Short Conceptual Overview

Maria Francesca Secchi, Valentina Masola, Gianluigi Zaza, Antonio Lupo, Giovanni Gambaro and Maurizio Onisto*

Recent data concerning heparanase: focus on fibrosis, inflammation and cancer

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Abstract: Heparanase (HPSE) is a multitasking protein characterized by enzymatic and non-enzymatic activities. By means of its enzymatic activity, HPSE catalyzes the cutting of the side chains of heparan sulfate (HS) proteoglycans, thereby inducing the remodeling of the extracellular matrix and basement membranes. Thanks to the cleavage of HS, HPSE also promotes the release and diffusion of several HS-linked molecules such as growth factors, cytokines and enzymes. In addition to degrading HS chains, HPSE has non-enzymatic functions that trigger several signaling pathways. This signaling activity is achieved by interacting with transmembrane proteins, activating kinases such as Akt and Src, or modulating the activity of factors such as FGF-2 and TGF-β. Several studies have recently highlighted a possible intracellular activity for HPSE, particularly at nuclear level. While HPSE activity is quite limited in physiological conditions, its demonstrated increasing involvement in various pathological conditions, such as in tumor progression and renal disease, have attracted the attention of a growing number of researchers. The fact that no other molecule is capable of performing the same function as HPSE makes this enzyme an attractive potential target of medical treatment. With this short conceptual overview, we aim to provide an update on current knowledge concerning the HPSE protein in the experimental and clinical settings, paying particular attention to its role in fibrosis, inflammation and cancer.

Keywords: cancer; extracellular matrix; fibrosis; heparan sulfate; heparanase; inflammation.

***Corresponding author: Maurizio Onisto,** Department of Biomedical Sciences, University of Padova, Viale G. Colombo 3, I-35121 Padova, Italy, e-mail: maurizio.onisto@unipd.it
Maria Francesca Secchi: Department of Biomedical Sciences, University of Padova, Viale G. Colombo 3, I-35121 Padova, Italy
Valentina Masola, Gianluigi Zaza and Antonio Lupo: Department of Medicine, University of Verona, Piazzale A.Stefani 1, I-37126 Verona, Italy
Giovanni Gambaro: Division of Nephrology and Dialysis, Columbus-Gemelli Hospital Catholic University, Via Moscati 31, I-00168 Rome, Italy

Introduction

The extracellular matrix (ECM) consists of a fibrous component (mainly collagen fibers and elastin) and an amorphous substance composed of proteoglycans and glycosaminoglycans (GAGs). GAGs are long linear chains of polysaccharides formed by disaccharide units, generally an amino sugar tied to a uronic acid, repeated many times. Due to their many negative charges, GAGs bind positively charged ions that, in turn, trap water molecules, forming hydrated gels and thereby providing mechanical and metabolic support for the ECM. When GAGs are synthesized from sites in the core of proteins, they give rise to proteoglycans. Important components of the ECM are the heparan sulfate proteoglycans (HSPGs), which are bound to the cell surface in the form of syndecans, glypicans and β-glycans, or occur in the basement membranes in the form of agrin, collagen type XVIII and perlecan (1, 2). By binding to other macromolecules such as fibronectin, laminin and collagen (type I and IV), HSPGs contribute to the structural integrity of the ECM and basement membranes, and they modulate interactions between cells and the matrix. Biochemically, via the domains of sulfated disaccharides, the HSPGs provide numerous docking sites for bioactive molecules such as cytokines, growth factors, enzymes, and/or their inhibitors. In this way, they interfere with the formation of receptor-ligand complexes or constitute a reserve of biofactors at ECM level (3). The enzymatic cleavage of the side chains of HSPGs thus results in ECM remodeling, as well as in the release of these bioactive mediators, producing a rapid tissue response to local or systemic stimuli. The enzymatic activity responsible for cutting the side chains of HS was

identified and named heparanase (HPSE). It is the only functional endo-glycosidase capable of cleaving heparan sulfate (HS) chains (4). Several review articles have been written on HPSE and its crucial role in the etiology of certain diseases. The purpose of this review is to provide an update on current experimental and clinical knowledge of this enzyme, with particular emphasis on its role in fibrosis, inflammation and cancer.

