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Role of Heparan Sulfate in Ocular Diseases

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Abstract

Heparan sulfate (HS), a ubiquitous and structurally diverse cell surface polysaccharide and extracellular matrix component, is a factor common to several major eye pathologies. Its multitude of functions and variable distribution among the different ocular tissues makes it an important contributor to a variety of disease states. Although HS facilitates the pathogenesis of many disorders, its role in each varies. Unique functions of HS have been particularly noted in viral and bacterial keratitis and age-related macular degeneration. Combined, these pathologies comprise a large portion of conditions leading to visual impairment worldwide. Given this prevalence of diseases facilitated by HS, it is prudent to take an in-depth look at this compound in the context of these pathologic states. While the initial part of the review will discuss the pathogenic aspects of HS, it is also important to consider the wider implications of such roles for HS. The remainder of the article will specifically address one such implication, the possibility for future use of novel HS-based therapeutics to combat these eye pathologies.

Keywords

heparan sulfate; herpetic keratitis; bacterial keratitis; age-related macular degeneration; choroidal neovascularization; proteoglycans

1. Introduction¹

Ocular diseases and the prevention of subsequent vision loss remain a top health concern. For the individual, vision loss can lead to significant social and financial ramifications, including an inability to perform one's occupation and an overall reduction in the quality of

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¹ABBREVIATIONS: age-related macular degeneration (AMD); α -L-iduronic acid (IdoA); β -D-glucosamine (GlcN); β -D-glucuronic acid (GlcA); chondroitin sulfate (CS); choroidal neovascularization (CNV); complement factor H (CFH); extracellular matrix (ECM); ganglion cell layer (GCL); glycoprotein B (gB); glycoprotein C (gC); glycoprotein D (gD); glycosaminoglycan (GAG); Galactose (Gal); heparan sulfate (HS); heparan sulfate proteoglycan (HSPG); herpes simplex virus-1 (HSV-1); herpetic stromal keratitis (HSK); iduronic acid (IdoA) nerve fiber layer (NFL); N-acetylated GlcN (GlcNAc); N-sulfo-GlcN (GlcNS); retinal pigment epithelium (RPE); Xylose (Xyl); 2-O-sulfotransferase (2-OST); 3-O-sulfated heparan sulfate (3-OS-HS); 3-O-sulfotransferase (3-OST); 6-O-sulfotransferase (6-OST)

life. Significant comorbidities are also associated with poor visual health, one example being an increased risk for falls and subsequent hip fracture (Ivers et al. 2000). On a national scale, the economic toll of major vision-related health issues among the adult United States population is estimated to be \$35.4 billion annually (Rein et al. 2006). In terms of population figures, vision impairment affects more than 3.4 million individuals 40 years and older in the U.S. (Congdon et al. 2004; Crews 2003; Saaddine et al. 2003). While the current prevalence rates are striking, the epidemiological forecasts for many of these diseases are perhaps more alarming. For example, the number of individuals with early age-related macular degeneration (AMD), a disease that affects the central part of the retina (macula), is predicted to rise to 17.8 million in the age 50 and older population by 2050, double its current prevalence (Rein et al. 2009). Although these ophthalmic conditions all ultimately lead to vision loss, there are considerable differences among their respective pathogenesises. Given this diversity within ocular pathology, it would be beneficial from a therapeutics discovery standpoint to identify any entity that links these various conditions.

An important, but poorly understood, contributor common to several major eye diseases is heparan sulfate (HS). Its wide reach should not be surprising given the near ubiquitous distribution of HS throughout the human body (Esko and Lindahl 2001). HS is a member of the glycosaminoglycan (GAG) family of polysaccharides and is composed of repeating disaccharide units (Rabenstein 2002). HS can be localized to the extracellular matrix (ECM) where it can attach to protein-based cores such as agrin, perlecan and collagen XVIII or can be embedded in cell membranes attached to core proteins such as syndecan and glypican (Rabenstein 2002; Tiwari et al. 2012). In addition to AMD, which affects 6.5% of the U.S. population over the age of 40 (Klein et al. 2011), HS is also known to facilitate both bacterial and viral keratitis, which are inflammatory conditions of the cornea (Bacsa et al. 2011; Hayashida et al. 2011; Oh et al. 2010; Salameh et al. 2012; Tiwari et al. 2004). This review focuses on the several major eye diseases for which HS has been shown in the literature to play an active role (Table 1). While we will present the currently available data on HS in ocular pathology, it is our hope that clarifying these unique functions of HS will encourage additional studies on the role of HS in ocular diseases and ultimately provide a platform from which therapeutics aimed at targeting multiple eye pathologies can be developed and successfully applied.

