



Cancer Metastasis: The Role of the Extracellular Matrix and the Heparan Sulfate Proteoglycan Perlecan

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Cancer metastasis is the dissemination of tumor cells to new sites, resulting in the formation of secondary tumors. This process is complex and is spatially and temporally regulated by intrinsic and extrinsic factors. One important extrinsic factor is the extracellular matrix, the non-cellular component of tissues. Heparan sulfate proteoglycans (HSPGs) are constituents of the extracellular matrix, and through their heparan sulfate chains and protein core, modulate multiple events that occur during the metastatic cascade. This review will provide an overview of the role of the extracellular matrix in the events that occur during cancer metastasis, primarily focusing on perlecan. Perlecan, a basement membrane HSPG is a key component of the vascular extracellular matrix and is commonly associated with events that occur during the metastatic cascade. Its contradictory role in these events will be discussed and we will highlight the recent advances in cancer therapies that target HSPGs and their modifying enzymes.

Keywords: cancer metastasis, heparan sulfate proteoglycan, perlecan, heparanase, therapeutic

CANCER METASTASIS

Metastasis of a tumor is the systemic dissemination and colonization of tumor cells from the primary tumor to a secondary site and is a major cause of cancer-related deaths (1). Cancer is a global epidemic with an estimated 18.1 million new cases and 9.6 million deaths occurring in 2018 (2). Metastasis is an inherently inefficient process, that involves spatial and temporal regulation by both intrinsic and extrinsic factors. It is generally assumed that a cancer cell's genetic mutational burden compounds with advancing malignancy, resulting in the acquisition of proliferative and invasive traits, and finally the capacity to metastasize and colonize, distant organs. However, mutational burden alone does not fully explain the capacity of cells to invade, disseminate, and metastasize to secondary sites (3–6). The role of the microenvironment is now becoming appreciated as a key element in cancer progression, which is driven by interactions between tumor cells and their microenvironment (7–9).

The extracellular matrix (ECM) is a non-cellular meshwork of crosslinked macromolecules including collagens, proteoglycans, and glycoproteins, that form a dynamic, supramolecular, scaffold. It provides cues, both physical and chemical, which influence cancer progression

and metastasis. Biochemical and biomechanical cues present in the ECM, such as sequestered growth factors, ECM biomechanics and ultrastructural organization, are sensed by cells and converted into downstream cellular responses. These downstream cellular responses act in concert to alter malignant progression. Modulation of ECM components, by way of disrupted turnover, and aberrant or absence of post-translational modification (10), are some of the changes common to many diseases, including cancer (11, 12). Moreover, the ECM is a highly ordered structure, and its functional properties are contingent upon the precise assembly of ECM components (13). Subtle changes in the stoichiometry of these components may have downstream biological ramifications which affect tissue function. Cancer associated fibroblasts (CAFs) are important stromal cells within the tumor microenvironment that can be educated and/or recruited by tumor secreted factors. The capacity of CAFs to synthesize and remodel ECM components critically affects tumor progression (14). Understanding the nature of the heterotypic interactions between tumor cells, the ECM, and CAFs within the tumor microenvironment will offer insights into the mechanisms underpinning tumor progression and metastasis.

The process of metastasis is typically represented as a series of interconnected, and overlapping events, whereby certain conditions must be met before tumor cells transition to the next stage (**Figure 1**). These events include invasion into adjacent tissue, intravasation into the bloodstream and lymphatics, cancer cell survival during transit and extravasation out of vessels, and finally secondary organ colonization. The ECM is a key component throughout this cascade of events, with its involvement in modulating the behavior of both tumor and non-malignant stromal cells at all steps along the metastatic cascade.

Epithelial-Mesenchymal Transition

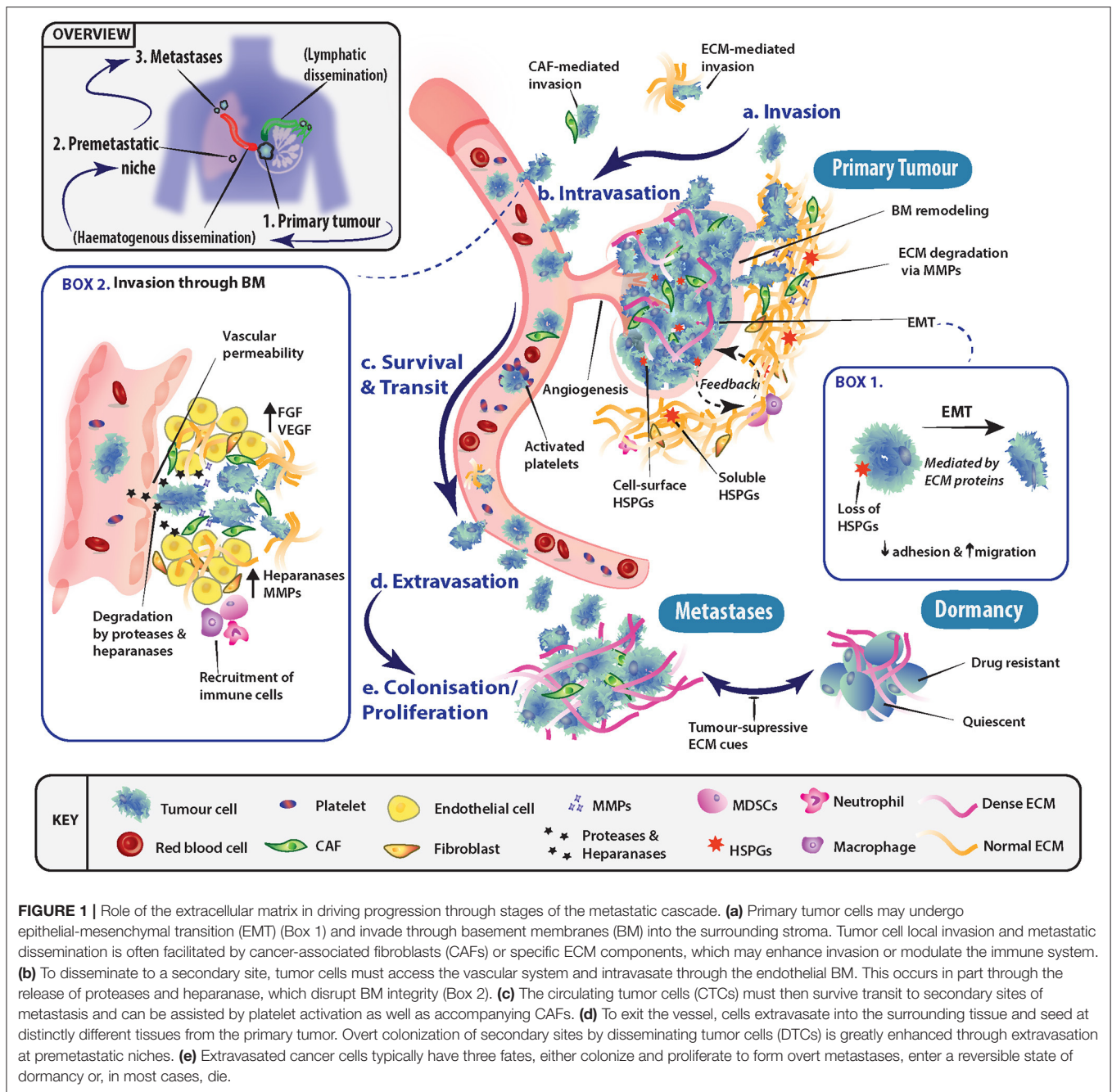
Epithelial-mesenchymal transition (EMT) is one of the key programs in cancer that is thought to facilitate the shift in tumor cell behavior from a static epithelial phenotype to a more migratory, invasive, and mesenchymal one (**Figure 1**, Box 1). EMT and its regulatory signaling pathways are influenced by biochemical cues within the ECM. For instance, ECM environments rich in the glycosaminoglycan (GAG) hyaluronan (HA), transduce signals through the membrane receptor CD44, triggering EMT (15–18). The glycoprotein tenascin C has also been shown to be elevated in late stage mammary invasive ductal carcinomas at the tumor-stromal border. Here, it induces EMT through the proto-oncogene tyrosine-protein kinase Src and focal adhesion kinase (FAK) axis (19, 20). Furthermore, the shift in expression of the heparan sulfate proteoglycan (HSPG) syndecan-1 from tumor cell expression to stromal cell expression (*viz.* vimentin positive CAFs) has been shown to feed back onto cancer cells and drive EMT in many solid tumors (21). However, in contrast, Shen et al. (22) demonstrated that tubulointerstitial nephritis antigen-like 1 (TINAGL1), an ECM protein which competitively binds to integrins $\alpha 5\beta 1$, $\alpha v\beta 1$, and epidermal growth factor receptor (EGFR), can inhibit fibronectin-mediated FAK/EGFR signaling. This highlights how the balance between multiple ECM molecules can regulate the same intracellular signaling networks.

