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REVIEW PAPER

Significance of Heparanase in Cancer and Inflammation

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Abstract Heparan sulfate proteoglycans (HSPGs) are primary components at the interface between virtually every eukaryotic cell and its extracellular matrix. HSPGs not only provide a storage depot for heparin-binding molecules in the cell microenvironment, but also decisively regulate their accessibility, function and mode of action. As such, they are intimately involved in modulating cell invasion and signaling loops that are critical for tumor growth, inflammation and kidney function. In a series of studies performed since the cloning of the human heparanase gene, we and others have demonstrated that heparanase, the sole heparan sulfate degrading endoglycosidase, is causally involved in cancer progression, inflammation and diabetic nephropathy and hence is a valid target for drug development. Heparanase is causally involved in inflammation and accelerates colon tumorigenesis associated with inflammatory bowel disease. Notably, heparanase stimulates macrophage activation, while macrophages induce production and activation of latent heparanase contributed by the colon epithelium, together generating a vicious cycle that powers colitis and the associated tumorigenesis. Heparanase also plays a decisive role in the pathogenesis of diabetic nephropathy, degrading heparan sulfate in the glomerular basement membrane and ultimately leading to proteinuria and kidney dysfunction. Notably, clinically relevant doses of ionizing radiation (IR) upregulate heparanase expression and thereby augment the metastatic potential of pancreatic carcinoma. Thus, combining radiotherapy with heparanase inhibition is an effective strategy to prevent tumor resistance and dissemination in IR-treated pancreatic cancer patients. Also, accumulating evidence indicate that peptides derived from human heparanase elicit a potent anti-tumor immune response, suggesting that heparanase represents a promising target antigen for immunotherapeutic approaches against a broad variety of tumours. Oligosaccharide-based compounds that inhibit heparanase enzymatic activity were developed, aiming primarily at halting tumor growth, metastasis and angiogenesis. Some of these compounds are being evaluated in clinical trials, targeting both the tumor and tumor microenvironment.

Keywords Extracellular matrix · Tumor microenvironment · Inflammation · Tumor associated macrophages · Heparan sulfate · Heparanase · Metastasis · Colitis · Colon carcinoma

Abbreviations

ECM	Extracellular matrix
HS	Heparan sulfate
HSPGs	Heparan sulfate proteoglycans
GAG	Glycosaminoglycan
MMP	Matrix metalloproteinase
VEGF	Vascular endothelial growth factor

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CAF	Cancer-associated fibroblasts
UC	Ulcerative colitis
IBD	Inflammatory bowel disease
AOM	Azoxymethane
DSS	Dextran sodium sulfate
IR	Ionoizing radiation
Egr1	Early growth response 1
CTL	Cytotoxic T lymphocyte
HAT	Histone acetyltransferase

Preface

The extracellular matrix (ECM) is a heterogeneous mixture of proteins and polysaccharides that surrounds cells, providing physical support for cellular organization into tissues and organs. Traditionally, the ECM was regarded as an inert scaffold providing a structural framework for cells to form tissues and organs. Back in 1979, we were among the first to realize that the ECM plays an active role in orchestrating cellular responses to both normal and pathological situations [1, 2]. The emerging notion was one of active interplay between cells and ECM where the cells synthesize matrix components which in turn dictate and regulate cell shape and function [1, 2]. The ECM network of proteins, glycoproteins and proteoglycans provides adherent cells with structural support and biochemical cues that regulate cell fate and function. We developed a straightforward approach to coat plastic surfaces with ECM deposited by cultured endothelial cells and demonstrated that this naturally produced ECM closely resembles the subendothelial basement membrane in vivo [2, 3]. This ECM and the more commonly used 3-dimensional tumor-derived basement membrane-like substrate (Matrigel) [4] are being applied to sustain cell proliferation, differentiation and survival in vitro, retaining the in vivo characteristics [5]. The ECM/Matrigel system is also widely used to study tumor cell invasion and vascular sprouting. In subsequent studies we have demonstrated that the ECM and basement membrane provide a storage depot for FGF2 and thereby regulates its bioavailability [6]. By now, the function of ECM as a reservoir for bioactive molecules is well recognized and highly important to the current appreciation of the tumor microenvironment and its significance in cancer progression and treatment.

Apart of studies on the biology of the ECM in general, our research has been focused on heparan sulfate (HS) glycosaminoglycan (GAG), one of the most important components of the ECM, basement membranes and cell surface molecules, shown to have a pronounced effect on fundamental biological processes, ranging from development and formation of blood vessels to cell invasion, inflammation and viral infection [7]. GAGs are linear polysaccharides consisting of a repeating disaccharide generally of an acetylated amino sugar alternating with uronic acid. Notably, while 4 and 20 building blocks make nucleic acids and proteins, respectively, 32 disaccharide building blocks make up these complex, highly acidic and information dense biopolymers. The chemical heterogeneity and structural complexity of GAGs make investigations of these molecules most challenging, raising fundamental questions as to how topological positioning and function of cells and tissues are regulated by GAGs. The biosynthesis of HS GAG takes place in the Golgi system and has been studied in great detail. Briefly, the polysaccharide chains are modified at various positions by sulfation, epimerization and N-acetylation, yielding clusters of sulfated disaccharides separated by low or non-sulfated regions [8, 9]. The sulfated saccharide domains provide numerous docking sites for a multitude of protein ligands, ensuring that a wide variety of bioactive molecules (i.e., cytokines, chemokines, growth factors, enzymes, protease inhibitors, ECM proteins) bind to the cell surface and ECM [10–12] and thereby function in the control of normal and pathological processes, among which are morphogenesis, tissue repair, inflammation, vascularization, and cancer metastasis [8–10, 12]. Unlike the well resolved biosynthetic pathway, the mode of HS breakdown is less characterized. Enzymatic activity capable of cleaving glucuronidic linkages and releasing polysaccharide chains resistant to further degradation by the enzyme was first identified by Ogren and Lindahl [13]. The physiological function of this activity was initially implicated in degradation of macromolecular heparin to physiologically active fragments [13, 14]. Subsequent studies revealed that the same enzyme (heparanase) is critically involved in various pathologies such as cancer progression [15], chronic inflammation [16, 17] and kidney dysfunction [18]. The present review summarizes our long term and ongoing research on the biology of the heparanase enzyme emphasizing its function in the tumor microenvironment and involvement in cancer and inflammation.

Heparan Sulfate Proteoglycans (HSPGs)

Proteoglycans are composed of a core protein to which GAGs side chains are covalently attached. Units of N-acetylglucosamine and glucuronic/iduronic acid form heparan sulfate (HS). HS biosynthesis is initiated by formation of a polysaccharide-protein linkage region, attaching four sugar units to a serine residue in the core protein. This tetrasaccharide sequence is extended by alternating addition of *N*-acetylglucosamine (GlcNAc) and *D*-glucuronic acid (GlcA) residues, forming repeating

disaccharides of (GlcAß1,4-GlcNAcα1,4)$_n$. During this polymerization process, the repeating disaccharide units are modified by a series of reactions in a sequential mode [19]. The first modification is *N*-deacetylation/*N*-sulfation of GlcNAc units, followed by C5-epimerization of GlcA to *L*-iduronic acid (IdoA) residues, and *O*-sulfation at C2 of IdoA and C6 of GlcNS residues. Tissue/cell specific regulation during HS biosynthesis [20, 21] dictates subtle and selective modulation of interactions between HS and various proteins [22, 23]. Two main types of cell-surface HS proteoglycans (HSPGs) core proteins have been identified: the transmembrane syndecan with four isoforms, carrying HS near their extracellular tips and occasionally also chondroitin sulfate chains near the cell surface [10], and the glycosylphosphatidyl inositol (GPI)-linked glypican with six isoforms, carrying several HS side chains near the plasma membrane and often an additional chain near the tip of its ectodomain [24]. Two major types of ECM-bound HSPGs are found: agrin, abundant in most basement membranes, primarily in the synaptic region [25]; and perlecan, endowed with a widespread tissue distribution and a very complex modular structure [26].

