Published in final edited form as:
FEBS J. 2013 May ; 280(10): 2294–2306. doi:10.1111/febs.12168.

The heparanase/syndecan-1 axis in cancer: mechanisms and therapies

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Abstract

Heparanase is an endoglucuronidase that cleaves heparan sulfate chains of proteoglycans. In many malignancies, high heparanase expression and activity correlate with an aggressive tumor phenotype. A major consequence of heparanase action in cancer is a robust up-regulation of growth factor expression and increased shedding of syndecan-1, a transmembrane heparan sulfate proteoglycan. Substantial evidence indicates that heparanase and syndecan-1 work together to drive growth factor signaling and regulate cell behaviors that enhance tumor growth, dissemination, angiogenesis and osteolysis. Pre-clinical and clinical studies have demonstrated that therapies targeting the heparanase/syndecan-1 axis hold promise in blocking the aggressive behavior of cancer.

Keywords

Heparanase, syndecan-1; heparan sulfates; hepatocyte growth factor; vascular endothelial growth factor; extracellular regulated kinase; heparin mimics

Introduction

Heparanase is a multi-functional molecule whose expression is closely associated with enhanced aggressive behavior of many types of tumors [1-4]. Heparanase drives tumor progression by up-regulating the expression and bioavailability of several key growth factors that flood the tumor microenvironment. Additionally, heparanase upregulates expression of the heparan sulfate-bearing proteoglycan syndecan-1 and also promotes its shedding from the cell surface. Shed syndecan-1 binds to the tumor-derived growth factors, concentrates them within the tumor microenvironment and potentiates their signaling activity. This coordinated action of heparanase and syndecan-1 provides a powerful mechanism to enhance tumor growth, angiogenesis, invasion and metastasis. In this review, we discuss the mechanisms regulating formation of the heparanase/syndecan-1 axis, its impact on tumor behavior and novel therapeutic strategies being employed to attack this axis with the goal of diminishing the growth and spread of tumors.

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Effect of the heparanase/syndecan-1 axis on growth factor signaling

There is increasing evidence that heparanase, by regulating the structure and function of heparan sulfate proteoglycans (HSPG), can regulate growth factor signaling and cell behavior [5-9]. The heparanase/syndecan-1 axis has been shown to augment signaling cascades in both tumor and host cells (i.e. endothelial cells, fibroblasts, immune cells) within the tumor microenvironment. Two of the best studied examples of growth factors strongly regulated by the heparanase/syndecan-1 axis are hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF).

HGF, also known as scatter factor, is a potent signaling molecule that signals exclusively via its interaction with c-met, a tyrosine kinase receptor. HGF is a mitogen that mediates mesenchymal-epithelial interactions [10] and regulates several critical biological processes [11, 12]. In cancers, aberrant HGF signaling has been reported to drive angiogenesis [13], cell migration and survival [14]. Among cancers, some of the highest levels of HGF are seen in multiple myeloma [15, 16]. HGF controls several aspects of myeloma disease including, cell proliferation, apoptosis [17], adhesion to matrix [18], and osteolytic bone disease [19]. Moreover, an elevated level of HGF in the serum of myeloma patients is associated with poor prognosis [20].

Interestingly, both heparanase and syndecan-1 regulate HGF function. Heparanase dramatically enhances expression of HGF by myeloma cells [21], and myeloma cell surface syndecan-1 binds strongly to HGF and this facilitates HGF-enhanced myeloma tumor cell growth [22]. The heparan sulfate chains of cell surface syndecan-1 bind to HGF, sequester it at the cell surface, and thereby elevate its availability for interacting with the c-met receptor [23]. In addition, shed syndecan-1 also binds to HGF and complexes of the two molecules are detected in the serum of myeloma patients [24]. Evidence suggests that shed syndecan-1/HGF complexes also stimulate c-met signaling in osteoblasts [24]. This elevates receptor activator of nuclear factor kappa-B ligand (RANKL) secretion and subsequent osteoclast activation providing at least one mechanism for the link between HGF and osteolysis in myeloma patients [21]. HGF is also a potent angiogenic factor and its binding to syndecan-1 may augment this activity within the myeloma bone marrow [13].

