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Review

Global impact of proteoglycan science on human diseases

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SUMMARY

In this comprehensive review, we will dissect the impact of research on proteoglycans focusing on recent developments involved in their synthesis, degradation, and interactions, while critically assessing their usefulness in various biological processes. The emerging roles of proteoglycans in global infections, specifically the SARS-CoV-2 pandemic, and their rising functions in regenerative medicine and biomaterial science have significantly affected our current view of proteoglycans and related compounds. The roles of proteoglycans in cancer biology and their potential use as a next-generation protein-based adjuvant therapy to combat cancer is also emerging as a constructive and potentially beneficial therapeutic strategy. We will discuss the role of proteoglycans in selected and emerging areas of proteoglycan science, such as neurodegenerative diseases, autophagy, angiogenesis, cancer, infections and their impact on mammalian diseases.

INTRODUCTION

General structural and functional considerations

The world of proteoglycan science has markedly expanded in recent decades and has now reached an international stature. A wider knowledge of proteoglycan biology is a desirable goal that poses worthy and timely objectives of this comprehensive review article. A recent search of the total number of patents between 2010 and 2022 filed and logged in the US Patent Public Search database (USPTO) has revealed many patents involving proteoglycans and related molecules (Figure 1). Data were accumulated from 2010 to 2022 using keywords such as heparin, proteoglycan, or hyaluronan within the published document with the following search engine: https://ppubs.uspto.gov/pubwebapp/. It is not surprising that heparin, one of the most important anti-coagulant factors, is the most frequently used with over 4,000 patents in this dataset.

Proteoglycans function in a variety of molecular networks[1–4] and act on distinct signaling pathways mainly due to their complex and composite nature. Indeed, proteoglycans are unique and specialized protein/polysaccharide hybrid molecules that harbor protein cores of diverse dimensions and glycosaminoglycan (GAG) side chains of varied chemical composition, size, and structure.[5,6]

It is noteworthy that the family of full-time proteoglycans comprises a very small percentage of the human genome. Currently there are about 20,000 protein-encoding genes in the main human databases, GENCODE/Ensembl, RefSeq, and UniProtKB. However, the proteoglycan family contains only about 50 individual protein cores, or ~0.25%. In addition, many protein cores are highly conserved across species.[7] These features suggest that proteoglycans are functionally essential to organismal structure and function, and consequently are involved in the pathophysiology of many diseases.

Proteoglycans can be comprehensively classified according to a taxonomy that integrates various characteristics including their cellular and subcellular localization, homology at the genetic and protein levels, and the presence of specific protein modules in their respective protein cores.[5] Proteoglycans are often named and categorized after their GAG chains—long, linear carbohydrate chains that are constituted of alternating, repeating disaccharides.[8] More specifically, these GAG chains cf. structural variation among proteoglycans as the quality, degree of sulfation, and number of attached chains can vary drastically,[9,10] providing these complex macromolecules with copious biological activities. An example of the initial steps of GAG biosynthesis, in this case heparan sulfate (HS) synthesis, involving chain initiation and elongation is shown in Figure 2A. The polymerization of typical GAG chains follows a general pattern of an amino sugar: N-acetylglucosamine, N-substituted glucosamine, or N-acetylgalactosamine followed by an uronic acid: glucoronic acid or iduronic acid or alternatively a galactose.[8] To better understand the variation within GAG chains, it is important to delve into the biosynthesis of these chains—more specifically HS and chondroitin sulfate (CS) as there is recent evidence for site-specific glycosylation of proteoglycans depending on the amino acid sequence of the specific attachment site.[11,12] The process begins in the endoplasmic reticulum-Golgi apparatus interface with the addition of xylose to a serine residue in the protein core (Figure 2A). This is catalyzed by xylosoltransferase 1,2. Next, two galactoses are added along with one glucuronic acid which forms the characteristic Xyl-Gal-Gal-GlcA tetrasaccharide linkage region. The difference between CS and HS

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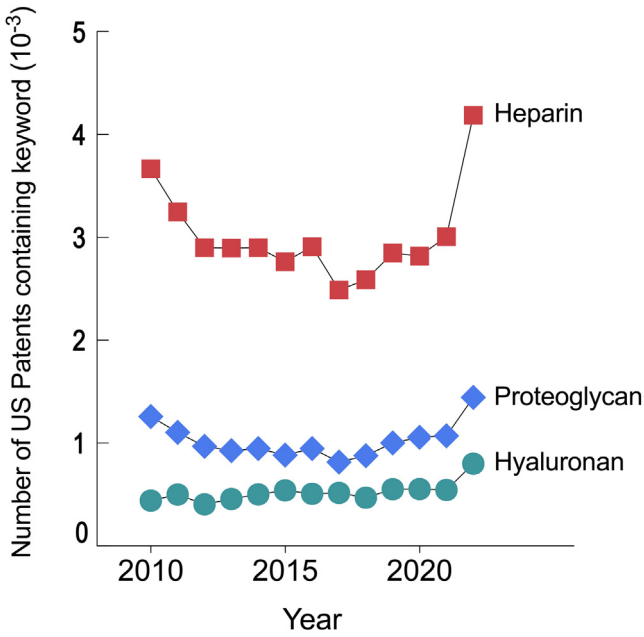

Figure 1. Graphic showing increased number of US patents containing key proteoglycan words in their title

Compilation of the total number of patents per year filed and logged in the US Patent Public Search database (USPTO) that contain the keywords: "heparin," "proteoglycan," or "hyaluronan" within the published document. Data were accumulated from 2010 to 2022 using the following search engine: https://ppubs.uspto.gov/pubwebapp/.

synthesis starts here, where there is either an addition of β1-4GalNAc for CS synthesis or α1-4GlcNAc for HS synthesis.[13] Subsequently, the nascent GAGs chains are elongated and, for HS synthesis, this process involves the use of EXTL3 to transfer a GlcNAc to the established tetrasaccharide linkage (Figure 2A). EXT1 and EXT2 form an HS polymerase complex and extend the chain by continually adding repeating GlcA and GlcNAc residues. Importantly, post-polymerization, the chain undergoes a series of modifications sometimes referred to as maturation (Figure 2B). These modifying enzymes include N-deacetylase/N-sulfotransferases (NDSTs), O-sulfotransferases (2OST, 6OSTs, and 3OSTs), and D-glucuronyl C5-epimerase (GLCE). NDSTs act on GlcNAc residues by replacing acetyl groups with sulfate groups. GLCE converts GlcA to IdoA residues primarily on GlcA residues near the N-sulfated glucosamine units. The aptly named sulfotransferases transfer sulfate groups to the respective positions of GlcA/IdoA and glucosamine residues. Because the NDSTs produce N-sulfated glucosamine which are preferred substrates for the aforementioned enzymes that perform epimerization and O-sulfation,[14] some regions become highly modified while others remain poorly modified; structural heterogeneity is achieved through this process.[15]

Different GAG chains are uniquely modified and contain different disaccharide units—small changes can determine categorization as epimerization of GlcA to IdoA in CS converts it to dermatan sulfate (DS). Very broadly, HS is constituted of GlcA/IdoA and GlcNAc, CS consists of GlcA and GalNAc, and DS is epimerized so naturally it is composed of IdoA and GalNAc (Figure 2B). HA lacks sulfate groups and does not attach to a core protein and is made up of GlcA and GlcNAc. Keratan sulfate (KS) has a different tetrasaccharide linkage region and specific amino acid residue. KS contains galactose and GlcNAc.

We believe that the strategic location of various proteoglycans has a biological meaning. For example, most of the heparan sulfate proteoglycans (HSPGs) are adjacent to most cells, either bound to the cell surface through a hydrophobic transmembrane domain or via a glycosyl-phosphatidyl-inositol (GPI) anchor.[10,16] In contrast, most of the chondroitin sulfate proteoglycans (CSPGs) are localized to the extracellular matrix spatially distant from the cellular compartment. In addition, both HSPGs and CSPGs bind to several morphogens and growth factors and present them in a bioactive form to their cognate receptors, during both developmental and pathological processes. Indeed, GAG modifications control general patterns of gene expression, cell specification and cell growth via morphogen regulation.[17] A wide-ranging deduction from these emerging proteoglycan functions is that HSPGs may have a more prominent role in cellular regulation because of their proximity to the cell surface, whereas CSPGs may have additional functions as structural and mechanical constituents.

