Heparanase as active player in endothelial glycocalyx remodeling

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Abstract

The surface of all animal cells is coated with a layer of carbohydrates linked in various ways to the outer side of the plasma membrane. These carbohydrates are mainly bound to proteins in the form of glycoproteins and proteoglycans and together with the glycolipids constitute the so-called glycocalyx. In particular, the endothelial glycocalyx that covers the luminal layer of the endothelium is composed of glycosaminoglycans (heparan sulphate -HS and hyaluronic acid -HA), proteoglycans (syndecans and glypicans) and adsorbed plasma proteins. Thanks to its ability to absorb water, this structure contributes to making the surface of the vessels slippery but at the same time acts by modulating the mechano-transduction of the vessels, the vascular permeability and the adhesion of leukocytes in thus regulating several physiological and pathological events. Among the various enzymes involved in the degradation of the glycocalyx, heparanase (HPSE) has been shown to be particularly involved. This enzyme is responsible for the cutting of heparan sulfate (HS) chains at the level of the proteoglycans of the endothelial glycocalyx whose dysfunction appears to have a role in organ fibrosis, sepsis and viral infection. In this mini-review, we describe the mechanisms by which HPSE contributes to glycocalyx remodeling and then examine the role of glycocalyx degradation in the development of pathological conditions and pharmacological strategies to preserve glycocalyx during disease pathogenesis.

Introduction

The endothelium is defined histologically as a typical example of a simple paving (squamous) epithelium lining the blood and lymphatic vessels. It is formed by a single continuous layer of flattened polygonal cells joined and aligned according to the direction of the blood or lymphatic flow. Especially at the level of blood capillaries, the phenotype of endothelial cells can change according to the organs and the function they perform there [1]. As a proof of concept in the kidney, the endothelial cells of the glomerulus capillaries are fenestrated to allow blood filtration while those of the larger diameter vessels are characterized by the presence of occluding junctions between contiguous cells [2]. Like all epithelial lining tissues, endothelial cells also present a gelatinous layer of a glucidic nature called glycocalyx at the lumen level, which effectively constitutes an element of the vascular barrier [3]. Initially considered to be a simple protective barrier for cells, the glycocalyx has in recent years been recognized as one of the main barriers responsible for endothelial func-

tions. It was also found that alterations of this structure are at the basis of some pathological situations such as sepsis, fibrosis, metabolic diseases and chronic cardiovascular and renal diseases [4–7]. The purpose of this review is to describe not only the composition and function of the endothelial glycocalyx, but also the responsible mechanisms for its degradation by heparanase-1(HPSE) and then to discuss possible strategies aimed at safeguarding this cellular structure.

Endothelial glycocalyx structure and functions

The glycocalyx of vascular endothelial cells is mainly composed of side chains of glycosaminoglycans (GAGs) conjugated with the protein axis of proteoglycans linked to the plasma membrane (basically syndecans 1, 2 and 4 and glypican 1), by HA bound by its receptor, as well as from glycoproteins and adsorbed plasma proteins are attached [8,9]. Structurally speaking, proteoglycans consist of a protein core to which long linear chains of negatively charged GAGs including heparan sulfate (HS), heparin (Hep), chondroitin sulfate (CS), keratan sulfate (KS) and dermatan sulfate (DS) [10]. Thanks to the numerous sulphate groups, the GAGs are negatively charged and this gives them the ability to bind considerable quantities of water, contributing to tissue hydration and mechanical resistance to compression. In addition, they can bind other molecules including growth factors, cytokines and enzymes with a storage function, thus protecting them from degradation [11]. They also help to create a gradient necessary for fluid transit and blood filtration in the kidney [12].

The core protein to which the GAGs of the endothelial glycocalyx bind belong to the group of transmembrane syndecans and to glypican-1 (GPC1) which binds to the membrane by means of GPI anchors. In particular, syndecans-1, -2, -4 (SDC1, SDC2, SDC4), have three attachment sites for HS in the N-terminal regions most distant from the cell surface while SDC1 also contains two additional sites for CS closer to the membrane [13]. The cytoplasmic tails of syndecans bind to various cytoskeletal proteins through connecting molecules helping to distribute pressure and shear blood forces over the entire endothelial cell [14]. GPC1 is the only glypican present in the endothelial glycocalyx and has three or four attachment sites exclusively for HS. GPC1 indirectly binds to the plasma membrane by anchoring with glycosylphosphatidylinositol (GPI) which localizes this proteoglycan at the level of the lipid rafts [13].