Heparanase has also been shown to stimulate VEGF secretion by both carcinoma and myeloma cells [25, 26]. Secreted VEGF forms a complex with shed syndecan-1 that positively modulates VEGF receptor signaling via activation of the extracellular regulated kinase (ERK) signaling pathway leading to enhanced endothelial invasion and angiogenesis [26]. Treatment of the VEGF-syndecan-1 complex with heparinase III, a bacterial enzyme that degrades heparan sulfate chains, or immunodepletion of the complex blocks the enhanced phosphorylation of ERK. This points to shed syndecan-1 as a key mediator of heparanase-enhanced signaling and invasion of endothelial cells. Shed syndecan-1 in addition to presenting VEGF to endothelial cells can also activate αvβ3 integrin, a key regulator of endothelial activation and angiogenesis [26-28]. It is intriguing to speculate that syndecan-1, which engages the αvβ3 integrin on endothelial cells and is essential for its activation, is also playing a secondary role in providing VEGF to enhance this activation mechanism. VEGF bound to syndecan-1, rather than to other HSPGs in the matrix, would be most effective at integrin activation as it would be directly supplied by syndecan-1 to VEGFR2 complexed with the integrin at the cell surface. Central to this process of VEGF signaling and integrin activation is the up-regulation of syndecan-1 shedding by heparanase.

Biology and mechanisms of syndecan-1 shedding

The core protein sequence for syndecan-1 is comprised of three major domains, i) an extracellular domain bearing the glycosaminoglycan chains (GAGs) that are predominantly

heparan sulfate, ii) a short transmembrane domain, and iii) a highly conserved cytoplasmic domain [29] (Fig. 1). Proteolytic cleavage of the extracellular domain in a region near the plasma membrane of the cell releases a soluble form of the proteoglycan containing intact heparan sulfate chains [30]. Because the heparan sulfate chains within the ectodomain often contain bound ligands (*e.g.,* growth factors) that promote signaling, it forms an autocrine signaling complex that upon shedding is transformed into a powerful paracrine regulator of cellular function [31]. The shed form of syndecan-1 can either remain soluble or bind and accumulate within the extracellular matrix [32]. Cells constitutively shed low levels of syndecan-1 but various stimuli such as chemokines, growth factors, bacterial virulence factors and insulin trigger signaling pathways that elevate the expression and/or activity of proteases to accelerate shedding [31, 33].

Syndecan-1 shedding is regulated by several known mechanisms. Phosphorylation of tyrosine residues present in the cytoplasmic domain [34] and the interaction of Rab5 with the cytoplasmic domain [35] have been shown to control cleavage of the ectodomain. In addition, it was recently demonstrated that the GAG chains of syndecan-1 are active modulators of its shedding in epithelial cells and in different tumor cell lines [36]. Reduction in the GAG content of syndecans renders their core protein highly susceptible to cleavage by metalloproteases. Reducing the amount of heparan sulfate either by addition of recombinant human heparanase or by addition of bacterial heparinase III elevates syndecan-1 shedding dramatically [37]. There are several potential means by which heparan sulfate chains of syndecan-1 may regulate its shedding. These include: i) physically blocking sheddases from accessing the cleavage sites, ii) stabilizing the core protein in a conformation that is less susceptible to proteolysis, and/or iii) helping to maintain the syndecan-Rab5 complex.

Shed syndecan-1 in cancer

Shed syndecans have been detected in a number of tumor types and represent a novel therapeutic target [38, 39]. High levels of shed syndecan-1 have been reported in cancers of lung [40], Hodgkin's lymphoma [41], and multiple myeloma [42]. Levels of serum syndecan-1 are a prognostic marker in lung cancer [40]. In myeloma, a high level of syndecan-1 in the serum is an independent predictor of poor prognosis for patients [43] and a reliable prognostic factor at different phases of the disease [44]. In cancers like multiple myeloma, the tumor cells constitutively shed high levels of syndecan-1 and are probably the major source of soluble syndecan-1 in this disease [45]. However, in breast cancer shed syndecan-1 is derived largely from the stromal fibroblasts present in the tumor [46, 47]. Shed syndecan-1 elevates the *in vitro* proliferation of T47D breast carcinoma cells [48]. In contrast, over-expression of a soluble form of syndecan-1 promoted an invasive phenotype but concomitantly inhibited the proliferation of MCF-7 breast cancer cells [49]. Synthetic peptides that mimic regions of soluble syndecan-1 have also been shown to enhance the invasion of tumor cell lines [50]. The earliest evidence that shed syndecan-1 can promote tumor growth *in vivo* came from studies using ARH-77 human lymphoblastoid cells [51]. When these cells were engineered to express soluble syndecan-1 and injected into human bone implanted in immunodeficient mice (SCID-hu model) they grew more aggressively and disseminated faster than their control-transfected counterparts. The soluble syndecan-1 from the ARH-77 cells accumulated extensively within the interstitial matrix of the human bone marrow. This closely resembles the pattern of syndecan-1 staining seen in myeloma patients where shed syndecan-1 becomes trapped in the bone marrow matrix and within the regions of marrow fibrosis [32]. Interestingly, the soluble form of syndecan-1 did not affect ARH-77 cell proliferation *in vitro* suggesting that the major effect of shed syndecan-1 *in vivo* is in regulating cross-talk between the tumor and host cells that promotes growth and dissemination of the tumor cells.

