Heparan Sulfate in the Developing, Healthy, and Injured Lung

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Abstract

Remarkable progress has been achieved in understanding the regulation of gene expression and protein translation, and how aberrancies in these template-driven processes contribute to disease pathogenesis. However, much of cellular physiology is controlled by non-DNA, nonprotein mediators, such as glycans. The focus of this *Translational Review* is to highlight the importance of a specific glycan polymer—the glycosaminoglycan heparan sulfate (HS)—on lung health and disease. We demonstrate how HS contributes to lung physiology and pathophysiology via its actions as both a structural constituent of the lung parenchyma as well as a regulator of cellular signaling. By highlighting current uncertainties in HS biology, we identify opportunities for future high-impact pulmonary and critical care translational investigations.

Keywords: heparan sulfate; glycosaminoglycan; glycocalyx; proteoglycan

Clinical Relevance

Heparan sulfate (HS) is a linear glycosaminoglycan that significantly impacts lung structure and cellular signaling. By reviewing HS and its impact on lung development, homeostasis, and injury, this *Translational Review* highlights critical knowledge gaps and opportunities for novel translational investigations relevant to diseases such as acute lung injury and pulmonary fibrosis.

Heparan sulfate (HS) is a linear glycosaminoglycan composed of repeating disaccharide units of glucosamine and a hexuronic acid (glucuronic acid or its epimer, iduronic acid). HS synthesis occurs within the Golgi apparatus, where disaccharide polymerization extends from a protein backbone. Together, this HS–protein complex is known as an HS proteoglycan (HSPG). HS undergoes sequential steps of epimerization and/or sulfation, as governed by a system of epimerases and sulfotransferases targeted to specific sites on component HS disaccharides (Figure 1) (1). This complex process of synthesis and modification dictates the biologic functions of HS, which arise largely from the localized negative charge imparted by clusters of sulfation-enriched domains. These domains enable HS to not only bind positively charged residues of soluble ligands and cell surface receptors (2), but also to sequester water (3)—characteristics that allow HS to function both as a structural component of the lung parenchyma and as a regulator of signaling pathways.

HS in Lung Development

Transgenic animal studies have demonstrated the importance of HS to proper organ development (4). As summarized in Table 1, the most severe pulmonary-relevant phenotypes described (embryonic lethal) arise from null mutants of *Glact1*, *Ext1*, and *Ext2*, which encode enzymes that build the initial unmodified HSPG. In addition, genes involved in the further modification of HS affect lung development. Null allele mutants of *Ndst1*, which encodes an enzyme that sulfates glucosamine at the N position, suffer from neonatal respiratory distress due to lung hypoplasia (5, 6). Genetic manipulations that result in HS containing less iduronic acid (*Glce/Hsepi*) or 6-O sulfation (*H6st1*) also produce animals that lack proper alveolar development (7, 8).

The importance of HS biosynthesis to lung development likely reflects the necessity of specific HS sulfation patterns (such as N sulfation) to enable interaction of HS with soluble ligands and their cognate cell surface receptors. Through these sulfation-dependent interactions, HS regulates growth factor and morphogen signaling that contributes to airspace and vascular lung development. Nearly complete desulfation of lung HS prevents fibroblast growth factor (FGF) 10 from binding to distal