2. Background on Heparan Sulfate

Heparan sulfate (HS) is a linear polysaccharide composed of repeating disaccharide subunits of uronic acid–(1→4)-D-glucosamine. From this core, N-sulfate, O-sulfate, and N-acetyl groups can be substituted for the disaccharide subunits, allowing for the generation of many complex heparan sulfate sequences (Rabenstein 2002). HS is conjugated to a core protein in the Golgi and can be expressed on cell surfaces as a heparan sulfate proteoglycan (HSPG). Nearly all mammalian cells are known to express and secrete HSPGs. In terms of distribution, the proteoglycans are found on cell surfaces as well as in the extracellular matrix (ECM). The HS chains themselves are rarely found alone, but rather are commonly attached to various core proteins. In fact, each of the families of HSPGs is characterized by the specific core protein they utilize. As briefly mentioned, syndecans, of which there are four members, and glypicans, of which there are six members, comprise the two biggest families of HSPGs expressed on cell surfaces (Iozzo 2001a). Of note, syndecan-1, -2, and -4 have been implicated in certain ocular diseases (Bacsa et al. 2011; Bartlett et al. 2007; Hayashida et al. 2011; Park et al. 2004; Regatieri et al. 2010) and their specific roles will be discussed in further detail later in this review. Others, such as perlecan, agrin, and collagen XVIII, comprise those HSPGs found in the ECM. A more detailed understanding of the HSPGs has become clear from recent advances in structural analysis. They appear to be in an ever-changing state, as they are targeted for the cell surface with subsequent quick

turnover rates. The specific structural modifications to HS are determined with the aim of achieving distinct and varying regulatory roles (Turnbull et al. 2001), thus generating a dynamic profile of available HSPGs.

This dynamic structural state of HS is critical to any discussion of this compound in the context of disease states. The structural complexity of HS is evident starting at its inception, during its biosynthesis in the Golgi apparatus. The initial steps in synthesis involve the placement of the tetrasaccharide β -D-glucuronic acid-galactose-galactose-xylose (GlcA-Gal-Gal-Xyl) onto serine residues of the eventual proteoglycan protein core. The HS chain itself begins with the addition of a β -D-glucoamine (GlcN) that is N-acetylated, N-acetylated GlcN (GlcNAc), to this core (Esko and Selleck 2002). As alternating GlcA and GlcNAc residues are added to this growing chain, the chain undergoes certain modifications as well (Figure 1). Three important classes of enzymes are crucial for the modifications to occur, and these include glycosyltransferases, epimerases, and sulfotransferases. The first modifications to occur are N-deacetylation and N-sulfation of the GlcNAc residue, a process that effectively creates N-sulfo-GlcN (GlcNS). Epimerase performs the next step of converting GlcA to α -L-iduronic acid (IdoA). Finally, the last modification step is that of O-sulfation performed by members of the sulfotransferases. Interestingly, this process employs three different sulfotransferases, the 2-O-sulfotransferases (2-OSTs), 6-O-sulfotransferases (6-OST) and 3-O-sulfotransferases (3-OST), with each enzyme utilized in the mentioned order (Esko and Lindahl 2001). As evidenced by the many steps involved in this modification of HS, there is ample opportunity for variations of HS to arise and, thus, for HS to play a multitude of different roles in disease states. Another detail worth investigating further is this final step of 3-O-sulfation of HS. The primary substrate utilized to make this modification is the sulfate compound of adenosine 3'-phosphate 5'-phosphosulphate (Xu et al. 2005). However, the final product, 3-O-sulfated heparan sulfate (3-OS-HS), can vary depending on the enzyme used in its modification. This is due to the fact that there are multiple members of the 3-OST family, the family of enzymes that carries out this 3-O-sulfation step. Unique variations among the members in their sulfotransferase domains allow for generation of distinct 3-OS-HS subtypes (Shworak et al. 1999; Yabe et al. 2001). An implication of this is that depending on the 3-OS-HS motif present, the functions of HS differ as well. For instance, the 3-OS-HS motif created via modifications by 3-OST-1 consists of sites that bind with high-affinity to anti-thrombin whereas other motifs created by 3-OST-2, 3-OST-3, 3-OST-4, and 3-OST-6 do not show this activity (Shworak et al. 1999). It is interesting to note that 3-O-sulfation is an extremely rare occurrence among HS modifications. This is another reason why HS is generally such a poor anticoagulant relative to heparin, which contains a significant proportion of 3-O-sulfated domains (Shworak et al. 1999).