Heparanase regulates syndecan-1 shedding and function

Up regulation of heparanase expression or addition of exogenous recombinant heparanase to myeloma cells stimulates syndecan-1 expression and shedding [37, 52]. Mechanistically, this enhanced shedding of syndecan-1 is due at least in part to heparanase-mediated activation of ERK signaling which leads to increased expression of MMP-9, a sheddase of syndecan-1 [6]. ERK activation by heparanase in myeloma cells is highly dependent on the heparan sulfate degrading activity of heparanase [6], although in other cell types, ERK signaling can be activated by latent heparanase that is devoid of enzymatic activity [53]. In myeloma cells the primary mediator of heparanase induced ERK activation is the insulin receptor signaling pathway [5]. In this pathway, heparanase plays a dual role by upregulating the phosphorylation of insulin receptors and by enhancing PKC activity. PKC in turn upregulates the expression of insulin receptor substrate-1 (IRS-1), the principal intracellular substrate of insulin receptor tyrosine kinase activity. IRS-1 is the most upstream molecule in the signal transduction cascade mediated by insulin, IL-4 and IGF-1. IRS-1 docks with the insulin receptor and undergoes phosphorylation and phospho-IRS1 engages multiple downstream signaling molecules resulting in ERK phosphorylation. These findings provide the first evidence for cooperation between heparanase expression and ERK activation in regulating expression of a protease that leads to shedding of syndecan-1. It is interesting that in multiple myeloma the activation of ERK requires the enzyme activity of heparanase. This suggests that stimulation of signaling occurs as the result of the clipping of heparan sulfate chains by heparanase. But how the trimming of syndecan-1 by heparanase can activate the insulin receptor is not clear. We speculate that heparanase remodeling of syndecan-1 heparan sulfate triggers clustering of the proteoglycan at the cell surface forming a molecular complex that enhances phosphorylation of the insulin receptor and stimulates PKC activity. Interestingly, one study has shown that heparanase facilitates the clustering of syndecan-1 and syndecan-4 on the surface of human glioma cells and thereby initiates signaling cascades that involve Rac1, Src and the PKC pathway resulting in enhanced cell adhesion and spreading [54]. Clustering of syndecan-1 and 4 is mediated by the heparin binding domains present in heparanase and this clustering does not require the heparan sulfate degrading activity of the enzyme.

There are multiple ways in which heparanase may regulate the function of syndecan-1 and other heparan sulfate proteoglycans. Heparanase degradation of heparan sulfate chains can initiate signaling cascades either by exposing cryptic sites on the heparan sulfate chains or on the core protein of HSPGs. This facilitates a close interaction of the binding partners with HSPGs. In melanoma cells heparanase stimulates FGF2 signaling by degrading the cell surface heparan sulfate chains [55]. Modification of heparan sulfate chains by heparanase enhances binding of FGF2 to cell surfaces and leads to stimulation of ERK and FAK phosphorylation [55]. High-affinity FGF2 binding and signaling require heparan sulfate chains of a minimum size and with some preference for specific structural features of the heparan sulfate. Depending upon the extent of heparan sulfate degradation by heparanase, sequences on the heparan sulfate chains, which bind to either FGF2 or FGFR, could be removed or cryptic sites could be revealed [56, 57]. Heparanase therefore can modify cellular heparan sulfate to support FGF2-stimulated signaling, potentially through modifying heparan sulfate structures to alter interactions with either FGF2 or FGFR, or both. Moreover, interplay between heparanase and syndecan-1 is required for renal tubular cells to undergo FGF2-induced epithelial mesenchymal transition [58].

