Chapter 36
The Good and Bad Sides of Heparanase-1 and Heparanase-2

Maria Aparecida Silva Pinhal, Carina Mucciolo Melo, and Helena Bonciani Nader

36.1 Extracellular Matrix: At the Crossroads of Cell-Cell and Cell-Microenvironment Relationships

The extracellular matrix (ECM) can be defined as the structure shared by all multicellular organisms, and it is composed of proteins and glycoconjugates that are synthesized and exported/secreted by the cells to the extracellular environment. The ECM is organized in a heterogeneous macromolecular network that does not only provide structural support, organization and tissue orientation (tissue biomechanical), but among other functions acts as substrate for cell growth, migration, proliferation, adhesion and differentiation and moreover, plays vital role in the various sensory crossroads of cell-cell and cell-environment interactions [1–5].

The ECM is composed of a wide variety of molecules, including glycosaminoglycan chains, which are generally covalently linked to a core protein giving rise to proteoglycans, fibrous structural proteins such as collagen and elastin; and fibrous adhesive proteins such as laminin and fibronectin. These components are organized into macromolecular networks that act in diverse cellular dynamics mentioned above. Such macromolecular glycoconjugates are the most abundant class of structurally diverse and heterogeneous molecules present in the ECM and cell surface, forming the so-called glycocalyx. Their diverse and heterogeneous structures are the result of the action of several glycosyltransferases, which are capable of polymerizing carbohydrate chains as well as other classes of enzymes such as sulfotransferases and epimerases, which alter their substitution pattern and stereochemistry at specific sites along the polymer. As a result, at the end of their biosynthesis, there will be structurally distinct functional chemical species.

M. A. S. Pinhal (✉) · C. M. Melo · H. B. Nader (✉)
Biochemistry Department, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil

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The ECM is at constant remodeling in response to various extracellular and intracellular stimuli, and the way such signals are transmitted, captured and interpreted, dictate and distinguish the fate of normal and pathological remodeling [3]. Hence, understanding and modulating the ECM information flux can deeply influence the development of new and improved therapeutic approaches of significance for life quality.

ECM, as previously mentioned, has a highly complex supramolecular structure, influencing the assembly, viability, and functions of cells and tissues. ECM components can influence multiple cell properties and functions directly or through its degradation products, being able to modify the cellular microenvironment and tissue function [6]. The epithelial-mesenchymal and epithelial-stromal interactions are critical in physiological and pathological processes, e.g., embryonic morphogenesis [7], tissue repair [8] and tumorigenesis [9], accompanied by dynamic changes and generating new cell-matrix interactions [10, 11]. The binding of cell surface receptors to ECM activates signal transduction pathways that regulate cell functions, including adhesion and migration [12, 13]. Several of these activities depend upon integrins, that are transmembrane glycoproteins composed of non-covalently linked heterodimers that may act at ECM receptors. Integrins require an activation process for interaction with ligands that may mediate reactions induced by their binding to ECM proteins [14–17].

Consequently, besides its structural role, ECM represents a microenvironment that can sequester growth factors and cytokines, which can facilitate rapid and localized changes in the activities of mediators in the absence of newly synthesized proteins. Moreover, the ECM plays an important role in cell-cell communication. Fibrillar and non-fibrillar components may limit or facilitate the transport of molecules across the extracellular space while regulating the interstitial hydrostatic pressure. Furthermore, extracellular matrix molecules are involved in cell signaling pathways, which are mediated by cell surface receptors. Once activated, this system can lead to ECM remodeling either by the production and activation of enzymes such as proteases and glucosidases or by de novo synthesis and structural modification of ECM components [1–3].

36.1.1 Glycosaminoglycans and Proteoglycans

Glycosaminoglycans (GAGs) are important constituents of both the ECM and cell surface. Apart from hyaluronic acid, all GAGs are present in tissues as proteoglycans, where the polysaccharide chains are covalently bound to a protein backbone. Heparan sulfate and heparin are composed of alternating units of D-glucosamine and uronic acid (β-D-glucuronic acid and α-L-iduronic acid), linked by α [1–4]-type glycosidic linkages. The glucosamine can be N-acetylated or N-sulfated and/or O-sulfated mainly at the C-6 position, and less at the C-3 position. Furthermore, the uronic acid moiety can be sulfated to various degrees at the C-2 position [18–21]. They participate in a variety of biological processes including cell-ECM interactions, cell growth, cell differentiation and malignant transformation due to their ability to bind and modulate key cell growth-related molecules, such as TGF-β (transforming

growth factor β), FGF (fibroblast growth factors), VEGF (vascular endothelial growth factor) and others [22–25].

Heparan sulfate proteoglycans (HSPGs) play an important role in cell-ECM interaction. Nearly all ECM molecules have heparan sulfate binding sites, suggesting that the balance between adhesion and cell motility rely on integrating PGs and integrin-mediated adhesion signals. HSPGs are composed of a protein backbone and one or more glycosaminoglycan chains of heparan sulfate (HS). There are subfamilies of HSPGs: transmembrane PGs (e.g., syndecans, betaglycan and CD44), PGs connected to the GPI anchor (e.g., glypicans), and PGs secreted into the ECM (e.g., agrin, several collagens, and perlecan) [26]. Syndecans are present at the cell surface and can also act as co-receptors along integrins by modulating interactions between the cytoskeleton and the ECM [25–27]. Syndecans can interact with a variety of ligands via HS chains. It is thought that these PGs are involved in vital cell functions, including cell proliferation, signaling, and recognition, as well as cell-matrix and cell-cell adhesion [28–30]. Numerous molecular interactions between heparan sulfate chains, growth factors, cytokines, and ECM molecules are known, associated in part with cell adhesion and migration mediated by integrins.