The complex GAG-protein interactions facilitate critical physiological and pathological processes, such as development, cancer invasion, angiogenesis, neuronal plasticity, and viral entry. However, mapping GAG-protein networks is a challenging task for biologists as these interactions need specific sulfation patterns often including ROS derived effects.[18] Moreover, these global interactions involve several transmembrane receptors and extracellular matrix-associated proteins.[19] Recent progress in establishing natural and synthetic GAG libraries has allowed us to decipher in detail specific GAG sequences interacting with proteins.[20] These GAGs are generally spotted on microarrays and have led to the discovery of novel binding partners while concurrently determining their size and chemical composition that promotes protein binding.[21] The recently updated GAG interactome contains about four times more GAG-binding proteins[2] than the originally

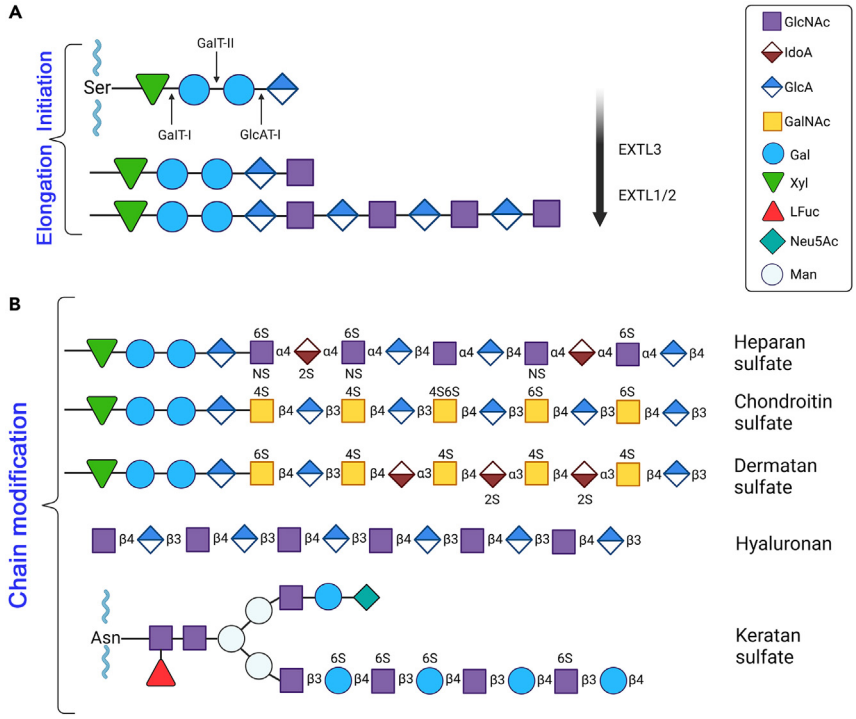

Figure 2. General proteoglycan biosynthetic steps involved in GAG chain initiation, elongation and modifications

(A) Biosynthesis of the backbone structure of heparan sulfate. The protein core provides a Ser residue that forms a linkage with a Xyl-Gal-Gal-GlcA tetrasaccharide. For HS biosynthesis, a GlcNAc residue is added to the tetrasaccharide linkage via exostosin like glycosyltransferase 3 (EXTL3). Subsequently, EXTL1 and EXTL2 add alternating GlcA and GlcNAc to the chain.

(B) The progressive GAG chain modification leads to diverse structures of sulfated and unsulfated GAGS. The representative structures of heparan sulfate, chondroitin sulfate, dermatan sulfate, hyaluronan, and keratan sulfate (which are linked to an Asp residue instead of a Ser) are shown respectively. We note that we are not discussing the detailed biosynthesis and structure of hyaluronan since this subject has been covered extensively in recent literature.[298,299,311] For additional information and details, please refer to the text. The schematic was adapted from Esko and Selleck[8] and McMillan et al.[15]

established GAG interactome,[22] with a marked increase in the number of DS and HS/heparin-binding proteins.[2] Moreover, advances in structural data related to GAG biosynthesis,[23] novel chemical and biochemical tools for studying GAG sequencing,[24] and chemical glycobiology including the generation of semi-synthetic proteoglycans[25] have allowed the dissection of the structural heterogeneity and have ultimately provided powerful insights into proteoglycan functions. Surely, the exploration of the GAG interaction networks could be used for therapeutic purposes especially in the design of specific inhibitors targeting specific GAG/protein interactions.

Another important general feature of proteoglycans is that they often undergo an irreversible post-translational modification via proteolytic processing of their protein cores.[26,27] While uncontrolled proteolysis is a noticeable feature of many diseases and pathological processes including cancer, angiogenesis and inflammation, proteolysis might also liberate protein core fragments with biological activity including endostatin and endorepellin.[28,29]

CELL SURFACE GPI-ANCHORED PROTEOGLYCANS: THE GLYPICAN FAMILY

Glypicans are HS proteoglycans linked to the outer surface of the plasma membrane via a lipid moiety composed of GPI anchor[30,31] (Figure 3A). There are six family members in mammals (GPC1 to GPC6) with no appreciable significant homology to other characterized structure, with exception of a short segment between residues 200 and 300 that is homologous to the Cys-rich domain of *Frizzled* proteins.[32] Notably, the glypican protein core is highly compact with 14 highly conserved Cys residues forming disulfide bonds in all six members of the family.[32] Another general structural feature of glypicans is that they almost exclusively carry HS chains, in contrast to Syndecans (see next section), and that the chains are near the C-terminus, near the plasma membrane (Figure 3A). This strategic localization suggests that these HS chains could mediate the interaction of glypicans with other cell-surface molecules, including growth factor receptors and co-receptors. Glypicans often undergo endoproteolytic cleavage of the proximal protein core by furin-like convertases (Figure 3A). The cleavage site is located at the C-terminal end of the Cys-rich domain generating two subunits that remain attached by disulfide bonds. Notably, the proteolytic processing of glypicans is required for modulation of cell survival, Wnt signaling, and gastrulation,[33] and has a remarkable impact on both the binding properties and GAG-chain sulfation suppression of *Hedgehog* (*Hh*) signaling.[34]

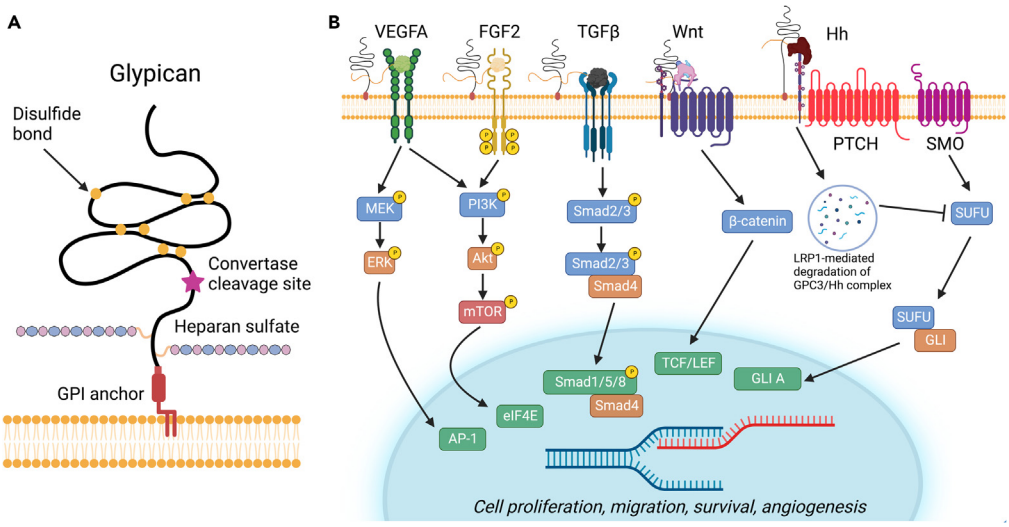

Figure 3. Graphical overview of the structure of glypican and downstream pathway signaling effects

(A) Structure of glypican and constituent parts. Glypicans are heparan sulfate proteoglycans attached to the cell surface via a GPI lipid anchor at the C-terminus. Glypican is composed of a ~450 kDa N-terminal protein domain containing fourteen conserved Cys residues that form seven disulfide bonds as well as a stalk region containing heparan sulfate glycosaminoglycan chains.