The cleavage of heparan sulfate chains by heparanase does not merely stimulate syndecan-1 shedding but may “de-protect” the syndecan from recognition by other proteins [36]. As discussed above, an example of this is enhanced MMP-mediated release of syndecan-1 from the cell surface when heparan sulfate chains have been trimmed by heparanase [6]. Another

example is the binding of lacritin, a prosecretory epithelial mitogen found in the tear ducts that bind directly to the syndecan-1 core protein, but only after heparan sulfate chains have been trimmed by heparanase [9]. This is highly specific for syndecan-1 and other syndecan family members like syndecan-2 or syndecan-4 cannot bind lacritin. The novel step in this is that the binding necessitates prior partial or complete removal of heparan sulfate chains of syndecan-1 by endogenous heparanase. Modification of the N-terminal domain of syndecan-1 therefore facilitates its interaction with the C-terminal mitogenic domain of lacritin [9]. Thus heparanase modification of syndecan-1 transforms a widely expressed HSPG into a highly selective surface binding protein. Further, cleavage of heparan sulfate chains can alter membrane localization of the proteoglycan, consequently altering the availability of heparan sulfate to interact with signaling molecules. This has been demonstrated with syndecan-1 and glypican, whose localization in the plasma membrane is affected by removing heparan sulfate chains [59, 60].

Nuclear function of heparanase and syndecan-1

In addition to their extracellular localization, both heparanase and syndecan-1 have been shown to be present within the nucleus of cells. Heparanase in the nucleus of cells is enzymatically active [61]. Nuclear heparanase is associated with increased cell differentiation [62]. Furthermore, heparanase localization within the nucleus also dictates its function. In brain metastatic breast cancer, heparanase localizes to the nucleolus after stimulation by epidermal growth factor (EGF) [63]. In the nucleolus, heparanase enhanced DNA topoisomerase I activity, which subsequently increased cellular proliferation. Moreover, heparanase preferentially associated with euchromatin, a lightly packed form of chromatin where gene transcription typically occurs, in T lymphocytes [64]. The data suggests that heparanase in the nucleus of the T lymphocytes can modulate histone H3 methylation through its interaction with a transcriptional complex [64]. The cellular localization of heparanase can also serve as a predictor of prognosis in some cancers. This has been demonstrated in head and neck cancers as well as gastric and esophageal cancers, where nuclear localization of heparanase predicted a favorable outcome for patients, but its cytoplasmic localization correlated with a poor outcome [65-67].

Several studies have demonstrated localization of heparan sulfate or heparan sulfate proteoglycans in the nucleus [68-71]. Here the heparan sulfate chains of proteoglycans can regulate expression of different genes, possibly by regulating the level of histone acetylation. One study demonstrated that free glycosaminoglycan chains can decrease histone acetylation by 50% [72]. The uptake of these glycosaminoglycans by tumor cells is a selective process; and their inhibition of histone acetylation is dependent upon heparan sulfate chain length and sulfation pattern [72, 73]. This indicates that there is some degree of specificity rather than just random inhibition by heparan sulfate. Syndecan-1 has been shown to be present in the nucleus of both mesothelioma and myeloma tumor cells [70, 74]. In mesothelioma, nuclear translocation of syndecan-1 was linked to specific points of the cell cycle indicating that syndecan-1 may have a specific function during cell division through interactions with microtubule structures [70]. Because the heparan sulfate chains present on the core protein of syndecan-1 bind a myriad of growth factors and regulatory proteins, it is likely that syndecan-1 transports cargo to the nucleus. In fact, studies have indicated that fibroblast growth factor-2 (FGF-2) binds to heparan sulfate proteoglycans and translocates to the nucleus [71, 75]. Additionally, syndecan-1 co-localizes with FGF-2 and heparanase in the nucleus of mesothelioma cells [76]. Furthermore, cell surface heparan sulfate chains have been implicated in the cellular uptake and nuclear translocation of several molecules including heparanase [61].

The heparanase/syndecan-1 axis functions very uniquely within the nucleus. Active heparanase enzyme decreases the level of nuclear syndecan-1 and thereby removes the block exerted by syndecan-1 on histone acetyl transferase enzyme (HAT) [74, 77]. This was first demonstrated in myeloma, where the elevation of heparanase expression in myeloma tumor cells is coupled with the loss of syndecan-1 in the nucleus resulting in an increase in HAT activity. This increase in HAT activity upon heparanase expression correlates with increased expression of several genes known to promote an aggressive tumor phenotype [77]. The precise mechanism behind how heparanase regulates nuclear levels of syndecan-1 is still unknown. One possibility is that heparanase modifies syndecan-1 in a manner that results in loss of syndecan-1 ability to translocate to the nucleus. The mechanism of action of heparanase/syndecan-1 axis on tumor cells and the microenvironment is summarized in Fig. 2.

