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Heparanase regulation of cancer, autophagy and inflammation: New mechanisms and targets for therapy

Ralph D. Sanderson[1], **Michael Elkin**[2], **Alan C. Rapraeger**[3], **Neta Ilan**[4], and **Israel Vlodavsky**[4]

[1]Department of Pathology; Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, USA

[2]Sharett Oncology Institute, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

[3]Department of Human Oncology, Wisconsin Institutes for Medical Research, Carbone Cancer Center, University of Wisconsin-Madison, Madison, WI, USA

[4]Cancer and Vascular Biology Research Center, Rappaport Faculty of Medicine, Technion, Haifa, Israel

Abstract

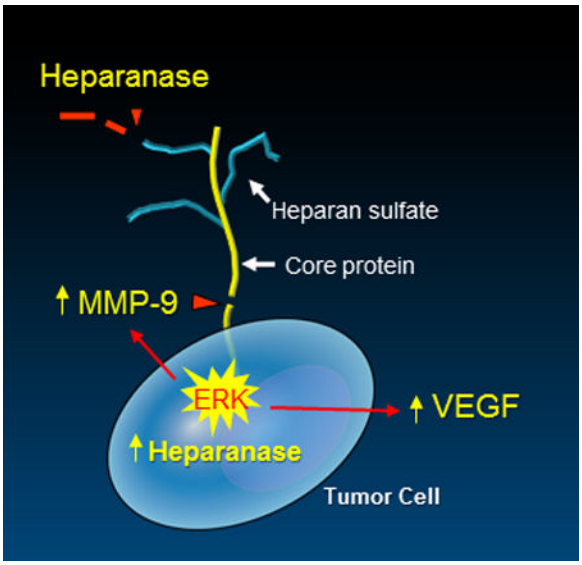
Because of its impact on multiple biological pathways, heparanase has emerged as a major regulator of cancer, inflammation and other disease processes. Heparanase accomplishes this by degrading heparan sulfate which regulates the abundance and location of heparin-binding growth factors thereby influencing multiple signaling pathways that control gene expression, syndecan shedding and cell behavior. In addition, heparanase can act via non-enzymatic mechanisms that directly activate signaling at the cell surface. Clinical trials testing heparanase inhibitors as anti-cancer therapeutics are showing early signs of efficacy in patients further emphasizing the biological importance of this enzyme. This review focuses on recent developments in the field of heparanase regulation of cancer and inflammation, including the impact of heparanase on exosomes and autophagy, and novel mechanisms whereby heparanase regulates tumor metastasis, angiogenesis and chemoresistance. In addition, the ongoing development of heparanase inhibitors and their potential for treating cancer and inflammation are discussed.

Graphical Abstract

Corresponding Author: Ralph D. Sanderson, 1720 Second Avenue South, WTI 602, University of Alabama at Birmingham, Birmingham, Alabama 35294, Telephone: 205-996-6226, ralphsanderson@uabmc.edu.

Author contributions

R.S., M.E., A.R., N.I. and I.V. wrote, reviewed and edited the manuscript. R.S. organized and submitted the manuscript.



By degrading heparan sulfate, heparanase impacts multiple signaling pathways that control gene expression, syndecan shedding and cell behavior. Heparanase also activates signaling at the cell surface via non-enzymatic mechanisms. This review focuses on recent developments that provide new insight into mechanisms of heparanase-mediated regulation of cancer and inflammation, including its impact on exosomes, autophagy, angiogenesis, chemoresistance, cell migration and metastasis.

Keywords

heparan sulfate; proteoglycan; heparanase; cancer; inflammation; autophagy; exosomes; heparanase inhibitors; metastasis; angiogenesis

Heparanase in cancer

Heparanase is an endoglucuronidase that cleaves heparan sulfate, thereby regulating the structure and function of heparan sulfate proteoglycans and remodeling cell surfaces and the extracellular matrix. Much of our current knowledge regarding heparanase function is related to cancer, and numerous comprehensive reviews are available on that subject [1–3]. There is abundant evidence that heparanase plays a role in cancer. Analyses at the RNA or protein level demonstrate that heparanase expression is enhanced in almost all cancers examined to date including, for example, ovarian, pancreatic, myeloma, colon, bladder, brain, prostate, breast, liver and rhabdomyosarcoma [4–14]. Numerous clinical studies have consistently demonstrated that upregulated heparanase expression correlates with increased tumor size, tumor progression, enhanced metastasis and poor prognosis [1, 14–18]. Knockdown of heparanase expression or treatment of tumor bearing mice with compounds that inhibit heparanase enzyme activity markedly impair tumor progression further underscoring the potential of anti-heparanase therapy for multiple types of cancer [19–25]. Importantly, there is only a single, enzymatically active form of heparanase in humans, it is expressed in very low levels in normal tissues and heparanase knock-out animals exhibit no

obvious deficits [17] implying that inhibition of heparanase will cause minimal side effects in cancer patients. Together these findings elevate heparanase as a highly desirable and druggable target for anti-cancer therapy, a view widely held by researchers in both academia and pharma and the topic of multiple reviews [1, 3, 15, 16, 21, 26–28].