(B) Glypicans serve a variety of functions and affect multiple signaling cascades including the Wnt and Hedgehog pathways. In the Wnt pathway, GPC3 acts as a co-receptor and attracts Wnt to the cell surface by forming a complex with Wnt and FZD, amplifying downstream β-catenin signaling. The HS chains are not necessary for activation but rather help with stabilization of the formation of the complex. In the Hedgehog signaling pathway, GPC3 competes with PTCH for *Hh* binding. Mediated by LRP1, the GPC3/*Hh* complex undergoes endocytosis and lysosomal degradation which results in an inhibition of Hedgehog signaling. Glypicans also act as co-receptors for various angiogenic growth factors, including VEGFA, FGF2, and this interaction is thought to stabilize assembly the multi-receptor complexes. This promotes downstream signaling leading to an increase in cell proliferation and migration. GPC1 also binds TGFβ as a co-receptor and promotes SMAD signaling. For additional details, please refer to the text. Figure adapted from Pam and Ho.[44]

Two seminal papers published in 1999 showed that the *Drosophila Division abnormally delayed gene* (*Dally*) encoded for a *Gpc* that regulates the morphogen *Wingless* signaling by cooperating with *Frizzled* 2 pathway.[35,36] Notably, recent evidence has shown that the palmitoylated Wingless is shielded by the core domain of a *Dally-like* glypicans (*Dlp*) thereby providing a new paradigm for *Gpc* signaling.[37] Specifically, in the presence of palmitoylated peptides, *Dlps* change conformation to create a hydrophobic space, thereby protecting the lipid of Wnt proteins from the aqueous environment and could function as a reservoir from which Wnt proteins can be handed over to their cognate receptors.[37,38]

Numerous genetic studies have clearly shown an important role for glypicans in developmental morphogenesis and patterning events.[39,40] *In vivo* evidence indicates that the main function of membrane-attached glypicans is to act as growth factor co-receptors including *Wnt* signaling (see previous text), Hedgehogs (*Hh*), vascular and fibroblast growth factors (VEGFA and FGF2), and bone morphogenetic proteins (BMPs). Glypicans have been implicated in the pathogenesis of several types of cancer, notably hepatocellular carcinomas, and as potential biomarkers and potential targets for personalized therapy.[41,42] The multiple signaling pathways where glypicans have been involved are schematically summarized in Figure 3B. As illustrated in the schematic, glypicans mostly act as co-receptors, that is, a cell surface receptor that binds a signaling molecule in addition to a primary receptor thereby facilitating ligand recognition and initiating biological processes regulating cell function.

Glypicans may have a stimulatory or inhibitory activity on signaling depending on the cellular context.[43,44] For example, in the case of Wnt, the stimulatory mechanism is based on the ability of glypicans to facilitate and/or stabilize the interaction of Wnts with their signaling receptors, the Frizzled proteins. Signaling by the Wnt proteins is finely regulated to ensure proper development and tissue homeostasis. This has been in part conceptualized by the idea of shedding of glypican/Wnt complexes by *Notum*, thought to be a phospholipase.[45] However, recent evidence in *Drosophila* has shown that *Notum* requires glypicans to suppress Wnt signaling but does not cleave their GPI anchor; it acts, instead, as a carboxylesterase that removes an essential palmitoylated moiety from Wnt proteins.[46]

A recent study has shown that targeting GPC1 expression by CRISPR/Cas9, RNAi or anti-GPC1 antibodies attenuates proliferation in a variety of cancer cells including bladder carcinomas, gliomas, and hepatocellular carcinomas.[47] Moreover, systematic Ingenuity Pathway Analysis suggests that suppression of proliferation of cancer cells results in ECM-mediated inhibition of specific pro-cancer signaling pathways involving TGFβ and p38 MAPK.[47] Indeed, it is well known that TGFβ affects ECM biology including various collagens and other proteoglycans.[48–51] Collectively, the aforementioned observations data suggest that GPC effects on specific cancer types could be utilized as potential diagnostic and prognostic factors.

As aforementioned, glypicans are involved in the pathogenesis of many solid tumors. What is especially striking is the involvement of GPC3 in the pathophysiology of hepatocellular carcinoma, where the proteoglycan stimulates canonical *Wnt* signaling[52] and can be a useful

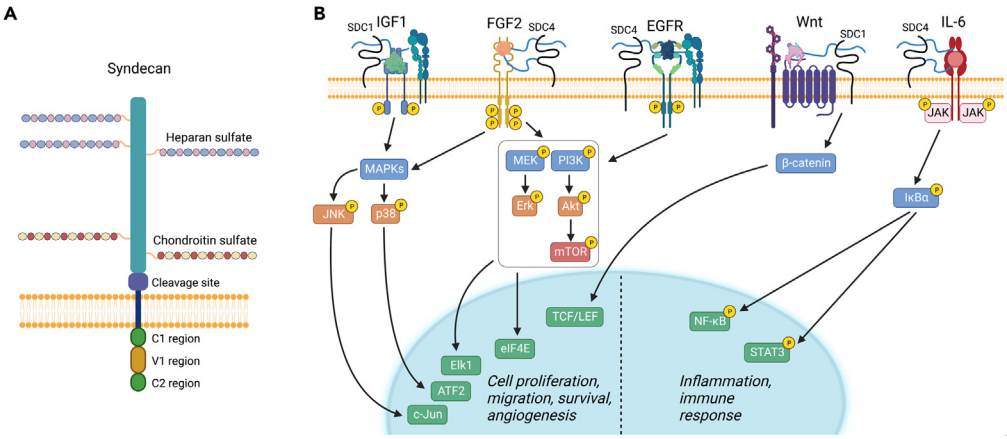

Figure 4. Overview of the structure of syndecan 1–4 (SDC) and downstream signaling pathways

(A) Graphical depiction of a typical syndecan. Syndecans are type I, single-pass transmembrane proteoglycans. All syndecans contain HS chains covalently linked to protein core ectodomain; however, SDC1 and 3 also contain CS chains. The relative sizes of SDC 1–4 are 33, 23, 41, and 22 kDa respectively. The cytoplasmic domain contains two conserved regions, C1 and C2, separated by a variable (V1) region. The crystal structures for syndecans remain obscure because the ectodomains contain a significant amount of disorder.