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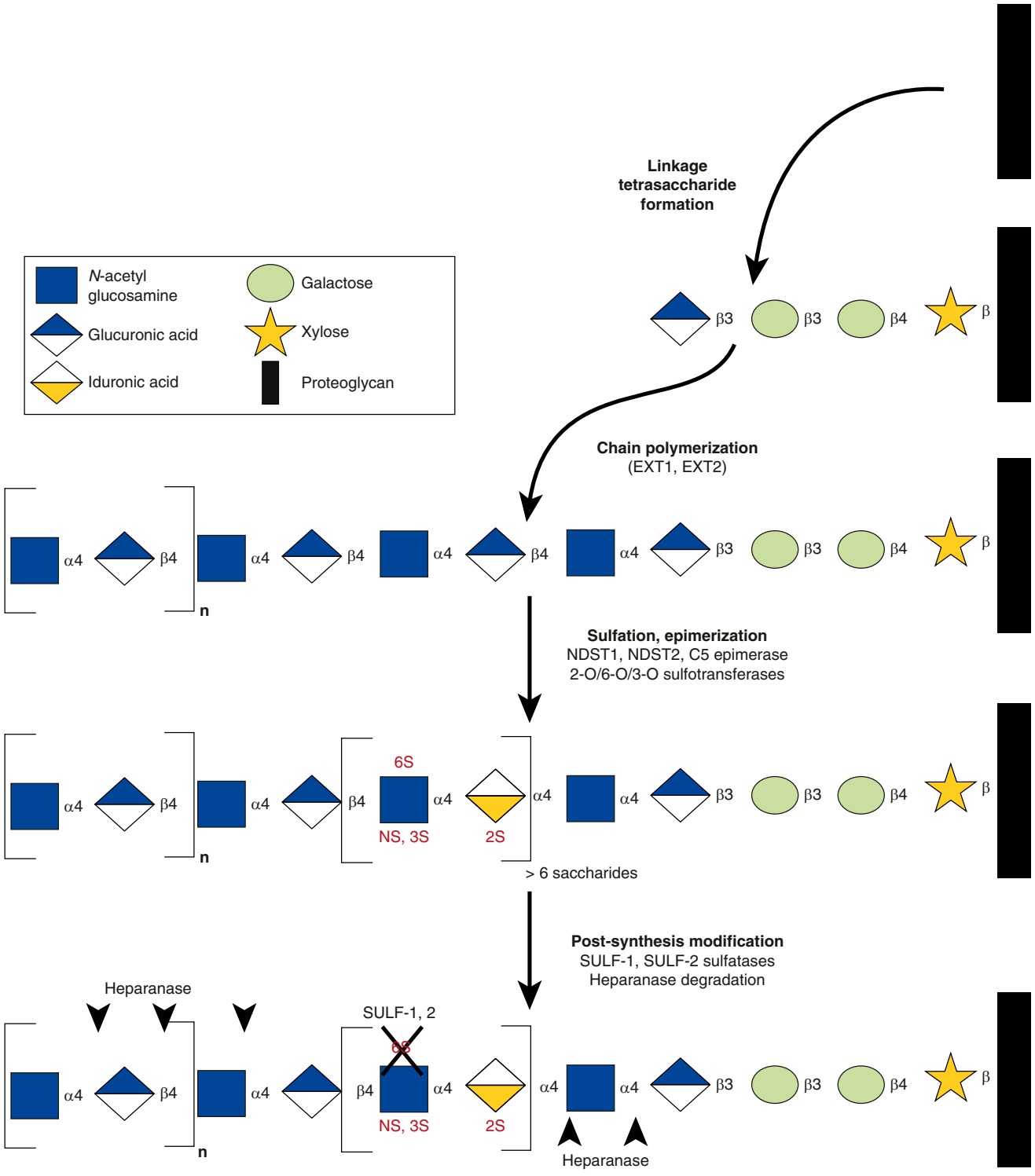

Figure 1. Heparan sulfate (HS) synthesis and modification. HS is synthesized within the Golgi apparatus via the actions of a complex biosynthetic machinery. After establishment of a linkage tetrasaccharide on a proteoglycan, heparan polymerization occurs via the sequential addition of uronic acid–glucosamine disaccharides. These disaccharides are rapidly modified by epimerization (glucuronic acid to iduronic acid) and/or targeted sulfation. Outside the Golgi apparatus, HS may be rapidly modified via endogenous sulfatases (e.g., SULF-1 and SULF-2, which desulfate 6-O HS) and heparanase (which generally acts to release highly sulfated fragments, although the exact target of cleavage remains the focus of active study). The coordination of this multistep process remains uncertain. EXT, exostosin glycosyltransferase; NDST, *N*-deacetylase/*N*-sulfotransferase; n, number of disaccharide repeats; NS, N-sulfation; S, sulfation at 2, 3, or 6 positions; SULF, sulfatase.