Mechanistically, by cleaving heparan sulfate chains, the heparanase enzyme alters the structure and function of heparan sulfate proteoglycans and contributes to tumor-mediated remodeling of both cell surfaces and the extracellular matrix. These actions dynamically impact multiple regulatory pathways, most notably by augmenting the bioavailability of growth factors and cytokines bound to heparan sulfate [26]. In addition, heparanase expression by tumor cells initiates upregulation of expression of multiple genes that promote aggressive tumor behavior including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and matrix metalloproteinase-9 (MMP-9) [29–33], among others. In addition, recent discoveries indicate that a major function of heparanase is to enhance the shedding of the cell surface proteoglycan syndecan-1 [18, 26, 34]. Shed syndecan-1 plays diverse roles in the tumor microenvironment including shuttling growth factors to both tumor and host cell surfaces and nucleating the formation of signaling complexes at the cell surface (discussed in detail below). For additional information on syndecans in cell signaling see ref. [35] in this minireview series.

Heparanase regulation of tumor progression via exosomes and autophagosomes

Heparanase regulates biogenesis, composition, and function of tumor cell-derived exosomes

Exosomes are powerful mediators of intercellular communication that drive tumor progression by regulating the behavior of tumor and host cells both locally within the tumor microenvironment and distally throughout the body [36]. Exosomes accomplish this regulatory function by docking with recipient cells and delivering their cargo of protein, DNA, mRNA and miRNA [36]. In cancer, secretion of exosomes often increases as tumors transit toward a more aggressive phenotype. The tumor-host crosstalk mediated by exosomes has multiple effects that can influence processes such as formation of the pre-metastatic niche, angiogenesis and host immune function [37–39]. Syndecan proteoglycans influence the biogenesis of exosomes through their interaction with the syntenin-ALG-2 interacting protein X (ALIX) complex [38, 40, 41]. Syntenin interacts with the syndecan core protein via two PDZ domains and to ALIX via three LYPXnL motifs [40]. ALIX then binds to ESCRT-III (endosomal-sorting complex required for transport), the machinery responsible for intraluminal vesicle formation at multivesicular endosomes. Importantly, heparanase activates the syndecan-syntenin-ALIX exosome pathway [40, 42]. Briefly, heparanase activity in endosomes trims long heparan sulfate chains into shorter ones, allowing clustering of syndecans through lateral interactions between their heparan sulfate chains [42, 43]. Heparanase-induced clustering is thought to stimulate the binding of syndecan cytoplasmic domains to the tandem PDZ domains of syntenin, driving ALIX-ESCRT-mediated sorting into exosomes [42–44]. Notably, heparanase activity also facilitates the recruitment of CD63 into exosomes, in a syntenin-dependent manner [42, 43]. Together, a

complex picture is emerging in which syndecans, CD63 and possibly other membrane proteins that associate with endosomal syndecan and/or tetraspanin-enriched microdomains, are sorted into exosomes by a shared heparanase-syndecan-syntenin-ALIX pathway machinery [43, 44]. Heparanase-mediated effects on exosome biogenesis are best explained by heparanase acting on syndecan, promoting the assembly of syndecan in complexes that, by recruiting syntenin and ALIX–ESCRT, promote endosomal membranes to bud [44]. From that perspective, heparanase inhibitors as well as specific syntenin-PDZ inhibitors might be of particular interest for cancer, where both exosome release and heparanase are often elevated in the more aggressive forms of disease [26, 45]. In support of the above considerations, we have reported that in human cancer cells, when expression of heparanase is enhanced, or when tumor cells are exposed to exogenous heparanase, exosome secretion is dramatically increased [45]. This appears to rely on its enzymatic cleavage of heparan sulfate, because enzymatically inactive forms of the enzyme do not suffice [45]. Thus, heparanase released by tumor or host cells (*e.g.*, macrophages) could diffuse within the microenvironment, impact neighboring tumor cells and enhance, among other effects, their secretion of exosomes. Heparanase also impacts exosome protein cargo as reflected by higher levels of syndecan-1, VEGF and HGF in exosomes secreted by heparanase-high expressing cells as compared to heparanase-low expressing cells [45]. In functional assays, exosomes from heparanase-high cells stimulated spreading of tumor cells on fibronectin and invasion of endothelial cells through extracellular matrix better than did exosomes secreted by heparanase-low cells, suggesting a role in promoting tumor cell spreading and angiogenesis [45].