(B) Syndecans serve a variety of functions but primarily act as co-receptors affecting multiple signaling pathways. SDC1 activates the IGF1 signaling pathway by stimulating docking of both IGF1-R and αvβ3/αvβ5 integrins and subsequently triggers angiogenesis. SDC4 mediates the assembly of EGFR and α6β4/α3β1 integrins which leads to downstream pro-migration signaling crucial for tumorigenesis. SDC4 binds FGF through heparan sulfate chains and is an essential co-receptor for FGF signaling. Interestingly, SDC4 can activate this signaling pathway in both an FGFR-dependent and FGFR-independent manner. Wnt signaling is modulated by SDC1 via its HS chains which bind Wnts and R-spondins to stimulate β-catenin signaling for cell growth. Syndecans play a role in recruiting pro-inflammatory molecules and modulating the progression of inflammation. This is done primarily through GAG chain binding to cytokines but there is evidence for a SDC1/IL-6 axis with the presence of a regulatory feedback loop that is yet to be elucidated. For additional details, please refer to the text. The graphic was generated with *BioRender.com*.

biomarker both in serum and tumor proper.[53] In support of these original observations, a recent study has shown that GPC3 is highly enriched in the small extracellular vesicles from patients with hepatocellular carcinoma.[54] Moreover, the furin site of GPC3 is exposed and readily available suggesting that furin-dependent GPC3 cleaved domains could be a promising biomarker for early detection of this lethal cancer and could also serve as a predictor for disease prognosis and progression.[54]

Another unexpected finding is the recent discovery that GPC1 is the main rate-limiting factor driving the unconventional secretion of FGF2.[55] It has been known for some time that FGF2 is secreted by a pathway of unconventional protein secretion based on direct self-translocation across the plasma membrane. Surprisingly, GPC1, but no other HS proteoglycans such as SDC or perlecan, is required for unconventional secretion of FGF2 into the extracellular space. Collectively, these novel findings provide not only a molecular mechanism underlying this process but also reveal an intimate relationship between GPC1 and FGF2 that could play a role in tumor progression and metastasis.[56]

INTERCALATED PROTEOGLYCANS: THE SYNDECAN FAMILY

The SDC family comprises four homologous type I transmembrane proteoglycans members. SDC1 (Figure 4A) and SDC3 are the largest members with protein cores of ~33 and ~41 kDa, respectively, whereas SDC2 harbors smaller with protein cores of ~23 and 22 kDa, respectively.[57] The general structure of syndecans includes an ectodomain, a single hydrophobic transmembrane domain, and a short C-terminal cytoplasmic domain with two constant and one intervening variable region (Figure 4A). An interesting feature of syndecans is their GAG attachment sites and type as they harbor both HS and CS in the ectodomain, in contrast to glypicans which preferentially harbor HS chains. Specifically, SDC1 and SDC3 bear CS chains near the plasma membrane whereas the HS chains are in a more distal region (Figure 4A). Recently it has been shown that the HS chains of SDC1 and SDC2 induce changes in conformational dynamics depending on the number of the HS chains and their location on the protein core.[58] The distal localization of the HS chains suggests a specific function of syndecans in modulating receptor activity vis-à-vis glypicans or other HSPGs such as perlecan, which is also located at the plasma membrane[59] via the α2β1 integrin which specifically binds its C-terminal domain V/endorepellin.[60–62]

Another general feature of syndecans is that the protease-mediated shedding of their protein cores at a site located proximal to the plasma membrane (Figure 4A) can relocate the ectodomain to more distal sites,[26] and the shed form of at least one transmembrane proteoglycan (SDC1) can be internalized and redirected to the nucleus.[63–65] Proteolytic processing can extend the inherent molecular diversity of proteoglycans via the generation of bioactive fragments with new functional properties. Shed SDC1 binds to hepatocyte growth factor (HGF) and stimulates tumor growth while promoting angiogenesis via VEGF.[66,67] The N-terminal SDC2 domain selectively enhances 6-O HS chains sulfation and promotes VEGFA-dependent neovascularization,[68] while SDC2 selectively regulates VEGF-induced vascular

permeability.[69] Moreover, there is evidence that chemotherapy stimulates SDC1 shedding, and this would have a negative effect on treatment by promoting tumor relapse.[70]

Syndecans are key regulators of cell signaling[71] by playing a major role in acting as co-receptors.[72] Syndecans have been involved in regulating at least five distinct signaling pathways (Figure 4B). Four of them at times utilize overlapping signaling components such as MAPK/MEK and PI3 kinase, but they ultimately and collectively promote cell proliferation, survival, migration, and angiogenesis. SCD1 mediates HGF binding and promotes MET signaling in multiple myeloma.[73] In addition, SCD1 promotes an aberrant Wnt/β-catenin signaling pathway by presenting Wnts and R-spondins to their receptors,[74] suggesting that targeting this pathway in the tumor microenvironment could be a potential therapeutic target for multiple myeloma.[75] Additionally, SDC4 has been recently implicated in regulating the IL-6/STAT3 signaling pathway in inflammatory breast carcinomas.[76]

Through utilization of the diverse and complex signaling pathways outlined previously, syndecans and its HS chains affect the homeostasis of various organs and the pathogenesis of several neoplastic diseases including those of the liver,[77] colon,[78] breast,[79] plasma cells,[74,75,80] and in bacterial infection.[81,82] Syndecans are directly involved in inflammation (see the following text) and are often upregulated in inflammatory diseases concurrent with enhancement of cytokine and chemokine levels. This dual activation boosts leukocyte adhesion to endothelial cells which is fortified by integrin-controlled signaling and other cell adhesion molecules, leading to the arrest of leukocyte rolling. The adhesion of leukocytes to endothelial cells results in transmigration through the endothelial cell barrier and active inflammatory infiltrate.[83] SDC2 and SDC4 engage the EGFR and RON tyrosine kinase receptors and promote carcinoma cell cycle progression.[84] SDC1 activates the IGF1/IGFR1 signaling pathway by promoting docking of both IGF1-R and αvβ3/αvβ5 integrins thereby triggering angiogenesis.[85] Notably, SDC4 mediates the assembly of EGFR and α6β4/α3β1 integrins which leads to a pro-migratory effect crucial for tumorigenesis. SDC4 binds FGF through its HS side chains and is a vital co-receptor for FGF signaling. Interestingly, SDC4 can activate this signaling pathway in both an FGFR-dependent and FGFR-independent manner. Recently, SDC4 has been recently implicated in the progression of gastric cancer and has been proposed to represent a potential therapeutic target for precision medicine.[86]

Another unique ability of SDC1 is its ability to act as a potential LDL receptor, by directly mediating ligand catabolism through a pathway distinct from classical coated pits, thereby acting as a receptor for atherogenic lipoproteins and other ligands *in vivo*.[87] SCD1 can also mediate apoE-VLDL uptake in human fibroblasts with little or no contribution from LRP and the endocytic path taken by SCD1 is clathrin-independent and relies upon lipid raft function. Genetic studies utilizing *Sdc1*$^{-/-}$ mice have shown that these mutant animals accumulate plasma triglycerides and exhibit protracted half-life of injected human VLDL and intestinally derived chylomicrons, suggesting that SCD1 is the primary proteoglycan mediating hepatic triglyceride clearance.[88] Indeed, shedding of SCD1 from human hepatocytes can alter VLDL clearance, thereby contributing to hypertriglyceridemia in septic patients.[89] Finally, there is evidence that SCD4 may also participate in the control of lipid metabolism as exercise evokes the secretion of hepatokines that promote SDC4 expression which in turn reduces fatty acid uptake and hepatic steatosis.[90]

PROTEOGLYCANS IN AXONAL GUIDANCE AND NEURODEGENERATIVE DISEASES

It is well established that the presentation of secreted axonal guidance factors plays a key function in shaping central nervous system (CNS) connectivity. One of the most intriguing roles of HSPGs is their direct involvement in regulating guidance factor activity. Through a genetic *in vivo* analysis it was shown that *Sdc* is critical for the fidelity of *Slit* repellent signaling at the midline of *Drosophila* CNS.[91] Moreover, the *Drosophila* glypican Dally-like protein (*Dlp*) is required for proper axon guidance and visual-system function.[92] Mosaic studies revealed that *Dlp* is necessary in both the retina and the brain for different aspects of visual system assembly and *Sdc* mutants also exhibited similar axonal guidance and visual-system defects.[92] Notably, rescue experiments using *Dlp*$^{+}$ transgenes were able to rescue some of the *Sdc* phenotypes, but the opposite was not effective.[92] Collectively, these findings suggest that both the HS chains and the protein cores play a role in this CNS function. In support of these findings is the observation that viable hypomorphic mutations in the two *C. ele*gans exostosin glycosyltransferase genes, *Rib1* and *Rib2*, evoke abnormal cell and axonal migration.[93] Moreover the *C. elegans Ephrin*4 functions non-cell autonomously with HSPGs to promote axonal growth and branching while *Sdc* and *Gpc* have cell-autonomous yet mutually antagonistic roles in ectopic neurite formation.[94] Another interesting finding is that synaptogenesis is modulated by HSPGs in *C. elegans* male neural network as loss of 3-O sulfation results disrupts the formation of synapses in a component of the mating circuits.[95] Overall, these studies point to a fundamental role for HSPGs not only in axonal guidance but also in the formation of synaptic connections.

