Published in final edited form as:
Trends Biochem Sci. 2014 June ; 39(6): 277–288. doi:10.1016/j.tibs.2014.03.001.

Heparan sulfate signaling in cancer

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Summary

Heparan sulfate (HS) is a biopolymer consisting of variably sulfated repeating disaccharide units. The anticoagulant heparin is a highly sulfated intracellular variant of HS. HS has demonstrated roles in embryonic development, homeostasis, and human disease via non-covalent interactions with numerous cellular proteins, including growth factors and their receptors. HS can function as a co-receptor by enhancing receptor-complex formation. In other contexts, HS disrupts signaling complexes or serves as a ligand sink. The effects of HS on growth factor signaling are tightly regulated by the actions of sulfyltransferases, sulfatases and heparanases. HS has important emerging roles in oncogenesis and heparin derivatives represent potential therapeutic strategies for human cancers. Here we review recent insights into HS signaling in tumor proliferation, angiogenesis, metastasis, and differentiation. A cancer-specific understanding of HS signaling could uncover potential therapeutic targets in this highly actionable signaling network.

Keywords

heparin; heparan sulfate; metastasis; sulfyltransferase; sulfatase; heparanase

Heparin sulfate proteoglycans

The anticoagulant heparin represents one of the oldest and most successful natural therapeutic agents. Heparin was discovered in 1916 and derives its name from its abundance in hepatic tissue [1]. Heparan sulfate (HS, originally called heparatin sulfate) is a member of the glycosaminoglycan family of carbohydrates initially identified as an impurity of heparin isolations that was found to be widely distributed in human tissues [2]. Heparin and HS both consist of repeating unbranched negatively charged disaccharide units variably sulfated at the 3-O, 6-O, or N-sites on glucosamine, and the 6-O site on glucuronic/iduronic acid (Box

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1). Heparin represents a highly sulfated intracellular variant of HS, though its physiologic roles remain unclear.

A critical pentasaccharide within heparin and endothelial HS binds specific basic residues of the circulating extracellular serine protease inhibitor antithrombin III, causing a conformational change that allows the enzyme to inactivate the pro-thrombotic proteases thrombin, factor IXa and factor Xa, thereby preventing clot formation [3] (Figure 1). Sulfation at each of the available sites shown in Figure 1 is necessary for heparin to recognize its binding site on antithrombin III.

Although heparin is synthesized primarily by mast cells [4], HS is found across mammalian cell types as a post-translational modification, generating heparan sulfate proteoglycans (HSPGs) that serve numerous biologic functions [5, 6]. Variation in saccharide length and number of attached sulfate groups provides important variability with functional consequences. Unlike heparin, HSPGs are often incompletely sulfated, providing an additional layer of regulation. Like many surface proteins, HSPGs are constantly internalized for lysosomal degradation or membrane recycling. The typical HSPG half-life is 4-24 hours, with complete turnover typically occurring by 48 hours [7]. HSPGs are classified as “full-time” if their function is restricted to HS effects on cell signaling, or “part-time” if they have additional structural features and roles in multiple signaling pathways. Full-time HSPGs include the four transmembrane syndecans (SDC), six GPI-anchored glypicans (GPC), and three basement membrane HSPGs (agrin, perlecan and collagen XVIII). The type III transforming growth factor β (TGF-β) receptor (TβRIII or betaglycan), neuropilins 1 and 2, and CD44 are part-time HSPGs with major roles as co-receptors in additional signaling pathways independent of their HS modification [8, 9]. As examples, TβRIII is required for TGF-β2 surface binding and downstream SMAD signaling in many cellular contexts including cancers and the neuropilins function as co-receptors for class 3 semaphorins.

The majority of the hundreds of protein interactions ascribed to HS are mediated by specific ionic binding to lysine/arginine residues aligned in “Cardin-Weintraub” sequences [10, 11]. A number of cytokines and growth factors contain these sequences. HS can bind cytokines (Box 2) to control their localization, set up gradients in the extracellular matrix, and alter their activity [6]. HS can also bind growth factors (Box 2). Fibroblast growth factor (FGF) binding interactions are the best characterized: the HS modifications on HSPGs, including SDC, GPC and TβRIII, bind both FGF ligands and receptors to form a ternary complex and enhance signaling (Figure 2), which can promote carcinogenesis [6, 12, 13]. By contrast, a high local concentration of cell surface HSPGs can function to disrupt growth factor signaling complexes or serve as a ligand sink. HSPGs can be found at the surface of cancer cells, and can also be shed by cancer and stromal cells to enhance or suppress cell signaling and influence cancer cell biology (Figure 3).

The ability of HS to bind growth factors leads to numerous biological and pathological roles for HSPGs, including demonstrated effects on tumor angiogenesis, proliferation and differentiation (Figure 4 and Box 2). Individual HSPGs have roles in specific cancers (Table 1). Some HSPGs, such as GPC1 and SDC2, are consistently up-regulated and serve similar

roles in promoting growth across cancer types [8]. Others, such as TβRIII, are down-regulated in most cancers and function to suppress tumor growth [14, 15]. A third group of HSPGs has conflicting roles in promoting or suppressing carcinogenesis depending on tumor cell of origin, illustrating the diversity of biological functions for this outwardly similar family of signaling molecules. Recent findings help to clarify the roles of HSPGs in tumor cell proliferation, metastasis, tumor angiogenesis and terminal differentiation, identifying novel therapeutic targets and heparin-based therapeutic strategies.

HS in cancer cell proliferation

The binding interactions between HS and mitogenic growth factors, including the fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), heparin-binding epidermal growth factor-like factor (HBEGF), and hepatocyte growth factor (HGF), could provide selective pressure resulting in increased expression of HSPGs in certain cancers. For instance, overexpression of the HSPGs GPC1 and SDC1 in breast cancer cells enhances the proliferative response to treatment with FGF2, HBEGF, and HGF [16]. GPC1 has similar effects in pancreatic cancer and gliomas [17]. In addition, knockdown of SDC1 and GPC1 in myeloma [18] and pancreatic cancer cells [19], as well as GPC5 knockdown in rhabdomyosarcoma cells [20], results in decreased proliferation, suggesting that HSPGs can potentiate heparin-binding growth factor signaling even in the absence of exogenous ligand treatment. These signaling effects could result from HSPG enhancement of autocrine growth factor binding or HSPG binding to growth factor receptors to promote dimerization and stimulate downstream signaling.

HSPGs also represent abundant and bulky points of contact for cell-matrix interactions by binding to fibronectin, laminin, thrombospondin, and collagen [6]. These interactions frequently depend on the sulfation characteristics of the binding HSPG and mediate roles in adhesion that can affect cancer cell proliferation. For example, SDC2 promotes cell adhesion and associated proliferation, and decreasing SDC2 expression results in cell cycle arrest and decreased colon and breast cancer tumorigenesis [21, 22]. SDC2 is overexpressed in tumors of the breast, colon, prostate, and bladder, as well as gliomas and sarcomas [17]. Recent work suggests methylated SDC2 could serve as a serum DNA biomarker to aid in the early detection of colon cancer [23].