Our finding that heparanase is present in exosomes raises the possibility that this is a means for its delivery to distal locations. Because of the known role of heparanase in promoting metastasis and angiogenesis, this may play a role in establishing niches to which tumor cells eventually home and grow. Our results indicate that heparanase promotes secretion of exosomes that interact with both tumor and host cells and drive them toward an aggressive tumor phenotype. Emerging data indicate that exosomes can act as barriers to anti-cancer therapy by interacting with tumor cells and enhancing their chemoresistance [46]. In fact, our ongoing studies reveal that heparanase enhances both exosome docking and exosome-mediated transfer of chemoresistance to tumor cells [47]. Collectively, it appears that heparanase helps drive exosome secretion, alters exosome composition and facilitates production and docking of exosomes that impact both tumor and host cell behavior thereby promoting tumor progression and chemoresistance [45].

Heparanase enhances tumor growth and chemoresistance by augmenting autophagy

In spite of its localization in a highly active protein degradation environment such as the lysosome, heparanase appears stable [48, 49] and exhibits a half-life of about 30 hours [50], relatively long compared with a $t_{1/2}$ of 2–6 hours for transmembrane HSPGs and 25 minutes for GPI-anchored HSPGs [51]. Residence and accumulation of heparanase in lysosomes indicate that the enzyme may function in the normal physiology of this organelle. In a search for such function we revealed a role of lysosomal heparanase in modulating autophagy [52]. Autophagy is an evolutionarily conserved catabolic pathway through which cytoplasmic components, including macromolecules such as proteins and lipids as well as whole

organelles, are sequestered into double-membrane vesicles called autophagosomes. Autophagosomes are subsequently fused with lysosomes, where the intracellular material is degraded and recycled. This process occurs at a basal level in every cell and is required to remove unfolded proteins and damaged organelles, thus maintaining cellular homeostasis. Autophagy is further induced by starvation and stress, promoting cancer cells survival by providing their metabolic needs [53, 54]. Our results indicate that heparanase promotes autophagy and that enhanced tumor growth and chemoresistance exerted by heparanase is mediated in part by augmenting autophagy [52]. This was concluded because reduced LC3-II (a protein that specifically associates with autophagosomes) levels are found in cells and tissues obtained from heparanase knockout mice as opposed to elevated LC3-II levels found in transgenic mice that over express heparanase [52]. Even higher induction of autophagy was evident in head and neck carcinoma and glioma cells over-expressing heparanase, in accordance with a strong pre-clinical and clinical correlation between heparanase expression and the progression of these malignancies [33, 55–60]. Notably, electron microscopy analyses of cells over-expressing heparanase revealed not only a higher number of autophagic vacuoles, but also abundant release of vesicles, likely exosomes, from the cell surface, further supporting the notion that heparanase enhances exosome secretion that contributes to tumor growth [45, 61] (Fig. 1).

The mechanism underlying autophagy induction by heparanase is not entirely clear, but likely involves mTOR1 that plays a pivotal role in nutrient-sensing and autophagy regulation [62]. mTOR1 activity inhibits autophagy but under starvation its activity is repressed, leading to autophagy induction. We found that heparanase over-expression correlates with reduced mTOR1 activity, evident by decreased levels of p70 S6-kinase phosphorylation, an mTOR1 substrate. In contrast, heparanase-knockout cells exhibited increased mTOR1 activity and p70 S6-kinase phosphorylation [52]. Notably, mTOR1 appeared more diffusely scattered in control cells, whereas in cells with high content of heparanase, mTOR1 is found mostly in perinuclear regions, co-localizing with heparanase and LysoTracker, a dye that labels acidic lysosomal vesicles (Fig. 1). Our results imply that autophagy induction contributes to the pro-tumorigenic function of heparanase. This emerges from *in vitro* and *in vivo* experiments utilizing inhibitors of autophagy (chloroquine) and heparanase (PG545) alone or in combination [52]. Thus, combining chloroquine and PG545 in a tumor xenograft model resulted in significantly smaller and more differentiated tumors, suggesting that heparanase activity drives cancer cell de-differentiation as part of its pro-tumorigenic properties. Equally important is the ability of heparanase over-expression to confer resistance to stress, chemotherapy and targeted drugs [63], mediated, at least in part, by enhancing autophagy [52]. Indeed, diverse classes of anticancer drugs induce autophagy [64], thus attenuating tumor cell elimination, while autophagy inhibitors overcome chemoresistance [65, 66]. Based on this concept, chloroquine is currently being evaluated in clinical trials in combination with different classes of chemotherapeutic agents [65].