During the past century, the rise in life expectancy has led to a significant increase in the incidence of age-related neurodegenerative disorders such as Alzheimer's disease (AD) and several amyloid related disorders.[96] Both senile plaques and vascular amyloid deposits harbor amyloid-β peptides[15] and these aggregates contain PGs, mostly HSPGs.[97] The central role played by HSPGs in AD has been demonstrated for a long time.[98] There is also ample evidence to support a key role for HSPGs in Tauopathies: HSPGs directly bind to co-deposit with Tau and modulate Tau secretion, internalization, and aggregation.[99] In support of these findings are several observations including: (1) Overexpression of heparanase (see the following) lowers amyloid burden in double transgenic mice overexpressing heparanase and β-amyloid precursor protein,[100] (2) heparanase expression is upregulated in the brain of AD patients,[101] and (3) heparanase overexpression impedes perivascular clearance of amyloid-β from murine brain.[102] Moreover, HSPGs are directly involved in propagation of proteopathic peptides[103] and in the prion-like spread of Tau pathology,[104] and can serve as mediators between monomeric Tau and its subsequent ERK1/2 activation.[105] Meanwhile, the expression of HSPGs is dysregulated in the disease, and this dysregulation may exacerbate the progression of Tauopathies.[99] Furthermore, neuronal HSPGs modulate amyloid-β clearance and aggregation in AD patients.[106] Specifically, GPC4 drives Tau hyper-phosphorylation suggesting that this HSPG can be a gateway for Tau spreading.[107] Both the cell surface HSPGs syndecans and glypicans

contribute to cellular uptake of α-synuclein and Tau,[108–110] and specific GAG chain length and sulfation appear to modulate this process in a differential way.[111] In an interesting *C. elegans* model of Parkinson's disease, where worms were fed α-synuclein preformed fibrils causing neurodegeneration, knockdown of *Sdc1* or enzymes involved in HS biosynthesis protected against α-synuclein aggregation, motor dysfunction and neurodegeneration.[112] Overall, these data indicate that HSPGs are directly involved in mediating prion-like α-synuclein neurotoxicity and could be a potential therapeutic target.

Several lines of additional evidence implicate HSPGs and specific sulfation of the HS chains in neurodegenerative disease. For example, GAGs from the hippocampus of AD patients exhibit abnormal ability to bind growth factor activities and to bind Tau proteins.[113] Internalization of Tau proteins is regulated by 6-O sulfation of HSPGs and this posttranslational modification can regulate Tau uptake in neuronal cells, IPS-derived neurons and in *ex vivo* murine brain slices.[114] In contrast, a recent paper has clearly shown that in AD brains there is a substantial increase of a specific 3-O-sulfatedHS domain, designated as Tetra-1, and that this modification is used in Tau internalization.[115] Moreover, overexpression of the *HS3ST1* gene, which encodes the enzyme responsible for the synthesis of the specific 3-O-sulfated Tetra-1, could indeed represent an AD-associated genetic risk factor.[115] These studies suggest that the 3-O-sulfated HS may serve as a new target for modifying AD and as a biomarker for early diagnosis.[115] Collectively, the studies summarized previously propose that a valuable therapeutic for AD patients could be a pharmacological targeting of HSPG/Tau interactions. As HSPGs can be novel AD biomarkers,[116] we need to define more precisely the spatiotemporal components of HS structural domains and their diversity in both normal and AD brains.

PROTEOGLYCANS IN INFECTION: NOVEL ROLES IN SARS-CoV-2

Over the past two decades, a major role for various proteoglycans in innate immunity has been established.[117–120] It is well known that viruses are obligate intracellular parasites and, consequently, viruses need to first bind a cell surface receptor and then enter permissive host cells ultimately hijacking their host intracellular machinery. It is also established that viruses can act as passive and metabolically inactive entities with incomplete available strategies to cross the plasma membrane of host cells. However, most mammalian cells are dynamic and offer several structures and processes allowing the uptake of macromolecular assemblies. The COVID-19 pandemic initially caused by an outbreak of SARS-CoV-2 in Wuhan, China has perturbed all facets of life globally since January 2020. As of February 2023, the virus has infected over 755 million people causing the death of >6.8 million people (https://covid19.who.int/). Although vaccinations and antiviral agents have been established, the virus continues to mutate, spike in incidence, and perturb society and the economy.[121] It is integral to understanding the mechanism by which viral infection occurs to further develop pharmaceuticals to minimize the risk of severe consequences of COVID-19 infection. As discussed in detail in the following, proteoglycans and specially cell surface HSPGs, are directly involved in SARS-CoV-2 infectivity and cellular invasion. Recent seminal studies have discovered that SARS-CoV-2 infection is dependent not only on angiotensin-converting enzyme 2 (ACE2) but also on HS proteoglycans, especially SDC1 and SDC4, and these are required for virus binding and infection.[122,123] SDC1 was shown to act as a co-receptor for SARS-CoV-2 binding.[123,124] It is also established that HS proteoglycans act as internalizing receptors for extracellular vesicles and SARS-CoV-2 lipid-enclosed particles.[125] The similarities in their biogenesis, biophysical property, size, and lipid composition could elucidate a shared dependence on HS proteoglycans for effective cell-surface attachment and uptake.[125]

Cell surface HS interacts with specific residues in the receptor-binding domain (RBD) region of the S1 subunit of the spike protein.[122] Molecular modeling highlights specific positively charged residues within the RBD region that likely bind to heparin which are highly acidic: R346, R355, K444, R466, and R509[122] (Figure 5A). Notably, the region containing these residues is inactive during the "closed" conformation of the RBD and fully exposed when "open." SARS-CoV-2 infection relies heavily on HSPG tissue expression. The SARS-CoV-2 S protein is cleaved into two subunits by pro-protein convertases in virus-producer cells. While the S1 subunit binds to ACE2, the S2 subunit consists of a fusion peptide that allows for immediate membrane fusion[126] (Figure 5B). This site contains an additional internal site dubbed the S2′ site which is exposed after ACE2 engagement. After ACE2-mediated endocytosis, S2′ is subsequently cleaved by transmembrane protease serine 2 (TMPRSS2) in the endosomal compartment[127] (Figure 5B). This causes a release of the fusion peptide which initiates fusion pore formation – a step that is vital for viral access to host cell machinery.[128] Moreover, cell surface HS interacts with the ectodomain of the SARS-CoV-2 spike proteins through the RBD in the S1 subunit of the spike protein. This in term shifts the structure of the protein to favor the RBD open conformation that in turn binds to ACE2. Additional findings also indicate HS polymers likely recruit endocytic ligands that further promote viral uptake.[129] HS interaction with the RBD to favor the "open" conformation is so essential in receptor accessibility and subsequent viral entry[125] that more recently, findings do suggest that HS could solely trigger or facilitate internalization, as SARS-CoV-2 infection is possible in the presence of HS and absence of ACE2.[130]

As a linear, highly acidic polysaccharide, HS can form weak ionic bonds with basic residues on viral capsid proteins which allows for augmentation of binding likelihood.[131] Though ACE2 is the functional receptor for SARS-CoV-2 viral entry—allowing for viral entry of the cell via endocytosis, HS acts as an adhesion receptor that is essential for SAR-CoV-2 viral binding and replication.[132] While ACE2 can indeed bind to the SARS-CoV-2 spike protein independently, the binding is enhanced significantly by HS.[122]

Heparin-protein interactions are heavily favored toward positive electrostatic surfaces. Molecular modeling has demonstrated that positively charged amino acids R346, R355, K444, R466, and R509 within the RBD region adjacent to the ACE2 binding domain likely interact directly with heparin. Notably, the site is inactive in the RBD "closed" conformation but fully exposed in the "open" conformation.[122] With the establishment of HS as an essential factor for viral entry and replication, pharmaceutical use of HS mimetics has been proposed to both reduce the severity and incidence of infection.[129,131,133,134] Exogenous heparin has also been proposed to compete with cell surface heparan interaction with viral proteins[135] as well as lactoferrin binding to HS proteoglycans in SARS pseudovirus infection.[136] More broadly, this could prove relevant for other similar exogenous sulfated polysaccharides.[124]

