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Ren, Z.

Publication date

2019

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Citation for published version (APA):

Ren, Z. (2019). *Heparan sulfate proteoglycans: key moderators of the interaction of multiple myeloma with the bone marrow niche*. [Thesis, fully internal, Universiteit van Amsterdam].

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CHAPTER

5

Syndecan-1 and stromal HSPGs: key moderators of communication between normal and myeloma plasma cells and the bone marrow niche

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Manuscript in preparation

Abstract

Upon antigen recognition by the B-cell-antigen receptor (BCR), B cells differentiate into plasma cells, which represent the effector cells of the humoral immune system. However, in contrast to their B cell precursors, plasma no longer express a cell-surface BCR and, hence, are deprived of the BCR-derived signals, which are crucial for survival throughout B cell development. Instead, for their survival, long-lived plasma cells heavily rely on communication with the bone-marrow (BM) microenvironment, which provides adhesion-mediated as well as multiple soluble signals. Plasma cells acquire strong expression of the cell-surface heparan sulfate proteoglycan (HSPG) syndecan-1, a phenotypic hallmark of plasma cells and their malignant multiple myeloma (MM) counterparts. In this review, we discuss data demonstrating how syndecan-1, in concert with HSPGs in the bone marrow microenvironment, mediates homing and survival of normal plasma cells, and promotes MM growth, by co-opting growth and survival factors from the BM niche and targeting them to their cognate receptors. The crucial function of syndecan-1 and of stromal HSPGs in the communication of MM with the bone marrow niche, designates these molecules and their synthesis machinery as potential treatment targets. Various venues to target syndecan-1/HSPGs in MM are currently being explored in preclinical and clinical studies.

Introduction

Antigen-specific B-cell differentiation is a highly complex multistep process, which ultimately results in the generation of plasma cells producing large quantities of high-affinity antibodies, specific for a given antigen. From the pre-B cell stage onwards, all steps in this differentiation process critically depend on the membrane expression and integrity of the B-cell antigen receptor (BCR) or its pre-BCR precursor, which provides essential signals for the survival, proliferation, and differentiation of the B cells. Loss of BCR expression or deletion of genes encoding BCR-signaling pathway components results in a strong reduction in mature B cell numbers¹⁻³. The BCR controls B cell survival largely through the PI3K/AKT pathway, which regulates the expression of genes involved in the induction of apoptosis and cell cycle³⁻⁵.

The BCR essentially is a membrane-bound immunoglobulin docking to an intracellular signaling cascade. A key difference between the plasma cell and its B-cell precursors is that, in plasma cells, the BCR is no longer membrane bound but secreted by deletion of the membrane-associated portion. This loss of BCR expression demands a complete make-over of the cellular signaling machinery, to enable plasma cells to survive in the absence of BCR mediated signals. As discussed in this review, expression of the transmembrane heparan sulfate proteoglycan (HSPG) syndecan-1 is an essential component of this metamorphosis of B cells to plasma cells. Syndecan-1, in collaboration with HSPGs expressed by bone marrow stromal cells, allows normal as well as multiple myeloma (MM) plasma cells to efficiently co-opt soluble factors produced in by cells in the BM-niche, promoting homing/retention, survival, and in MM, tumor growth. Therapeutic strategies to target HSPG/syndecan-1 in MM are discussed.

HSPGs

Numerous studies with genetically modified cells and animals, as well as genetic defects in humans, give evidence of the crucial role of HSPGs and their modification enzymes in development, differentiation, and disease, including multiple types of cancer⁶⁻¹². HSPGs are a class of extracellular matrix or cell-membrane-bound glycoproteins consisting of a protein core and covalently attached HS glycosaminoglycan (GAG) chains, which are composed of alternating N-acetylated glucosamine (GlcNAc) and D-glucuronic acid (GlcA) units¹³⁻¹⁶. Three major families of HSPGs are the membrane-spanning syndecans, the membrane glycosylphosphatidylinositol (GPI)-linked glypicans, and the basement membrane or extracellular matrix proteoglycans perlecan, agrin, and collagen XVIII^{11,14}. The HS chains are crucial in HSPGs function. Unlike the synthesis of proteins and nucleic acids, which is template-guided, HS-chain synthesis involves a complex series of processing reactions, regulated by multiple enzymes^{11,17}. This process can be divided into three steps: 1) chain