While traditional thinking envisions heparanase as an enzyme that functions extracellularly to cleave heparan sulfate and facilitate remodeling and ‘priming’ of the extracellular matrix (ECM), our results indicate that heparanase may also function inside cells [67]. From a translational point of view, targeting heparanase in the lysosome may be as important as its inhibition extracellularly, but the ability of currently available heparanase inhibitors to cross

the plasma membrane and enter the cell is unclear. Alternatively, the pro-autophagy function of heparanase can be inhibited by inhibiting its cellular uptake and hence decreasing its lysosomal content [67]. This opens the way for the development of a new class of highly specific inhibitors (i.e., monoclonal antibodies) that prevent heparanase uptake by targeting its heparin-binding domain. Involvement of heparanase in exosome formation, autophagy and activation of innate immune cells (discussed below) indicate that it fulfills normal functions associated, for example, with vesicular traffic, lysosomal secretion, stress response, heparan sulfate turnover and immune surveillances. Unraveling these aspects of heparanase biology is ongoing and critical to our understanding of its multiple roles in health and disease. Interestingly, in addition to heparanase, proteoglycans have also been implicated in regulation of autophagy and inflammation and are the subject of a minireview within this series [68].

A novel heparanase-driven mechanism promoting both metastasis and angiogenesis

Metastasis is a multi-step process regulated by enzymes, growth factors and signaling from adhesion receptors [69, 70]. Historically, heparanase is thought to stimulate metastasis and angiogenesis by degrading extracellular matrix, thereby liberating heparan sulfate-bound growth factors and chemokines from the extracellular matrix or cell surfaces. These growth factors are then free to interact with high affinity signaling receptors on the surface of tumor or host cells. Using human myeloma cells as a model, we recently discovered a mechanism that shines new light on how heparanase promotes both metastasis and angiogenesis. Key to this mechanism is the ability of heparanase to promote shedding of syndecan-1. The heparan sulfate degrading activity of heparanase shortens the length of heparan sulfate chains on syndecan-1 leaving the core protein vulnerable to attack by proteases [71]. Heparanase also mediates upregulation of MMP-9 expression by tumor cells. MMP-9 cleaves the juxtamembrane region of syndecan-1 thereby releasing an intact ectodomain from the cell surface [29] [23]. (Fig. 2).

Shedding exposes a cryptic domain on the syndecan-1 core protein that contains binding sites for very late antigen 4 (VLA-4) and vascular endothelial growth factor receptor 2 (VEGFR2). Coupling of these receptors by shed syndecan-1 activates VEGFR2 and localizes the receptor complex to the leading edge of the tumor cell where it stimulates invasion [72] (Fig. 2). Remarkably, this same heparanase-dependent mechanism is in play on endothelial cells where it potentiates endothelial tube formation. This mechanism adds to a growing list of receptor tyrosine kinases (IGF-1R, HER2, and EGFR) that rely on syndecan-mediated docking to an integrin in order to carry out critical roles in tumorigenesis and angiogenesis. As is the case here, the interactions are extracellular and accessible to synthesized peptides that mimic the capture site in the syndecan. These peptides are called “synstatins” or “SSTNs” and act to competitively disrupt the signaling mechanism [73–77]. In the case of VEGFR2 coupling to VLA-4, peptides based on either the VEGFR2 docking site ($SSTN_{VEGFR2}$) or the VLA-4 docking site ($SSTN_{VLA-4}$) in syndecan-1 serve to prevent tumor cell invasion or endothelial tube formation [72] (Fig. 2). This signaling mechanism is also highly dependent on heparanase as the initiating step, identifying a key and

unanticipated modulating role for this enzyme. This role was confirmed by use of the heparanase inhibitor Roneparstat, a chemically modified non-anticoagulant heparin that diminishes syndecan-1 shedding and subsequent tumor invasion and angiogenesis [72].

Framing this information into the known steps of tumor cell metastasis, we envision the following. Syndecan-1 shed by tumor cells nucleates the coupling of VEGFR2 to VLA-4, initiating tumor cell migration out of an established lesion and into the circulation. To extravasate to a new site, the circulating tumor cells must bind and migrate through the endothelial cell layer of bone marrow capillaries, enter the bone marrow stroma, stimulate angiogenesis and grow. Binding of tumor cells to the vascular endothelium occurs via interaction of VLA-4 with vascular cell adhesion molecule 1 (VCAM-1), an adhesion receptor abundant on bone marrow endothelia [78, 79]. Although binding of tumor cells to VCAM-1 can occur in the absence of heparanase, the invasion through the endothelial layer and throughout the bone marrow stroma is likely to depend on heparanase, as it facilitates the invasive phenotype arising from the coupling of VEGFR2 to VLA-4 [72]. In addition, as cells invade, degradation of the heparan sulfate-rich subendothelial basement membrane is also facilitated by heparanase. The invading cells not only rely on VCAM-1 as they transit the endothelium, but also rely on it within the bone marrow as VCAM-1 is highly expressed on bone marrow stromal cells, along with fibronectin, another VLA-4 ligand, that is enriched in the bone marrow matrix. This same mechanism is likely to potentiate local angiogenesis, induced in part by heparanase expression and stimulation of syndecan-1 shedding in bone marrow endothelial cells, and potentially supplemented in part by syndecan-1 shed into the local environment from the tumor cells. In addition, heparanase can also potentiate VEGF dependent angiogenesis by enhancing VEGF expression in the tumor cells [30]. Finally, tumor cells that acquire this invasive phenotype due to heparanase-mediated shedding of syndecan-1 within the bone marrow may display a heightened ability to rely on it to re-enter the circulation, and engage and invade through the vascular endothelium at a distant site in a cyclic process that drives the spread of blood-borne tumor cells throughout the body.

