Biology of the Heparanase–Heparan Sulfate Axis and Its Role in Disease Pathogenesis

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Abstract

Cell surface proteoglycans are important constituents of the glycocalyx and participate in cell–cell and cell–extracellular matrix (ECM) interactions, enzyme activation and inhibition, and multiple signaling routes, thereby regulating cell proliferation, survival, adhesion, migration, and differentiation. Heparanase, the sole mammalian heparan sulfate degrading endoglycosidase, acts as an "activator" of HS proteoglycans, thus regulating tissue hemostasis. Heparanase is a multifaceted enzyme that together with heparan sulfate, primarily syndecan-1, drives signal transduction, immune cell activation, exosome formation, autophagy, and gene transcription via enzymatic and nonenzymatic activities. An important feature is the ability of heparanase to stimulate syndecan-1 shedding, thereby impacting cell behavior both locally and distally from its cell of origin. Heparanase releases a myriad of HS-bound growth factors, cytokines, and chemokines that are sequestered by heparan sulfate in the glycocalyx and ECM. Collectively, the heparan sulfate–heparanase axis plays pivotal roles in creating a permissive environment for cell proliferation, differentiation, and function, often resulting in the pathogenesis of diseases such as cancer, inflammation, endotheliitis, kidney dysfunction, tissue fibrosis, and viral infection.

Keywords
- heparanase
- syndecan
- glycocalyx
- signal transduction
- exosomes

Heparan Sulfate Proteoglycans

Heparan sulfate proteoglycans (HSPGs) are ubiquitously present in every tissue compartment, particularly the extracellular matrix (ECM), cell surface, intracellular granules, and nucleus.[1] They consist of a core protein to which several linear heparan sulfate side chains are covalently linked. Heparan sulfate is composed of repeating disaccharide units of glucosamine and uronic acid, with variable additions of sulfate groups and other modifications.[1,2] Because of their heterogeneity and versatility, proteoglycans serve as important functional components at the cell surface and ECM. Extensive posttranslational modifications on heparan sulfate chains of proteoglycans, particularly the addition of sulfate groups, not only impart negative charge but also increase their high degree of structural diversity and functional heterogeneity. The largest secreted proteoglycan is perlecan, carrying up to three heparan sulfate side chains. Perlecan is an integral component of the ECM and basement membrane[1,2] and a key component of the vascular ECM, where it interacts with a variety of other matrix components and helps maintain the endothelial barrier function and vascular homeostasis.[3] Perlecan has been implicated in various pathological processes, such as tumor development, osteoarthritis, muscle hypertrophy, atherosclerosis, and diet-induced obesity.[1,2,4] Heparan sulfate functions not only as structural protein but also as anchor due to the high content of charged

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groups in heparan sulfate.[5] The latter property is used to bind several bioactive proteins that can be rapidly released when required, endowing proteoglycans with the ability to fine-tune signal transduction. The glycosaminoglycan (GAG) chains of proteoglycans interact with a plethora of GAG-binding proteins including ECM proteins, enzymes and enzyme inhibitors, chemokines, growth factors, membrane receptors, lipid-binding proteins, pathogen surface proteins, and viral envelope proteins. To a large extent, the majority of GAG-binding proteins interact with HS due to the greater sequence heterogeneity and more variable sulfation patterns. This is of particular functional importance in the case of cell surface proteoglycans, where the extracellular GAG chains can act as coreceptors.[6] In this context, the heparan sulfate chain immobilizes the ligand and increases its local concentration, resulting in increased receptor dimerization and stimulation of intracellular signaling.[7,8] The major classes of cell surface proteoglycans comprise four members of the transmembrane HSPGs of the syndecan family and six members of the GPI-anchored glypican family of HSPG. Cell surface proteoglycans are an important constituent of the glycocalyx and participate in cell–cell and cell – ECM interactions, enzyme activation and inhibition, and pleiotropic signaling routes, thereby regulating cell proliferation, adhesion, migration, and differentiation.

Syndecans and Glypicans

The syndecans form a family of four type I single-pass transmembrane HSPGs expressed by all adherent cells and capable of carrying chondroitin sulfate and heparan sulfate chains in a cell-type and developmental-specific pattern. The presence of heparan sulfate chains allows interactions with a large number of heparin/heparan sulfate-binding proteins.[7,9–11] Binding of many of the ligands leads to syndecan-mediated endocytosis and catabolism, or orchestration of signaling cascades.[12] Syndecans act as receptors for the ECM, as endocytic receptors, and most importantly as signaling coreceptors that modulate receptor tyrosine kinase, chemokine, and morphogen signaling. In this way, they play an important role in regulating inflammation and angiogenesis, cell proliferation, differentiation, and cell adhesion and motility. Via their heparan sulfate chains, syndecans are capable of forming a ternary complex with the ligand (e.g., FGF2) and its tyrosine kinase receptor (e.g., FGFR).[8,13] HSPGs also modulate signaling via a different class of receptors, G-protein–coupled receptors (GPCRs),[14] as exemplified by chemokine signaling. In this case, cell surface HSPGs not only assist in forming chemokine gradients at the endothelial surface, which serve to guide leukocytes from the circulation to sites of inflammation[14] but also are thought to facilitate signaling through the GPCRs by promoting multimerization of the ligand.[15,16] Structurally, all syndecans are composed of an extracellular ectodomain harboring two or three consecutive Ser-Gly sequences flanked by hydrophobic and acidic residues, which serve as attachment sites for heparan sulfate. All syndecans also contain a highly conserved transmembrane region containing the dimerization motif GxxxG that enables homo- and hetero-oligomerization of syndecans. The conserved short C-terminal cytoplasmic domain is divided into the first conserved region (C1), variable domain (V) that is specific for individual syndecan members, and the second conserved region (C2). Each of these cytoplasmic domains has been shown to interact with specific adaptor molecules that mediate cellular function.[17–20] Functional data suggest that the hydrophobic amino acid sequence AVAAV, located 26 AA from the transmembrane domain, is involved in regulating cancer cell invasiveness,[21] whereas a sequence covering amino acid 93 – 120 in human syndecan-1 termed "synstatin" mediates the lateral association with αv integrins and formation of a complex with insulin-like growth factor receptor.[22]