From mice to worms, embryos that lack HS die during gastrulation [27], suggesting a critical developmental role for HSPGs. HSPGs function is not limited to developmental processes but play key roles in numerous biological settings, including cytoskeleton organization, cell-cell and cell-ECM interactions [8, 28, 29]. HSPGs exert their multiple functional repertoires via several distinct mechanisms that combine structural, biochemical and regulatory aspects. By interacting with other macromolecules such as laminin, fibronectin, and collagens I and IV, HSPGs contribute to the structural integrity, self-assembly and insolubility of the ECM and basement membrane, thus intimately modulating cell-ECM interactions [10, 30, 31]. Accumulating evidence indicate that HSPGs act to inhibit cellular invasion by promoting tight cell-cell and cell-ECM interactions, and by maintaining the structural integrity and self assembly of the ECM [32, 33]. Notably, one of the characteristics of malignant transformation is down regulation of GAGs biosynthesis, especially of the HS chains [32, 33]. Low levels of cell surface HS also correlate with high metastatic capacity of many tumors. Biochemically, HSPGs often facilitate the biological activity of bound ligands by actively participating in receptor-ligand complex formation [34]. In other cases, HSPGs mediate cellular uptake and catabolism of selected ligands [34], and/or sequester polypeptides to the ECM and cell surface, generally as an inactive reservoir [6, 35–38]. Cleavage of HSPGs would ultimately release these proteins and convert them into bioactive mediators, ensuring rapid tissue response to local or systemic cues.

Mammalian Heparanase

Heparanase is an endo-β-glucuronidase that cleaves HS side chains presumably at sites of low sulfation, releasing saccharide products with appreciable size (4–7 kDa) that can still associate with protein ligands and facilitate their biological potency. Mammalian cells express primarily a single dominant functional heparanase enzyme (heparanase-1) [39, 40]. A second heparanase (heparanase-2) has been cloned and sequenced but has not been shown to have HS degrading activity [41, 42]. For simplification, throughout this review we will refer to heparanase-1 as heparanase. Enzymatic degradation of HS leads to disassembly of the ECM and is therefore involved in fundamental biological phenomena associated with tissue remodeling and cell migration, including inflammation, angiogenesis and metastasis [15, 39, 40]. Normally, heparanase is found mainly in platelets, mast cells, placental trophoblasts, keratinocytes and leukocytes. Heparanase released from activated platelets and cells of the immune system facilitates extravasation of inflammatory and tumor cells [43]. It also stimulates endothelial mitogenesis, primarily through release of HS-bound growth factors (i.e., FGF, HGF, VEGF) residing in the ECM [44].

The heparanase mRNA encodes a 61.2 kDa protein with 543 amino acids. This pro-enzyme is post translationally cleaved into 8 and 50 kDa subunits that non-covalently associate to form the active heparanase [45, 46] (Fig. 1). Heterodimer formation is essential for heparanase enzymatic activity [45, 47]. The heparanase structure delineates a TIM-barrel fold harboring the enzyme' active site and a C-terminus domain that is critical for heparanase secretion and signaling function [48] (Fig. 1). Site-directed mutagenesis revealed that similar to other glycosyl hydrolases, heparanase has a common catalytic mechanism that involves two conserved acidic residues, a putative proton donor at Glu^{225} and a nucleophile at Glu^{343} [49] (Fig. 1). Cellular processing of the latent 65 kDa pro-heparanase into its active 8+50 kDa heterodimer involves removal a 6 kDa linker segment and is inhibited by a cell permeable inhibitor of cathepsin L [50]. Moreover, multiple site-directed mutagenesis and cathepsin L gene silencing and knockout experiments indicate that cathepsin L is the predominant enzyme responsible for processing and activation of pro-heparanase [51]. Applying a structural model, it has been demonstrated that the linker segment, or even a small 1 kDa portion at its C-terminus, render the active site inaccessible to the HS substrate [51].

Heparanase in Cancer

In early studies heparanase activity was shown to be associated with the metastatic potential of tumor-derived

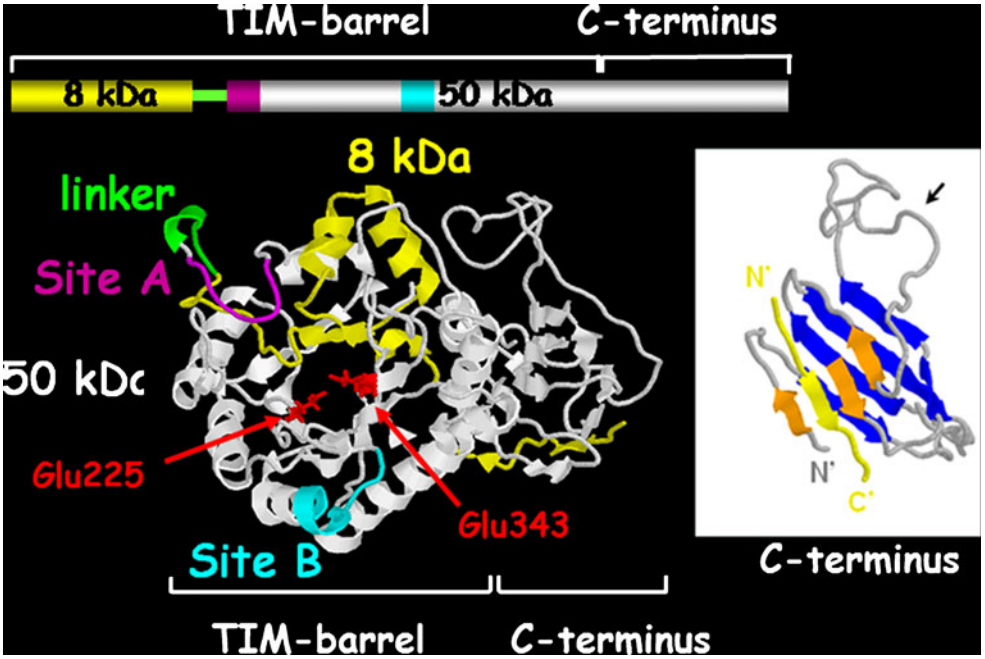

Fig. 1 Predicted model of the active heparanase heterodimer showing the 50+8 kDa heparanase subunits, TIM-barrel and C-terminus domains, active site (Glu_{225} & E_{343}, Red), and heparin binding domains (sites A and B). Right: Predicted structure of the C-terminus domain

cells such as B16 melanoma [52] and T-lymphoma [53]. These observations gained substantial support when specific molecular probes became available shortly after cloning of the heparanase gene. Both over-expression [54, 55] and silencing [55, 56] of the heparanase gene clearly indicate that heparanase not only enhances cell dissemination, but also promotes the establishment of a vascular network that accelerates primary tumor growth and provides a gateway for invading metastatic cells [40, 57]. These studies enabled researchers to critically approve the notion that HS cleavage by heparanase is required for structural remodeling of the ECM underlying tumors and endothelial cells, thereby facilitating cell invasion, and providing a proof-of-concept for the pro-metastatic and pro-angiogenic capacity of heparanase. The clinical significance of the enzyme in tumor progression emerged from a systematic evaluation of heparanase expression in primary human tumors. Immunohistochemistry, in situ hybridization, RT-PCR and real time-PCR analyses revealed that heparanase is up-regulated in essentially all major types of human cancer, namely carcinomas, sarcomas and hematological malignancies [40, 48, 57, 58] (Table 1). Notably, increased heparanase levels were most often associated with reduced patients' survival post operation (Fig. 2), increased tumor metastasis and higher microvessel density [40, 57, 59] (Table 1), thus critically supporting the intimate involvement of heparanase in tumor progression and encouraging the development of heparanase inhibitors as anti-cancer therapeutics [59–63].

