More than a biomarker: the systemic consequences of heparan sulfate fragments released during endothelial surface layer degradation (2017 Grover Conference Series)

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Abstract

Advances in tissue fixation and imaging techniques have yielded increasing appreciation for the glycosaminoglycan-rich endothelial glycocalyx and its in vivo manifestation, the endothelial surface layer (ESL). Pathological loss of the ESL during critical illness promotes local endothelial dysfunction and, consequently, organ injury. Glycosaminoglycan fragments, such as heparan sulfate, are released into the plasma of animals and humans after ESL degradation and have thus served as a biomarker of endothelial injury. The development of state-of-the-art glycomic techniques, however, has revealed that these circulating heparan sulfate fragments are capable of influencing growth factor and other signaling pathways distant to the site of ESL injury. This review summarizes the current state of knowledge concerning the local (i.e. endothelial injury) and systemic (i.e. para- or endocrine) consequences of ESL degradation and identifies opportunities for future, novel investigations.

In 1966, John Luft used electron microscopy to observe a 20-nm-thick "endocapillary layer" projecting from the apical surface of mouse diaphragmatic capillary endothelial cells into the vascular lumen.[1] This layer was believed to be biologically insignificant, potentially representing a vestigial remnant of the basolateral membrane reflecting onto the apical endothelial surface. In the decades since this observation, however, major advances were made in not only electron microscopy approaches, but also in endothelial-protective tissue fixation techniques.[2,3] These advances allowed for the realization that this "insignificant" endocapillary layer was in fact a substantial endothelial glycocalyx (Fig. 1a) that can dwarf the size of the endothelial cell itself.[4,5] Furthermore, the emerging use of intravital microscopy revealed that in vivo, the endothelial glycocalyx forms a massive endothelial surface layer (ESL), reaching thicknesses >1 μm and occupying a substantial proportion of the cross-sectional area of the vessel.[6]

This increasing appreciation of ESL size, coupled with the development of novel techniques capable of interrogating ESL function, has led to an explosion of interest in the impact of ESL integrity on both vascular homeostasis and disease. This review seeks to summarize the current state of knowledge (and identify opportunities for further investigation) concerning ESL structure during health, the impact of

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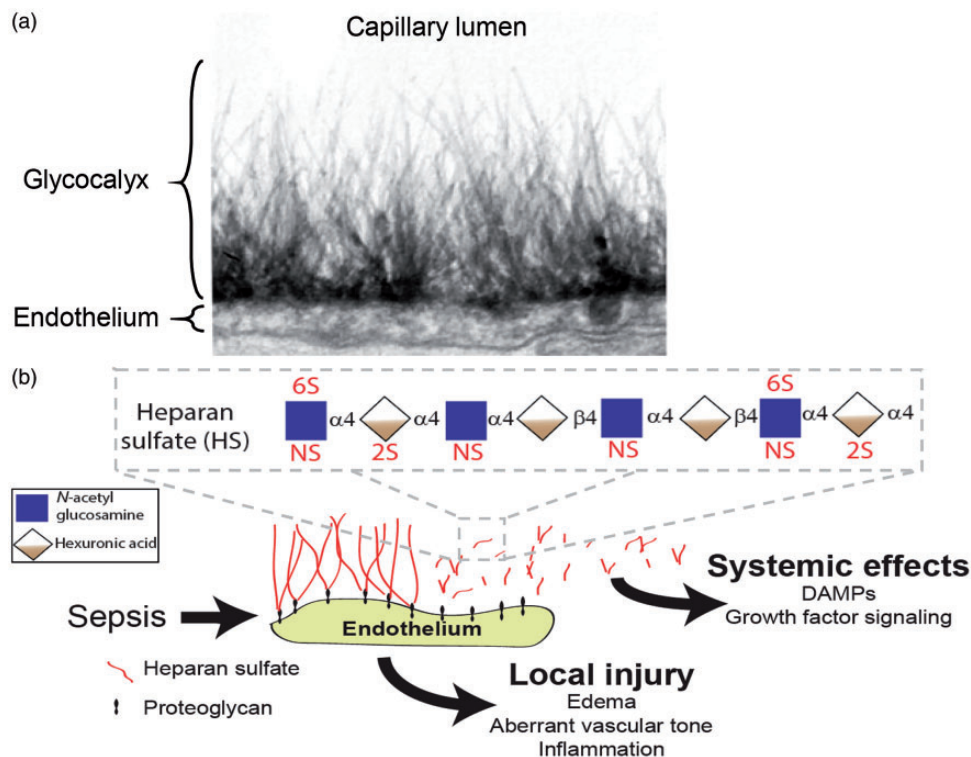Email: eric.schmidt@ucdenver.edu