Modeling and remodeling of the matrix are driven by the local cellular milieu, including secreted and cell-associated components in a framework of dynamic reciprocity. The current collection of expertly-written chapters aims to relay state-of-the-art information concerning the mechanisms of matrix modeling and remodeling in normal physiology and disease. Even though there are many results showing that glycosaminoglycans from the extracellular matrix and cell surface play a fundamental role in controlling the proteolytic activity of several metalloproteases [31–34], as well as cysteine proteases, such as cathepsins [35–38], in this review we will focus on the role played by heparanase in the cell environment.

The interactions between cells and ECM are crucial for cell events such as growth, death, differentiation, and motility, which have importance in various biological processes such as morphogenesis, inflammation, immune response, parasitic and virus invasion, cell transformation and metastasis. The ECM composition is not static and changes in response to internal and external cell stimuli occur in order to maintain the metabolic equilibrium. Changes in ECM structure and organization are implicated in many pathobiological states, including cancer, neurodegenerative diseases, and fibrosis, among others [1–4, 39].

36.2 Heparanase: A Key Modulator of ECM Architecture at the Crossroads of Homeostasis and Diseases

36.2.1 General Aspects

Heparan sulfate chains are degraded by hydrolase, known as heparanase, which is an endo-β-D-glucuronidase that cleaves β-D-glucuronyl(1 → 4)D-N-acetylated glucosamine. Heparanase (HPSE, Hpa or Hpa1) is capable of cleaving heparan sulfate side chains of heparan sulfate proteoglycans on cell surfaces and the extracellular

matrix. As previously mentioned, heparan sulfate proteoglycans are crucial elements for normal cell physiology due to their strategic localization and interaction with ECM components, integrins, and the cytoskeleton. Heparanase, by altering heparan sulfate structure at the cell surface and ECM leads to a cascade of cellular events that affect a diversity of physiological processes, such as cell growth, adhesion, migration, and death. The repertoire of physiological and pathological activities of heparanase is growing steadily, being implicated in inflammation, neovascularization, and tumor development [40–42].

Heparanase-1 resides in the endosomal/lysosomal compartment for a relatively long time and is likely to play a role in the normal turnover of heparan sulfate [43]. Furthermore, heparanase secretion kinetics resembles that of cathepsin D, a known lysosomal enzyme, validating its lysosomal origin. Extracellular signals activate protein kinases signaling pathways leading, among other effects, to heparanase secretion [44]. Also, the uptake of heparanase is dependent on the presence of heparan sulfate proteoglycans (syndecans) at the cell surface [45].

At present, there are more than 1500 papers focusing on heparanase. Therefore, the purpose of this chapter is to give a general view of the putative biological roles of heparanase and its implications in normal homeostasis and disease situations. Heparanase-1, as an enzyme, plays a role in remodeling the ECM and basement membrane by degrading heparan sulfates and thus liberating heparan sulfate-bound proteins, such as cytokines and growth factors. On the other hand, heparanase also exhibits non-enzymatic activities due to its capacity to interact with membrane proteins such as tissue factor and tissue factor pathway inhibitor, thus playing a role in the coagulation cascade [46]. The identification of heparanase-1 in tumor cells and platelets, as well as characterization of its substrate specificity, type of uronic acid recognized by the enzyme, and inhibition by non-anticoagulant heparin molecules goes back to the ‘80s [47–56]. Other papers reported its activity and secretion by degranulating mast cells [57, 58], T and B lymphocytes, granulocytes, and macrophages [59, 60], suggesting a role for heparanase in vessel wall injuries, atherosclerosis, neovascularization, and immune responses.

36.2.2 Heparanase Favors Blood Coagulation

Heparanase-1 has been implicated in coagulation in a non-enzymatic manner. Heparanase overexpression in human leukemia, glioma, lung cancer, and breast carcinoma cells leads to increased levels of tissue factor (TF) and tissue factor pathway inhibitor (TFPI). Heparanase-1 was demonstrated to enhance tissue factor activity. Hence heparanase procoagulant activity in the plasma of patients with lung cancer reveals a new mechanism by which the coagulation system is activated in malignancy [46]. Additionally, the interaction of heparanase-1 with TFPI at the cell surface of endothelial and tumor cells, increases the coagulability due to dissociation of TFPI from the cell membrane, thus resulting in increased coagulation activity,

supporting a prothrombotic function of heparanase [61]. Peptides generated from tissue factor pathway inhibitor, which inhibit heparanase procoagulant activity, attenuate inflammation in a sepsis mouse model. Likewise, peptides inhibiting heparanase procoagulant activity significantly reduced tumor growth, vascularisation, and relapse. The procoagulant domain in heparanase-1 protein may thus play a role in tumor progression, suggesting a new mechanism for the involvement of the coagulation system in cancer [62].

Also, the ability of von Willebrand factor (VWF) to trap platelets contributes to inflammation, infection, and tumor progression. Overexpression of syndecan-1 (SDC-1) significantly supports the binding of VWF to endothelial cells. However, heparanase degradation of heparan sulfate chains or impaired synthesis of heparan sulfate, a major component of the endothelial glycocalyx, reduce platelets recruitment by VWF [63]. Therefore, the first step of hemostasis, platelet aggregation, appears to be dependent on heparanase modulation.

