Glycocalyx Gone Awry: Pathologic Cell Signaling during Endotheliopathy

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Abstract

The endothelial glycocalyx is a viscus mesh that lines the vascular lumen and serves a variety of functions in health and disease. This gelatinous matrix of proteoglycans and glycosaminoglycans is the critical contact point between the circulation and end organs; transmitting physical and biochemical signals through the endothelial surface layer to affect endothelial cell activity that subsequently impacts organ function. In this review we explore the roles of the major structural components of the glycocalyx in mediating cell signaling events during inflammatory-induced endotheliopathy. We also discuss proposed therapeutic strategies to attenuate glycocalyx disruption and promote its regeneration.

Keywords: Vascular Endothelium; Heparan Sulfate; Hyaluronan; Syndecan; Glypican; Inflammation; Heparanase; Matrix Metalloproteinase

Abbreviations: Agpt-2: Angiopoietin-2; ATIII: Antithrombin III; CD44: Cluster of Differentiation 44; CS: Chondroitin Sulfate; eNOS: Endothelial Nitric Oxide Synthase; GAG: Glycosaminoglycan; GCX: Glycocalyx; Erk: Extracellular Signal-Regulated Kinase; FGF: Fibroblast Growth Factor; FGFR: Fibroblast Growth Factor Receptor; HA: Hyaluronan; HMW: High Molecular Weight; HPSE: Heparanase; HS: Heparan Sulfate; HSPG: Heparan Sulfate Proteoglycan; ICAM-1: Intercellular Adhesion Molecule-1; IL-1β: Interleukin-1 Beta; LMW: Low Molecular Weight; MAP kinase: Mitogen-Activated Protein Kinase; MMP: Matrix Metalloproteinase; NF-kB: Nuclear Factor Kappa-Light-Chain Enhancer Of Activated B Cells; NO: Nitric Oxide; PECAM-1: Platelet Endothelial Cell Adhesion Molecule-1; PI3K: Phosphoinositide-3-Kinase; PKC: Protein Kinase C; RHAMM: Receptor For Hyaluronan Mediated Motility; ROS: Reactive Oxygen Species; Syn: Syndecan; TLR: Toll-Like Receptor; VCAM-1: Vascular Cell Adhesion Molecule-1; VE-cadherin: VEGF: Vascular Endothelial Growth Factor; VEGFR: Vascular Endothelial Growth Factor Receptor

Introduction

The vascular endothelial glycocalyx (GCX) is the interface of blood-endothelial interactions and an active regulator of vascular tone and permeability. Overwhelming inflammatory states like trauma, sepsis, and burns precipitate a cascade of events that culminate in multiorgan dysfunction, primarily through endothelial cell activation and GCX disruption. Degradation of the GCX has been shown to increase vascular permeability, resulting in tissue edema; propagate local inflammation; and activate microvascular coagulation with resultant microthrombi and vascular occlusion. Though much attention has been dedicated to understanding the pathophysiology of these mechanisms leading to GCX disintegration, our understanding of the precise endothelial cell signaling mechanisms affected by GCX degradation and their implications on vascular homeostasis remains incomplete. Our focus in this review will be on the known and posited downstream extra- and intracellular signaling events that contribute to vascular dysfunction after endothelial GCX disruption secondary to inflammation. We will also discuss the work that has been done to therapeutically target these mechanisms in an effort to restore vascular integrity.

Background

The GCX of the vascular endothelium is a matrix of luminal cell-surface proteoglycans (e.g., syndecan, glypican, versican, decorin, mimecan, biglycan) decorated with a variety of glycosaminoglycans (GAG) (e.g., heparan sulfate, hyaluronic acid, chondroitin sulfate, dermatan sulfate, and keratan sulfate) [1,2]. The thickness and composition of the endothelial surface layer varies significantly across tissues, tailoring the function of the endothelial surface layer (ESL) to its particular native tissue and local blood flow [1,3-5].

The major structural proteoglycans in the vascular ESL are the membrane-anchored heparan sulfate proteoglycans (HSPG) syndecan and glypican1. Syndecans (Syn) are a transmembrane family of four proteoglycans (Syn1-Syn4) that uniquely cooperate with a host of extracellular...
signaling to the intracellular environment through their highly conserved transmembrane domain. Nuanced intracellular communications are dictated by the conserved C1 and C2 and variable V cytoplasmic domains of the Syn protein that associate with distinct enzymes and cytoskeletal elements [6]. The extracellular ectodomain of Syn is much more variable in its length compared to the cytoplasmic domain and predominantly expresses heparan sulfate, though post-translational substitutions with chondroitin sulfate moieties may also be expressed [6]. The diverse expression of HS lengths and sulfation patterns on Syn allows the GCX to serve as a large reservoir of associated plasma proteins and participate in a wide array of cell signaling pathways [7]. Glycans only express an extracellular domain that is anchored to the endothelial cell membrane through a glycosylphosphatidylinositol anchor imbedded in lipid rafts and caveolae [8,9]. The remaining proteoglycans are expressed in the ESL as secreted molecules within the GCX and contribute significantly less its structure and function [10].