initiation, after the core protein is translated, xylosyltransferase-I and/or -II transfer xylose from UDP-xylose to specific serine residues of the core protein. Subsequently, two D-galactose residues are attached by galactosyltransferase-I and -II and a GlcA is attached by glucuronosyltransferase-I to form the core tetrasaccharide (Xyl-Gal-Gal-GlcA-); 2) chain elongation or polymerization, HS chain is polymerized by the alternating addition of GlcA and GlcNAc residues, to a final length up to 200 disaccharides^{18,19}. This elongation of HS is carried out by exostin (EXT) family enzymes, including EXT1 and EXT2²⁰. Reduced function of either EXT1 or EXT2 results in the bone exostosis phenotype, while a complete loss of function of either enzyme, resulting in absence of HS chains, is lethal at gastrulation^{21,22}; 3) chain modification, after elongation HS chains undergo a series complex modifications. These include GlcNAc deacetylation and sulfation by the N-deacetylase/N-sulfotransferases, epimerization of GlcA to its epimer iduronic acid by glucuronyl C5-epimerase (GlcE), and sulfation at the 2-O-, 3-O-, and 6-O-position by different sulfotransferases^{14,15,19} (Figure 1). After this synthesis and modifications process, the structure and function of the HS chain can still be modified by a number of endo- and exogenous enzymes. Thus, heparanase, which is expressed by many human cancers but hardly in normal tissues^{23,24}, cleaves HS chains into small fragments, which have been implicated biological processes such as wound repair, angiogenesis, and cancer²⁵⁻²⁷. Similar to the endogenous HS digesting enzyme heparanase, the bacterial heparinase (or heparitinase) can also cleave the HS chains^{13,28}. Furthermore, the sulfation status of HS, which is crucial for proteins interaction, can be modified by the extracellular endosulfatases sulfatase-1 (Sulf-1) and sulfatase-2 (Sulf-2), which remove the 6-O sulfate moieties from disulfated GlcA-GlcNS6S and trisulfated IdoA2S-GlcNS6S^{23,29}. Sulf-1 and Sulf-2 knockout mice show multiple defects in development, regeneration, and differentiation³⁰⁻³², illustrating the importance of extracellular HS modification.

Due to the complex synthesis and modifications, HS chains show a high degree of heterogeneity with respect to chain length, disaccharide composition, and sulfation pattern. Importantly, these HS-modifications determine the binding capacity and specificity of HS-chains for a given protein and are dynamically regulated during development, differentiation, and tumor progression^{11,15,16,33-35}, hence, the functions of HSPGs are both cell-type and differentiation-status dependent. Among the growth-factors, morphogens and cytokines that have been to be shown to be regulated by HSPGs are fibroblast growth factor (FGF), vascular-endothelial growth factor (VEGF), hepatocyte growth factor (HGF), heparin-binding epidermal growth factor (HB-EGF), Wnts, a proliferation-induced ligand (APRIL), interleukin-6 (IL-6), and multiple chemokines. HSPGs act by binding and concentrating these protein ligands and act as scaffolds facilitating the formation of receptor complexes^{13,36-40}. Due to their unique capacity to regulate growth factor-mediated signaling pathways, HSPGs have emerged as players in a variety of cellular activities, affecting many physiological and patho-

B-cell maturation, diminished baseline and antigen-stimulated immunoglobulin levels, and reduced plasma cell numbers. *Glce* encodes glucuronyl C5-epimerase, an enzyme that converts D-glucuronic acid to its stereoisomer L-iduronic acid. This releases the conformational constraints of the polysaccharide, allowing chain flexibility, thereby providing access of protein ligands to specific regions of the HS side chains^{52,53}. Interestingly, the *Glce* defect was shown to mitigate the binding of APRIL to murine BM plasma cells and to attenuate the response to APRIL-mediated survival signals, which may explain the reduction of BM plasma cell numbers⁵³. McCarron et al. recently reported that mice lacking syndecan-1 expression display deficient antigen-specific antibody responses and that syndecan-1 is required for plasma cell maturation and survival in a cell-intrinsic manner⁹. Taken together, these studies demonstrate that both syndecan-1 and its correct modification by HS modifying enzymes are critical for the survival of normal plasma cells in the bone marrow niche.