Invasion and Intravasation

Tumor cell invasion is initiated through the breakdown of the interactions (i.e., cell-cell and cell-ECM) at the primary tumor site, allowing cells to invade into the adjacent tissue (**Figure 1a**), in conjunction with local remodeling of the adjacent basement membrane (BM). As tumor cells pass through the local microenvironment of the primary site, they are exposed to a milieu of biomechanical cues within the ECM such as tissue stiffness, density and porosity (23–25), which regulate tumor cell fate. Seminal work demonstrated the ECM's importance at initial stages of metastasis, where interactions between tumor cells and a fibrotic and stiff extracellular matrix induced a malignant and invasive phenotype, which could be blocked to re-establish tissue order (26). At the tissue organizational level, the alignment of collagen fibers has been shown to have prognostic value in breast cancer whereby collagen fibers aligned perpendicular to the tumor periphery, known as tumor-associated collagen signature-3 (TACS-3), are prognostic of patient survival (27, 28).

Hydration of tumor tissue is strongly influenced by the presence of specific glycosaminoglycans (GAGs) within the tissue, due to their anionic structure and their ability to attract water. As hydration increases, increased intratumoral hydrostatic pressure rises and alters the biomechanical properties of the tissue which is known to be crucial to invasiveness (29, 30). Perfusion of nutrients, growth and chemotactic factors are also affected leading to changes in cancer cell invasion (31). Finally, matrix metalloproteinases (MMPs) released from both tumor and stromal cells degrade the ECM and facilitate local invasion (32, 33). The release and activation of ECM-sequestered growth factors [e.g., transforming growth factor (TGF)- β , fibroblast growth factors (FGFs)] may also play a part in this malignant process (34).

Following local invasion at the primary site, tumor cells typically spread around the body via the hematogenous or lymphatic networks which requires traversing the vascular and/or lymphatic BMs (**Figure 1b**, Box 2). However, tumors need not be clinically advanced for this to occur, as dissemination has been observed very early in tumor formation, even before clinical symptoms of disease are evident (35, 36). BMs are specialized tissues underlying epithelial and endothelial structures. BMs are membrane like structures with low porosity and their constituents are densely arranged together. Thus, for cells to traverse BMs, known as intravasation, they require the activation of specific protease-dependent and -independent programs (37–39). BMs impart polarity and survival signals to cells in contact with them, in addition to acting like a molecular sieve for the perfusion of nutrients and molecules from the blood through to the interstices. As such, the structural integrity of vessels and their BMs presents a major obstacle to invading tumor cells. However, in cancer, disruption of BMs is commonly observed. A series of recent studies (40, 41) demonstrated that the ECM molecule hyaluronan and proteoglycan link protein-1 (HAPLN1) decreased with aging of the ECM. This resulted in disruption of the vascular BM and increased vessel permeability, leading to subsequently enhanced



melanoma metastasis in mice. In addition, HA has been shown to be important in the regulation of vascular endothelial barrier permeability, through stabilization of cell-cell junctions (42, 43). Furthermore, high molecular weight HA secreted by tumors has been shown to negatively regulate hyaluronan binding protein 2 (HABP2), a serine protease, which is known to compromise vessel integrity (44). Along with the release of proteases by tumor cells, invasion through BMs can be affected by the release of heparanase (45, 46), which degrades the HS chains of HSPGs located in the BM and ECM, as reviewed by (47) (Figure 1, Box 2).

Survival and Transit Through the Circulatory System

Once tumor cells enter the circulation, their survival in the absence of cell-cell and cell-ECM cues is a crucial factor determining metastatic outcome (Figure 1c). Various mechanisms have been uncovered which facilitate cancer cell survival in the circulation. For example, circulating tumor cell (CTC) clusters exploit mechanisms such as tropomyosin receptor kinase B (TrkB) signaling to combat apoptosis induced by the lack of cell-ECM interactions, termed “anoikis” (48, 49). In addition, the close association of stromal elements with tumor

cells in circulation, namely CAFs and their secreted factors (e.g., FGFs) enhance survival and facilitate metastasis (50). Platelet derived TGF- β signaling also protects against the lack of cell-ECM interactions present in circulation, through inducing a mesenchymal-like phenotype (51). The activation of platelets provides CTCs with fibrinogen (52) and tissue factor (53), which protects against immune clearance within the circulation and at secondary sites. The cues provided may temporarily be substituting for the absence of correct tissue and ECM contacts, and therefore likely provide survival signals that protect cancer cells (7).

Extravasation

Tumor cells that survive within the circulation and lodge in the vasculature of secondary organs, must extravasate into the parenchyma in order to begin the colonization process (Figure 1d). The site of extravasation may be determined to some extent by the formation of “pre-metastatic niches” (54), which can in part explain metastatic organotropism (55). Of note, secreted factors from the primary tumor, such as MMP-3, -9 and -10 (56, 57), can induce the production of vessel destabilizing factors at secondary sites of future metastasis, which act to enhance extravasation. Once extravasated into secondary organs, tumor cells must adapt to the new local cues (i.e., ECM molecules as well as locally secreted growth factors) in order to persist and go on to form overt metastases (Figure 1e). At this stage, the alternatives are entry into a dormant state, or ultimately death. Therefore, this phase in the cascade relies on the interaction between the extravasated tumor cells and the characteristics of the host tissue microenvironment for the successful establishment and outgrowth of overt metastases.