The most ubiquitously expressed GAGs in the endothelial GCX are heparan sulfate and hyaluronan [11]. Heparan sulfate (HS) is a highly variable GAG covalently bound to Syn and glypican on the endothelial cell surface and comprises over 50% of the GAG composition in the GCX. [11] The diversity of glucosamine and hexuronic acid (i.e., iduronic acid or glucuronic acid) polymerization and varied sulfation pattern allows the HS molecule to associate with a host of plasma proteins with specificity [12]. Imbedded within the HSPG mesh of the GCX are cytokines and chemokines that form gradients for endothelial cell signaling and leukocyte chemotaxis [7]. Additionally, the interaction of growth factors with HS on parent HSPGs governs the activation of the parent cell surface growth factor receptors.6. Hyaluronan, or hyaluronic acid (HA), exists as a high molecular weight (HMW) GAG (over 1MDa) attached to the ESL through interactions with CD44 and other GAGs [11]. Its function is related to its uniquely non-sulfated, very large structure that provides both a lubricating and structure-stabilizing role in the GCX. As a relative proportion, the expression of chondroitin, dermatan, and keratan sulfates is approximately four times less than that of heparan sulfate [11]. Dermatan sulfate (also classified as chondroitin sulfate type B) and keratan sulfate are primarily bound to secreted proteoglycans and exist more prevalently in the extracellular matrix of endothelial cells [11]. These lesser understood GAGs appear to contribute little to ESL function.

In health, the intact GCX serves several vital homeostatic functions. The negatively charged, highly proteoglycan composition of the GCX provides an electrostatic and physical barrier to circulating proteins to limit their extravasation and concomitantly limit the flow of circulating water to the extravascular space via modified Starling forces [13]. Endothelial cell surface receptors are buried within this luminal matrix and rely on proteoglycans and GAGs as co-receptors for cell signal activation [14]. Circulating cell interactions with the endothelium are regulated by cytokine and chemokine gradients within the GCX and by the various cell adhesion molecules hidden within the GCX [15,16]. The ESL also contains expressed cofactors for coagulation homeostasis, including antithrombin III (ATIII), thrombomodulin, tissue factor, and tissue factor pathway inhibitor. Thus, the GCX serves as a key touchpoint between and regulator of systemic inflammation and coagulation. Moreover, the gelatinous-like nature of the GCX transmits shear stress and hydrostatic pressure to the endothelial cells, resulting in endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) regulation that coordinates vascular tone and permeability [17].

In diseases of local and systemic inflammation, degradation of the GCX results in a predictable cascade of inflammation, coagulation activation, and increased vascular permeability. Due to the intricate involvement of the endothelial GCX in the cell surface signaling, disruption of the GCX results in significant perturbations in extra- and intracellular signaling pathways that propagate the pathophysiology of the inflamed state. The components of the GCX contribute in unique ways. Elucidating the individual contributions of the GCX components to the pathophysiology of endotheliopathy could prove instrumental in the development of successful therapies that restore vascular integrity in the setting of systemic inflammation.

Role of Heparan Sulfate

In adults with respiratory failure or sepsis, circulating HS levels are significantly elevated compared to controls and correlate with illness severity [18,19]. Plasma HS levels are also higher in patients after traumatic injury [20] or major vascular procedures that induce systemic ischemia/reperfusion [21]. The circulating HS is presumably shed from the endothelial GCX among other sources, suggesting a mechanistic linkage between inflammatory-mediated ESL perturbation and organ injury.