36.2.3 Heparanase and the Tumor Microenvironment

Increased expression of heparanase-1 seems to be a marker for various tumors [51, 64–67]. Furthermore, high levels of heparanase expression correlate with poor survival rates, as in gliomas, breast cancer, gastrointestinal tumors, and esophageal carcinomas [68–70]. The crosstalk between heparanase-1 and macrophages propitiates chronic inflammatory conditions creating a pro-tumorigenic microenvironment, as is the case for chronic inflammatory bowel disease and colon cancer, among others [42, 71]. Also, the protagonist of heparanase-1 in inflammation, neuronal disorders, and viral infection is becoming more evident [70, 72–79].

36.2.4 Exosomes

Exosomes are extracellular vesicles produced in the endosomal compartment of most eukaryotic cells and play a role in intercellular communication and signal transduction [80, 81]. Recently, the pathophysiological effects of exosomes on diseases, especially cancer, have emerged. Heparanase-1 enhances exosome secretion, alters its composition, and thereby promotes tumor progression [81–84].

Exosomes participate in multiple mechanisms that support tumorigenesis, such as ECM remodeling, angiogenesis, thrombosis, and tumor cell proliferation, being implicated in the promotion and establishment of a pro-tumorigenic metastatic niche due to their cargo, including oncoproteins and heparanase [82–84]. Using CAG cells (plasma cell myeloma), it has been shown that several exosome cargoes such as syndecan-1, VEGF and hepatocyte growth factor, are regulated by high levels of activated heparanase-1, reflecting in the spreading of tumor cells and invasion

of endothelial cells in vitro [84]. The biogenesis of a class of these vesicles depends on syntenin and syndecans [85]. Heparanase-1 acts as a regulator of the syndecan-syntenin-exosome biogenesis pathway, and the upregulation of both syntenin and syndecan has been demonstrated in cancer [86–88]. Syndecan heparan sulfate proteoglycans were found to control exosome biogenesis and endosomal-sorting complex through syntenin-1 and ALIX [87, 88]. The fact that both syntenin and heparanase are upregulated in tumors favors the proposed role of exosomes in carcinogenesis. Recent data show that anti-myeloma drugs used in the treatment of myeloma upregulate heparanase through the nuclear factor-kappa B (NF-κB) signaling pathway [89]. Additional studies demonstrated increased exosome secretion when myeloma cells were exposed to the same drugs. The chemotherapy-induced exosomes display a proteomic profile distinct from cells not exposed to the drug. Furthermore, besides an increase in the levels of heparanase, it shows a distinct localization, being present at the exosome surface and hence act on the surrounding ECM. Exosomes secreted by tumor cells, together with high levels of heparanase-1, not only alter the behavior of tumor cells but also promote alterations to nonneoplastic host cells [82]. Thus, macrophages exposed to these exosomes increase the secretion of myeloma growth-promoting factors [90] and exosomes secreted by tumor cells containing heparan sulfate, modulate the expression of heparanase-1 in circulating T-lymphocytes [92]. These and other results bring new insights into the understanding of chemoresistance [91].

36.2.5 Heparanase Inhibitors

Since heparanase-1 is known to be involved in tumor progression, inhibitors of this enzyme have been produced as novel cancer therapeutics [93]. An improved understanding of the molecular contexts favoring the action of these agents against cancer would allow a full application of their potential. Current approaches for heparanase-1 inhibition include the development of chemically modified heparins, small molecule inhibitors, natural products, synthetic oligonucleotides, and neutralizing antibodies [94–101].

Development of heparanase-1 inhibitors focused on carbohydrate-based compounds of which few are being evaluated in clinical trials for various types of cancer, including myeloma, pancreatic carcinoma, and hepatocellular carcinoma [103]. Low-sulfate oligosaccharides were less effective heparanase inhibitors than medium- and high-sulfated fractions of the same-size saccharide. While O-desulfation abolished the heparanase-inhibiting effect of heparin, O-sulfated, N-substituted (e.g., N-acetyl or N-hexanoyl) species of heparin retained high inhibitory activity [102]. Therapeutic potential of a supersulfated low molecular weight heparin (ssLMWH) showed potent anti-heparanase activity in preclinical models [104]. Synthetic glycopolymers that mimic heparin structure with reduced anticoagulant activity is another strategy to generate heparanase-1 inhibitors.

Among these glycopolymers, a sulfated poly-2-aminoethyl methacrylate grafted heparin disaccharide has shown potent efficacy in inhibition of heparanase-1 activity and microvascular endothelial cell proliferation, protecting against tumor metastasis [105]. Several heparan sulfate glycomimetics demonstrated heparanase-1 inhibition comparable to the compounds in clinical development and also inhibit metastasis and growth of human myeloma cells in mouse xenografts [106].

Roneparstat (=SST0001), a chemically-modified heparin saccharide with 100% N-acetylation and 25% glycol split with non-anticoagulant activity is known to decrease the uptake and the effects of soluble heparanase-1 [89].

PI-88 is a mixture of highly sulfated, monophosphorylated mannose oligosaccharide a heparanase inhibitor showed efficacy as an adjunct therapy for hepatocellular carcinoma [107].

PG545, a synthetic mixture of tetrasaccharide derived from heparin is also an inhibitor of the heparan sulfate-degrading enzyme heparanase-1 (Hammond & Dredge, Chap. 22 in this volume). Using a murine model of lymphoma, it was observed that the antitumor effect of PG545 is dependent on natural killer cells [108, 109]. Moreover, PG545 exhibits a strong anti-lymphoma activity, eliciting lymphoma cell apoptosis, and involving ER stress response [110].