HSPGs located at the cell surface are also shed, creating soluble proteins that affect proliferation. HSPGs are often expressed in the tumor stroma [6] and their release can influence cancer cell biology (Figure 3). For instance, stromal SDC1 released into the tumor microenvironment can promote breast carcinoma growth via enhanced FGF2 signaling [24]. This effect is enhanced by heparanase expression [25], showing that interactions between HS signaling components can coordinately promote carcinogenesis. Conversely, surface expression of HSPGs and release of soluble forms from the stroma promote FGF2 signaling to suppress proliferation in neuroblastoma [26, 27]. In other circumstances, the surface and soluble forms of an HSPG have opposing effects. For example, although GPC3 is overexpressed in hepatocellular carcinoma (HCC) and promotes tumor growth via Wnt and IGF signaling [28], soluble GPC3 blocks Wnt signaling to inhibit HCC growth [29]. Likewise, GPC1 promotes proliferation and anchorage-independent growth in pancreatic

cancer cells [19, 30], whereas release of GPC1, caused by cleaving the GPI anchor that tethers it to the membrane, inhibited the mitogenic response to FGF2 and HBEGF [30]. The HS chains on glypicans are located close to the GPI anchor and cellular plasma membrane, a proximity that could facilitate formation of growth factor signaling complexes, and help to explain the divergent roles of surface and soluble glypicans.

HS in tumor angiogenesis

In addition to interactions with mitogenic factors, HS also binds growth factors with demonstrated roles in angiogenesis, including FGFs, PDGF, and vascular endothelial growth factors (VEGFs) [6, 31]. Syndecans, glypicans, perlecans and neuropilins are known to influence angiogenesis via growth factor binding [32]. These binding interactions typically enhance tumor angiogenic signaling due to HS modifications. For example, perlecan at the surface of tumor cells and secreted into the extracellular matrix can bind ligand and adaptor proteins via its three N-terminal and one C-terminal HS chains to enhance FGF signaling and tumor angiogenesis [33]. Conversely, fragments of the C terminus of perlecan, known as endorepellin or LG3, lack these HS-mediated signaling effects and actually suppress tumor angiogenesis by repressing VEGF production [34]. Although the HSPG collagen XVIII does not play a significant role in tumor angiogenesis C-terminal fragments of collagen XVIII, known as endostatin, weakly bind other HSPGs and can prevent FGF-induced endothelial cell growth, angiogenesis, and tumor progression [35, 36]. Recombinant human endostatin has proven a successful antiangiogenic therapeutic strategy in preclinical models and clinical trials in NSCLC [37], however it remains unclear whether these effects are dependent upon HS modifications and/or HSPG interactions.

Neuropilins (Nrp1 and Nrp2) are part-time HSPGs that were initially identified as regulators of nervous system development and were subsequently found to play critical roles in tumor angiogenesis [38]. Nrp1 binds VEGFA and B via discrete domains in the core protein to promote tumor angiogenesis and progression [39]. Nrp1-targeting strategies have shown promise in preclinical models and might serve as adjuvants to VEGF-targeting antiangiogenic agents [39]. Nrp2 binds VEGFC and D to promote lymphangiogenesis, which facilitates tumor progression [38, 40]. Thus, therapeutic strategies that are able to block both Nrp1 and 2 could offer enhanced clinical benefit by inhibiting both angiogenesis and lymphangiogenesis. This strategy has recently shown promise in a preclinical model of breast cancer [41]. Although Nrp HS is thought to facilitate Nrp-VEGF-VEGFR complex formation [42], the precise roles of Nrp HS modifications remain unclear. Future studies should clarify which actions of Nrp on cancer cell signaling and biology are due to HS modifications.

Canonical HS binding to antithrombin III (Figure 1) suppresses platelet activation, aggregation, and thrombus formation. This activity explains the clinical use of heparin, and endothelial HSPGs have been demonstrated to have similar functions [43], though their precise identities remain unclear. The effects of heparin on platelet signaling and biology extend beyond this simplistic anticoagulation mechanism. This complexity is illustrated by a counterintuitive side effect of heparin: a pathologic immune response that leads to platelet activation and the clinical disorder heparin-induced thrombocytopenia [44]. Recently,

heparin has been shown to have additional effects on platelet biology that influence tumor angiogenesis. Heparin-treated platelets released less VEGF and more endostatin than control cells, suggesting an additional mechanism for observed antitumorigenic effects [45]. These studies demonstrate the complex roles of heparin and HSPGs in tumor angiogenesis, which can affect disease progression.

HS in tumor metastasis

Heparin derivatives have been proposed as anti-metastasis agents with cancer-specific mechanisms of action. It can be challenging to separate the proliferative and angiogenic effects of individual HSPGs from their effects on tumor metastasis, since local growth and vascularization are critical steps in the metastatic cascade. As expected, the mitogenic activity of SDC1 and GPC1 in pancreatic cancer cells leads to enhanced metastasis in mouse models and high expression of these HSPGs is associated with increased metastasis in patient data [19]. *In vitro* cell systems have helped delineate additional specific roles for HSPGs in tumor cell adhesion, migration, intravasation, and survival during bloodstream transit.

In contrast to the role of SDC1 in promoting proliferation, HS chains on syndecans can bind matrix proteins to promote adhesion, maintenance of cell polarity and reduced cell invasiveness [8, 17]. Decreases in SDC1 expression in colon, lung, liver, ovarian, cervical, head and neck, and squamous cell cancers, as well as mesothelioma, and myeloma are thought to disrupt these HS signaling functions to promote disease progression [17]. The observation that SDC1 can promote tumor growth in some settings but decrease metastasis in others encapsulates the complexity of HSPG co-receptor signaling. It remains unclear why expression of a given HSPG would affect one biology but not another in a particular tumor. To further complicate matters, increased adhesion does not uniformly suppress metastasis and can in fact promote extravasation of circulating tumor cells. For example, SDC2 and SDC4 promote adhesion to enhance invasion in lung and liver cancer. Interestingly, glypicans do not appear to influence invasiveness [46], demonstrating specificity amongst HSPGs that is likely related to distinct HS structures.

The “part-time” HSPG CD44 was initially identified as a lymphocyte-homing receptor that binds the matrix protein hyaluronan [8]. CD44 is poorly expressed in non-transformed epithelia but highly expressed in cancer cells, where it has diverse roles in tumor dissemination, cancer stem cell biology, and circulating tumor cell survival [47]. Similar to other HSPGs, CD44 can bind FGF2, HBEGF, VEGF, and HGF to promote cancer cell metastasis (Box 1). Additionally, HGF can enhance CD44 expression in a prometastatic positive feedback loop [47]. Particular splice variants (especially v6) have been implicated in the progression of breast, endometrial, cervical, ovarian, colon, and liver cancers, and oral squamous cell carcinoma. It remains unclear which of these functions can be ascribed to HS modifications on CD44. A comprehensive characterization of HS modifications in CD44 variants has not been undertaken, however CD44 v3 displays an additional sulfation site that could further promote growth factor signaling [48], suggesting that CD44 splice variants have distinct sulfation characteristics. In colon cancer cells, CD44 v6 appears critical to tumorigenic HGF signaling [49], suggesting that HS modifications could be responsible for

CD44 effects on cancer progression. Loss of expression of CD44 has been reported with progression of bladder, squamous cell, and endometrial cancers, and neuroblastoma [8, 47, 50]. Contradictory reports of CD44 involvement in progression and simultaneous loss of expression in certain cancer types, including endometrial and squamous cell cancers, illustrate the complex roles of this HSPG in tumor metastasis, with many functions still undefined.

