin-4-sulfation negatively regulates axonal guidance and growth. *J. Cell Sci.* 121:3083–91
62. Bayliss MT, Osborne D, Woodhouse S, Davidson C. 1999. Sulfation of chondroitin sulfate in human articular cartilage. The effect of age, topographical position, and zone of cartilage on tissue composition. *J. Biol. Chem.* 274:15892–900
63. Hickery MS, Bayliss MT, Dudhia J, Lewthwaite JC, Edwards JCW, Pitsillides AA. 2003. Age-related changes in the response of human articular cartilage to IL-1α and transforming growth factor-β (TGF-β). Chondrocytes exhibit a diminished sensitivity to TGF-β. *J. Biol. Chem.* 278:53063–71
64. Mania VM, Kallivokas AG, Malavaki C, Asimakopoulou AP, Kanakis J, et al. 2009. A comparative biochemical analysis of glycosaminoglycans and proteoglycans in human orthotopic and heterotopic bone. *IUBMB Life* 61:447–52
65. Smith AJ, Singhrao SK, Newman GR, Waddington RJ, Embery G. 1997. A biochemical and immuno-electron microscopical analysis of chondroitin sulphate–rich proteoglycans in human alveolar bone. *Histochem. J.* 29:1–9
66. Schachter H, Freeze HH. 2009. Glycosylation diseases: Quo vadis? *Biochim. Biophys. Acta* 1792:925–30
67. Thiele H, Sakano M, Kitagawa H, Sugahara K, Rajab A, et al. 2004. Loss of chondroitin 6-*O*-sulfotransferase-1 function results in severe human chondrodysplasia with progressive spinal involvement. *PNAS* 101:10155–60
68. Hermanns P, Unger S, Rossi A, Perez-Aytes A, Cortina H, et al. 2008. Congenital joint dislocations caused by carbohydrate sulfotransferase 3 deficiency in recessive Larsen syndrome and humero-spinal dysostosis. *Am. J. Hum. Genet.* 82:1368–74
69. Lewis D, Davies Y, Nieduszynski IA, Lawrence F, Quantock AJ, et al. 2000. Ultrastructural localization of sulfated and unsulfated keratan sulfate in normal and macular corneal dystrophy type I. *Glycobiology* 10:305–12
70. Saito T, Nishida K, Nakayama J, Akama TO, Fukuda MN, et al. 2008. Sulfation patterns of keratan sulfate in different macular corneal dystrophy immunophenotypes using three different probes. *Br. J. Ophthalmol.* 92:1434–36
71. Akama TO, Nishida K, Nakayama J, Watanabe H, Ozaki K, et al. 2000. Macular corneal dystrophy type I and type II are caused by distinct mutations in a new sulphotransferase gene. *Nat. Genet.* 26:237–41
72. Cabral RM, Kurban M, Wajid M, Shimomura Y, Petukhova L, Christiano AM. 2012. Whole-exome sequencing in a single proband reveals a mutation in the *CHST8* gene in autosomal recessive peeling skin syndrome. *Genomics* 99:202–8
73. Miyake N, Kosho T, Mizumoto S, Furuichi T, Hatamochi A, et al. 2010. Loss-of-function mutations of CHST14 in a new type of Ehlers–Danlos syndrome. *Hum. Mutat.* 31:966–74
74. Malfait F, Syx D, Vlummens P, Symoens S, Nampoothiri S, et al. 2010. Musculocontractural Ehlers–Danlos syndrome (former EDS type VIB) and adducted thumb clubfoot syndrome (ATCS) represent a single clinical entity caused by mutations in the dermatan-4-sulfotransferase 1 encoding *CHST14* gene. *Hum. Mutat.* 31:1233–39
75. Dundar M, Müller T, Zhang Q, Pan J, Steinmann B, et al. 2009. Loss of dermatan-4-sulfotransferase 1 function results in adducted thumb–clubfoot syndrome. *Am. J. Hum. Genet.* 85:873–82
76. Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, et al. 2011. Heparan sulfate 6-*O*-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. *PNAS* 108:11524–29
77. Castillo GM, Lukito W, Wight TN, Snow AD. 1999. The sulfate moieties of glycosaminoglycans are critical for the enhancement of β-amyloid protein fibril formation. *J. Neurochem.* 72:1681–87
