Chapter 11
Heparanase in Cancer Metastasis – Heparin as a Potential Inhibitor of Cell Adhesion Molecules

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11.1 Introduction

Cancer progression – metastasis is a process encompassing multiple steps including successful escape of tumor cells from the primary tumor sites, survival in the circulation, evading immune responses, seeding in distant organs, and most importantly initiation and sustained growth at these sites. Even though sustained proliferation of tumor cells is likely the most fundamental trait in tumor cells, the capacity to "modulate" the tumor microenvironment consisting of non-tumorigenic stromal cells significantly contributes to metastasis, virtually at every step of this process [1, 2]. Particularly, the capacity of tumor cells to secrete extracellular matrix-degrading enzymes, such as heparanase or proteases, profoundly contributes to migratory properties of tumor cells, or to the release of factors promoting tumor growth and angiogenesis [3, 4].

Heparanase expression is linked to an invasive phenotype of a variety of cancer types in patients and has been confirmed in numerous animal models [3, 5, 6]. This book covers all known aspects of heparanase biology in great details. Thus the focus of this chapter is on heparanase action in leukocyte recruitment and cell-cell interactions during cancer progression. In addition, the activity of heparin and heparin derivatives on cancer progression will be discussed with respect to their anti-heparanase and anti-adhesive activities; and current developments towards therapeutic applications.

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11.2 Cell Adhesion Promotes Tumor Cell and Leukocyte Migration

The immune system "responds" only when leukocytes are able to cross blood vessels. Circulating leukocytes do not interact with the endothelial cells. Endothelial activation is required to initiate leukocyte adhesion and to enable the migration of leukocytes through the endothelium. The formation of chemokine gradient is a prerequisite for driving the cell recruitment and the capacity of cells to adhere to the vessel wall and to initiate trans-endothelial migration [7]. Selectins are likely the first cell adhesion molecules involved in leukocytes recruitment and transendothelial migration. Inflammatory stimuli activate endothelial cells that increase expression of adhesion molecules including P-selectin, E-selectin and increases vascular permeability [8, 9]. Interactions of vascular selectins with their ligands lead to the leukocyte rolling on activated endothelium, followed by integrin-mediated firm adhesion and finally leukocyte extravasation into the parenchyma, where leukocytes can execute their effector functions [10].

Extravasating leukocytes need to engage the endothelium, and after diapedesis they face the basement membrane surrounding most of postcapillary venules, which is a complex meshwork of collagens interconnected with glycosaminoglycans, such as heparan sulfate. Heparanase is a hydrolytic enzyme enabling degradation of heparan sulfate, thus likely promoting leukocyte migration after extravasation [11, 12]. Interestingly, neither neutrophils nor T cells require heparanase expression for efficient extravasation as has been shown using heparanase deficient mice [11]. In contrast, efficient monocyte extravasation required heparanase expression, in a peritoneal inflammation model. In the pulmonary vasculature, which represents the largest capillary bed in our body, the transendothelial migration of neutrophils also does not require heparanase expression [12]. However, in chronically inflamed lungs, neutrophil accumulation required heparanase, while T cell recruitment remained heparanase independent but was mediated by ICAMs instead. Thus, contrary to tumor cells, where enhanced heparanase expression is linked to invasive behavior, leukocyte transendothelial migration does not require heparanase.

During cancer metastasis, selectin-mediated interactions were shown to be essential for the recruitment of myeloid cells and monocytes that enhances tumor cell extravasation and facilitate metastasis in several mouse models. The metastatic microenvironment further promotes recruitment of monocytes and myeloid cells through enhanced presence of chemokines, e.g., CCL2, CCL5 [9, 13]. These observations show that metastatic tumor cells "highjack" the physiological function of selectins and leukocyte recruitment to promote tumor growth and metastasis.

11.3 Cell Adhesion as Determinant of Metastasis

Cell adhesion defines the physiological function of any cell in the body through contacts to other cells or to extracellular matrix (ECM) component within the tissue environment. Since cell adhesion is connected to signal-transduction pathways affecting cell phenotype, survival, differentiation and migration, alteration in cell adhesion frequently observed in tumor cells directly contributes to cancer progression. During cancer progression, several families of adhesion molecules including cadherins, integrins, junctional-adhesion molecules, and selectins have been studied. The topic of cell adhesion in cancer progression is too extensive to be covered in this chapter and excellently reviewed elsewhere [14, 15]. Thus, we focus on the role of cell adhesion in steps of metastasis in the context of potential involvement of heparanase.

Hematogenous metastasis is a highly orchestrated process describing the ability of tumor cells to enter in the blood circulation, survive the circulation and extravasate in secondary sites, where they can form metastases. Tumor cells in circulation undergo multiple interactions with blood cellular components, such as platelets and leukocytes that are essential for tumor cell survival and further for metastasis. Two major families of cell adhesion molecules, selectins and integrins, have been identified to facilitate cell-cell interactions essential for the metastatic spread (reviewed in [10, 16–19]. Here we will discuss selectins and integrins both as mediators of metastasis and as targets of heparin-based therapeutic approaches.

11.3.1 Selectin as Mediators of Metastasis

Selectins have been identified as key adhesion molecules that mediate adhesive events between leukocytes, endothelial cells, and platelets during leukocyte trafficking and hemostasis [20]. There are three members of the selectin family: P-, L- and E-selectin. Platelets and endothelial cells express P-selectin that is stored in α-granules and Weibel-Palade bodies, respectively; and upon activation rapidly translocates on the cell surface of these cells. E-selectin is expressed in endothelial cells, whereupon activation a *de novo* transcription is initiated, and its cell surface expression lasts longer than of P-selectin. L-selectin is constitutively expressed on cell surfaces of almost all leukocyte subpopulations [21]. Selectins mediate adhesion by heterotypic interactions of their C-type lectin domain with glycan-bearing ligands. The minimal recognition motif for all selectins is the sialyl-Lewis^x sLe^x and its isomer sialyl-Lewis^a (sLe^a) tetrasaccharide that is sequentially synthesized by N-acetyl-glucosaminyltransferases, galactosyltransferases, α1,3-fucosyltransferases IV or VII, and α2,3-sialyltransferases [10, 21]. Due to the relatively low binding affinity towards a single carbohydrate domain, physiological ligands represent a scaffold of clustered domains to increase the avidity of binding. In addition, P- and L-selectin, but not E-selectin, can bind to sulfated glycans including heparin,

heparan sulfate, fucoidan and sulfated glycolipids [22], indicating that selectins recognize rather a carbohydrate "patch" generated in different ways.

The relevance of selectin involvement in hematogenous metastasis has been deduced from the observations that particularly epithelial cancers (carcinomas) undergo profound changes in cell-surface glycosylation [23, 24]. The cancer-induced aberrant glycosylation often goes along with enhanced presence of selectin ligands enabling tumor cells to interact with other blood constituents once they enter the blood circulation [23]. Selectin-mediated interactions of tumor cells with leukocytes, platelets, and endothelial cells provide a mechanistic explanation for the clinical association with poor prognosis of cancer patients [23, 25, 26]. Tumor cells in blood circulation are often covered by platelets, as has been observed in patients and animal models. The first evidence that this platelet-tumor cell interaction is P-selectin dependent has been described in a mouse model with P-selectin deficiency [27]. Platelet's P-selectin binds to carcinoma mucins since glycan removal inhibited platelet-tumor cell interaction and thus also metastasis [28]. The role of platelets in malignancy and metastasis has been thoroughly investigated since then and is excellently reviewed elsewhere [29–31]. Of note, P-selectin expression on endothelial cells also contributes to metastasis as has been shown in bone marrow reconstituted P-selectin-deficient mice [32]. Similarly, E-selectin has been associated with formation of a premetastatic niche, where the recruitment of tumor cells, as well as myeloid-derived cells, facilitates metastasis [33, 34]. Interestingly, endothelial activation is essential for the recruitment of monocytes and tumor cell extravasation [9, 35].

Leukocytes are a key component of the tumor microenvironment, and they exert many activities promoting metastatic dissemination and metastatic niche formation. Particularly, myeloid-derived cells such as monocytes and neutrophils expedite tumor cell extravasation and formation of metastatic niche [36–38]. L-selectin facilitates the recruitment of myeloid cells to tumor cells in the circulation and enables their extravasation through the endothelial cells [13, 39]. The inhibition or the absence of L-selectin resulted in attenuation of metastasis due to the lack of leukocyte-induced endothelial activation [9, 13]. There is little knowledge about the role of L-selectin on lymphocytes in cancer progression, despite the canonical function of L-selectin in facilitating the recruitment of lymphocytes into lymph nodes [21]. In an inflammation model, L-selectin was shown to mediate the recruitment of activated $CD8^+$ T cells to virus-infected organs and thereby confers protective immunity [40]. Whether L-selectin facilitates activated cytotoxic $CD8^+$ T cell recruitment during tumor progression remains unclear.

