

# Chapter 4

## Involvement of Syndecan-1 and Heparanase in Cancer and Inflammation



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### 4.1 Introduction

#### 4.1.1 *The Syndecan Family of Heparan Sulfate Proteoglycans*

Glycosaminoglycans (GAG) are sulfated polysaccharides composed of unbranched chains of repetitive disaccharide units of uronic acid and glucosamine. One of the most studied GAGs is Heparan sulfate (HS), a complex GAG present in virtually all animal cells [1, 2] (Fig. 4.1). Via a linkage tetrasaccharide, HS can be O-glycosidically attached to serine or threonine residues of proteins in the cell membrane, or to proteins which are secreted into the extracellular matrix (ECM), thus forming Heparan Sulfate Proteoglycans (HSPG)[1, 2]. The HS chains present a vast structural complexity due to the possibility of inserting different disaccharide units during biosynthesis. The insertion of sulfate radicals residues largely contributes to its negative charge characteristic, and the degree of sulfation usually ranges between 0.6–1.5 sulfates per disaccharide [3]. The iduronic or hexuronic acid can be sulfated at the position 2, while the glucosamine can be N-sulfated, N-acetylated and/or O-sulfated in positions 6 and 3. Besides its sulfation pattern, the molecular weight can widely vary between HS chains, ranging frequently between 5–70 kDa [4]. Another important characteristic is the considerable amount of non-sulfated regions of glucuronic acid and N-acetyl glucosamine, allowing the HS chains to organize itself in

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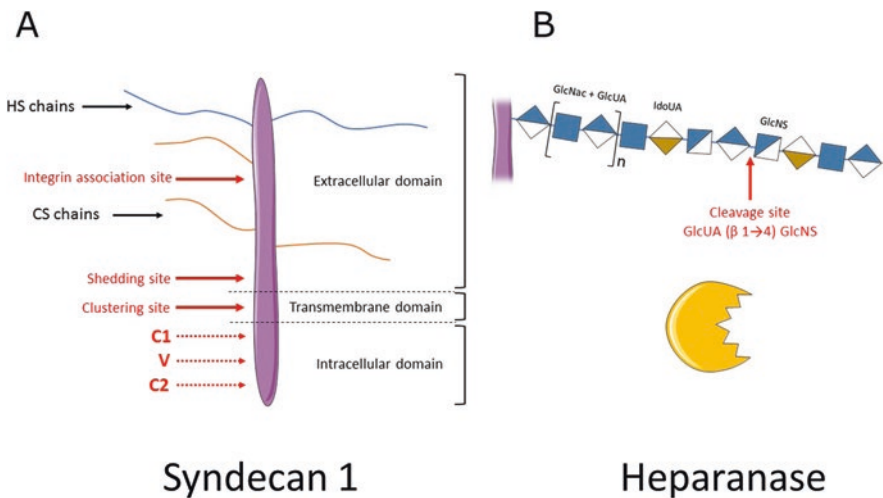
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N-acetylated and N-sulfated domains. Each domain has distinct structural characteristics that dictate the kinds of molecules that can interact with them [2]. The synthesis of HSPGs occurs in the Golgi apparatus and is dependent on many enzymes which catalyze the different steps of HS elongation and modification in a sequential manner. The majority of HS deregulation in disease occurs as a result of alterations in the expression of enzymes involved in its synthesis, however, in some cases, the deregulation is also due to alterations in the core protein [5].

Syndecans (Sdc) are a family of transmembrane HSPGs expressed at the surface of several cell types, although they have also been found in the nucleus [6]. Moreover, their intact extracellular domains can be shed into the extracellular environment [7]. In mammals, this family is composed of four members that are expressed in a highly regulated cell-specific manner [8]. Among the syndecans, Sdc1 is the most highly expressed HSPG on the cell membrane of epithelial and plasma cells. Both the GAGs heparan sulfate and chondroitin sulfate (CS) are bound to its protein core [9]. Neuronal cells and cartilage express mainly Sdc3, while mesenchymal cells express Sdc2 and Sdc4, but have Sdc1 as well [10]. Sdc4 is expressed by most cell types [8]. All syndecans are composed of three different domains: an extracellular N-terminal domain where several glycosaminoglycan chains are covalently attached, a single transmembrane domain and a cytoplasmic C-terminal domain, which is subdivided into two constant domains separated by a variable region (Fig. 4.1). The extracellular domains of syndecans mediate various cell-cell