Protein tyrosine phosphorylation plays a pivotal role in various growth factors signaling to induce cell proliferation, differentiation, and survival. Protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) are the two counteracting proteins, which regulate tyrosine phosphorylation. PTP1B is a ubiquitously expressed non-transmembrane phosphatase that belongs to the protein tyrosine phosphatases superfamily, and the implication of PTP1B in dephosphorylation of Src (Y530) is well documented in the progression of oncogenesis in various cancers. Therefore, PTP1B has been emerged as a promising next-generation therapeutic target to design novel, effective, and bioavailable drugs to fight against cancer [111]. A synthetic strategy that could generate libraries of biologically active condensed-bicyclic triazolo-thiadiazoles identified inhibitors of PTP1B. Among such compounds, 1,2,4-triazolo-1,3,4-thiadiazoles presents human heparanase-1 inhibitory activity [112].

Compound 1-(2-hydroxyethyl)-2-imidazolidinone was synthesized as an inhibitor of both heparanase-1 and metalloproteinase 9 (MMP-9). The inhibition of basement membrane degrading enzymes such as heparanase-1 and MMP-9 may improve the epidermal barrier function of facial skin, which is exposed to the sun on a daily basis. Therefore, 1-(2-hydroxyethyl)-2-imidazolidinone is an effective way to care for regularly sun-exposed facial skin [113].

Heparanase-1 neutralizing monoclonal antibodies profoundly attenuated myeloma and lymphoma tumor growth and dissemination in preclinical models by targeting heparanase in the tumor microenvironment [97, 103]. As previously mentioned, peptides derived from TFPI-2 inhibitory site were shown to inhibit tissue factor/heparanase-1 complex and to attenuate sepsis severity and tumor growth in a mouse model [46]. Interestingly, aspirin binds to Glu225 at the active site of heparanase-1 and inhibits its enzymatic activity, preventing tumor metastasis and angiogenesis [114].

36.3 Heparanase-2 the Ugly Duckling or the Beautiful Swan

36.3.1 Heparanase-2 Cloning

Mackenzie and coworkers cloned a new heparanase, nominated human heparanase-2 or Hpa2 which differs from Hpa1 since it does not present catalytic activity. The gene encoding heparanase-2 is located on chromosome 10q23–24. There are three isoforms of heparanase-2 originated by alternative splicing containing amino acids 592, 534 and 480, termed Hpa2c, Hpa2b and Hpa2a, respectively. These isoforms are all membrane-associated proteins containing the C-terminal portion facing the cytoplasm. Heparanase-2c is the only variant capable of being secreted, possibly because it contains specific glycosylation sites, which are absent in Hpa2a and Hpa2b. It was also observed that unlike Hpa1, heparan sulfate proteoglycans on the cell surface (i.e., syndecans) are not able to promote internalization and proteolytic processing of heparanase-2 [115].

The coding region alignment of heparanase-1 and heparanase-2 shows 40% identity, including amino acid residues critical to the catalytic activity (Glu225 and Glu343). Moreover, heparanase-2 has a high affinity for heparan sulfate. The segment comprising the HS-binding regions and catalytic site of heparanase-1 are not conserved in heparanase-2, preventing heparanase-2 from being processed by proteolysis [115, 116] (McKenzie, Chap. 34 in this volume). The HPSE2 gene presents 12 exons, comprises approximately 630 kb and is located in a region (10q23–24) that is predisposed to loss of heterozygosity, characteristic of genomic instability in cancer. Molecular defects in the occurrence of loss of heterozygosity are derived from tumor suppressor genes, which protect DNA integrity or involve chromosome segregating genes that mediate correct separation of sister chromatids into daughter cells during mitotic cell division. Segregating genes may include genes involved in the determination of centromere structure, sister chromatid cohesion and genes involved in formation of the anaphase promoter complex [117]. Therefore, loss of heterozygosity, as well as segregating genes, are potentially involved in carcinogenesis [118].

The determination of genomic segments susceptible to loss of heterozygosity in solid tumors allowed the delineation of specific regions of the genome as tumor suppressor genes favoring a molecular profile of accumulation of genetic changes in a multi-step process during cancer progression [119, 120].

36.3.2 Heparanase-2 and Urofacial Syndrome

The urofacial syndrome, or hydronephrosis, comprises an autosomal recessive congenital disorder characterized by inverted facial expressions, an unusual facial expression, in association with obstructive urinary tract disease. The clinical symptoms of the urofacial syndrome are largely confined to the urinary tract, and patients appear to grimace when they smile. The main urologic features include urinary incontinence, bladder enlargement, renal complications, and many patients also

experience repeated episodes of urinary tract infections [121, 122]. The genetic characterization of urogenital syndrome identified HPSE2 gene as the primary candidate for such pathology [123, 124, 125] (Roberts and Woolf, Chap. 35 in this volume). It was identified as a great variety of mutations (deletions and missense) in all 12 exons of the HPSE2 gene in the human genome. However, it should be noted that many of the mutations in the HPSE2 gene are not related to the clinical features of the urogenital syndrome. Thus, the urogenital syndrome is probably underestimated, particularly when urinary tract characteristics are mild. Such phenotypic variability present in the urogenital syndrome may also be the result of environmental influences and other genetic modifications [123].