78. Iannuzzi C, Irace G, Sirangelo I. 2015. The effect of glycosaminoglycans (GAGs) on amyloid aggregation and toxicity. *Molecules* 20:2510–28
79. van Horssen J, Wesseling P, van den Heuvel LPWJ, de Waal RMW, Verbeek MM. 2003. Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. *Lancet Neurol.* 2:482–92
80. Díaz-Nido J, Wandosell F, Avila J. 2002. Glycosaminoglycans and β-amyloid, prion and tau peptides in neurodegenerative diseases. *Peptides* 23:1323–32

81. Lindahl B, Eriksson L, Lindahl U. 1995. Structure of heparan sulfate from human brain, with special regard to Alzheimer's disease. *Biochem. J.* 306:177–84
82. Lindahl B, Westling C, Giménez-Gallego G, Lindahl U, Salmivirta M. 1999. Common binding sites for β-amyloid fibrils and fibroblast growth factor 2 in heparan sulfate from human cerebral cortex. *J. Biol. Chem.* 274:30631–35
83. McLaurin J, Franklin T, Zhang XQ, Deng JP, Fraser PE. 1999. Interactions of Alzheimer amyloid-β peptides with glycosaminoglycans. Effects on fibril nucleation and growth. *Eur. J. Biochem.* 266:1101–10
84. Morawski M, Brückner MK, Riederer P, Brückner G, Arendt T. 2004. Perineuronal nets potentially protect against oxidative stress. *Exp. Neurol.* 188:309–15
85. Viapiano MS, Matthews RT. 2006. From barriers to bridges: chondroitin sulfate proteoglycans in neuropathology. *Trends Mol. Med.* 12:488–96
86. Fernández-Vega I, García O, Crespo A, Castañón S, Menéndez P, et al. 2013. Specific genes involved in synthesis and editing of heparan sulfate proteoglycans show altered expression patterns in breast cancer. *Breast Cancer Res.* 13:R58
87. Cooney CA, Jousheghany F, Yao-Borengasser A, Phanavanh B, Gomes T, et al. 2011. Chondroitin sulfates play a major role in breast cancer metastasis: a role for *CSPG4* and *CHST11* gene expression in forming surface P-selectin ligands in aggressive breast cancer cells. *Breast Cancer Res.* 13:R58
88. Backen AC, Cole CL, Lau SC, Clamp AR, McVey R, et al. 2007. Heparan sulphate synthetic and editing enzymes in ovarian cancer. *Br. J. Cancer* 96:1544–48
89. Vallen MJE, Massuger LFAG, ten Dam GB, Bulten J, van Kuppevelt TH. 2012. Highly sulfated chondroitin sulfates, a novel class of prognostic biomarkers in ovarian cancer tissue. *Gynecol. Oncol.* 127:202–09
90. Kalathas D, Theocharis DA, Bounias D, Kyriakopoulou D, Papageorgakopoulou N, et al. 2009. Alterations of glycosaminoglycan disaccharide content and composition in colorectal cancer: structural and expressional studies. *Oncol. Rep.* 22:369–75
91. Ricciardelli C, Mayne K, Sykes PJ, Raymond WA, McCaul K, et al. 1997. Elevated stromal chondroitin sulfate glycosaminoglycan predicts progression in early-stage prostate cancer. *Clin. Cancer Res.* 3:983–92
92. Ricciardelli C, Sakko AJ, Stahl J, Tilley WD, Marshall VR, Horsfall DJ. 2009. Prostatic chondroitin sulfate is increased in patients with metastatic disease but does not predict survival outcome. *Prostate* 69:761–69
93. Teng YHF, Tan PH, Chia SJ, Zam NABM, Lau WKO, et al. 2008. Increased expression of non-sulfated chondroitin correlates with adverse clinicopathological parameters in prostate cancer. *Mod. Pathol.* 21:893–901
94. Sakko AJ, Butler MS, Byers S, Reinboth BJ, Stahl J, et al. 2008. Immunohistochemical level of unsulfated chondroitin disaccharides in the cancer stroma is an independent predictor of prostate cancer relapse. *Cancer Epidemiol. Biomark. Prev.* 17:2488–97
95. Theocharis AD, Vynios DH, Papageorgakopoulou N, Skandalis SS, Theocharis DA. 2003. Altered content composition and structure of glycosaminoglycans and proteoglycans in gastric carcinoma. *Int. J. Biochem. Cell Biol.* 35:376–90