**Fig. 4.1** Structure and specific protein and glycosidic domains of Syndecan-1 and Heparanase function. **(A)** The representative structure of the Syndecan-1 core protein. The different domains are represented on the right side and the GAG chains are represented in blue (Heparan Sulfate) and orange (Chondroitin Sulfate). The red arrows represent the various protein domains important for the interaction with other proteins. **(B)** Example of a Heparan Sulfate chain. The different monosaccharidic components are represented. The Heparanase cleavage site is represented in red

and ECM-cell interactions dependent on their GAG composition. This domain, in turn, varies significantly between the members of the syndecan family, unlike the transmembrane and cytoplasmic domains, which are highly conserved [11]. Although most studies focus on describing the importance of the extracellular and intracellular domains of syndecans, the transmembrane region was described to be sufficient in reducing cell migration via alterations in focal adhesion dynamics [12]. The GAGs attached to syndecans have a structure capable of directly interacting with many growth factors, cytokines, chemokines, and other macromolecules and enzymes found in the ECM, leading to the presentation to their receptors on the cell membrane. These interactions mediated by the GAG chains imply several physiological activities attributed to syndecans, such as cell proliferation, migration, and invasion [13]. The cytoplasmic domains of syndecans are capable of interacting with various intracellular kinases and the cytoskeleton, promoting important intracellular signaling [11]. Syndecans can also interact with other family members as structural analysis has shown that Sdc1 is less likely to form homodimers than the other syndecans, and can form heterodimers with Sdc2 and Sdc3 but not with Sdc4 [14, 15]. Further studies are needed to better understand the implications of these interactions. These diverse intra- and extracellular interactions with different molecules characterize the syndecans as key molecules in various physiological and pathological cellular functions. In this review, we will mainly focus on the role of the prototype member of the family, Sdc1, which is best characterized for its functional interplay with heparanase in the context of inflammation and cancer.

The extracellular domain of Sdc 1 harbors five GAG-attachment sites. Three are close to the N-terminus and two are close to the cell membrane. While HS and CS can be located near the N-terminus, only CS is found near the plasma membrane. Cells can vary the number of HS and CS chains added to Sdc1, but it always has at least one HS chain at its N-terminus [10]. Studies have shown that the number of GAGs attached to Sdc1 also affects its function, such as reducing its ability to mediate cell-cell interaction and cell invasion through the ECM [16]. The extracellular domain of Sdc1 can be shed by many proteases, such as matrix metalloproteinases (MMPs) and A-disintegrin and matrix metalloproteinases (ADAMs) [17]. Currently, there are no reports of endogenous extracellular trimming of CS chains in proteoglycans of mammalian cells. The presence of extracellular CS chains close to the plasma membrane can alter its susceptibility to cleavage and shedding by proteases, as well as change its ability to associate with receptors and other syndecans. Injuries or bacterial infections may lead to upregulation of Sdc1, heparanase, and proteases that can release the ectodomain fragment of Sdc1 [18–20]. In vitro, Sdc1 ectodomain has been shown to decrease proliferation of tumor cells [21], whereas another study using tumor cells expressing uncleavable membrane-bound Sdc1 showed increased proliferation and decreased invasion [22]. These events are regulated by HS chains in Sdc1. The length and sulfation of these chains can vary between cell types due to the action of several enzymes that regulate glycan elongation and modification [23]. Of these enzymes, cleavage by heparanase is of particular importance.

### 4.1.2 Heparanase – A Key Enzyme in ECM Remodeling

Heparanase is an endo- $\beta$ -glucuronidase that degrades specific domains of HS chains (Fig. 4.1). After being produced in the rough endoplasmic reticulum, it is sent to the Golgi apparatus and secreted to the ECM in its inactive, latent form [24]. Subsequently, it can interact with Sdc1 at the plasma membrane and be endocytosed to reach the lysosomes, where it can be proteolytically activated [25]. Following this processing step, heparanase can reach the ECM again, where it executes extracellular functions [25, 26], or act inside the cell and modulate intracellular processes [26]. Despite its enzymatic activity, studies using mutated inactive heparanase have demonstrated that it can also exert activities unrelated to HS degradation. Enzymatically inactive heparanase can facilitate adhesion and migration of endothelial cells [27], promote phosphorylation of signaling molecules such as Akt and Src [27–29], as well as activate receptor tyrosine kinases such as EGFR [30]. For example, in head and neck carcinoma, heparanase was also shown to facilitate the formation of lymphatic vessels and the migration of tumor cells toward these vessels [31].