Secondary Organ Colonization

More recently, it has become increasingly apparent that secondary sites may not simply be naïve recipients of disseminated cells, and instead, the ECM and local microenvironment may be remodeled prior to the arrival of tumor cells. This concept has been termed the pre-metastatic niche (54, 58–60), and encompasses the idea that primary tumors were capable of remodeling the tissue microenvironment of secondary organs prior to their arrival in order to facilitate metastatic colonization (Figure 1). This was first demonstrated by Kaplan et al. (60) who showed that bone marrow derived hematopoietic progenitor cells, activated by secreted factors from the primary tumor, are capable of remodeling secondary lung tissue to produce a fibronectin-rich environment prior to tumor cell arrival. This environment then acts to support overt colonization by the seeding tumor cells. Cell-ECM interactions not only supply an anchorage point for seeding, but also activate survival and proliferative signaling programs transduced through integrin complexes and their associated downstream signaling (61–63). These cell-ECM interactions, and signaling networks are potential targets for therapeutic intervention, such as has recently been shown for ROCK inhibition (64, 65). CTCs arriving in secondary organs typically initiate and drive ECM remodeling at these sites. For example, breast cancer cells metastasizing to the lung produce their own tenascin C that promotes survival

and macrometastatic outgrowth via NOTCH and WNT stem cell pathways (66). This is further perpetuated by secretion of TGF- β by cancer cells, which stimulates fibroblasts to secrete periostin (POSTN), further activating WNT signaling (67). Additionally, when secreted at elevated levels, bone morphogenic protein (BMP)-4 and -7 have been demonstrated to cause cancer cell dormancy in both lung (68, 69) and bone (70), which is driven by secreted protein acidic and rich in cysteine (SPARC) in the prostate cancer setting (71).

Another example of ECM induced dormancy has been observed within the “perivascular niche,” which, in some tissues, such as bone and lung, produce a source of quiescing thrombospondin 1 (TSP1) (72). Upon vascular disruption, in situations such as inflammation or wounding, TSP1 secretion is disrupted and the generation of a tumor-promoting microenvironment ensues and facilitates metastatic outgrowth (72–74). Additionally, vascular endothelial cell secretion of perlecan has also been shown to influence lung cancer cell dormancy in the perivascular niche (75). Perlecan has also recently been shown to be upregulated in CAFs in pancreatic cancer through secretion of TNF α from p53 gain-of-function (but not p53 loss-of-function) cancer cells. Cancer cell education of CAFs and the elevated secretion of perlecan was responsible for the generation of a prometastatic microenvironment (76).

It is clear that the ECM is a key regulatory determinant of tumor cell phenotype and behavior, which is dynamically modified throughout the different stages of metastatic progression. The inherent nature of a patient's ECM and the particular modifications accrued by the ECM throughout tumorigenesis may be viewed as either necessary and/or sufficient to enable malignant progression. Thus, the tumor ECM represents a vast territory of underexploited therapeutic targets in treating cancer and cancer metastasis.

PROTEOGLYCANS AND THEIR GLYCOSAMINOGLYCAN CHAINS

Glycosaminoglycans (GAGs) are well-established regulators in the metastatic spread of cancer (77–82). GAGs are negatively charged glycan structures comprised of repeat disaccharide units and belong to one of four subgroups: (1) heparin/HS, (2) chondroitin/dermatan sulfate (CS/DS), (3) keratan sulfate, and (4) hyaluronic acid or HA. All GAGs, other than HA, are covalently attached to the core protein of proteoglycans (PGs). HSPGs are ubiquitously expressed and consist of a protein core to which HS chains are covalently linked. Biological activities associated to HSPGs are mediated through interactions with various ligands, via the protein core or the HS side chains, where the specificity and affinity of these interactions is related to the HS chain structure and position of sulfate groups (83, 84). HSPGs are involved in multiple roles ranging from structural development and maintenance, to organization of the ECM and BM via binding with matrix molecules including collagen IV, fibronectin, and laminin (85, 86). In particular, HS modulates cell-cell interactions by acting as a co-receptor for different cell surface receptors as well as influencing cell-ECM interactions.

HS also mediates the sequestering of various growth factors, chemokines, cytokines, morphogens, and enzymes by forming protected “reservoirs” that upon release can promote receptor-ligand signaling complexes to mediate crucial regulatory roles in cellular processes to maintain tissue homeostasis (87). Structural modification of HS can occur post-translationally by the actions of sulfotransferases, sulfatases (Sulfs), heparanase, MMPs and other proteolytic enzymes (e.g., plasminogen) can modify the protein core of HSPGs and can therefore regulate HSPG-dependent signaling pathways (88, 89). Heparanase is the only mammalian derived enzyme that is capable of degrading HS (90) as well as heparin (91). HSPGs regulate a myriad of activities including; cell adhesion and migration, proliferation, differentiation and morphogenesis, vascularization, cytoskeletal organization, and tissue repair (92). These phenomena are essential for metastasis onset and success.

Heparan Sulfate Proteoglycans

HSPGs have intracellular, cell surface, and ECM localizations, including the BM (93). The BM PGs, perlecan, agrin, and collagen XVIII are primarily substituted with HS GAGs. Endothelial, epithelial, immune cells, and fibroblasts all synthesize these HSPGs, though HSPGs produced by different cell types will be decorated with HS chains that differ in structure, and thus their biological interactions will also differ (94). Hence, HSPGs have been reported to have both pro-angiogenic and anti-angiogenic properties due to heterogeneous HS structures and thus, their interactions with numerous growth factors differ (95). Cell surface HSPGs belong to members of the transmembrane syndecan (SDC) and the glycosylphosphatidyl-inositol (GPI)-anchored glypican (GPC) families. There are four mammalian SDCs (SDC 1-4) and six GPCs (GPC 1-6). The location of HS chains on the PG protein core with respect to the cell surface differs between SDCs and GPCs. The HS chains that decorate GPCs are located close to the plasma membrane. In the SDCs, the HS chains are located at sites further away from the cell surface. The SDC family members are differentially expressed on different cell types, SDC-1 is found on epithelial cells, SDC-2 on fibroblasts and endothelial cells, SDC-3 is on neural cells, and SDC-4 is ubiquitously produced by most cell types but in relatively low abundance (96). Shedding of cell surface HSPGs provides another mechanism to control HSPG distribution, as SDCs can be enzymatically released by MMPs, where GPCs are shed by GPI-specific lipases (97, 98). While, HSPG shedding downregulates their functions at the cell surface, the shed, and now soluble, HSPGs may facilitate the transfer of bound ligands to signaling receptors on neighboring cells conveying positive or negative effects in cancer progression (99). Opposing roles for anchored vs. shed GPCs have been demonstrated. Overexpression of GPC-3 in hepatocellular carcinoma (HCC) promotes tumor growth via WNT (100) and insulin-like growth factor (IGF) signaling (101). However, soluble GPC-3 blocks WNT signaling and inhibits HCC growth (102). Similarly, transmembrane GPC-1 promotes proliferation and metastatic growth of pancreatic cancer cells (103, 104), whereas, soluble GPC-1, inhibits the mitogenic response to FGF-2 and heparin-binding EGF-like growth factor (HBEGF) (104). Additionally, glycoproteins such as betaglycan

and CD44v3 are part-time HSPGs, and may have potential roles in cancer (105, 106).