Cell-cell interactions are critical to metastasis. P-selectins on platelets bind sialylated fucosylated mucins on tumor cells to facilitate interactions that provide an immunoprotective shielding effect [51]. Cancer cell mucin expression also mediates interactions with L-selectins on endothelial cells that can promote intravasation, extravasation, and metastasis. Soluble HS binding to selectins prevents mucin binding. These observations have led to the therapeutic strategy of heparin treatment to interfere with mucin-selectin interactions [52]. Since heparin also inhibits the actions of heparanase, therapeutics based on HS might target both selectins and heparanase to suppress metastasis [51].

HSPGs also influence cell polarity, changes in morphology during cancer progression, and the process of epithelial-to-mesenchymal transition (EMT). This is not surprising given that HS binds growth factors implicated in EMT, including HGF and VEGF [9], and “part-time” HSPGs can bind additional EMT factors including TGF-β [9]. HSPGs can become upregulated during EMT, along with heparanase to cleave them, leading to enhanced HSPGs in the extracellular matrix that serve as a depot for EMT-promoting growth factors [53]. SDC1 and SDC2 might serve in this capacity in prostate cancer, as expression of both proteins is associated with disease progression [54]. Additionally, SDC1 expression shifts from the tumor to the stroma during breast, lung, colon, and bladder cancer progression [53]. This change in expression could function to remove the anti-metastatic effects of SDC1 at the cancer cell surface, shifting to a higher concentration of SDC1 in stroma cells and the extracellular matrix, where it can promote EMT. In support of this location-specific role, knockdown of SDC1 in breast cancer cells led to morphologic and gene expression changes consistent with EMT and return of SDC1 expression in cells with a mesenchymal phenotype caused restoration of epithelial morphology and reduced growth in soft agar [8]. Expression of a cleaved form of SDC1, however, increased EMT, as did treatment with heparanase, suggesting that surface and soluble SDC1 have opposing actions on EMT signaling [55]. Interestingly, FGF2 increased SDC1 shedding to drive cells toward GPC1-dependent EMT signaling [56]. These studies demonstrate the interconnectivity of HSPG signaling in tumor cells.

As discussed above for cancer cell proliferation, coordinated HS signaling effects can also influence tumor metastasis. Increased heparanase expression, which is associated with increased metastasis and decreased survival in patients with pancreatic cancer [57], promotes metastasis through enhancing SDC1 shedding [25]. Heparanase cleavage of SDC1 also promotes metastasis in breast cancer [25] and breast cancer cells cause systemic increases in heparanase expression to further increase SDC1 cleavage and metastasis [58]. As detailed below, coordinated HS signaling effects can also influence cancer cell differentiation.

HS in cancer cell differentiation

Tumor histology, cell-of-origin, and cancer stem cell studies have demonstrated that cancer cells are de-differentiated or un-differentiated versions of normal cells. These insights have led to the development of differentiating agents used in the clinical management of acute promyelocytic leukemia and neuroblastoma. Through growth factor binding, HS also has roles in cancer cell differentiation.

SDC1 regulates skin homeostasis, as it is readily expressed by normal squamous epithelia and keratinocytes but lost in squamous malignancies including mesothelioma, head and neck, and cervical cancers [59, 60]. SDC1 expression is induced by keratinocyte differentiation and suppressed by malignant transformation; consistent with this, SDC1 expression is decreased in poorly differentiated head and neck and cervical tumors. These effects of SDC1 are believed to result from it acting as a co-receptor for FGF2 in squamous epithelial differentiation. SDC1 expression is also decreased in lung cancer, especially in poorly differentiated non-small-cell and squamous-cell lung tumors [61].

GPC3 is classified as an oncofetal protein, signifying restricted expression during embryonic development and deregulated return of expression in oncogenic settings including testicular germ cell tumors, HCC, and the x-linked Simpson-Golabi-Behemel syndrome, which predisposes to Wilm's tumor [17]. Although oncofetal proteins typically do not play a role in tumor pathogenesis, they can serve as diagnostic biomarkers. In HCC, GPC3 can promote cell growth via HS-independent enhancement of IGF and Wnt signaling [28]. In contrast to its function in HCC, GPC3 suppresses cell growth in breast cancer cells [17, 62]. Once again, tumor context plays an important role in HSPG function.

HSPGs have important roles in neuronal development via effects on FGF signaling. HSPGs, including TβRIII, GPC1, GPC3, SDC3, and SDC4, have recently been demonstrated to promote neuronal differentiation in neuroblastoma cells to suppress proliferation and tumor growth [26, 27]. These effects were critically dependent on HS functioning as a co-receptor for FGF2 signaling. Expression of these HSPGs and CD44 [50] is decreased in advanced-stage disease. As has been described in other cancers, HSPGs are highly expressed in the neuroblastoma tumor stroma [6, 27], where they can be released in soluble form to promote neuroblast differentiation. Heparin and non-anticoagulant 2-O, 3-O-desulfated heparin (ODSH) have similar differentiating effects and represent potential therapeutic strategies for neuroblastoma [27]. These results contrast with the opposing roles of soluble and surface SDC1 discussed previously, and the opposing roles of soluble and surface TβRIII in breast cancer [63]. In neuroblastoma, soluble and surface HSPGs function similarly to enhance FGF signaling and neuroblast differentiation, identifying a setting where heparin derivatives could serve as therapeutic agents.

Heparins as therapeutic agents in cancer

Data from epidemiologic studies and clinical trials demonstrate a protective and therapeutic effect for heparin treatment on tumor growth and metastasis [64]. In certain tumors, such as small-cell lung cancer, a portion of the survival benefit can clearly be ascribed to antithrombotic effects [65]. However, the benefits of heparin treatment exceed the effects of

anticoagulation, suggesting that other mechanisms are involved [66]. Multiple mechanisms likely contribute to the therapeutic effects of heparin, including inhibition of selectin binding [66], inhibition of heparanase [51] and sulfatases [67], decreased platelet signaling to suppress tumor angiogenesis [45], and enhanced terminal differentiation of cancer cells [27]. For a comprehensive review of 50 years of heparin treatment in animal models of metastasis, see [68].

As discussed previously, selectins mediate tumor cell interactions with platelets and endothelial cells to promote metastasis. These interactions are suppressed in tandem with heparanase inhibition during heparin treatment [51], leading to decreased metastasis in preclinical models of colon cancer and melanoma [66, 69, 70]. Future studies should clarify which anti-metastasis mechanisms are critical to the effects of heparin, though it is likely that multimodal inhibition is the most effective therapeutic strategy. The selectin-inhibitory effects of heparin were influenced by sulfation at the N-, 2-O-, and 6-O-positions; however, non-anticoagulant “glycol-split” heparins still showed antimetastatic activity [70], supporting heparin activity beyond antithrombotic effects while identifying alternate heparin-based therapies without anticoagulation side effects. The non-anticoagulant heparin ODSH also inhibited selectin-mediated lung metastasis in an animal model of melanoma [71] and is currently being tested in a phase II trial in metastatic pancreatic cancer.