Most of what is known about heparanase function are based on its activity in cancer, where heparanase RNA and protein levels are increased in many different forms of malignant diseases [32] (Table 4.1). By degrading HS chains, heparanase alters many regulatory paths, mainly by augmenting the bioavailability of growth factors previously bound to the HS. This diffusion of growth factors and cytokines affects many different physiological processes, such as cell migration, angiogenesis, inflammation, coagulation, autophagy, and exosome production [33, 34]. Most members of the syndecan family are involved in these activities. In angiogenesis, for instance, Sdc1, Sdc2, and Sdc3 have important effects related to their ability to bind and present pro- and anti-angiogenic factors [35–37].

## 4.2 The Heparanase-Syndecan Axis

There is ample evidence that heparanase can affect cell behavior by regulating the structure and function of HSPGs. The Heparanase/Syndecan Axis (commonly referred as the Heparanase/Syndecan-1 axis due to most evidence showing a major role for Sdc1 on this process) has been shown to affect signaling cascades in healthy or malignant cells and biological systems (Fig. 4.2). The most studied growth factors that are strongly regulated by this axis are hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). Other than just stimulating syndecan shedding, the cleavage of heparan sulfate chains by heparanase also makes it easier for additional proteins to recognize and bind to syndecan [38]. One example is the binding of the protein lacritin, a prosecretory epithelial mitogen that is expressed in the tear ducts and can bind directly to syndecan-1 core protein only after the trimming of HS chains by heparanase [39]. The heparin-binding domains of heparanase also

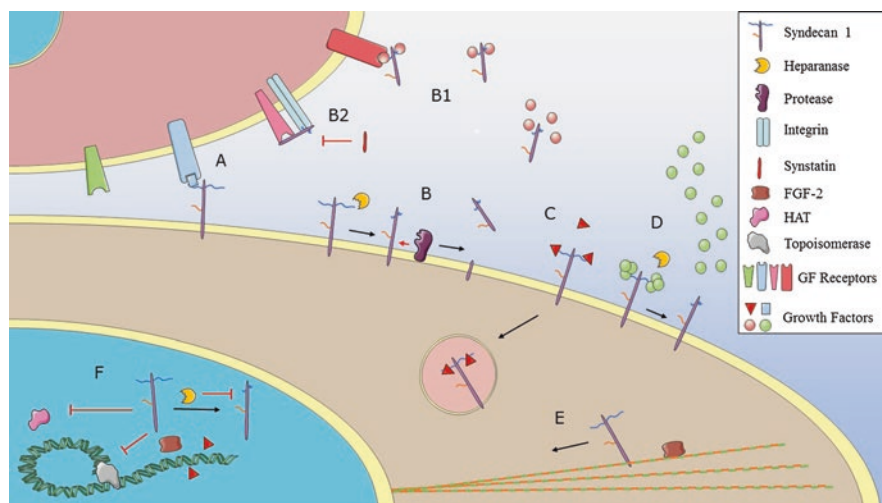
**Table 4.1** Dysregulation and prognostic value of Syndecan-1 and heparanase in cancer

Cancer type	Syndecan-1			Heparanase
	Cell-surface	Stromal	Serum	
Bladder	Decreased expression related to poor prognosis [202, 203]	Higher expression related to poor prognosis [203]	Higher expression related to poor prognosis [203]	Higher expression related to poor prognosis [204, 205]
Breast	High expression is associated with poor prognosis in most subtypes [206–210]	High expression is associated with poor prognosis [206]		Higher expression related to poor prognosis [211, 212]
Colon	Downregulation is associated with more aggressive clinicopathological parameters [213–215]	Syndecan-1 is upregulated in the stroma of a fraction of tumors [214]		Higher expression related to poor prognosis [216]
Gallbladder	Higher expression related to poor prognosis [217]			Higher expression related to poor prognosis [219]
Gastric	Decreased expression related to poor prognosis [219, 220]	Higher expression related to poor prognosis [219, 220]		Higher expression related to poor prognosis [221]
Head and neck	Decreased expression related to poor prognosis [222, 223]	Higher expression related to poor prognosis [224]		Higher expression related to poor prognosis [225]
Kidney	In clear-cell renal cell carcinomas, decrease with increasing nuclear grade [226].			Higher expression related to poor prognosis [227, 228]
Liver	Decreased expression related to poor prognosis [229]		Higher expression related to poor prognosis [230]	Higher expression related to poor prognosis [231]
Lung	Decreased expression related to poor prognosis [232]		Higher expression related to poor prognosis [233, 234]	Higher expression related to poor prognosis [235, 236]
Melanoma		Low infiltration with Sdc1-positive plasma cells is related to good prognosis [237]		Higher expression related to poor prognosis [238–240]

