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Editorial

Heparanase in health and disease: The neglected housekeeper of the cell?

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1. Introduction

The membrane of most cells in the body is surrounded by a dynamically regulated, highly-hydrated fibrous meshwork of carbohydrates, known as pericellular matrix or glycocalyx. Its main constituents are several glycoconjugates (see nomenclature and definitions in Box 1), including glycolipids, the polysaccharide heparan sulfate (HS) linked to proteoglycan core proteins (*e.g.* glypican, syndecan, betaglycan), the polysaccharide hyaluronan and proteins bound to these polysaccharides.

2. Heparan sulfate proteoglycans: key components of the extracellular matrix

HS is a sulfated glycosaminoglycan polysaccharide produced by nearly all animal species; it is a linear polysaccharide essential for the organization of protein-receptor interactions at the cell surface and for the creation of chemotactic gradients of growth factors and chemokines. Besides being a crucial component of basement membranes, HS proteoglycans regulate the interaction of numerous signaling molecules (including growth factors, cytokines, chemokines, morphogens,

Box 1
Nomenclature and definitions

Glycoconjugates: Various types of compounds consisting of carbohydrates covalently linked with other types of chemical constituent.

Glycolipids: lipids with a carbohydrate attached by a covalent glycosidic bond. The carbohydrate portion may be a single monosaccharide or a linear or branched chain.

Glycoproteins: proteins containing oligosaccharide chains (glycans) covalently attached to amino acid side-chains; the carbohydrate portion consists of short chains, often branched.

Proteoglycans: proteins that are heavily glycosylated; the protein portion of the molecule represents only a small portion of the total molecular weight; the carbohydrate portion consists of long unbranched repeating disaccharide units (glycosaminoglycans, a.k.a. mucopolysaccharides). The three major classes of proteoglycans, characterized by their side chains, are the heparan sulfate proteoglycans, chondroitin sulfate proteoglycans, and keratan sulfate proteoglycans.

Polysaccharides: polymeric carbohydrate molecules composed of relatively long chains of monosaccharide units bound together by glycosidic linkages.

Lipopolysaccharides: large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core, and inner core joined by a covalent bond; they are typically found in the outer membrane of Gram-negative BACTERIA. They are also known as lipoglycans and endotoxins.

Peptidoglycans: polymers consisting of amino acids and specific sugars — alternating residues of β-(1,4) linked N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) — that form a mesh-like layer outside the plasma membrane of most BACTERIA; they are also known as mureins.

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proteases, and other proteins) with their specific receptors [1].

HS proteoglycans can be classified into four different families based on their location, their attachments to membranes, and their core protein structures. Glypicans are membrane-associated glycosyl-phosphatidylinositol (GPI)-anchored proteins; syndecans are transmembrane spanning proteins; secreted forms (including agrin, perlecan, collagen XVIII) are associated with the extracellular matrix (ECM); serglycin granules are present in intracellular storage granules.

3. Heparanase: a powerful multitasking enzyme

The only mammalian endoglycosidase capable of degrading HS is known as heparanase (HPSE); when released into the ECM, HPSE degrades HS cleaving polymeric HS molecules; additionally, this ubiquitously expressed enzyme is involved in a number of intracellular processes including autophagy, endocytosis, gene transcription, and secretion of exosomes [2–4]. HPSE is an endo-β-glucuronidase of the Carbohydrate Active Enzymes (CAZy) Glycoside Hydrolase (GH)79 family.

Within lysosomes and late endosomes, HPSE performs an essential housekeeping role in catabolic processing of internalized HS proteoglycans. Furthermore, HPSE can be trafficked to the cell surface or released into the ECM where it mediates breakdown of extracellular pools of HS: thus, latent pools of growth factors stored by HS are released, promoting increased cell proliferation, motility, and angiogenesis. Therefore, in addition to being an enzyme, HPSE has also non-enzymatic functions, like the ability to trigger different signaling pathways by interacting with specific transmembrane proteins.

4. Structural insights

Two genes encode for HPSE, *HPSE1* and *HPSE2*, although the latter lacks enzymatic activity [5] and seems to act predominantly as a negative regulator of heparanase. The *HPSE1* gene is located on chromosome 4 in humans (chromosome 5 in mice); it is expressed as two mRNA transcripts by alternative splicing, HPSE1a (5 kb) and HPSE1b (1.7 kb), which have the same open reading frames and encode the same 543 amino-acid polypeptides. HPSE is synthesized as a 68 kDa pre-proHPSE, the expression of which is mainly transcriptionally regulated. Pre-proHPSE is targeted to the endoplasmic reticulum via its N-terminal signal peptide, which is then cleaved to produce a 65 kDa proHPSE; the proHPSE is eventually transported to the Golgi apparatus where it is packaged into vesicles and secreted [6].