Processing, localization, enzymatic and non-enzymatic activities of heparanase

Mammalian HPSE is the only endoglycosidase capable of cleaving HS chains of HSPGs. It exerts its enzymatic activity by catalyzing the cleavage of the β(1,4)-glycoside bond between glucuronic acid and glucosamine residues, generating fragments of about 5–7 kDa. Human HPSE gene (*HPSE1*) encodes a polypeptide of 543 amino acids and 68 kDa that is synthesized in the endoplasmic reticulum. Cutting of the N-terminal signal peptide gives rise to the inactive latent HPSE precursor of 65 kDa (pro-HPSE), which is readily secreted (5). Activation of the precursor into the mature enzyme demands the reuptake of the latent protein and intracellular proteolytic processing. Endocytosis is mediated mainly by syndecans and requires the syndecan-interacting proteins syntenin and α-actinin (6). Once inside the lysosome, the precursor undergoes the cleavage of a linker region (by cathepsin-L), giving rise to 8- and 50-kDa subunits that form the mature dimeric enzyme (7). Within the lysosome, HPSE is implicated in HSPGs turnover. Outside the cell, HPSE degrades cell surface HS and matrix and promotes ECM remodeling and the release of HS-linked molecules, including growth factors and cytokines. Apart from its primary perinuclear localization within lysosomes and late endosomes, several studies have found mature HPSE in the nucleus during *in vitro* cancer cell differentiation (8, 9). It emerged from subsequent studies that, after lysosomal permeabilization, HPSE is released in the cytoplasm and shuttled into the nucleus (probably by heat shock protein 90), where it is involved in nuclear HS degradation and in regulating gene expression (10, 11). HPSE induces the expression of genes implicated in glucose metabolism and inflammation in fatty acid-treated endothelial cells (12) (Figure 1). In addition to degrading HS chains, HPSE also has non-enzymatic and HS-independent functions. Evidence has been found of cell-membrane receptor(s) by means of which both the 65-kDa precursor and the activated form of HPSE activate signaling pathways and regulate several cellular processes. Migration and invasion are induced in endothelial cells overexpressing HPSE or exposed to latent HPSE precursors by the induction of the PI3K/Akt pathway (13, 14). Recent characterizations of Akt activation by HPSE have revealed that it involves Ras and is integrin-dependent (15). In a model of occlusive vascular disease it was shown that angiogenesis is promoted by HPSE secreted from mesenchymal stem cells via the integrin β1/HIF2 pathway (16). The adhesion of lymphoma and T-cells to ECM and endothelium was found to be mediated by HPSE in an integrin-dependent manner (17, 18). Angiogenesis via VEGF upregulation is promoted by Src activation in various cell lines overexpressing HPSE (19). No HPSE receptors have been identified to date. It seems that a receptor resident in the lipid raft is recruited by HPSE to induce Akt phosphorylation (20), and that signaling activation is mediated by the C-terminus domain (21).

Heparanase and fibrosis

Tissue fibrosis is an unregulated wound-healing response characterized by a gradual accumulation and decreased remodeling of ECM. Chronic injuries are the main triggers of this process in major organs (22). Common pathways and effector cells are implicated in fibrosis in various parenchymal organs. Persistent parenchymal cell injury leads to chronic inflammation that, in turn, stimulates the activation of effector cells into fibrogenic myofibroblasts. Myofibroblasts express the alpha isoform of smooth muscle actin (α-SMA) and secrete large amounts of ECM proteins (primarily collagen, fibronectin and laminin), which are responsible for tissue scarring and organ architecture deformation and failure. The source of the myofibroblast pool varies, including resident fibroblasts, fibrocytes, pericytes and epithelial cells undergoing epithelial-to-mesenchymal transition (EMT) (23). The transdifferentiation of epithelial cells into myofibroblast-like cells (i.e. EMT) is characterized by the loss of epithelial markers and the acquisition of mesenchymal ones, and by an increased migration and matrix protein secretion (24). Increasing interest has been paid to HPSE in this field in the last decade, since its involvement in the EMT of renal tubular cells in kidney fibrosis was discovered. HPSE is overexpressed by injured glomerular and tubular cells exposed to albuminuria, high glucose levels and advance glycosylation end products (25). Once secreted, HPSE is a key regulator of FGF-2 and TGF-β activity, the main pro-fibrotic factors and inducers of EMT in the kidney (26, 27). HPSE knock-down in a proximal tubular cell line prevents the