The second gene that characterizes the urogenital syndrome is LRIG2, leucine-rich repeats and immunoglobulin-2-like domains, which encodes a transmembrane family of proteins that modulate a variety of signaling pathways [126]. Surprisingly, LRIG2 gene mutant mice present a normal survival rate without detectable phenotype or exhibit slowed growth and a slight increase in spontaneous mortality [127, 128]. Both HPSE2 and LRIG2 represent proteins that co-localize with a neuronal marker, β3-tubulin, present in the human bladder, which justifies their potential involvement in neuronal modulation [125] (Roberts and Woolf, Chap. 35 in this volume). However, the fact that heparanase-2 knockout mice have been able to develop urofacial syndrome strongly suggests that HPSE2 is independently related to the development of such pathology. Furthermore, the presence of HPSE2 gene in a chromosome region susceptible to loss of heterozygosity may reinforce its involvement in carcinogenesis [129].

36.3.3 What Can we Learn from Heparanase-2 Knockout/ Knockdown Studies?

The HPSE2 analog gene was identified in frogs, showing that the protein is localized in the embryos neural tube region, where the motor neurons develop. Heparanase-2 knockdown in frogs was performed by morpholino; the *Xenopus tropicalis* embryos developed skeletal muscles paralysis, and motor neurons showed significant morphological alteration. Biochemically, HPSE2 knockdown in frogs caused an increase in FGF-2 expression, enhancement in cell signaling mediated by kinases, and transcription alterations of genes associated with neurons and muscles. It was hypothesized that the primary role of heparanase-2 in this model was modulation of FGF-2 and signal transduction during neural development, corroborating the phenotype found in urofacial syndrome [130]. Deletion of both HPSE2 alleles in mice caused a phenotype similar to urofacial syndrome, with bladder distended, abnormal voiding behavior, slow growth rate, renal dysfunction, malnutrition, and the animals die one month after birth. The mice also presented severe urological alterations, and the bladder is characterized by excessive presence of fibrotic tissue correlated with an increased level of transforming growth factor-beta (TGF-β), indicating that tissue remodeling involving such mutation is also related to the signaling of TGF-β [129].

Urogenital carcinoma is highly frequent in California sea lions (*Zalophus californianus*), and the etiology of such carcinoma was extensively studied and is

clearly multifactorial [131]. Interestingly, a genetic analysis of many generations of sea lions showed a single locus associated with the occurrence of bladder carcinoma, and in a case-control study, it was shown that bladder carcinoma in sea lions was significantly associated with homozygosity at the locus of the Pv11 microsatellite. Pv11 was mapped as a microsatellite of intron 9 of the HPSE2 gene, evidencing the relationship between this microsatellite and the HPSE2 gene and suggesting that HPSE2 gene alteration could be related to bladder carcinogenesis in sea lions [132].

Microsatellites are DNA repeated sequences and the most common microsatellite in humans is a dinucleotide repeat of nucleotides C and A, which occurs tens of thousands of times throughout the genome. Microsatellites are also known as single-sequence repeats. Although the length of these microsatellites is highly variable in different persons, the number of repetitions of such sequences contributes to the fingerprint of individual DNA. Therefore, each individual has microsatellites of definite length which occur at thousands of sites within a genome. Microsatellites are high spots for mutations compared to other areas of DNA, leading to high genetic diversity. Microsatellites are widely used for DNA profiling in cancer diagnosis, fingerprint analysis, paternity test, and forensic identification, to locate a gene or mutation responsible for a particular disease. Microsatellite instability is the condition of genetic hypermutability (predisposition to mutation) that results from repair of DNA incompatibility. The presence of microsatellite instability represents phenotypic evidence that repair mechanisms are not functioning normally. The evidence of HPSE2 gene mapped as a microsatellite and correlates with bladder carcinoma in California sea lions suggest that heparanase-2 might be involved with cancer.

36.3.4 Colorectal Cancer

The first article evidencing increased expression of heparanase-2 in human cancer revealed an enhancement of heparanase-2 in colorectal carcinoma compared to the non-neoplastic tissue and inverse correlation between heparanase-2 overexpression and downregulation of syndecan-1 [133]. Considering this article Giordano questioned whether heparanase-2 would be the ugly duckling representing a tumor marker or whether heparanase-2 could be the beautiful swan acting as a mechanism to compensate for the loss of syndecan-1 from the tumor tissue [134]. By this time, other authors have reported a decrease in syndecan-1 as a worse prognosis for colorectal carcinoma with a higher incidence of liver and lymph node metastases, as well as decreased patient survival and poor histological differentiation of tumors [135, 136]. Additionally, it was known that heparanase-1 could modulate adhesion and invasion of neoplastic cells by activation of Rho, independently of its enzymatic activity, corroborating the notion that heparanase-2, due to its similarity with heparanase-1, might be associated with carcinogenesis even without having a catalytic activity [137].

The presence of heparanase-1, heparanase-2, and syndecan-1 in colorectal adenomas, suggested a possible role of these molecules in progression of benign tumors. There was an inverse correlation between heparanase-2 and syndecan-1, as well as heparanase-1 and heparanase-2, while heparanase-1 and syndecan-1 showed a direct correlation [138]. The inverse correlation between heparanase-2 and heparanase-1 in

benign colorectal tumor suggests that heparanase-2 may be related to good prognosis since heparanase-1 is directly involved in tumor metastasis.