11.3.2 Selected Aspects of Integrins during Cancer Metastasis

Integrins are ubiquitously expressed trans-membrane glycoproteins with important functions in cellular adhesion and signaling. The structure of integrins comprises non-covalently bound heterodimers with α- and a β-subunit. 18 α- and 8 β-integrin

subunits have been characterized that combine to form 24 unique canonical α/β receptors identified so far. Integrins mediate cell adhesion, primarily to components of the ECM, such as fibronectin, vitronectin, laminin, or collagen, thus contributing to cellular anchorage but also cell motility and invasion. Furthermore, integrins also mediate certain aspects of cell-cell interactions relevant to tumor cell metastasis. Integrins are important mediators of bidirectional cellular signaling due to their anchorage to the cytoskeletal structures (e.g., α-actinin, talin, and vinculin). As a consequence, ligation of extracellular ligands can influence intracellular processes (outside–in signaling) through activation of kinases, GTPases of the Ras/Rho signaling pathways. On the contrary, intracellular signals can induce alterations in the integrin conformation and thus change ligand-binding properties (inside–out signaling).

An immense body of knowledge has been accumulated covering the multiple roles of integrins in oncology and tumor cell metastasis [41]. To name just a few aspects of integrin-triggered interaction during metastasis, integrins mediate growth factor receptor signaling [42], tumor cell chemoresistance [43], epithelial to mesenchymal transition [44] and angiogenesis [45]. For further details, we refer the readers to excellent reviews in this field [41, 46]. In general, the expression pattern of integrins has been associated with the malignant progression of certain tumors and correlated with altered patient's survival. In this context, we will address here only the functions on integrins, which are directly related to cell-cell interaction during metastasis and discuss the potential targets for heparin to interfere in these processes.

The role of platelets in cancer metastasis has been outlined regarding P-selectin. However, platelets also express five different integrins, which contribute to the formation of tumor cell-platelet emboli during metastasis [47]. For instance, α6β1 and αIIbβ3 integrins are directly involved in platelet adhesion to tumor cells, and pharmacological interference with these integrins resulted in attenuated metastasis [48, 49]. The α4β1 integrin, very-late antigen-4 (VLA-4), is found on many cells of hematopoietic origin referring to their role in immune response by binding to the endothelial ligand VCAM-1. In addition, VLA-4 is also aberrantly expressed and active in different tumor types, such as melanoma [50]. The current evidence shows that VLA-4 facilitates melanoma cell binding to activated endothelial cells expressing VCAM-1 at distant sites, and the interference in VLA-4 attenuates melanoma metastasis in different model systems [51–54]. Furthermore, the aberrant expression of the VLA-4 ligand, VCAM-1, by certain tumor cells was shown to mediate trafficking and binding of macrophages into the forming micrometastases and thereby promotes the formation of a permissive microenvironment for tumor cell outgrowth [55]. Integrins have recently been identified to contribute to organ-specific metastasis through their expression on tumor-derived exosomes [56]. Proteomic analyses of tumor-derived exosomes with tropism towards lungs and liver were shown to express predominantly the integrins α6β4 and α6β1 (lungs) and αvβ5 (liver), driving the formation of a pre-metastatic niche in the respective organ.

Taken together, selectin and integrin adhesion receptors promote cancer progression through various mechanisms (reviewed in [16]). As a consequence,

pharmacological interference with adhesion receptor activities remains an attractive option to attenuate metastasis. Although the inhibition of selectins in experimental settings has been efficient in numerous cancer models, there are no clinical studies focusing on selectins. In the case of integrins, the ubiquitous expression of the different integrin subtypes and partly overlapping ligand recognition complicate a specific targeting. Interestingly, heparin and heparin derivatives have been shown to inhibit P- and L-selectin as well as VLA-4 integrin in experimental settings. Since heparins are currently used in the treatment of cancer patients with thrombosis, the question remains to which extent heparin may affect cell adhesion or heparanase activity during metastasis.

11.4 Heparin as an Inhibitor of Cell Adhesion

Heparin, low-molecular-weight heparin (LMWH) and heparin derivatives (further named as heparins) were tested in many different animal models for its potential to attenuate cancer progression (reviewed in [57, 58]). Interestingly, various heparins tested in animal models have shown primarily attenuating effect on metastasis rather than tumor growth. Most of these studies were performed in an experimental metastasis model where tumor cells were directly injected into the blood circulation. Despite many limitations of this experimental approach, the timely defined presence of tumor cells in the circulation has allowed the evaluation of cellular and molecular mechanisms during the hematogenous phase of metastasis. A variety of heparins efficiently reduced cancer progression when applied shortly before or shortly after the intravenous injection of tumor cells [57]. However, the application of heparin twenty-four hours before or after the tumor cell injection had no effect on metastasis [32, 59]. While in the majority of early studies heparins were used at concentrations exceeding the therapeutic dosage rage, later publications have confirmed the efficacy of heparin to inhibit metastasis also at clinically relevant concentrations [60, 61]. The fact that heparins showed anti-metastatic activity only when tumor cells were still in circulation strongly indicate that cellular and molecular events occurring during this phase are potential targets of heparin instead of solely affecting the coagulation pathway [16, 26]. This conclusion is further supported by the observation that half-life of heparin in circulation is rather limited, not exceeding six hours, thus affecting processes like angiogenesis or tumor growth is less likely.

Heparin is a complex natural glycosaminoglycan extracted from porcine intestine, which in clinical preparation is enriched for the ability to inhibit the clotting cascade. Also, heparins have a wide variety of potential biological effects including the ability to interact with integrins, inhibit P- and L-selectin interactions, inhibit heparanase, attenuate angiogenesis, and affect growth factors and chemokines [26, 62]. Despite these many potential effects, experimental data indicate that heparins affect processes during the hematogenous phase of metastasis. Within blood vessels, circulating tumor cells ultimately interact with the endothelium and other

blood constituents (e.g., platelets and leukocytes) that might lead to tumor cell arrest and extravasation also through increased heparanase activity. Thus, the antimetastatic activities of heparins were analyzed by various groups for their potential to affect coagulation, inhibit cell-cell interactions and to block heparanase as depicted in Fig. 11.1 (reviewed in [63]). Although the anticoagulant activity may contribute to reduced metastasis, several studies clearly indicated that heparins without anticoagulant activity attenuated equally well cancer progression in various cancer entities; e.g., lung, colon and breast cancers (reviewed in [64]). The initial finding that P- and L-selectins can effectively bind to heparins [65, 66] initiated a series of studies testing the hypothesis that heparin treatment inhibits selectin-mediated interactions and thereby metastasis [28, 39, 60]. In parallel, it was shown that integrin-mediated interactions during inflammation could be inhibited by heparin [67, 68], suggesting that heparin may also block integrin-mediated interactions during metastasis. Indeed, heparin was shown to block αIIbβ3-integrin-mediated interactions of platelets with melanoma cells [69]. In another study, heparin was shown

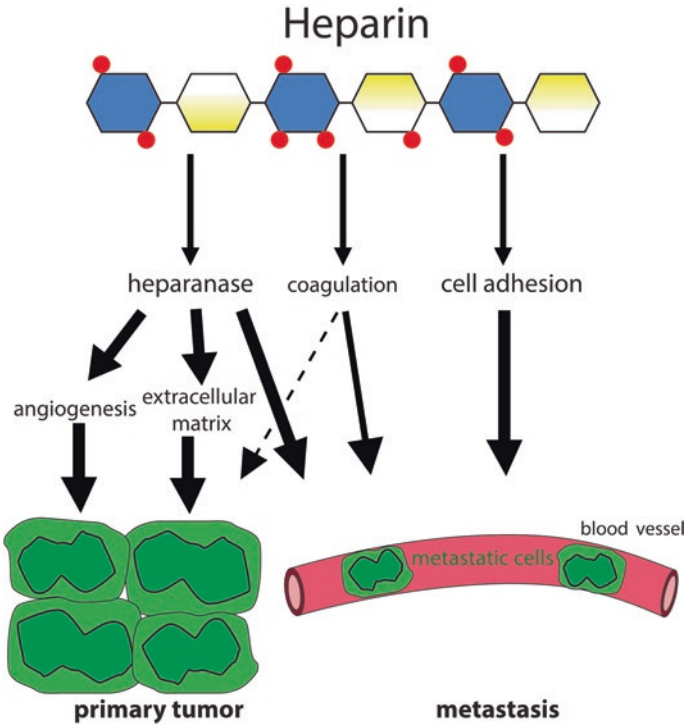

Fig. 11.1 Heparin contains diverse biological activities that affect cancer progression. The main three biological activities affecting heparanase (HPSE), coagulation and cell adhesion as discussed in this chapter. In addition, heparanase positively influences primary tumor growth through the promotion of angiogenesis, and extracellular matrix remodeling also associated with the release of growth factors. Many of these activities have been confirmed *in vitro* and *in vivo*. A direct effect of heparin on metastasis has been confirmed through inhibition of heparanase and cell adhesion

to block α4β1-integrin-mediated adhesion of melanoma cells to the endothelium [70]. Taken together, these studies provided a rationale to explore heparin as an inhibitor of cell adhesion during metastasis, albeit the heparanase-inhibitory activity remained to be resolved.