Fig. 1. Structure of the endothelial glycocalyx/endothelial surface layer. (a) Endothelial glycocalyx thickness is larger than the endothelial cell itself, as demonstrated by electron microscopy of ruthenium-red labeled rat myocardial capillaries. Figure used with permission from van den Berg et al. *Circ Res*.[5] In vivo, the glycocalyx forms an even more substantial ESL, with thickness > 1 µm. (b) Pathological degradation of the glycocalyx/ESL during critical illnesses (such as sepsis) causes not only local endothelial injury, but also releases biologically active heparan sulfate fragments into the circulation that may influence signaling processes in an endocrine fashion. For simplicity, chondroitin sulfate and hyaluronic acid are not shown. α4 and β4 refer to glycosidic bonds connecting constituent saccharides. Inset: structure of a heparan sulfate octasaccharide fragment, demonstrating potential sites of sulfation within constituent disaccharide units.

ESL degradation on endothelial dysfunction during critical illness, and the emerging appreciation for the paracrine and endocrine signaling capabilities of ESL fragments released during vascular injury. Particular attention will be given to the critical roles played by the glycosaminoglycan heparan sulfate (HS) in the maintenance of ESL integrity and the local and systemic responses to endothelial injury.

The importance of the ESL in endothelial health and injury

As suggested by the name "glycocalyx" (a "sugar layer"), the ESL is enriched in glycosaminoglycans including HS, chondroitin sulfate (CS), and hyaluronic acid (HA).[2] HS and CS are anchored to the endothelial surface via covalently binding to cell surface proteins known as proteoglycans.[7] HS represents the most common ESL glycosaminoglycan, with HS proteoglycans (HSPGs) accounting for 50–90% of endothelial-associated proteoglycans[8] (Fig. 1b). Additionally, HA intercalates itself throughout the ESL via non-covalent interactions with cell surface proteins such as CD44.[2] These glycosaminoglycans have the ability to sequester water,[9] imparting substantial size and measurable rigidity to the ESL.[10] The lungs, known to have particularly

high concentrations of HS,[11] feature a uniquely thick ESL (1.6–1.7 µm) compared to other vascular beds (e.g. 0.6 µm in the cremaster or mesentery).[12,13]

This substantial size and gel-like consistency enables the ESL to serve several critical functions relevant to vascular homeostasis:[6]

(a) Barrier function. By virtue of its enrichment in highly sulfated glycosaminoglycans (such as HS and CS), the ESL forms a negatively-charged fiber "mesh" that overlies cell–cell junctions, limiting protein permeability and (in accordance with Starling forces) opposing fluid flux out of the vascular lumen.[14] Accordingly, enzymatic degradation of HS[15] and HS-associated proteoglycans[16] from isolated perfused vessels can lead to endothelial barrier dysfunction. Comparatively less is known about the role of ESL CS in transvascular fluid flux.