Regarding MM, Yang *et al.* demonstrated that treatment with bacterial heparinase III, which digests HS chains, dramatically inhibits the growth of primary MM cells in a human severe-combined immuno-deficient (HU-SCID) MM model⁴⁶. Although this study indicated a role for HS, it did not allow discrimination between the effects of heparinase on either MM cells or niche cells. To address this question, the same authors employed shRNA-mediated gene silencing to knockdown syndecan-1. This resulted in a delayed development and strongly reduced myeloma tumor *in vivo*, establishing the important role of syndecan-1 in MM pathobiology⁴⁶. In a subsequent study, to address the role of the HS-chains decorating syndecan-1, Reijmers *et al.* inducibly silenced *EXT1*, thus interfering with HS-side chain synthesis^{51,54}. Knockdown of *EXT1* caused loss of cell-surface HS and significantly inhibited MM cell growth *in vitro*. Moreover, in a xenotransplant model, induction of *EXT1* knockdown dramatically reduced MM growth and significantly increased survival. In xenotransplanted mice with already established tumors, *EXT1* knockdown resulted in massive tumor necrosis⁵¹. Taken together, these studies demonstrate that syndecan-1 and its HS side chains are essential for MM cell growth and survival.

Mechanisms underlying protective functions of syndecan-1

For their survival in the BM, plasma cells are critically dependent on growth factors produced in the microenvironment or niche⁵⁵⁻⁵⁷. Many of these factors have also been implicated in the growth and survival of MM cells. The plasma cell niche is composed of different cell types including BMSCs, endothelial cells, various hematopoietic cells, including granulocytes, monocyte/macrophages, and megakaryocytes, osteoclasts, and osteoblasts^{14,58,59}. These cells produce various cytokines, chemokines, and growth factors, including APRIL, IL6, HGF, EGFs, insulin-like growth factor (IGF)-1, FGFs, Wnts, R-spondins, and CXCL12^{13,14,59-62}. The presence of HS-binding domains in many of these soluble niche-derived factors^{13,14,63}, suggests a role for

syndecan-1 and/or stromal HSPGs in controlling their distribution and activity.

APRIL plays an important role in the survival of long-lived plasma cells. Mice deficient in APRIL or its receptor, B-cell maturation antigen (BCMA), have significantly decreased numbers of plasma cells in the BM^{64,65}. In the BM microenvironment, APRIL is secreted by various types, the main sources being osteoclasts⁶⁶, eosinophils⁶⁷, and megakaryocytes^{58,68}. Unlike the closely related TNF-family member B-cell activating factor (BAFF), which can auto-multimerize, APRIL requires HSPG interaction for multimerization and signaling. APRIL binds to HS via its N-terminal lysine-rich region, leaving its TNF-like region free to interact with the BCMA and TACI receptors^{69,70}. HSPGs interaction leads to multimer formation at the cell surface, a process important for the signaling of many TNF superfamily members^{71,72}. APRIL secreted by BM niche cells was shown to bind to syndecan-1 on the cell surface of plasma cells, promoting survival^{53,64,73}. In MM, APRIL also promotes cell survival and, moreover, mediates growth^{70,74}. Like in normal plasma cells, this involves the receptors BCMA and TACI, as well as interaction with syndecan-1^{70,75,76}. The growth-stimulating effect of APRIL on MM cells was abrogated by heparin⁷⁰, indicating that HS-side chains are essential in APRIL induced MM cell growth and survival.