An emerging role for heparanase and syndecan-1 in exosome biogenesis and function

In addition to the mechanisms described above, heparanase and syndecan-1 appear to play important roles in regulating exosomes, lipid bilayer-bound extracellular vesicles 30-100 nm in diameter. Exosome secretion is upregulated as tumors become increasingly aggressive, and the cargo contained within exosomes, including proteins, mRNA and miRNA, can provide an important mechanism for intercellular communication between tumor and host cells [78, 79]. For example, exosomes derived from tumor cells have been shown to promote immune evasion [80], angiogenesis [81] and metastasis [82, 83]. Interestingly, a number of different heparan sulfate proteoglycans have been found in exosomes derived from various tissues (Table 1). Delivery to recipient cells of these exosomes bearing heparan sulfate proteoglycans along with their binding partners (*e.g.,* FGFs, VEGF, and HGF) may represent an important means of heparan sulfate-assisted signaling. In addition, syndecan-1 plays a key role in regulating the formation of exosomes through the interaction of the syndecan-1 cytoplasmic domain with both syntenin and ALIX to form a complex that supports the budding of intraluminal vesicles within endosomal membranes [84]. This study also revealed that heparan sulfate was essential for robust exosome biogenesis. Heparanase has been found in exosomes isolated from ascites fluid of ovarian cancer patients [85], and recent work in our lab has shown that heparanase significantly upregulates exosome biogenesis and alters the protein composition and function of exosomes secreted by myeloma tumor cells (unpublished observation). Although the mechanism by which heparanase enhances exosome biogenesis is unknown, it is reasonable to speculate that remodeling of the heparan sulfate chains of syndecan-1 by heparanase enhances formation of the syndecan-1-syntenin-ALIX complex which in turn drives exosome biogenesis. Given the potential importance of exosomes in regulating the progression of cancer and other diseases, it will be important to further explore how heparanase and syndecan-1 participate in regulating the formation and function of exosomes.

Therapeutic strategies to target the heparanase/syndecan-1 axis

Because of the importance of the syndecan-1/heparanase axis in driving cancer, therapeutic agents that disrupt this axis could potentially be useful in the clinic. Due to its multiple functions in driving the aggressive behavior of many tumor types, heparanase has received substantial attention as a therapeutic target while syndecan-1 presents a more difficult molecule to exploit therapeutically.

Several approaches hold potential for inhibition of heparanase including use of modified heparins, small molecule inhibitors and function-blocking monoclonal antibodies. Heparin is an inhibitor of heparanase enzyme activity, but cannot be used at high concentrations as an

anti-tumor drug because of its anti-coagulant activity. Thus, modified heparins or heparin mimics have been developed and have taken on many forms with predictably wide-ranging results [86]. A comprehensive overview of heparin mimics as drugs has recently been published [87]. Here we will focus on several heparin mimics that were developed with an eye on their ability to inhibit heparanase enzyme activity. These have been tested in preclinical models and have moved, or are moving toward human trials in patients with cancer.

PI-88 is a phosphosulfomannoligosaccharide obtained by hydrolysis of yeast mannan that yields a heterogeneous mixture of highly sulfated di- to hexasaccharides [88]. This compound has anti-heparanase and anti-angiogenic activity presumably due to its binding to heparanase and to binding factors such as VEGF. PI-88 does have some anti-coagulant activity and in human subjects can cause thrombocytopenia, thrombosis, injection site hemorrhage and other bleeding problems [89]. However, it is reasonably well-tolerated and is efficacious against some cancers, most notably hepatocellular carcinoma [89]. The clinical development of PI-88 was initiated by Progen Pharmaceuticals Ltd. and was recently licensed to Medigen Biotechnology Corporation which is now conducting a prospective randomized, double blinded, multicenter, phase III trial in subjects with hepatitis virus-related hepatocellular carcinoma after surgical resection. A second generation of anti-heparanase compounds developed by Progen, including PG545, showed promise in preclinical studies using murine models of breast, prostate, liver, lung, colon, head and neck cancers and melanoma [90]. PG545 is a fully sulfated, synthetic tetrasaccharide that is homogenous in composition [91]. Recent studies demonstrated that PG545 inhibited both heparanase activity and expression and blocked tumor growth and metastasis in animal models [92]. Unfortunately, phase I trials in humans had to be halted due to an unexpected reaction at the site of injection so the future prospects for this drug remain unknown.