(continued)

**Table 4.1** (continued)

Cancer type	Syndecan-1			Heparanase
	Cell-surface	Stromal	Serum	
Oral	Decreased expression related to poor prognosis [241]	Higher expression related to poor prognosis [242]		Higher expression related to poor prognosis [243]
Ovarian	Decreased expression related to poor prognosis [244]	Higher expression related to poor prognosis [244, 245]		Higher expression related to poor prognosis [246]
Pancreas	Higher expression related to favorable prognosis [247]	Higher expression related to poor prognosis [247]		Higher expression related to poor prognosis [248]



**Fig. 4.2** The different functions of Syndecan-1 and how it can be affected by Heparanase. Syndecan-1 participates in many important physiological functions, such as (A) presenting growth factors to receptors on the cell surface or on the membrane of neighboring cells. (B) The trimming of HS chains mediated by heparanase exposes Sdc1 to the recognition and shedding by proteases, what can lead to (B1) binding and accumulation of factors in the extracellular matrix and presentation to receptors in distant cells, or (B2) binding to both integrins and directly to some receptors, activating them and leading to activation of intracellular pathways, what can be inhibited by Synstatins. (C) Some extracellular factors can trigger Sdc1 endocytosis, this endosome can be degraded or translocated into the cell nucleus and the factors can act as transcription factors. (D) Sdc1 can sequester factors from the extracellular environment, binding them to their GAG chains. Heparanase releases them into the extracellular matrix allowing them to act on their specific receptors. (E) The translocation of Sdc1 to the nucleus is highly dependent on tubulin, and it is also responsible for FGF-2 shuttling to the nucleus. (F) Sdc1 known nuclear actions include inhibiting DNA topoisomerase I activity and blocking the enzyme histone acetyltransferase (HAT) activity, controlling cell proliferation and differentiation. The trimming of the HS chains by heparanase decreases the level of nuclear Sdc1

facilitate the clustering of Sdc1 and Sdc4 on the membrane of human glioma cells, initiating signaling cascades that lead to augmented cell adhesion and spreading. Notably, this activity does not require the HS degrading activity of heparanase [40].

### ***4.2.1 Heparanase Mediated Sdc1 Shedding***

The proteoglycans present in the cell membrane have many important roles in health and disease. One example is their strong interaction with the tumor stroma, allowing the activation of numerous pathways in cancer cells [7]. Several of these proteoglycans can also undergo an enzymatic shedding of their extracellular domains by proteases known as ‘sheddases’. In this process, the intact ectodomain of the proteoglycan is converted to a soluble molecule, allowing them to stay functional even after solubilization [7]. The shedding of membrane-bound proteoglycans is a controlled process that regulates many physiological and pathological conditions. With the exception of glycosylphosphatidylinositol (GPI)-anchored proteoglycans, such as the Glypican family, all membrane-bound proteoglycans are shed by a protease [1, 7].

The shedding of proteoglycans can be regulated at different levels. One of the mechanisms is the regulation of the expression of sheddases and protease inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs), or the binding of these inhibitors to the GAG chains, either inhibiting or enhancing the proteoglycan activity, or even protecting them from degradation [41, 42]. The shedding occurs constitutively in cells, but it can also be enhanced by several stimuli, such as growth factor signaling, bacterial and viral stimuli, cell stress, among others [43, 44]. One of the most well-characterized actions of heparanase is its ability to facilitate Sdc1 shedding [45–47]. By trimming the HS chains on Sdc1, the core protein becomes more vulnerable to the actions of proteases [38]. All syndecans can be shed *in vivo* and *in vitro* by a process mediated by plasmin, thrombin, MMPs and/or ADAMs, and its proteolytic cleavage can be modulated by different signaling pathways such as protein kinase C (PKC), and the protein tyrosine kinase (PTK) MAP kinase [41]. After Sdc1 shedding, its remaining cytoplasmic C-terminal fragment (cCTF) undergoes proteolysis by  $\gamma$ -secretase and the proteasome. Nevertheless, this fragment has also been shown to inhibit lung cancer cell migration and invasion through the phosphorylation of Src, FAK, and Rho-GTPase, which blocks Sdc1-dependent migration and invasion, and also reduces lung tumor formation by these cells [48].