Importantly, heparanase up-regulation in human tumors (i.e., head & neck, tongue, hepatocellular, breast and gastric carcinomas) is associated with large tumors [64–68] (Table 1). Likewise, heparanase over-expression enhanced [54, 69–72], while local delivery of anti-heparanase siRNA inhibited [55] the progression of tumor xenografts, altogether implying that heparanase function is not limited to tumor metastasis but is also engaged in accelerated vascularization and growth of the primary lesion [54] (Table 1). Evidence indicates that heparanase not only assists in the breakdown of ECM but also is involved in regulating the bioavailability and activity of growth factors and cytokines. Briefly, as discussed above, various heparin-binding growth factors are sequestered by HS in the ECM, providing a localized, readily accessible depot, protected from proteolytic degradation [6, 73], yet available to activate cells after being released by heparanase. Local release and activation of tissue-specific growth factors is clearly involved in creating a favorable 'soil' for growth of the primary tumor as well as in dictating the organ selectivity of metastasis.

Other ECM-Degrading Enzymes

Tumor cell invasion and spread through the blood and lymphatics is the hallmark of malignant disease and the greatest impediment to cancer cure. Metastasis is a multistage process that requires cancer cells to escape from the primary tumor, survive in the circulation, seed at distant sites and grow. Each of these processes involves rate-limiting steps that are influenced by the malignant and non-malignant cells of the tumor microenvironment [74–76]. Numerous studies have shown that metastases formation depends on the ability of tumor cells to invade blood vessel

Table 1 Correlation between heparanase expression and key clinical parameters in human cancer

Author (Ref.)	Carcinoma	Patient number	% Positive	Positive correlation with			
Gohji et al., 2001 [197]	Bladder	40	85 (17/20)	[a]MVD		Metastasis	Survival
Gohji et al., 2001 [198]	Bladder	67	48 (32/67)			Metastasis	
Maxhimer et al., 2002 [66]	Breast	53	36 (19/53)		Tumor size		
Shinyo et al., 2003 [199]	Cervical	92	49 (45/92)	MVD			Survival
Friedman et al., 2000 [178]	Colon	17	100				
Nobuhisa et al., 2005 [200]	Colon	54	69 (37/54)			Metastasis	Survival
Sato et al., 2004 [201]	Colorectal	130	25 (33/130)	MVD		Metastasis	Survival
Watanabe et al., 2003 [202]	Endometrial	40	50 (20/40)	MVD			
Endo et al., 2001 [203]	Gastric	63	49 (31/63)			Metastasis	
Tang et al., 2002 [68]	Gastric	116	83 (96/116)		Tumor size	Metastasis	Survival
Takaoka et al., 2003 [204]	Gastric	44	80 (35/44)			Metastasis	Survival
Doweck et al., 2006 [64]	Head & Neck	74	86 (64/74)		Tumor size		Survival
El-Assal et al., 2001 [65]	Hepatocellular	55	47 (26/55)	MVD	Tumor size		
Takahasi et al., 2004 [205]	Lung	76				Metastasis	Survival
Cohen et al., 2008 [206]	Lung	114	75 (85/114)			Metastsis	Survival
Kelly et al., 2003 [207]	Multiple Myloma	100	86 (86/100)	MVD			
Bar-Sela et al., 2006 [208]	Nasopharyngeal	46	35 (16/46)				Survival
Zheng et al., 2009 [209]	Neuroblastoma	42	62 (26/42)				Survival
Koliopanos et al., 2001 [146]	Pancreatic	33	75 (25/33)				Survival
Kim et al., 2002 [210]	Pancreatic	89	78 (69/89)				Survival
Rohloff et al., 2002 [148]	Pancreatic	50	76 (38/50)			Metastasis	Survival
Mikami et al., 2008 [211]	Renal	70				Metastasis	Survival
Ben-Izhak et al., 2006 [212]	[b]Salivary gland	60	70 (42/60)				Survival
Shafat et al., 2010 [58]	Ewing's Sarcoma	69	100		Tumor size		
Nagler et al., 2007 [67]	Tongue	60	92 (55/60)		Tumor size		Survival

% Positive = Number of cases positively stained with anti-heparanase antibodies

[a] *MVD* Microvascular density

[b] Exemplified in Fig. 2

walls and tissue barriers in a process involving enzymes capable of digesting ECM components. Attention focused on serine (i.e., plasminogen activators) and cysteine (i.e., cathepsins) proteases as well as matrix metalloproteinases (MMPs) [77]. These enzymes, whose substrates include major components of the ECM are often upregulated in metastatic cancers [77, 78]. It was originally thought that their role was simply to break down tissue barriers, enabling tumor cells to invade through stroma and blood vessels at primary and secondary sites. Subsequent studies revealed that MMPs also participate in angiogenesis [79] and their substrate repertoire goes far beyond ECM elements [77, 80, 81]. While MMPs attracted much attention, other proteases constitute the tumor milieu. A large family of proteases consists of cysteine proteases named cathepsins. Like MMPs, some cathepsins are often upregulated in cancer and, once secreted or localized to the cell surface, can degrade components of the ECM [82–84]. In addition, as discussed above, cathepsin L is held responsible for the processing and activation of heparanase, found to be strongly implicated in cell dissemination associated with tumor metastasis, angiogenesis, and inflammation.

Notably, proteases can contribute to the sustained growth of established tumor foci by cleavage of the ectodomain of membrane-bound pro-forms of growth factors, releasing peptides that are mitogens for tumor cells and/or vascular endothelial cells [77]. On the other hand, some ECM degradation products (i.e., tumstatin, endostatin) suppress endothelial cell proliferation and thereby inhibit tumor angiogenesis [85], further emphasizing the significance of the tumor microenvironment in the control of cell growth and function. Moreover, some ECM-degrading enzymes (i.e., cathepsins, plasminogen activators, MMPs, heparanase) are also engaged in multiple signaling pathways, primarily by means of non-enzymatic activities that affect both the tumor cells and the tumor microenvironment [40, 77]. Both normal and tumor cells appear to use the same set of ECM-degrading enzymes and molecular