(b) Nitric oxide synthesis. The ESL projects into the vascular lumen and may deform in the presence of shear stresses that accompany increases in vascular flow. This stress is transduced into the endothelial cell, triggering induction of endothelial nitric oxide synthesis (eNOS).[17] The subsequent NO-mediated

vasorelaxation allows for accommodation of the increased vascular flow responsible for the inciting shear forces. Enzymatic degradation of HS and HA (but not CS) led to loss of flow-mediated dilatation in vivo.[18,19]

(c) Leukocyte-endothelial adhesion. An intact ESL projects into the vascular lumen beyond the span of cell-surface adhesion molecules.[2] Furthermore, its gel-like consistency may impart resistance to penetration by circulating leukocytes. Accordingly, the ESL is anti-adhesive, opposing leukocyte-endothelial adhesion in both systemic[20,21] and pulmonary[12] vascular beds. Paradoxically, the intact ESL may promote leukocyte rolling (an important step in adhesion in non-pulmonary vascular beds) by serving as a ligand for L-selectin as well as promoting chemokine availability.[22] It is unclear how these findings are reconciled with the pro-adhesive effects of enzymatic HS degradation observed in vivo.

Local endothelial impact of ESL degradation during critical illness

Given that an intact ESL contributes to the maintenance of endothelial barrier function, mechanotransduction of shear stress, and prevention of leukocyte-endothelial adhesion, pathological degradation of the ESL would be expected to induce tissue edema, microcirculatory tone dysfunction, and inflammation. These putative consequences of ESL degradation mirror the known pathophysiologic stigmata of several critical illnesses, including sepsis, the acute respiratory distress syndrome, trauma, and ischemia-reperfusion injury. Indeed, animal and human studies have demonstrated a pathogenic role of ESL degradation in the onset of vascular injury during these disease states. Sepsis-associated induction of heparanase triggers degradation of vascular HS, leading to the collapse of the pulmonary[12,23] and renal[24–26] ESL. The downstream consequences of this degradation appear to vary based upon the affected vascular bed. While pulmonary ESL loss contributes to lung injury via promotion of lung edema and neutrophil adhesion,[12,17,23] we observed that heparanase-mediated glomerular HS degradation induces an early loss of glomerular filtration in the absence of kidney edema or inflammation,[24] potentially indicating a role for ESL integrity in the control of afferent and efferent glomerular tone (and, consequently, glomerular filtration pressure). These and other local consequences of ESL degradation (as well as the potential roles played by other "sheddases") have been extensively reviewed elsewhere.[13,27,28]

Importance of HS structure in cell signaling

While significant effort has been dedicated to understanding the local effects of ESL loss as a mediator of injury to the underlying endothelium, little is known about the distant, systemic consequences of this degradation. ESL loss has been demonstrated to release glycosaminoglycan fragments into the circulation.[29–31] While these circulating fragments have been largely regarded as either simple biomarkers of endothelial injury[27] or potentially damage-associated molecular patterns,[32] emerging data indicate that circulating HS (enriched in highly sulfated hexa- to octasaccharides[29]) may have a significant impact on both local and systemic signaling pathways.[33] These effects may be long-lasting, given that circulating HS fragments may persist for >5 days in patients with respiratory failure.[29] Understanding paracrine/endocrine-like effects of circulating HS fragments requires an understanding of HS structure, with particular attention to the localization of sulfation within the disaccharide units that comprise a HS chain.

Heparan sulfate structure

HS is a linear polysaccharide, composed of repeating disaccharide units of N-glucosamine and a hexuronic acid (either glucuronic acid or iduronic acid). This structure of HS is ubiquitous and is conserved across both invertebrates and vertebrates, suggesting evolutionarily important biological functions.[34] Although the precise size of HS in vivo is uncertain, chain length is estimated to be in the range of 50–200 saccharides.[35,36] As detailed later in this review, constituent disaccharides may be sulfated at distinct sites, enabling a substantial variety of possible HS structures, as determined by variables such as chain length and disaccharide sulfation pattern.[37] Indeed, this potential structural heterogeneity is so great that it is feasible that no two glycosaminoglycans in the body are identical.[38] Despite this variability, there are organ-specific trends in HS lengths and compositions.[36] Despite these organ-specific similarities in HS, there still remains heterogeneity of HS across individual organ substructures, such as the lung airways and alveoli.[39] Adding to this complexity, there may be temporal shifts in HS structure, as HS length and sulfation may be dynamically modified in response to cellular and environmental cues.[7] The development of new analytical techniques has allowed for an increasing appreciation of the staggering complexity of HS structure and its putative biological relevance.