Another major survival factor for plasma cells is IL-6^{58,77-80}. IL-6-deficient mice show dramatically decreased IgG level comparing to wild type mice, indicating its critical role in plasma cells survival⁷⁷. *In vitro* experiments demonstrated that the protective effect of BMSCs for plasma cells was inhibited by anti-IL-6 antibody⁷⁹ or deletion of IL-6 in BMSCs⁸⁰. Most plasma cell survival studies were performed in mouse models. However, a recent study showed that IL-6 is also mandatory for the generation of human long-lived plasma cells, in combination with other soluble factors, in particular, APRIL⁸¹. Syndecan-1, in addition to APRIL signaling, also promotes IL-6 signaling, contributing to plasma cell maturation and survival⁹. Whether or not this interaction of IL6 with HS is of importance for the biology of MM is currently unknown.

In addition to APRIL and IL6, which both are recognized physiological mediators of normal plasma cell survival as well as MM growth factors, several other soluble factors implicated in MM growth and survival have also been shown to interact with syndecan-1. HGF, an autocrine and paracrine MM growth factor, has been shown to interact with syndecan-1. This interaction strongly enhances activation of the HGF receptor Met and the downstream RAS/MAPK and PI3K/AKT pathways thereby promoting MM cell growth and survival^{38,82}. Similarly, MM cells can express several EGF-family proteins. Among these proteins, only the EGF-members that are able to bind HS, such as HB-EGF, neuregulin-1, and amphiregulin, can activate PI3K/AKT and promote MM cell growth. Removal of cell surface syndecan-1 abrogated HB-EGF induced cell growth in MM^{37,83}.

A distinct class of ligands that has recently been shown to be regulated by syndecan-1 in MM are Wnts. Previous studies from our own and other laboratories demonstrated that

aberrant Wnt signaling plays an important role in the pathogenesis of MM, promoting proliferation^{13,62,84}. This Wnt pathway activity was shown to depend on autocrine and paracrine Wnt ligands produced by MM cells and BMSCs and to be unleashed by the genetic and epigenetic loss of negative Wnt pathway regulators as well as by overexpression of (co-)receptors^{13,62,85,86}. In earlier studies in model organisms like *Xenopus* and *Drosophila*, it was already shown that HSPGs are involved in the control of Wnt signaling activation^{87,88}. Furthermore, syndecan-1 was shown to be required for Wnt1-induced mammary tumorigenesis in mice¹⁰. A recent study from our laboratory has extended these findings to MM. By means of CRISPR mediated knockout and inducible shRNA-mediated knockdown of *EXT-1*, a critical enzyme for syndecan-1 HS side chain synthesis^{20,89}, we demonstrated that the HS side chains of syndecan-1 mediate aberrant Wnt signaling activation in MM and Wnt signaling-dependent MM cell growth. Mechanically, syndecan-1 was shown to promote Wnt signaling by binding Wnts and R-spondins via its HS side chains, which facilitated interaction with corresponding receptors, i.e. Frizzled and LGR4, which are overexpressed by MM cells^{13,62}. R-spondins contain thrombospondin protein domain, which mediates their HSPG binding^{90,91}.

IGF-1 is another major growth factor for MM. Similar to HGF and EGF, IGF-1 promotes MM cell growth and survival via RAS/MAPK and PI3K/AKT pathway⁹²⁻⁹⁴. In addition, IGF-1 plays an important role in MM cell trafficking and localization in the BM microenvironment by inducing adhesion and migration via activation of β 1-integrins⁹⁵. In MM, high expression of IGF1R correlates with poor prognosis. Unlike many other growth factors, IGF-1 does not bind to HSPGs⁹⁶. Instead, syndecan-1 was recently shown to regulate IGF-1 signaling by capturing the IGF-1R to form a receptor complex and activate IGF-1R, which suppress apoptosis by inhibiting apoptosis signal-regulating kinase-1^{97,98}. Disruption of the interaction between syndecan-1 and IGF-1R by a truncated peptide significantly reduce MM cell growth⁹⁸.