Zhang and coworkers reported that heparanase-2 represents a favorable prognosis in colorectal cancer, observing intense cytoplasmic labeling of heparanase-2 in gastric cancer compared to non-neoplastic tissue; however overexpression of heparanase-2 indicated higher survival of patients affected by such neoplasm [139]. Otherwise, among tissues collected from patients with colon carcinoma heparanase-1 was overexpressed specifically in cases of tumor metastasis, indicating that the most poorly differentiated carcinoma tissues presented the highest expression of heparanase-1 [140]. Thus, while heparanase-1 appears to be directly related to metastatic tumors, heparanase-2 appears to be overexpressed in benign tumors and does not increase in more advanced stages. The expression of both heparanase-1 and heparanase-2 isoforms (heparanase-2a, 2b, and 2c), was determined in plasma samples from 21 patients with gastrointestinal cancer and 43 healthy individuals. The results showed a significant increase in all heparanase species in the plasma of cancer patients compared to the control group. Additionally, the enzymatic activity of heparanase-1 was increased in all individuals affected by cancer compared to the control group [141], suggesting a potential noninvasive new diagnostic assay to detect both heparanase-1 and heparanase-2.

36.3.5 Breast Cancer

The interactions between tumor cells and ECM components are essential during invasion and metastasis. Tumor cells must destruct the basement membrane in order to be able to migrate into the connective tissue. Degradation of the ECM and basement membrane releases HS-bound active cytokines, growth factors, and angiogenic factors. In fact, degradation of HS chains by heparanase-1 generates oligosaccharides that intensify the action of such growth factors, cytokines, and angiogenic factors, thus inducing cellular proliferation, inflammation, and formation of new blood vessels, contributing to the carcinogenic process. While heparanase-2 lacks HS-degrading activity, it has a high affinity towards heparan sulfate, which can alter ECM dynamics leading to deregulated cancer cell proliferation and invasion. In fact, decreased heparanase-2 expression appears to represent an excellent diagnostic marker for the molecular subtypes of luminal A, luminal B, and triple negative breast cancer. Therefore, heparanase-2 by interacting intensively with HS chains prevents the deleterious action of heparanase-1. It was reported that heparanase-2 is downregulated in tumor tissues from patients with luminal A, luminal B, and triple negative breast cancer compared to non-neoplastic tissue, while metalloprotease-11 was overexpressed in all subtypes of breast cancer [142].

The evaluation of infiltrating ductal adenocarcinomas (metastatic and non-metastatic adenocarcinomas) evidenced a significant decrease of heparanase-2 [143]. Heparanase-2, as well as heparanase-1, are overexpressed in the mononuclear fraction of peripheral blood cells in patients with breast cancer. Circulating lymphocytes obtained from healthy individuals when incubated with plasma from patients with breast cancer express a significantly higher amount of both heparanases [144].

The mechanism of induction of heparanase-1 and heparanase-2 expression in circulating lymphocytes appears to be mediated by heparan sulfate secreted by tumor cells, proving a cross-talk between the tumor, tumor microenvironment and circulating lymphocytes [92].

36.3.6 Cervical and Endometrial Cancer

Immunohistochemical analyses showed a progressive increase of heparanase-2 according to the severity of cervical lesions comparing low-grade squamous intraepithelial lesions and invasive carcinoma, while the group of non-affected individuals presented lower expression of heparanase-2. This study revealed that heparanase-2 can be used as an auxiliary biomarker and contribute to improving the histopathological diagnosis of benign cervical lesions [145]. Labeling of heparanases in endometrial tissue has demonstrated that while heparanase-1 strongly targets advanced cancer in glandular tissue, heparanase-2 revealed strong staining at the endometrial stroma that is not affected by neoplasia [146]. Again, these results corroborate the notion that heparanase-2 is involved in early stages of tumor development and is present in benign tumors.

36.3.7 Ovarian Cancer

There was no significant difference in heparanase-2 expression between benign and malignant ovarian tumors, indicating that heparanase-2 is not exclusive for malignant tumors [147]. Interesting data were obtained using fertilization methods that involve superovulation. Fertilization induces the expression of several genes which participate in endometrial remodeling and affect trophoblast migration, embryo implantation, and endovascular invasion. A study that evaluated genes related to superovulation showed a significant reduction in heparanase-2 expression [148].

36.3.8 Bladder Cancer

Immunohistochemical analysis revealed that Heparanase-2 is expressed by bladder normal transitional epithelium and its expression level decreases substantially in bladder cancer. Notably, tumors that retain high levels of heparanase-2 have been diagnosed as low grade and low stage, suggesting that Hpa2 is possibly essential to preserve cell differentiation and disrupt cellular motility. In vitro, addition of recombinant heparanase-2 inhibited bladder carcinoma cell migration. Moreover, tumors produced by bladder carcinoma cells that overexpress heparanase-2 were smaller and of lower grade than tumors produced by mock-transfected cells [149]. Interestingly, the expression of Hpa2 in bladder stromal cells correlates with collagen deposition and a marked increase in lysyl oxidase (LOX) staining. The association between heparanase-2 and LOX expression was clinically confirmed by staining of bladder

cancer biopsy samples [149]. In summary, heparanase-2 seems to function in bladder tissue to maintain cell differentiation and decrease cell motility in a manner that appears to be independent of heparanase enzymatic activity.