HS synthesis largely occurs within the Golgi apparatus.[40] HS biosynthesis begins with the xylosyltransferase-mediated addition of an anchoring xylose (from a UDP-xylose donor) onto a serine residue of a putative proteoglycan. The efficiency of xylosylation is controlled by the availability of UDP-xylose, xylosyltransferase activity, and other competing reactions, yielding variable numbers of glycosaminoglycan chains attached to a proteoglycan backbone.[41] After xylosylation, a core tetrasaccharide is formed by the sequential addition of two galactose residues (by β1-4 galactosyl and β1-3 galactosyltransferases) followed by a glucuronic acid (by β1-3 glucuronosyltransferase).[41] This tetrasaccharide then undergoes modifications such as phosphorylation and sulfation by an undiscovered process. At this point,
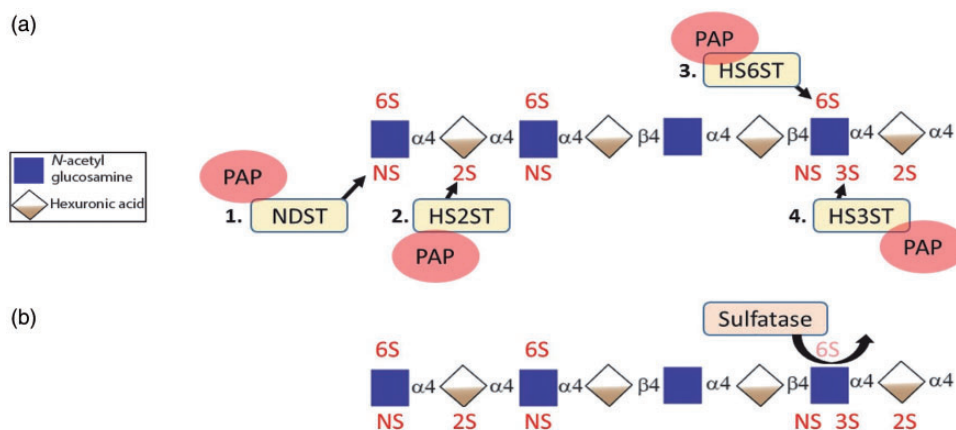
Fig. 2. HS sulfation patterns and catalyzing enzymes. (a) Sulfate groups from the high energy donor, 3'-phosphoadenyl-5'-phosphosulfate (PAP) are transferred to specific positions of HS. Sulfation generally progresses in order as depicted. (b) HS is further modified post-synthetically at cell membrane and extracellular space by sulfatase which specifically cleaves 6-O sulfates from N-glucosamine residues. NDST, N-deacetylase/N-sulfotransferase; HS2ST, heparan sulfate-2-sulfotransferase; HS6ST, heparan sulfate-6-sulfotransferase; HS3ST, heparan sulfate-3-sulfotransferase; α4 and β4: glycosidic bonds.

biosynthetic processes diverge, allowing for the development of either CS or HS proteoglycans. The addition of α1-4 N-acetyl-glucosamine by exostosin-like glycosyltransferase 3 (EXTL3) commits the nascent glycosaminoglycan strand to becoming a HS proteoglycan.[41] Subsequent polymerization of HS with alternating N-glucosamine and hexuronic acid are catalyzed by a complex of exostosin 1 (EXT1) and 2 (EXT2).[42]

As HS polymerization progresses, there is immediate (and coordinated) modification of constituent disaccharides including sulfation and epimerization (Fig. 2). N-glucosamine may be sulfated at the N-position by N-deacetylase/N-sulfotransferases (NDSTs).[42] These sites of N-sulfation are most likely to become highly sulfated regions of the mature HS polysaccharide. N-sulfation may be followed by epimerization of glucuronic acid to iduronic acid by C5-epimerase, at sites typically adjacent to N-sulfated glucosamines.[42] Hexuronic acids (iduronic acid and, rarely, glucuronic acid) are then sulfated at 2-O position by 2-O sulfotransferases (HS2ST), which stops further epimerization of glucuronic acid.[41] Sulfation of N-glucosamine residues at the 6-O position follows, catalyzed by heparan sulfate-6-sulfotransferases (HS6ST). The final step of HS modification is the rare (but important) 3-O sulfation of glucosamine residues by 3-O sulfotransferases (HS3ST). Despite the rarity of 3-O sulfation, HS3STs represent the largest family of sulfotransferase enzymes.[43] These modifications of HS occur in clusters along the chain, separated by regions devoid of sulfation.[41] Accordingly, HS chains may be geographically divided into NS domains (N-sulfated), NA domains (N-acetylated), and NS/NA domains (mixed).[41]