11.5 The Role of Heparin in Cancer Treatment – Clinical Evidence

The close relationship between cancer and hypercoagulability has been observed already in the nineteenth century by the French physician Armand Trousseau (reviewed in [71, 72]) and is under constant investigation since then (reviewed in [73, 74]). Cancer patients with malignant diseases are at a higher risk of developing thromboembolic complications contributing to morbidity and mortality of the disease when compared to a healthy population. This includes venous thromboembolism (VTE) which encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE); and also arterial thrombosis. Cancer-associated thrombosis in numbers is reflected in six times higher chance to develop VTE compared to non-cancer subjects, that is further increased in patients receiving chemotherapy [75]. Epidemiologically, about 15% of cancer patients will develop VTE, and about 20% of patients with VTE have an unknown neoplasm at the time of diagnosis. Consequently, VTE complications contribute significantly to morbidity and mortality of cancer patients, where VTE is the second leading cause of death. There are several mechanisms being identified to be involved in cancer-triggered thromboembolism enabling the development of specific therapies (reviewed in [72, 75]).

Antithrombotic prophylaxis or treatment is an essential component of clinical therapy especially for patient populations with an increased risk to develop VTE. A multitude of studies and meta-analyses have been performed to define an optimal pharmacological interference with the activated hemostatic system in cancer diseases (reviewed in [63]). The treatment guidelines of first-line therapy for the short- and long-term management of cancer-associated VTE, including those of the European Society of Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN), or the American Society of Clinical Oncology (ASCO) currently recommend LMWH as first choice [76, 77]. The clinical handling of LMWH in oncology is experienced for several decades and displays a balanced safety profile, e.g., superiority over vitamin K antagonists. The guidelines recommend the treatment of acute symptomatic VTE by LMWH for longer periods, up to six months. In terms of VTE prophylaxis, LMWH is also guideline-based recommended for certain cancer patients for short term application [78]. Although heparin and particularly LMWH are used for the treatment of cancer patients with thrombotic complication for decades, the question, whether heparin treatment has other anti-cancer activities, going beyond anticoagulation, remains open.

A potential antitumor effect of an antithrombotic treatment using heparin and LMWH has been suggested based on several retrospective and prospective clinical trials of cancer patients at risk of VTE (summarized in [79]). Several controlled prospective studies in the early 2000 showed promising results especially in patients with early, non-metastatic stage of the disease. While there was no effect on overall survival in a variety of solid tumors, the analysis of a subset of patients, who were metastasis-free at the beginning of the trial, demonstrated a significant increase in survival with the LMWH dalteparin in that population [80–83]. An open-label controlled study in small cell lung cancer patients, receiving standard treatment plus/minus LMWH in a therapeutic dose for 18 weeks, showed a significant increase in the overall survival and progression-free survival in the treatment arm [84]. A comprehensive review on all clinical trials conducted by 2007 has been re-evaluated, confirming certain benefits for LMWH-treated cancer patients especially in the early stage of lung cancer [85]. Nonetheless, several further clinical studies could not, or not completely confirm this beneficial LMWH effect on overall survival, while the reduction in VTE as a primary endpoint by LMWH was significant [86–88]. Three of these studies have been performed in non-small cell lung cancer patients using three different LMWH preparations: nadroparin, dalteparin, and tinzaparin, respectively [86, 87, 89].

The reasons for this non-favored outcome in light of LMWH are likely multifaceted due to methodological issues, tumor entities, or treatment regimens; and the use of different LMWH preparations. Furthermore, it remains open to which extent the chemotherapy, which was given in parallel, interferes with any effect of LMWH on patient's survival. Finally, it needs to be considered that all LMWH preparations are tested only for anticoagulant activity, but not for the other biological activities, such as inhibition of cell adhesion or heparanase. There is a considerable difference in the effectiveness of different LMWH preparations on cell adhesion and subsequently on metastasis as tested in preclinical models [60]. Nevertheless, further studies on LMWH are warranted to address the question, how heparins affect cancer progression, beyond coagulation. The design of a clinical study should take into account the biological activity of heparins that has been proven in numerous preclinical studies.

11.6 Heparanase – Another Player in Cancer Progression

Heparanase (HPSE), an endoglycosidase, is the sole enzyme in mammalian organisms able to cleave heparan sulfate (HS) chains into HS fragments [90]. HS macromolecules have an essential role in cell signaling and communication, primarily due to their capacity to bind growth factors and cytokines and thereby create a reservoir of signaling molecules in the ECM and on the surface of cells. Thus, the capacity of HPSE to degrade HS has important implications for remodeling of the cellular microenvironment as has been shown during inflammation and cancer progression [91]. HPSE affects tumor growth and metastasis in various ways (e.g., by fostering

tumor cell extravasation, angiogenesis, bioavailability of HS-bound growth factors, etc.) discussed in other chapters of this book (Vlodavsky et al., Ilan et al.). Many types of cancer show upregulated expression of HPSE that is a marker of poor prognosis in cancer patients [92]. Experimental evidence using transgenic expression of HPSE both in tumor cells or in mice as well as HPSE knock-down strategies convincingly linked HPSE to metastasis [93, 94]. Interestingly most but not all activities of HPSE during tumorigenesis and metastasis were shown to be related to its enzymatic functionality altering the tumor microenvironment. Here we briefly recapitulate the most important aspects of HPSE concerning metastasis, while a thorough discussion of HPSE protumorigenic activities can be found in other chapters of this book and are reviewed elsewhere [5, 95].

The enzymatic function of HPSE was shown to promote tumor angiogenesis and production of exosomes that significantly contribute to the formation of metastases [96, 97]. Recently it was shown that HPSE-mediated shedding of syndecan-1 liberates peptide fragments with VEGF receptor activity [98]. Exosomes are dominant mediators of intercellular communication that drive metastasis by regulating the tumor-host cell interactions both locally within the tumor microenvironment and distally at metastatic sites [99]. The enhanced expression of HPSE in human cancer cells, as well as tumor cell exposure to exogenous HPSE possibly derived from the tumor microenvironment, was shown to dramatically increase exosome secretion through modulation of syndecan-1 signaling [97, 100]. This process relies on HPSE enzymatic cleavage of heparan sulfate and also impacts exosome protein cargo as reflected by higher levels of syndecan-1, VEGF and HGF [97] (David and Zimmermann; Sanderson et al., Chaps. 10 and 12 in this volume). HPSE was identified as a key factor in myeloma cells driving survival and resistance against chemotherapy via activating the ERK signaling pathway [101]. Treatment of colon carcinoma cells with heparanase induced expression of inflammatory cytokines such as CCL2, CCL5, and CXCL1, indicating that heparanase from stromal cells has the capacity to directly activate tumor cells and thereby modulate the tumor microenvironment [102]. Similarly, heparanase activity was shown to activate macrophages in the tumor microenvironment through the Erk, p38 signaling pathway [103]. (Hulett et al., Elkin et al., Chaps. 7 and 17 in this volume).

Concerning the non-enzymatic activity of HPSE, latent HPSE induces adhesion receptor activity for spreading and/or migration of different tumor cell types by inducing a signaling axis via binding and clustering the cellular HSPGs. Latent HPSE facilitates integrin binding and thereby promotes adhesion and metastasis of melanomas, which can be antagonized by LMWH [104]. Interestingly, latent HPSE on endothelial and cancer cells induces tissue factor (TF) expression and thus contributes to coagulation [105]. Furthermore, the latent HPSE binds and displaces tissue factor pathway inhibitor from the endothelial surface, providing another way to modulate thrombosis in cancer [106] (Nadir et al., Chap. 33 in this volume).

The multiple functional mechanisms as to how HPSE facilitates tumor progression and metastasis make it an excellent target for pharmacological inhibition.

Manifold experimental approaches exist at the preclinical and even clinical level to target HPSE from a therapeutic perspective. Not surprisingly, heparins have been one of the first compounds tested in several *in vitro* and *in vivo* models.