Also important to a discussion of HS in a particular disease or set of diseases is that HSPG expression is known to be tissue dependent and to fluctuate at various time points depending on stage of embryological development, aging, and of coexisting pathological states (Brickman et al. 1998; Feyzi et al. 1998; Jayson et al. 1998; Lindahl et al. 1998; Molist et al. 1998; Safaiyan et al. 1998). Given its wide distribution, it is also not surprising that HS has a large number and diversity of known functions. These include roles in cell adhesion (Lindahl et al. 1994), modulating cell growth, regulating development (Castellot et al. 1985; Perrimon and Bernfield 2000), inhibiting blood coagulation (Marcum et al. 1986), angiogenesis (Iozzo and San Antonio 2001b; Sasisekharan et al. 1997), viral infection (Chen et al. 1997; Shukla et al. 1999), bacterial infection (Aquino et al. 2010; Bartlett and Park 2010; Chen et al. 2008; Wadström and Ljungh 1999), atherosclerosis (Varki and Freeze 2009) and tumor metastasis (Hulett et al. 1999; Vlodaysky et al. 1999).

3. Heparan Sulfate in the Eye

An examination of the distribution of HS in the various ocular tissues gives a clue to its possible roles in different ophthalmologic conditions. HS is known to be present throughout all the retinal layers, as well as in the choroid. Presumably, HS is also fairly abundant in structures with basement membranes, such as in the inner limiting membrane, which is the boundary between the retina and vitreous, in blood vessels, and in Bruch's membrane (Clark et al. 2011). In these structures with basement membranes, HS is thought to be present as HSPGs, including perlecan, agrin, and type XVIII collagen (Call and Hollyfield 1990; Witmer et al. 2001). HS also has a strong presence in various retinal layers such as the nerve fiber layer (NFL), ganglion cell layer (GCL), and retinal pigment epithelium (RPE) (Clark et al. 2011). The degree of sulfation HS possesses in each of these different tissues of the eye also differs. For instance, N- and 2-O-sulfation are more common to HS found in the inner limiting membrane, whereas 3-O-sulfation is common to HS in the ILM and the choroidal and neurosensory retinal vasculature (Clark et al. 2011). HS is also present throughout the corneal stroma (Tiwari et al. 2006) and basal laminae of ocular blood vessels (Tezel et al. 1999). The syndecan family of HSPGs, specifically syndecan-1, has also been shown to be present on corneal epithelial cells (Park et al. 2004). The perlecan family has also been shown to be an integral part of the corneal epithelial basement membrane (Inomata et al. 2012). Unlike those studies examining the specific sulfation patterns of HS across different retinal layers, those looking at these variations of HS present within the anterior chamber have yet to be performed.

4. Role of Heparan Sulfate in Herpetic Stromal Keratitis

Herpetic stromal keratitis (HSK) is a deteriorating lesion of corneal eye tissue that results from ocular infection by herpes simplex virus-1 (HSV-1) (Kim et al. 2006) and has been reported to be the principal cause of infectious blindness in developed nations (Liesegang 2001). The role of HS in HSK is demonstrated at several points throughout the HSV lifecycle. These include during viral entry into ocular cells, viral "surfing", and virus-mediated cell-to-cell fusion (Figure 2A).

HSV-1 utilizes its glycoprotein C (gC) for initial contact with the host cell as this will bind to HSPGs located on the plasma membrane of a host cell. It should be noted that the presence of gC is not essential for this interaction as another HSV-1 glycoprotein, glycoprotein B (gB), will assume gC's role in the latter's absence. The presence of HSPG, however, is crucial (Salameh et al. 2012). After this initial contact, subsequent steps allow HSV-1 to be completely internalized into the body of the host cell. One such process is "surfing," an extracellular process where the virus is able to migrate across membrane extensions shaped like filopodia in order to position itself for internalization into the cell body. HS that is present on the filopodia appears to be important in moderating viral surfing as the HSV-1 gB has been evidenced to bind to it during this process (Oh et al. 2010).