The strategic location of HSPGs in tissues are critical to their functional roles. Localization of SDCs and GPCs in the plasma membrane regulates intracellular and cell-ECM signaling. Localization of HSPGs in the BM regulates their barrier functions and co-ordinates cell-cell/ECM-cell interactions. Localization of perlecan at the interface of tissues and tissue layers, coupled with their sequestered growth factors, has been hypothesized as on-site “depots” that assist with the restoration of those borders when compromised (107). Cell surface HSPGs can also act as docking modules for MMPs (108, 109), which promote invadopodia and enable cells to move in specific directions through the ECM (110). MMPs secreted by invadopodia promote the invasion of breast carcinoma cells into the ECM (111). Endothelial cells also release granules containing MMP-2 and MMP-9 at focal sites, and their focal MMP activation can contribute to directed angiogenic events (112). It has been proposed that cell surface HSPGs generate a tract in the ECM for the migration of cells. Weak interactive properties between cells and HS allow the cell to “walk” along the cell surface or ECM HS chains facilitating cellular migration (108). Shed fragments of cell surface HSPGs can also influence cell proliferation by amassing in intracellular spaces and sequestering growth factors (86). Degradation of HS, by heparanase, on SDC-1 produces heparin-like fragments that activate FGF-2 mitogenicity (113). The biological role of a HSPG therefore depends on the properties of its protein core, the number of GAG chains attached, its localization in cells and tissues, as well as the biosynthetic modifications its GAG chains receive *in situ*.

The vast range of biological functions attributed to GAGs in cancer metastasis, and numerous other biological events, is due to their non-templated controlled, highly heterogeneous and complex structure, which enables the regulation of tissue-specific functions. Biosynthesis of GAGs is a sequential process that occurs in the endoplasmic reticulum and the Golgi apparatus (114). This process is governed by a large family of enzymes, and while the function of these enzymes is known, the process that controls sulfate specific GAG structure, as well as the degree and position of sulfate motifs is not. HS, the major GAG discussed herein, consists of a glucuronic acid-galactose-galactose-xylose-linker region (GlcA-Gal-Gal-Xyl) which is initiated by the enzymatic transfer of xylose to specific serine-glycine residues of core protein sequences (115). HS assembly occurs by sequential addition of N-acetyl glucosamine (GlcNAc) to the linkage tetrasaccharide acceptor, then GlcA to form GlcA-GlcNAc disaccharide repeats (**Figure 2**). As the chain polymerizes, HS is also enzymatically modified by sulfotransferases and an epimerase at various positions in a coordinated manner, with the product of one modification serving as substrate for the next step (116). The enzyme, N-deacetylase/N-sulfotransferase (NDST), substitutes the N-acetyl group with a sulfate group in between clusters of GlcNAc, leaving regions of the chain unmodified. Further modifications include; epimerization of GlcA to iduronic acid (IdoA) and 2-O-sulfation of IdoA, O-sulfation of GlcNAc by sulfotransferases at C6 or less commonly, at C3. Thus, sulfation along HS chains is not uniform and contains highly sulfated

regions (NS domains) and largely unmodified regions (NA domains). Ligand binding to HS depends on the arrangements of NS and NA domains, and on the modified residues within the NS domains. The HS-FGF-2 interaction exemplifies a GAG-growth factor interaction and demonstrates how specific HS structures facilitate FGF-2/FGFR-mediated signaling. HSPGs play a vital role in the FGF-2/FGFR interactions by assembling FGF-2 near the receptor, which forms a ternary complex that stabilizes the ligand-receptor complex, thereby promoting signal transduction (117). HS chains require N-sulfated glucosamine and 2-O-sulfated IdoA units to bind to FGF-2 (118). At the same time, for HS chains to bind FGFR, they require 6-O-sulfated GlcN residues along with 2-O-sulfated IdoA with N-sulfated GlcN residues also reported to be involved in this interaction (119).

A number of studies have highlighted that HS dysregulation in cancer can occur when the expression and behavior of HS-synthesizing and HS-modifying enzymes are altered (120–124). For instance, Weyers et al. reported on the structural differences found in sulfation patterns between normal and breast cancer tissues in addition to differences in sulfation between patients with non-lethal and lethal cancer (121). Specifically, patients with lethal cancer presented with decreased levels of 6-O sulfation of HS, and increased levels of unsulfated disaccharides. Furthermore, observed increases in HS chain length suggested that the breast tissue underwent changes in the HS polymerization pathway. A similar study assessing transcriptional patterns in panels of breast, prostate, colon cell lines, and isolated tumors confirmed that changes in HS biosynthetic enzyme levels occurred in a tissue-specific manner and particularly affected modification enzymes which undertake HS sulfation (120); supporting previous studies in animal models (125, 126). Interestingly, the authors also discovered that there was no difference in the biosynthetic enzymes between normal and metastatic cell lines and proposed that the cells maintain relatively normal PG expression pattern at the cell surface in order to avoid immune detection.

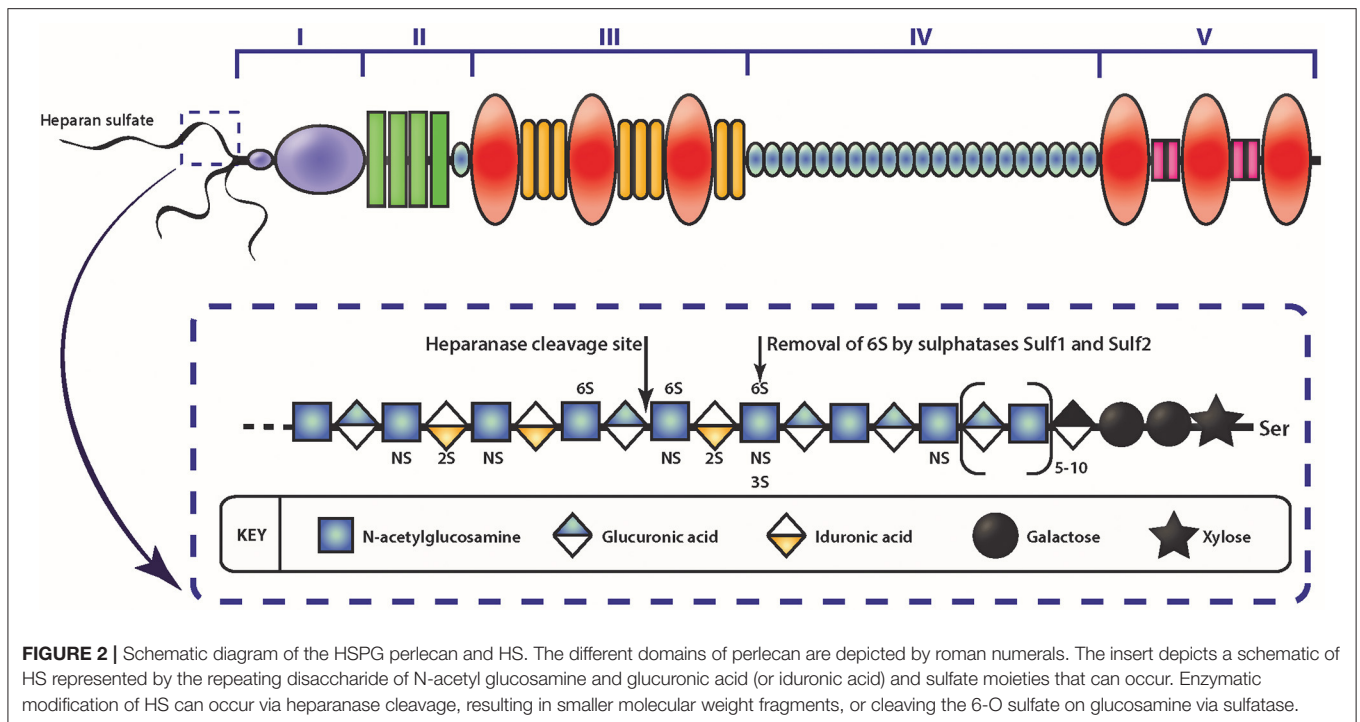
The two known human orthologs of sulfatases (HSulf-1 and HSulf-2) are released as soluble enzymes capable of cleaving the 6-O sulfate on glucosamine (127). Despite similarities in their structural organization and mechanistic action, these sulfatases have been shown to have opposite roles in cancer progression. HSulf-1 suppresses FGF-2-mediated tumor cell proliferation and invasion, HSulf-2 augments these activities to progress disease, as examined in HCC (128). HSulf-1 is downregulated in breast, pancreatic, ovarian, head and neck cancers according to a tumor suppressor effect (129). HSulf-2 has additional roles in the pathogenesis of non-small-cell lung carcinoma (NSCLC), pancreatic cancer and glioblastoma despite unaltered expression levels (130, 131). In contrast, prostate cancer cells overexpressing HSulf-2 present with reduced levels of the trisulfated disaccharide UA(2S)-GlcNS(6S) in conjunction with an increase in EMT markers and WNT signaling (132). In this regard, the role of HS-modifying enzymes in regulating EMT is noteworthy, given its important role in metastatic progression (133, 134). For instance, Maupin et al. consistently found upregulation of the HSulf-2 enzyme in various *in vitro* models mimicking aspects of pancreatic cancer EMT (135). Furthermore, increased