36.3.9 Thyroid and Head and Neck Cancer

One of the major challenges for the diagnosis of thyroid cancer is to identify ideal markers that can distinguish between differentiated thyroid carcinoma and benign lesions. Ultrasound-guided aspiration is the most appropriate method to evaluate thyroid nodules. However, a significant percentage of the cytological examination has an indeterminate classification with malignancy proportions ranging from 10 to 30%. The anatomopathological evaluation of tissues obtained by surgical resection allows good diagnosis, but in this case, the patient has already undergone thyroidectomy, and often the thyroid ablation was unnecessary because the tumor is benign. Heparanase-2 seems to be an excellent marker to differentiate benign tumors from malignant thyroid tumors. Intense labeling of heparanase-2 in the colloid secreted by follicular thyroid cells along with negative stroma staining characterizes benign tumors. Conversely, negative colloid staining and intense labeling of the extracellular matrix by heparanase-2 indicates differentiated thyroid carcinoma [150].

Levy-Adam and coworkers proposed an elegant model of heparanase-2 action and demonstrated that the interaction of heparanase-2 with HS induces inhibition of heparanase-1 activity [116]. Cells obtained from head and neck tumors that overexpress heparanase-2 are abundantly decorated with stromal cells and collagen deposition, correlating with a marked increase in lysyl oxidase expression. In this study, it was observed that the enzymatic activity of heparanase-1 was not affected in cells that over-express heparanase-2, suggesting that reduced tumor growth is not due to the regulation of heparanase-1 by heparanase-2. Furthermore, the growth of xenografts that overexpress heparanase-2 was unaffected by administration of anti-Hpa2 monoclonal antibodies that inhibit the interaction of Hpa2 with HS, together indicating that the function of heparanase-2 does not depend on heparanase-1 activity or HS binding [151]. Hpa2 overexpression in head and neck cancer cells markedly reduces tumor growth due to inhibition of vascularization. Restrained tumor growth was associated with a prominent decrease in tumor vascularity (blood and lymph vessels), likely due to reduced Id1 expression, a transcription factor highly implicated in VEGF-A and VEGF-C gene regulation [151].

36.3.10 Heparanase-2 and Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disease with a high incidence that causes progressive loss of memory and cognitive dysfunction and causes death due to chronic complications. Alzheimer's disease is caused by abnormal accumulation of cytotoxic peptides called amyloid-β (Aβ) that form senile plaques and intracellular accumulation of hyperphosphorylated forms of the microtubule-associated tau protein. HS proteoglycans favor Aβ or tau fibrillization and promote resistance to proteolytic

degradation of such protein aggregates [152]. Both heparanases (heparanase-1 and heparanase-2) are overexpressed and co-localized with Aβ aggregates in degenerate neurons and are also present in the extracellular matrix at different stages of Alzheimer's disease. While heparanase-1 is present in fragmented nuclei of senile plaques composed of β-amyloid deposition, heparanase-2 is found around senile compact plates [153].

Studies have shown that the enzymatic activity of heparanase-1 appears to decrease β-amyloid deposition or block the intracellular formation of tau fibrils by promoting degradation of HS chains, whereas heparanase-2 seems to act as a heparanase-1 inhibitor, competing for HS binding. Thus, it appears that both heparanases are involved in Alzheimer's disease [154, 74] (Li and Zhang, Chap. 25 in this volume).

36.3.11 Heparanase-2 as a Tumor Suppressor

Cancer is caused by sequential pathological variations or mutations, the transformation of proto-oncogenes into oncogenes and loss of function of tumor suppressor genes. However, it is also important to consider epigenetic changes that may alter the expression pattern of certain genes. Such epigenetic changes include DNA methylation as well as histone modifications. The complex between histones and DNA comprises the structural unit of chromatin. The organization of chromatin is regulated in part by post-translational modifications of histones. The complex of proteins called Polycomb act as transcriptional repressors that promotes the silencing of specific genes by chromatin modifications. Specifically, EZH2 protein belonging to the Polycomb complex is capable of promoting methylation of Lys27 residues of histone H3, leading to repression of target genes [155]. Together, the Polycomb complex plays central roles in epigenetic silencing of stem cell target genes, tumor metastases, and cancer [156–158]. Target genes of the Polycomb complex have been extensively investigated. In a study that evaluated the signature repression of the Polycomb complex in metastatic prostate cancer, 87 genes were described as down-regulated genes that were associated with worse clinical prognosis. Strikingly, HPSE-2 gene expression is repressed by the Polycomb complex, strongly supporting the tumor suppressor activity of heparanase-2 [156].

36.4 Conclusions

HPSE gene is located on chromosome 4q21.2 and the enzyme heparanase (Hpa-1) degrades HS chains and plays a role in the normal turnover of HS proteoglycans. The oligosaccharides generated by heparanase at the cell surface and ECM lead to a cascade of cellular events that affect a diversity of physiological processes, such as cell growth, adhesion, migration, and death. Hence, heparanase is being implicated in diverse cellular systems, including inflammation, neovascularization, tissue remodeling, carcinogenesis, tumor cell metastasis, and blood coagulation. Additionally, heparanase acts as a regulator of the syndecan-syntenin-exosome biogenesis pathway and enhances exosome secretion. Inhibitors of heparanase include

chemically modified heparins, small molecules, natural products, synthetic oligonucleotides, and neutralizing antibodies, and some of these compounds are currently in clinical trials and have been produced as novel cancer therapeutics.