After completion of polymerization, sulfation, and epimerization, HS proteoglycans are transported from the Golgi to the cell membrane, where they contribute to the endothelial glycocalyx or other extracellular matrix structures. At this point, HS can be further modified by either heparanase-mediated cleavage or selective removal of 6-O sulfates by sulfatase-1 and -2.[44,45] Notably, other sites of sulfation (N-, 3-O, 2-O) do not have known extracellular sulfatases, suggesting an evolutionarily conserved function of dynamic regulation of HS 6-O sulfation.

Functional significance of HS sulfation

The importance of HS synthesis and modification is demonstrated not only by the redundancy of enzymes in the aforementioned biosynthetic processes, but also by the fact that mutations that interfere with these processes have dramatic phenotypes. Indeed, Ext1 null mice (which lack the ability to polymerize HS) are embryonically lethal,[46] and human mutations in EXT1 and 2 cause hereditary multiple exostoses, a disorder characterized by the formation of multiple cartilaginous benign tumors in bones.[47]

Similarly, the importance of HS sulfation is demonstrated by the critical roles played by sulfotransferases in development. While *D. melanogaster* and *C. elegans* only have one ortholog of NDST, vertebrates have four isoforms of NDST.[48] Vertebrate isoforms of NDSTs are differentially expressed in a tissue-specific and developmental stage-specific manner, and most cells express two isoforms.[49] NDST-1 is widely expressed and its deficiency in mice is lethal.[50] Most NDST-1 deficient mice survive to birth, but become cyanotic and die within 10 h from neonatal respiratory distress.[50,51] Other NDST-1 knockout mice die prenatally with various severe defects in skull and eye development.[48,52] In NDST-1 deficient mouse embryos, abnormal vascular development is evident. This results from the inability of PDGF-BB to bind to HS, which causes impaired pericyte recruitment.[53] Deletion of NDST2 in mice yields an abnormal phenotype in mast cells, which is the site of heparin production.[48,54,55] Double knockout of NDST-1 and 2 is

embryonically lethal.[48,56] However, mutation of NDST3 or NDST4 does not appear to affect development, yielding nearly normal and fertile mice, suggesting that loss of these isoforms is well-compensated by other isoforms.[57,58]

Other sites of sulfation similarly have importance in development. Germline deletion of HS2ST (responsible for 2-O sulfation) in mice is also lethal.[59] These mice survive until birth, but die perinatally due to kidney agenesis.[59,60] Deletion of HS2ST is also shown to affect FGF2 signaling in brain development.[61] There are three isoforms of HS6STs, which are responsible for 6-O sulfation.[62,63] Most HS6ST-1 deficient mice die during embryonic and perinatal stages, and the mice that survive show developmental abnormalities such as reduction of vasculature in the placenta, mediated by reduction of Wnt signaling, and enlarged alveoli in lungs.[64] Vascular branching pattern defects have been reported in HS6ST-2 knockout zebrafish.[65]

The importance of 3-O sulfation is reflected by the presence of seven (seemingly redundant) HS3ST isoforms in vertebrates. HS3ST-1 and HS3ST-5 are necessary to create the pentasaccharide sequence essential for antithrombin binding, as described later in this review.[66,67] Knocking out HS3ST-1 causes postnatal lethality that is genetic-background-dependent.[68] No obvious phenotype was reported in HS3ST-2 knock out mice.[69]

Finally, knocking out sulfatase-1 and 2 (that selectively remove 6-O sulfation from mature HS proteoglycans post-synthetically) results in viable mice, but they show postnatal growth defects and an enlarged, dysfunctional esophagus, and double knockout results in reduced fertility.[70]