The potent effects of the heparan-modifying enzymes heparanase and sulfatase in promoting cancer metastasis (Box 1) have generated interest in therapeutic targeting of their activity. In a mouse model of melanoma, heparin treatment reduced heparanase activity and lung metastasis via decreased release of FGF2 from the extracellular matrix [72]. These effects were dependent on N- and O-sulfation of heparin. As discussed above, heparanase targeting strategies may also inhibit sulfatases [67].

In addition to preventing the binding of platelets to selectins and integrins [69], which shields cancer cells from immune surveillance, heparin suppresses platelet release of tumor angiogenic signals [45]. The combined effects of heparin in inhibiting prometastatic platelet biology represent a relatively new field with promising therapeutic potential. The precise mechanisms and characteristics of an ideal platelet-inhibitory heparin remain to be elucidated.

A recent report has identified a role for HSPGs and heparin derivatives, including ODSH, in neuroblast differentiation to suppress xenograft growth and metastasis [27], and clinical trials are currently being organized. ODSH has been proven safe in adult clinical trials, though its safety in children and efficacy in neuroblastoma remain unknown. Future studies will determine whether the differentiating effects of heparin are seen in other neuroendocrine tumors. Heparin might also have differentiating activity in squamous cell cancers based on the activity of SDC1 in skin development and observed suppression of SDC1 expression in cervical, head and neck, and lung squamous tumors [60]. Terminal differentiation currently represents a theoretical approach for most tumors; insights into HS signaling will help identify additional novel differentiating strategies for clinical development.

Heparin has been shown to act as a growth factor co-receptor in a similar manner as HSPGs [13], and high doses of heparin or soluble HSPGs inhibit growth factor signaling by acting as a ligand sink [27, 73]. Future studies should investigate whether heparin treatment alters growth factor signaling in cancer cells. In addition to therapeutic effects on selectins, heparanase, sulfatase, platelet biology, and differentiation, heparin and its derivatives may mimic certain HSPGs in suppressing tumor growth and metastasis in specific cancers.

Concluding remarks

We are entering an exciting period for tumor glycobiology. A large number of high-quality mechanistic studies have demonstrated important roles for HS signaling in cancer biology, including cell proliferation, tumor angiogenesis, metastasis, and differentiation. Although the roles for individual HSPGs in specific cancers are clear in some cases (e.g., SDC1 in breast and pancreatic cancer), most remain unclear and require further investigation. The importance of this approach is underscored by recent studies using an anti-GPC3 antibody to decrease tumor growth in a mouse model of HCC and preliminary clinical trial data [74, 75]. Similar therapeutic strategies can be devised once the roles of individual HSPGs in specific cancers are clarified. One of the greatest challenges in the field is parsing out the individual contributions of HS signaling components in a dynamic and highly integrated tumor microenvironment. “Part-time” HSPGs present an additional challenge, as they also affect HS-independent signaling pathways. *In vitro* model systems will provide important insights, and future experiments should address the extent to which ligands, HSPGs, and modifying enzymes including sulfotransferases, sulfatases and heparanases, can counteract or compensate for one another or synergize to influence tumor cell proliferation and invasion.

Although many preclinical studies and clinical trials support the investigation of heparins as anti-metastasis agents, not all results agree with this trend. Some animal models suggest heparin can alter metastasis distribution or even accelerate dissemination [68]. It remains unclear whether the levels of heparin necessary for metastasis inhibition in mouse models are achievable in human patients without prohibitive anticoagulation [66]. Heparin, HSPGs, and their modifying enzymes can have immunomodulatory effects that alter tumor growth and metastasis [76, 77]. Though not discussed here, the effects of heparin and HSPGs on tumor immunology represent an important area for future exploration.

Modifications in saccharide length and sulfation have generated heparin derivatives that lack anticoagulant properties while potentially retaining oncotherapeutic efficacy [27, 70, 78]. As our understanding of metastasis evolves, we will be able to rationally design heparin-based therapeutic strategies using one or more of these derivatives. These strategies will likely depend on cancer cell-of-origin, stage of disease, and even patient-specific characterization of heparanase or selectin expression. The essential roles of HS in cancer make these pathways promising areas for translational research and drug development, especially as we move into an era of precision and personalized cancer therapy.

Acknowledgments

We thank Angela L. Gaviglio for critical reading of this manuscript. This work was supported in part by NIH grants F30 CA168043-01 (EHK), R01-CA136786 (GCB), and R01-CA135006 (GCB), as well as a Reach Award from Alex's Lemonade Stand.

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Box 1: Synthesis and modification of HSPGs

A specific amino acid motif directs protein glycanation in the Golgi apparatus to form an HSPG [79] (Figure I). HS polymers stretch from 5-70 kDa [5] and HSPGs contain from one to greater than one hundred HS chains [7]. Following saccharide extension by the enzymes *ext1* and *ext2* [6], HS is further modified by sulfation at the 3-O, 6-O, and N-sites on glucosamine, as well as the 2-O site on glucuronic acid [6]. These modifications impart functional specificity to HS and proceed in a highly regulated and orderly sequence.

The role of sulfotransferases in carcinogenesis has recently been explored. Expression of HS3ST2 is epigenetically silenced in lung cancers, where it functions to suppress tumor growth and invasion [80]. By contrast, HS2ST1, HS3ST3B1, HS3ST4, and HS6ST1 and 2 promote cell proliferation, invasiveness and tumor angiogenesis [77, 81-83], presumably via increased HS sulfation and enhanced growth factor signaling.

HS modifications continue after synthesis and sulfation due to the actions of heparanase and sulfatase enzymes [17, 84, 85]. Heparanase at the cell surface or in the extracellular matrix recognizes an HS sulfation motif and hydrolyzes the glycosidic bond between glucuronic acid and glucosamine (Figure I), enabling rapid alterations with demonstrated roles in tumor metastasis and angiogenesis in neuroblastoma, breast, prostate, colon, lung, liver, ovarian, and pancreatic cancer [84, 86]. Heparanase-targeting strategies, including PI-88, SST0001, M402, and PG545, have shown promise in suppressing tumor growth and metastasis in preclinical models and early clinical trials [87-92].

The two known human sulfatases, Sulf1 and Sulf2, are released as soluble enzymes that can cleave the 6-O sulfate on glucosamine (Figure I),[85]. Despite mechanistic similarities, the sulfatases have opposing roles in carcinogenesis, which is best demonstrated in HCC [93]; Sulf1 suppresses FGF2-mediated tumor cell proliferation and invasion, whereas Sulf2 enhances these processes to promote disease progression [94]. Sulf1 is down-regulated in breast, pancreatic, ovarian, and head and neck cancers, where it functions to suppress tumor cell proliferation and invasion by inhibiting the co-receptor function of HSPGs [85]. Consistent with its role in promoting tumor progression, Sulf2 has additional roles in the pathogenesis of non-small-cell lung cancer (NSCLC), pancreatic cancer, and glioblastoma despite unaltered expression levels [95, 96]. The heparanase-inhibiting compound PI-88 also suppresses sulfatase-2 activity, representing a therapeutic strategy for tumors where Sulf2 drives carcinogenesis [67]. These studies demonstrate the critical importance of heparan sulfate modifying enzymes in the growth factor signaling effects of HS in cancer cells.