Glypicans are a family of six GPI-anchored cell surface HSPG characterized by an approximately 50-kDa domain that contains 14 highly conserved cysteine residues. Glypicans are modified with up to three HS chains near the juxtamembrane region and are expressed mainly by mesenchymal and epithelial cells. By presenting GAG chain–bound morphogens and growth factors to their cognate receptors, glypicans modulate numerous signaling processes with relevance to tumor cell proliferation and angiogenesis.[1]

Glycocalyx

Vascular endothelial cells are lined with glycocalyx, consisting primarily of membrane-bound proteoglycans and sialic acid–containing glycoproteins. The glycocalyx is highly important in endothelial function, as it is involved in microvascular reactivity and modulates interaction of the endothelium with blood constituents.[23] It contributes to a multitude of endothelial functions, including regulation of vascular tone and permeability, coagulation, leukocyte and platelet adhesion, and inflammation propagation.[24] On one hand, it protects the endothelial cell from shear stress caused by blood flow and serves as a vascular permeability barrier. On the other hand, it harbors various chemokines, receptors, growth factors, and enzymes that play a central role in endothelial function and blood/vascular/tissue interactions. Glycocalyx is continuously renewed via the balanced and rather intense processes of degradation and synthesis. In the course of diverse diseases, this balance is shifted toward degradation, leading to the net loss of glycocalyx. The syndecan family of HSPG is the most ubiquitous in the endothelial glycocalyx and serves critical functions in endothelial cell signaling. Through intracellular associations with syntenin, synectin, and actin stress fibers,[25,26] the syndecans coordinate mechanotransduction to the intracellular environment to regulate cellular alignment and ligand interactions with cell surface receptors, thereby contributing to a myriad of transmembrane signaling pathways.[27–34]

Heparanase

Heparanase is the sole heparan sulfate degrading endoglycosidase present in mammals.[35–38] Human heparanase mRNA encodes for a 61.2-kDa protein containing 543 amino acids. This proenzyme form is then cleaved by cathepsin L to generate the active form consisting of 8 and 50 kDa subunits that associate noncovalently.[39] Structurally, heparanase is

composed of a TIM-barrel fold that contains the active site of the enzyme and a C-terminus domain required for secretion and signaling function of the protein.[40,41] Heparanase has a catalytic mechanism which includes a putative proton donor at Glu225 and a nucleophile at Glu343[35] as well as the heparin/heparan sulfate binding domains (HBD1 = Lys158-Asp171 and HBD2 = Gln270-Lys280) that are situated in close proximity to the active site micro-pocked fold.[42] The C-domain is composed of 8 β strands arranged in two sheets, as well as a flexible, unstructured loop that lies in between. Notably, the 8-kDa subunit enfolds the 50-kDa subunit, contributing β/α/β unit to the TIM-barrel fold and one of the 8 β strands that comprise the C-domain.[40,43,44] Heparanase cleaves heparan sulfate chains of proteoglycans, thereby releasing 4- to 7-kDa fragments of heparan sulfate that can remain biologically active. Heparanase enzyme activity also releases heparin-bound growth factors, cytokines, chemokines, and other ligands stored within the ECM. By cleaving heparan sulfate, it contributes to the reorganization of the ECM and glycocalyx resulting in enhanced cell adhesion, motility, and invasion. Moreover, heparanase is involved in signal transduction and gene expression via its enzymatic and nonenzymatic activities.[36,45,46] Heparanase acts as an "activator" of HSPG and therefore is a pivotal player in creating a permissive environment for cell growth and differentiation. Heparanase regulates multiple biological activities that enhance tumor growth, metastasis, and angiogenesis.[38] Numerous studies have demonstrated that heparanase is enhanced in many cancers and its expression is associated with poor patient prognosis.[38] Heparanase knockout mice exhibit resistance to sepsis-induced degradation of pulmonary endothelial glycocalyx and endothelial hyperpermeability.[47] On the contrary, transgenic mice overexpressing heparanase exhibit reduced leukocyte crawling, adhesion, and infiltration of inflamed sites,[48] reportedly due to the defective ability of truncated HS chains in the glycocalyx to ligate chemokines.

Syndecan Mediates Cellular Uptake of Heparanase

Secreted or exogenously added latent heparanase rapidly interacts with normal and tumor-derived cells, followed by internalization and processing into a highly active enzyme,[49–54] collectively defined as heparanase uptake. Heparanase uptake is regarded a prerequisite for the delivery of latent 65-kDa heparanase to lysosomes and its subsequent proteolytic processing and activation into 8 and 50 kDa protein subunits. Heparanase interacts with syndecans by virtue of the typical high affinity that exists between an enzyme and its substrate. This high-affinity interaction directs rapid and efficient cellular uptake of the heparanase–syndecan complex.[51,55] Peptide corresponding to the heparin/heparan sulfate binding domain of heparanase (termed KKDC) similarly associates with the plasma membrane and induces clustering of syndecans, resulting in Rac1 activation and improved cell spreading.[42,56] Notably, syndecan clusters formed by the KKDC peptide were exceptionally large and failed to be internalized (►**Fig. 1A**).[56] Likewise,