HS also interacts with another HSV-1 glycoprotein that is important in promoting HSV-1's ability to fuse with the cell membrane, glycoprotein D (gD). gD mediates its effects through interactions with any of three receptors, one of them being a member of the HS family, 3-OS-HS (O'Donnell et al. 2006; Shukla et al. 1999; Tiwari et al. 2005; Xia et al. 2002). Fusion of HSV-1 with the host cell is also facilitated by two specific types of HSPGs, syndecan-1 and syndecan-2 proteoglycan core proteins. Although these proteoglycans are composed of structurally distinguishable entities - GAGs, such as heparan sulfate, and the core proteins -, the HS moieties are the components of this compound that appear to have specific roles in this viral-cell fusion step. Evidence for this is found in the fact that although both syndecan-1 and syndecan-2 down regulation inhibit HSV-1 infection, that of

syndecan-2 does so to a greater degree (Bacsa et al. 2011). This is significant because syndecan-2 is comprised entirely of HS chains. Syndecan-1 on the other hand, consists of both HS and chondroitin sulfate (CS) chains (Rapraeger et al. 1985; Shworak et al. 1994; Su et al. 2007). Thus, a plausible explanation for greater inhibition by syndecan-2 down regulation may lie in the greater number of HS binding sites lost with syndecan-2 down regulation compared to those lost following the down regulation of syndecan-1 (Bacsa et al. 2011).

The role of HS in the HSV-1 ocular infection does not end when the virus enters the cell. Important in the propagation of HSV-1 infection is its ability to reach uninfected neighboring cells. This process of cell-to-cell fusion requires the merging of the plasma membranes from both the infected and uninfected cells, a process that forms a syncytial cell (Salameh et al. 2012). For the infected cell, HSV-1's gD mediates this process. However, for the uninfected cell, 3-OS-HS has been shown to be an important regulator of this step. Cells without 3-OS-HS have demonstrated a significant disruption in cell-to-cell fusion (Tiwari et al. 2004). *In vivo* significance of HS and 3-OS-HS in HSV-1 corneal infection have also been demonstrated recently using a mouse model (Tiwari et al. 2011). Thus, from aiding in the initial attachment of HSV-1 to the host cell to viral "surfing" and this later step of cell-to-cell fusion, HS facilitates the pathogenesis of HSK.

5. Role of Heparan Sulfate in Bacterial Keratitis

Bacterial keratitis is a major contributor to permanent vision loss worldwide as it carries with it significant risks for permanent corneal scarring and decreased visual acuity (Bourcier et al. 2003; Jett and Gilmore 2002; Limberg 1991). The pathogen *Staphylococcus aureus* continues to be the leading cause of bacterial keratitis, as 10–25% of cases have implicated this bacterial species (Green et al. 2008; Ly et al. 2006; Schaefer et al. 2001). The syndecan family of HSPGs is important in the pathogenesis of *S. aureus* corneal infection (Figure 2B). Both syndecan-1 and -4 are present in the corneal epithelium, although syndecan-1 is expressed at higher levels comparatively (Hayashida et al. 2011). Previously it had been proposed that syndecan-1 regulates bacterial infections by functioning as an HSPG ectodomain following its shedding, a process that is promoted *in vivo* in certain disease states (Bartlett et al. 2007; Bartlett and Park 2010; Bernfield et al. 1999; Sanderson 2001). It has been suggested that organisms such as *S. aureus* are able to induce the host cell's metalloproteinase-mediated shedding apparatus at the cell surface (Park et al. 2004). It has been further demonstrated that once *S. aureus* infects mouse corneal tissue, it is able to promote the shedding of syndecan-1 from corneal epithelial cell surfaces via release of its alpha- and beta- toxins (Hayashida et al. 2011). These HSPG ectodomains are then able to facilitate *S. aureus* corneal infection via a blocking effect on neutrophils in a fashion that is HS-dependent. Those affected neutrophils are no longer able to kill *S. aureus*. However, syndecan-1 is not necessary for *S. aureus* to make its initial contact with the injured corneal epithelium. This role may be filled by other HSPGs or other components of the ECM (Hayashida et al. 2011).

6. Role of Heparan Sulfate in Age-Related Macular Degeneration

AMD is a major contributing factor to vision loss worldwide. In the Western world, AMD is the number one cause of irreversible blindness (Kelly et al. 2010). AMD is characterized by destruction of the macula, the central region of the retina. This subsequently leads to impairment in vision. AMD occurs predominantly as two types, the neovascular or "wet" form and the atrophic or "dry" form (Coleman et al. 2008) and the roles of HS in the pathogenesis of this disease are evident in both forms. As a primer for a discussion on a particular role for HS in the development of AMD, it is important to note that previous

studies have localized HS to Bruch's membrane (Call and Hollyfield 1990), an important area for pathogenesis of AMD. In addition to the presence of HS, Bruch's membrane has been noted to display dynamic changes that are dependent on age and existing disease states (Nadanaka and Kitagawa 2008; Oppermann et al. 2006; Rabenstein 2002).