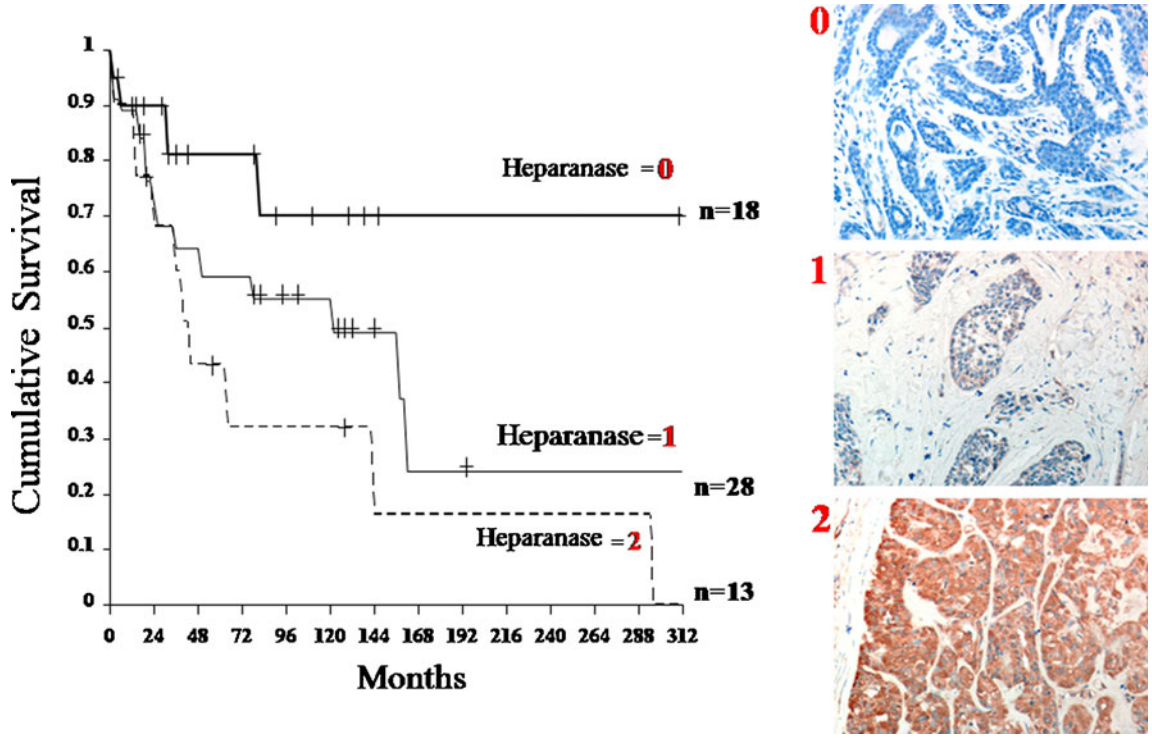

Fig. 2 Overall survival curves of patients with salivary gland tumors according to heparanase immunostaining levels. *Right*: Immunohistochemical staining of heparanase in patients with salivary gland tumors. Formalin-fixed paraffin-embedded sections of salivary gland tumors were subjected to immunostaining of heparanase, applying anti-heparanase pAb 733. Staining was graded as 0 (negative), 1 (weak) and 2 (strong). *Left*: Kaplan-Meier analysis showed poor survival of patients with positive (score 1 & 2) heparanase expression, compared with patients who were diagnosed as heparanase-negative (score 0). After 300 months (25 years) of follow-up, 0% of heparanase-positive patients survived compared with 70% of patients with no detectable heparanase expression. [Reproduced from Ben-Izhak et al., (2006) *Neoplasia* **8**, 879–884]

machinery to perform their biological functions. Thus, the normal physiological functions of proteases and heparanase in embryonic morphogenesis, wound healing, tissue repair and inflammation have been effectively 'hijacked' by tumor cells.

Heparanase-Protease Cooperation

A cross-talk between heparanase and MMPs has been demonstrated. Thus, enhanced expression of heparanase leads to increased levels of MMP-9, while heparanase gene silencing resulted in reduced MMP-9 activity [86]. Moreover, not only MMP-9 but also urokinase-type plasminogen activator (uPA) and its receptor (uPAR), molecular determinants responsible for MMP-9 activation, are up-regulated by heparanase [86]. These findings provided the first evidence for cooperation between heparanase and MMPs in regulating HSPGs on the cell surface and likely in the ECM, and are supported by our generation and characterization of heparanase knockout (KO) mice. Despite the complete lack of heparanase gene expression and enzymatic activity, heparanase-KO mice develop normally, are fertile, and exhibit no apparent anatomical or functional abnormalities [87]. Notably, heparanase deficiency was accompanied by a marked elevation of MMP family members such as MMP-2, MMP-9, and MMP-14, in an organ-dependent manner, suggesting that MMPs provide tissue-specific compensation for heparanase deficiency [87]. These and other results [86] suggest that heparanase is intimately engaged in the regulation of gene transcription and acts as a master regulator of protease expression, mediating gene induction or repression depending on the biological setting. These effects of heparanase are attributed to its nuclear uptake, degradation of nuclear HS and the associated activation of histone deacetylase (HDAC) activity [88].

Inflammation–A Major Determinant of the Tumor Microenvironment and Cancer Progression

According to Paget's 'seed and soil' hypothesis [89], metastatic spread cannot simply be explained by blood vessels anatomy, but is rather directed in a specific manner toward a 'congenital soil' [89]. The 'seed and soil' hypothesis is now widely accepted [90], and the terms

'seed' and 'soil' have been characterized to some extent at the cellular and molecular levels. The 'soil' is defined as biologically unique microenvironment that enables the 'seed' (metastatic cancer cell) to strike roots, germinate, and grow (i.e., proliferate). The 'congenital soil' can be made, for example, of endothelial cells expressing specific adhesion molecules in different vascular beds [91], or of tumor cells expressing receptors that respond to specific ligands, leading to their traffic and arrest in a specific tissue. The CXCR4 receptor, for example, plays a role in the bone marrow, lymph nodes, and pulmonary homing of metastases from breast tumor that expresses its ligand, CXCL12 (SDF-1) [90, 92]. In addition, emerging evidence indicates that bone marrow-derived hematopoietic progenitor cells positive for vascular endothelial growth factor receptor (VEGFR) arrive at a distant organ, alter the tissue microenvironment and promote the recruitment of metastatic tumor cells [92, 93]. Accordingly, treatment with neutralizing VEGFR during the early phases of primary tumor development or following the formation of the premetastatic niche inhibits metastasis [94].

While the 'soil' is still insufficiently characterized, the 'seed' (i.e., metastatic cancer cell) has been studied in far greater detail. It is generally accepted that in addition to the dynamic adhesive and motility capabilities intrinsic to tumor cells, tumors are supported continuously by host (stromal) cells which are recruited to the neoplastic lesion and affect all stages of tumor progression and metastasis. The tumor microenvironment includes epithelial cells, fibroblasts, endothelial cells, infiltrated leukocytes, and bone marrow-derived (stem) cells of the above lineages. Endothelial cells lining blood and lymph vessels are major component of the tumor microenvironment, and anti-angiogenesis therapy, targeting vascular endothelial growth factor (VEGF) or its receptor (VEGFR) is implemented clinically [95]. Cancer-associated fibroblasts (CAF) comprise another important component of the tumor microenvironment [96, 97]. CAF are phenotypically and functionally distinct from their normal counterparts in their increased rate of proliferation and expression of ECM components and growth factors [98–100]. By targeting HPV early region that includes the E6/E7 oncogenes to the skin tissue (K14-HPV16), it was demonstrated that CAF are pro-inflammatory and enhance tumor growth, angiogenesis, and recruitment of macrophages [101]. Thus, CAF functionally link major components of the tumor microenvironment (macrophages, endothelial cells) in a cross-talk that enhances tumor development and cell dissemination.

The last decade critically revealed the decisive role of inflammatory responses in different stages of tumor development and metastasis [102]. The most frequently found immune cells within the tumor microenvironment are tumor-associated macrophages [102, 103]. Notably, high density of these cells correlates with poor prognosis, while removal of macrophages almost completely ablated metastasis in a mouse of breast cancer [103–106]. In a recent study (discussed in detail below) we have demonstrated the importance of mucosal macrophages and heparanase in sustaining immune-epithelial crosstalk underlying colitis-associated colon tumorigenesis [16].

Heparanase and Heparan Sulfate in Inflammation

Upregulation of heparanase was reported in different inflammatory conditions, often associated with degradation of HS and release of chemokines anchored within the ECM network and cell surfaces. Moreover, remodelling of the ECM facilitates transmigration of inflammatory cells towards the injury sites. Prior to cloning of the heparanase gene, heparanase activity originating in activated cells of the immune system (T-lymphocytes, neutrophils) has been found to contribute to their ability to penetrate blood vessels and accumulate in target organs [43]. In a more recent study we have demonstrated that up-regulation of heparanase, locally expressed (i.e., by vascular endothelium, skin keratinocytes) at the site of inflammation, is an essential step of delayed type hypersensitivity [107]. Degradation of HS in the subendothelial basement membrane resulted in vascular leakage, a hallmark of delayed type hypersensitivity skin reactions [107]. Upregulation of heparanase has also been found in colonic epithelium of patients with inflammatory bowel disease (IBD) [16, 108] (Fig. 3), and in skin lesions of psoriasis patients (our unpublished results). Notably, heparanase staining was primarily detected in epithelial rather than immune cells, altogether indicating that immunocytes are not the primary source of the enzyme in inflammation. Heparanase activity was also found to be dramatically elevated in sinovial fluid from rheumatoid arthritis patients [17], suggesting an important role for heparanase in promoting joint destruction and indicating heparanase as an attractive target for the treatment of rheumatoid arthritis [17].