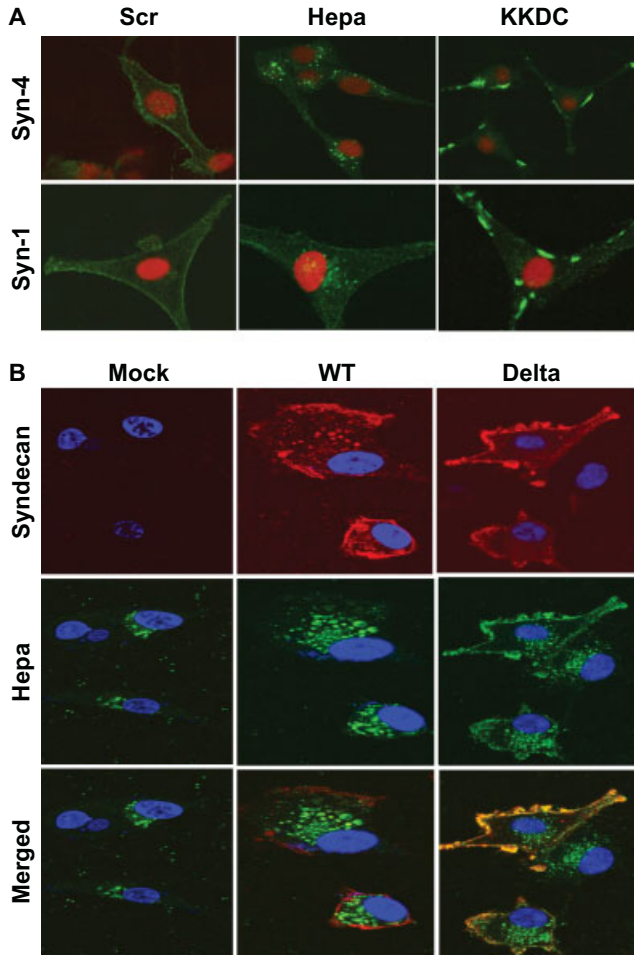

Fig. 1 Syndecan mediates cellular uptake of heparanase. (**A**). The KKDC peptide induces syndecan clustering. U87 cells were serum-starved for 24 hours and were then incubated with the KKDC/Scr peptides (50 μM) or heparanase (1 μg/mL) for 1 hour. Cells were then gently washed, fixed with 4% paraformaldehyde, and subjected to fluorescent staining with anti-syndecan-4 (upper panels) and anti-syndecan-1 (lower panels) antibodies (modified from Levy-Adam et al[56]). (**B**). Heparanase uptake requires the syndecan-1 cytoplasmic tail. Heparanase (1 μg/mL) was added to U87 glioma cells overexpressing WT syndecan-1 or syndecan-1 lacking its cytoplasmic tail for 1 hour at 37°C. Cells were then fixed with cold methanol and subjected to immunofluorescent staining applying antiheparanase mouse monoclonal antibody (middle panels, green). Merged images with rat anti-syndecan staining (upper panels, red) are shown in the lower panels. Note retention of heparanase at the cell membrane, colocalizing with syndecan lacking the entire cytoplasmic tail (delta), and increased heparanase-positive endocytic vesicles in cells overexpressing wild-type (WT) syndecan-1 (Image Courtesy: Shteingauz et al[61]).

heparanase-2 (Hpa2), a close homolog of heparanase, binds HS with high affinity but fails to get internalized,[57] implying that interaction and clustering per se are not sufficient for the internalization of syndecan and its cargo. Syndecans get internalized via diverse routes depending on their ligand cargo, utilizing different syndecan-1 domains and adaptor molecules. Large multivalent ligands such as lipoprotein lipase (LPL) elicit syndecan-1 internalization via rafts that involve Erk and Src signaling because inhibitors of these kinases attenuated internalization while ligand binding was not changed.[58] Unlike

LPL, heparanase seems to utilize coated pits rather than rafts as the primary endocytosis route.[59,60]

We examined the role of syndecan-1 cytoplasmic domain in heparanase processing.[61] To this end, we transfected cells with a full-length mouse syndecan-1 or deletion constructs lacking the entire cytoplasmic domain (delta), the conserved (C1 or C2), or variable (V) regions. Heparanase uptake was markedly increased following syndecan-1 overexpression. In contrast, heparanase was retained at the cell membrane and its processing was impaired in cells overexpressing syndecan-1 deleted for the entire cytoplasmic tail (►**Fig. 1B**).[61] Subsequent studies revealed that the C2 and V regions of syndecan-1 cytoplasmic tail mediate heparanase endocytosis, processing, and activation. Furthermore, syntenin, known to interact with syndecan C2 domain, and α actinin are essential for heparanase processing,[61] illustrating the tight regulation of heparanase activation and syndecan-mediated endocytosis. α-Actinin was colocalized with syndecan-1 at the cell membrane and α-actinin gene silencing was associated with reduced and more diffused heparanase-positive endocytic vesicles, likely due to disruption of syndecan-1 interaction with the actin cytoskeleton.[61] Syntenin is a PDZ-domain–containing protein that interacts with a plethora of proteins including the C2 domain of syndecans.[62] Reduced heparanase uptake following syntenin gene silencing indicates that this adaptor molecule is involved in syndecan-mediated endocytosis, in agreement with the necessity of the C2 domain of syndecan-1 for heparanase uptake.[61] Notably, the decrease in heparanase processing following syntenin or α-actinin gene silencing exceeded the decrease in internalization, suggesting that syntenin and α-actinin may direct heparanase to the lysosome. It appears that like syndecan, syntenin levels may promote tumor progression by modulating heparanase uptake, activation, and extracellular retention. Altogether, our results suggest that heparanase utilizes a raft-independent route for internalization by syndecan-1 that leads to its processing rather than total destruction in lysosomes. Thus, at least two different syndecan-1-mediated endocytic pathways exist, one that relies on the C1 domain and utilizes rafts–MKKK–cortactin axis[58] and a second, raft-independent, route that is mediated by the C2 and V domains of syndecan-1 (►**Fig. 1**) and involves α-actinin and syntenin, leading to heparanase processing and activation. Interestingly, as discussed later, syndecans and syntenin, via interaction with ALIX, have also been implicated in regulating the biogenesis of exosomes.[63]