Impact of HS sulfation on cellular signaling

The importance of HS sulfation to development has been attributed to the influence of HS on growth factor signaling. One of the well-described pathways that illustrates the importance of HS sulfation on cellular signaling is the fibroblast growth factor (FGF) pathway.[42] HS facilitates FGF signaling (thereby promoting cell migration, proliferation, and differentiation) by binding to FGFs and their cognate FGF receptors (FGFRs), bringing these ligands and receptors into close proximity.[71] Alternatively, HS binding may sequester FGFs away from their receptors, creating a repository that can be released by HS degradation during physiological or pathological stimuli.[71] The interaction between HS and FGF2 required HS sequences of five to six saccharides enriched in N-sulfation of glucosamine and 2-O sulfation of iduronic acid.[72,73] In fibroblasts with impaired heparan sulfate synthesis, Guimond et al. reported that FGF-induced mitogenic activity required HS > 10 saccharides in length with 2-O and 6-O sulfation.[74] Heparin (a highly sulfated variant of HS) depleted of 6-O sulfation competitively inhibited FGF-2 mediated mitogenic activity. Furthermore, FGF-1 or FGF-4 activity was sensitive to saccharides of various sulfation patterns.[74,75] In vivo, FGF2-mediated angiogenesis within chick embryos was inhibited

by excess 6-O desulfated heparin, attributed to binding and sequestering of FGF2.[76] Binding of FGFR to HS is also required for effective signaling and is dependent upon 6-O sulfation of glucosamine.[77]

Antithrombin is another well-described example of a protein whose function is shaped by electrostatic interactions with HS. Antithrombin inhibits thrombin, Factor Xa, and other procoagulant factors in a slow, progressive manner.[78] Antithrombin activity is accentuated when bound to specific forms of HS (such as heparin), but a specific pentasaccharide sequence with 3-O sulfation of glucosamine residues is a requisite for this interaction to occur. This sequence variant is observed in only 1–10% of glycosaminoglycans.[42,78–80] The inhibitory effect of antithrombin on Factor Xa only requires interaction with this pentasaccharide HS sequence,[81] whereas an additional, separate hexasaccharide sequence is required for accelerating inactivation of thrombin.[42]

Systemic consequences of circulating HS fragments released during ESL degradation

Endothelial glycocalyx degradation, as happens during sepsis, ischemia-reperfusion injury, and other hyper-inflammatory states, induces not only local endothelial dysfunction (as described previously) but also releases fragments of HS and HSPG extracellular domains (ectodomains) into the circulation.[29,31,82] These now-soluble factors are thought to be biologically active, having the ability to affect endothelial signaling processes in an autocrine, paracrine, and/or endocrine manner.[83] As described above, HS (both soluble and cell-surface bound) is able to bind to over 100 different signaling mediators in a oligosaccharide length and sulfation-dependent manner, in part through electrostatic interactions between negatively charged sulfate groups on HS and positively charged amino acids within signaling mediators.[84] These interactions between HS and proteins (i.e. signaling ligands and receptors) allow soluble HS to both positively and negatively regulate various endothelial signaling pathways. While there are few studies that describe the effect of circulating HS and HSPGs on endothelial signaling in vivo, there are several in vitro studies that demonstrate the ability of soluble HS and HSPGs to affect endothelial cell signaling.

As previously noted, endothelial growth factor signaling is heavily influenced by the presence or absence of HS. The effect of HS on endothelial FGF and vascular endothelial growth factor (VEGF) signaling have been the most extensively investigated. Given sufficient sulfation and chain length, HS binds both growth factor ligands and receptors and acts as a scaffolding molecule to facilitate growth factor ligand-receptor binding.[84] Indeed, the formation of a HS-growth factor ligand-growth factor receptor ternary complex is required for receptor activation and downstream signaling of several pathways including FGF2-FGFR1 signaling.[85] However, despite HS being a requirement for several signaling pathways, soluble HS and HSPGs can both

activate and inhibit FGF and VEGF signaling under different circumstances.