HS is selectively cleaved from its associated proteoglycan into varying fragment lengths by heparanase-1 (HPSE), the only known mammalian endogluconidase to have enzymatic activity on HS [22]. Consistent with the observed elevation of plasma HS levels described above during systemic inflammation, HPSE levels and enzymatic activity are concomitantly elevated in organs injured during inflammation [4,23]. Secreted by activated leukocytes, platelets, and endothelial cells [24,25] in the setting of hyperglycemia [26], angiopeptin-2 [27], TNFα [28], or IL-1β [28], HPSE acts at the GCX to cleave HS from its associated proteoglycan into various fragment lengths [29,30] (Figure 1). This HS trimming releases stereotypically associated proteins (e.g., chemolines, growth factors) into circulation and the local microenvironment [27]. Now free, these proteins activate a myriad of receptors that propagate inflammation, endothelial permeability, and leukocyte transcytosis [31]. Soluble HS fragments can also independently...
serve as damage-associated molecular patterns recognized by toll-like receptor 4 (TLR4), resulting in the unpropitious activation of NF-κB, increased expression of cytokines and cell adhesion molecules, and mitochondrial dysfunction [32-34]. Conversely, Zhang Y et al. [35] demonstrated that soluble HS of varying lengths attenuate circulating histone-mediated damage of human pulmonary microvascular endothelial cells in vitro independently of TLR-signaling. Thus, the local effects of soluble HS are likely contextually dependent on the characteristics of the HS fragments present, the presence of bound signaling molecules, and the surrounding inflammatory milieu.

**Role of Hyaluronan**

Adults admitted to the intensive care unit with septic shock demonstrate elevated plasma HA levels compared to controls [19]. Schmidt et al. [18] similarly reported elevated circulating HA fragments in patients with lung injury. This group went on to describe that in two adult cohorts admitted for sepsis or acute respiratory distress syndrome, increased urinary levels of HA early in the presentation of systemic illness were predictive of the development of acute kidney injury and in-hospital mortality [36]. Taken together, these data suggest that soluble HA and its degradation products promote proinflammatory signaling that culminates in organ dysfunction.
HA is shed from the GCX and further degraded by hyaluronidase and/or reactive oxygen species released from activated leukocytes, resulting in the formation of low molecular weight (LMW)-HA [37]. LMW-HA fragments produce a proinflammatory cellular phenotype, demonstrated by the activation of the M1 phenotype in macrophages in vitro [38,39] and exacerbation of lung injury in in vivo models of airway hyperreactivity [40]. HA signaling appears to be fragment size-dependent as soluble HMW-HA promotes an anti-inflammatory M2 phenotype in macrophages [38]. Several endothelial cell surface receptors that recognize LMW-HA have been identified, including TLR4, CD44, and the receptor for HA-mediated motility (RHAMM) (Figure 2). Human dermal microvascular endothelial cells treated with LMW-HA upregulate proinflammatory cytokine production in a TLR4-dependent manner [41]. Evidence for this signaling pathway is supported by the protection of TLR4 knockout mice from LMW-HA-induced lung injury [42]. The interaction of HA with cell surface CD44 has been extensively studied, demonstrating a wide array of signaling cascades terminating in cytokine upregulation, cytoskeletal remodeling, and cell proliferation that can occur independently of or in concert with RHAMM [43]. The role of RHAMM in HA signaling appears to be more nuanced as its expression depends on various factors, including induction by CD44/protein kinase Cδ activation [44]. After being expressed on the cell membrane, RHAMM binds with soluble LMW-HA, thereby activating the ERK1/2-MAP kinase pathway to promote cellular proliferation [45] and Ras/Raf/Rac to effect cytoskeletal rearrangement [43].

Role of Other Glycosaminoglycans

The other GAGs expressed in the endothelial GCX have received much less attention, limiting our understanding of their role in the inflammatory response. When shed, chondroitin sulfate appears to exhibit anti-inflammatory properties through histone-binding and NF-kB downregulation [46,47]. However, soluble chondroitin sulfates may also exhibit untoward antibacterial peptide inhibition in the setting of sepsis [48]. Dermatan sulfate may exhibit more tissue-specific local inflammatory regulation by mediating FGFR-dependent cell proliferation and NF-kB activation with resultant increased ICAM-1 expression in and around epidermal wounds [49,50]. Keratan sulfate does not have a known role in inflammatory signaling at present.

Role of Surface Heparan Sulfate Proteoglycans

The HSPGs are the only known endothelial surface-anchored proteoglycans, making them integral in maintaining GCX integrity and endothelial responsiveness to luminal physical and biochemical stimuli. Loss of endothelial surface expression of HSPGs and their covalently bound GAGs therefore has dire consequences on shear stress mechanosignaling and other cellular signaling mechanisms that regulate vasoactivity, vascular permeability, and endothelial cell homeostasis. Clinical studies bear this to be true. Circulating levels of Syn, particularly Syn1, have repeatedly been shown to significantly rise in systemic inflammatory conditions like trauma [20,51,52] sepsis [48,53] and burns [54,55] and correlate with illness severity [48,51,54,56]. Though the glypicans have been less well studied, circulating glypicans levels are elevated in the setting of sepsis and correlate with disease severity [57]. Adults with severe direct lung injury from pneumonia also demonstrate elevated levels of plasma glypican [58].