methylation of the HSulf-1 promotor was found to be present in samples from gastric cancer patients (55%) as compared to healthy patients (19%) (136). This was measured using cell-free serum samples taken from patients and the authors advised that methylation-induced silencing of HSulf-1 showed potential as an early diagnostic tool for cancer. Likewise, other studies have proposed that specific biosynthetic trends for each tumor type (121) or proteoglycan staining patterns based on associated GAGs could serve as potential prognostic biomarkers in various histological types (123). Certainly, this area of research will continue to evolve as new analysis tools become available to study GAG structure and identify key structure-function relationships. Significantly, tumor cells have been reported to actively manipulate the binding capacity of their HSPGs for FGF-2 and other growth factors, by modifying the overall density and sulfation pattern of their HSPGs (81). Since natural killer (NK) cells recognize particular HS fine structural patterns, explicitly 6-O-sulfonation and N-acetylation patterns, cancer cells can change their HS patterns to evade NK cells and immune surveillance (137, 138). Studies of breast and pancreatic cancer cells that express increased extracellular heparanase and aberrant HSulf activity have also been shown to affect recognition by NK cells (139).

The Role of Perlecan in Cancer Metastasis

Among the various contributory factors so far identified to be involved in the various stages of cancer progression, perlecan, a modular HSPG stands out as an important player. Perlecan contains multiple domains (**Figure 2**) which allows participation in a variety of roles, as well as being a major structural constituent of BMs (85, 107, 140–143). Perlecan is encoded by the HGPS2 gene, and is predominately substituted with HS chains, though depending on the cell type it originates from, it may be substituted with CS, DS, a combination of HS, CS, and/or DS, or as a GAG-free glycoprotein (144, 145). The N-terminal Domain I is most commonly decorated with three HS chains, whereas at the C-terminal, Domain V can also be substituted with HS and/or CS chains (146). The protein core is divided into five domains, with each domain involved in binding to various partners, from classical ECM components such as collagen IV, nidogen-1, and fibronectin, to growth factors, including FGF-2, -7, vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) (85, 147, 148). While it is present in the BM of most endothelial and epithelial cells, perlecan also associates with the cell surface via interaction with $\alpha 2 \beta 1$ integrin (149). The c-terminal fragment of perlecan can exist as a separate fragment to the perlecan protein core, known as endorepellin, though it is not separately synthesized but rather is a result of proteolytic cleavage of secreted perlecan by proteases (150).

Interestingly, the two other HSPGs of BMs, agrin, and collagen XVIII, do not share much structural homology with perlecan, with the exception of Domain V of agrin (142). Although Domain I is unique to perlecan (151), it does contain the SEA (Sperm protein, Enterokinase, Agrin) module, which is present within other ECM proteins. GAG decoration on perlecan has been shown to be modulated by the presence of the SEA module since its deletion results in a recombinant protein



with decreased HS content and an increase in CS (152). The importance of GAG decoration on perlecan has been further demonstrated in *Hspg2*^{Δ3/Δ3} mice, whereby deletion of exon 3 of the *Hspg2* gene removes the GAG attachment sites in Domain I and the mice presented with impaired angiogenesis, delayed wound healing, and retarded tumor growth (153). The functions that perlecan Domain I plays in various cellular functions cannot be overstated, most notably in angiogenesis (141–143, 154) and is predominantly due to the GAG chains that decorate this domain. The HS moieties of perlecan can bind a variety of pro-angiogenic factors including FGF-1, -2, -4, -7, -10, hepatocyte growth factor and TGF-β (85, 142, 154, 155). The pro-angiogenic activity of perlecan is achieved primarily through the interaction between HS, that decorate the protein core, FGF, and its corresponding receptors. These interactions actively coordinate cell proliferation, motility and adhesion (94, 156, 157). Conversely, and despite being a key region within a pro-angiogenic parent molecule, endorepellin is a potent inhibitor of angiogenesis (158, 159). Endorepellin, via the protein core, binds to both VEGFR-2 and α2β1 on endothelial cells triggering a signaling cascade that disrupts cell actin cytoskeleton and inhibits cell motility (149, 158, 160). Endorepellin is also reported to have transcriptional control by suppressing HIF-1α, a key transcription factor involved in promoting angiogenesis (159). Endorepellin is comprised of three laminin-like globular domains (LG1-LG3) with most of the biological activity attributed to LG3, cleaved from the parent molecule by protease digestion (161, 162). Circulating LG3 levels have been shown to be reduced in breast cancer patients and are being explored as a biomarker for cancer progression and invasion (163). The expression of perlecan has been investigated

in various cancer types both *in vitro* and *in vivo* (Table 1). Although the findings are inconsistent, it is apparent that perlecan controls cancer progression by regulating interactions between cells and signaling molecules during the various stages, including ECM dysregulation, angiogenesis and invasion, which will be discussed in the following sections.

Extracellular Matrix Dysregulation

Cells interact with the ECM to regulate their activities and behavior. This interaction can occur directly through cell surface receptors, including integrins and discoidin domain receptors, and indirectly, via the release of growth factors and cytokines sequestered in the GAG chains (88, 178). ECM remodeling is instrumental to these essential functions including a fundamental role in angiogenesis (179). ECM remodeling removes the restrictive physical barrier, liberating endothelial cells to proliferate and migrate, which is coupled with the release of sequestered pro-angiogenic growth factors from HS chains of perlecan. The ECM is constantly deposited, remodeled, and degraded during development through to maturity to maintain tissue homeostasis (180, 181). Tissue inhibitor of metalloproteinase 3 (TIMP-3) inhibits ECM turnover and has been associated with cancer (182). This enzyme binds to sulphated GAGs on perlecan; further highlighting the significance of sulfation patterns in modulating protein activity (183). The highly dynamic nature of the ECM plays a crucial role in cancer progression and is the first barrier to developing metastasis. ECM remodeling is hijacked by tumor cells and invading stromal cells, resulting in dysregulated remodeling and dynamics (184, 185). This alters the composition and organization of the ECM and eventually leads to changes in its

TABLE 1 | Summary of *in vivo* observations for perlecan expression in various cancer types.