Regarding the use of heparanase inhibitors to block tumor progression, at present there are three heparan sulfate mimics in early stage clinical trials in cancer patients, Roneparstat, Necuparanib and PG545 [22, 23, 25], for reviews see [1, 16, 26] [2]. Recently, using animal models, Roneparstat was found to be highly effective when used in combination with front line chemotherapeutic agents like bortezomib or melphalan against established and aggressive myeloma tumors growing within bone, or when used in combination with lapatinib to treat brain metastatic breast cancer [80, 81]. Additional opportunities for development of anti-heparanase therapeutics include monoclonal antibodies and use of the recently published crystal structure of heparanase for identification of small molecule inhibitors [82].

Heparanase in inflammation

The involvement of heparanase in immune reactions was first suggested by studies demonstrating heparan sulfate-degrading activity in immunocytes (neutrophils, macrophages, activated T-lymphocytes) that contribute to immune cell diapedesis and

accumulation in target organs [83–89]. A role for heparanase in inflammatory responses was further supported by the finding that inhibitors of heparanase enzyme activity (i.e., heparin, synthetic heparin-mimicking compounds) had anti-inflammatory effects both in experimental and clinical settings [90–95].

The majority of early studies on the role of heparanase in inflammation focused on its ability to promote extravasation of immune cells. However, because heparan sulfate controls inflammatory responses at multiple levels, including sequestration of cytokines/chemokines in extracellular space, modulation of leukocyte interactions with endothelium and ECM, and initiation of innate immune responses through interactions with toll-like receptors (TLR) [96–104], enzymatic remodeling of heparan sulfate by heparanase appears to affect several aspects of inflammatory reactions. These include leukocyte recruitment, migration toward sites of inflammation, release of cytokines and chemokines anchored within the ECM or cell surfaces, as well as activation of innate immune cells.

Expanding a previous notion that immunocytes represent the principal source of the enzyme, more recent reports reveal a variety of cellular sources of heparanase in inflammation. Induction of heparanase was found to occur largely in epithelial and/or endothelial compartments in numerous inflammatory settings, including *in vivo* models of delayed type hypersensitivity [94], vascular injury [105], inflammatory bowel disease [106, 107], sepsis-associated lung injury [108] and autoimmune diabetes [109]. In addition, heparanase induction was found in auto-immune and auto-inflammatory human disorders, including rheumatoid arthritis [110], chronic obstructive pulmonary disease [111], Dengue disease [112], pleural empyema [113], inflammatory lung disease [108], ulcerative colitis and Crohn’s disease [107, 114]. Heparanase expression is induced in the presence of inflammatory cytokines [94, 107, 108, 115] or bacterial/viral infection [112, 113, 116].

In parallel with elucidation of cellular sources of the enzyme, accumulating experimental data reveal a complex picture of mechanisms employed by heparanase to modulate inflammatory responses. While many of these mechanisms (discussed below) are mediated by its well-characterized enzymatic function performed at the cell surface and within the extracellular compartment, other actions involve transcriptional regulation of the inflammatory phenotype in endothelial and immune cells by intracellular heparanase localized to the nucleus [117, 118]. Additionally, by virtue of heparanase ability to influence cell signaling independently of its enzymatic function, it was suggested that heparanase affects inflammatory cell responses via an unidentified cell surface receptor [119].

Mounting evidence suggests that heparanase profoundly influences the molecular physiology of innate immunocytes, including phagocytes (i.e., neutrophils, macrophages, dendritic cells), mast cells, and eosinophils [107, 108, 111, 120–125]. Inflammatory conditions in lungs seem to be one of the most extensively investigated anatomic sites in this respect [108, 111, 113, 126]. A recent report focused on heparanase-mediated degradation of the endothelial glycocalyx [108]. The glycocalyx, a thin gel-like layer that covers the luminal surface of endothelial cells lining blood vessels, is composed of heparan sulfate proteoglycans and glycoproteins [127, 128]. It acts as a barrier to circulating cells by limiting the availability of endothelial surface adhesion molecules to leukocytes [127, 128].