At low concentrations, soluble HS is thought to activate FGF2 and VEGF signaling by facilitating ligand-receptor binding as previously described.[86–88] However, at high concentrations, soluble HS compete to bind both growth factor ligand and receptor, reducing the formation of ternary HS-growth factor ligand-growth factor receptor complexes and inhibiting downstream signaling.[89] Furthermore, if HS is present on the endothelial cell surface, as occurs in healthy endothelial cells and endothelial cells with retained and recovered glycocalyces, soluble HS can also compete with endothelial cell-surface HS for growth factor ligand, sequestering the ligand away from its cognate receptor and inhibiting signaling.[90]

As such, the effect of circulating HS may not only depend on the presence of biologically active fragments (with activity determined by specific sulfation pattern and length), but also the absence of competing cell-surface HS (Fig. 3). We recently reported that circulating HS has the ability to activate endothelial FGF2 signaling in mice after septic endothelial glycocalyx degradation, facilitating endothelial glycocalyx recovery.[83] The effect of circulating HS on FGF2 and VEGF signaling on endothelial cells with a replete ESL is uncertain. Directly testing this hypothesis is limited by the absence of an intact ESL in cultured cells;[91,92] thus, most in vitro models would be expected to approximate an injured, ESL-degraded endothelial surface as opposed to a healthy endothelium.

While there are many studies that highlight the varied effects of HS on endothelial growth factor signaling, there are far fewer publications studying the effect of soluble shed HSPG ectodomains on endothelial FGF and VEGF signaling and angiogenesis. However, from these limited studies it appears that soluble HSPGs also have the ability to both activate and inhibit endothelial growth factor signaling. In a study by Kato et al., the soluble extracellular domain of syndecan-1 (a HSPG) was shown to inhibit FGF2 signaling and cell proliferation; however, heparitinase treatment of the ectodomain transformed syndecan-1 into a facilitator of FGF2 signaling.[93] The authors suggest that the degradation of undersulfated HS regions and release of heavily sulfated HS domains from the syndecan-1 ectodomain allow the freed heavily sulfated HS to bind and facilitate FGF2-FGFR1 signaling.[93] Similarly, another group has recently shown that shed syndecan-2 inhibits angiogenesis, although the inhibitory effect of syndecan-2 studied may be independent of the HS chains on syndecan-2 and growth factor signaling.[94] In contrast to these studies, Purushothaman et al. have reported that shed syndecan-1 can enhance endothelial cell invasion and angiogenesis. In this study, the authors show that while both the syndecan-1 core protein and the associated HS may each be partially responsible for this effect, that the HS attached to the shed syndecan-1 enhances VEGF signaling in endothelial cells, as this effect can be abolished by HS degradation from syndecan-1 by heparinase-III.[95] Although the studies performed by Purushothaman et al. and Kato et al. examine the effect of syndecan-1 associated HS on signaling initiated by two different growth factor ligands, VEGF and FGF2, these findings starkly contradict each other. As such, more research is needed to understand the complex role of shed HSPG ectodomains on endothelial growth factor signaling and elucidate the distinct roles of the HSPG core protein, the associated HS, and HS after cleavage from the core protein.