Cancer type	Assessment technique	Observations	References
Melanoma	Immunohistochemistry	Increased in BM at tumor-stroma interface and surrounding blood vessels Increased levels in tissue	(164)
	mRNA expression	Increased levels in tissue	(165)
Colon	Immunohistochemistry	Increased in stroma	(166)
Lung	Immunohistochemistry	Decreased to undetected in BM at tumor-stroma interface	(167)
	mRNA expression	Increased levels in tissue	(165)
Breast	Immunohistochemistry	Decreased to undetected in BM at tumor-stroma interface	(168, 169)
	mRNA expression (<i>in situ</i>)	Increased levels in tumor and stromal cells	
	Immunohistochemistry	Increased in stroma	(166)
Hepatocellular carcinoma (HCC)	Immunohistochemistry	Increased in BM at tumor-stroma interface and blood vessels in stroma	(170)
	Immunoelectron microscopy	Increased at BM at tumor-stroma interface	
Intrahepatic cholangiocarcinoma (ICC)	Immunohistochemistry	Decreased to undetected in stroma	(171)
	mRNA expression (<i>in situ</i>)	Increased levels in tumor cells and stromal fibroblasts	
Ameloblastoma	mRNA expression (<i>in situ</i>)	Increased levels in stromal cells	(172)
Prostate	Immunohistochemistry	Increased in stromal cells	(173)
Ovarian	Immunohistochemistry	Decreased to undetected in BM at tumor-stroma interface	(174)
		Unaltered in BM of surrounding blood vessels or stroma	
Pancreatic	Immunohistochemistry	Increased in BM and stroma	(175)
Oral squamous cell carcinoma (SCC)	Immunohistochemistry	Decreased to undetected in BM at tumor-stroma interface	(176)
		Increased in stroma	
Glioblastoma	mRNA expression	Increased levels in tissue	(177)

essential properties (23, 25). However, the exact interactions and the role of BM components such as perlecan in mediating the abnormalities remain unstudied.

The breaching mechanism by which tumor cells invade the BM has not been clearly determined but has been proposed to involve a number of ECM-distinct and most likely complementary mechanisms: proteolytic degradation of the ECM in parallel with abnormal ECM synthesis (186). Degradation of ECM is mediated by multiple proteases including MMPs, ADAMs, and ADAM-TS (short for a disintegrin and metalloproteinase, and a disintegrin and metalloproteinase with thrombospondin motifs), in addition to heparanase, liberating pro-angiogenic factors that in turn activate angiogenesis and promote the proliferation of tumor cells (185, 187). Stromal cells, including CAFs, along with infiltrating immune cells and tumor cells, results in a sustained presence of these proteinases. This situation overall leads to the progressive destruction of normal ECM and establishment of the cancer-associated ECM. Remarkably, it is the same set of proteins, in different structural configurations and likely altered interactions with each other and the surrounding environment, that results in the abnormal ECM. Certain regions within the ECM have been identified to be important for tumor cell proliferation and survival but can be partially hidden or “cryptic,” only becoming unmasked upon enzymatic digestion (142). At present, no cryptic epitopes have been identified for perlecan but undoubtedly the fine structural sequences of the HS chains may be accountable.

Angiogenesis

Angiogenesis is a key requirement for cancer growth and progression (188); this multi-step process is dependent on ECM

remodeling and endothelial cell activation for the coordinated differentiation into functional vessels. HSPGs have long been acknowledged to control angiogenesis via the sequestering and release of growth factors which regulate endothelial cells, smooth muscle cells, and fibroblasts (189). The role of perlecan in pro-angiogenic and anti-angiogenic functions place it center stage. Both tumor cells and host stromal cells synthesize perlecan; confirmed by a series of early xenograft immunostaining and transcriptional studies (166, 168, 190). The secretion of perlecan by tumor cells was proposed by the authors to facilitate formation of blood vessels during tumor expansion through the binding and interaction between perlecan and angiogenic growth factors. The incorporation of tumor perlecan into host blood vessels is likely mobilized by proteases easing the recruitment and diffusion of angiogenic growth factors into the tumor stroma (89). Gradients of perlecan expression have been observed in tumor vessels with the most reactive areas located at or around the sprouting edges, suggesting that tumor-derived perlecan can favor or induce the neovascularization of tumors (166, 190). Alternatively, host cells are proposed to synthesize perlecan as a defensive mechanism, with HS acting as a “sink” for growth factors by limiting their diffusion (154). The HS chains may be key elements that direct the intermolecular interactions that occur between perlecan and other BM components. The diverse substructure of HS chains might influence not only the growth factor-binding ability of perlecan but mediate roles in adhesion that can affect cancer cell proliferation and migration (86).

Tumor cells can also upregulate the production of several angiogenic factors such as FGF and VEGF in order to support their altered growth patterns and metabolism (154). For example, tumor vessels formed as a result of VEGF upregulation are

abnormal; these vessels are variably fenestrated and leaky, accompanied by a disorganized or loose BM (191) (**Figure 1**, Box 2). These conditions typically lead to high interstitial pressures, escalated tissue hypoxia and production of additional VEGF (192). Human prostate cancer cells, depleted of perlecan and grafted in mice, produced tumors of decreased size and vascularization, where the effects were correlated to reduced secretion of VEGF-A in the xenografts (193). The occurrence of hypoxia during the early stages of tumor growth has been shown to regulate a number of angiogenic growth factors and cytokines, including VEGF (194). The expression of regulatory enzymes responsible for HS chain synthesis is also subject to hypoxic influence with preferential synthesis of HS resulting in increased responsiveness of hypoxic endothelial cells to FGF-2 (195). The release of heparanase from tumor cells into the ECM promotes cleavage of HS fragments, which in turn liberates bound growth factors that act to further support tumor angiogenesis (196). Perlecan also plays a role in establishing cytokine gradients in the ECM which are utilized by cells to migrate through tissues, as in the case of angiogenesis (87, 197).

Invasion

Malignant tumors are characterized by their invasiveness into nearby tissues, followed by metastasis to distal locations away from the primary tumor site. In order for these processes to take place, a series of signaling mechanisms contribute to the breakdown of the surrounding ECM by activating or releasing various proteolytic enzymes. A key enzyme involved in HSPG processing is heparanase, which recognizes a HS sulfation motif to hydrolyze the glycosidic bond between glucuronic acid and glucosamine (198). Heparanase activity digests HSPGs, resulting in increased endothelial permeability that enables the passage of invading cells through established boundaries, and the release of sequestered growth factors and soluble HS fragments that support angiogenesis and tumor growth (196). It has also been proposed that reduced adhesion of tumor cells to the underlying ECM, as well as increased cell motility, is due to cleavage of cell surface HS by heparanase produced by the tumor cell itself (108). Notably, heparanase has also been recognized to participate in some non-enzymatic activities, separate from its involvement in ECM degradation and remodeling (199–201).