Box 2: Growth factor signaling pathways affected by HS in cancer

HS-binding growth factors activate signaling pathways with demonstrated roles in cancer cell proliferation, tumor angiogenesis, metastasis, and differentiation. Mitogenic growth factors including hepatocyte growth factor (HGF) and heparin-binding epidermal growth factor-like factor (HBEGF) lead to changes in expression in downstream transcription factors such as myc, jun, and fos, which lead to cell cycle progression via p27, cyclin-dependent kinases and inactivation of Rb [97, 98]. Angiogenic growth factors such as platelet-derived growth factor (PDGF) support blood vessel formation, encouraging tumor growth [99]. Metastasis promoting growth factors such as hedgehog (Hh) enhance invasiveness and pluripotency, leading to tumor cell dissemination [100].

Many HS-binding growth factors have roles in multiple aspects of carcinogenesis. For example, vascular endothelial growth factor (VEGF) stimulates angiogenesis and has also been implicated in promoting metastasis [101]. Fibroblast growth factors (FGFs) signal via mitogen-activated protein kinases (MAPK) to drive proliferation while also promoting angiogenesis, and in some contexts terminal differentiation [102]. Other HS-binding growth factors can suppress carcinogenesis. Bone morphogenetic protein (BMP)-7 can inhibit bone metastases [103]. Therefore, binding interactions between HS-binding growth factors and their respective receptors can trigger both tumor-promoting and tumor-suppressing signaling cascades.

Highlights

1. Heparan sulfate represents a saccharide biopolymer family including the anticoagulant heparin
2. Heparan sulfate signaling is highly ordered and tightly regulated, involving numerous modifying enzymes
3. Heparan sulfate signaling influences tumor proliferation, angiogenesis, metastasis, and differentiation
4. Heparins represent emerging therapeutic strategies for human cancers

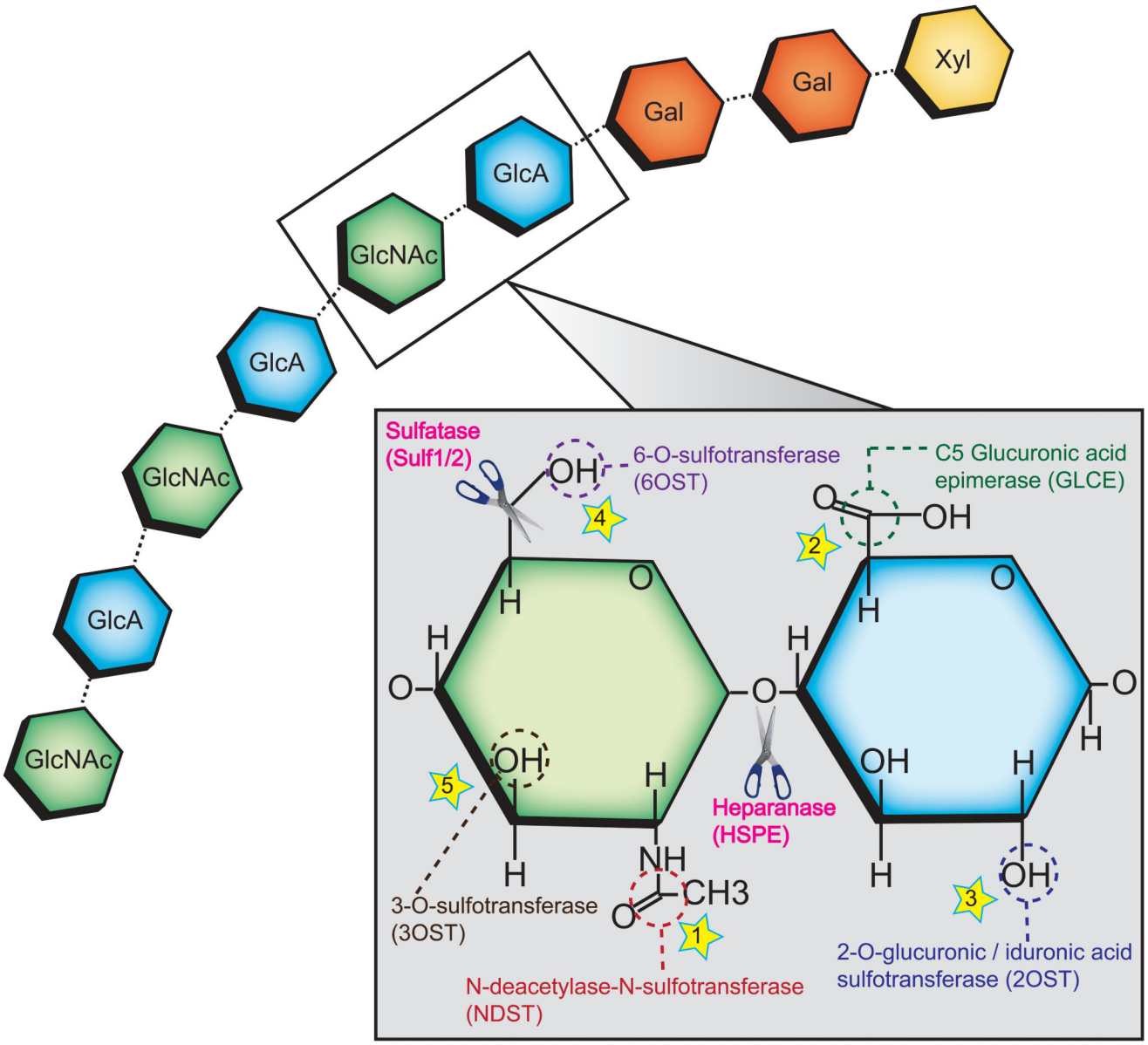

Figure I. HS structure and modification

Heparin and HS consist of a xylose(Xyl)-galactose(Gal)-galactose-glucuronic acid (GlcA) linkage tetrasaccharide followed by repeating disaccharide units (inset) variably sulfated at the 3-O, 6-O, or N-sites on glucosamine (GlcNAc), and the 6-O site on glucuronic acid. Dashed circles indicate sulfation reactions. Starred numbers indicate the highly regulated order of reactions. Heparanases and sulfatases further modify HS structure (scissors).