Heparanase Powers a Chronic Inflammatory Circuit that Promotes Colitis-Associated Tumorigenesis

The most feared long-term complication of IBD (in particular, UC) is colon carcinoma, as patients with ulcerative colitis (UC) have a risk of colorectal cancer which is an order of magnitude higher than the normal population [109]. In fact, colon carcinoma represents a paradigm for the association between inflammation and cancer [110]. While significant progress has been made in deciphering the role of inflammatory cytokines (TNFα,

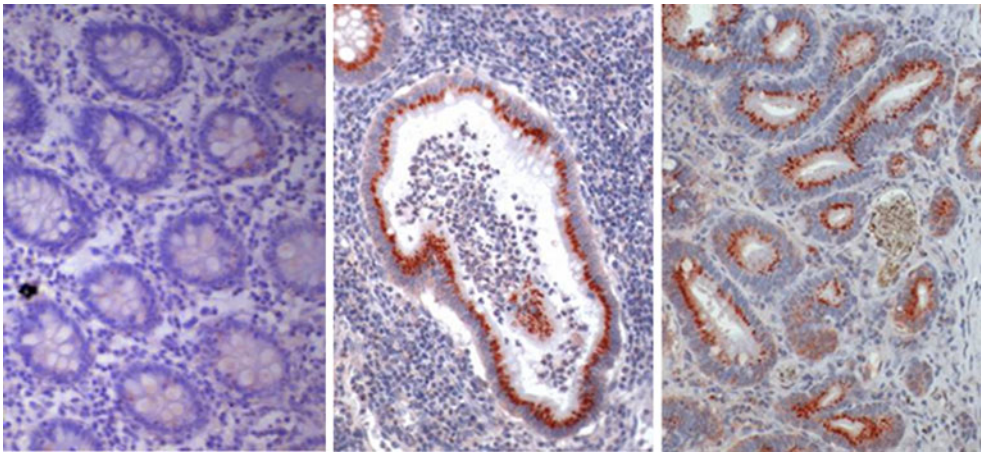

Fig. 3 Heparanase expression in acute and chronic phases of UC. Tissue specimens derived from normal colon tissue (*left*), UC patients in acute (*middle*) and chronic (*right*) phases of the disease were stained with anti-heparanase antibody (reddish staining). Photographs are representative of control (n=29) and UC (n=10) samples (original magnification, ×200). [Reproduced from Lerner et al., (2011) *J Clin Invest* 121:1709–1721]

IL-1β, IL-6) and their downstream transcription factors (NFκB, STAT3) in tumor-stimulating cross-talk between immune and epithelial cells [110–112], little is known about the role of ECM-degrading enzymes in this cross-talk. Based on the preferential expression of heparanase in chronically inflamed colonic epithelium (Fig. 3) and increased incidence of colon cancer in colitis patients, we hypothesized that stimulation of heparanase expression plays an important role in the pathogenesis of UC, representing a mechanistic link between inflammation and cancer. Utilizing UC tissue specimens, along with a mouse model of colitis-associated cancer induced by the carcinogen azoxymethane (AOM), followed by the inflammatory agent dextran sodium sulfate (DSS) [113], we found that heparanase is constantly overexpressed by the colonic epithelium in UC and experimental colitis during both the acute and chronic phases of the disease. Moreover, heparanase over-expression preserves a chronic inflammatory conditions in DSS colitis and thus creates a tumor-promoting microenvironment characterized by enhanced NF-κB signaling [111, 114], STAT 3 induction [115], and increased vascularization. Furthermore, we identified a novel biological mechanism contributing to chronic colitis and the associated colon tumorigenesis. This mechanism involves a self-sustained cycle through which heparanase of epithelial origin, acting synergistically with the local flora and cytokine milieu, facilitates abnormal activation of innate immune cells (i.e., macrophages) which, in turn, stimulate further production of the enzyme by the colonic epithelium. Moreover, in chronic colitis activated macrophages represent a primary source of cathepsin L responsible for proteolytic activation of latent heparanase [16]. A detailed analysis of the functional importance of heparanase in modulating inflammatory responses and sustaining immune-epithelial crosstalk underlying the pathogenesis of chronic colitis-associated tumorigenesis, is presented below.

Immunohistochemical staining revealed that while heparanase expression was practically not detected in healthy colon epithelium, in UC specimens the epithelial lining showed a marked expression of heparanase in both the acute (Fig. 3, middle) and chronic (Fig. 3, right) phases of the disease. In all phases, inflammatory cells in the involved areas, showed little or no heparanase expression. We investigated the tempo-spatial pattern of heparanase expression in experimental DSS-induced colitis. DSS causes an acute inflammatory reaction and ulceration in the colon [116] and when three cycles of DSS administration are applied, acute inflammation is followed by chronic colitis [116]. Moreover, three cycles of DSS subsequent to a single pretreatment with the chemical carcinogen AOM result in development of colon tumors in ~100% of the treated mice [113], representing a well established model of colitis-associated cancer. A marked increase in heparanase mRNA, protein and enzymatic activity was readily detected in the colon during all phases of DSS-induced colitis. In accordance, increased levels of cathepsin L mRNA and protein were detected in the mouse colonic tissue at corresponding time points [16].

Heparanase Over-Expression Preserves a Chronic Inflammatory Condition and Increases the Incidence and Severity of Colitis-Associated Tumors

Unlike ulcerative colitis (UC) patients, in which increased heparanase expression is constantly preserved (Fig. 3), in DSS colitis, heparanase levels gradually decreased during the chronic phase. Thus, to assess the precise role of heparanase in colitis-related tumorigenesis, we utilized heparanase transgenic (*Hpa*-tg) mice previously shown to express elevated levels of heparanase in colonic epithelium, but not in splenocytes [117], closely resembling heparanase expression pattern in UC patients. Applying the AOM-DSS protocol, we noted a marked increase in tumor incidence, vascularization and severity

in *Hpa*-tg mice vs. their *wt* littermates. Moreover, when three DSS cycles were administered alone, without AOM pretreatment, none of the *wt* mice developed colonic tumors, whereas in 90% of the *Hpa*-tg mice colonic tumors were easily seen, emphasizing the essential role of heparanase in this experimental system [16].

In contrast to the acute phase, heparanase over-expression profoundly affected the chronic phase of DSS-induced colitis, as demonstrated by microscopic and biochemical analyses of inflammatory phenotypes expressed by *Hpa*-tg vs. *wt* mice on experimental day 80 (1 month after cessation of the last DSS cycle). Altogether, our results indicate that the constantly elevated levels of heparanase in *Hpa*-tg mice (resembling the observations in UC patients, Fig. 3) markedly affect the **chronic** phase of DSS colitis and create a tumor-promoting inflammatory microenvironment primarily via increased vascularization, enhanced NF-κB signaling, augmented levels of TNFα, and STAT 3 activation [16].