SST0001 (formerly designated as G4000) is a modified heparin that is 100% N-acetylated and 25% glycol split [86, 93] and has a molecular mass averaging 20 kDa. N-acetylation renders it non-anticoagulant and glycol splitting appears to enhance its affinity for heparanase where it affectively blocks heparanase enzyme activity [53]. SST0001 in pre-clinical models has been shown to have efficacy against Ewing's sarcoma, myeloma and pancreatic cancer [94-97]. Pharmacodynamic studies indicate that SST0001 effectively inhibits heparanase activity *in vivo* and can regulate levels of growth factors (*e.g.,* HGF, VEGF) and inhibit angiogenesis [96]. Moreover, SST0001 works well in combination with dexamethasone against myeloma tumors growing in mice [96]. Importantly, SST0001 is not toxic to cells growing *in vitro*. This suggests that its anti-tumor effects *in vivo* are due to disruption of the tumor promoting effects that heparanase has within the tumor microenvironment. Sigma-tau Research Switzerland S.A. recently initiated a phase I clinical trial of SST0001 in patients with advanced multiple myeloma. Another glycol-split heparin compound similar to SST0001 is M402. M402 is smaller than SST0001, having a molecular mass averaging 6 kDa [98]. In addition, it differs from SST0001 in that it was not N-acetylated and thus may have broader activity in binding growth factors than does SST0001. Nonetheless, given that M402, SST0001, PG545 and PI-88 are all highly sulfated, it is likely that they all have biological activity beyond inhibition of heparanase enzyme activity. M402 showed efficacy in a melanoma model of experimental metastasis and in spontaneous metastasis using the 4T1 murine mammary carcinoma model [98]. A phase 1/2 proof-of-concept clinical trial of M402 in combination with gemcitabine in patients with advanced metastatic pancreatic cancer was begun in July 2012.

Now that multiple heparin mimics have reached clinical trials in humans, it will be interesting to see which, if any, of the strategies have resulted in a clinically relevant drug. For those that show safety and tolerability in phase I studies, the challenge will be to

determine when during the progression of cancer is the best time to begin their administration, and how to use them in combination with other treatments. If these heparin mimics do disrupt the tumor microenvironment in human cancers, they may be effective in combination with drugs that target tumor cells. This strategy would effectively target both the host environment and the malignant cell itself. Interestingly, heparanase appears to play an active role in inflammatory conditions and kidney dysfunction and thus inhibitors of heparanase may be of therapeutic use in diseases such as colitis, sepsis [99], autoimmune diabetes [100] and diabetic nephropathy [101].

Although proteoglycans are more difficult to target therapeutically than is heparanase, several approaches have shown promise. One possibility is to interfere with normal assembly of glycosaminoglycan chains on the core proteins of proteoglycans using hydrophobic aglycones. These aglycones, depending on their structure, can drive formation of antiproliferative glycosaminoglycans or inhibit glycosaminoglycan synthesis or proteoglycan synthesis [73, 102, 103]. This can result in inhibition of tumor growth and angiogenesis [73, 102]. Another approach is to generate fragments of heparan sulfate that have anti-tumor activity. This is accomplished by using bacterial enzymes to degrade heparan sulfate *in vitro* followed by administration of these fragments to animals bearing tumor. This approach has been successful in blocking tumor growth in murine models of melanoma and myeloma [57, 95]. Both of these studies used a pool of degraded heparan sulfate but did not identify the specific structures within that pool having anti-tumor activity. Precise identification of these anti-tumor structures within heparan sulfate could provide clues in how to better prepare heparin mimics that will effectively inhibit tumor growth and progression.

Recent studies have revealed that syndecan-1docks with integrins and the IGF1 receptor to form a ternary complex that activates integrin signaling [104, 105]. This docking occurs through a specific region of the syndecan-1 core protein extracellular domain including amino acids 92-119. Synstatin, a synthetic peptide composed of amino acids 92-119 of the syndecan-1 core protein, inhibits angiogenesis and blocks growth of carcinomas *in vivo* [104]. This growth inhibition *in vivo* is likely due at least in part to inhibition of αvβ3 integrin signaling required for endothelial cell migration and angiogenesis. It will be interesting to determine if targeting both arms of the syndecan-1/heparanase axis using synstatin in combination with heparin mimics will have additive or synergistic effects in murine models of cancer. Recent advances in RNAi technology also offer an opportunity to perturb the expression of key molecules or the signaling pathway in the syndecan-1/heparanase axis. Finally, recent studies have shown the potential for expressing specific micro RNAs, such as miRNA-1258 which blocks heparanase expression and diminishes metastasis of breast cancer cells [106].