6.1 HS Links Choroidal Neovascularization and AMD

Among the various etiologies leading to AMD, choroidal neovascularization (CNV) remains the leading cause (Dreyfuss et al. 2010a). CNV is a process that occurs in the neovascular type of AMD when newly formed capillaries extend from the choroid through Bruch's membrane and gain entry into the retina (Gass 1973; Teeters and Bird 1973). If this process is left uninterrupted, AMD will eventually lead to fibrosis beneath the macula, which ultimately leads to a decrease in macular photoreceptors and sensory retina degeneration (Teeters and Bird 1973). To understand the role of HS in CNV, it is important to look at the regulatory factors of this CNV process. CNV is kept in check in large part by various angiogenic agents, including growth factors, cytokines and ECM components, such as GAGs (Campochiaro 2000; Dreyfuss et al. 2009). HS has a particular role in promoting the angiogenesis that occurs in both healthy and disease states (Dreyfuss et al. 2010b; Matavelli et al. 2009; Sampaio et al. 2006; Soler et al. 2008; Tkachenko et al. 2005). HS mediates its regulatory role of CNV mainly via its interaction with various angiogenic growth factors, including FGF, VEGF, TNF- α , TGF- β , and IFN- γ (Dreyfuss et al. 2010a) (Figure 2C). Further evidence is documented from an observation of the expression profiles of two specific HSPGs, perlecan, a basement membrane HSPG, and syndecan-4, an endothelial cell surface HSPG, in response to a laser-induced CNV. Rat retinas with laser-induced CNV showed significant upregulation of expression profiles for both perlecan and syndecan-4 when compared to control retinas. The expression profiles of these HSPGs were found not only to depend on the presence or absence of CNV, but also on the size of the CNV lesion (Regatieri et al. 2010). A previous study had suggested that the higher expression of HSPGs in CNV lesions may be linked to the endothelial dysfunction and increased capillary permeability (Jiang and Couchman 2003). Interestingly, perlecan located on basement membranes was the most overexpressed of the HSPGs. Of significance, this particular HSPG can serve as a scaffold for blood vessel formation (Jiang and Couchman 2003), regulating the effects of angiogenic growth factors (Regatieri et al. 2010). Furthermore, the upregulated expression of these HSPGs were noted on the outer layers of the retina, an area which is related to choroidal damage (Regatieri et al. 2010).

6.2 HS Links the Complement Activation System and AMD

HS also implicates itself in the pathogenesis of AMD via its regulation of an integral component of the body's innate immune system, the complement activation system. Previous findings have linked the complement system to the development of AMD. These include an initial finding that the extracellular deposit drusen consists of factors involved in activating complement (Anderson et al. 2010; Hageman et al. 2001). Accumulation of drusen between the retinal pigment epithelium and Bruch's membrane, an ECM separating the retina from the choroidal blood vessels, can lead to blindness. Drusen buildup is also correlated to the amount of inflammation that occurs when there is inadequate inactivation of the complement system where the RPE and Bruch's membrane meets (Hageman et al. 2001). In addition, genetic variations of certain complement proteins have been strongly linked to increased risk of developing AMD. This association is pertinent among the genetic etiologies of AMD as specific genetic alterations of complement factor H (CFH) are potentially responsible for nearly half of the population-based risk for developing AMD (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005; Klein et al. 2005). CFH is a glycoprotein that modulates the complement system's alternative pathway (Clark et al. 2010a). CFH is also essential in balancing the inactivation and activation states of the