Table 1. Lung Developmental Defects in Heparan Sulfate Biosynthesis Genetic Mutants

Gene	Role in HS Biosynthesis	Effect on Lung Development (Reference)
Glact1	Forms HS/CS/DS–protein tetrasaccharide link	Null allele: embryonic lethal (56)
Ext1/Ext2	Elongates HS chain	Null allele: embryonic lethal; aberrant endoderm development (57, 58)
Ndst1	Deacetylates and sulfates glucosamine residues at the N position	Null allele: perinatal lethal; pulmonary hypoplasia and neonatal respiratory distress (5, 6)
H6st1	Sulfates glucosamine residues at the 6 position	Null allele: embryonic/perinatal lethal; enlarged alveoli (7)
Glce (Hsepi)	Epimerizes glucuronic acid to iduronic acid	Targeted disruption: perinatal lethal; neonatal respiratory distress and thickened poorly inflated alveoli (8)

Definition of abbreviations: CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate.

epithelial cells, inhibiting airway branching (9). More specifically, *N*-sulfated HS is required to bind bone morphogenic protein 2 and 4, regulating lung epithelial proliferation and alveolar epithelial type (AT) II to ATI cell differentiation (10). HS also contributes to lung vascular development by interacting with two murine isoforms of vascular endothelial growth factor regulating blood–air barrier formation and proper vessel density (11). These interactions described are only a few of the many HS-dependent signaling pathways important for lung development.

HS in Lung Physiology

HS as a Structural Molecule

Sulfated glycosaminoglycans (such as HS) readily sequester water, forming large, gel-like structures with measurable rigidity (12). In addition, sulfated regions of HS bind both cell matrix proteins and cell adhesion proteins, allowing HS to contribute to structures within the pulmonary vasculature, interstitium, and alveolar epithelium (13) (Figure 2). Interestingly, there is significant heterogeneity of HS sulfation across different compartments within the lung, potentially reflecting context-specific contributions of this glycosaminoglycan to pulmonary function (14).

HS in the pulmonary endothelial glycocalyx/endothelial surface layer. The endothelial glycocalyx is an intraluminal matrix enriched in glycoproteins and proteoglycans. *In vivo*, glycocalyx glycosaminoglycans (including HS, chondroitin sulfate, and hyaluronic acid) interact with plasma proteins and avidly sequester water, forming an endothelial surface layer (ESL) of substantial thickness. This gel-like, fully hydrated ESL contributes to the endothelial barrier to fluid and protein, transduces vascular shear stress into endothelial nitric oxide (NO) synthesis, and regulates the availability of cell membrane adhesion molecules to circulating leukocytes (15). Accordingly, enzymatic HS degradation causes collapse of pulmonary ESL thickness (16), leading to lung edema (17, 18), aberrant pressure-induced endothelial NO synthesis (17, 18), and lung inflammation (16).

The contribution of HS to endothelial barrier function has been attributed to its physical presence as a charged meshwork overlying endothelial cells (15). In contrast, the mechanism by which an intact ESL regulates NO synthesis is less certain, with investigators speculating the importance of interactions between HSPGs (glypican) and endothelial NO synthase within endothelial caveoli (19). Furthermore, the impact of HS on leukocyte adhesion is complex. Loss of ESL thickness exposes endothelial surface adhesion molecules, facilitating neutrophil adhesion within alveolar capillaries (16). In contrast, endothelial surface HS can serve as an L-selectin ligand and regulator for chemotactic agent availability (20); as such, aberrance of pulmonary ESL HS structure or sulfation might be expected to decrease L-selectin–mediated neutrophil–endothelial interaction. It remains unclear how these seemingly disparate roles of ESL HS on leukocyte diapedesis are reconciled *in vivo*.