Syndecan Shedding

Syndecan-1 is highly expressed on the surface of many types of tumor cells and in some cases within tumor stroma as well.[64–66] For example, overexpression of syndecan-1 has been observed in pancreatic, gastric, and breast carcinomas, correlating with increased tumor aggressiveness and poor clinical prognosis; syndecan-2 is often overexpressed in colon carcinoma, and syndecan-4 is upregulated in hepatocellular carcinoma,[17] possibly leading to increased heparanase uptake and activation. Indeed, heparanase expression in these carcinomas often coincides with disease progression and reduced patients' survival.[55,67,68] In other cases (i.e., head and neck and lung carcinomas, laryngeal cancer, malignant mesothelioma), however, loss of syndecan-1 was associated with disease progression,[12,17] thus illustrating the complexity of heparan sulfate function in cancer and possibly a redundancy among HSPGs. Syndecans are cleaved within the 15 amino acid juxtamembrane region, thus explaining the requirement for sheddases to acquire the membrane-proximal conformation of the catalytic center. Shedding of the ectodomain is followed by intramembrane proteolysis by γ-secretase of the remaining fragment of syndecans. Shedding can be stimulated by an array of agonists that initiate the shedding process through upregulation of protein sheddases.[69] In most cases, shedding results in release of an intact ectodomain containing fully functional GAG chains capable of binding to the same extracellular ligands and acting in either autocrine or paracrine fashion.[6,7] In addition to heparan sulfate chains on the shed syndecan-1, the core protein is also shown to be involved in the activation of signaling pathways that contribute to tumor growth and metastasis. Furthermore, shed syndecan-1 binds to the ECM where it can act to trap tumor cells contributing to the initiation of a tumor niche. It also can form growth factor and chemokine concentration gradients that contribute to tumor progression and inflammation. Shedding of a membrane proteoglycan is a means of eliminating the proteoglycan coreceptor function for various signaling processes at the cell surface, a mechanism for modulating cell adhesion and cell–matrix interactions. Shedding can be constitutive or induced by a wide range of stimuli which involve signaling through the PKC, tyrosine kinase, and the MAP kinase pathways. While studying heparanase, the Sanderson's laboratory made the surprising discovery that this enzyme dramatically enhances syndecan-1 shedding from the surface of myeloma or/and breast cancer cells.[70] Heparanase-induced shedding of syndecan-1 was the result of two distinct mechanisms: (1) heparanase-stimulated ERK signaling in the tumor cells leading to upregulation in the expression of the syndecan-1 sheddase MMP-9[71,72] and (2) heparanase via its enzyme activity shortens the heparan sulfate chains of syndecan-1, thereby rendering the core protein increasingly susceptible to cleavage by MMP-9.[73] This unique link between heparanase and syndecan-1 shedding was further confirmed in a variety of pathological states.[71,74] The cleavage of heparan sulfate chains by heparanase also makes it easier for additional proteins to recognize and bind to syndecan. One example is the binding of the protein lacritin, a prosecretory epithelial mitogen that is expressed in the tear ducts and whose active form is selectively deficient in dry eye. Lacritin can bind directly to syndecan-1 core protein only after the trimming of heparan sulfate chains by heparanase, acting as a novel "on-switch" for lacritin ligation of syndecan-1 necessary to trigger basal tearing.[75]

The Heparanase–Syndecan Axis Regulates Multiple Aspects of Tumor Progression

In experiments with human myeloma cells engineered to express a high level of heparanase it was found that when syndecan-1 is shed, it exposes a cryptic juxtamembrane site

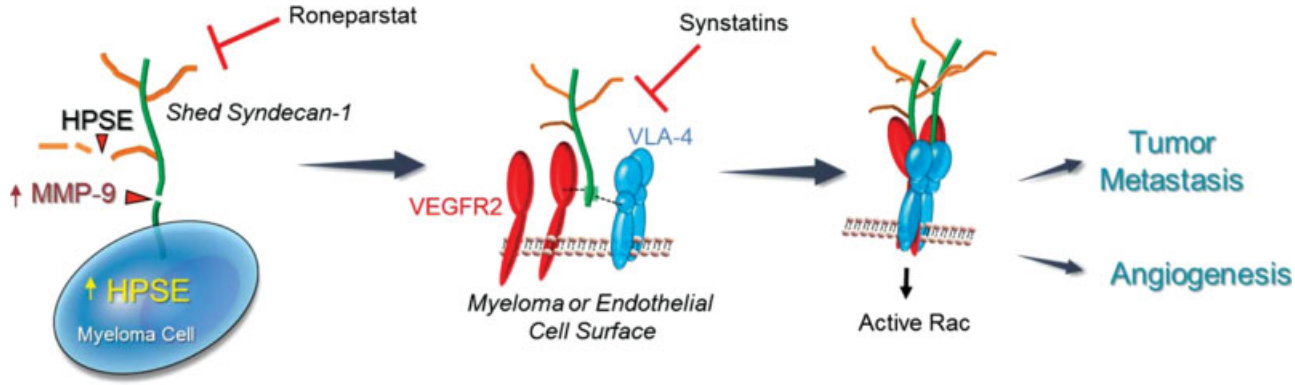

Fig. 2 Heparanase activates a signaling mechanism that drives both tumor cell invasion and angiogenesis. (Left panel) Myeloma cells express syndecan-1 on their cell surface composed of a core protein (green) and heparan sulfate chains (brown). Upregulation of heparanase (HPSE) expression by myeloma cells leads to trimming of syndecan-1 heparan sulfate chains, shortening their length, and allowing increased access of proteases to the exposed syndecan-1 core protein. One such protease is MMP-9, a syndecan-1 sheddase whose expression is upregulated when heparanase is expressed by myeloma cells. MMP-9 cleaves the syndecan-1 core protein and the proteoglycan is shed from the cell surface. (Center panel) Shedding of syndecan-1 exposes a cryptic domain within the juxtamembrane region of the core protein (green). Within this cryptic domain are amino acid sequences that bind to clustered VLA-4 (blue) and VEGFR2 (red) on the surface of myeloma cells or endothelial cells. (Right panel) The coupling of VLA-4 and VEGFR2 receptors by shed syndecans activates VEGFR2 signaling that stimulates both cell invasion and endothelial tube formation. This signaling mechanism is inhibited by Roneparstat, a heparanase inhibitor that diminishes syndecan-1 shedding, or by synstatin peptides, peptide mimics of the syndecan-1 core protein that competitively inhibit binding of either VLA-4 or VEGFR-2 to shed syndecan-1. Image Courtesy: Sanderson et al.[176]

on the syndecan-1 core protein that then complexes with VEGFR2 and very late antigen-4 (VLA-4) on the myeloma cell surface (►**Fig. 2**).[76] This leads to VEGFR2 receptor signaling and triggering of downstream activation of Rac-GTPase resulting in polarized migration and myeloma cell invasion (►**Fig. 2**). Peptides known as synstatins that mimic regions within the cryptic core protein domain of syndecan-1 can selectively block binding to either VEGFR2 or VLA-4, thereby inhibiting the formation of the syndecan-1/VEGFR2/VLA-4 complex and subsequent cell spreading and invasive phenotype (►**Fig. 2**). These results further confirm the crucial role of shed syndecan-1 in promoting the spread and invasive phenotype of myeloma cells seen when heparanase level is elevated.[71] Early studies examining heparanase in myeloma patients revealed that high levels of heparanase in the myeloma bone marrow correlate closely with elevated microvessel density.[77] In vitro studies revealed that shed syndecan-1 binds to VEGF and facilitates activation of the VEGF receptor on endothelial cells.[78] It has also been shown that heparanase can enhance expression of VEGF by myeloma, breast cancer, and glioma cells,[78,79] thus providing mechanistic evidence for the role of heparanase in angiogenesis. Moreover, just as with the myeloma cells, shed syndecan-1 induced by heparanase can complex with VLA-4 and VEGFR2 on endothelial cells to stimulate their invasive behavior and this can be inhibited by synstatins.[76] Thus, via a single mechanism initiated by heparanase enhancement of syndecan-1 shedding, heparanase promotes both tumor cell metastasis and angiogenesis.