Summary

Although it is well known that heparanase and syndecan-1 individually can regulate the behavior of tumors, it has recently become clear that these two molecules work in concert to drive tumor progression. Heparanase not only enhances syndecan-1 expression, it also dramatically influences syndecan-1 location by increasing its shedding from the cell surface, altering its position on the plasma membrane and diminishing its abundance in the nucleus. In addition, heparanase upregulates expression of growth factors such as HGF and VEGF which then bind to syndecan-1 heparan sulfate forming a complex that protects the growth factor from degradation; retains the growth factor within the tumor microenvironment and potentiates interaction of the growth factor with its high affinity signaling receptor. Heparanase and syndecan-1 both, and perhaps by working together, drive exosome biogenesis and regulate exosome function. In addition, both heparanase and syndecan-1 are

retained as cargo within exosomes where they again may act together to influence the behavior of cells within the tumor microenvironment and distally within niches that may nurse the growth of metastasizing cells. Due to the decades of prior work on heparanase and proteoglycans, the field has moved closer to the exciting possibility of translating basic findings into new cancer therapies. Several drug candidates, designed to block heparanase or syndecan-1 function are now in various stages of pre-clinical and clinical investigation with the potential to significantly blunt tumor progression.

Acknowledgments

This work was supported by NIH CA135075, CA138340 and CA138535 to RDS, by Grant No. 2009230 from the United States-Israel Binational Science Foundation (jointly to IV and RDS). The authors apologize for the inability, due to space limitations, to reference all studies relevant to this review.

Abbreviations

EGF	Epidermal growth factor
ERK	Extracellular regulated kinase
FAK	Focal adhesion kinase
FGF2	Fibroblast growth factor 2
FGFR	Fibroblast growth factor receptor
GAG	Glycosaminoglycans
HAT	Histone acetyl transferase
HGF	Hepatocyte growth factor
HSPG	Heparan sulfate proteoglycan
IGF	Insulin-like growth factor
IRS-1	Insulin receptor substrate-1
MMP-9	Matrix metalloproteinase-9
PKC	Protein kinase C
RANKL	Receptor activator of nuclear factor kappa-B ligand
SDC-1	Syndecan-1
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2

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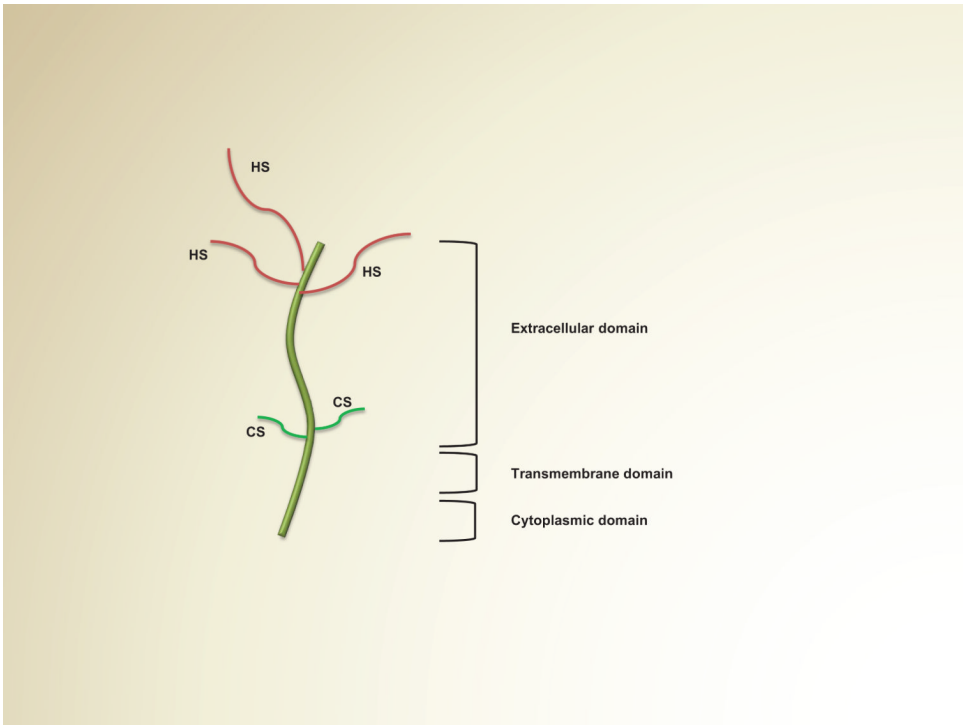

Figure 1.
Schematic model of syndecan-1 structure – Syndecan-1 core protein consists of three major domains, 1) a long extracellular domain that bears the heparan sulfate (HS) and chondroitin sulfate (CS) chains at distinct sites, 2) a short transmembrane domain, and 3) a cytoplasmic domain that is highly conserved among different syndecans.