complement system in plasma and, thus, is critical in host cell and tissue protection (Rodríguez de Córdoba et al. 2004). Previous studies have identified a specific CFH gene polymorphism, Y402H, which is a variation has been associated with increased risk for AMD development (Despriet et al. 2006; Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005; Klein et al. 2005) and is thought to comprise the major genetically-based risk factor for AMD (Hecker et al. 2010). An examination of the association between this specific allotypic variant and increased risk of AMD helps bring another potential role of HS in AMD pathogenesis to the forefront. Clark et al. examined CFH binding specifically in the human macula (Clark et al. 2010b). The allotypic variant 402H was found to have decreased binding sites within Bruch's membrane and the choroidal blood vessels when compared to the number of binding sites for the wild type variant 402Y. The fact that there was decreased binding in Bruch's membrane was thought to be particularly important because this is where drusen forms and accumulates (Clark et al. 2010b). Later studies, however, did not indicate a decreased number of binding sites within Bruch's membrane between the allotypic variants of CFH (Kelly et al. 2010). However, these later studies also did not rule out differences in activity levels between the variants. It is possible that minor region-dependent GAG alterations might induce varying activity levels between the mutant and wild type CFH (Kelly et al. 2010) (Figure 2D). Although there are conflicting data on the effect on number of HS binding sites between the variants, a role of HS in the binding of either allotypic CFH is likely. Tissue sections pretreated with heparinase enzymes, enzymes that degrade HSPGs, demonstrated reduced CFH binding sites in both the RPE and Bruch's membrane by 70% (Clark et al. 2010b), verifying that HS is the receptor CFH primarily binds. Not only is the presence of HS essential for CFH to properly bind within the RPE and Bruch's membrane, but its sulfation patterns also play a key function (Clark et al. 2010b). It was further demonstrated in fluid-phase assays that the addition of highly sulfated HS moieties slows the inactivation rate of complement attributed to CFH (Kelly et al. 2010). Addition of the least sulfated HS moiety, however, increased the rate of complement inactivation by CFH (Kelly et al. 2010). The 402H CFH relied on the interactions with N-, 2-O-, and 6-O-sulfate moieties. The 402Y variant did not demonstrate this reliance (Clark et al. 2006). Studies that can demonstrate how CFH distribution is affected endogenously by these changes in GAG interactions still need to be determined before a clear link can be established between HS and a form of pathogenesis of AMD (Clark et al. 2010b). In addition, data explaining why the clinical manifestations of this CFH polymorphism are restricted to the eye need to be obtained. It is possible that the variable expression of specific GAG structures across multiple tissue types could provide a clear explanation for this (Clark et al. 2010a). Age may also play a factor in determining when this polymorphism manifests itself phenotypically, as GAG structures are known to be prone to age-dependent changes (Clark et al. 2006; Feyzi et al. 1998).

7. Potential Heparan Sulfate-Based Therapeutics in Ocular Disease

One benefit of elucidating a more defined role of HS in ocular diseases is the opportunity for advances in therapeutics. With greater understanding of the key roles of HS in the pathogenesis of these diseases, researchers have already begun to target the specific HS-based interactions that allow for vision impairment. Gains in deducing the structural complexities of HS have also aided this in progress. Targeting HS-based interactions may be a clinically novel and promising approach to combating these pathologies.

Current therapies for HSV-1 keratitis consist of various regimens of antiviral and anti-inflammatory drugs (Vajpayee et al. 1996). Given the role of HS in HSV-1 ocular infection, it is possible new therapeutics can exploit the importance of HS for HSV-1 to help decrease incidence rates of HSK. One proposed method utilizes the application of fibroblast growth factor-2 (FGF-2) (Kim et al. 2006). Although it has been proposed that the FGF-2 protein

exerts its therapeutic effects by inducing ulcer healing following SK lesions, previous studies have shown that FGF-2 can inhibit the binding of HSV-1 to HS (Baird et al. 1990; Kaner et al. 1990). FGF-2 possibly does this by competitively binding to HS on host cells (Kaner et al. 1990; Mirda et al. 1992). However, long-lasting antiviral effects upon FGF-2 application in corneas were not detected (Kim et al. 2006). Only temporary antiviral effects were noted. This inability to achieve permanent inhibition of HSV-1 may be related to an inefficient delivery mechanism, one which does not currently allow for sufficient concentrations of FGF-2 to reach the corneal epithelium where the receptors are located (Kim et al. 2006). In addition to an incomplete picture of FGF-2, potential side effects of FGF-2 should be noted, including disruption of cytokine expression (Zhang and Issekutz 2001), production of prostaglandins (Kawaguchi et al. 1995), nitric oxide induction (Goureau et al. 1995; Murphy et al. 2001), and neutrophil interactions with endothelial cells (Zhang and Issekutz 2001). While these candidates may show promise, other therapeutic development approaches based on knowledge of the role of HS in HSV-1 infection have led to the development and successful use of peptides that bind to specific receptors of the HS family. One investigation examined two peptides, G1 and G2, which bind to HS and 3-OS-HS, respectively (Tiwari et al. 2011). Mouse corneas were treated with these peptides or control peptides and then subsequently infected with HSV-1. Compared to controls, mouse corneas with either G1 or G2 peptide had intact epithelium and essentially no trace of HSV-1 protein expression. Controls had noticeable damage to the corneal epithelium. In addition to playing a role in HSV-1 ocular infection *in vivo*, these peptides serve as potential means for prophylaxis against this viral infection of the eye (Tiwari et al. 2011).