11.7 Heparin as an Inhibitor of Heparanase in Metastasis

The role of ECM-bound or cell surface-bound proteoglycans in cancer progression has granted the development of HPSE inhibitors based on heparin structure. Structural evaluations have led to the identification of two high-affinity heparin recognition domains of HPSE, one close to the N-terminus of the enzyme, where blockade of binding to heparin inhibits the enzymatic activity [107]. Meanwhile, in 2015 the crystal structure of HPSE has been solved, providing further insight into the mapping of substrate recognition sites [108].

Heparin has served as a structural scaffold for optimization of the structural requirements for the development of glycosidic inhibitors of HPSE. First structural modifications of heparin identified the requirements for sulfation and N-acetylation to differentiate between anticoagulant and HPSE inhibitory properties of heparin during hemostasis [109, 110]. Later on, the capacity of heparin and heparin derivatives to attenuate lung colonization by melanoma cells has been confirmed using experimental metastasis approach in mice [59]. The effectivity of heparins to attenuate metastasis was restricted to a period shortly before or after tumor cell inoculation, which was considered to inhibit predominantly the tumor cell-derived HPSE during this phase. Another group has confirmed the impact of sulfation degree of heparin derivatives on HPSE inhibition in the same melanoma experimental metastasis model [111]. Notably, in this study, the opening of the iduronic acid ring by oxidative/reductive procedures has been applied and tested for its HPSE inhibitory activity. This approach has been later identified as one of the key methods to optimize heparin for its HPSE inhibitory activity [112] (Naggi et al., Giannini et al., Noseda et al.).

A comprehensive library of heparin derivatives as potential HPSE inhibitors has been assessed *in vitro* [112]. The essential structural requirements of heparin for HPSE inhibition has been linked to its sulfation grade. A partial N-desulfation (replaced by acetylation) is well tolerated and considered as a discriminating factor between anticoagulant and HPSE-inhibiting activity of heparin. However, the preparation of a series of glycol-splitting derivatives (RO-heparins) with various degrees of N-desulfation resulted in the identification of highly efficient compounds [112]. These derivatives were used as scaffolds for the development of HPSE-specific inhibitors such as Roneparstat that has been tested in multiple myeloma patients [113] (Noseda et al., Chap. 21 in this volume). The elimination of conformational restrains by glycol-splitting of the iduronic acid leads to rotational freedom of the heparin molecule, which together with N-acetylation strongly enhanced HPSE binding and thereby inhibitory potential. The structural modifications of heparin discussed with respect to its specific biological activities

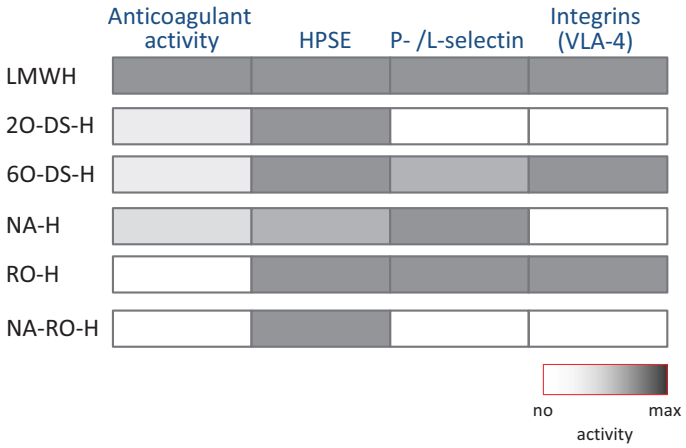

Fig. 11.2 Structural modifications of heparin and their impact on relevant targets during tumorigenesis and metastasis. The anticoagulant activity of heparin is strongly attenuated or minimalized by 2O- or 6O-desulfation; or by opening the iduronic acid ring (glycol-split heparin/RO-H), inhibitory capacity towards HPSE, P- and L-selectin or the integrin VLA-4 are largely preserved. Notably, N-acetylation of glycol-split heparin (NA-RO-H) appears to be a key to differentiate HPSE-inhibitory potential from the other targets

are schematically shown in Fig. 11.2. Further insight into the role of structural flexibility of glycol-split derivatives has recently been provided by an NMR-based conformational analysis and molecular dynamic study [114] (Naggi et al., Chap. 20 in this volume).

Glycol-split heparin derivatives with various degree of N-desulfation/N-acetylation have been tested for their antimetastatic activity [115]. RO-heparin as a specific inhibitor of HPSE inhibited melanoma metastasis, while had little effect on colon carcinoma metastasis. Interestingly, melanoma cells express high levels of HPSE while there was little expression detected in colon carcinoma cells, indicating the efficacy of HPSE inhibition only in cancers associated with high HPSE expression. Further studies investigated heparin-like polymers from mollusk origin for their capacity to inhibit HPSE besides other potential targets and thereby metastasis [116]. Novel strategies to develop HPSE inhibitors based on heparin-like structures resulted in the preparation of a glycopolymer with specific sulfation pattern that appears suitable for attenuating breast cancer metastasis [117].

Several preclinical studies using a variety of animal models confirmed the role of HPSE in metastasis (reviewed in [64, 118]). Based on many studies developing heparin-based inhibitors of HPSE devoid of anticoagulant activities resulted in the development of two drugs: roneparstat and necuparanib, that were subjected to clinical trials as a treatment for cancer with enhanced HPSE activities [113, 119]. The relevance of HPSE inhibition and its impact on cancer progression is well- defined. Nevertheless, heparin-based inhibitors require further analysis to determine the contribution of targeting other biological activities (e.g., cell adhesion) to their antimetastatic activity.

11.8 Dissecting the Role of Heparin in Cancer Progression

Heparin and its derivatives are being studied for their potential to treat cancer in numerous preclinical models, and the results of these studies indicate the capacity of heparin to inhibit cancer progression independently of its anticoagulant activity. Since heparin, particularly LMWH, is still used for the treatment of cancer patients with thrombotic complications, and based on several studies is associated with prolonged survival of some patient groups, heparin remains an attractive option for further development. Many pre-clinical studies have shown that heparin derivatives or non-anticoagulant heparin-based analogs attenuate metastasis irrespective of the animal model or cancer entity [59, 60, 115, 120–122]. As discussed in this chapter, inhibition of cell adhesion and of HPSE are likely the two biological activities contributing most significantly to inhibition of cancer progression. While several studies characterized heparin derivatives for their HPSE-inhibitory activity (e.g., 59, 120), other studies evaluated the selectin inhibitory activity [28, 60]. Later on, heparin derivatives were tested for both HPSE and selectin-inhibitory activity using experimental metastatic mouse models [115, 122]. The selectin-specific heparin derivative (57% N-acetylated-heparin) attenuated metastasis both with colon carcinoma and melanoma cells [115]. On the contrary, HPSE specific heparin derivative (100% N-acetylated, 25% glycol-split heparin) effectively inhibited melanoma metastasis but was ineffective for attenuation of colon carcinoma metastasis. When we used semisynthetic sulfated trimannose C-C dimers, HPSE specific derivative again attenuated only melanoma metastasis, while selectin specific-derivative inhibited both melanoma and colon carcinoma metastasis [122]. Of note, while melanoma cells (B16-BL6) and many other tumor cell types express relatively high levels of HPSE [115, 123, 124], colon carcinoma cells (MC-38) showed low amounts [115]. Taken together, these studies revealed that heparin-based selectin inhibition attenuated metastasis in both B16-BL6 and MC-38 cells, while the inhibition of HPSE affected metastasis of those tumor cells with enhanced HPSE activity.

In recent work, HPSE-neutralizing antibodies were tested as inhibitors of cancer progression using Burkitt's lymphoma and glioma cells [123]. While Raji cells do not produce any detectable HPSE activity, the anti-HPSE antibody significantly reduced tumor growth and metastasis of these cells. These findings indicate that heparanase derived from the tumor microenvironment significantly contributes to tumor progression [123].

11.9 Conclusions

Metastasis is in ninety percent of cancer patients the ultimate cause of death, yet there is no specific anti-metastatic therapy currently available. Based on the current understanding of metastasis, invasiveness, and mechanisms related to tumor cell

survival, HPSE, as the principal modifier of the tumor microenvironment and invasiveness; and cell adhesion mechanisms involving selectins and integrins and enabling tumor cell interactions with other cells during the metastatic process, offer most relevant targets for exploration of future therapies. While specific targeting of HPSE, based on both heparin-glycomimetics and specific antibody-based therapies are ongoing, particularly in hematopoietic malignancies [5, 123, 125], there is little progress on cell adhesion-based approaches. However, heparin derivatives have been extensively studied for their capacity to interfere with cancer progression (reviewed in [10, 16, 26]).