Alveolar basement membrane. The alveolar basement membrane is a structure enriched in HSPGs (including collagen XVIII, agrin, and perlecan) that can directly connect the alveolar endothelium and epithelium. Basement membrane HSPGs function not only to form a charged molecular "sieve" that contributes to the alveolocapillary barrier, but are also essential to the HS-dependent anchoring of endothelial and epithelial cells to extracellular matrix (ECM) proteins (3, 21, 22). These interactions, however, are complex. Perlecan HS, for example, demonstrates both adhesive and antiadhesive functions, and is necessary for mechanical stretch–induced alveolar epithelial signaling, indicating that HS facilitates matrix–cellular coupling (23, 24).

Pulmonary interstitium. Although a shared basement membrane can directly connect alveolar epithelium with capillary endothelium, these cellular compartments may additionally be separated by an interstitium enriched in nonfibrillary proteins, including chondroitin sulfate proteoglycans and, to a lesser extent, HSPGs (3, 25). These sulfated proteoglycans contribute to the "viscoelasticity" of the lung parenchyma (26), allowing for alveolar stability at low lung volumes (27). HSPGs also safeguard against lung edema formation by maintaining a noncompliant lung interstitium, which responds to fluid accumulation with a rapid increase in interstitial hydrostatic pressure that opposes ongoing vascular leak (3). Accordingly, experimental proteoglycan degradation triggers a transition from interstitial edema to alveolar flooding (28).

Alveolar epithelial surface layer. Although the importance of HS to the pulmonary ESL is well described, the structural significance (and very existence) of alveolar epithelial surface HS is uncertain. HS can be identified on the surface of mouse alveolar type II–like epithelial 12 cells (Figure 3) as well as in human ATII-like A549 cells (29). Although the liberation of HSPGs (including syndecan-1) into the alveolar space during lung injury implies the presence of HS lining the alveolar lumen, these findings are only suggestive, and the original source of the shed HSPGs *in vivo* has yet to be determined (30).

HS in Cellular Signaling

Sulfation of HS is essential for its influence on cellular signaling, as the imparted negative charge allows HS to bind and regulate other bioactive ligands (Table 2). Some of the