Macrophages Represent Both a Cellular Target for Heparanase Action and Regulators of Heparanase Expression/Activity in Chronic Colitis

Macrophages are known to have a dual role in inflammation. In the scenario of inflammation resolution, macrophages perform phagocytosis and produce anti-inflammatory cytokines, thereby preventing inflammatory responses from lasting too long. However, if inflammation resolution is deregulated, macrophage response switches to the pattern of chronic inflammation. Recruitment and activation of macrophages within the intestinal mucosa play a key role in the pathogenesis of both human UC [118, 119] and murine DSS colitis [120, 121]. Moreover, activated macrophages are candidate cells linking between inflammation and cancer [106, 122]. Notably, the tumor promoting cytokines IL-1, IL-6 and TNFα [111, 112] are produced by activated macrophages and, along with macrophage-derived growth factors and reactive oxygen species, foster tumor initiation [119, 122]. This notion led us to examine the involvement of macrophages in our system. In fact, we detected increased macrophage infiltration in the colon of *Hpa*-tg vs. *wt* mice on day 80 of the chronic DSS colitis model [16]. Macrophages infiltrating the *Hpa*-tg colon manifested a striking pro-inflammatory profile, evidenced by increased number of TNFα expressing macrophages and elevated NFκB signaling. These findings led us to assume that heparanase over-expression directly affects macrophage activation. To recapitulate conditions occurring in UC (i.e., heparanase-rich environment and abundant microbial flora), we isolated mouse peritoneal macrophages and stimulated them with lipopolysacharide (LPS) in the absence or presence of recombinant active heparanase. Pretreatment with heparanase strongly sensitized macrophages to activation by LPS, as indicated by a marked increase in TNFα, IL-6 and IL-12p35 [16], all macrophage-derived cytokines known to be induced by TLR-4 signaling and tightly involved in the pathogenesis of UC [119]. These findings are in agreement with previous reports showing that intact extracellular HS inhibits LPS-mediated TLR4 signaling and macrophage activation, and that its removal relieves this inhibition [123]. Given that one of the unique aspects of colorectal cancer development is the involvement of lumenal flora and TLR signaling [124, 125], the observed ability of heparanase to sensitize macrophages to LPS activation is of particular significance in light of the increased epithelial permeability to lumenal microbial products, characteristic of UC [126]. Altogether, the above data suggest that up-regulated heparanase enables enhanced activation of macrophages, reprogramming their response from resolution of inflammation to unresolved chronic colitis.

On the other hand, we found that activated macrophages are capable of inducing heparanase expression in colonic epithelial cells, most likely through TNFα-mediated stimulation of Egr1, a powerful inducer of heparanase transcription in colonic tumor cells [127] and DSS colitis [16]. Notably, TNFα was shown to stimulate Early growth response 1 (Egr1 transcription factor regulating heparanase promoter activity) expression in IBD patients [128]. In addition, due to their unique ability to secrete mature cathepsin L and allow extracellular accumulation of the active enzyme [129], activated macrophages appear to be responsible for proteolytic activation of latent proheparanase in colitis. Thus, macrophages not only represent a cellular target for heparanase action, but also decisively upregulate heparanase in chronic colitis, both at the transcriptional and posttranslational levels. These results demonstrate and highlight the cooperation between two cellular compartments (i.e., colon epithelium and activated macrophages) in heparanase activation during inflammation-associated colon carcinogenesis. Collectively, our results [16] indicate that heparanase generates a self-sustaining connection between chronic colitis and tumorigenesis (Fig. 4). Briefly, macrophages activated by influx of the luminal flora secrete TNFα and stimulate production of heparanase by the colon epithelium. The secreted 65 kDa latent heparanase is processed into its active form by cathepsin L, supplied by the activated macrophages. Enzymatically-active heparanase sensitizes macrophages to further activation by microbial flora, thus preventing inflammation resolution, switching macrophage responses to the chronic inflammation pattern and creating tumor-inducing inflammatory environment [16]. In addition, high heparanase levels support tumor progression via stimulation of angiogenesis, release of ECM-bound growth factors and

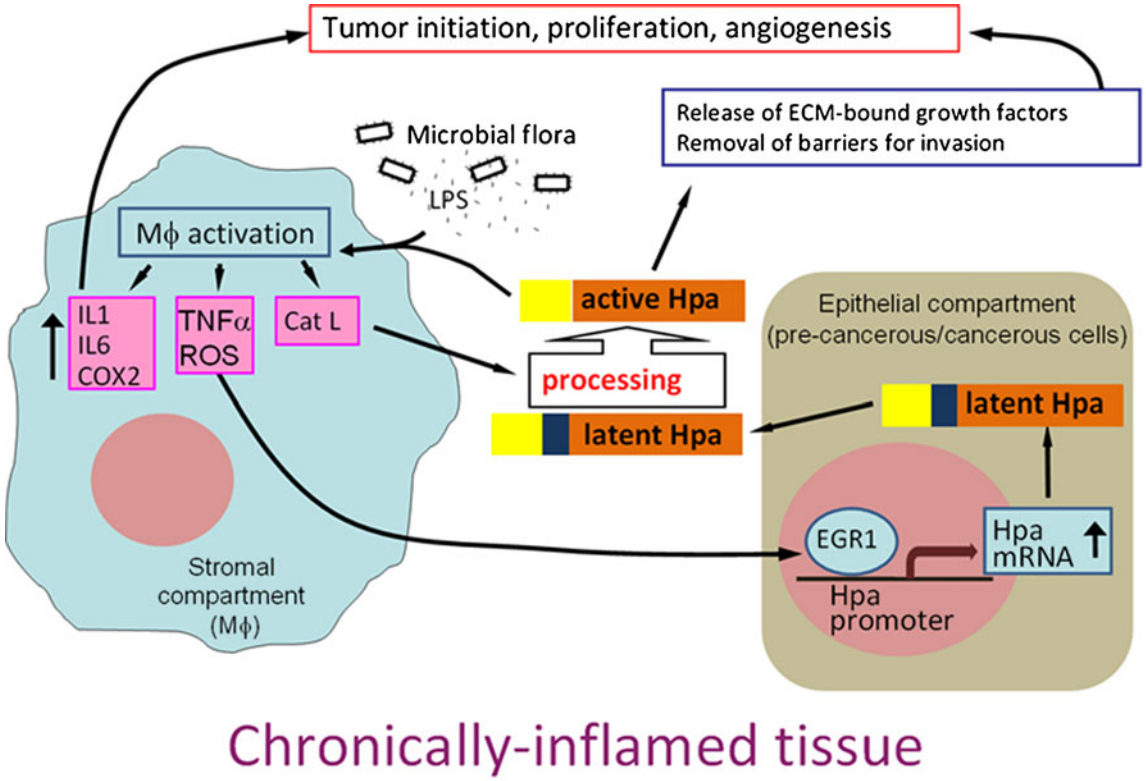

Fig. 4 A model of heparanase-driven vicious cycle that may power chronic inflammation and the associated tumorigenesis. Increased levels of TNFα secreted by activated macrophages induce heparanase expression in the epithelial compartment via Egr1-dependent mechanism. Secreted latent heparanase is processed into its active form by cathepsin (supplied by the macrophages), and in turn sensitizes macrophages to further activation (i.e., in case of colitis by luminal flora) thus preventing inflammation resolution, switching macrophage responses to the chronic inflammation pattern and creating tumor-inducing inflammatory environment. In addition, heparanase promotes tumor progression via stimulation of angiogenesis, release of ECM-bound growth factors and bioactive HS fragments, and removal of extracellular barriers for invasion. [Reproduced in part from Lerner et al., (2011) *J Clin Invest* 121:1709–1721]

bioactive HS fragments and removal of extracellular barriers for invasion (Fig. 4). Importantntly, as presented in Fig. 4, the above described self-sustaining tumor promoting inflammatory circuit may occur in other organs where inflammation plays a significant role in tumorigenesis and preferential expression of heparanase has been reported during cancer progression (Fig. 4).

The newly identified heparanase-powered vicious cycle may explain a yet poorly understood "multiplier effect" in IBD inflammation, in which even a small initial elevation in 'initiating' inflammatory stimuli gives rise to large increases in downstream cytokines [130]. Thus, disruption of the heparanase-driven chronic inflammatory circuit is highly relevant to the design of therapeutic interventions in colitis and the associated cancer.