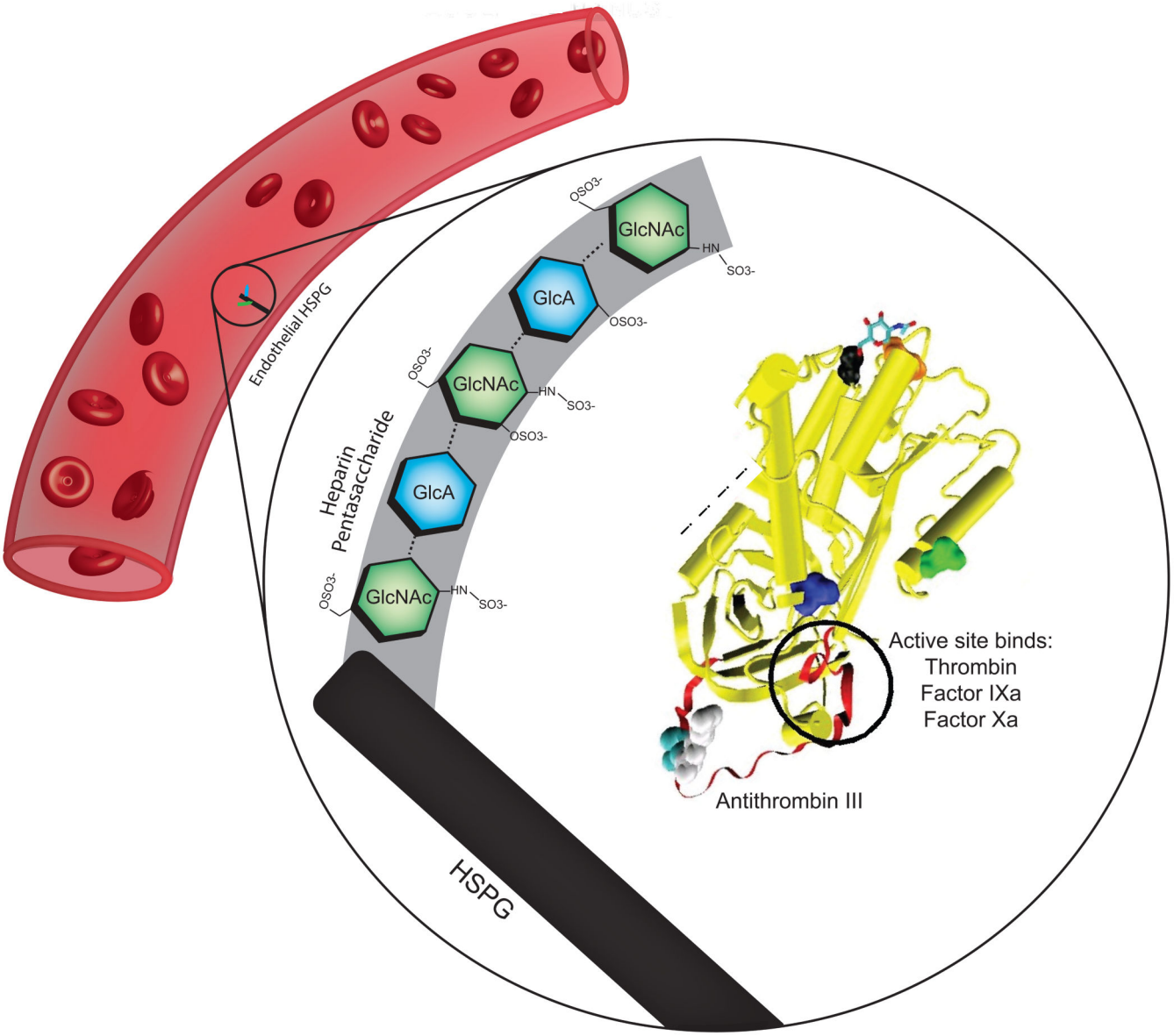

Figure 1. Anticoagulant effects of heparin and HS

Endothelial heparan sulfate proteoglycans (HSPGs) and heparin bind antithrombin III via the sulfated glucosamine (GlcNAc) and glucuronic acid (GlcA) heparin pentasaccharide recognition sequence shown in the inset. Antithrombin in turn binds thrombin, factor IXa, and factor Xa to prevent coagulation. Antithrombin monomer reproduced with permission from K. Murphy (http://en.wikipedia.org/wiki/File:Antithrombin_monomer.jpeg).