HA is the only non-sulfated GAG found mainly in the luminal part of the glycocalyx and which is synthesized on the cell surface by specific enzymes (HAS1, 2 and 3) [15]. Unlike HS and CS, it is a longer disaccharide polymer which does not covalently bind to any protein but is anchored to the cell membrane by specific surface receptors such as CD44. Although lacking in sulphate groups, HA contains numerous negative charges due to the presence of carboxylic groups which give it considerable hydration capacity [16].

Furthermore, at the endothelial glycocalyx level, glycoproteins with short oligosaccharide chains attached to their protein nucleus can also be found. These oligosaccharides are covered at their ends by molecules of sialic acid (SA), a 9-carbon monosaccharide that contributes to the net negative charge of the glycocalyx [17]. Other glycoproteins are considered to be part of the glycocalyx and include some important membrane receptors, including mechano-sensors and transducers such as selectins and integrins, and members of the immunoglobulin superfamily. Some plasma proteins such as albumin, antithrombin and alpha 1 acid glycoprotein can get trapped at GAG levels and thus further extend the glycocalyx layer [18,19].

Mechanisms of glycocalyx degradation

Several acute and chronic conditions such as sepsis, ischemia/reperfusion (I/R) injury, diabetes, trauma, atherosclerosis, hypertension, kidney and acute lung injury are characterized by endothelial glycocalyx degradation [20–25]. Endothelial glycocalyx degradation determines the release of its components, i.e. HA, HS and/or proteoglycans (PGs) such as SDC1, into the bloodstream, where they can be measured to provide information about endothelial glycocalyx damage [26–28] since most of these parameters correlate with the severity of disease and mortality [23,29]. The mechanisms prompting endothelial glycocalyx are various, and they impact each other fueling the degradation of the glycocalyx: reactive oxygen/nitrogen species (ROS/RNS), matrix metalloproteinases (MMPs), hyaluronidases (HYAL) and HPSE. (Fig. 1).

Oxidative stress and ROS/RNS have a central role in endothelial glycocalyx degradation in an inflammatory context [30]. Two typical ROS components are peroxides and radicals; small molecules that are able to form chemical bond with DNA, lipids and proteins. ROS and RNS are also able to damage the GAGs in the glycocalyx by modifying the saccharide residues. The modified sugars are unstable and susceptible to hydrolytic cleavage which leads to the fragmentation of the polysaccharides. The unsulfated high-molecular weight polymer of HA are particularly vulnerable to chemical modifications and the derived low-molecular weight HA fragments have a pro-inflammatory activity sustaining, in turn, ROS production [31–35].