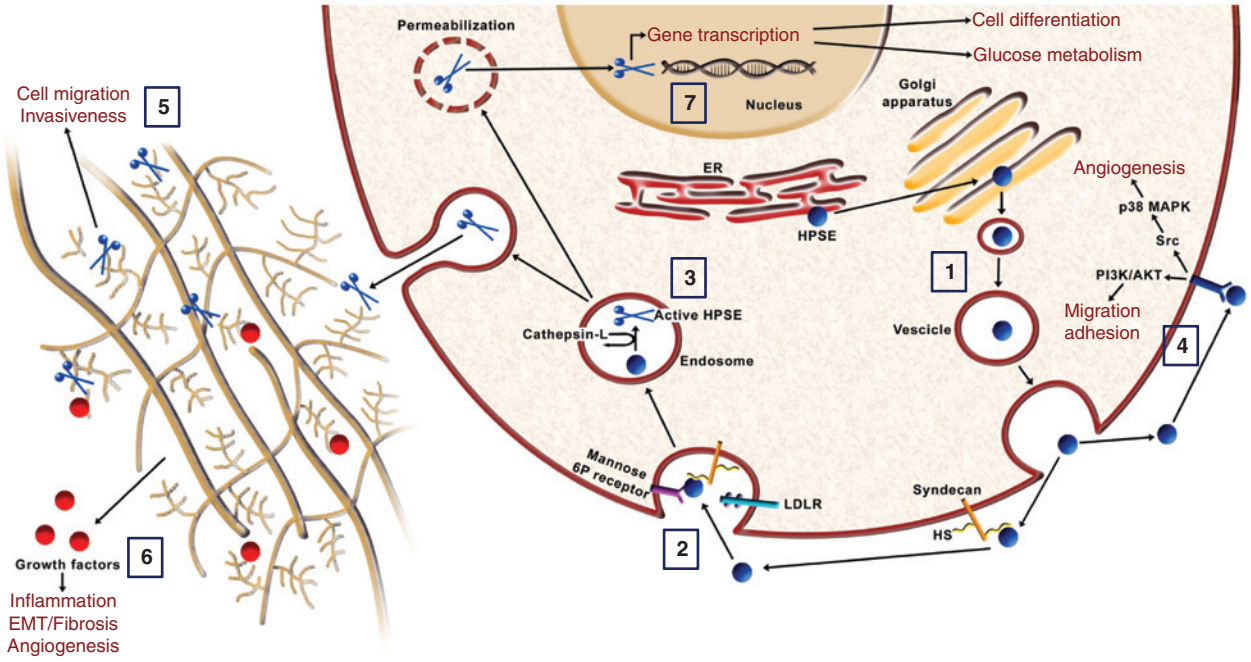

Figure 1: HPSE: processing, localization, enzymatic and non-enzymatic activities.
HPSE is synthesized in the endoplasmic reticulum as a latent precursor (pro-HPSE). After moving to the Golgi apparatus, pro-HPSE is secreted outside the cell [1]. HPSE uptake [mediated by syndecans, mannose-6-phosphate-receptor and low-density lipoprotein receptor (LDLR)-related protein] [2], and its delivery to lysosome enables the proteolytic processing (by cathepsin-L) and activation of the precursor into the dimeric active enzyme [3]. The interaction of latent HPSE with HPSE-binding proteins activates various intracellular signaling pathways implicated in angiogenesis, cell adhesion and migration [4]. HPSE secretion or translocation into the nucleus may occur under the effect of different stimuli. Extracellular HSPGs degradation encourages cell migration, thus enhancing tumor cell invasiveness and metastasis [5]. Angiogenesis, EMT and inflammatory response are indirectly regulated by HPSE via HS-linked growth factors that are released after HS cleavage [6]. Nuclear HPSE translocation is implicated in cell differentiation, inflammation and glucose metabolism through gene transcription regulation mechanisms [7].

EMT induced by FGF-2 (26), and delays the EMT induced by TGF-β (27). A lack of HPSE also prevents TGF-β overexpression by injured tubular cells *in vitro* (27), and HPSE-ko mice show no increase in TGF-β in injured kidneys, and no onset of fibrosis (28). These findings point to HPSE as a potential pharmacological target in chronic renal disease (29). There are few and sometimes conflicting data on the role of (HPSE) in promoting fibrosis in organs other than the kidney. HPSE regulates TGF-β availability by shedding syndecan-1, and its upregulation has consequently been found associated with intestinal fibrosis *in vivo* (30).