As discussed in this chapter, heparin carries several biological activities that are beneficial for the attenuation of metastasis. In fact, most of heparin preparations used both in preclinical and clinical studies carried out thus far contained various activities such as HPSE and selectin-inhibitory activity. Yet, only the anti-coagulation and HPSE-inhibiting activity of heparin were mostly assessed. Despite many studies, the identification of the critical biological activities of heparin for its anti-metastatic behavior is still not achieved, largely because heparin has not been rigorously tested for all or at least several known activities. Clearly, it is of scientific interest to dissect the role of heparin as an inhibitor of cancer progression, but from the clinical perspective, inhibition of multiple mechanisms involved in cancer progression by heparin might prove beneficial for cancer patients. Thus, further clinical studies designed based on the current knowledge of the potential mode of action of heparins in cancer setting are warranted.

References

1. Hanahan, D., & Coussens, L. M. (2012). Accessories to the crime: Functions of cells recruited to the tumor microenvironment. *Cancer Cell, 21*, 309–322.
2. Quail, D. F., & Joyce, J. A. (2013). Microenvironmental regulation of tumor progression and metastasis. *Nature Medicine, 19*, 1423–1437.
3. Levy-Adam, F., Ilan, N., & Vlodavsky, I. (2010). Tumorigenic and adhesive properties of heparanase. *Seminars in Cancer Biology, 20*, 153–160.
4. Cohen, I., Pappo, O., Elkin, M., San, T., Bar-Shavit, R., Hazan, R., Peretz, T., Vlodavsky, I., & Abramovitch, R. (2006). Heparanase promotes growth, angiogenesis and survival of primary breast tumors. *International Journal of Cancer, 118*, 1609–1617.
5. Vlodavsky, I., Singh, P., Boyango, I., Gutter-Kapon, L., Elkin, M., Sanderson, R. D., & Ilan, N. (2016). Heparanase: From basic research to therapeutic applications in cancer and inflammation. *Drug Resistance Updates, 29*, 54–75.
6. Vlodavsky, I., Gross-Cohen, M., Weissmann, M., Ilan, N., & Sanderson, R. D. (2018). Opposing functions of Heparanase-1 and Heparanase-2 in Cancer progression. *Trends in Biochemical Sciences, 43*, 18–31.
7. Muller, W. A. (2011). Mechanisms of leukocyte transendothelial migration. *Annual Review of Pathology, 6*, 323–344.
8. Wolf, M. J., Hoos, A., Bauer, J., Boettcher, S., Knust, M., Weber, A., Simonavicius, N., Schneider, C., Lang, M., Sturzl, M., et al. (2012). Endothelial CCR2 signaling induced by colon carcinoma cells enables extravasation via the JAK2-Stat5 and p38MAPK pathway. *Cancer Cell, 22*, 91–105.

9. Hauselmann, I., Roblek, M., Protsyuk, D., Huck, V., Knopfova, L., Grassle, S., Bauer, A. T., Schneider, S. W., & Borsig, L. (2016). Monocyte induction of E-Selectin-mediated endothelial activation releases VE-cadherin junctions to promote tumor cell extravasation in the metastasis Cascade. *Cancer Research, 76*, 5302–5312.
10. Läubli, H., & Borsig, L. (2010). Selectins promote tumor metastasis. *Seminars in Cancer Biology, 20*, 169–177.
11. Stoler-Barak, L., Petrovich, E., Aychek, T., Gurevich, I., Tal, O., Hatzav, M., Ilan, N., Feigelson, S. W., Shakhar, G., Vlodavsky, I., et al. (2015). Heparanase of murine effector lymphocytes and neutrophils is not required for their diapedesis into sites of inflammation. *The FASEB Journal, 29*, 2010–2021.
12. Petrovich, E., Feigelson, S. W., Stoler-Barak, L., Hatzav, M., Solomon, A., Bar-Shai, A., Ilan, N., Li, J. P., Engelhardt, B., Vlodavsky, I., et al. (2016). Lung ICAM-1 and ICAM-2 support spontaneous intravascular effector lymphocyte entrapment but are not required for neutrophil entrapment or emigration inside endotoxin-inflamed lungs. *The FASEB Journal, 30*, 1767–1778.
13. Läubli, H., Spanaus, K. S., & Borsig, L. (2009). Selectin-mediated activation of endothelial cells induces expression of CCL5 and promotes metastasis through recruitment of monocytes. *Blood, 114*, 4583–4591.
14. Cooper, J., & Giancotti, F. G. (2019). Integrin signaling in Cancer: Mechanotransduction, Stemness, epithelial plasticity, and therapeutic resistance. *Cancer Cell, 35*, 347–367.
15. Rodrigues, J. G., Balmana, M., Macedo, J. A., Pocas, J., Fernandes, A., de-Freitas-Junior, J. C. M., Pinho, S. S., Gomes, J., Magalhaes, A., Gomes, C., et al. (2018). Glycosylation in cancer: Selected roles in tumour progression, immune modulation and metastasis. *Cellular Immunology, 333*, 46–57.
16. Bendas, G., & Borsig, L. (2012). Cancer cell adhesion and metastasis: Selectins, integrins, and the inhibitory potential of heparins. *International journal of cell biology, 2012*, 676731.
17. Borsig, L. (2018). Selectins in cancer immunity. *Glycobiology, 28*, 648–655.
18. Witz, I. P. (2008). The selectin-selectin ligand axis in tumor progression. *Cancer Metastasis Reviews, 27*, 19–30.
19. Marsico, G., Russo, L., Quondamatteo, F., & Pandit, A. (2018). Glycosylation and integrin regulation in Cancer. *Trends Cancer, 4*, 537–552.
20. Ley, K., Laudanna, C., Cybulsky, M. I., & Nourshargh, S. (2007). Getting to the site of inflammation: The leukocyte adhesion cascade updated. *Nature Reviews. Immunology, 7*, 678–689.
21. Kansas, G. S. (1996). Selectins and their ligands: Current concepts and controversies. *Blood, 88*, 3259–3287.
22. Varki, A. (1997). Selectin ligands: Will the real ones please stand up? *The Journal of Clinical Investigation, 99*, 158–162.
23. Hauselmann, I., & Borsig, L. (2014). Altered tumor-cell glycosylation promotes metastasis. *Frontiers in Oncology, 4*, 28.
24. Pinho, S. S., & Reis, C. A. (2015). Glycosylation in cancer: Mechanisms and clinical implications. *Nature Reviews. Cancer, 15*, 540–555.
25. Kannagi, R., Izawa, M., Koike, T., Miyazaki, K., & Kimura, N. (2004). Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. *Cancer Science, 95*, 377–384.
26. Borsig, L., Stevenson, J. L., & Varki, A. (2007). Heparin in Cancer: Role of Selectin interactions. In A. A. Khorana & C. W. Francis (Eds.), *Cancer-associated thrombosis* (pp. 97–113). New York: Informa Healthcare.
27. Kim, Y. J., Borsig, L., Varki, N. M., & Varki, A. (1998). P-selectin deficiency attenuates tumor growth and metastasis. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 9325–9330.
28. Borsig, L., Wong, R., Feramisco, J., Nadeau, D. R., Varki, N. M., & Varki, A. (2001). Heparin and cancer revisited: Mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 3352–3357.