96. Weyers A, Yang B, Yoon DS, Park JH, Zhang F, et al. 2012. A structural analysis of glycosaminoglycans from lethal and nonlethal breast cancer tissues: toward a novel class of theragnostics for personalized medicine in oncology? *OMICS* 16:79–89
97. Vynios DH, Theocharis DA, Papageorgakopoulou N, Papadas TA, Mastronikolis NS, et al. 2008. Biochemical changes of extracellular proteoglycans in squamous cell laryngeal carcinoma. *Connect. Tissue Res.* 49:239–43
98. Fuster MM, Esko JD. 2005. The sweet and sour of cancer: glycans as novel therapeutic targets. *Nat. Rev. Cancer* 5:526–42
99. Iozzo RV, Sanderson RD. 2011. Proteoglycans in cancer biology, tumour microenvironment and angiogenesis. *J. Cell. Mol. Med.* 15:1013–31
100. ten Dam GB, van de Westerlo EMA, Purushothaman A, Stan RV, Bulten J, et al. 2007. Antibody GD3G7 selected against embryonic glycosaminoglycans defines chondroitin sulfate E domains highly up-regulated in ovarian cancer and involved in vascular endothelial growth factor binding. *Am. J. Pathol.* 171:1324–33

101. Kleeff J, Ishiwata T, Kumbasar A, Friess H, Büchler MW, et al. 1998. The cell-surface heparan sulfate proteoglycan glypican 1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. *J. Clin. Investig.* 102:1662–73
102. Wade A, Robinson AE, Engler JR, Petritsch C, James CD, Phillips JJ. 2013. Proteoglycans and their roles in brain cancer. *FEBS J.* 280:2399–417
103. Nurcombe V, Smart CE, Chipperfield H, Cool SM, Boilly B, Hondermarck H. 2000. The proliferative and migratory activities of breast cancer cells can be differentially regulated by heparan sulfates. *J. Biol. Chem.* 275:30009–18
104. Sanderson RD. 2001. Heparan sulfate proteoglycans in invasion and metastasis. *Semin. Cell Dev. Biol.* 12:89–98
105. Goldshmidt O, Zcharia E, Abramovitch R, Metzger S, Aingorn H, et al. 2002. Cell surface expression and secretion of heparanase markedly promote tumor angiogenesis and metastasis. *PNAS* 99:10031–36
106. Pashkuleva I, Reis RL. 2010. Sugars: burden or biomaterials of the future? *J. Mater. Chem.* 20:8803–18
107. Pomin VH. 2015. A dilemma in the glycosaminoglycan-based therapy: synthetic or naturally unique molecules? *Med. Res. Rev.* 35:1195–219
108. Karst NA, Linhardt RJ. 2003. Recent chemical and enzymatic approaches to the synthesis of glycosaminoglycan oligosaccharides. *Curr. Med. Chem.* 10:1993–2031
109. Boltje TJ, Buskas T, Boons GJ. 2009. Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research. *Nat. Chem.* 1:611–22
110. Petitou M, van Boeckel CAA. 2004. A synthetic antithrombin III binding pentasaccharide is now a drug! What comes next? *Angew. Chem. Int. Ed.* 43:3118–33
111. Disney MD, Seeberger PH. 2004. The use of carbohydrate microarrays to study carbohydrate–cell interactions and to detect pathogens. *Chem. Biol.* 11:1701–7
112. Soares da Costa D, Pires RA, Frias AM, Reis RL, Pashkuleva I. 2012. Sulfonic groups induce formation of filopodia in mesenchymal stem cells. *J. Mater. Chem.* 22:7172–78
113. Araújo AR, Soares da Costa DP, Amorim S, Reis RL, Pires RA, Pashkuleva I. 2016. Surfaces mimicking glycosaminoglycans trigger different response of stem cells via distinct fibronectin adsorption and reorganization. *ACS Appl. Mater. Interfaces* 8:28428–36
114. Freudenberg U, Liang Y, Kiick KL, Werner C. 2016. Glycosaminoglycan-based biohybrid hydrogels: a sweet and smart choice for multifunctional biomaterials. *Adv. Mater.* 28:8861–91
115. Brockhausen I, Anastassiades TP. 2008. Inflammation and arthritis: perspectives of the glycobiologist. *Expert Rev. Clin. Immunol.* 4:173–91