Upregulation of heparanase occurs in essentially all human tumors and is closely correlated with an invasive phenotype in experimental models and has been linked to worse outcomes in cancer patients (196, 202, 203). A few examples are presented. Lung metastatic melanoma cells overexpress heparanase isoform 1 (Hpa1) mRNA (up to 29-fold) compared to normal lung tissue (204). Hpa1 enzyme was identified around vascularized regions, as well as blood vessels near the invasion front in various representative models (204, 205). Heparanase over-expressing breast tumors are seven times larger and present significantly more vascularization (206). Friedmann et al. presented high levels of heparanase mRNA in lymph, liver, and lung tumor metastases with the highest amounts of both mRNA and enzyme detected in deeply invading colon carcinoma cells (207). Heparanase activity is upregulated in lung and brain cancers, with melanoma cells that are highly metastatic to the

brain overexpressing Hpa1 (208, 209). Specimens from breast cancer patients showed that lymphocytes express heparanase and when serum collected from these patients was introduced to fresh lymphocytes, heparanase expression was stimulated in the normal lymphocytes (210). Furthermore, a non-metastatic cell type, transfected with the gene that encodes heparanase, acquired a metastatic phenotype (211). Hypoxia was found to augment heparanase activity and consequently invasion in ovarian cancer cell lines (212). Inversely, anti-sense targeting of heparanase weakens the invasive ability of carcinoma cells (213).

The importance of HSPG structure in tumor biology was demonstrated in a study where Liu et al. injected bacterial recombinant heparinase (Hep) I (which cleaves highly sulfated regions) and Hep III (which cleaves unsulfated regions) into melanoma challenged mice and found that the specificity of the enzymes dictated whether tumors regressed (Hep III) or advanced (Hep I) due to where the different enzymes cleaved HS (214). This finding demonstrated both the heterogeneity of HS and the fine control of biological function due to these different HS structures. They found that the resulting tumor cell GAG fragments were distinct following treatment with the different heparinase isoforms, with Hep III digestion causing up to 75% inhibition in tumor growth whereas fragments as a result of Hep I digestion significantly enhanced growth. Furthermore, the demonstrated effects were modulated by FGF-2 signaling, as Hep I-generated fragments promoted FGF-2 activity, whereas Hep III-generated fragments inhibited signaling, with additional implication of MAP kinase and FAK pathways. It should be noted that there is a difference in the mechanism by which mammalian-derived heparanase and bacterial-derived heparinase cleave HS; heparanase is a hydrolase, as opposed to heparinase which is an eliminase (215). In some instances, the overexpression of heparanase is linked to other enzyme activities. In addition to heparanase overexpression, melanoma cells were reported to exhibit 3-O-sulfotransferase gene hypermethylation and subsequent gene silencing (216). A study by Ma and Geng, showed that the cell adhesion molecule P-selectin, present on endothelial cells and activated platelets, was still capable of binding to a HS-like molecule displayed on melanoma cells despite the absence of its recognition motif (217). Interplay between a series of enzymes including 3-, 6-O-sulfotransferase and HSulf enzymes may transform HS to confer P-selectin binding ability and hence promote the migration of cells to secondary sites (81). Additionally, heparanase mediates upregulation of MMP-9, expressed from tumor cells, to indirectly stimulate invasion (218). In addition to the biological effects modulated by the HS chains of perlecan, perlecan-rich borders can resist cell invasion and serve as tissue boundaries (107). These borders include the glandular BM (219), the reactive stromal compartment (173), the vasculature (220), and bone marrow reticular matrix (221). Perlecan and MMP-7 co-localize at tissue boundaries when surveyed in prostate cancer sections, with MMP-7 proposed to act as a molecular switch by altering cancer cell behavior to favor cell dispersion and invasiveness (222, 223).

While increased expression of perlecan is shown in a number of tumor types (**Table 1**), its levels are also undetectable in other instances. Several early studies reported strong mRNA levels of

perlecan with the overexpressed perlecan protein deposited in the ECM and in tumor cells at the invading front (164, 171, 172, 224). These studies were supported by observations of inhibited tumor growth and angiogenesis (193, 225) or reduced cell proliferation and invasiveness (226) when perlecan was downregulated by anti-sense targeting. This is contrary to the findings reported by Mathiak et al. where anti-sense targeting of perlecan resulted in stimulation of tumor cell growth *in vitro* and *in vivo* accompanied with increased invasiveness in the ECM (227). It has been suggested that the lack of perlecan in these cases could perhaps be related to the tissue microenvironment preferentially favoring the diffusion of growth factors, which encourages tumor growth and metastasis (142, 154). Alternatively, Nerlich et al. reported high levels of perlecan mRNA in both tumor and stromal cells but then very low levels of perlecan protein present in tumor-associated BM (168, 169). Similarly, differences were observed between perlecan mRNA and secreted protein measured from stably transfected anti-sense perlecan targeting subclones, with reduction of >50% compared to the untransfected parental cell line (193). A recent study exploring the localization of perlecan in squamous cell carcinoma (SCC) reveals that perlecan and its binding growth factors namely VEGF [binds to HS chains (85)], Sonic Hedgehog (SHH) [HS and protein core (228)], and FGF-7 [protein core (147)] co-localize within the epithelial layer before invasion (176). Once the carcinoma cells started to invade, perlecan and FGF-7 were identified in the stromal space while VEGF and SHH remained at the epithelial layer. This correlates with other studies that suggested biosynthesis of perlecan was switched over from carcinoma cells to stromal cells (174, 190, 229, 230). The discrepancy between significantly enhanced mRNA synthesis and loss in protein deposition may also point to the activity of proteolytic enzymes or a post-translational block of protein synthesis or both (154).

Overexpression of perlecan in prostate cancer stroma has been linked to TNF α -mediated transcriptional induction (173). This suggests that perlecan transcription could be a part of cytokine-mediated innate immune response to cancer invasion. Perlecan has also been implicated in regulating prostate cancer progression via the SHH pathway (231). Franses and colleagues explored the role of endothelial cells in regulating cancer cell behavior, where perlecan silencing eliminated the ability of endothelial cells to suppress cancer invasiveness in both *in vitro* and *in vivo* models of breast and lung cancer (75). These findings indirectly contrast with the early work (discussed above) showing that perlecan depletion (albeit in cancer cells) slows tumor growth and reduces metastasis (193, 225, 226). The fact that perlecan acts in a cell context-specific manner could be a consideration for the contradicting data (142). It is important to note that perlecan derived from different cellular sources carries different HS structures and as such different growth factor binding and functional capabilities (94, 157). For example, Lord and colleagues have shown that the GAG chains differ between perlecan enriched from human coronary artery smooth muscle or endothelial cells and this influences their roles in mediating cell adhesion and proliferation, as well as FGF binding and signaling (157). Therefore, it can be summarized that tumor subtype, stage, degree of tumor differentiation, and/or various

histological location and identifying reagent (i.e., primer region of interest or antibody epitope) may result in the different distribution of perlecan across the reported studies.

THERAPEUTIC TARGETING OF HEPARAN SULFATE PROTEOGLYCANS AND THEIR FUNCTION IN CANCER METASTASIS

Therapies that target HSPGs in cancer metastasis cover a range of modalities, highlighted in **Table 2**. Most therapies that target metastasis and the role of HS revolve around the inhibition of heparanase. The inhibition of heparanase eliminates the cleavage of HS chains and the release of bioactive molecules such as, FGFs, and VEGF, to disrupt the downstream events that are associated not only with the progression of cancer but also with cancer metastasis. Given the prevalence of cancer and the role of HSPGs in multiple events there is an extensive amount of literature, including a number of recent reviews (203, 247, 248), that detail the mechanisms of action of the range of therapeutics that are being developed. The following section will review the most recent advances in the field.