Many of the current medical therapies for AMD exert their actions after neovascularization has already occurred. Inhibitors of angiogenesis may hold much promise in regards to CNV and AMD. These are quickly gaining reputations as novel potential drugs in the fight against cancer and other degenerative pathologies (Loges et al. 2009). One particular angiostatic drug class comes from a class of compounds that can mimic HS structurally, the sulfate oligosaccharides. By interfering with the ability of HS to facilitate angiogenesis, these compounds can achieve therapeutic potential (Dreyfuss et al. 2010a). Currently, anti-VEGF monoclonal antibodies are being administered intravitreally (Loges et al. 2009; Regatieri et al. 2009) to prevent the interactions between HS and these growth factors that are known to lead to angiogenesis. Based on experimental data, it is not out of the realm of possibility that other agents, such as anti-TNF- α monoclonal antibodies, can be applied in a similar fashion for use in AMD afflicted individuals (Regatieri et al. 2009). Dreyfuss et al. also examined possible HS-based therapeutics by applying a shrimp heparinoid as a possible anti-CNV agent (Dreyfuss et al. 2010a). The shrimp heparinoid is a compound that is similar to HS, but with the distinguishing factor of having a low number of 2-O-sulfate moieties. This is important as this property has been suggested as a possible explanation for the insignificant anticoagulant or hemorrhagic effects of this shrimp heparinoid compared to other heparin compounds. When applied, shrimp heparinoid induces disruptions in the interactions that various growth factors would normally have with HSPGs found in the ECM. Apparently, the shrimp heparinoid can bind to VEGF, but is unable to facilitate its ability to induce mitogenesis due to the lack of these 2-O-sulfate groups. The results from the study show significant decreases in the degree of CNV in the shrimp heparinoid treated versus untreated control groups. Results also indicate that introduction of shrimp heparinoid leads to decreased levels of VEGF and TGF- β within the area affected by CNV. These angiogenic factors were also decreased in retinal and choroidal tissues. This compound's angiostatic effect may occur via competitive inhibition with cell surface HSPGs for growth factors (Dreyfuss et al. 2010a).

8. Discussion

Although HS has a near ubiquitous presence throughout mammalian cells, the intricacies of HS's composition have kept HS-based therapeutics at bay. Ironically, it is this same structural complexity that now can provide a unique advantage to both researchers and clinicians in targeting this compound for therapeutic benefit. The reason for this initial hesitancy has partly been due to the dilemma of how to target specific HS moieties without incurring the adverse effects targeting such a ubiquitous compound might reasonably entail. One of the more systemic adverse effects necessary to avoid is one which pertains to the body's coagulable state. Heparin, a close analogue of HS, and, as mentioned earlier, the 3-OS-HS motif modified by 3-OST-1 both have important roles in anticoagulation (Rabenstein 2002; Shworak et al. 1999). With recent advances that have further deciphered the enigma that has been the structural composition of HS, a new understanding of the diversity of HS has emerged. As mentioned earlier, in the example of 3-O-sulfation, the body utilizes up to seven different enzymes to create this modification, with the end result being different 3-OS-HS motifs that vary in functionality (O'Donnell et al. 2010). Thus, although HS is ubiquitous, there is a sense of novelty to each type of HS. This fosters the ability to specifically isolate the motif of interest and allows for the targeting of specific functions. Recent work in our lab with G1 and G2 peptides targeting 3-OS-HS interactions with HSV-1 gD is just one example of progress that has benefited from this targeted specificity. The creation of novel peptides to block HSV-1 interaction with cell surface 3-OS-HS is critical in showing the potential that can be realized with such therapeutics (Tiwari et al. 2011).