The role of BM-niche HSPGs in the pathogenesis of MM

HSPGs are not only expressed on MM cells, but also by the niche cells such as BMSCs, endothelial cells, osteoblasts or osteoclasts⁹⁹⁻¹⁰¹. Thus, syndecan-2 expressed by osteoblasts is a key constituent of the osteoblast environment, involved in the control of osteogenesis, osteoclast activity and bone remodeling in mice, regulating Wnt signaling¹⁰². Furthermore, bone marrow endothelial cells also express HSPGs^{101,103} and mediate adhesion of hematopoietic progenitor cells. In concert with adhesion molecules such as L-selectin and integrins, HSPGs play an important role in the homing of hematopoietic progenitor cells to the BM¹⁰³. It was demonstrated that long-lived BM plasma cells are in close contact with VCAM+/CXCL12+ BMSC¹⁰⁴. These BMSCs abundantly express HSPG on their cell surface^{105,106},

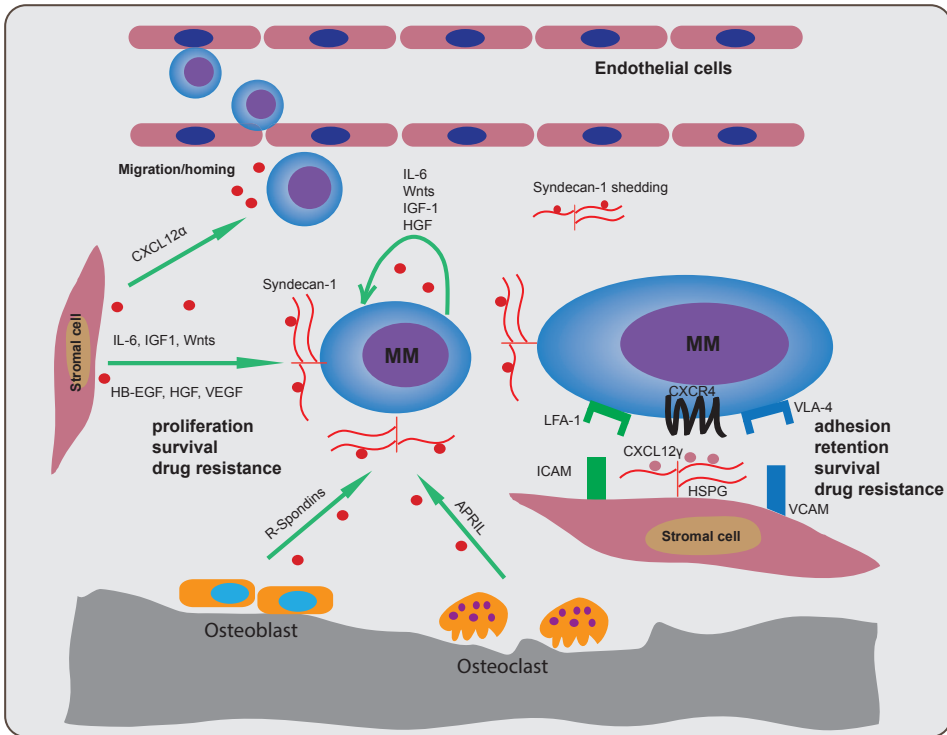


Figure 2. HSPG/syndecan-1 in the interaction of MM cells with the BM microenvironment. A schematic overview of an MM cell interacting with different components of the BM microenvironment. BMSCs secrete high level of CXCL12, which induced MM cells migration/homing back to the BM. In BM, the niche cells including BMSCs, osteoblasts, osteoclasts and MM cells themselves produce many growth factors and cytokines. The HS chains of syndecan-1 can bind many of these soluble factors, thereby promoting MM cell proliferation, survival and drug resistance. In addition, BMSC expressed HSPGs are involved in CXCL12/CXCR4 mediated MM-BMSC interaction. And this direct adhesion also plays an important role in MM retention, survival and drug resistance.

but the function of BMSC-derived HSPG in the biology of normal and MM plasma cells remains largely unexplored.