116. Altgarde N, Nileback E, de Battice L, Pashkuleva I, Reis RL, et al. 2013. Probing the biofunctionality of biotinylated hyaluronan and chondroitin sulfate by hyaluronidase degradation and aggrecan interaction. *Acta Biomater.* 9:8158–66
117. Migliorini E, Thakar D, Sadir R, Pleiner T, Baleux F, et al. 2014. Well-defined biomimetic surfaces to characterize glycosaminoglycan-mediated interactions on the molecular, supramolecular and cellular levels. *Biomaterials* 35:8903–15
118. Thakar D, Migliorini E, Coche-Guerente L, Sadir R, Lortat-Jacob H, et al. 2014. A quartz crystal microbalance method to study the terminal functionalization of glycosaminoglycans. *Chem. Commun.* 50:15148–51
119. Novoa-Carballal R, Silva C, Möller S, Schnabelrauch M, Reis RL, Pashkuleva I. 2014. Tunable nanocarriers from clicked glycosaminoglycan block copolymers. *J. Mater. Chem. B* 2:4177–84
120. Salbach-Hirsch J, Ziegler N, Thiele S, Möller S, Schnabelrauch M, et al. 2014. Sulfated glycosaminoglycans support osteoblast functions and concurrently suppress osteoclasts. *J. Cell. Biochem.* 115:1101–11
121. Hintze V, Samsonov SA, Anselmi M, Möller S, Becher J, et al. 2014. Sulfated glycosaminoglycans exploit the conformational plasticity of bone morphogenetic protein 2 (BMP-2) and alter the interaction profile with its receptor. *Biomacromolecules* 15:3083–92
122. Mhanna RF, Voros J, Zenobi-Wong M. 2011. Layer-by-layer films made from extracellular matrix macromolecules on silicone substrates. *Biomacromolecules* 12:609–16
123. Francesko A, Soares da Costa D, Lisboa P, Reis RL, Pashkuleva I, Tzanov T. 2012. GAGs-thiolated chitosan assemblies for chronic wounds treatment: control of enzyme activity and cell attachment. *J. Mater. Chem.* 22:19438–46

124. Teixeira R, Reis RL, Pashkuleva I. 2016. Influence of the sulfation degree of glycosaminoglycans on their multilayer assembly with poly-L-lysine. *Colloids Surf. B* 145:567–75
125. Salbach J, Kliemt S, Rauner M, Rachner TD, Goettsch C, et al. 2012. The effect of the degree of sulfation of glycosaminoglycans on osteoclast function and signaling pathways. *Biomaterials* 33:8418–29
126. van der Smissen A, Hintze V, Scharnweber D, Möller S, Schnabelrauch M, et al. 2011. Growth promoting substrates for human dermal fibroblasts provided by artificial extracellular matrices composed of collagen I and sulfated glycosaminoglycans. *Biomaterials* 32:8938–46
127. Azevedo HS, Pashkuleva I. 2015. Biomimetic supramolecular designs for the controlled release of growth factors in bone regeneration. *Adv. Drug Deliv. Rev.* 94:63–76
128. Levett PA, Melchels FPW, Schrobback K, Hutmacher DW, Malda J, Klein TJ. 2014. A biomimetic extracellular matrix for cartilage tissue engineering centered on photocurable gelatin, hyaluronic acid and chondroitin sulfate. *Acta Biomater.* 10:214–23
129. Wang DA, Varghese S, Sharma B, Strehin I, Fermanian S, et al. 2007. Multifunctional chondroitin sulphate for cartilage tissue–biomaterial integration. *Nat. Mater.* 6:385–92
130. Grenier S, Donnelly PE, Gittens J, Torzilli PA. 2015. Resurfacing damaged articular cartilage to restore compressive properties. *J. Biomech.* 48:122–29
131. Conovaloff A, Panitch A. 2011. Characterization of a chondroitin sulfate hydrogel for nerve root regeneration. *J. Neural Eng.* 8:056003
132. Cai SS, Liu YC, Shu XZ, Prestwich GD. 2005. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials* 26:6054–67
133. Kim M, Lee JY, Jones CN, Revzin A, Tae G. 2010. Heparin-based hydrogel as a matrix for encapsulation and cultivation of primary hepatocytes. *Biomaterials* 31:3596–603