29. Labelle, M., & Hynes, R. O. (2012). The initial hours of metastasis: The importance of cooperative host-tumor cell interactions during hematogenous dissemination. *Cancer Discovery, 2*, 1091–1099.
30. Coupland, L. A., & Parish, C. R. (2014). Platelets, selectins, and the control of tumor metastasis. *Seminars in Oncology, 41*, 422–434.
31. Schlesinger, M. (2018). Role of platelets and platelet receptors in cancer metastasis. *Journal of Hematology & Oncology, 11*, 125.
32. Ludwig, R. J., Boehme, B., Podda, M., Henschler, R., Jager, E., Tandi, C., Boehncke, W. H., Zollner, T. M., Kaufmann, R., & Gille, J. (2004). Endothelial P-selectin as a target of heparin action in experimental melanoma lung metastasis. *Cancer Research, 64*, 2743–2750.
33. Hiratsuka, S., Goel, S., Kamoun, W. S., Maru, Y., Fukumura, D., Duda, D. G., & Jain, R. K. (2011). Endothelial focal adhesion kinase mediates cancer cell homing to discrete regions of the lungs via E-selectin up-regulation. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 3725–3730.
34. Kang, S. A., Hasan, N., Mann, A. P., Zheng, W., Zhao, L., Morris, L., Zhu, W., Zhao, Y. D., Suh, K. S., Dooley, W. C., et al. (2015). Blocking the adhesion cascade at the premetastatic niche for prevention of breast cancer metastasis. *Molecular Therapy, 23*, 1044–1054.
35. Shi, H., Zhang, J., Han, X., Li, H., Xie, M., Sun, Y., Liu, W., Ba, X., & Zeng, X. (2017). Recruited monocytic myeloid-derived suppressor cells promote the arrest of tumor cells in the premetastatic niche through an IL-1beta-mediated increase in E-selectin expression. *International Journal of Cancer, 140*, 1370–1383.
36. Kitamura, T., Qian, B. Z., & Pollard, J. W. (2015). Immune cell promotion of metastasis. *Nature Reviews. Immunology, 15*, 73–86.
37. Borsig, L., Wolf, M. J., Roblek, M., Lorentzen, A., & Heikenwalder, M. (2014). Inflammatory chemokines and metastasis-tracing the accessory. *Oncogene, 33*, 3217–3224.
38. Wang, Y., Ding, Y., Guo, N., & Wang, S. (2019). MDSCs: Key criminals of tumor premetastatic niche formation. *Frontiers in Immunology, 10*, 172.
39. Läubli, H., Stevenson, J. L., Varki, A., Varki, N. M., & Borsig, L. (2006). L-selectin facilitation of metastasis involves temporal induction of fut7-dependent ligands at sites of tumor cell arrest. *Cancer Research, 66*, 1536–1542.
40. Mohammed, R. N., Watson, H. A., Vigar, M., Ohme, J., Thomson, A., Humphreys, I. R., & Ager, A. (2016). L-selectin is essential for delivery of activated CD8(+) T cells to virus-infected organs for protective immunity. *Cell Reports, 14*, 760–771.
41. Seguin, L., Desgrosellier, J. S., Weis, S. M., & Cheresh, D. A. (2015). Integrins and cancer: Regulators of cancer stemness, metastasis, and drug resistance. *Trends in Cell Biology, 25*, 234–240.
42. Seguin, L., Kato, S., Franovic, A., Camargo, M. F., Lesperance, J., Elliott, K. C., Yebra, M., Mielgo, A., Lowy, A. M., Husain, H., et al. (2014). An integrin beta(3)-KRAS-RalB complex drives tumour stemness and resistance to EGFR inhibition. *Nature Cell Biology, 16*, 457–468.
43. Jakubzig, B., Baltes, F., Henze, S., Schlesinger, M., & Bendas, G. (2018). Mechanisms of matrix-induced Chemoresistance of breast Cancer cells-deciphering novel potential targets for a cell sensitization. *Cancers (Basel)*, 10.
44. Parvani, J. G., Gujrati, M. D., Mack, M. A., Schiemann, W. P., & Lu, Z. R. (2015). Silencing beta3 integrin by targeted ECO/siRNA nanoparticles inhibits EMT and metastasis of triple-negative breast Cancer. *Cancer Research, 75*, 2316–2325.
45. Zhang, Z., Ramirez, N. E., Yankeelov, T. E., Li, Z., Ford, L. E., Qi, Y., Pozzi, A., & Zutter, M. M. (2008). alpha2beta1 integrin expression in the tumor microenvironment enhances tumor angiogenesis in a tumor cell-specific manner. *Blood, 111*, 1980–1988.
46. Hamidi, H., & Ivaska, J. (2018). Every step of the way: Integrins in cancer progression and metastasis. *Nature Reviews. Cancer, 18*, 533–548.
47. Lavergne, M., Janus-Bell, E., Schaff, M., Gachet, C., & Mangin, P. H. (2017). Platelet Integrins in tumor metastasis: Do they represent a therapeutic target? *Cancers (Basel), 9*.
48. Mammadova-Bach, E., Zigrino, P., Brucker, C., Bourdon, C., Freund, M., De Arcangelis, A., Abrams, S. I., Orend, G., Gachet, C., & Mangin, P. H. (2016). Platelet integrin alpha6beta1

controls lung metastasis through direct binding to cancer cell-derived ADAM9. *JCI Insight, 1*, e88245.
49. Lonsdorf, A. S., Kramer, B. F., Fahrleitner, M., Schonberger, T., Gnerlich, S., Ring, S., Gehring, S., Schneider, S. W., Kruhlak, M. J., Meuth, S. G., et al. (2012). Engagement of alphaIIbbeta3 (GPIIb/IIIa) with alphanubeta3 integrin mediates interaction of melanoma cells with platelets: A connection to hematogenous metastasis. *The Journal of Biological Chemistry, 287*, 2168–2178.
50. Schlesinger, M., & Bendas, G. (2015). Contribution of very late antigen-4 (VLA-4) integrin to cancer progression and metastasis. *Cancer Metastasis Reviews, 34*, 575–591.
51. Okahara, H., Yagita, H., Miyake, K., & Okumura, K. (1994). Involvement of very late activation antigen 4 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) in tumor necrosis factor alpha enhancement of experimental metastasis. *Cancer Research, 54*, 3233–3236.
52. Garofalo, A., Chirivi, R. G., Foglieni, C., Pigott, R., Mortarini, R., Martin-Padura, I., Anichini, A., Gearing, A. J., Sanchez-Madrid, F., Dejana, E., et al. (1995). Involvement of the very late antigen 4 integrin on melanoma in interleukin 1-augmented experimental metastases. *Cancer Research, 55*, 414–419.
53. Schlesinger, M., Roblek, M., Ortmann, K., Naggi, A., Torri, G., Borsig, L., & Bendas, G. (2014). The role of VLA-4 binding for experimental melanoma metastasis and its inhibition by heparin. *Thrombosis Research, 133*, 855–862.
54. Rebhun, R. B., Cheng, H., Gershenwald, J. E., Fan, D., Fidler, I. J., & Langley, R. R. (2010). Constitutive expression of the alpha4 integrin correlates with tumorigenicity and lymph node metastasis of the B16 murine melanoma. *Neoplasia, 12*, 173–182.
55. Chen, Q., Zhang, X. H., & Massague, J. (2011). Macrophage binding to receptor VCAM-1 transmits survival signals in breast Cancer cells that invade the lungs. *Cancer Cell, 20*, 538–549.
56. Hoshino, A., Costa-Silva, B., Shen, T. L., Rodrigues, G., Hashimoto, A., Tesic Mark, M., Molina, H., Kohsaka, S., Di Giannatale, A., Ceder, S., et al. (2015). Tumour exosome integrins determine organotropic metastasis. *Nature, 527*, 329–335.
57. Borsig, L. (2010). Antimetastatic activities of heparins and modified heparins. *Experimental evidence. Thromb Res, 125*(Suppl 2), S66–S71.
58. Niers, T. M., Klerk, C. P., DiNisio, M., Van Noorden, C. J., Buller, H. R., Reitsma, P. H., & Richel, D. J. (2007). Mechanisms of heparin induced anti-cancer activity in experimental cancer models. *Critical Reviews in Oncology/Hematology, 61*, 195–207.
59. Vlodavsky, I., Mohsen, M., Lider, O., Svahn, C. M., Ekre, H. P., Vigoda, M., Ishai-Michaeli, R., & Peretz, T. (1994). Inhibition of tumor metastasis by heparanase inhibiting species of heparin. *Invasion & Metastasis, 14*, 290–302.
60. Stevenson, J. L., Choi, S. H., & Varki, A. (2005). Differential metastasis inhibition by clinically relevant levels of heparins—Correlation with selectin inhibition, not antithrombotic activity. *Clinical Cancer Research, 11*, 7003–7011.
61. Ludwig, R. J., Alban, S., Bistrian, R., Boehncke, W. H., Kaufmann, R., Henschler, R., & Gille, J. (2006). The ability of different forms of heparins to suppress P-selectin function in vitro correlates to their inhibitory capacity on bloodborne metastasis in vivo. *Thrombosis and Haemostasis, 95*, 535–540.
62. Smorenburg, S. M., & Van Noorden, C. J. (2001). The complex effects of heparins on cancer progression and metastasis in experimental studies. *Pharmacological Reviews, 53*, 93–105.
63. Garcia-Escobar, I., Beato-Zambrano, C., Munoz Langa, J., Brozos Vazquez, E., Obispo Portero, B., Gutierrez-Abad, D., Munoz Martin, A. J., & Cancer, and Thrombosis Working Group of the Spanish Society of Medical, O. (2018). Pleiotropic effects of heparins: Does anticoagulant treatment increase survival in cancer patients? *Clinical & Translational Oncology, 20*, 1097–1108.
64. Casu, B., Vlodavsky, I., & Sanderson, R. D. (2008). Non-anticoagulant heparins and inhibition of cancer. *Pathophysiology of Haemostasis and Thrombosis, 36*, 195–203.
65. Norgard-Sumnicht, K. E., Varki, N. M., & Varki, A. (1993). Calcium-dependent heparin-like ligands for L-selectin in nonlymphoid endothelial cells. *Science, 261*, 480–483.