We have recently found that BMSC expressed HSPGs are involved in CXCL12/CXCR4 mediated MM-BMSC interaction. The chemokine CXCL12 and its receptor CXCR4 are pivotal for homing of HSC and plasmablasts to the BM. By alternative splicing, up to 6 isoforms of CXCL12 are generated¹⁰⁷. CXCL12 α is the most studied isoform and to date, virtually all functional *in vitro* studies, including those on cell migration and adhesion, have exclusively used this CXCL12 α isoform. Interestingly, the recently characterized isoform CXCL12 γ has been shown to bind HS with an unprecedentedly high affinity. This enhanced binding affinity to HSPG of CXCL12 γ is mediated by its extended C-terminal domain, which contains 3 overlapping HS-binding BBXB motives and is predicted to immobilize CXCL12 γ to HSPGs once secreted^{108,109}. Interestingly, it was reported that mutant mice lacking

the c-terminal HS-binding domains of CXCL12 contain increased levels of circulating HSC, suggesting a role for the HS-binding domains of CXCL12 in stem cell homing and/or retention¹¹⁰. Indeed, it was demonstrated that CXCL12 γ is expressed in murine BM¹⁰⁸, including distinct expression on capillary endothelial cells (our unpublished observation). Moreover, by immunohistochemistry employing a CXCL12 γ specific monoclonal antibody, we observed expression on stromal cells in both normal and MM-involved human BM. Functionally, CXCL12 γ immobilized by HS on the cell surface of BMSCs induced MM cell adhesion to BMSCs, while removal of stromal cell-surface HS, by either knockout of *EXT1* or by heparitinase treatment, attenuated MM adhesion. Furthermore, this loss of HS reduced the cell-adhesion mediated drug resistance to bortezomib. Our data suggest that the differences in affinity for HS of various CXCL12 isoforms may be associated with distinct roles in the regulation MM and plasma cell (and perhaps HSC) homing to and retention in the BM. CXCL12 α shows a relatively low affinity to HS and, upon secretion by BMSCs, will create a chemo-attractive gradient attracting CXCR4 positive plasmablasts and MM cells to the BM. By contrast, CXCL12 γ , having an extremely high affinity for HS, will be immobilized by HSPGs on the cell surface of BMSCs. This HSPG-immobilized CXCL12 γ plays an important role in MM cell retention in the BM niche as well as in cell adhesion-mediated drug (CAM-DR) resistance in MM.

Finally, it should be noted that HSPG localization and function are not restricted solely to the cell surface¹¹¹. Proteolytic cleavage of the extracellular domain of syndecan-1 releases a soluble form of HSPG containing intact HS side chains to the BM microenvironment^{46,112-114}. This shedded syndecan-1 can still bind many cytokines and growth factors and serve as a reservoir by accumulating these bioactive molecules in the MM BM niche. This may promote the growth and survival of MM *in vivo*^{14,28,115}. In addition, soluble syndecan-1 can be detected in the serum of some MM patients and high serum levels is associated with poor prognosis^{116,117}. Furthermore, syndecan-1 shedding may also play an important role in the crosstalk between tumor cells and the niche. Stewart *et al.* recently reported that syndecan-1 shedded from MM cell-surface can be taken up by BMSCs cells and transported to the nucleus. It was proposed that MM cells may shuttle growth factors such as HGF to the nucleus of neighboring cells resulting in altered histone acetylation in the host cells¹¹². *Vice versa*, since BMSCs also express HSPGs, HS moieties shedded from BMSC could also target MM cells.

HSPGs as targets for the treatment of MM

Given the role of HSPGs in MM and other human cancers, HSPGs and their synthesis and modification enzymes have emerged as potential therapeutic targets. To date, the pharmacological potential of these targets was investigated in a number of studies using

various strategies. These include monoclonal antibodies (mAbs) or HS mimetics to block HSPG-ligand interaction and small molecule inhibitors targeting HS synthesis or modification enzymes^{24,118-121} (Table 1). Currently, most of these studies are still at a preclinical stage but several potential therapeutics are under clinical investigation.

HSPG functions mainly through their HS side chains. Hence, monoclonal antibodies have been developed to directly target HS-side chains. Gao and colleagues developed a mAb (HS20), which targets the HS side chain of Glypican-3, an HSPG which is highly expressed in hepatocellular carcinoma, but not in normal adult tissues¹²². This mAb was found to inhibit the growth of hepatocellular carcinoma xenografts in mice, by impeding Wnt signaling. There was no detectable *in vivo* toxicity, indicating that targeting of HSPGs by

Table 1. Current HSPG-targeting therapeutic options in cancer.