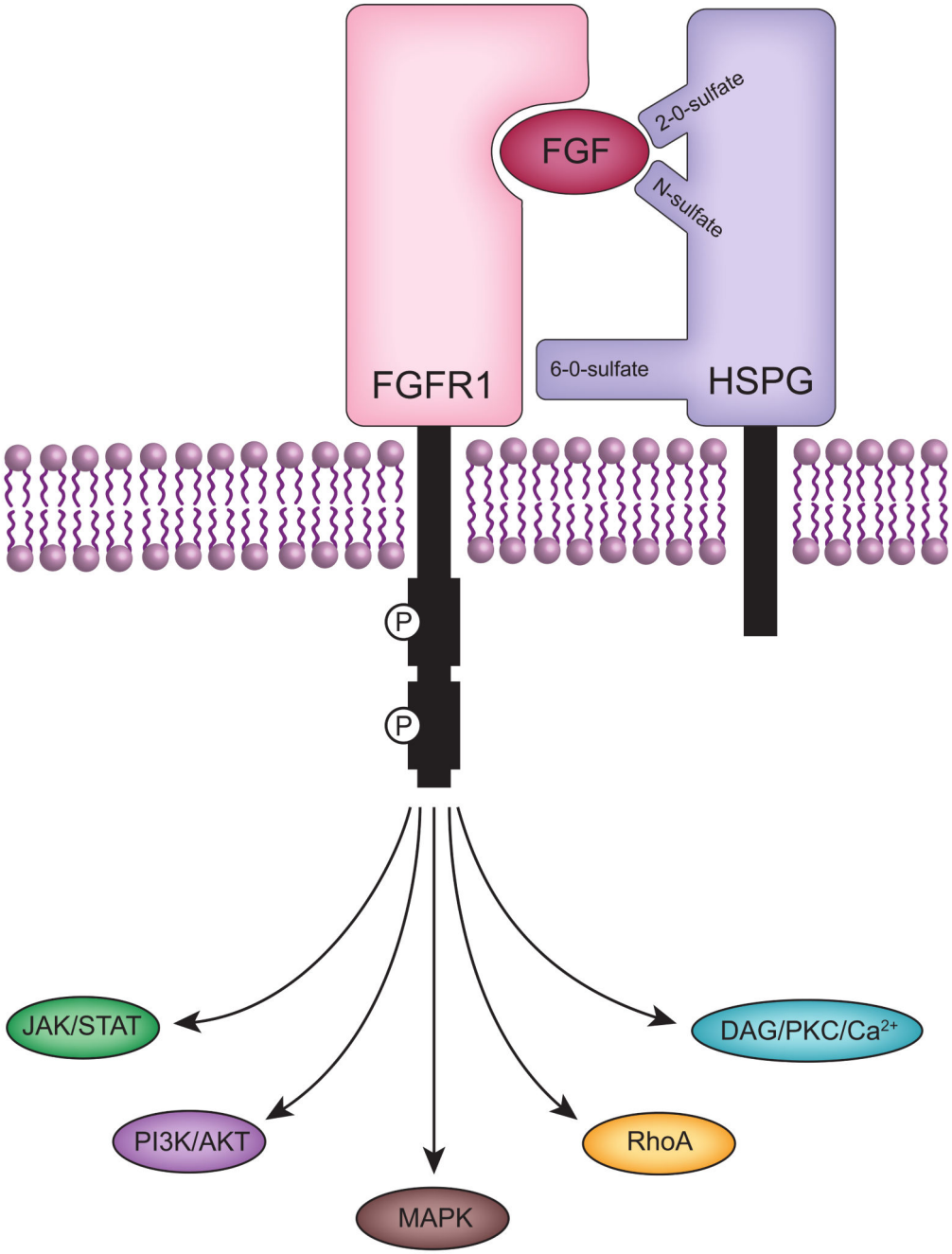

Figure 2. HS ternary complex formation

Heparan sulfate proteoglycans (HSPGs) and heparin bind fibroblast growth factor (FGF)-2 via 2-O-sulfate on glucuronic acid and N-sulfate on glucosamine, as well as FGF receptors (FGFR1) via 6-O-sulfate on glucosamine to enhance downstream signaling via Janus kinase/signal transducers and activators of transcription (JAK/STAT), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), mitogen-activated protein kinases (MAPK), Ras homology (RhoA), or diacylglycerol/protein kinase C/calcium (DAG/PKC/Ca^{2+}).

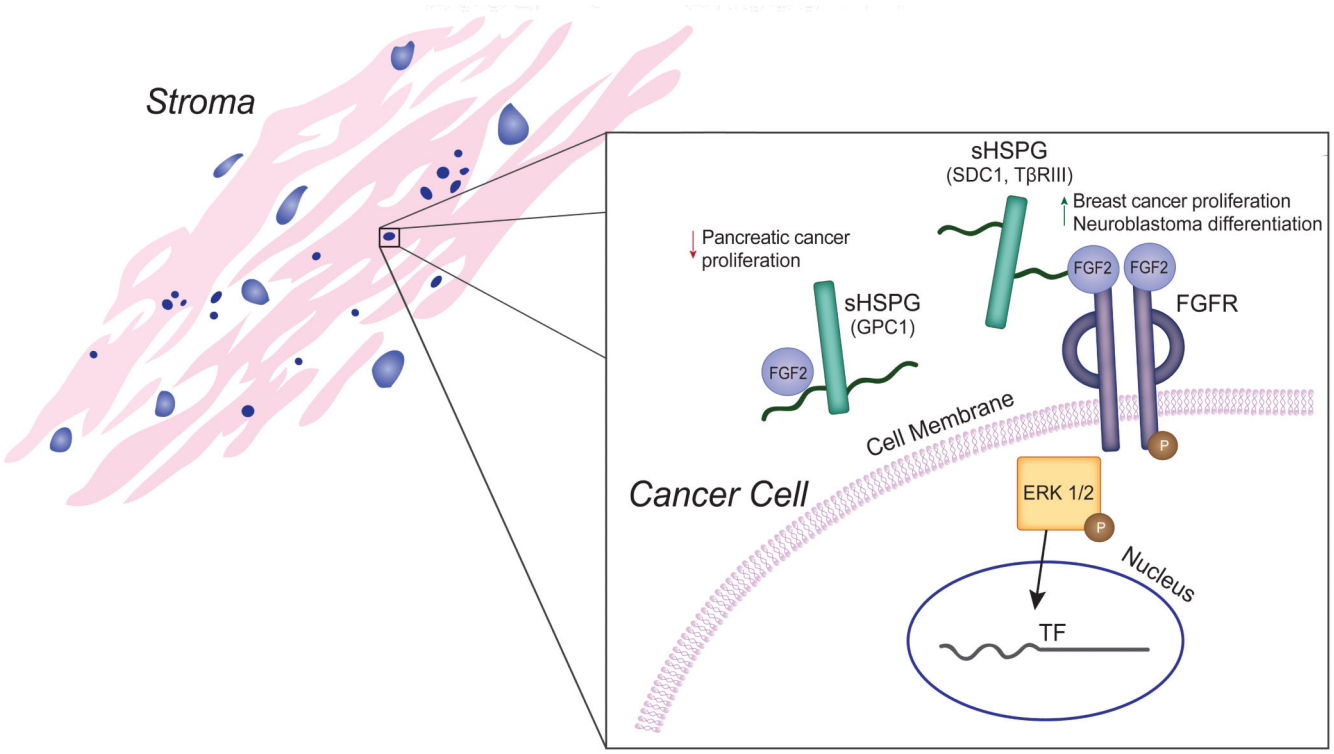

Figure 3. Soluble HSPGs released from the tumor stroma alter cancer cell signaling

Heparan sulfate proteoglycans (HSPGs) cleaved from the stromal cell surface and released in soluble form can bind ligands including FGF2 and receptors including FGFR1 to alter cancer cell signaling (inset). Soluble HSPGs (sHSPG) can decrease **(A)** or increase **(B)** extracellular signal-regulated kinase (ERK 1/2) phosphorylation, translocation to the nucleus and activation of transcription factors (TF). Examples from pancreatic cancer, breast cancer, and neuroblastoma are shown.