66. Koenig, A., Norgard-Sumnicht, K., Linhardt, R., & Varki, A. (1998). Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *The Journal of Clinical Investigation, 101*, 877–889.
67. Diamond, M. S., Alon, R., Parkos, C. A., Quinn, M. T., & Springer, T. A. (1995). Heparin is an adhesive ligand for the leukocyte integrin mac-1 (CD11b/CD1). *The Journal of Cell Biology, 130*, 1473–1482.
68. Vorup-Jensen, T., Chi, L., Gjelstrup, L. C., Jensen, U. B., Jewett, C. A., Xie, C., Shimaoka, M., Linhardt, R. J., & Springer, T. A. (2007). Binding between the integrin alphaXbeta2 (CD11c/CD18) and heparin. *The Journal of Biological Chemistry, 282*, 30869–30877.
69. Zhang, C., Liu, Y., Gao, Y., Shen, J., Zheng, S., Wei, M., & Zeng, X. (2009). Modified heparins inhibit integrin alpha(IIb)beta(3) mediated adhesion of melanoma cells to platelets in vitro and in vivo. *International Journal of Cancer, 125*, 2058–2065.
70. Fritzsche, J., Simonis, D., & Bendas, G. (2008). Melanoma cell adhesion can be blocked by heparin in vitro: Suggestion of VLA-4 as a novel target for antimetastatic approaches. *Thrombosis and Haemostasis, 100*, 1166–1175.
71. Callander, N., & Rapaport, S. I. (1993). Trousseau's syndrome. *The Western Journal of Medicine, 158*, 364–371.
72. Varki, A. (2007). Trousseau's syndrome: Multiple definitions and multiple mechanisms. *Blood, 110*, 1723–1729.
73. Falanga, A., & Marchetti, M. (2007). Heparin in tumor progression and metastatic dissemination. *Seminars in Thrombosis and Hemostasis, 33*, 688–694.
74. Rickles, F. R., & Falanga, A. (2001). Molecular basis for the relationship between thrombosis and cancer. *Thrombosis Research, 102*, V215–V224.
75. Vignoli, A., Marchetti, M., & Falanga, A. (2017). Heparins inhibit the endothelial prothrombotic features induced by tumor cells. *Thrombosis Research, 157*, 55–57.
76. Mandala, M., Labianca, R., & European Society for Medical, O. (2010). Venous thromboembolism (VTE) in cancer patients. ESMO clinical recommendations for prevention and management. *Thrombosis Research, 125*(Suppl 2), S117–S119.
77. Lyman, G. H., Bohlke, K., Falanga, A., & American Society of Clinical, O. (2015). Venous thromboembolism prophylaxis and treatment in patients with cancer: American Society of Clinical Oncology clinical practice guideline update. *Journal of Oncology Practice/ American Society of Clinical Oncology, 11*, e442–e444.
78. Prisco, D., Tufano, A., Cenci, C., Pignatelli, P., Santilli, F., Di Minno, G., & Perticone, F. (2019). Position paper of the Italian Society of Internal Medicine (SIMI) on prophylaxis and treatment of venous thromboembolism in patients with cancer. *Internal and Emergency Medicine, 14*, 21–38.
79. Smorenburg, S. M., Hettiarachchi, R. J., Vink, R., & Buller, H. R. (1999). The effects of unfractionated heparin on survival in patients with malignancy--a systematic review. *Thrombosis and Haemostasis, 82*, 1600–1604.
80. Lee, A. Y., Rickles, F. R., Julian, J. A., Gent, M., Baker, R. I., Bowden, C., Kakkar, A. K., Prins, M., & Levine, M. N. (2005). Randomized comparison of low molecular weight heparin and coumarin derivatives on the survival of patients with cancer and venous thromboembolism. *Journal of Clinical Oncology, 23*, 2123–2129.
81. Lebeau, B., Chastang, C., Brechot, J. M., Capron, F., Dautzenberg, B., Delaisements, C., Mornet, M., Brun, J., Hurdebourcq, J. P., & Lemarie, E. (1994). Subcutaneous heparin treatment increases survival in small cell lung cancer. "petites cellules" group. *Cancer, 74*, 38–45.
82. Kakkar, A. K., Levine, M. N., Kadziola, Z., Lemoine, N. R., Low, V., Patel, H. K., Rustin, G., Thomas, M., Quigley, M., & Williamson, R. C. (2004). Low molecular weight heparin, therapy with dalteparin, and survival in advanced cancer: The fragmin advanced malignancy outcome study (FAMOUS). *Journal of Clinical Oncology, 22*, 1944–1948.
83. Klerk, C. P., Smorenburg, S. M., Otten, H. M., Lensing, A. W., Prins, M. H., Piovella, F., Prandoni, P., Bos, M. M., Richel, D. J., van Tienhoven, G., et al. (2005). The effect of low

molecular weight heparin on survival in patients with advanced malignancy. *Journal of Clinical Oncology, 23*, 2130–2135.
84. Altinbas, M., Coskun, H. S., Er, O., Ozkan, M., Eser, B., Unal, A., Cetin, M., & Soyuer, S. (2004). A randomized clinical trial of combination chemotherapy with and without low-molecular-weight heparin in small cell lung cancer. *Journal of Thrombosis and Haemostasis, 2*, 1266–1271.
85. Akl, E. A., Barba, M., Rohilla, S., Terrenato, I., Sperati, F., Muti, P., & Schunemann, H. J. (2008). Low-molecular-weight heparins are superior to vitamin K antagonists for the long term treatment of venous thromboembolism in patients with cancer: A cochrane systematic review. *Journal of Experimental & Clinical Cancer Research, 27*, 21.
86. van Doormaal, F. F., Di Nisio, M., Otten, H. M., Richel, D. J., Prins, M., & Buller, H. R. (2011). Randomized trial of the effect of the low molecular weight heparin nadroparin on survival in patients with cancer. *Journal of Clinical Oncology, 29*, 2071–2076.
87. Macbeth, F., Noble, S., Evans, J., Ahmed, S., Cohen, D., Hood, K., Knoyle, D., Linnane, S., Longo, M., Moore, B., et al. (2016). Randomized phase III trial of standard therapy plus Low molecular weight heparin in patients with lung Cancer: FRAGMATIC trial. *Journal of Clinical Oncology, 34*, 488–494.
88. Perry, J. R., Julian, J. A., Laperriere, N. J., Geerts, W., Agnelli, G., Rogers, L. R., Malkin, M. G., Sawaya, R., Baker, R., Falanga, A., et al. (2010). PRODIGE: A randomized placebo-controlled trial of dalteparin low-molecular-weight heparin thromboprophylaxis in patients with newly diagnosed malignant glioma. *Journal of Thrombosis and Haemostasis, 8*, 1959–1965.
89. Meyer, G., Besse, B., Doubre, H., Charles-Nelson, A., Aquilanti, S., Izadifar, A., Azarian, R., Monnet, I., Lamour, C., Descourt, R., et al. (2018). Anti-tumour effect of low molecular weight heparin in localised lung cancer: A phase III clinical trial. *The European Respiratory Journal*, 52.
90. Vlodavsky, I., Ilan, N., Nadir, Y., Brenner, B., Katz, B. Z., Naggi, A., Torri, G., Casu, B., & Sasisekharan, R. (2007). Heparanase, heparin and the coagulation system in cancer progression. *Thrombosis Research, 120*(Suppl 2), S112–S120.
91. Iozzo, R. V., & Sanderson, R. D. (2011). Proteoglycans in cancer biology, tumour microenvironment and angiogenesis. *Journal of Cellular and Molecular Medicine, 15*, 1013–1031.
92. Ilan, N., Elkin, M., & Vlodavsky, I. (2006). Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. *The International Journal of Biochemistry & Cell Biology, 38*, 2018–2039.
93. Vlodavsky, I., Friedmann, Y., Elkin, M., Aingorn, H., Atzmon, R., Ishai-Michaeli, R., Bitan, M., Pappo, O., Peretz, T., Michal, I., et al. (1999). Mammalian heparanase: Gene cloning, expression and function in tumor progression and metastasis. *Nature Medicine, 5*, 793–802.
94. Edovitsky, E., Elkin, M., Zcharia, E., Peretz, T., & Vlodavsky, I. (2004). Heparanase gene silencing, tumor invasiveness, angiogenesis, and metastasis. *Journal of the National Cancer Institute, 96*, 1219–1230.
95. Sanderson, R. D., Elkin, M., Rapraeger, A. C., Ilan, N., & Vlodavsky, I. (2017). Heparanase regulation of cancer, autophagy and inflammation: New mechanisms and targets for therapy. *The FEBS Journal, 284*, 42–55.
96. Elkin, M., Ilan, N., Ishai-Michaeli, R., Friedmann, Y., Papo, O., Pecker, I., & Vlodavsky, I. (2001). Heparanase as mediator of angiogenesis: Mode of action. *The FASEB Journal, 15*, 1661–1663.
97. Thompson, C. A., Purushothaman, A., Ramani, V. C., Vlodavsky, I., & Sanderson, R. D. (2013). Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes. *The Journal of Biological Chemistry, 288*, 10093–10099.
98. Jung, O., Trapp-Stamborski, V., Purushothaman, A., Jin, H., Wang, H., Sanderson, R. D., & Rapraeger, A. C. (2016). Heparanase-induced shedding of syndecan-1/CD138 in myeloma and endothelial cells activates VEGFR2 and an invasive phenotype: Prevention by novel synstatins. *Oncogene, 5*, e202.