Drug name or therapeutic strategy	Mechanisms of action	Stage of study	Reference
HS20 mAb	Inhibits Wnt signaling dependent growth by directly targeting Glypican-3 HS side chains; Inhibit migration and motility	Pre-clinical in hepatocellular carcinoma	122
BT062 mAb	Syndecan-1-specific mAb; Induce cell cycle arrest and apoptosis	Phase I/IIa in MM	123,124
EXT1 shRNA	Disrupt HS chain biosynthesis; Inhibit Wnt, HGF, HB-EGF signaling pathway dependent cell growth.	Pre-clinical in MM	13,51
OKN-007	Sulf-2 inhibitor; Inhibits tumor cell proliferation and increases apoptosis	Phase I in malignant glioma	130-133
SST0001	Heparin mimetics; Inhibits the heparanase activity and expression of HGF, VEGF, and MMP-9 in MM, resulting in decreased angiogenesis; Impairs the shedding of syndecan-1.	Phase I in MM	121,138,139
PI-88	Heparin mimetics; Inhibits the heparanase activity; Inhibit angiogenesis by antagonizing the interactions between pro-angiogenic growth factors and HS	Phase III in hepatocellular carcinoma	140-142
PG545	Heparin mimetics; Inhibits the heparanase activity; Inhibits Wnt/ β -catenin signaling dependent growth.	Phase I in solid tumor	143-147
Necuparanib (M402)	Heparin mimetics; Inhibits the heparanase activity; inhibit multiple factors involved in tumor-host cell interactions, including VEGF, FG2, CXCL12 α , and P-selectin.	Phase I/II in metastatic pancreatic cancer	148,149

mAbs has the potential for liver cancer treatment¹²². In MM, the syndecan-1 specific mAb BT062 conjugated to cytotoxic maytansinoids was shown to inhibit the MM tumor growth *in vivo*, leading to prolonged host survival¹²³. Recent pre-clinical studies demonstrated that BT062 (indatuximab ravtansine) acts additively or even synergistically with clinically approved therapies for MM treatment¹²⁴. Currently, a phase I/IIa study with BT062, in combination with lenalidomide or pomalidomide and dexamethasone, in relapsed and refractory MM is ongoing (NCT01638936).

Apart from the HSPG itself, the enzymes of the HS synthesis and modification machinery also present promising therapeutic targets for MM and other cancer types. The finding that knockdown of *EXT1*, which is essential for HS chains synthesis, dramatically reduces the growth of MM tumor xenotransplants, even in mice with already established tumors⁵¹, provides proof-of-concept for this notion. Notably, HSPG sulfation is a major determinant of the specificity and the affinity of growth factors interactions with HS^{11,19,125,126}. Thus, the enzymes which regulate the sulfation of HS potentially present key therapeutic targets. As already discussed, in addition to sulfate modifications during the biosynthesis by sulfotransferases, HS sulfation is also regulated post-synthetically by the extracellular sulfatases Sulf-1 and Sulf-2, which specifically remove 6-O-sulfate from the HS chains¹²⁷⁻¹²⁹. Interestingly, a small molecular inhibitor (OKN-007) targeting Sulf-2 has been reported to inhibit tumor cell proliferation and have anti-tumor activity in several different types of cancer¹³⁰⁻¹³³. Currently, OKN-007 is under phase I clinical trial in human recurrent malignant glioma patients (NCT01672463).