Role of Heparanase in Radiation Enhanced Invasiveness of Pancreatic Carcinoma

Pancreatic cancer is one of the most aggressive neoplasm with an extremely low 5-year survival rate [131–133]. Currently, pancreaticoduodenectomy is the only curative form of treatment, however ~90% of pancreatic cancer patients miss the opportunity for complete surgical resection at the time of diagnosis [131, 134]. Thus, radiotherapy remains a major component of treatment modalities for controlling pancreatic tumor progression [135]. However, pancreatic cancer often shows resistance to ionoizing radiation (IR), and randomized trials could not demonstrate benefit from radiation, revealing rather conflicting results [136, 137]. Accumulating preclinical and clinical data suggest that IR may stimulate tumor aggressiveness [138–144], although the identity of downstream effectors acting at the cell or tissue levels and responsible for this effect, remains poorly investigated. Our recent results indicate that IR augments heparanase expression and thereby aggressiveness of pancreatic carcinoma both in vitro and in vivo [145]. Causal involvement of heparanase in pancreatic carcinoma progression is well-documented. There was a 30-fold increase in heparanase mRNA in pancreatic cancer tissue samples, in comparison to normal pancreatic tissue [146]. Moreover, elevated levels of the enzyme have been found in body fluids of patients with active pancreatic

cancer disease [147] as compared to healthy donors. Pancreatic cancer patients whose tumors exhibit high levels of the heparanase mRNA had a significantly shorter postoperative survival time than patients whose tumors contained relatively low levels of heparanase [146, 148]. A recent finding that heparanase positivity is a highly significant independent variable for pancreatic adenocarcinoma dedifferentiation and lymph node metastasis further demonstrate a crucial role of the enzyme in the aggressiveness of pancreatic cancer [149].

We have demonstrated that clinically relevant doses of IR augment invasive ability of pancreatic carcinoma cells in vitro and in vivo through upregulation of heparanase expression, and revealed that the molecular mechanism responsible for IR-induced heparanase transcription involves the Early growth response 1 (Egr1) transcription factor [145]. As a transcriptional regulator, Egr1 can both induce and repress the expression of its target genes, including heparanase [127, 150, 151]. Egr1 was previously shown to activate heparanase expression in T lymphocytes, prostate, breast, and colon carcinomas, but to inhibit its transcription in melanoma cells [127]. Transactivation studies using Egr1 expression vector, co-transfected with a reporter construct encoding for LUC under the heparanase promoter, showed that in pancreatic carcinoma cells Egr1 acts to repress heparanase transcription. In fact, IR treatment of PANC1 pancreatic carcinoma cells resulted initially in a transient increase in Egr1, followed by profound and continuous decline in Egr1 levels, as compared to its basal levels in untreated PANC1 cells [145]. Moreover, ChIP analysis revealed a marked decrease in occupancy of the heparanase promoter by Egr1 following IR treatment. We have found that pancreatic carcinoma cells express high basal levels of Egr1 and of the NGFI-A/Egr1-binding protein NAB2. NAB2 is a transcriptional co-repressor that directly interacts with Egr1 and represses activation of its target promoters [152]. It is therefore plausible that in the presence of both Egr1 and NAB2 the heparanase promoter is repressed in pancreatic cells. However, following IR-associated temporal decrease in Egr1, this repression is no longer operative, allowing for activation of the heparanase promoter.

Combination of radiotherapy with drugs that inhibit IR-induced tumor aggressiveness may be an attractive strategy to diminish adverse pro-metastatic action while retaining the therapeutic benefit of radiation, thus reducing resistance of pancreatic cancer to treatment. We have demonstrated that compound SST0001 (Sigma-Tau Research Switzerland, SA), a specific potent heparin-based inhibitor of heparanase enzymatic activity which lacks anti-coagulant activity [59, 153–155], attenuated radiation-induced invasiveness in vitro [145] in an orthotopic model of pancreatic cancer. Thus, spread of orthotopically growing pancreatic tumors was significantly reduced in mice treated with a combination of SST0001 and IR, as compared with either modality alone [145]. Taken together, our results support the combination of radiotherapy with a specific heparanase inhibitor as an effective strategy to prevent tumor resistance and progression, observed in many IR-treated pancreatic cancer patients. Notably, inducibility of heparanase by radiation appears not to be limited to pancreatic tissue, as it was recently demonstrated that heparanase is upregulated by IR in liver [156] and by ultraviolet B radiation in human skin [157].

Heparanase, a Promising Antigen for Tumor Immunotherapy

Heparanase expression at the early stages of tumor initiation and progression, and by the majority of tumor cells, can be utilized to turn the immune system against the very same cells. Accumulating evidence indicate that peptides derived from human heparanase can elicit a potent anti-tumor immune response, leading to lysis of heparanase-positive human gastric (KATO III), colon (SW480), and breast (MCF-7) carcinoma cells, as well as hepatoma (HepG2) and sarcoma (U-2 OS) cells [158–160]. In contrast, no killing effect was noted towards autologous lymphocytes [158–160]. Notably, the development of tumor xenografts produced by B16 melanoma cells was markedly reduced in mice immunized with peptides derived from mouse heparanase (i.e., aa 398–405; 519–526) compared to a control peptide in both immunoprotection and immunotherapy approaches [158, 161]. To increase the immunogenicity of these peptide vaccines, Wang et al. designed four-branched multiple antigenic peptides based on HLA-A2-restricted cytotoxic T lymphocyte (CTL) epitopes of human heparanase. These multiple antigenic peptides vaccines were capable of inducing HLA-A2-restricted and heparanase-specific CTL in vitro and in mice, exerting no effect on autologous lymphocytes and dendritic cells. Moreover, compared to their corresponding linear peptides, heparanase multiple antigenic peptide vaccines elicited a much stronger lysis of tumor cells by activating CD8+ T lymphocytes and increasing the release of IFN-γ. Given the broad spectrum, high effectiveness, high specificity and safety, it was concluded that multiple antigenic peptide vaccines based on CTL epitopes of human heparanase can be used as potent immunogens for tumor immunotherapy [161].

Beckhove et al. elucidated the role of the bone marrow as an important organ for the priming and memory formation of T cell mediated anti-tumor immune response [162–165]. Upon reactivation in vitro and adoptive transfer, tumor antigen-reactive bone marrow memory T cells from