Heparanase-2 is located on chromosome 10q23–24, and coding region alignment with heparanase-1 showed 40% identity. Additionally, heparanase-2 has no enzymatic activity but has a higher affinity for HS compared to Hpa-1. Heparanase-2 appears to be overexpressed in benign tumors and less aggressive tumors. Apart from attenuation of heparanase-1 enzymatic activity, heparanase-2 inhibits neovascularization mediated by VEGF, independent of heparanase-1 modulation and HS binding. The fact that the HPSE2 gene is located in a chromosomal region susceptible to loss of heterozygosity, and is under the control of the Polycomb complex, strongly suggests that HPSE2 may function as a tumor suppressor. It also plays important roles in embryogenic development and survival. Altogether, heparanase-2 may be looked upon as a beautiful swan, while heparanase-1 is the ugly duckling (Figs. 36.1 and 36.2).

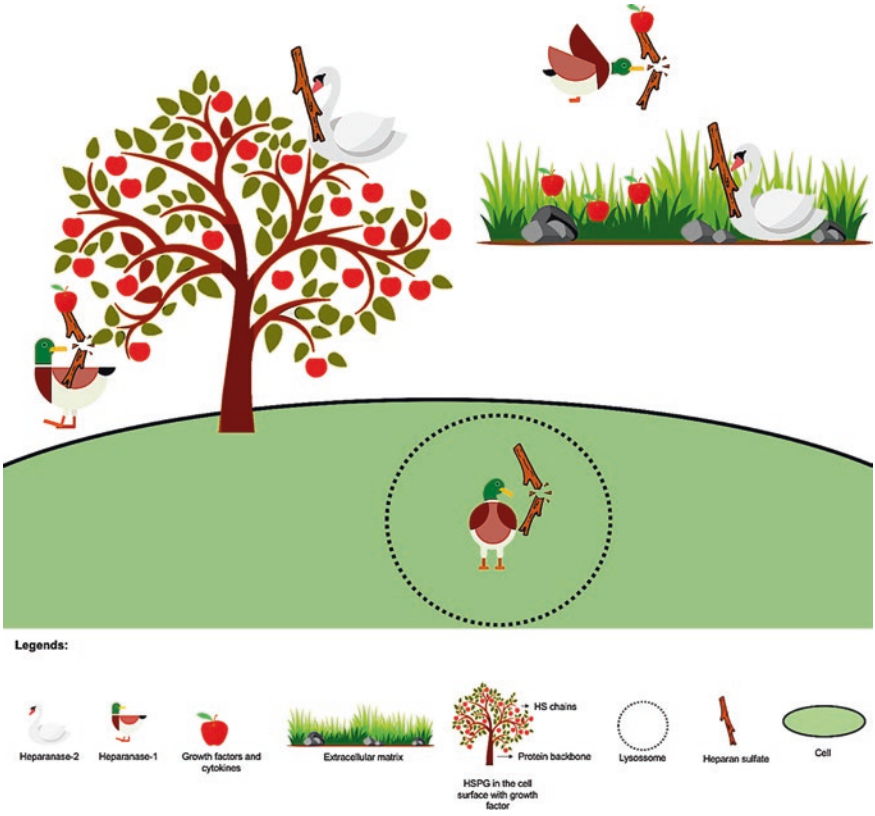

Fig. 36.1 Interaction of heparan sulfate with heparanase-1 and heparanase-2. Both heparanases interact with heparan sulfate side chains of proteoglycans (HSPG) at the cell surface and extracellular matrix. Heparanase-1 binds and degrades HS chains generating oligosaccharides with high affinity to growth factors, cytokines, and angiogenic factors, affecting cell proliferation, cell migration, angiogenesis, and inflammation. Heparanase-2 binds with high affinity to HS chains but does not cleave them. Heparanase is also involved in the lysosomal turnover of HSPG. Heparanase-2c is the only variant capable of being secreted, possibly because it contains specific glycosylation sites, that are absent in Hpa2a and Hpa2b. heparanase-2 is does not promote the internalization of HSPG.

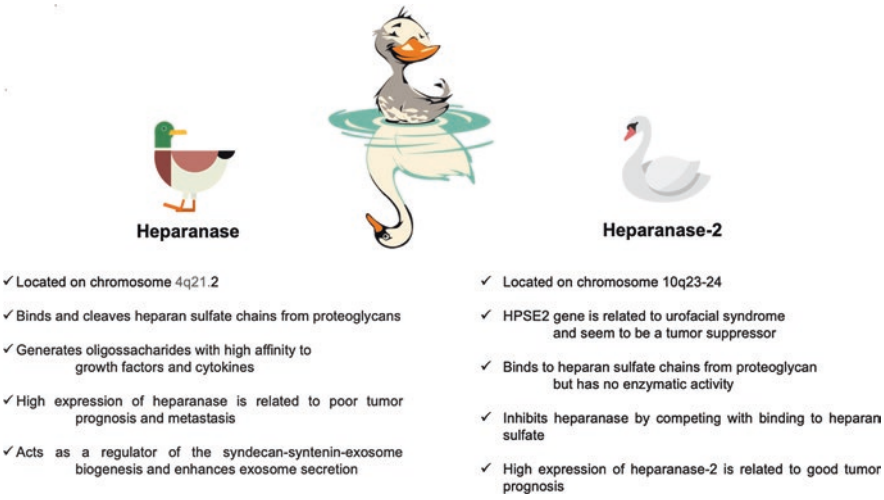

Fig. 36.2 Effects of heparanase and heparanase-2. The scheme summarizes the main characteristics and principal roles of both heparanases. While heparanase-1 is strongly associated with tumorigenesis and tumor metastasis, heparanase-2 plays a role in tissue development and appears to function as a tumor suppressor

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