A second family of signaling mediators that HS is known to regulate is inflammatory signaling mediators, thereby affecting endothelial cell activation and inflammation. As is seen with growth factor signaling, HS may both
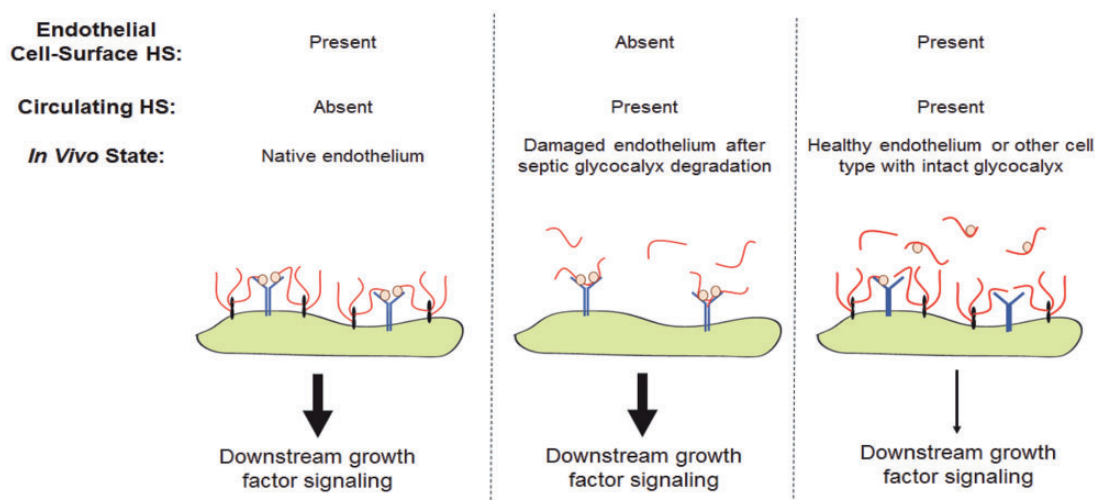

Fig. 3. Growth factor signaling may be shaped by both cell-surface and soluble HS. During homeostasis, cell-surface HSPGs provide cis-activation of growth factor signaling by stabilizing ligand-receptor interactions. In the absence of cell-surface HSPGs, this activation may be salvaged by the presence of soluble HS fragments of sufficient size and sulfation to engage growth factor ligands and receptors. In the presence of both cell-surface HSPGs and circulating HS, we hypothesize that the excess of HS sequesters ligands, attenuating downstream growth factor signaling.

enhance and reduce inflammatory signaling depending on its mode of action. HS is thought to enhance inflammation by binding to interferon-γ (IFN-γ) and protecting it from degradation, thus increasing its half-life and activity in circulation.[96] Additionally, HS fragments generated by heparanase digestion are thought to act as damage-associated molecular pattern (DAMP) ligands themselves, binding to Toll-like receptor 4 (TLR-4) and increasing pro-inflammatory cytokine release.[32] Furthermore, HS in serum from septic shock patients has been shown to induce mitochondrial dysfunction in cardiomyocytes in a TLR-4 dependent manner.[97]

Alternatively, soluble HS is also able to reduce inflammation by binding and inhibiting downstream signaling of circulating inflammatory mediators, including histones and high mobility group protein B1 (HMGB1), in a mechanism similar to that of high concentration soluble HS-inhibited growth factor signaling. During tissue injury, dying cells and neutrophils (via neutrophil extracellular traps) release nuclear proteins, including histones and HMGB1, which, when extracellular, can be detected as DAMPs. Endothelial cell-surface HS is known to facilitate the binding of both histones and HMGB1 to the cell surface where they can signal through TLR-2, TLR-4, and/or the receptor for advanced glycation end products (RAGE) to enhance inflammatory signaling and cytokine release.[98–101] Preliminary data suggest that soluble HS, like HS released during endothelial glycocalyx degradation, can intercept HMGB1 and histones and prevent their binding to the endothelial cell-surface.[100,102,103] In a model of histone-induced lung injury, we recently reported that HS attenuates lung injury initiated by intravascular histone injection; however, this affect was observed with HS oligosaccharides incapable of directly binding histones, suggesting that there may be alternative protective roles of HS against histone-induced injury.[103]

Given their important and promiscuous roles, circulating HSPGs and HS in vivo likely impart complex, context-dependent roles in health and disease. These roles remain to be further defined.

Summary and future directions

In the past 50 years, our understanding of the ESL has evolved from being an insignificant remnant of the basolateral membrane to a critical (and massive) contributor to endothelial function. With the advent of new analytical techniques, we are poised to make additional discoveries about the significance of ESL degradation in disease: not only does ESL loss cause local endothelial dysfunction, but the release of ESL components into the circulation shapes systemic responses to critical illness. Further mechanistic investigations are needed not only to understand these systemic responses, but also to identify therapeutic targets that can improve meaningful, multi-system outcomes in critical illness.

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