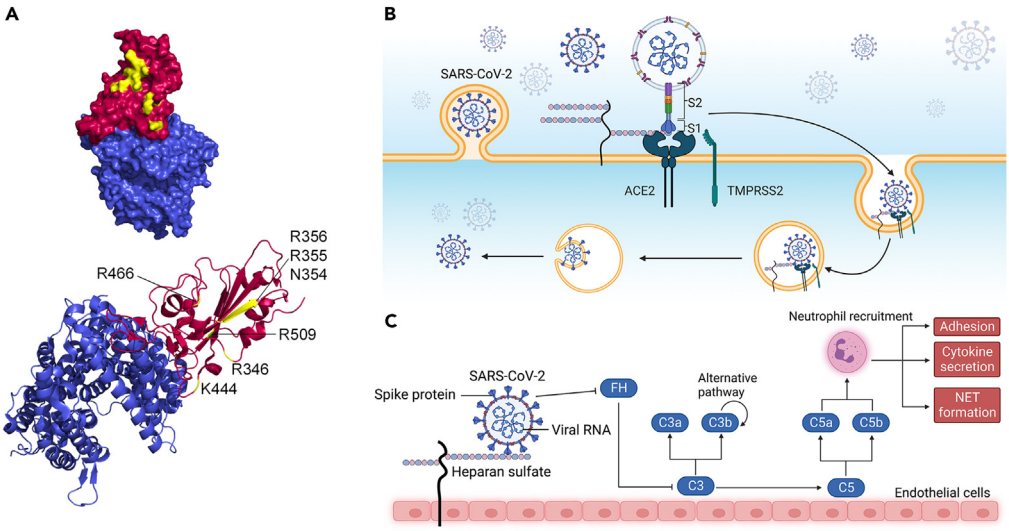

Figure 5. SARS-CoV-2 binding to ACE2 is enhanced with heparan sulfate acting as an adhesion receptor

(A) PyMOL rendering of the crystal structure of SARS-CoV-2 spike including the receptor-binding domain bound to ACE2 receptor. Heparin binding sites are highlighted in yellow in the RBD region of SARS-CoV-2. (PDB ID: 6M0J).

(B) Graphical depiction of the binding and internalization of SARS-CoV-2.

(C) Graphical depiction of proposed activation of the complement cascade. Viral uptake results in activation of the canonical and alternative complement pathways which contribute to the pathology of viral infection in various tissues. For additional details, please refer to the text. Panels (B and C) were generated with *BioRender.com*.

The complement pathway plays an essential role in the severity of respiratory symptoms caused by SARS-CoV-2. The virus competes with C3b-regulatory factor H (FH) binding to HS.[137] This removes the FH-mediated inhibition of C3 which further downstream activates the alternative pathway[137] (Figure 5C). Thus, the virus is a non-canonical alternative pathway activator which gradually evokes complement activation through interactions with HS.[138] In addition to complement activation via the alternative pathway, SARS-CoV-2 induces complement activation via the classical and mannose-binding lectin pathways simply as a pathogen. Overall, complement activation causes cleavage of C3 and C5 complexes into bioactive components C3a, C3b, C5a, and C5b[139] (Figure 5C). Cytokines are released, neutrophils are activated, and inflammation at the infection site is subsequently generated. Targeting the complement system activation appears to be key to lessening the risk of severe COVID-19 symptoms and HS offers a target of interest for future therapeutics.

As the field of corona viruses is constantly expanding, we should point out that other co-regulators have been recently identified, including the transmembrane leucine-rich repeat-containing protein 15 (LRRC15)[140–142] and Neuropilin-1.[143,144] Notably, LRRC15, which is often expressed in various solid tumors, can inhibit adenoviral infection[145] and can hinder SARS-CoV-2 cellular entry in *trans*.[146] In agreement with this anti-viral activity, decreasing LRRC15 plasma levels in infected patients are associated with a more severe clinical outcome.[147] It is not yet established whether HS is a co-factor for LRRC15 or neuorpilin-1, but we should point out that neuropilin-1 is a HS-binding protein. Specifically, neuropilin-1 binds to a rare modification of HS, the 3-O-sulfate groups,[148] and by doing so it enhances its biological activity.[149]

PROTEOGLYCANS AND HEPARANASE AS PROAUTOPHAGIC SECRETED FACTORS

Autophagy is an essential modulator of both tumor suppression and promotion; currently, rapamycin and chloroquine, two potent autophagic modulators, are frequently used to regulate autophagy in anticancer therapy. In terms of cancer development, autophagy is also important in prevention in both cell autonomous and non-cell autonomous methods.[150] Notably, the extracellular matrix contains several constituents that can affect intracellular digestive machinery as well as macroautophagy.[151,152,152–157]

Decorin is a well-studied small leucine-rich proteoglycan (SLRP) with a complex genomic structure and complex promoter region regulated by various growth factors[158–161] Decorin has been implicated for a long time in the regulation of collagen fibrillogenesis,[162–166] cartilage and tendon homeostasis,[165–168] regulation of TGFβ activity, wound healing and fibrosis,[169–177] angiogenesis,[154,171,178–180] Lyme disease,[181] and ocular pathophysiology.[166,175,182–184] Soluble decorin inhibits cancer growth primarily by acting as a pan-RTK inhibitor[185,186]; it binds to EGFR and downregulates its biological activity[187–190] while elevating cytosolic Ca^{2+} in A431 carcinoma cells.[191] Decorin suppresses the growth of various tumors with different histopathological backgrounds,[192–204] but also plays a role in developmental and genetic diseases.[205–212] Notably, reduced expression of stromal decorin is linked to a poor outcome in invasive breast cancer patients.[213–215] Thus, evaluation of stromal decorin may be a good biomarker to predict breast cancer patient outcome.[214,215] One way through which decorin may affect prostate cancer growth and metastatic potential is via MEIS transcription factors that regulate HOX genes. Indeed, MEIS1 expression hinders proliferation of prostate cancer cells *in vitro* and in murine xenografts by evoking an HOXB13-mediated upregulation of decorin.[216]

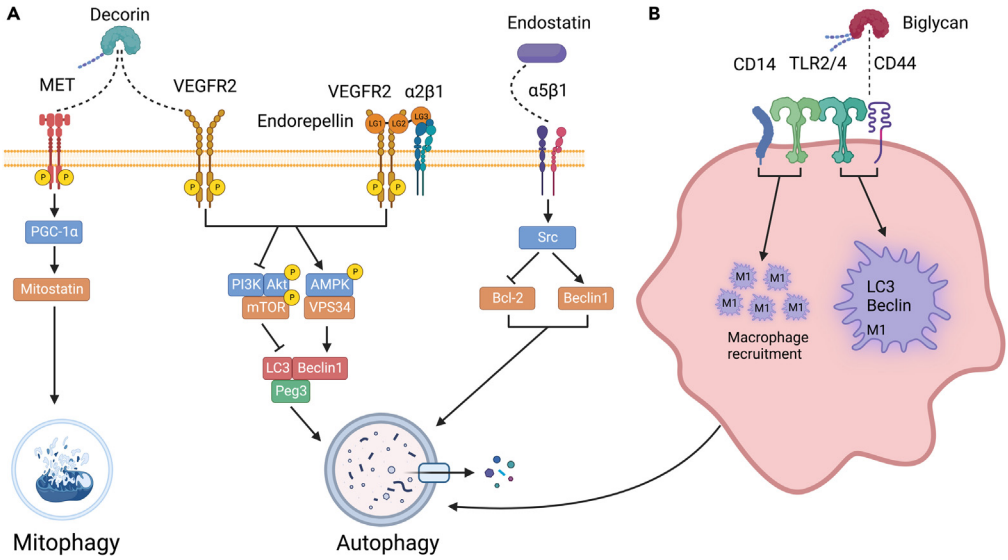

Figure 6. Schematic representation of the various proteoglycans and proteoglycan fragments evoking autophagy

(A) Decorin and endorepellin share VEGFR2 to target endothelial cells. However, decorin uses the MET receptor to evoke mitophagy in cancer cells. Instead, endorepellin requires the co-receptor α2β1 integrin to evoke autophagy in endothelial cells sharing similar pathways. Endostatin acts via the α5β1 integrin in endothelial cells.