The first reports of heparanase inhibitors in an anti-cancer or anti-metastatic activity, stemmed from the use of heparin and low molecular weight heparins (LMWHs) (249). As heparin has a similar structure to HS, though a higher sulfated version, it competes with endogenous HS for both heparanase binding and substrate activity. However, the risk to patients regarding bleeding due to anticoagulant activity of heparin has limited their use as therapeutics for cancer and cancer metastasis, particularly as a long term therapeutic. Given the potential of both heparin and LMWHs, much effort has been directed toward either modifying or mimicking the structure heparin/LMWHs to remove the anticoagulant activity whilst retaining the ability to inhibit heparanase. The success of HS mimetics is clear through the number of these materials that have made it through to clinical trials. Modification of heparin through desulfation and glycol splitting has seen the development of roneparstat (250) and its investigation in a Phase I trial as a therapeutic for myeloma (232). In addition to roneparstat, HS mimetics muparfostat (PI-88) (233), neuparanib (N-402) (234), piixatimod (PG545) (251), have been, or are currently in clinical trials for use as a therapy targeting metastasis of melanoma or pancreatic cancer. More recent reports have detailed the use of these HS mimetics not only in the development of therapeutics, but the development of more representative models for testing anti-cancer/anti-metastatic therapeutics including patient-derived xenografts (237) and organoid models (238). Neuparanib has been shown to reduce tumor cell proliferation and invasion in an organoid model, and plasma levels of patients within a clinical trial cohort reported increased levels of tissue inhibitor of MMP-3 (238). The attempt at mimicking the structure of HS has seen the development of glycopolymers with well-defined sulfation patterns and the ability to optimize disaccharide length for peak heparanase inhibition (252), which reduced metastasis of breast cancer in a rodent model. The ability to design and synthesize HS mimicking structures that eliminate anti-coagulation activity

TABLE 2 | Summary of therapeutics that target heparan sulfate proteoglycans.

Therapeutic	Results or observations (specific compound reported in brackets)	References
HS mimetic/ heparanase inhibitor	In a Phase I clinical trial demonstrated safety though anti-myeloma efficacy was minimal (Roneparstat)	(232)
	Demonstrated safety in a Phase I clinical trial for melanoma [Muparfostat (PI-88)]	(233)
	Acceptable safety and encouraging signals of activity in patients with metastatic pancreatic cancer in Phase I clinical trial [Neuparanib (N-402)]	(234)
	Anti-metastatic effects in murine models of melanoma and lung cancer	(235)
	Inhibition of primary tumor growth and reduced metastasis in murine breast cancer model	(236)
	Acceptable safety and encouraging signals of activity in patients with metastatic pancreatic cancer in Phase I clinical trial	(234)
	Inhibition of metastasis from primary tumor in a lung cancer patient derived xenograft model	(237)
LMWH	Reduced MMP1 expression and increased TIMP3 expression in pancreatic cancer patients	(238)
	Reduced primary tumor and pulmonary metastasis in a murine melanoma model. LMWH was incorporated into a hydrogel system	(239)
Heparanase inhibitor	Benzoxazole derivatives demonstrated anti-metastatic potential via reduced expression levels of FGF-1, FGF-2, VEGF, and MMP-3 in a fibrosarcoma derived cell line	(240)
Sulfatase inhibitor	Inhibition of TGFβ1/SMAD and Hedgehog/GL1 pathways in hepatocellular carcinoma cell lines	(241)
	Reduced tumor size in mice implanted with xenograft pediatric glioblastomas	(242)
Immunotherapy	GPC-2 targeting antibody-drug conjugate reduced proliferation of GPC-2 expressing cells derived from neuroblastomas	(243)
	Monoclonal antibody that binds to GPC-3 demonstrated safety in a Phase I clinical trial for hepatocellular carcinoma	(244)
	GPC-3 CAR-T cells eliminated GPC-3 positive tumors in murine model of hepatocellular carcinoma.	(245, 246)

and target heparanase has more recently been facilitated with use of computational modeling to predict the anti-cancer/anti-metastatic potential (253–255).

In addition to the issues associated with anticoagulant activity, heparin also has a short half-life which can mean when administered intravenously that high dosages are required for a therapeutic effect or that there is the need for multiple injections. More recent reports have demonstrated the therapeutic use of heparin via incorporation or tethering to a substrate for targeted delivery. Reduction of metastasis in a lung cancer model was achieved with incorporation of heparin into a hydrogel system for local administration of the therapeutic (256). Tethering heparin to oligonucleotides via a cleavable linker that is pH

sensitive (239), has also been demonstrated as a method of targeted delivery and the reduction of pulmonary metastasis in a melanoma model. Furthermore, delivery of LMWH, through tethering to micelles, reduced pulmonary metastasis in a breast cancer model, which was further reduced by using a delivery system that facilitated targeted co-delivery of the LMWH with the chemotherapy agent doxorubicin (257).

Despite their anti-metastatic properties, HS mimetics and polysaccharide derivatives have limitations due to their relatively high molecular weights, and rather heterogenous structures. More recently, there has been the exploration of small molecular inhibitors of heparanase, that overcome these limitations, for example benzimidazole and benzoxazole derivatives (258–260). Benzimidazole and benzoxazole derivatives have been long studied in medicinal chemistry (261). Most recent advances in these derivatives include the synthesis of symmetrical analogs that demonstrated superior anti-heparanase activity as compared to non-symmetrical analogs (240), with the ability to not only inhibit heparanase, but also bind and sequester HS interacting growth factors and chemokines that modulate angiogenesis.

In addition to heparanase, sulfatases can modify HS via the removal of 6-*O*-sulfate groups and as such have been investigated as a targeting molecule. The compound designated OK-007, 2,4-disulfophenyl-*N*-tert-butyl nitron, inhibits the enzymatic activity of Sulf2. This compound was initially explored as a treatment for acute ischemic stroke (262), though has since been investigated as a potential therapeutic for HCC (241) and glioblastoma (242). Coutinho de Souza et al. (242) demonstrated the ability for OKN-007 to reduce cell proliferation and the expression of the receptor for platelet derived growth factor, and the authors speculated potential anti-angiogenic properties of OKN-007.

More recently, monoclonal antibody therapy, a form of immunotherapy, has been explored as a route to target HSPGs. Though, these therapies have been mainly focused toward targeting primary rather than secondary tumors. Monoclonal antibodies targeting GPC-2 have been developed as a therapeutic for neuroblastoma (243), and antibodies targeting GPC-3 have progressed to phase I trials in HCC (244). More recently GPC-3 in HCC has been used as a target in chimeric antigen receptor, or CAR T-cell therapy (245, 246, 263), with the therapy demonstrating the ability to reduced HCC tumors in a xenograft model (245).

CONCLUSIONS

The role of HSPGs in cancer metastasis is through the interaction of the HS chains or PG protein core with key biological molecules associated with metastatic events. The non-templated heterogeneous structure of HS modulates these specific interactions between mediators, influencing events in the metastatic cascade. Furthermore, the increase in heparanase expression in multiple cancer types results in the cleavage of HS chains and release of mediators involved in these events. HSPGs, including perlecan, have antithetic roles in

cancer and metastasis through the interaction with biological molecules. The subtle differences in HSPG structure, particularly that of HS, results in a family of molecules that behave as both pro- or anti-metastatic factors. Thus, due to the structure specific interactions between HS and mediators of metastatic events, future therapeutics that target HSPGs and their cleaving enzymes need to target specific HS or heparanase binding structures, and ideally have targeted delivery, to ensure both efficacy and reduced off-target effects to truly improve patient outcomes.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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