134. Lee H, McKeon RJ, Bellamkonda RV. 2010. Sustained delivery of thermostabilized chABC enhances axonal sprouting and functional recovery after spinal cord injury. *PNAS* 107:3340–45
135. Persiani S, Roda E, Rovati LC, Locatelli M, Giacovelli G, Roda A. 2005. Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline glucosamine sulfate in man. *Osteoarthr. Cartil.* 13:1041–49
136. Dodge GR, Jimenez SA. 2003. Glucosamine sulfate modulates the levels of aggrecan and matrix metalloproteinase 3 synthesized by cultured human osteoarthritis articular chondrocytes. *Osteoarthr. Cartil.* 11:424–32
137. Fransen M, Agaliotis M, Nairn L, Votrubec M, Bridgett L, et al. 2015. Glucosamine and chondroitin for knee osteoarthritis: a double-blind randomised placebo-controlled clinical trial evaluating single and combination regimens. *Ann. Rheum. Dis.* 74:851–58
138. Hochberg MC, Martel-Pelletier J, Monfort J, Möller I, Castillo JR, et al. 2016. Combined chondroitin sulfate and glucosamine for painful knee osteoarthritis: a multicentre, randomised, double-blind, non-inferiority trial versus celecoxib. *Ann. Rheum. Dis.* 75:37–44
139. Yamada S, Sugahara K. 2008. Potential therapeutic application of chondroitin sulfate/dermatan sulfate. *Curr. Drug Discov. Technol.* 5:289–301
140. Salbach J, Rachner T, Rauner M, Hempel U, Anderegg U, et al. 2012. Regenerative potential of glycosaminoglycans for skin and bone. *J. Mol. Med.* 90:625–35
141. Ariga T, Miyatake T, Yu RK. 2010. Role of proteoglycans and glycosaminoglycans in the pathogenesis of Alzheimer's disease and related disorders: amyloidogenesis and therapeutic strategies—a review. *J. Neurosci. Res.* 88:2303–15
142. Leveugle B, Scanameo A, Ding W, Fillit H. 1994. Binding of heparan sulfate glycosaminoglycan to β-amyloid peptide: inhibition by potentially therapeutic polysulfated compounds. *NeuroReport* 5:1389–92
143. Aisen PS, Saumier D, Briand R, Laurin J, Gervais F, et al. 2006. A Phase II study targeting amyloid-β with 3APS in mild-to-moderate Alzheimer disease. *Neurology* 67:1757–63
144. Santa-Maria I, Hernández F, Del Rio J, Moreno FJ, Avila J. 2007. Tramiprosate, a drug of potential interest for the treatment of Alzheimer's disease, promotes an abnormal aggregation of tau. *Mol. Neurodegener.* 2:17
145. Dudas B, Rose M, Cornelli U, Pavlovich A, Hanin I. 2008. Neuroprotective properties of glycosaminoglycans: potential treatment for neurodegenerative disorders. *Neurodegener. Dis.* 5:200–5

146. Kisilevsky R, Lemieux LJ, Fraser PE, Kong XQ, Hultin PG, Szarek WA. 1995. Arresting amyloidosis in vivo using small-molecule anionic sulfonates or sulfates—implications for Alzheimer's disease. *Nat. Med.* 1:143–48
147. Lee AY, Levine MN, Baker RI, Bowden C, Kakkar AK, et al. 2003. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *N. Engl. J. Med.* 349:146–53
148. Schnoor R, Maas SLN, Broekman MLD. 2015. Heparin in malignant glioma: review of preclinical studies and clinical results. *J. Neuro-Oncol.* 124:151–56
149. Varki NM, Varki A. 2002. Heparin inhibition of selectin-mediated interactions during the hematogenous phase of carcinoma metastasis: rationale for clinical studies in humans. *Semin. Thromb. Hemost.* 28:53–66
150. Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. 2001. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. *PNAS Biol. Sci.* 98:3352–57
151. Tanaka Y, Miyamoto S, Suzuki SO, Oki E, Yagi H, et al. 2005. Clinical significance of heparin-binding epidermal growth factor–like growth factor and a disintegrin and metalloprotease 17 expression in human ovarian cancer. *Clin. Cancer Res.* 11:4783–92