In a mouse model of sepsis-associated inflammatory lung disease, rapid induction of heparanase activity (via a tumor necrosis factor alpha-dependent mechanism) was demonstrated in pulmonary microvascular endothelial cells and correlated with neutrophil recruitment [108]. Heparanase induction was also found in biopsies of human inflammatory lung disease [108]. According to this report, sepsis associated loss of the pulmonary glycocalyx and endothelial hyperpermeability were attenuated in heparanase-null mice and in mice treated with inhibitors of heparanase enzymatic activity [108]. Another study, utilizing a dorsal air pouch inflammation model demonstrated that heparastatin (an iminosugar-based inhibitor of heparanase) potentially suppresses extravasation of neutrophils and monocytes by impairing the degradation of basement membrane heparan sulfate [129]. On the other hand, several recent reports combining heparanase knock out approach and/or inhibitors with *in vivo* models of airway inflammation found no significant effect of the enzyme in neutrophil recruitment/entrapment in the lung vasculature [111, 126]. Nevertheless, heparanase was critical for neutrophil accumulation in smoke-exposed lungs [126]. Even more perplexing, constitutive overexpression of heparanase in heparanase transgenic (Hpa-tg) mice was shown to attenuate intraluminal crawling of neutrophils in microvessels toward an extravascular chemokine source, reportedly due to reduction in endothelial surface heparan sulfate chain length and altered ability of truncated heparan sulfate to serve as a ligand for chemokines [120]. Additionally, studies exploring acute inflammatory phenotypes [130, 131] in Hpa-tg mice demonstrated that neutrophil recruitment and activation were attenuated in the presence of constitutively increased levels of heparanase. In light of the reported anti-inflammatory effects of heparin [90], increased levels of highly sulphated, "heparin-resembling" heparan sulfate fragments, which are constantly present in Hpa-tg as compared to wild type mice [132], may offer an explanation for the inhibitory effects of continuous heparanase overexpression on neutrophils in these settings [130, 131].

Unlike heparanase influence on neutrophils, its ability to modulate pro-inflammatory macrophage action remains less disputable and was highlighted in the setting of inflammatory bowel disease [107, 114], diabetic complications [133], pancreatic carcinoma associated inflammation [134], neointimal lesions following vascular injury [105] and atherosclerotic plaque progression toward vulnerability [121]. Modulation of toll-like receptor (TLR) signaling provides an attractive explanation for heparanase-mediated change in macrophage phenotype. Intact extracellular heparan sulfate inhibits TLR4 responses and macrophage activation, while its removal relieves this inhibition [102]. Indeed, incubation with active heparanase enzyme reduces the amount of intact heparan sulfate on the macrophage cell surface by 50% and significantly increases binding of fluorescent-labeled LPS (TLR4 ligand) by macrophages *in vitro* [107, 134], suggesting that degradation of cell-surface heparan sulfate by heparanase increases accessibility of the TLR. On the other hand, soluble heparan sulfate fragments released by heparanase degradation [135, 136], were found to stimulate TLR (in particular TLR4) signaling *in vitro* [102, 103, 137, 138] and *in vivo* [104].

The complexity of heparanase action in inflammatory processes is best exemplified by multiple levels of the enzyme involvement in type 1 diabetes and diabetic complications. In autoimmune diabetes, multiple roles were identified for heparanase produced by islet

autoreactive T cells and inflammatory leukocytes. These roles include promotion of leukocyte migration from pancreatic blood vessels (i.e., across the sub-endothelial basement membrane and through the pancreatic ECM), aiding the passage of leukocytes across the islet basement membrane and depleting islet beta cells of the intracellular heparan sulfate needed for their survival [109, 139, 140]. In addition, heparanase was implicated in several inflammation-related complications of diabetes, notably – diabetic retinopathy [141] and diabetic nephropathy [133]. The latter condition is characterized by activation of immune cells and there is clear evidence for a significant role of chronic inflammation in its pathogenesis, highlighting the role of kidney-infiltrating macrophages [142–145]. In the diabetic kidney, macrophages activated by various elements of the diabetic milieu (*e.g.*, high glucose [146], AGE [147, 148], albumin [149], free fatty acids [150]), release reactive oxygen species and proinflammatory cytokines such as tumor necrosis factor alpha or IL- 6 causing injury to podocytes and tubular cells [143, 144, 151]. Under these conditions heparanase that is overexpressed and post-translationally activated by Cathepsin L sustains continuous activation of kidney-damaging inflammatory macrophages eventually fostering chronic inflammation and renal injury [133].