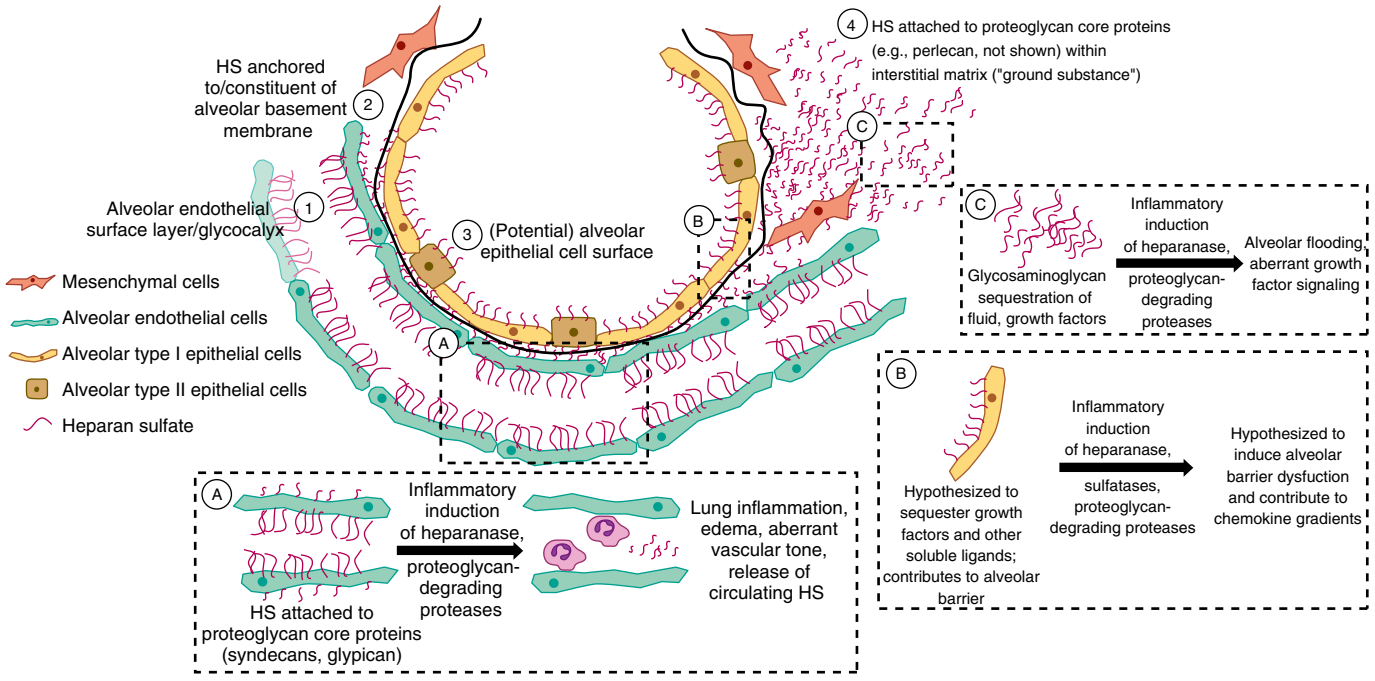

Figure 2. HS in the alveolus. HS is abundant within the alveolus, contributing to: (*1*) the pulmonary microvascular glycocalyx/endothelial surface layer; (*2*) the alveolar basement membrane; (*3*) the alveolar epithelial surface layer; and (*4*) the lung interstitial extracellular matrix. Although the size of the pulmonary endothelial surface layer has been measured *in vivo*, its size relative to other HS-containing structures in the lungs is unknown. Indeed, even the existence of an alveolar epithelial surface layer is uncertain. During inflammation, HS degradation and/or modification at various alveolar sites contributes to lung injury (*A–C*).

best-described interactions of HS are those with FGF ligands and FGF receptors. HSPGs on the cell surface may bind and oligomerize FGF ligands and provide a *cis*-scaffold for FGF ligand–receptor binding (31). Within the ECM, HS binds and stores FGF ligands, which can be released upon ECM degradation during injury (4). In a similar manner as with FGF signaling, HS binds and regulates many other soluble ligands that play critical roles in physiologic cellular signaling in the pulmonary vasculature and epithelium.

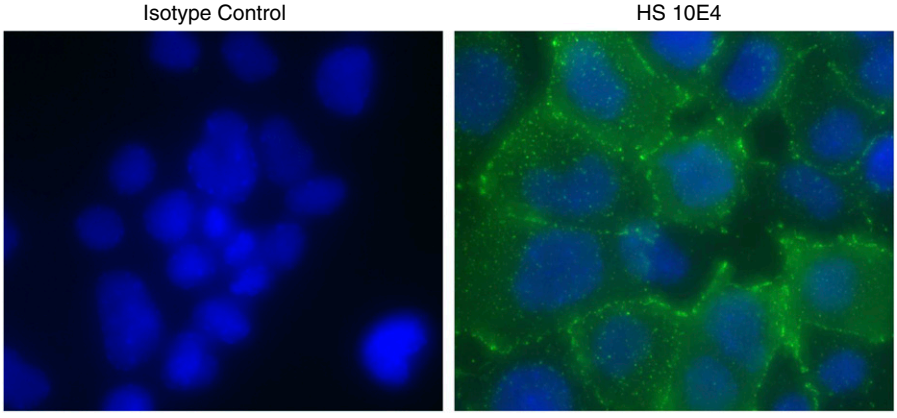

Figure 3. Mouse alveolar type II–like epithelial 12 (MLE-12) cells express cell surface HS. Before fixation, live MLE-12 cells were treated with the HS 10E4 antibody at 4°C, which recognizes *N*-sulfated glucosamine residues of HS. This approach limits staining to the cell surface. An isotype antibody serves as a control. Z series images were taken, and a maximum intensity projection image was created. *Green*, HS 10E4; *blue*, 4',6-diamidino-2-phenylindole (DAPI).