tumour patients infiltrated autologous tumors and mediated their complete rejections in xenotransplant mouse models, suggesting their therapeutic potential [162, 163, 166, 167]. These results demonstrate a selective homing of memory T cells from the bone marrow into human tumors suggesting that tumor rejection is based on the recognition of tumor-associated antigens on tumor cells and dendritic cells by specifically activated, central and effector memory T cells [162, 168]. Moreover, it has been demonstrated in a clinical study that adoptive transfer of *ex vivo* reactivated pre-existing bone marrow memory T cells provides a therapeutic option for the treatment of advanced metastasized breast cancer patients [169]. Of high significance is the evaluation of tumour antigens that are major target structures of spontaneous effector and regulatory T cell responses since the repertoire of such pre-existing tumour specific T cells may have a major influence on the immunological and clinical efficiency of immunotherapeutic strategies. Importantly, HLA-I restricted epitopes from heparanase were characterized as target antigens of spontaneous CD8 T cell responses in breast cancer patients [170]. Briefly, high levels of heparanase specific T lymphocytes were identified in breast cancer patients using heparanase peptide-MHC class I tetramers. Moreover, in a high proportion of these patients, memory T cell responses to heparanase derived HLA-A2 restricted peptides, were demonstrated, leading to generation of anti-tumor cytotoxic T lymphocytes. Thus, heparanase emerged as a new metastasis-associated antigen recognized in breast cancer patients by spontaneously induced memory T lymphocytes [170], indicating that heparanase is an attractive tumor-associated antigen in cancer patients. In addition, the immune system appears capable of sensing ECM degradation and responding to heparan sulfate degradation fragments released by heparanase from the ECM. Thus, heparanase activity might directly activate antigen presenting cells and allow for tumor antigen specific T cell responses in the absence of foreign inflammatory stimuli – a fundamental new mechanism for induction of anti tumor immune responses. Applying newly synthesized long peptides of heparanase that can be processed by dendritic cells, heparanase ranked among the most frequently recognized tumour antigens in patients with pancreatic, colorectal or breast cancer [171]. Together with its selective expression on a small subpopulation of highly malignant metastasizing tumour cells, heparanase provides a unique opportunity for a tumour type independent, metastasis-selective tumour immunotherapy. Taken together, heparanase appears to represent a highly promising target antigen for immunotherapeutic approaches against a broad variety of tumours. While causing the generation of high frequencies of specific CD4 and CD8 memory T cells, heparanase, in contrast to most other tumour antigens (i.e., CEA or MUC1), did not induce spontaneous regulatory T cell responses [171]. Anti-heparanase immunotherapy is thus expected to be prolonged and more efficient due to the absence of T-suppressor cells. These results provide a basis for a vaccination treatment approach that is currently being tested in advanced metastasized breast cancer patients [172]. In fact, studies are underway (Beckhove et al., DKFZ, Heidelberg) to characterize the most promising heparanase-derived long peptides and generate these for clinical use in an immunotherapeutic trial of adoptive T cell transfer of heparanase-reactive, *ex vivo* activated T cells.

Conclusions and Perspective

Ten years following its cloning, the repertoire of heparanase functions is only starting to be revealed. From activity mainly implicated in cell invasion associated with tumor metastasis, heparanase has turned into a multifaceted protein that appears to participate in essentially all major aspects of tumor progression [39, 48, 173]. Genetic tools (i.e., heparanase knock-out and over-expressing mice [87, 117]) are now available and being utilized to examine the possibility that heparanase is not only intrinsic to tumor cell invasion and angiogenesis, but also modifies, enzymatically and/or by virtue of its pro-adhesion function [173, 174], the site of cell seeding and hence participates in establishing a 'congenital soil' for successful homing and colonization of disseminated tumor cells.

Evidence now supports a concept by which growth of the primary tumor is fueled by circulating metastatic tumor cells [175, 176]. According to this notion, tumor cells are present in the circulation in large numbers even at the early stages of cancer and long before metastatic growth at distant sites can be detected [176]. These cells can reinfiltrate and promote growth and angiogenesis of the primary tumor [175]. The possible involvement of heparanase in tumor self-seeding is supported by the timing of its induction during tumorigenesis and its pro-metastatic function. Using the RIP-Tag2 tumor model, it was demonstrated that heparanase mRNA and protein are elevated upon the transition from normal to angiogenic islets, followed by a further increase when solid tumors were detected [177]. Furthermore, heparanase expression is elevated already at the early stages of human neoplasia. In the colon, heparanase gene and protein are expressed already at the stage of adenoma [178], and during esophageal carcinogenesis heparanase expression is induced in Barrett's epithelium, an early event that predisposes patients to formation of dysplasia which may progress to adenocarcinoma [179]. Tumor self-seeding also facilitates the recruitment of stromal components. The possible involvement of heparanase in this aspect is supported by its pro-angiogenic and pro-inflammatory effects.

There is growing evidence that heparanase upregulates expression of genes that participate in creating aggressive behavior of tumors. These include VEGF, MMP-9, uPA/uPAR and tissue factor and likely other effectors that condition the tumor microenvironment to promote an aggressive cancer phenotype [47, 86, 180]. Although HS has been associated with several functional roles within the nucleus, its inhibition of gene transcription via inhibition of topoisomerase I and histone H3 acetyltransferase (HAT) activity are particularly intriguing [181, 182]. HATs regulate gene expression by catalyzing acetylation of the N-terminal region of histones, thereby modifying chromatin structure in a manner that facilitates transcriptional activation. It was recently shown that both heparin and HS can act as potent inhibitors of p300 and pCAF HAT activities [181]. Notably, a marked reduction in the level of nuclear syndecan-1 was found following upregulation of heparanase in myeloma cells [88], possibly associated with nuclear localization of the enzyme [183–185]. This could lead to increased histone acetylation with an associated increase in gene transcription. In fact, heparanase-mediated loss of nuclear syndecan-1 enhances HAT activity to promote expression of genes that drive an aggressive tumor phenotype [186]. Heparanase regulation of gene expression may thus be related to its ability to inhibit accumulation of HSPGs within the nucleus. Hence, strategies to enhance nuclear HS levels may prove effective in blocking at least some of the heparanase-mediated effects that promote tumor growth and metastasis.

While most attention was addressed in recent years to heparanase function in tumor biology, emerging evidence indicate that heparanase is also engaged in several other pathological disorders. A most interesting example is the apparent role of heparanase in glomerular diseases [187, 188]. HSPGs are important constituent of the glomerular basement membrane and its permselective properties. Loss of HSPGs was observed in several experimental and human glomerulopathies, including diabetic nephropathy, minimal change disease, and membranous glomerulophathy [187]. In addition, expression of heparanase was up-regulated in the course of these diseases [189, 190], likely destructing the permselective properties of HS. Notably, PI-88 (a heparanase inhibitor) was effective as an antiproteinuric drug in experimental nephropathy model [191].

Upregulation of heparanase was reported in different inflammatory conditions such as inflammatory bowel disease [16], rheumatoid arthritis [17], and psoriasis (Lerner et al., our unpublished results), often associated with degradation of HS and release of chemokines anchored within the ECM network and cell surfaces. We have demonstrated that heparanase of epithelial origin induces abnormal activation of innate immune cells (i.e., macrophages) which, in turn, stimulate further production of the enzyme by the epithelial compartment, thereby preserving chronic inflammation and creating a tumor-promoting microenvironment [16]. Mechanistic features of this vicious cycle await further investigation.

Novel heparanase inhibitors such as non-anticoagulant glycol-split heparin (SST0001) [59, 192], PG545 [195] or rationally designed structure-based compounds are hoped to enter the clinic and relief patients' condition. Compound SST0001 effectively inhibited myeloma growth in vivo, even when confronted with an aggressively growing tumor within human bone [192]. SST0001 also diminishes heparanase-induced shedding of syndecan-1, known to be a potent promoter of myeloma growth, indicating that SST0001 inhibits myeloma growth and angiogenesis via disruption of the heparanase/syndecan-1 axis [192]. Notably, inhibition of cathepsin L by serpin was markedly augmented by heparin and HS [193], suggesting that compound SST0001 will not only inhibit heparanase activity but also heparanase processing through cathepsin L inhibition [194]. A highly promising compound is PG545, a synthetic, fully sulfated HS mimetic that has recently entered Phase I trials for advanced cancer [195]. PG545 exhibits preclinical anti-tumor and anti-metastatic efficacy with a pharmacokinetic profile that supports less frequent dosing compared with other HS mimetics [195]. Ectopic miR-1258 was recently reported to suppress heparanase expression and activity, resulting in inhibition of experimental breast cancer brain metastasis [196]. Clearly, heparanase-inhibiting compounds are broad acting, targeting both the tumor and the tumor microenvironment (i.e., inflammation, angiogenesis) [194].

Although much has been learned in the last decade, the repertoire of heparanase functions in health and disease is only starting to emerge. Clearly, from activity implicated mainly in cell invasion associated with tumor metastasis, heparanase has turned into a multifaceted protein that appears to participate in essentially all major aspects of tumor progression, inflammation and kidney dysfunction.

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Conflict of Interest The authors declare that they have no conflict of interest.

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