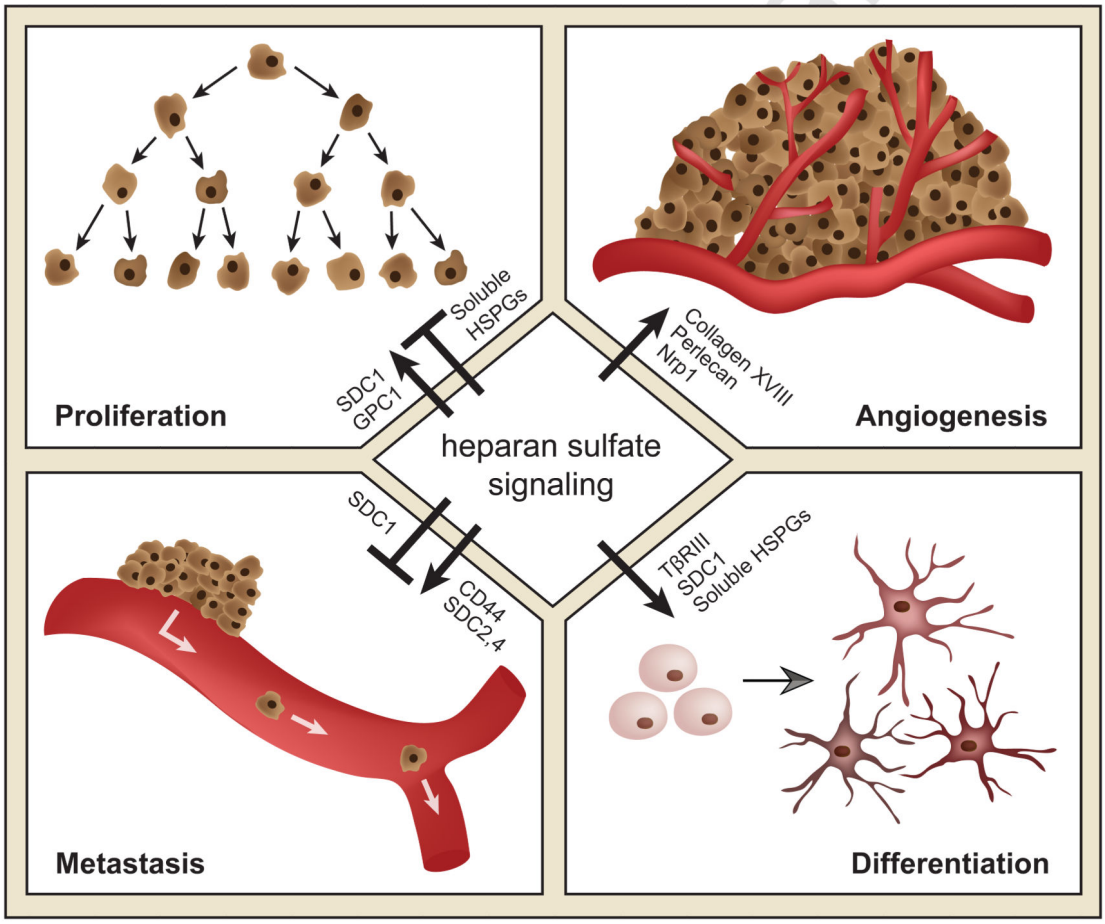

Figure 4. Heparan sulfate effects on cancer cell biology

Heparan sulfate has demonstrated roles in tumor cell proliferation, tumor angiogenesis, metastasis and terminal differentiation. The roles of specific heparan sulfate proteoglycans (HSPGs), including syndecans (SDC), glypicans (GPC), the type III transforming growth factor β receptor (TβRIII), neuropilin 1 (Nrp1), perlecan, collagen XVIII, and CD44, are depicted.

Table I

HSPGs and their binding interactions

Receptor	Cytokines	Growth Factors	Refs
Unspecified HSPG	IL-5,6,8,10, CXCL12/SDF-1, TNF-α, and PF-4	**FGF-1, -2, -4, -7, -8, -10 and -18, HGF, PDGF, HBEGF, Neuregulin-1, VEGF, BMP-7, Noggin, Hh**	[6, 104, 105]
SDC1-4	CXCL12/SDF-1	**FGF-2, HGF, VEGF, HBEGF, Hh,** Midkine, Pleiotrophin, TGF-β, Wnt	[8, 9]
GPC1-8		**FGF-1, -2, HGF, VEGF, BMP-7, Hh,** Wnt, TGFβ, Midkine, IGF	[8, 20, 106]
Agrin		**FGF-2**	[107]
Perlecan		**FGF-2, -7**	[108, 109]
TβRIII		**FGF2**, TGFβ-1, -2, -3, inhibin, **BMP-7**, -2, -4, GDF-5	[8, 9]
CD44	MCP-1	**FGF-2, VEGF, HBEGF, HGF**	[110, 111]
NRP1-2		**FGF-2, -4, VEGF**, PIGF, PDGFB, semaphorins, TGFβ	[8]

Heparin-binding growth factors are bolded. Abbreviations: BMP—bone morphogenetic protein, CXCL12—chemokine C-X-C motif ligand, FGF—fibroblast growth factor, GDF—growth and differentiation factor, HBEGF—heparin-binding epidermal growth factor, HGF—hepatocyte growth factor, Hh—Hedgehog, HS—heparan sulfate, TGF—transforming growth factor, IGF— insulin growth factor, IL—interleukin, MCP—monocyte chemoattractant protein, PDGF— platelet derived growth factor, PF—platelet factor, PIGF—placental growth factor, SDF—stroma cell-derived factor, TNF—tumor necrosis factor, VEGF—vascular endothelial growth factor.

Table 1

Individual HSPGs in cancer

HSPG	Expression Change	Cancer	Biology	Refs
SDC1	Elevated	BrCa, PDAC, OC, MM, TCC	Proliferation (*c*,*m*)	[17, 18, 112]
	Reduced	HCC, SCC, CRC, NSCLC, CC, EC, MSTO, OC	Adhesion, polarity, invasion, EMT (*c*)	[9, 59]
SDC2	Elevated	BrCa, PC, CRC, TCC, glioma, sarcoma	Adhesion, proliferation (*c*)	[17, 21, 112-114]
SDC3	Elevated	TCC		[112]
	Reduced	NB	Differentiation (*c*)	[27]
SDC4	Reduced	NB	Differentiation (*c*)	[27]
GPC1	Elevated	BrCa, PDAC, glioma	Proliferation	[17]
	Reduced	NB	Differentiation (*c*)	[27]
GPC3	Elevated	HCC, OC, GC, FTC, TGCT, NB, Wilms', YST, lung SCC, HB	Proliferation (*c*,*m*)	[17, 74, 115-118]
	Reduced	BrCa, OC, MSTO, NSCLC, NB	Proliferation, differentiation (*c*)	[17, 27, 119]
GPC5	Elevated	RMS, NSCLC	Proliferation, invasion (*c*)	[20, 120]
	Reduced	NSCLC	Initiation	[121]
Agrin	Elevated	HCC, glioma, cholangiocarcinoma	Angiogenesis (*c*)	[122-124]
Perlecan	Elevated	CRC, PC, HB, PDAC, melanoma	Proliferation, angiogenesis (*c*,*m*)	[17, 125, 126]
	Reduced	BrCa, OC, NSCLC	Invasion (*c*,*m*)	[61, 127 129]
Collagen XVIII			Angiogenesis (*c*,*m*)	[35, 130]
TβRIII	Elevated	CRC, NHL, BCLL	Migration, proliferation (*c*,*m*)	[131, 132]
	Reduced	BrCa, PC, OC, MM, NB, NSCLC, PDAC, EC, RCC, melanoma	Invasion, proliferation, differentiation, immune response (*c*,*m*)	[14, 15, 27, 133, 134]
CD44	Elevated	BrCa, CRC, OC, EC, CC, TCC, oral SCC, melanoma	Adhesion, invasion, CSC (*c*,*m*)	[8, 47]
	Reduced	NB		[50]
NRP1,2	Elevated	BrCa, PC, CRC, OC, NB, NSCLC, AML, glioma	Angiogenesis (*c*,*m*)	[38, 40, 41, 135, 136]

Abbreviations: AML—acute myeloid leukemia, BCLL—B-cell chronic lymphocytic leukemia, BrCa—breast cancer, CC—cervical cancer, CRC—colorectal cancer, CSC—cancer stem cell, EC—endometrial cancer, EMT—epithelial-to-mesenchymal transition, FTC—follicular thyroid cancer, GC—gastric cancer, HB—hepatoblastoma, HCC—hepatocellular carcinoma, MM—multiple myeloma, MSTO—mesothelioma, NB—neuroblastoma, NHL—non-Hodgkin's lymphoma, NSCLC—non-small-cell lung cancer, OC—ovarian cancer, PC—prostate cancer, PDAC—pancreatic ductal adenocarcinoma, RCC—renal cell carcinoma, RMS—rhabdomyosarcoma, SCC—squamous cell carcinoma, TCC—transitional cell carcinoma (bladder), TGCT—testicular germ cell tumor, YST—yolk sac tumor. Within the biology column: c—in vitro studies in cells, m—in vivo studies in mice.