Mechanosensing of vascular shear stress to the endothelial cytoskeleton is primarily mediated through the HSPGs [59] (Figure 3). Syn1 and Syn4, in particular, directly influence cytoskeletal remodeling through syntenin and synectin linkages to the actin cytoskeleton [60-64]. As blood flow shear stress deforms the GCX, Syn transmits this torque to the actin cytoskeleton [65] via, in part, activation of RhoA GTPases [66]. Due to the intricate cytoplasmic connections of the cytoskeleton to adherens and gap junctions and intracellular organelles, the surface Syn-mechanotransduction becomes “decentralized” and propagates throughout the cytoplasm [67]. The resultant cytoskeletal reorganization influences cellular shape and distribution along the vasculature, inter- and intracellular molecular transport, cell-cell adhesions, and nuclear activation [65,67]. Syn4 also activates protein kinase Ca in a calcium-independent fashion to maintain proper cytoskeletal stress fiber alignment in response to blood flow [60,68]. Moreover, Syn4, through its interaction with Rho, activates focal adhesion kinase to maintain focal adhesion integrity [69]. Thus, loss of Syn expression from the ESL would be expected to culminate in destabilized cytoskeletal arrangement with poor endothelial responsiveness to changes in surface shear as demonstrated by Basyens et al. [70]. Degradation of the HSPGs appears to also significantly alter the endothelium’s ability to regulate vasoreactivity to vascular shear stress by disrupting eNOS activation. Voyvodic et al. [66] have shown that loss of Syn1 from the GCX suppresses the endothelial cell’s ability to activate the PI3/Akt pathway in response to luminal shear, resulting in decreased phosphorylated Akt. Dammeler et al. [71] have demonstrated that eNOS is activated in response to Akt phosphorylation. Taken together, the results of these studies suggest that loss of Syn1 expression from the GCX may result in decoupling of eNOS activation from vascular blood flow. HS trimming by heparinase III, a bacterial enzyme with nonspecific HS-degrading activity at the ESL, has also been shown to abrogate shear-induced eNOS activation [59,72]. Likewise, glypicans influences caveolae protein 1 expression that subsequently regulates eNOS expression in caveolae [73-75]. Thus, deranged glypicans expression suppresses eNOS activity in response to shear via decreased eNOS expression [9,76]. Interestingly, HA also participates in mechanosignaling to influence eNOS activation [77,78]. Thus, removal of HSPGs and HA impairs shear-induced vasoactivity of the endothelial cell by attenuating eNOS expression and activation.

HSPGs protect the endothelium from leukocytes and platelets by shielding cell adhesion molecules and glycoproteins used by these circulating cells for endothelial attachment [79,80]. HPSE-
mediated removal of HS from Syn opens the proteoglycan up to the enzymatic activity of MMPs [81-83], thereby exposing P- and E-selectins and VCAM-1, PECAM-1, and ICAM-1. Now available, circulating leukocytes and platelets adhere to these cell adhesion molecules and become activated [4,80,84,85]. Indeed, the result of loss of Syn1 endothelial surface expression has been shown to promote a proinflammatory phenotype that is abrogated by re-expression of Syn1 [66]. Similarly, treatment of various endothelial cell lines with heparinase III has been shown to significantly increase leukocyte adhesion with resultant tissue injury [4,86]. Release of chemokines from the cell surface during GCX degradation into the local microenvironment may serve to attract leukocytes to and through the endothelium. Conversely, chemokine release from the ESL may also act as a mechanism to reduce leukocyte attachment to the endothelium [87]. Furthermore, like HSPGs, cell adhesion molecules may undergo enzymatic cleavage by MMPs [88]. Shapiro et al. [89] found plasma levels of the soluble form of several cell adhesion molecules (e.g., sE-selectin, sICAM-1, and sVCAM-1) to be significantly elevated in adults presenting to the emergency department with sepsis. While the purpose for cell adhesion molecule cleavage is not entirely clear, it may provide a protective mechanism for the endothelium to limit leukocyte attachment and activation or it may serve as an essential step in the active invasion of the leukocyte through the endothelium by dissociating from the ESL. Thus, the degree to which the GCX prevents or aids in leukocyte chemotaxis, attachment, and activation during inflammatory-related ESL disruption may be dictated by the degree of remaining HS and cell adhesion molecule expression after GCX degradation [90].