The crystal structures of human HPSE has been recently solved at 1.6–1.9 Å resolution [7], revealing a (β/α) 8 domain and a β-sandwich domain, and showing that an endo-acting binding cleft is exposed by proteolytic activation of latent proHPSE; the recognized cleavage site is a trisaccharide accommodated into the HPSE binding cleft and sulfation is essential for HPSE interaction with HS. The C-terminal domain of the 50 kDa subunit has been demonstrated to regulate protein secretion, enzymatic and non-enzymatic activity of HPSE.

5. Activation of HPSE

The *HPSE1* promoter in normal cells and tissues is constitutively silenced by methylation and the actions of p53. Instead, *HPSE1* is constitutively active in trophoblast, keratinocytes, and activated leukocytes and mast cells. HPSE expression can also be induced in other cell types by reactive oxygen species (ROS), components of the renin–angiotensin–aldosterone system (RAAS), endothelin 1, glucose, estrogens, certain p53 variants, and inflammatory mediators such as IL-1β, TNFα, and NF-kB [8]. Conversely, cellular quiescence induced by nitric oxide (NO) suppresses HPSE production, as does anti-inflammatory intracellular signaling through the vitamin D receptor. The ECM remodeling mediated by HPSE elicits the diffusion of cytokines, lipoproteins, and growth factors, facilitate cell motility, inflammation, angiogenesis, and coagulation.

Importantly, as mentioned above, HPSE also exhibits non-enzymatic activity: for instance, the pro-enzyme of 65 kDa stimulates signaling cascades that enhance phosphorylation of specific proteins including Akt, Src, and ERK. These HPSE-mediated processes have been demonstrated to play a pivotal role in cell adhesion, migration, and invasion in different cell types. More recently, HPSE has been also shown to regulate exosome production [4,9,10] and autophagy [11]. Low density lipoprotein (LDL) receptor-related proteins and mannose 6 phosphate receptors mediate the uptake of secreted proHPSE [12]; intracellular HPSE can localize to autophagosomes, where it positively stimulates the autophagic process through a non-enzymatic mechanism that involves downregulation of mTOR1 activity [6,11]. Notably, HPS activity is dependent on an acidic environment; indeed, bafilomycin A1 and chloroquine, which are known to raise the lysosomal pH, block HPSE enzymatic activity [13].

Upon lysosome permeabilization and via interaction with the chaperone heat shock protein 90, active HPSE can also translocate in the nucleus, where it degrades nuclear HS and regulates gene expression.

6. Mechanistic roles of HPSE in human disease

HPSE has been mainly involved in cancer progression, with studies demonstrating its contribution to motility, invasion, and metastasis [14]. The functional role of HPSE in cardiovascular disease has been especially investigated in the context of vascular injury: indeed, HPSE has been shown to be a critical mediator of thrombosis following angioplasty and stent-induced flow disturbance [15]. Aldi and colleagues in this issue of Atherosclerosis elegantly show that HPSE may have dual functions in vascular calcification, depending on the stage of the disease and the presence of inflammatory cells [16]: while HPSE plausibly enhances mineralization and osteogenic differentiation of vascular smooth muscle cells, it is associated with inflammation-induced osteoclast differentiation and activity in advanced atherosclerotic plaques.

In the nervous system, HS proteoglycans are crucial for the regulation of synaptic plasticity and have been shown to modulate the activity of calcium channels [17] and thereby neuronal firing rates; moreover, brain endothelial HS proteoglycans can modulate leukocyte migration across the blood-brain barrier and contribute to the formation of neuro-inflammatory lesions; a role for HPSE has been also inferred in multiple sclerosis and in prion disease [18]. Furthermore, HPSE has been implied in the pathogenesis of glomerulonephritis [6], pancreatic β cell failure and diabetes mellitus and its complications [19]. By regulating HS-bioavailability, HPSE is also involved in the pathogenesis of viral-mediated disorders: indeed, numerous human and non-human viruses utilize HS as an attachment co-receptor to entry into host cells [20].

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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