In tumors, shed Sdc1 plays multiple roles such as the delivery of growth factors to both tumor and host cells and triggering of signal transduction events at the cell surface [1, 41]. Most known functions of Sdc in the tumor context are mediated by the membrane-bound Sdc on cancer cells. However, tumor stroma syndecan has been shown to also have an important role in this context, either bound in the membrane of stromal cells or soluble ectodomains, which can be generated by cleavage from the tumor cells or other cell types such as cancer-associated fibroblasts, endothelial cells or leukocytes. In myeloma cells, the up-regulation of heparanase or



the addition of recombinant heparanase to the media was shown to lead to enhanced Sdc1 expression and shedding [45, 46, 49]. This was mostly due to the heparanase mediated activation of ERK signaling, leading to enhanced expression of MMP-9. This proteinase cleaves Sdc1 in the juxtamembrane region, releasing an intact ectodomain to the extracellular matrix [50, 51]. This effect in myeloma cells is interesting because it is highly dependent on the HS degrading activity of heparanase, whereas in other cells types ERK signaling can be triggered by heparanase, independent of its enzymatic activity [52, 53]. In a lung inflammation model in mice, soluble Sdc1 has been shown to be required for the formation of chemotactic gradients regulating leukocyte-endothelial interactions and angiogenesis [54, 55]. Another interesting finding regarding the shed ectodomain of Sdc1 is the observation that it can be transported to the nucleus, where it can influence transcription by the inhibition of histone acetylation [6, 10, 56] (see also Purushothaman and Sanderson, Chap. 12 in this volume).

#### ***4.2.2 Heparanase and Sdc1 in the Nucleus***

Sdc1 has been found in the nucleus of many cell types such including malignant mesothelioma, myeloma, neuroblastoma, lung adenocarcinoma, and breast carcinoma [57]. In addition, Sdc2 has been found in the nucleus of injured cerebral cortex neurons, astrocytes, and chondrosarcoma [58, 59]. Apart from localization to the cytoplasm and cell membrane, heparanase can also be found in its active form in the nucleus and this localization is correlated with cell differentiation [60]. From a mechanistic perspective, a lot remains to be uncovered about how HSPGs enter the nucleus and regulate nuclear processes. In the nucleus of mesothelioma cells, Sdc1 has been shown to co-localize with heparanase and fibroblast growth factor-2 (FGF-2)[61]. The amount of HSPGs in the nucleus is increased after inhibition of PKC and drastically diminished after its stimulation [62]. Apparently, FGF-2 seems to trigger its nuclear translocation by stimulating the dephosphorylation of the protein core [63].

The nuclear translocation of Sdc1 has been shown to be highly dependent on tubulin both in malignant and normal cells. Sdc1 co-localizes with tubulin at the mitotic spindle during all phases of mitosis and the interference with tubulin integrity blocks the transport of Sdc1 to the nucleus [6]. During mitosis in malignant mesothelioma cells, Sdc1 accumulates in the nucleus by associating with tubulin structures and inhibits cell cycle progression, proliferation, and migration [57]. Nuclear FGF-2 and HS also seem to regulate cell cycle in many cell types. The nuclear entry of FGF-2 happens in the G1 restriction point of the cell cycle and exogenous HS arrests cells in the G1 phase in a transient manner [64, 65]. A decrease in nuclear HS allows the regular cell cycle progression. The arrest of cells in the G2/M phase of the cell cycle efficiently blocks the nuclear translocation of Sdc1. It has been shown that the entire molecule is present in the nucleus, with both ecto- and endodomains and the HS chains [6]. The HS chains of HSPG were found to be



important for the uptake and nuclear translocation of many different molecules, including heparanase [60]. The mechanism of FGF-2 shuttling into the nucleus is dependent on both the FGF receptor and HSPGs [66]. Sdc1 and FGF-2 share the same tubulin-mediated route to the nucleus, where they co-localize with heparanase. The minimal peptide sequence required for the tubulin-dependent nuclear translocation of Sdc1 is the juxtamembrane RMKKK motif that acts as a nuclear localization signal (NLS) [61]. Replacement of arginine (R) in the RMKKK motif dramatically reduces the amount of nuclear Sdc1, and complete deletion of this sequence abrogates the nuclear translocation of Sdc1 [67].