Figure 3: GCX-mediated mechanosignaling. The cytoplasmic domain of Syn is associated with the actin cytoskeletal network via linkages with syntenin and syndecan, which allows for propagation of mechanotransduction signaling from the cellular surface to intercellular junctions and throughout the cytoplasm. In response to shear stress, Syn initiates cytoskeletal alignment and focal adhesion formation via activation of RhoA. In some cell types, Syn1 may also regulate shear stress-induced activation of PI3K/Akt signaling. Glypican, HS, and HA mediate cellular activation of eNOS and NO production in response to shear stress via associations with caveolar/lipid raft regions that are enriched with signaling molecules (e.g., inactive eNOS). Loss of any or all of these GCX constituents impairs the endothelial cell response to intra-luminal forces.

Lastly, decreased HSPG expression can disrupt critical receptor-mediated signaling pathways important for maintaining endothelial homeostasis. Downregulation of Syn1 in murine glomerular endothelial cells affects VEGF-VEGFR2 signaling with resultant reduction in downstream Akt, ERK1/2, and Rac1 activation [91]. FGF2-FGFR signaling is heavily influenced by Syn4 stabilization of the growth factor-receptor complex to affect a multitude of complex intracellular pathways that result in maintenance of HS expression in the GCX and stabilization of endothelial cell-cell adhesions [92]. Constitutive expression of Syn4 also contributes to the tightly controlled release of angiotensin-2 (Agpt-2) from the endothelium [93]. Agpt-2 is a cytokine that antagonizes the endothelial cell surface Tie2 receptor, resulting in destabilization of cell-cell junctions [94]. Thus, loss of Syn-4 ESL expression may contribute to dysregulated FGFR signaling and Agpt-2 release that would result in increased endothelial permeability and impaired GCX restoration. The role that glypican disruption plays in endothelial cell receptor-mediated signaling during inflammation remains to be studied [95].

Therapeutic Strategies Targeting GCX Restoration

As our knowledge of the signaling mechanisms influenced by the GCX is continually growing, so too is the development of novel strategies to (1) prevent GCX damage, (2) inhibit the downstream detrimental signaling cascades initiated by GCX shedding, and/or (3) promote GCX restoration. Over the past two decades, various studies have demonstrated the impact that fluid resuscitation strategies have on clinical outcomes, due in part to the recognition of the role of endothelial GCX integrity in mediating organ function. Well-intentioned goal-directed clinical practices (e.g., early high-volume crystalloid resuscitation for hemorrhagic or septic shock) may actually worsen clinical outcomes, [96,97] in part through GCX perturbation [98,99]. The use of fresh frozen plasma (FFP) as a resuscitation fluid has received much attention due to its ability to upregulate GCX synthesis, promote repair of EC junctions, and restore microvascular function after hemorrhagic shock [98,100-102]. More recently, the GCX-protective benefits of FFP resuscitation have been attributed to its adiponectin [103], ATIII [104-106].
Other therapies that demonstrate GCX-protection include hydrocortisone [104] via mitigation of TNFα-induced endothelial activation; dexamethasone [107] and sphinogosin-1-phosphate by downregulating endothelial cell MMP expression; doxycycline [108] via MMP inhibition; and heparin/heparan sulfate mimetics derivatives [4,109,110] via HPSE inhibition. To prevent aberrant downstream signaling events after GCX injury, purified HMW-HA may be administered systemically [111]. Lastly, ESL resolution may be promoted by FFP as described above; sphinogosin-1-phosphate by increasing ESL HS expression [112]; sulodexide [113,114] by re-coating the ESL with highly purified GAGs; and FGFR-receptor agonists to promote exostosin-1 expression and HS synthesis [115]. Although promising, these therapies remain theoretical at present and require further study with prospective randomized clinical trials [116].

Conclusions

Component of the GCX structure, namely HS, HA, and the HSPGs, contributes uniquely to the function of the ESL both in health and inflammatory pathophysiology. However, further study is needed to more clearly understand how GCX-component degradation contributes to endotheliopathy in the setting of inflammation. There is compelling evidence to suggest that strategies targeting the prevention of GCX damage and the restoration of its integrity will improve vascular function, which has the potential to meaningfully improve clinical outcomes. Given the progress in the field of endothelial GCX research over the last several years, we anticipate significant translational developments of therapies aimed at protecting the GCX in the years to come.

References