Unlike the well-known role of HPSE as a pro-metastatic agent in hepatocellular carcinoma, the involvement of HPSE in the pre-cancerous condition of liver fibrosis/cirrhosis is still not clear. Ikeguchi et al. found an inverse correlation between HPSE expression and stage of liver fibrosis in human tissue (31). Xiao et al. reported no difference in HPSE expression between cirrhotic and normal liver tissue (32). Discordant data have also emerged from a rat model of chronic liver disease: Ohayon et al. found an increase of HPSE in advanced fibrosis (33), whereas Goldshmidt et al. had previously described HPSE protein levels peaking in the early stages of fibrosis (34). Further studies will be needed to ascertain whether the mechanism by which HPSE exerts its pro-fibrotic action in the kidney is tissue-specific or applies to other organs too.

Heparanase and inflammation

There is now strong evidence of HS being involved in several aspects of inflammation. In particular, it controls the binding and bioavailability of cytokines in ECM (35, 36), it modulates the interaction of leukocytes with endothelium and ECM (37), and it regulates the activation of toll-like receptors (TLRs) (38, 39). Given these properties, HS degradation by HPSE may regulate inflammation in several steps, via chemokine release and modulation of the chemoactive gradient, leukocyte recruitment, extravasation and activation.

Recent findings suggest that HPSE can either facilitate or limit inflammatory responses, seemingly depending on

tissue- or cell-specific contextual cues that may dictate the direction taken by its action in inflammation (40). In colitis (characterized by an abundant luminal flora and the activation of TLR signaling pathways), HPSE of epithelial origin modulates the inflammatory phenotype of macrophages, preventing the inflammation from regressing, and switching macrophage response to a chronic inflammatory pattern (41). HPSE also strongly increases the *in vitro* activation of macrophages by lipopolysaccharide (LPS, a specific stimulator of TLR4 signaling), with a secondary increase in TNFα, IL-6 and IL-12 production. Activated macrophages can, in turn, induce epithelial HPSE expression (via a TNFα-dependent mechanism), and post-translational processing of the pro-enzyme via an increased secretion of cathepsin-L, thereby fueling a self-sustaining inflammatory circuit (41). Other studies have confirmed that HPSE participates in regulating TLRs and the subsequent release of pro-inflammatory cytokines by generating soluble HS fragments (42). The involvement of HPSE in renal inflammation has recently been demonstrated in mouse models of sepsis (43), and diabetic nephropathy (28). As regards the latter, diabetic HPSE-ko mice do not develop diabetic nephropathy, and macrophage infiltration is lower than in controls (28). Under diabetic conditions, the latent HPSE overexpressed by glomerular cells and post-translationally activated by cathepsin-L of tubular origin sustains the continuous activation of kidney-damaging macrophages, giving rise to chronic inflammatory conditions and fostering macrophage-mediated renal injury (44). One of the latest studies also proved that HPSE contributes significantly to pulmonary inflammatory cell recruitment in a model of allergic asthma (45). Another important parameter contributing to inflammation is macrophage polarization vs. an inflammatory (M1) or regenerative (M2) phenotype, and a recent work suggested that HPSE may be responsible for macrophage polarization in cancer (46).

Indirect but strong evidence of the role of HPSE in inflammation in humans emerges from several clinical trials on the efficacy of heparin as an anti-inflammatory agent. Heparin and heparin-derived compounds compete with the HS chain in binding to HPSE, so they have proved highly efficient HPSE inhibitors. Heparin and its derivatives have been shown to exert anti-inflammatory effects in the treatment of asthma and in patients undergoing cardiopulmonary bypass and or cataract surgery (47).

Heparanase and cancer

The first studies demonstrating an enzymatic activity attributable to (HPSE) date back to 1983, when Nakajima et al. (48) found a relationship between HS degradation and the invasive and metastatic potential of B16 melanoma cells, and Vlodavsky et al. (49) demonstrated the same link in lymphomas. After the HPSE gene had been identified and cloned in the late 1990s, several experimental studies showed that this enzyme's overexpression was instrumental in increasing the dissemination and metastatic potential of cancer cells, partly as a result of the establishment of a new vascular network (4, 50). Various gene silencing strategies for HPSE also coincided with a reduction in the invasive and metastatic properties of tumor cells (51, 52). HPSE promotes tumor progression not only thanks to HS cleavage in the ECM, but also due to the release and diffusion of HS-linked pro-angiogenic factors (e.g. VEGF, FGF-2), cytokines and enzymes (e.g. MMPs) (53). When released by HPSE activity, these molecules contribute to the creation of a microenvironment favorable to cancer cell growth. On the whole, these experimental data have supported the formulation of the fundamental concept (or hypothesis) that enzymatic cleavage of HS side chains by HPSE is a necessary condition for ECM remodeling by cancer cells for the purpose of invasive and metastatic processes (54). This hypothesis was subsequently confirmed by clinical evidence in human patients. HPSE expression was upregulated and correlated with reduced survival in all the major types of tumors analyzed (carcinomas, sarcomas, hematological and CNS tumors). These data have been widely reported and discussed in several recent reviews (5, 53–56), so we will not dwell further on these aspects here.