Conclusions and perspectives

As investigation of heparanase continues, new and important roles for the enzyme are emerging. Recent studies demonstrating a role for heparanase in exosome formation, autophagy and activation of innate immune cells have further widened the scope of its influence. In addition, even though heparanase has long been associated with enhanced tumor metastasis and angiogenesis, the surprising discovery of a novel mechanism whereby heparanase induces shedding of syndecan-1 that then couples VEGFR2 and VLA-4 at the cell surface to promote metastasis and angiogenesis reminds us that there is still much to learn about mechanisms of heparanase action. Also, central to many of the downstream impacts of heparanase is its ability to regulate expression of genes for effectors such as HGF, MMP-9 and VEGF, yet our understanding of how heparanase regulates gene expression is not complete. Lastly, a remaining challenge in the field rests in the development of clinically effective inhibitors of heparanase that can be used to treat cancer and inflammatory diseases. The inhibitors currently in clinical trials are all modified heparins or heparin mimics and although they may prove effective, other more highly specific inhibitors such as monoclonal antibodies and small molecule chemical inhibitors are yet to be exploited. Further unraveling the mechanisms of action of heparanase and developing effective inhibitors of this enzyme are critical to our understanding of its multiple roles in health and disease.

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Abbreviations

ALIX	ALG-2 interacting protein X
ECM	extracellular matrix
ESCRT	endosomal-sorting complex required for transport
HGF	hepatocyte growth factor
HSPG	heparan sulfate proteoglycan
MMP-9	matrix metalloproteinase 9
SSTNs	synstatins
TLR	toll-like receptor
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
VEGFR2	vascular endothelial growth factor receptor 2
VLA-4	very late antigen 4

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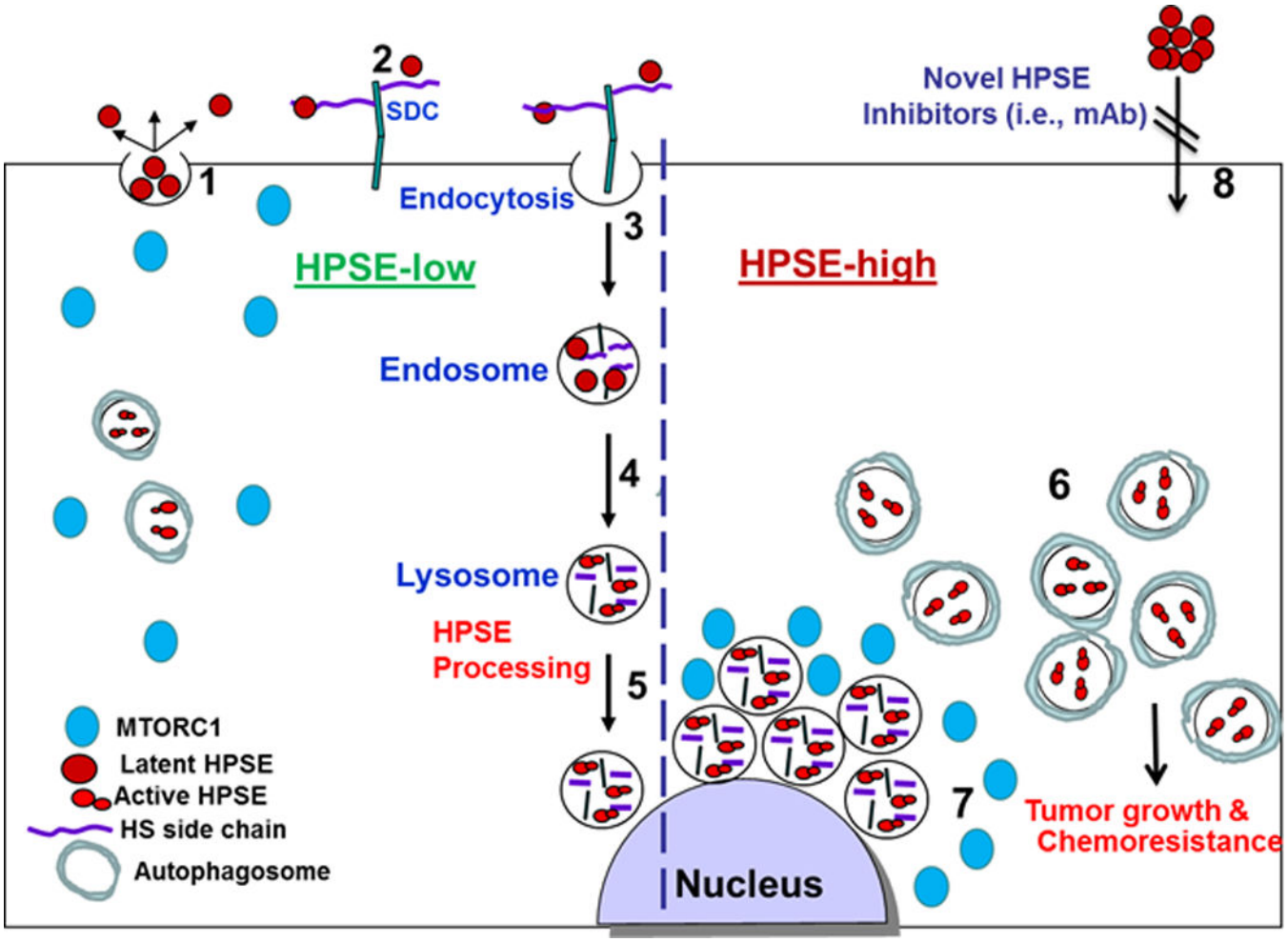

Figure 1.