As advances in HS-therapeutics continue to be made, certain challenges must be concurrently overcome. A main challenge would be to further uncover the specific modifications of the HS molecules involved in the major eye diseases for which HS has generally been shown to play a role. This would allow for more specific targeting of only those HS moieties that are implicated. Another obstacle is that certain pathologies appear to invoke HS in order to carry out its pathogenesis. In other pathologic states, it is a deficiency of HS that promotes visual impairment. For any therapeutic to achieve clinical benefit, it would have to maneuver this fine line between over- and under- activity of HS. Given the varying distribution profiles of HS among the different anatomical and physiological regions of the eye, prospects of overcoming this barrier are promising. Another challenge is that in cases where HS activity must be targeted, there is evidence to suggest that the structure of HS may undergo active conformational changes during pathogenic conditions. This has recently been noted specifically with observations of HS changes during microbial infections (Ali et al. 2012). It is not out of the realm of possibility, then, that HS may also demonstrate non pathogen-related, pathology-dependent changes in structure that render precise targeting of its compound more difficult. Furthermore, investigation into more precise and effective delivery mechanisms would also be prudent in efforts to minimize adverse pharmaceutical effects. Additionally, the roles of HS in the pathogenesises of these diseases must be understood in the context of the entire pathogenic picture. Similar to how HS has a potential interaction with CFH in its facilitation of AMD, it is possible other important players in HS-mediated ocular conditions have yet to be uncovered. There have also been reports on the upregulation of certain HSPGs in AMD (Regatieri et al. 2010) and work in our laboratory suggests increased expression of HS during HSV infection (Ali et al. 2012). Thus, it is also possible that HS may serve as a potential marker for the diseased state.

Goals of future studies, then, should work toward addressing these challenges. If these can elucidate further insights into HS's roles in these diseases, we may be able to realize the clinical potential of the already promising initial investigations. Given the progress made

thus far, we remain optimistic regarding this largely untapped and promising source of novel therapeutics against the major conditions that threaten vision health today.

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Highlights

- We reviewed the literature to identify roles of heparan sulfate in ocular diseases
- Heparan sulfate aids in the pathogenesises of herpetic and bacterial keratitis
- Heparan sulfate has roles in the pathogenesis of age-related macular degeneration
- Targeting heparan sulfate may be a novel way to prevent vision-threatening diseases

Figure 1.

HS chain modifications. An unmodified HS chain, shown at top, consists of repeating disaccharide subunits of uronic acid $-(1\rightarrow4)-D$ -glucosamine. From this unmodified chain, specific modifications occur in the order demonstrated: 1) N-Deacetylation and N-Sulfation. 2) C5 epimerization. 3) 2-O-Sulfation. 4) 6-O-Sulfation. 5) 3-O-Sulfation.

Figure 2.

Role of HS in various eye pathologies. (A) At the initial stages of herpes stromal keratitis, HSV utilizes either of its glycoproteins, gB or gC, to bind to HS on filopodia. Viral surfing subsequently follows via gB-HS interactions. At the cell surface, HSV interacts via gD with 3-OS-HS, an interaction that leads to cell uptake of the HSV structure. Not shown in the figure is the eventual cell-to-cell spread that HSV undertakes to mediate its propagation. (B) Release of alpha/beta-toxins by *S. aureus* activates the host cell's metalloproteinase shedding mechanism, which leads to shed ectodomains of HSPGs that render neutrophil inactivation of the bacterial pathogen ineffective. (C) HSPGs located throughout the choroidal layer of the retina play an important role in CNV as they interact with various angiogenic factors. (D) A proposed impaired binding of CFH to HS in the choroidal blood vessels and in BM, either through a decreased number of binding sites or alterations in HS, is believed to play a role in decreased CFH inactivation of the CAS. Abbreviations: BM, Bruch's membrane; CAS, complement activation system; CFH, complement factor H; GCL, ganglion cell layer; gB, glycoprotein B; gC, glycoprotein C; HS, heparan sulfate; HSPG, heparan sulfate proteoglycans; HSV, herpes simplex virus; NFL, nerve fiber layer; RPE, retinal pigment epithelium; 3-OS HS, 3-O sulfated heparan sulfate.

Table 1

Role of HS in Ocular Diseases

Disease	Role	Pathologic Implications	References
AMD	CNV regulation CHF binding	Unregulated angiogenesis Varying abilities to bind to HS lead to decreased inactivation of complement activation system	Dreyfuss et al. 2010a; Jiang and Couchman 2003; Regatieri et al. 2010 Clark et al. 2010a; Clark et al. 2010b; Kelly et al. 2010
Bacterial keratitis	Shed HSPG ectodomain	Blocking effect on neutrophils targeting <i>S. aureus</i>	Hayashida et al. 2011
Herpetic stromal keratitis	HSV-1 gB, gC, gD binding	Mediates HSV-1 viral-cell fusion and cell-to-cell spread	Bacsa et al. 2011; O'Donnell et al. 2006; Oh et al. 2010; Salameh et al. 2012; Shukla et al. 1999; Tiwari et al. 2004; Xia et al. 2002

Abbreviations: AMD, Age-related Macular Degeneration; CHF, complement factor H; CNV, corneal neovascularization; gB, glycoprotein B; gC, glycoprotein C; gD, glycoprotein D HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; HSV-1, herpes simplex virus-1