(B) Effects of biglycan on macrophage autophagy are mediated by a complex of receptors including TOLL2/4, CD14, and CD44. For additional details, please refer to the text. The graphic was created with *BioRender.com*.

Decorin evokes Peg3-dependent autophagy in endothelial cells of various genetic lineages including human, mouse, and porcine endothelial cells.[151,217,218] We have previously shown using wild-type mice starved for 2 days that decorin is an autophagy-inducible proteoglycan and is required for proper *in vivo* autophagic progression in various organs.[219] Moreover, *Dcn*$^{-/-}$ mice display an aberrant response to fasting compared to wild-type mice.[219] Decorin can also promote autophagy by chronic suppression of Met bioactivity in trophoblasts.[220] Decorin binds to vascular endothelial growth factor receptor 2 (VEGFR2) resulting in the phosphorylation of AMPK at Thr^{172} which among its regulation of a plethora of catabolic processes, is capable of initiating autophagy[221–223] (Figure 6A). Concurrently, AMPK phosphorylation results in an attenuation of the mTOR axis, an anti-autophagic coordinator of anabolic pathways.[224] The combination of these signaling cascades causes an accumulation of Peg3 which co-localizes with Beclin 1 and LC3 to associate with autophagosomes in both microvascular and macrovascular endothelial cells. More recently, decorin has been characterized to interact via the MET/mitostatin/Parkin pathway to sustain tumor cell mitophagy[225] (Figure 6A).

Of interest, there is a link between decorin-evoked autophagy and cancer as decorin suppresses glioma cell invasion by inducing autophagy via the Met/Akt/mTOR axis.[226] Decorin can also induce prolonged respiratory complex turnover and mitochondrial DNA depletion in triple negative breast cancer cells.[227] The beneficiary effect on "cellular protection" is also shown by the finding that decorin protects intestinal cells via its pro-autophagic action in inflammatory bowel disease[228] and seemingly protects retinal pigment epithelial cells from oxidative stress and apoptosis via AMPK-mTOR-regulated autophagy.[229] Moreover, decorin evokes reversible mitochondrial depolarization in carcinoma and vascular endothelial cells.[230] This pathway is novel and requires further elucidation; decorin-evoked mitophagy requires TCHP/mitostatin mRNA interaction with PGC-1α which results in accumulation of mitostatin.

It has been known for a long time that large modular proteoglycans, such as perlecan and collagen XVIII, can be processed by various proteases to generate bioactive fragments such as endostatin and endorepellin.[231–234] Endorepellin is the C-terminal domain of perlecan HSPG2, a very large HSPG with a multifaceted gene organization[235] and a complex promoter region.[236,237] Endorepellin has been known for two decades now as a key inhibitor of angiogenesis, in both developmental and cancer associated angiogenesis.[29,60,238–241] In contrast, the parent proteoglycan perlecan is required for vascularization and is potently pro-angiogenic, a function mainly mediated by its N-terminus cluster of three HS chains.[242–250] One of the main features of perlecan is its ability to interact with various extracellular matrix proteins, growth factors and surface receptors via either the HS chains or the modular protein core[251–259] (see the following text).

Endorepellin potently induces autophagy via VEGFR2 in a similar fashion to decorin. We found that endorepellin interacts with both the α2β1 integrin and VEGFR2 (Figure 6A), and we introduced the concept of dual receptor antagonism,[260] We further discovered that endorepellin by interacting with the ectodomain of VEGFR2 induces significant phosphorylation of AMPK, which leads to an increase in the autophagic markers Beclin 1 and LC3-II.[261] Like decorin, concurrent inhibition of the anti-autophagic mTOR pathway via phosphorylation of mTOR at Ser^{2448} further drives autophagy.[262] Notably, differing from decorin-mediated autophagy via VEGFR2, the kinetics of phosphorylation is markedly slower for endorepellin: AMPK phosphorylation peaks at 4 h of treatment when compared to the 30 min of decorin. More recently we have shown that conditional expression of endorepellin in the tumor vasculature attenuates breast cancer growth, angiogenesis and

hyaluronan deposition.[263] The proposed mechanism occurs through an endorepellin evoked downregulation of HAS2, the main hyaluronan synthase of most cancer and endothelial cells.[264] Thus, angiostatic cues from the matrix link endothelial cell autophagy to hyaluronan biology and its role as a pro-angiogenic and pro-inflammatory polysaccharide.[28,265]

Another matrix component that has been involved in autophagy in vascular endothelial cells is endostatin, the C-terminal monomeric fragment of type VIII collagen which is a hybrid HS proteoglycan. One of endostatin primary targets is integrin α5β1 (Figure 6A). Because endostatin has been characterized to induce autophagy in a nutrient-stress independent manner in endothelial cells, downstream signaling from α5β1 integrin binding is hypothesized to reduce Bcl-2 levels via Src tyrosine kinase signaling which concomitantly results in an increase in Beclin 1.[266] The physiological disruption of the Bcl-2/Beclin 1 ratio causes an induction of autophagy.[266]

Biglycan, another versatile SLRP, in addition to being a structural component of the extracellular matrix, is a multifaceted signaling molecule regulating a plethora of cellular functions including innate immunity and inflammation. Furthermore, soluble biglycan is a damage-associated molecular pattern[267] that promotes M1 macrophage recruitment and inflammation via Toll-like receptor (TLR) 2 and 4 together with the coreceptor cluster of differentiation 14 (CD14)[268–271] (Figure 6B). Transgenic biglycan promotes C-X-C motif chemokine ligand (CXCL)1, CXCL2, CCL2, and CCL5-mediated neutrophil and macrophage infiltration via (MyD)88/TRIF pathways to trigger a potent pro-inflammatory milieu in the renal parenchyma.[272] Moreover, soluble biglycan is a potentially useful biomarker for inflammatory renal diseases.[273] Collectively, the ability of biglycan to modulate and aggravate inflammatory signaling through various receptors makes it a novel therapeutic target for inflammation associated diseases.

Biglycan was first hypothesized to modulate autophagy as mice stably overexpressing soluble biglycan were found to have a significant increase in autophagic macrophages.[272] Upon further investigation, it was discovered that biglycan TLR2/4 as well as co-receptors CD44/CD14 which initiates inflammatory response.[270] CD44 binding affinity is significantly higher for biglycan when compared to CD14.[274] CD14 interaction induces recruitment of M1 macrophages. Biglycan induces Beclin 1 expression along with LC3-II and p62 accumulation by engaging CD44 and TLR4 in these M1 macrophages, which directly drives phagophore/autophagosome formation and assembly.[274] Biglycan also exhibits protective effects toward doxorubicin in osteosarcoma cells by activating the Wnt/β-catenin pathway and suppressing autophagy.[275] Enhancing chemotherapeutic efficacy is also mediated by an ability of biglycan to bind and activate insulin-like growth factor 1 receptor (IGF-IR) signaling.[276]

In addition to its role in inflammation and autophagy, biglycan signaling carries clinical implications in the modulation of tumorigenesis.[271,277,278] For example, targeted suppression of stromal biglycan inhibits metastasis, impairs tumor angiogenesis, and enhances tumor $CD8^+$ T cell infiltration, thereby improving chemotherapeutic efficacy in breast cancer.[279] The multitude and diversity of tumors where biglycan serves as a protumorigenic/prognostic marker and as a promising therapeutic target indicate its potential role in cancer biology.[278,280]