In physiological conditions, ROS is also essential for signaling mechanisms, regulating e.g. proliferation, differentiation and migration under physiological conditions [36] and their levels are controlled by antioxidant enzymes for ROS inactiva-
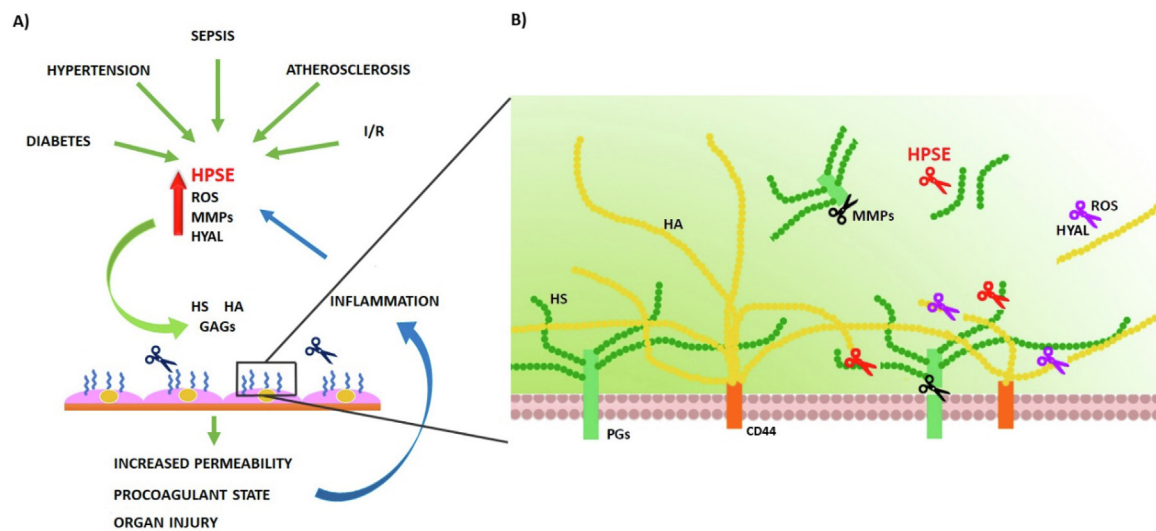
Fig. 1. A) Several chronic and acute pathological conditions are responsible of endothelial glycocalyx damage. During sepsis, global and regional I/R, diabetes mellitus, hypertension, atherosclerosis a series of factors (HPSE, MMPs, HYAL and ROS) are produced and they contribute to glycocalyx shedding/depolymerization targeting HS, HA and proteoglycans. Glycocalyx dysfunction impairs the local microcirculation increasing procoagulant state leading to organ injury. Sub-endothelial damage together with cytokines released by glycocalyx shedding induce an inflammatory state which in a vicious loop sustain glycocalyx degradation. B) In detail, different glycocalyx components are degraded by different agents. HS is cleaved by HPSE, PGs are shedded by MMPs and HA is cleaved by HYAL and degraded by ROS.

tion, such as SOD, catalases and glutathione per-oxidases [37]. However, under pathological conditions such as atherosclerosis and I/R injury this balanced state is altered.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which cleave extracellular matrix components such as collagen, gelatin and elastin, thereby promoting vascular remodelling [38]. During vascular pathologies MMPs are mainly produced by inflammatory cells, but also by stimulated vascular smooth muscle cells [39]. Important activators of MMPs and inactivators of tissue inhibitors of MMPs (TIMPs) are ROS [40], but cytokines, shear stress, hypoxia and hormones may also lead to enhanced proteinase activity in atherosclerosis, hypertension, chronic venous disease, and aneurysm formation [41,42].

MMPs degrade endothelial glycocalyx by cleaving the protein core of proteoglycans, such as syndecans [43]. Shedding of syndecans can represent a measure of glycocalyx degradation [44–46]. Specifically, SDC-1 is shed by MMP-2, MM-9, MT1-MMP and ADAM-17 and, SDC-2 is cleaved by MMP-9 [22,47]. MMPs can also degrade chondroitin sulphate [48]. The action of MMPs reduces glycocalyx thickness [25] and causes the release of heparan sulphate and syndecans into the bloodstream sustaining thrombosis and endothelial inflammation [47,49].

Another important constituent of endothelial glycocalyx is hyaluronic acid (HA), a linear, non-sulfate, negatively-charged glycosaminoglycan that consists of glucuronic acid and n-acetylglucosamine

repeating units. HA within the glycocalyx binds non-covalently to the receptor CD44 [50]. The principal human hyaluronidases are HYAL1 and HYAL2 whose expression is increased in hyperglycemic and/or inflammatory settings as proved by several studies [51–53]. Another important regulator of hyaluronidases is shear stress which specifically increases HYAL4 expression which in turn downregulates NO expression [54]. The action of hyaluronidase on the glycocalyx produces low-molecular-weight HA fragments. High- and low-molecular-weight HA have very dissimilar properties. High-molecular weight HA enhances the barrier function of endothelial cells, while low-molecular weight HA is dangerous for endothelial cells, since it activates toll-like receptors 2 and 4, which triggers inflammation [47]. Low-molecular-weight HA fragments can also stimulate ROS production in a size-dependent manner and inducing the expression of VCAM-1 and CM-1, fueling inflammation and damage to endothelial cells [47].

Considering that between 50 % and 90% of the GAGs in the glycocalyx consist of HS [55], it is not difficult to predict how HPSE, an enzyme involved in the degradation of HS, can play a fundamental role in the turnover of the glycocalyx associated with different pathological situations.