Heparin, a highly sulfated version of HS, has been shown to have anti-tumor activity in several experimental and clinical studies^{118,134,135}. However, its strong anti-coagulant activity hampered its use as an anti-tumor drug²⁸. Several heparin mimetics, which contain specific structural modifications in their GAG domains, have been developed to reduce the anticoagulant activity but retain the anti-tumor activity of heparin. These heparin mimetics function by inhibiting heparanase and by competing with HS for binding of essential tumor growth and survival factors^{118,136}. SST0001, a chemically modified HS mimetic with a very low anticoagulant activity, was shown to inhibit myeloma growth and angiogenesis via disruption of the heparanase/syndecan-1 axis¹²¹. SST0001 inhibits the heparanase activity and expression of HGF, VEGF, and MMP-9 in MM, resulting in decreased angiogenesis. In addition, it also impairs the shedding of syndecan-1 and reduces heparanase-mediated degradation of syndecan-1 HS chains^{121,137}. Ramani et al. have shown that SST0001 sensitizes MM xenografts to chemotherapy and prevent regrowth of tumors after therapy¹³⁸. Currently, a phase I clinical trial for SST0001 has been completed in patients with advanced MM (NCT01764880). This study identified SST0001 doses within the range from 300 to 400 mg/day to be safe¹³⁹.

Another heparin mimetic, PI-88, a mixture of highly sulfated mannose oligosaccharides, inhibits heparanase activity and interferes with angiogenesis by antagonizing the interactions between pro-angiogenic growth factors and heparan sulfate^{140,141}. PI-88 is the most extensively studied heparin mimetic in clinical trials¹³⁷. In a recent phase II study, PI-88 treatment yielded a clinically significant survival advantage for patients with hepatocellular carcinoma¹⁴². However, a subsequent phase III trial in hepatocellular carcinoma was stopped because it failed to reach the primary objective at interim analysis (NCT01402908).

PG545 is a synthetic fully sulfated HS mimetic with strong anti-metastatic and anti-angiogenesis activity in cancer^{143,144}, activities mainly attributed to inhibition of heparanase activity and competition for HS-binding growth factors. A recent study reported that PG545 exerts anti-tumor activity by inhibiting Wnt/ β -catenin signaling in pancreatic cancer cells¹⁴⁵. Interestingly, in murine models of B cell lymphomas, PG545 treatment also significantly delayed tumor growth and prolonged survival¹⁴⁶. Currently, a phase I clinical trials for PG545 has been completed in patients with advanced solid tumors (NCT02042781), showing this compound was well tolerated up to 100 mg via intravenous infusion¹⁴⁷.

Concluding Remarks and Future Perspectives.

Upon differentiation of B cells into plasma cells, expression of the BCR is lost as a key source of survival signaling. Consequently, plasma cells are highly dependent on survival signals emanating from the BM niche. The fact that they have acquired expression of syndecan-1, enables plasma cell to communicate effectively with this niche, by binding growth factors, cytokines, and chemokines via the syndecan-1 HS chains, which promotes signals mediating survival, homing, and, in MM plasma cells, proliferation. In addition to syndecan-1 expressed by the MM cells, HSPGs expressed by BMSCs also contribute to the interaction of MM cells with the BM niche. These HSPGs endorse plasma cell and MM homing, adhesion, and retention, and mediate MM drug resistance. Hence, combining conventional MM treatment with a therapy targeting HSPGs could present an effective strategy to attenuate MM growth and survival and to overcome drug resistance in MM. Although this strategy has been successfully tested in animal models, a very limited number of clinical studies with mAbs, and HS-mimetics have thus far been performed, which have not yet shown convincing benefit. Notably, given the fact that HSPGs are expressed by many other cell types, in addition to MM cells, it will be very important to carefully monitor the potential side effects of targeting HSPG/syndecan-1. To minimize these side effects, identifying the specific HS modifications which are essential for binding of ligands that are of key importance in the pathogenesis of MM, specifically APRIL, IL6, and Wnts, is of great importance as it will

allow targeting of specific HS-modifying enzymes. In conclusion, a vast amount of studies establish a crucial role of HSPGs, specifically syndecan-1, in the biology of MM. In concert with data showing efficacy of HSPG-targeting treatments in various animal cancer models, they designate HSPG/syndecan-1 as highly promising targets for MM treatment, which needs further exploration and fine-tuning.

Acknowledgments

Supported by grants from the Dutch Cancer Society and Lymph&Co to S.T.P. and M.S. and a CSC Chinese Government Scholarship to Z.R.

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