In addition to proteoglycans, enzymes involved in the catabolism and modification of HS chains such as heparanase,[281,282] can be involved in regulation of autophagy. Heparanase is a multifunctional molecule harboring both enzymatic and non-enzymatic functions.[283,284] Through its endo-β-glucuronidase activity, heparanase cleaves HS chains in various HSPGs releasing relatively large HS fragments of 4–7 kDa fragments that are biologically active.[285] Heparanase also releases HS-bound growth factors, cytokines, and chemokines stored within the microenvironment of remodeling tissues and growing cancers.[286,287] Moreover, heparanase can mediate tumor-host crosstalk enhancing protumorigenic gene transcription ultimately activating pro-survival signaling pathways and the formation of exosomes.[288] Together, the enzymatic and non-enzymatic biological activities of heparanase establish this unique enzyme as a multifunctional molecule that increases the aggressiveness of tumor cells.[288,289] Notably, heparanase has been recently shown to evoke autophagy in cancer cells, leading to tumor growth and chemo-resistance.[281] Specifically, heparanase localizes to autophagosomes and concurrently possesses the ability to positively regulate the autophagic process.[281] Notably, along with increasing absolute autophagosome number, heparanase overexpression also causes an increase in the number of vesicles released from the cancer cells.[281] It is highly likely that these vesicles are indeed exosomes as heparanase can evoke exosome secretion, composition, and function of tumor cell-derived exosomes which could amplify tumor development.[290] In human cancer cells of various histogenetic backgrounds, including myeloma, lymphoblastic, and breast cancer cells, when expression of heparanase is enhanced or when tumor cells are exposed to exogenous heparanase, exosome secretion is dramatically increased.[290] Exosomes evoked by hypoxic conditions, often a hallmark of cancer, transport heparanase and enhance macrophage migration, endothelial tube generation and cancer cell stemness.[291] Thus, targeting heparanase in tumors using synthetic or chemically modified inhibitors,[292] seems a reasonable approach to combat cancer. Collectively, these observations provide a potential mechanism for heparanase to increase tumor growth through the induction of autophagy, and further propose a functional link between heparanase activity, autophagy, and exosome secretion, culminating in cancer progression. In conclusion, proteoglycans, together with bioactive proteolytic fragments of their protein cores and processed GAGs by heparanase, and perhaps other lyases, are emerging as potent regulators of autophagy and could become even more relevant as potential therapeutics for cancer, neurodegenerative diseases, and inflammatory processes.

HEPARAN SULFATE PROTEOGLYCANS AS ANTIAUTOPHAGIC FACTORS

In contrast to the proautophagic proteoglycans described previously, decorin and biglycan, and C-terminal unglycanated fragments of HSPHs, perlecan and collagen XVIII, the parent molecule, perlecan/Hspg2, functions as a suppressor of autophagy. An elegant genetic study has utilized *Hspg2*$^{-/-}$ mice,[293] which are usually embryonic lethal,[239,294] by rescuing them via transgenic expression of *Hspg2* into the cartilage (*Hspg2*$^{-/-}$Tg). This rescue is based on the high expression of *Hspg2* in cartilage and its loss causes early respiratory failure.[294] Genetic ablation of *Hspg2* resulted in tenotomy-induced atrophy of the soleus muscle while concurrently increased autophagy vis-à-vis wild-type mice that had undergone the same procedure.[293] In addition to a decrease in autophagic substrates such as p62 and an increase in LC3-II levels,

perlecan deficiency caused a reduction in phosphorylated p70S6k and Akt, while concurrently inducing phosphorylation of AMPKα, suggesting that Hspg2 inhibits autophagy in through the mTORC1 pathway.[293] The main conclusion of this study posits perlecan as a necessary factor for maintaining muscle mass through proper regulation of autophagy, a concept also supported by zebrafish studies where perlecan morphants show a severe myopathy characterized by abnormal actin filaments and disorganized sarcomeres.[246]

It is well established that neuromuscular junctions display developmental and activity dependent plasticity that can be directly affected by HSPGs. Notably, in *Drosophila*, muscle-specific knockdown of HS biosynthetic machinery increased autophagy at the neuromuscular junctions.[295] Moreover, HS production was also essential for maintaining normal autophagic processes in the fat body, the principal energy storage and nutritional sensing organ of *Drosophila*. These data are further supported by an elegant study using *Drosophila Parkin* mutants where globally suppressing HS biosynthesis led to increased lifespan and resistance to oxidative stress.[296] Collectively, these findings demonstrate that HS is a critical regulator of on autophagy, a biological process essential for natural assembly of postsynaptic membrane specializations.[295] The mechanism by which HS inhibits autophagy has yet to be fully elucidated and we do not know which HSPG is primarily responsible for this activity. Moreover, how these extracellular polysaccharides influence intracellular catabolic events and the cell surface receptors and signaling molecules at play is an interesting question, and its answer would benefit both the proteoglycan and autophagy fields.[265]

Limitations of the study

In this review we have not covered the expanding field of hyaluronan science due to space limitations. Although not a true proteoglycan, hyaluronan is mostly bound to proteins such as the cell surface receptors CD44 and RHAMM and, in tissues, hyaluronan is non-covalently linked to the G1 domains of the hyaluronan- and lectin-binding proteoglycans known as hyalectans[297] which include aggrecan, versican, brevican, and neurocan.[5] The hyaluronan field has expanded enormously in the past two decades primarily because of its involvement in cancer, angiogenesis, inflammation, and innate immunity. We refer the readers to several recent reviews covering this subject.[298–302] Other topics not covered here are the proteoglycan roles in cartilage and bone diseases, and their functions during development and organogenesis. There are several excellent reviews covering these important topics as well.[303–305]

UNANSWERED QUESTIONS AND FUTURE CHALLENGES

The current proteoglycan arena is exciting, powerful, and promising regarding both diagnostic and therapeutic applications. Nevertheless, several outstanding questions regarding the molecular mechanisms and machinery regulating proteoglycan/growth factor interactions and their role in development and diseases remain to be addressed in the future.

Another key question is how can we exploit high-throughput sequencing, such as single-cell RNAseq, to attain an improved understanding of proteoglycan heterogeneity and detect new mutations/isoforms of various proteoglycan genes in patients for precision medicine?

On a more practical and achievable level, what is the present and future role of biomarkers that could be used to identify patients that could benefit from proteoglycan-targeted therapy? How will distinct combinatorial therapies be established, and which treatment will act synergistically with therapies targeting tumor-specific antigens?

What is the role of the immune system in regulating proteoglycan activity and vice versa? How could we activate the immune system against specific proteoglycans such as glypican, for example,[42] to penetrate the highly immunosuppressive tumor microenvironment and eliminate solid tumors?

Another important bioactivity that needs further attention is on the role of protease-evoked release of source proteoglycans and their dual roles as true and decoy receptors. Unraveling the complex mechanism of action and the potential commitment of pro-inflammatory signaling pathways triggered by these shed proteoglycans is a clear gap in our knowledge.

Will recombinant decorin be used as an oncosuppressive therapeutic and could it be utilized as effective adjuvant therapy for human malignancies? The fact that decorin affects both epithelial and bone marrow-derived malignancies,[306–308] and its role in evoking epithelial mesenchymal transition,[309] infers a more general role for this proteoglycan in curtailing mammalian malignancies.

Another intriguing recent technological advance is the ENCODE4 RNAseq that can read long transcripts, thereby discovering numerous transcripts and many alternative splice variants.[310] Thus, an important question is how many previously unrecognized transcripts will be found in the proteoglycan encoding genes? And, if so, how many new biological roles will be uncovered in the future? Given the global impact of proteoglycan science on human physiology and diseases, the future of proteoglycans is so bright " …. *we will have to wear shades*" (Pat MacDonald).

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AUTHOR CONTRIBUTIONS

R.V.I. and C.X. were responsible for conceptualization, drafting, and editing of the manuscript, as well as the design and generation of all the figures. L.S. contributed to writing and editing. R.V.I. was responsible for the final editing and proofing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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