The role of heparanase in glycocalyx degradation

Heparanase (HPSE) is defined as an *endo*-β-D-glucuronidase belonging to the group of glycosidic

hydrolases or enzymes capable of processing carbohydrates. The cleavage site on which HPSE intervenes is the β 1,4 glycosidic bond between GlcA and GlcNS in the chains. Only a limited number of sites are cut by HPSE thus releasing 5–10 kDa fragments of HS (10–20 sugar units). The human gene encoding heparanase-1 (HPSE) has been mapped to chromosome 4q21.3 and can give rise to two mRNAs containing the same open reading module as a result of alternative splicing [56,57]. Shortly thereafter, a second homologous gene with HPSE, named heparanase-2, was identified. It encodes the HPSE-2 protein which has an homology sequence of 40%. Unlike HPSE, this variant does not exhibit glycosidase activity but appears to have an inhibitory role against HPSE thus hypothesizing a role as a tumor suppressor [58].

HPSE is synthesized as a 65 kDa proenzyme which undergoes a post-translational cut, generating two subunits of 50 kDa and 8 kDa which are not covalently connected and which constitute the active form of the enzyme [59]. The glycosylation of HPSE is fundamental for its transport through the endoplasmic reticulum and the Golgi apparatus and its secretion [60]. The conversion and activation of the inactive pro-HPSE into the active dimeric enzyme then requires its reabsorption by endocytosis where at the lysosomal level the cathepsin L catalyzes the cut that originates the two subunits that form the mature enzyme. Mannose 6-phosphate and low-density lipoproteins receptors have been described as high-affinity receptors for HPSE while membrane HSPGs (in particular, syndecans) as low-affinity receptors [61]. In non-pathological tissues and in normal physiological conditions, HPSE usually shows low levels of protein expression limited mostly to keratinocytes, trophoblast, platelets and leukocytes [62]. On the contrary, in pathological conditions such as in tumor progression and metastasis, in inflammation, during the epithelial-mesenchymal transition (EMT) and in fibrosis there is a notable increase in expression for HPSE [63,64,65].

In the vascular endothelium it has been reported that the expression of HPSE is upregulated at the site of inflammation in multiple organs [65]. Its expression is up-regulated in endothelial cells by several factors: ROS [66], inflammatory cytokines [67], high glucose [68] and advanced glycosylation products [69]. HPSE is also released by inflammatory cells and platelets [70] within the vasculature.

HPSE can modulate glycocalyx damage in various ways: first, by degrading HS, HPSE controls the communication of endothelial cells with blood cells [71]. In fact, the degradation of heparan sulfate chains renders the proteoglycan core protein more accessible to enzymatic cleavage by MMPs and thrombin [72,73]. By cleaving HS, HPSE also regulates vascular permeability [74], and makes adhesion molecules and cytokine receptors more accessible for binding and activation, thus propagating inflammation [75,76,77,78]. Specifically, in inflammation, factors such as TNF-α promotes the activation of vascular endothelial cells by increasing P- and E-selectin expression. Subsequently, leukocytes begin to roll and attach to endothelial cells, and chemokines on endothelial cells start to activate followed by chemokine activation of the leukocytes [79,80]. Following leucocyte attachment to the endothelial cell surface, they traverse into the interstitial tissue and inflict inflammatory damage to the organs [81]. The release of pro-inflammatory cytokines and growth factors linked to HS sustain oxidative stress with an additional fueling of inflammation [82]. Moreover, HS fragments released by HPSE activate toll-like receptor (TLR) 4 signaling [77] and concentrate growth factors and cytokines to an easier ligand recognition by cognate cell surface receptors [83]. TLR activation on macrophages activates Nuclear factor-kB (NF-kB) leading to expression of additional inflammatory cytokines (TNF-α, IL-1β, IL-8) [84]. The cytokines can also stimulate HPSE expression on endothelial cells [68] and potentiate release of ROS and MMPs [85].

HPSE also has non-enzymatic activity on endothelial cells through ERK activation and intracellular regulation of SDC-1 expression [86]. HPSE may contribute to MMP-9 and VEGF upregulation and trauma [87,88,89]. It has been established that an altered profile of circulating exosome levels is associated with the onset of critical illness [90,91] and HPSE may sustain secretion of exosomes by activating the syndecan–syntenin–ALIX complex that engages the endosomal-sorting complex required for transport (ESCRT) [92,93].