Shed Syndecan-1 in the Nucleus

Syndecan-1 has been found in the nucleus of several different types of cancers and can be localized there via a nuclear localization sequence (NLS) found in its cytoplasmic tail.[64,80–82] Likewise, shed syndecan-1 present in the conditioned medium of heparanase-high myeloma cells was rapidly translocated into the nucleus of a human lymphoblastoid cell line (ARH77) that lacks syndecan-1 expression.[83] Indeed, several membrane proteoglycans contain amino acid motifs that represent NLSs.[84] In the case of syndecan-1, the conserved juxtamembrane sequence RMKKK represents an NLS, in which the arginine residue plays a particularly important role for tubulin-dependent internalization of the HSPG.[84] Nuclear heparan sulfate chains in conjunction with heparanase can influence numerous events, including the promotion of mitotic spindle formation and subsequent chromosome stability, inhibition of DNA topoisomerase-I activity, and regulation of cell proliferation.[35,80,85–90] The majority of the nuclear shed syndecan-1 was observed in discrete patches within the euchromatin, the area of active gene transcription. Moreover, it was found that shed syndecan-1 in the nucleus of bone marrow–derived stromal cells binds to the histone acetyltransferase (HAT) enzyme via HS chains and diminishes HAT activity and histone H3 acetylation. This is of functional relevance, as a reduction of nuclear syndecan-1, likely by nuclear heparanase, enhances HAT activity, and this epigenetic mode of regulation results in an increased expression of genes which drive an aggressive tumor phenotype (i.e., MMP-9, VEGF, vimentin, TGF-β, and hepatocyte growth factor)[91] while repressing expression of the major cellular iron transporter, hepcidin.[92] Heparanase may also regulate gene expression by binding directly to gene promoters, with evidence of promiscuous binding to DNA independent of its enzymatic activity.[87] Also, shed syndecan-1 via its heparan sulfate chains binds to HGF and shuttles the growth factor into the nucleus.[83] This represents a previously unappreciated mechanism, whereby the heparanase–shed syndecan-1 axis can regulate growth factor trafficking and activity.

Exosomes

Exosomes are extracellular vesicles between 40 and 100 nm in diameter released from the endosome of cells. Exosomes can carry payloads of cytoplasmic and membrane components, including DNA, proteins, enzymes, mRNA, miRNA, lipids, and activated receptors.[93–97] Recipient cells endocytose the exosome, releasing their contents and therefore playing a vital role in cell–cell communication. The finding that heparanase enhances exosome biogenesis came initially in human myeloma cells transfected with the cDNA for HPSE.[98] This resulted in an approximately fourfold increase in HPSE expression by the cells and stimulated an approximately sixfold increase in the amount of exosomes secreted. The addition of recombinant, enzymatically active heparanase to myeloma cells enhanced exosome biogenesis in a dose-dependent fashion, thereby confirming the findings in transfected cells. Similarly, recombinant heparanase enhanced exosome secretion by MDA-MB-231 human breast cancer cells, indicating that the effect of heparanase on stimulation of exosome biogenesis was not cell-type specific. Myeloma cells transfected with a cDNA coding for a mutated, enzymatically inactive form of heparanase failed to enhance exosome secretion. Thus, the stimulatory effect that heparanase has on exosome biogenesis is related to its ability to enzymatically alter the heparan sulfate chains of syndecan, thereby facilitating intraluminal budding and formation of exosomes.[35,98,99] Mechanistically, heparanase enhances exosome biogenesis by stimulating endocytosis of syndecans and by acting through the syntenin–Alix pathway to stimulate shortening the heparan sulfate chains of syndecan-1 and thereby intraluminal budding of vesicles.[99] Analysis of exosome cargo secreted by myeloma cells expressing heparanase at a high level revealed more syndecan-1, VEGF, HGF, and fibronectin than was present in exosomes secreted by cells expressing at low level of heparanase. The finding that heparan sulfate chains are retained on the surface of exosomes[71,100] raised the possibility that cargo bound to syndecans via heparan sulfate is retained on exosomes and available to mediate intercellular communication when the exosomes dock with recipient cells. It is likely that VEGF and HGF detected are bound to the exosome surface through interaction with syndecan heparan sulfate.[101] Interestingly, heparan sulfate can play a dual role in exosome–cell interaction; heparan sulfate on the exosome surface captures fibronectin, and heparan sulfate on recipient cells acts as a receptor for fibronectin thereby docking the exosome to the cell.[102] This is consistent with the previously reported role of heparan sulfate acting as a receptor for exosome docking with recipient cells.[103] Moreover, when added to myeloma cells, the exosomes secreted by cells expressing elevated heparanase enhanced cell spreading on fibronectin-coated surfaces and increased the capacity of endothelial cells to invade through Matrigel.[98]

Taken together, these findings established that syndecans, their heparan sulfate chains, and heparanase all play important roles in regulating exosome biogenesis, exosome protein composition, exosome function, and exosome docking to recipient cells. Moreover, heparanase localizes to the surface of exosomes where it becomes activated and degrades heparan sulfate within the ECM.[101] Given the importance of exosomes in intercellular communication in disease states such as cancer and inflammation, syndecans and heparanase represent viable targets for blocking exosome biogenesis and function. Roneparstat, a heparanase inhibitor composed of modified heparin,[104] can block exosome docking with recipient cells and antiheparanase monoclonal antibody can inhibit exosome-mediated migration of macrophages.[101,102] Future studies on the role of syndecans and heparanase in the regulation of exosome biogenesis, composition, and function will likely uncover additional ways in which to target exosomes therapeutically.