Recently, Hermano et al. (46) highlighted a new role for HPSE in the development of pancreatic adenocarcinoma. As mentioned above in the section on HPSE and inflammation, their results underscore the involvement of HPSE in directing the tumor-promoting behavior of tumor-associated macrophages (TAMs). Moreover, the analysis of human pancreatic adenocarcinoma specimens revealed a direct correlation between HPSE expression and macrophages infiltration. These findings suggested that the degree of HPSE expression may be highly significant when it comes to defining a target patient subgroup that may benefit from pharmacological treatment targeting TAMs.

In addition to its well-documented enzymatic activity in the extracellular environment, several studies have recently suggested a possible intracellular activity for HPSE, with an importance in the course of tumor progression that has yet to be well clarified. HPSE has been shown to have a role at nuclear level by shedding syndecan-1 in mesenchymal tumor cells (57), and the loss of syndecan-1 in the nucleus promotes the transcription of genes that contribute to the tumor phenotype (e.g. MMP-9)

as a result of histone acetyltransferase activity (11). Other studies have suggested a link between HPSE and NF-kB-dependent gene regulation (58, 59). On the other hand, Cohen-Kaplan et al. found that an increase in HPSE at nuclear level correlated with a slower tumor growth and a longer survival in patients with head and neck tumors (60). Nuclear HPSE was also found to down-regulate the expression of pro-tumorigenic genes in a melanoma model, pointing to a tumor-suppressive role instead of the secreted enzyme's well-known pro-tumorigenic function (61). These relatively recent findings have led to the suggestion that the ability of HPSE to regulate gene transcription and cell signaling depends not only on the combination of its enzymatic and non-enzymatic activities, but also on its extracellular and/or intracellular location. Further studies will be needed to fully clarify the role of nuclear HPSE in tumorigenesis.

The pro-tumorigenic properties of HPSE seems to be mediated also by its pro-autophagic function, as it was demonstrated in tumor xenograft models of human cancer. The cells overexpressing HPSE were more resistant to stress and chemotherapy as a consequence of an increased autophagy, effects that were reversed by lysosome inhibitor treatment. On the whole, these results establish a new role for HPSE in modulating autophagy in normal and malignant cells, and thereby conferring growth advantages under stress as well as resistance to chemotherapy (62).

Beside numerous experimental data concerning HPSE as pro-tumorigenic molecule, it is of interest to mention the work of Caruana and colleagues (63) who first demonstrated that the ECM degradation by means of HPSE can be exploited for anti-tumor strategies. Indeed, it was shown that inducing HPSE expression in long-term *ex vivo*-expanded T cells co-expressing a tumor-specific CAR (chimeric antigen receptor) increased their capacity to degrade the ECM and their anti-tumor activity. This experimental strategy could enhance the anti-tumor activity of CAR-redirected T cells in patients with stroma-rich solid tumors.

Conclusions

It is now evident from all the above contributions that HPSE can be considered as a protein with multiple functions in various diseases. In the light of its dual, enzymatic and non-enzymatic function, and intra- and extracellular localization, future molecules with an inhibitory function will need to act selectively, inhibiting one function but not the other, depending on the context. When the crystal structure of the enzyme becomes available, it will be possible to identify the protein domain responsible for a given function, and then design its potential inhibitors. Bearing in mind that, once HPSE has been deactivated, no other molecules are capable of exerting its same function, this enzyme seems particularly interesting as a drug target. Several HPSE inhibitors are currently the object of clinical trials, and some have already shown some anti-tumor efficacy (64). It is to be hoped that future HPSE-targeting drugs will bring benefits not only for cancer patients, but also for those with other disorders in which the enzyme has now revealed an important etiological role.

List of abbreviations

ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
GAGs	glycosaminoglycans
HPSE	heparanase
HSPG	heparan sulfate proteoglycan
HS	heparan sulfate

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