The shed glycocalyx layer prejudices intracellular production of endothelial nitric oxide synthase (eNOS) [94,95]. Reduced eNOS activity diminishes nitric oxide (NO) production leading to impaired vascular reactivity. NO then promotes modifications in the exocytosis of Weibel–Palade bodies [96,97] which favors the release of von Willebrand factor and angiopietin-2. In the end, these molecules activate circulating platelets and promote endothelial destabilization [98,99].

HPSE also contributes to the pro-coagulant state by increasing tissue factor (TF) activity resulting in increased activation of the coagulation system [85]. Moreover, HPSE up-regulates TF expression in endothelial cells and the release of the protein tissue factor pathway inhibitor (TFPI) from the cell surface [100].

In summary, the consequences of HS cleavage lead to an activated and inflamed endothelium that results in tissue edema and hypoperfusion that sustain end-organ injury.

HPSE dependent glycocalyx dysfunction conditions

Glycocalyx dysfunction arises in response to mechanical cellular stress, endotoxins,

inflammatory mediators, atrial natriuretic peptide, ischemia–reperfusion injury, free oxygen radicals and hyperglycemia, and also the novel coronavirus disease-2019 (COVID-19) [47,101].

Glycocalyx degradation occurs in infective (sepsis) and non-infective (trauma) inflammation [102] and in these setting TNF-α is a central player [73]. Sepsis induces glycocalyx degradation and leads to delayed regeneration [103]. Afterwards, this setting also stimulates the recruitment and phenotype alterations of macrophages [104,105] as well as leukocyte adhesion and focal vascular inflammation [74]. HPSE enzymatic activity and syndecan-1 shedding are increased in trauma [106,107,108] and sepsis [29,109,110] patients and they correlate with the degree of illness [44] and these results has been validated in a study of human and murine study [111]. Also, both septic patients with lung injury have increased levels of circulating HS [112] and sepsis-induced acute kidney injury (AKI) patients show augmented urinary HS [113] that may reflect increased HPSE levels due to the inflammatory state [114]. The central action of HPSE in sepsis is also confirmed by the fact that its inhibition in preclinical models mitigates lung injury and renal dysfunction [73,115].

Hemorrhagic shock is one of the main responsible of endothelial glycocalyx derangement and endothelial injury, characterized by the disruption of junctional structures [116,117]. Ischemia/reperfusion (I/R) event exerts a damage at two timepoints; firstly, glycocalyx damage arises during the hypoxic phase, and secondly during blood reperfusion. Consequently, endothelial cells become swollen and detached from the basement membrane. I/R injury also induces the release of a series of molecules (HPSE, histamine, cathepsin and oxygen-free radicals)which contribute to glycocalyx impairment [118,119]. This evidence has also been confirmed by the fact that glycocalyx components, such as heparan sulfate and SDC-1, can be detected in soluble form in the plasma of patients undergoing major vascular surgery with global or regional ischemia [24].

Endothelial dysfunction is generally considered to be one of the initial events in atherosclerosis and HPSE also participates in this process. Inflammatory mediators present in atherosclerotic lesions lead to the upregulation of angiopoietin 2 by endothelial cells and foam macrophages; angiopoietin 2, in turn, increases the expression of HPSE, leading to the degradation of heparan sulfate [47,120]. Then, shed HS fragments activate leukocytes and platelets, increase the expression of ICAM1 and VCAM1, and the consequent leucocyte adhesion and extravasation [47,76,121].

Alterations in the endothelial glycocalyx occur early in the onset of diabetes, a pathology in which the vasculature is disrupted globally [122,123,124]. HPSE has been deeply implicated in diabetes and its consequences [125]

The coronavirus disease-19 (COVID-19) pandemic has resulted from infection of human with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In the last two decades, other members of the coronavirus family have emerged: the severe acute respiratory syndrome coronaviruses (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) [126]. Globally, as of 13 October 2021, there have been 238,521,855 confirmed cases of COVID-19, including 4,863,818 deaths (World Health Organization).