Syndecan-1 Generates Chemokine Gradients in Inflammatory Diseases

The interaction of chemokines with heparan sulfate chains of syndecan-1 gathers chemokines to endothelial cell surfaces at sites of inflammation and creates chemotactic gradients that lead the directional migration of leukocytes.[105,106] Similarly, shed syndecan-1 (Sdc1) from the surfaces of epithelial cells directs the transepithelial migration of leukocytes.[107] Shed syndecan-1 is responsible for resolving chemokine gradients by removing sequestered chemokines from inflammatory sites, thus preventing prolonged leukocyte inflammation and tissue damage.[108] The vascular endothelium serves as the interface between the immune system and end organs to modulate tissue perfusion and function. However, during overwhelming states of infection or injury, the unregulated activity of local and circulating enzymes causes damage to the endothelial glycocalyx which compromises vascular function and propagates multiorgan failure.[47,109,110] In the process of glycocalyx degradation, unexposed cell adhesion molecules and cytokine receptors become available for binding and activation, thus propagating inflammation.[47,111–113] Moreover, shed heparan sulfate fragments activate toll-like receptor (TLR)-4 signaling[114] and accompany growth factors and cytokines to facilitate ligand recognition by cognate cell surface receptors.[115] Several clinical evaluations of circulating GAG signatures demonstrate increased levels of plasma HS early in sepsis[116–118] that correlate with degree of illness.[116,119] These findings are corroborated in a study of human and murine sepsis describing increased heparanase activity in plasma from adults with sepsis and increased heparanase expression in tissues harvested from septic mice.[120] Taken together, these and other findings suggest that heparanase is active at the endothelial surface during sepsis and is clinically important. Indeed, when inhibited in preclinical models of sepsis, lung injury and renal dysfunction are attenuated.[47,74,121]

Heparanase and Syndecan in Acute and Chronic Inflammation

HSPGs have multiple functions in inflammation, including (1) building morphogen, growth factor, chemokine, and cytokine gradients; (2) sequestering and protecting chemokines and cytokines in the extracellular space; (3) acting as coreceptors to stabilize receptor–ligand complexes; (4) initiation of innate

immune responses and signal transduction independently or by engaging inflammatory receptors such as TLRs; (5) regulating immune cell adhesion, migration, and activation; and (6) modulation of leukocyte interactions with endothelium and ECM.[14,122–129] Thus, heparan sulfate remodeling by heparanase may affect several aspects of inflammatory reactions, such as leukocyte recruitment, extravasation, and migration toward inflammation sites; release of cytokines and chemokines anchored within the ECM or cell surfaces; as well as activation of innate immune cells.[130–132] Via degradation of heparan sulfate in the ECM, heparanase activity facilitates the recruitment of immune cells.[122,133–135] It also leads to upregulation of cytokine expression in macrophages[114,136,137] and its expression, in turn, is induced by a variety of inflammatory cytokines, fatty acids, and high glucose.[138–140] Soluble heparan sulfate fragments generated by heparanase have been shown to promote TLR-4 signaling in dendritic cells[141] and human peripheral blood monocytes.[114,122] Several in vitro and in vivo studies have highlighted the diverse roles of syndecans in inflammation.[142,143] Syndecans bind and retain multiple HS-binding proteins resulting in attenuation or propagation of their functions, including properties of chemokines/cytokines and their interactions with leukocytes and endothelial cells. For example, insulin promotes shedding of syndecan-1 ectodomain[22] and increased inflammatory mediators, and proinflammatory monocytes in patients with type-1 diabetes and nephropathy have been correlated with increased plasma syndecan-1 levels.[139]

Reduced expression of Sdc1 has been observed in patients with ulcerative colitis, attributed to disrupt healing of colonic ulcers. Likewise, Sdc1 KO mice revealed a substantial increase in mortality versus wild-type (WT) mice subjected to the dextran sodium sulfate (DSS) model of inflammatory bowel disease.[144] Sdc1 KO mice developed more severe inflammation during chronic DSS colitis, associated with increased recruitment of inflammatory cells, elevated IL-6 expression and activation of STAT3, increased crypt damage, and increased weight loss compared with WT mice. Notably, colitis-associated cancer can be experimentally modeled in mice by application of the carcinogen azoxymethane and subsequent induction of chronic colitis with DSS. In this model, Sdc1 KO mice formed larger tumors than their WT controls.[144] It appears that the increased inflammation and tissue damage in the absence of Sdc1 drive colon cancer progression via enhanced signaling through the IL-6/STAT pathway. When transgenic mice that overexpress heparanase were subjected to DSS colitis, high heparanase expression preserved the inflammatory conditions, along with increased expression of tumor necrosis factor-α (TNFα) and cyclin D1, and a substantially increased recruitment of TNFα-expressing macrophages. Overall, these and other data indicate that heparanase drives a vicious cycle that promotes colitis and chronic inflammation-associated tumorigenesis.[144,145]

Heparanase Contributes to the Pathogenesis of Kidney Disease

Heparan sulfate is one of the main polysaccharides in the glomerular endothelial glycocalyx and contributes to the glomerular filtration barrier. Heparanase-mediated degradation and subsequent remodeling of heparan sulfate in the ECM of the glomerulus is a key mechanism in the development of glomerular disease, as exemplified by the resistance of heparanase-deficient animals to diabetes and immune-mediated kidney disease.[139,146] Albuminuria, the hallmark of glomerular diseases, occurs through a multistep process in which heparanase, immune cells, and dysregulated signaling between podocytes and endothelial cells lead to loss of endothelial glycocalyx barrier function. Increased glomerular heparanase activity associated with renal loss of heparan sulfate has been demonstrated in a wide variety of human proteinuric glomerular diseases, including diabetic nephropathy, the major life-threatening complication of diabetes.[147] Patients with these diseases have increased urinary excretion of heparanase[148] and heparan sulfate.[149] Given that the diabetic milieu is one of the strongest inducers of heparanase release, blocking heparanase activity (i.e., Roneparstat, Sulodexide) is a potential approach to the treatment of kidney diseases. One mechanism by which heparanase might contribute to the development of diabetic nephropathy is through its effects on inflammation. Increased levels of monocyte chemoattractant protein-1 (MCP-1) in renal tissue and urine in patients with diabetic nephropathy suggests that macrophages have a pathogenic role in the progression of renal diseases in humans.[150] Indeed, association between renal macrophages and pathological lesions has been demonstrated in human diabetic nephropathy.[139] Macrophages can secrete cathepsin L which facilitates the processing and activation of proheparanase in the extracellular environment. Active heparanase itself can further activate macrophages by generating heparan sulfate fragments that can function as ligands for TLR and/or receptor for advanced glycation end products (RAGE) signaling, thereby contributing to a vicious feedback loop.[151] Inhibition of glomerular cathepsin L and heparanase may, therefore, lead to restoration of the glomerular glycocalyx and barrier function.[139]