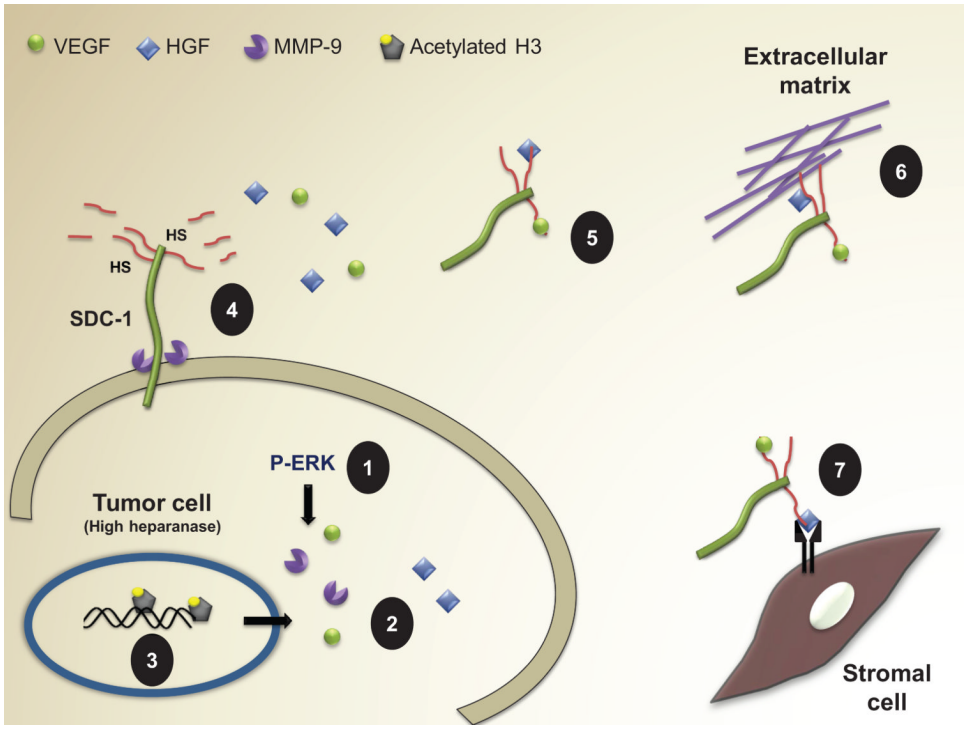

Figure 2.

Model of the heparanase/syndecan-1 axis in cancer – The model shows the series of molecular events triggered by heparanase in tumor cells that establishes the heparanase/syndecan-1 axis. When heparanase is elevated in tumors the following events occur, **(1)** Levels of active ERK (P-ERK) are elevated in the cells. **(2)** P-ERK up-regulates the cellular expression of VEGF and MMP-9. HGF is also elevated in these cells but via signaling pathways that are independent of P-ERK. **(3)** With the increase in heparanase expression, syndecan-1 levels in the nucleus are diminished leading to an increase in the levels of acetylated histone H3. This facilitates the transcription of MMP-9 and VEGF. **(4)** Due to heparanase activity, the HS chains of syndecan-1 (SDC-1) on the cell surface are trimmed leading to enhanced cleavage of the core protein by MMP-9 which is now present in abundance. **(5)** The heparan sulfate chains of the shed syndecan-1 bind and complex with growth factors including HGF and VEGF whose expression is also stimulated by the expression of heparanase. **(6)** Shed syndecan-1 bearing the growth factors binds to extracellular matrix proteins (e.g., fibronectin, collagens) and sequesters these growth factors in the tumor microenvironment as well as at distal sites. **(7)** Shed syndecan-1 binding potentiates the signaling of the bound growth factors. This results in a strong, sustained downstream signaling in the host cells (e.g., stromal cells, endothelial cells), priming the microenvironment to support aggressive tumor growth.

Table 1

Heparan sulfate proteoglycans found in exosomes

Molecule	Cell / Tissue/ Biological Fluid of origin
Heparanase	Ovarian cancer ascites [85]
Syndecan-1	Bladder cancer cells [107], colorectal cancer cells [108], urine [109]
Syndecan-4	Hepatocytes [110], colorectal cancer cells [108], saliva [111]
Glypican-1	Saliva [111]
Glypican- 4	Reticulocytes [112], saliva [111]
Glypican- 5	Mast cells [113]
Perlecan	Embryonic fibroblasts [114], bladder cancer cells [107], colorectal cancer cells [108], colon cancer cell lines [110], saliva [111], urine [109]

*Table created using information from exocarta.org, an exosome content database