- *Pulmonary vasculature*: basement membrane HSPGs serve as important regulators of angiogenesis and smooth muscle cell activation. Intact perlecan accelerates FGF2-induced angiogenesis, presumably via HS-mediated FGF2–FGF receptor 1 signaling (32). Accordingly, perlecan knockout mice exhibit aberrant vascular development (21). Interestingly, peptide fragments derived from basement membrane HSPGs, such as endorepellin (the C-terminus fragment of perlecan) and endotstatin (the C-terminus fragment of collagen XVIII) are strongly antiangiogenic, potentially due to their ability to bind cell surface HSPGs and limit endothelial interactions with proangiogenic ligands (33, 34).
- *Pulmonary epithelium*: although it is unclear if alveolar epithelial cell surface HS contributes to an epithelial surface layer, epithelial HSPGs do influence alveolar intercellular signaling and epithelial cell phenotype. When added to ATII cells in combination with FGF1, heparin, a heavily sulfated form of HS, enhances the RNA expression of the ATII cell marker, surfactant protein B, and the ATI cell marker, aquaporin-5 (35). This

Table 2. Signaling Proteins Regulated by Heparan Sulfate that Influence Lung Development, Homeostasis, and Injury

Binding Partner	Impact of Heparan Sulfate (References)
Growth factors	
FGF	Facilitates ligand–receptor binding, augmenting GF signaling (31, 59) FGF10 influences airway branching during lung development (9) FGF2 induces angiogenesis (32) FGF1 and -2 enhances ATII cell proliferation (60)
BMP2/4	May inhibit signaling by preventing binding to the receptor, but also facilitates signaling by enhancing stability of ligand (10) In the lung HS has been shown to inhibit BMP signaling during development and facilitate alveolar dilation (10)
VEGF	Co-receptor for VEGFs/VEGFRs (61) Contributes to vascular development and blood–air barrier formation (11)
Cytokines/chemokines	
IL-6/IL-8	HS binds to IL-8 in lung tissue (62) Soluble heparin attenuates the secretion of IL-8 from endothelial cells when treated with LPS; however, cell-surface HS released by heparanase induces expression of IL-8 (40, 44)
CXCL10	Syndecan-4 binds CXCL10 and reduces fibroblast migration and fibrosis in animals treated with bleomycin (54)
Nuclear proteins	
Histones	Endothelial glycocalyx binds circulating histones and soluble heparin, competitively inhibits binding of histones to endothelial cells, and improves survival during sepsis (41, 42)
HMGB1	Soluble heparin inhibits the binding of HMGB1 to macrophages and reduces mortality in a sepsis model (43)

Definition of abbreviations: AT, alveolar epithelial type; BMP, bone morphogenic protein; CXCL10, C-X-C motif chemokine 10; FGF, fibroblast growth factor; GF, growth factor; HMGB1, high-mobility group box 1; HS, heparan sulfate; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

effect is partially dependent on the degree of heparin sulfation (35). Furthermore, HS in both the ECM and on the epithelial cell surface affects epithelial cell viability and growth in a sulfation-dependent manner, consistent with influence on growth factor signaling (36).

HS in Lung Injury

As with lung development and physiological homeostasis, HS can impart a multifactoral influence over pathogenic processes during both acute and chronic lung injury.

Acute Lung Injury

Known clinically as the acute respiratory distress syndrome (ARDS), acute lung injury is a heterogeneous entity representing the end result of any number of direct (e.g., pneumonia) or indirect (e.g., nonpulmonary sepsis) pulmonary insults. Interestingly, different ARDS-triggering events and pathophysiologic pathways may demonstrate unique patterns of HS dysfunction.