Given that heparanase facilitates syndecan shedding, its involvement in acute kidney injury (AKI) is not surprising. The enzyme is induced and activated in AKI regardless of its causes, whether ischemic, nephrotoxic, septic, or transplantation related. This event unleashes a host of sequelae characteristic of the pathogenesis of AKI, such as induction and reinforcement of innate immune responses, predisposition to thrombosis, activation of monocytes/macrophages, triggering epithelial–mesenchymal transition, and remodeling of the ECM, thus setting up the stage for future fibrotic complications and development of chronic kidney disease.[152] Experimental sepsis is associated with upregulation of glomerular heparanase and loss of glomerular heparan sulfate. Shed syndecan-1, a marker of glycocalyx damage, can thus be utilized as a potential biomarker to predict impending renal failure. In mice, administration of heparanase inhibitors attenuated the sepsis-induced reduction in glomerular filtration rate, whereas the systemic inflammatory response was unaltered, demonstrating that heparanase activation contributes specifically to early septic renal glomerular dysfunction.[121] Moreover, inhibition of heparanase (Roneparstat, PG545, sulodexide) was found to minimize AKI

and protect against chronic kidney dysfunction, inflammation, and profibrotic damages induced by ischemia/reperfusion injury.[153–155] Together, these findings indicate that upregulation of extracellular heparanase contributes to the inflammatory response of AKI and its long-term outcomes, such as fibrosis and the eventual development of chronic kidney disease. These and other studies underscore the emerging therapeutic strategies of inhibiting heparanase, as well as the diagnostic value of detecting products of heparanase activity for prognostication and treatment.

Atherosclerosis

Heparin and HSPG are important regulators of cellular and molecular processes in atherogenesis. They mediate lipoprotein clearance, recruitment of inflammatory cells, and smooth muscle cell function.[156,157] Also, HSPGs bind LPL to the endothelial cell surface where it promotes cellular uptake of chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids.[156] While the vast majority of atherosclerotic lesions remain stable, some undergo alterations that make them vulnerable to rupture. Briefly, the inflammatory process creates a thin cap of fibrous tissue over a lipid-rich and metabolically active core which is the hallmark of vulnerable, high-risk plaques, associated with acute coronary syndrome and sudden cardiac death.[158] Activated immune cells are abundant at sites of rupture, producing inflammatory molecules and proteolytic enzymes that transform the stable plaque into vulnerable, unstable structures.[159] Overexpression or exogenous addition of heparanase activates macrophages likely involving TLR-2, TLR-4, and NFκB.[137] A growing body of evidence indicates that TLR signaling can be elicited in the absence of infection through endogenous ligands generated at the sites of tissue remodeling and inflammation. Of note, ECM components and their degradation products generated during tissue injury or remodeling have been found to function as TLR ligands.[126] Examples are hyaluronic acid, decorin, and soluble biglycan recognized as ligands for TLR-2 and TLR-4,[160] and versican which activates tumor-infiltrating myeloid cells through TLR-2 and its coreceptors TLR-6 and CD14. Thus, while activation of TLRs does not require binding or cleavage of HSPG, heparanase may activate TLRs by introducing conformational changes in cell membrane HS proteoglycans (i.e., syndecans, glypicans) following their clustering and activation, or by heparan sulfate cleavage products.[126] Our results indicated that in both colitis and atherosclerosis models, heparanase not only functions to recruit but also activates macrophages, reprogramming their response from resolution of inflammation to unresolved chronic inflammation. Moreover, activated macrophages are capable of inducing heparanase expression in cells, most likely through TNFα-mediated stimulation of the Egr1 transcription factor, a powerful inducer of heparanase transcription.[145] It appears that macrophages not only represent a cellular target for heparanase action (i.e., macrophage activation) but can also upregulate heparanase, both at the transcriptional and posttranslational levels.[145] Taken together, our results emphasize the clinical relevance of heparanase in plaque progression and rupture, and identify TLR family members as mediators of this function.[137,158] Signals initiated by heparanase are transmitted to the cell nucleus, actively inducing the transcription of proinflammatory genes that further fuels the inflammatory reaction.[37,46]

Role of the Heparanase–Syndecan-1 Axis in Viral Pathogenesis

Heparan sulfate serves as the first point of contact between target cells and a large number of human viruses (i.e., dengue, hepatitis C, HIV, papilloma, herpes)[45,161] including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[162] Focusing on herpes simplex virus (HSV), Agelidis and Shukla expanded the established role of heparan sulfate from a viral attachment molecule to an essential receptor required for entry.[161] Heparin/heparan sulfate mimicking compounds compete with heparan sulfate and thereby inhibit viral infection. This is relevant to coronavirus disease (COVID)-19 pandemic as SARS-CoV-2 binds to heparin[163] and HS has been found to function as adhesion molecule that increases the virus density on the cell surface, thereby facilitating the interaction between the virus and its receptor.[164]

HSV-1 and HSV-2 are a family of enveloped, double-stranded DNA viruses that infect the large majority of humans worldwide. Syndecan-1 and syndecan-2 heparan sulfate chains are critical for initial binding of HSV-1 to target cells.[165] These proteoglycans are upregulated upon viral infection and reducing their presence diminishes HSV-1 binding to cells and infection. Interestingly, heparanase is also upregulated in cells following their infection with HSV-1 where it facilitates viral egress by degrading heparan sulfate chains leading to release of newly made virus attached to the cell surface.[161,164] This dynamic relationship between viral attachment, viral egress, and regulated expression of syndecan-1 and heparanase provides new therapeutic targets that could be attacked to regulate viral infection that may also be relevant to the current COVID-19 pandemic. Heparin and heparan sulfate are composed of the same saccharide building blocks. However, while heparin is composed of extended, heavily sulfated saccharide sequences, heparan sulfate displays distinct domains, the sulfation patterns of which are regulated in cell-autonomous fashion.[166] Low-molecular-weight heparin (LMWH) and heparin-like compounds have been shown to efficiently compete with heparan sulfate and thereby attenuate viral attachment and infection,[167] providing a straightforward explanation for the antiviral effect of LMWH in clinical settings. Indeed, a recent article showed that the SARS-CoV-2 Spike S1 receptor-binding domain interacts with heparin.[163] Heparin, LMWH, and non-anticoagulant species of heparin are also known to inhibit the enzymatic activity of heparanase[168] shown recently to promote viral infection and spread.[45,161,169] Chemically modified non-anticoagulant species of heparin and related compounds are therefore regarded as promising candidates given their dual capacity to (1) compete with heparan sulfate and thereby inhibit virus-cell adhesion and entry and (2) inhibit heparanase enzymatic activity and thereby attenuate virus detachment/release and spread.[161] In support of these observations are recent clinical studies