A schematic model of heparanase trafficking and function in autophagy. Once secreted (**1**), heparanase rapidly interacts with cell membrane HSPGs such as syndecans (SDC) (**2**), followed by a rapid endocytosis of the heparanase-HSPG complex (**3**). Conversion of endosomes to lysosomes (**4**) results in heparanase processing and activation (**5**). Typically, heparanase appears at perinuclear lysosomal vesicles (**5**). Lysosomal heparanase regulates the basal level of autophagy and resides within autophagosomes (HPSE-low). Cancer cells that exhibit high content of heparanase (HPSE-high) are endowed with increased autophagy (**6)** that promotes tumor growth and chemo resistance. Enhanced autophagy by heparanase is associated with reduced p70 S6-kinase phosphorylation levels and accumulation of mTOR1 at peri-nuclear areas (**7**) vs. more diffused distribution in control (HPSE-low) cells. Function of heparanase within the cell encourages the development of new class of inhibitors that will prevent heparanase uptake and lysosomal accumulation (**8**).

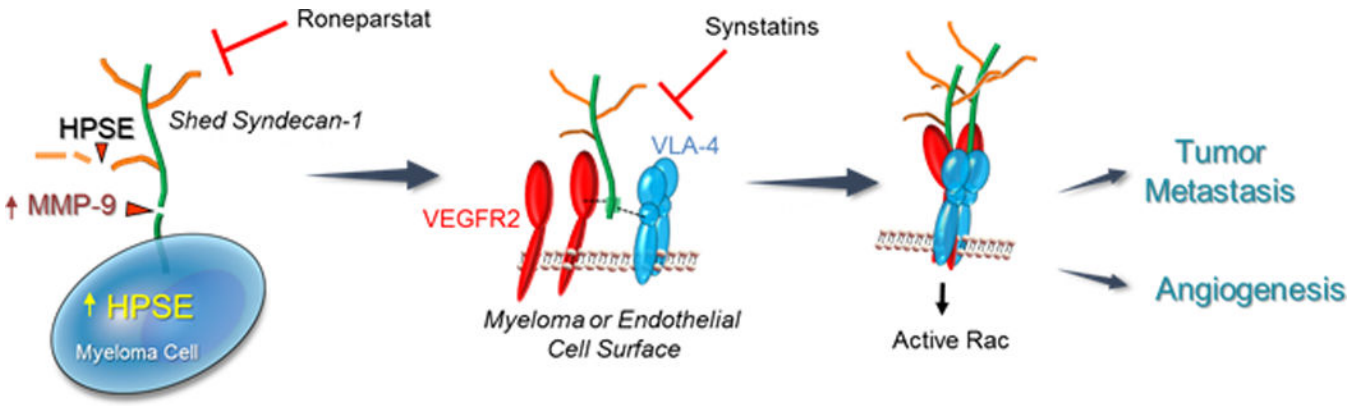

Figure 2.

Heparanase activates a signaling mechanism that drives both tumor cell invasion and angiogenesis. **(Left Panel)** Myeloma cells express syndecan-1 on their cell surface composed of a core protein (green) and heparan sulfate chains (brown). Upregulation of heparanase (HPSE) expression by myeloma cells leads to trimming of syndecan-1 heparan sulfate chains, shortening their length and allowing increased access of proteases to the exposed syndecan-1 core protein. One such protease is MMP-9, a syndecan-1 sheddase whose expression is upregulated when heparanase is expressed by myeloma cells. MMP-9 cleaves the syndecan-1 core protein and the proteoglycan is shed from the cell surface. **(Center Panel)** Shedding of syndecan-1 exposes a cryptic domain within the juxtamembrane region of the core protein (green). Within this cryptic domain are amino acid sequences that bind to clustered VLA-4 (blue) and VEGFR2 (red) on the surface of myeloma cells or endothelial cells. **(Right Panel)** The coupling of VLA-4 and VEGFR2 receptors by shed syndecans activates VEGFR2 signaling that stimulates both cell invasion and endothelial tube formation. This signaling mechanism is inhibited by Roneparstat, a heparanase inhibitor that diminishes syndecan-1 shedding, or by synstatin peptides, peptide mimics of the syndecan-1 core protein that competitively inhibit binding of either VLA-4 or VEGFR-2 to shed syndecan-1.