- *Indirect lung injury*: systemic insults (such as nonpulmonary sepsis) can induce diffuse, pulmonary endothelial–centered lung injury. Accordingly, sepsis-induced lung injury is characterized by pulmonary ESL degradation, potentially mediated by induction of endothelial heparanase, an HS-specific endoglucuronidase (16). Other potential ESL "sheddases" include lysosomal constituents and matrix metalloproteinases (37–39). HS degradation rapidly reduces ESL size and rigidity, allowing neutrophils to access their cognate endothelial adhesion molecules, extravasate, and cause inflammatory lung injury (16). This injury may be additionally influenced by HS fragments released during ESL degradation. Soluble heparin has been shown to be antiinflammatory by inhibiting LPS-induced NF-κB signaling and IL-8 secretion from pulmonary endothelium, and by binding and sequestering circulating damage-associated molecular patterns, such as extracellular nuclear proteins, high-mobility group box 1 protein, and histones (40–43). Paradoxically, HS fragments released by enzymatic heparanase degradation may themselves propagate injury as a damage-associated molecular pattern (44).
- *Direct lung injury*: in contrast to indirect/systemic lung injury, direct lung injury arises from insults that primarily target the lung epithelium. Accordingly, patients with pneumonia-induced respiratory failure have less circulating HS than patients with sepsis- or pancreatitis-induced respiratory failure (45). These differences may reflect insult-dependent roles of heparanase in lung injury: although heparanase knockout mice were protected from polymicrobial sepsis-induced lung injury (16), no such protection was enjoyed after intranasal LPS (46). In contrast, protease induction during direct lung injury onset sheds HSPGs into the alveolar space where they affect lung injury severity by releasing a sulfation-dependent chemokine gradient necessary for alveolar neutrophilic influx and by facilitating resolution of alveolar inflammation (30, 47, 48).
- *Ventilator-induced lung injury*: although mechanical ventilation is necessary for the supportive care of patients with ARDS, improper use can induce injury across the alveolar endothelium, epithelium, and interstitium. High tidal volume ventilation induces matrix metalloproteinase activation, leading to proteoglycan cleavage and loss of whole-lung soluble and protein-bound glycosaminoglycan content (49). This fragmentation may be offset by induction of HSPG expression within the alveoli of high tidal volume–ventilated lungs (26). This degradation of interstitial proteoglycans removes a safeguard against alveolar flooding, as described previously here.

Chronic Lung Injury

Idiopathic pulmonary fibrosis (IPF) is thought to arise from chronic, unresolving lung injury. In comparison to the normal lung, HS is expressed in a different structural and spatial pattern during chronic injury, potentially regulating signaling pathways that contribute to the pathogenesis of fibrosis. During fibrosis, there is both increased amounts of lung HSPGs and alterations in HS sulfation (e.g., increased 6-O sulfation) (50, 51). This increased 6-O sulfation may contribute to IPF progression by enhancing pathogenic transforming growth factor-β signaling in both lung fibroblasts and ATII

cells (29, 51). In addition, as observed during direct acute lung injury, HSPGs are shed into the alveolar space in animal models of pulmonary fibrosis and can be detected in the bronchoalveolar lavage fluid from patients with IPF (52); inhibition of this shedding with antioxidants attenuates fibrosis (53). Although HSPG shedding may enhance fibrosis, one HSPG, syndecan-4, appears to play an antifibrotic role, perhaps via sequestration of antifibrotic ligands, such as CXCL10 (54). Futhermore, heparin (in conjunction with FGF1) decreases collagen production and increases apoptosis and cell migration in IPF fibroblasts (55). These findings highlight the various and conflicting effects of HS on the pathogenesis of IPF, identifying the need for further investigation.

Conclusions

HS is critical to pulmonary homeostasis, maintaining parenchymal structure and facilitating cellular signaling necessary for lung development and function. The translational importance of HS is demonstrated by the critical contributions of changes in HS structure to pathologic events during acute and chronic lung injury. Accordingly, therapeutics targeted against HS degradation (16), sulfation (29), or modulation of HS–chemokine interactions (54) may have therapeutic value in ARDS or pulmonary fibrosis, warranting further translational study. ■

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