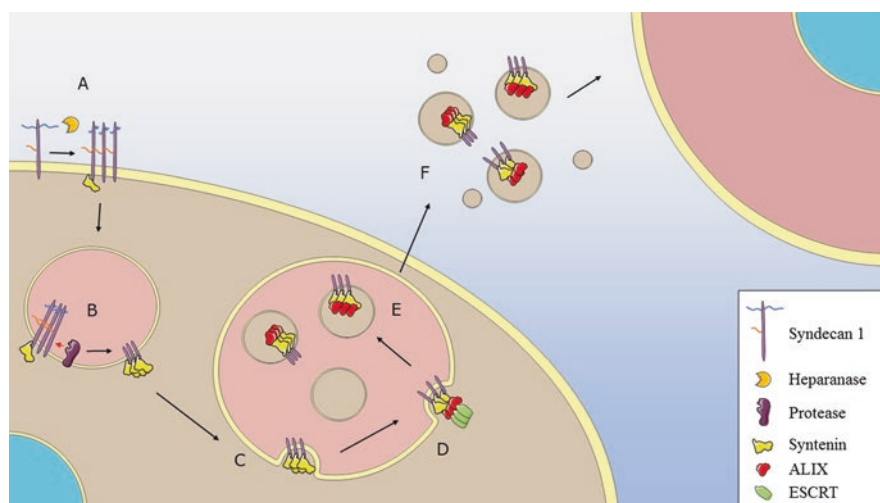
Many of the known nuclear functions of Sdc1 are associated with the HS interaction with various growth factors and nuclear structures. It is known that Sdc1 can be found in the nucleus as an intact HSPG, but it is still unclear if its actions are dependent only on the GAG chains or the entire molecule. The HS chains are important for nuclear translocation or degradation of Sdc1 and its negative charges facilitate their nuclear interactions. HS can compete with DNA for the binding of proteins such as transcription factors and enzymes. Sdc1 main known nuclear actions are to shuttle FGF-2 into the nucleus, inhibit DNA topoisomerase I activity and inhibit the activity of the enzyme histone acetyl transferase (HAT), controlling cell proliferation and differentiation [63, 64, 68]. Trimming of the HS chains by heparanase decreases the level of nuclear Sdc1, leading to an enhanced HAT activity and, consequently, augmented expression of genes that promote an aggressive phenotype in tumor cells such as MMP-9, VEGF, HGF and RANKL [69, 70]. It has been shown that HS competes with the DNA for topoisomerase I binding in the nucleus and is also able to dissociate the DNA-topoisomerase I complex, that is necessary for the unwinding of supercoiled DNA during transcription. This would suggest a negative effect of nuclear HS on gene transcription [68]. Activation of EGFR leads to nuclear translocation of heparanase and degradation of HS, increasing the activity of topoisomerase I and cell proliferation [71].

### ***4.2.3 Effects on Exosome Formation and Function***

Exosomes are secreted to the extracellular matrix by virtually all cell types [72]. They are very small vesicles ranging between 30–100 nm in diameter, but their classification comes from their endosomal origin rather than size. In mammalian cells, the biogenesis of exosomes begins with the invagination of the cell membrane forming the primary endocytic vesicles, which leads to the fusion of these vesicles and the formation of the early endosomal compartment [72]. During its maturation into late endosomes, it changes the protein composition of its membrane and receives many components derived from the trans-Golgi network. In the meantime, a second invagination occurs at the interior of the endosome forming the intraluminal vesicles (ILVs), that, consequently, have the same topology of the cell membrane (inside-in/outside-out) [73]. The late endosome containing many ILVs is called a multivesicular body (MVB). When it fuses with the plasma membrane, their ILVs