revealing favorable effects in COVID-19 patients who were treated with LMWH to cope with their hypercoagulable condition.[170] Given that one of the most important mechanisms underlying the deterioration of COVID-19 disease is cytokine storm,[171] it is appealing to note that many of the LMWH-treated patients had lower levels of proinflammatory cytokines (i.e., IL-6) resulting in less aggressive disease.[172] We attribute this result to inhibition of heparanase rather than to the anticoagulant effect. Thus, besides its anticoagulant activity, there are other routes to explain a favorable effect of LMWH on COVID-19 patients, including prevention of viral adhesion and also anti-inflammatory activity based on inhibition of neutrophil chemotaxis and leukocyte migration.

The story of heparanase in viral infection has roots in the longstanding connection between heparan sulfate and a large number of viruses, including the SARS-CoV-2. Direct regulation of heparan sulfate levels on the cell surface by heparanase enzymatic activity dictates the extent of virus release after replication has occurred. Additionally, virus-induced heparanase activation and nuclear translocation result in higher expression of proinflammatory factors leading to worsened disease (i.e., cytokine storm),[171] together indicating that active heparanase drives hallmark features of viral pathogenesis.[45] Heparan sulfate levels at the cell surface dramatically decrease with progression of infection due to concomitant NFκB-mediated upregulation of host *HPSE* gene expression.[164] Active heparanase is also an important immunomodulatory factor that is harnessed by the virus to enhance pathogenesis in a tissue environment. In fact, heparanase inhibitor is effective in decreasing viral spread and proinflammatory factor production in response to viral infection.[169] It appears that heparanase behaves as a molecular switch in viral infection, which transforms the cell from a virus-permissive mode, in which viral attachment and entry are favored, to a virus-deterring mode which allows for viral detachment and egress from cells.[164] Notably, upregulation and activation of heparanase is a strategy common to a broad range of viral species to increase egress, spread, and transmission.[169] Interestingly, it appears that heparanase plays a role also in driving the undesirable cytokine storm discussed earlier. In individuals with SARS-CoV-2 infection, the level of inflammatory cytokines is markedly higher than normal, mitigated by LMWH and held responsible for the severity of the diseases.[172] Agelidis et al documented that upon HSV-1 infection, heparanase translocates to the nucleus of the infected cells and promotes inflammatory signaling, mediated primarily via NFκB.[169] A recent study reported an increase of heparanase activity and heparan sulfate levels in the plasma of patients with COVID-19, which was also associated with the severity of the disease, suggesting potential treatment utility of LMWHs.[173] It was further found that pixatimod (PG545), heparanase-inhibiting HS mimetic, is a potent inhibitor of SARS-CoV-2 infectivity.[174] Collectively, heparanase emerges as a master host-encoded virulence factor that once activated enhances viral spread and triggers downstream inflammatory cascades.[175]

Perspectives and Conclusion

The take-home messages from this review are summarized in ►**Table 1**. An intact endothelial barrier is pivotal for the regulation of fluid and protein extravasation, particularly in the lungs and kidneys. Several research studies propose a paramount role for endothelial cell dysfunction in the pathogenesis of inflammatory disease conditions (e.g., sepsis, acute respiratory distress syndrome, and proteinuric kidney disease) as well as in COVID-19 complications. These are attributed, among other aspects, to increased activity of the endothelial glycocalyx-degrading heparanase enzyme that can compromise the function of the endothelial barrier. Heparin, which is a prototypical anticoagulant, exerts significant anti-inflammatory and antiviral effects in animal models and clinical conditions, primarily through interference with heparan sulfate and probably due to its inhibition of heparanase enzymatic activity. Intact endothelial barrier function is pivotal for the regulation of fluid and protein extravasation, particularly in the lungs and kidneys. Notably, the loss of endothelial barrier function due to heparanase activity has been established for inflammatory disease conditions, including sepsis, acute respiratory distress syndrome, and proteinuric kidney disease. Likewise, heparanase and endothelial cell dysfunction appear to

Table 1 Take-home messages from this review

• Heparanase acts as an "activator" of heparan sulfate proteoglycans, together regulating the bioavailability of heparan sulfate-bound growth factors, cytokines, and chemokines
• Heparanase utilizes a raft-independent route for internalization by syndecan-1 that leads to its processing and activation in lysosomes
• Heparanase enhances syndecan-1 shedding from the surface of cancer cells, thereby promoting tumor cell metastasis and angiogenesis. The heparanase–shed syndecan-1 axis also regulates growth factor trafficking and activity
• Cell surface proteoglycans are important constituents of the glycocalyx, participating, among other effects, in pleiotropic signaling routes and hence regulating cell adhesion, migration, proliferation, and differentiation
• Syndecans and heparanase play important roles in regulating exosome biogenesis, protein composition, function, and docking to recipient cells
• The heparanase–syndecan axis drives a vicious cycle that induces inflammatory responses, promoting, among other effects, tissue fibrosis, chronic kidney disease atherosclerosis, and inflammation-associated tumorigenesis
• Heparanase emerges as a major host-encoded virulence factor that once activated enhances viral spread and triggers downstream inflammatory cascades

play an important role in the pathogenesis of COVID-19 complications. Given the protective anticoagulant, anti-inflammatory, and antiheparanase activity of heparin and LMWH, prospective studies that evaluate the clinical outcomes of COVID-19 patients treated with LMWH are needed. Similarly, the beneficial effect of heparanase-inhibiting heparin/HS mimicking compounds should be examined in COVID-19 patients. An important feature is the ability of heparanase to promote syndecan-1 shedding, mediated in part by regulating the expression of genes that drive MMP production. Shed syndecan impacts cell behavior both locally and distally from its cell of origin, contributing to disease pathogenesis in cancer, inflammation, and viral infection. In this regard, the function of nuclear heparanase remains an area ripe for investigation and may provide further clues as to how the heparanase–HS axis affects gene transcription and helps drive disease pathogenesis.

Authors' Contributions

I.V. and N.I. designed and wrote the review. U.B., H.M.N., and S-M.Y. contributed some sections and revised the review.

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Conflict of Interest

None declared.

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