The glycocalyx is an essential component of cell surfaces and it is the first barrier against all pathogens. Given its location, viruses and other pathogens have evolved to utilize glycocalyx components as attachment factors which facilitate their interaction with cell surface receptors [127]. Different tissues and cell types show differences in HS structure [128] and HS structure can vary between individuals and age groups [129]. These differences may contribute to tissue tropism from different pathogens. For example, several viruses use salicylic acids, while other viruses, such as coronaviruses, interact with heparan sulfate (HS) [130]. The angiotensin-converting enzyme 2 (ACE2), previously identified as the cellular receptor for SARS-CoV, also acts as a receptor of the new coronavirus (SARS-CoV-2) [131]. ACE2 is necessary but insufficient for infection, whereas interaction with both ACE2 and HS is required to initiate a productive infection [130]. Clausen et al 2020 have shown that the interaction of the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein with cell surface HS promote a conformational change in an "open" active conformation that favors ACE2 binding. Competition studies, enzymatic removal of HS and genetic studies confirm that binding of HS to S protein enhances binding to ACE2 receptor [132].

After infection, replication and dissemination of SARS-CoV-2, COVID-19 patients primarily present common flu symptoms such as fever, muscle pain and cough. In severe cases, they have acute respiratory distress syndrome (ARDS) [133] and progression of illness can cause a multi-organ dysfunction in which a common complication is acute kidney injury (AKI) and proteinuria [134]. In COVID-19 patients, HPSE contribute to the progression of illness both for the disruption of barriers and also for cytokines storm that drive the massive inflammatory response. In fact, HPSE promotes endothelial barrier disruption, by degrading glycocalyx, an event that has been previously described both for ARDS and proteinuric diseases [7,135]. In addition, HPSE stimulates the expression and release of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, IL-10 and TNF-α, that stimulate the recruitment of leukocytes [136,137]. It has been demonstrated that HPSE levels are up-regulated in COVID-19 infected people and it is

associated with disease severity [138]. In addition, it has been reported that the use of low molecular heparin can reduces HPSE activity in COVID-19 patients [138]. In line with increased HPSE activity, also plasma HS and IL-6 levels up-regulated [138]. Interestingly, viral dissemination is supported by high HPSE expression levels [139].

Heparanase inhibition as a therapeutic option to prevent glycocalyx degradation

As described so far, HPSE has been shown to play a fundamental role in promoting the degradation of the glycocalyx associated with various pathologies.

Experimental and clinical evidences have shown that HPSE can be a very promising therapeutic target, considering that this enzyme is unique in the mammalian genome and that its expression in physiological conditions is very low. In recent years, several classes of HPSE inhibitors have been developed ranging from monoclonal antibodies to polysulfated saccharides. The development of these compounds began with the observation that heparin had the ability to inhibit the activity of HPSE due to its competition with HS for binding with the enzyme [140] So, the use of heparin and related compounds such as low molecular weight heparin (LMWH) or ultra-fractionated heparin (UFH), is related to: competition with the cell surface HS binding site reducing viral infection, inhibition of circulating HPSE enzymatic activity, anti-inflammatory and anti-coagulative effects [132,138,141,142]. In this regard, heparin have been the most widely used drugs for the treatments and prevention of endothelial cell disorders. On the other hand, the mimetics of heparan sulfate have a lower anticoagulant activity and a greater selectivity for HPSE than heparin, thus broadening the spectrum of the therapeutic activity of these compounds.

Currently, the only HPSE inhibitors that have reached the clinical trial stage belong to the class of polysaccharides and are: PI-88, PG545, Roneparstat and M402. These inhibitors have so far been used as potential anticancer compounds on the assumption that HPSE has been shown to play a key role in promoting the proliferation, angiogenesis and growth of tumor cells by acting both at the level of the tumor and its microenvironment [143]. Some of them have been shown to be effective in countering the neoplastic growth of some tumors but, more importantly, they have been shown to have a safety profile and good tolerability in prolonged treatments without particular side effects.

It will therefore be desirable that the next drugs aimed at inhibiting the activity of HPSE prove to have therapeutic efficacy not only in the vast field of cancer therapy but also in the treatment of other pathologies such as those affecting the vascular endothelium and for which HPSE is an important factor that determines the disease.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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