99. Peinado, H., Zhang, H., Matei, I. R., Costa-Silva, B., Hoshino, A., Rodrigues, G., Psaila, B., Kaplan, R. N., Bromberg, J. F., Kang, Y., et al. (2017). Pre-metastatic niches: Organ-specific homes for metastases. *Nature Reviews. Cancer, 17*, 302–317.
100. Baietti, M. F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F., Coomans, C., Vermeiren, E., et al. (2012). Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nature Cell Biology, 14*, 677–685.
101. Ramani, V. C., Zhan, F., He, J., Barbieri, P., Noseda, A., Tricot, G., & Sanderson, R. D. (2016). Targeting heparanase overcomes chemoresistance and diminishes relapse in myeloma. *Oncotarget, 7*, 1598–1607.
102. Tsunekawa, N., Higashi, N., Kogane, Y., Waki, M., Shida, H., Nishimura, Y., Adachi, H., Nakajima, M., & Irimura, T. (2016). Heparanase augments inflammatory chemokine production from colorectal carcinoma cell lines. *Biochemical and Biophysical Research Communications, 469*, 878–883.
103. Gutter-Kapon, L., Alishekevitz, D., Shaked, Y., Li, J. P., Aronheim, A., Ilan, N., & Vlodavsky, I. (2016). Heparanase is required for activation and function of macrophages. *Proceedings of the National Academy of Sciences of the United States of America, 113*, E7808–E7817.
104. Gerber, U., Hoss, S. G., Shteingauz, A., Jungel, E., Jakubzig, B., Ilan, N., Blaheta, R., Schlesinger, M., Vlodavsky, I., & Bendas, G. (2015). Latent heparanase facilitates VLA-4-mediated melanoma cell binding and emerges as a relevant target of heparin in the interference with metastatic progression. *Seminars in Thrombosis and Hemostasis, 41*, 244–254.
105. Nadir, Y., Brenner, B., Zetser, A., Ilan, N., Shafat, I., Zcharia, E., Goldshmidt, O., & Vlodavsky, I. (2006). Heparanase induces tissue factor expression in vascular endothelial and cancer cells. *Journal of Thrombosis and Haemostasis, 4*, 2443–2451.
106. Nadir, Y., Brenner, B., Gingis-Velitski, S., Levy-Adam, F., Ilan, N., Zcharia, E., Nadir, E., & Vlodavsky, I. (2008). Heparanase induces tissue factor pathway inhibitor expression and extracellular accumulation in endothelial and tumor cells. *Thrombosis and Haemostasis, 99*, 133–141.
107. Levy-Adam, F., Abboud-Jarrous, G., Guerrini, M., Beccati, D., Vlodavsky, I., & Ilan, N. (2005). Identification and characterization of heparin/heparan sulfate binding domains of the endoglycosidase heparanase. *The Journal of Biological Chemistry, 280*, 20457–20466.
108. Wu, L., Viola, C. M., Brzozowski, A. M., & Davies, G. J. (2015). Structural characterization of human heparanase reveals insights into substrate recognition. *Nature Structural & Molecular Biology, 22*, 1016–1022.
109. Bar-Ner, M., Eldor, A., Wasserman, L., Matzner, Y., Cohen, I. R., Fuks, Z., & Vlodavsky, I. (1987). Inhibition of heparanase-mediated degradation of extracellular matrix heparan sulfate by non-anticoagulant heparin species. *Blood, 70*, 551–557.
110. Bar-Ner, M., Kramer, M. D., Schirrmacher, V., Ishai-Michaeli, R., Fuks, Z., & Vlodavsky, I. (1985). Sequential degradation of heparan sulfate in the subendothelial extracellular matrix by highly metastatic lymphoma cells. *International Journal of Cancer, 35*, 483–491.
111. Lapierre, F., Holme, K., Lam, L., Tressler, R. J., Storm, N., Wee, J., Stack, R. J., Castellot, J., & Tyrrell, D. J. (1996). Chemical modifications of heparin that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, anti-tumor and anti- metastatic properties. *Glycobiology, 6*, 355–366.
112. Naggi, A., Casu, B., Perez, M., Torri, G., Cassinelli, G., Penco, S., Pisano, C., Giannini, G., Ishai-Michaeli, R., & Vlodavsky, I. (2005). Modulation of the heparanase-inhibiting activity of heparin through selective desulfation, graded N-acetylation, and glycol splitting. *The Journal of Biological Chemistry, 280*, 12103–12113.
113. Galli, M., Chatterjee, M., Grasso, M., Specchia, G., Magen, H., Einsele, H., Celeghini, I., Barbieri, P., Paoletti, D., Pace, S., et al. (2018). Phase I study of the heparanase inhibitor roneparstat: An innovative approach for ultiple myeloma therapy. *Haematologica, 103*, e469–e472.
114. Ni, M., Elli, S., Naggi, A., Guerrini, M., Torri, G., & Petitou, M. (2016). Investigating glycol-Split-heparin-derived inhibitors of Heparanase: A study of synthetic Trisaccharides. *Molecules*, 21.

115. Hostettler, N., Naggi, A., Torri, G., Casu, B., Vlodavsky, I., & Borsig, L. (2007). P-selectin- and heparanase-dependent antimetastatic activity of non-anticoagulant heparins. *The FASEB Journal, 21*, 3562–3572.
116. Gomes, A. M., Kozlowski, E. O., Borsig, L., Teixeira, F. C., Vlodavsky, I., & Pavao, M. S. (2015). Antitumor properties of a new non-anticoagulant heparin analog from the mollusk Nodipecten nodosus: Effect on P-selectin, heparanase, metastasis and cellular recruitment. *Glycobiology, 25*, 386–393.
117. Loka, R.S., Sletten, E.T., Barash, U., Vlodavsky, I., and Nguyen, H.M. 2018. Specific inhibition of Heparanase by Glycopolymer with well-defined Sulfation pattern prevents breast Cancer metastasis in mice. ACS Applied Materials & Interfaces (in press):doi: https://doi.org/10.1021/acsami.1028b17625.
118. Läubli, H., & Borsig, L. (2009). Heparins attenuate cancer metastasis: Are selectins the link? *Cancer Investigation, 27*, 474–481.
119. Zhou, H., Roy, S., Cochran, E., Zouaoui, R., Chu, C. L., Duffner, J., Zhao, G., Smith, S., Galcheva-Gargova, Z., Karlgren, J., et al. (2011). M402, a novel heparan sulfate mimetic, targets multiple pathways implicated in tumor progression and metastasis. *PLoS One, 6*, e21106.
120. Parish, C. R., Freeman, C., Brown, K. J., Francis, D. J., & Cowden, W. B. (1999). Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. *Cancer Research, 59*, 3433–3441.
121. Kragh, M., Binderup, L., Vig Hjarnaa, P. J., Bramm, E., Johansen, K. B., & Frimundt Petersen, C. (2005). Non-anti-coagulant heparin inhibits metastasis but not primary tumor growth. *Oncology Reports, 14*, 99–104.
122. Borsig, L., Vlodavsky, I., Ishai-Michaeli, R., Torri, G., & Vismara, E. (2011). Sulfated hexasaccharides attenuate metastasis by inhibition of P-selectin and heparanase. *Neoplasia, 13*, 445–452.
123. Weissmann, M., Arvatz, G., Horowitz, N., Feld, S., Naroditsky, I., Zhang, Y., Ng, M., Hammond, E., Nevo, E., Vlodavsky, I., et al. (2016). Heparanase-neutralizing antibodies attenuate lymphoma tumor growth and metastasis. *Proceedings of the National Academy of Sciences of the United States of America, 113*, 704–709.
124. Koliopanos, A., Friess, H., Kleeff, J., Shi, X., Liao, Q., Pecker, I., Vlodavsky, I., Zimmermann, A., & Buchler, M. W. (2001). Heparanase expression in primary and metastatic pancreatic cancer. *Cancer Research, 61*, 4655–4659.
125. Ritchie, J. P., Ramani, V. C., Ren, Y., Naggi, A., Torri, G., Casu, B., Penco, S., Pisano, C., Carminati, P., Tortoreto, M., et al. (2011). SST0001, a chemically modified heparin, inhibits myeloma growth and angiogenesis via disruption of the heparanase/syndecan-1 axis. *Clinical Cancer Research, 17*, 1382–1393.