are released to the extracellular environment and, upon release, the ILVs are called exosomes [72, 73]. Both MVB and ILV formation is dependent on the endosomal sorting complex required for transport (ESCRT), but also on specific membrane domains and oligomerization/clustering processes [74, 75]. ESCRT-0 leads the clustering of mono-ubiquitinated cargo proteins into the endosome. ESCRT-I and ESCRT-II are related to the membrane deformation and the intraluminal budding of these cargos. ESCRT-I recruits ESCRT-III via ESCRT-II or through the adaptor protein ALG-2 interacting protein X (ALIX), resulting in ESCRT-III stabilization. ESCRT-III induces vesicle scission, and the vacuolar protein sorting (Vps) 4 ATPase mediates the final step of dissociation and recycling of ESCRT-III leading to IVL formation [74].

The way by which Syndecans influence the biogenesis of exosomes is through the interaction with the syntenin/ALIX complex [76–78] (Fig. 4.3). Syntenin is a small cytosolic protein that contains two PSD95/Dlg/Zonula occludens 1 (PDZ) domains. Both PDZ domains are necessary for syntenin membrane localization and the high-affinity interaction with syndecans [79, 80]. Syntenin can interact with both Sdc core protein and ALIX, which leads ALIX to bind to ESCRT-III, the complex required for the IVL formation in multivesicular bodies. The HS chains in Sdc are essential for this activity [76]. In some human cancer cell types, when the cells are exposed to exogenous heparanase or the endogenous expression of heparanase



**Fig. 4.3** Syndecan-1 and Heparanase mediated exosome formation. (A) Heparanase acts by trimming the HS chains in Sdc1, which facilitates its clustering. This, stimulates the binding of the cytoplasmic domains of syndecan to syntenin and its internalization. (B) This cluster of Sdc1 is recognized by proteases and cleaved. (C-D) After cleaving of the core protein, syntenin interacts with both Sdc1 and ALIX, which leads ALIX to bind to ESCRT-III and (E) intraluminal vesicles formation in the multivesicular bodies. (F) After releasing to the extracellular environment, these vesicles are called exosomes and can carry many different factors to distant sites throughout the body

is enhanced, exosome secretion increases dramatically. This process is dependent on the HS trimming activity of heparanase, as enzymatically inactive forms of the enzyme do not have the same effect [81]. Heparanase acts in this pathway by trimming the long HS chains in Sdc into shorter ones, which facilitates the clustering of syndecans [82, 83]. This clustering stimulates the binding of the cytoplasmic domains of syndecan to syntenin, driving ALIX-ESCRT-mediated sorting into exosomes [82–84]. This action of heparanase also facilitates the recruitment CD63 to exosomes, in a syntenin dependent fashion [82, 83] (Fig. 4.3). Consequently, heparanase inhibitors or syntenin inhibitors could be of particular interest for the treatment of cancer patients, as both exosome release and heparanase expression are frequently elevated in more aggressive subtypes [47, 81]. Heparanase has been shown to up-regulate the biogenesis of exosomes and affect its composition and function in myeloma cells [81]. Heparanase has also been found in exosomes isolated from ascites of ovarian cancer patients [85], and the levels of Sdc1, VEGF and HGF in exosomes derived from heparanase high expressing cells seem to be higher compared to heparanase low expressing cells [81]. Interestingly, exosomes secreted from heparanase high expressing cells were shown to better stimulate spreading of tumor cells on fibronectin and also stimulate invasion of endothelial cells compared to exosomes from heparanase low expression cells, suggesting a role in cancer angiogenesis and the spreading of cancer cells [81] (see also David and Zimmermann; Purushothaman and Sanderson, Chaps. 10 and 12 in this volume).

#### ***4.2.4 Effects on Growth Factor Signaling***

The shedding of syndecans exposes domains in the core protein that can bind to different receptors. One well-described mechanism that affects signaling processes in the cell membrane is the one mediated by syndecan binding to integrin and to a tyrosine kinase receptor such as HER2, EGFR or IGF-1R. This tertiary complex activates various functions in the cells such as cancer progression and angiogenesis [86–90]. In these cases, the signaling mechanism can be disrupted by synthetic peptides called Synstatins (SSTNs), that mimic the binding sites of syndecan and compete with coupling to the tyrosine kinase receptors. Sdc1 shedding exposes a domain on the core protein that can bind to very late antigen 4 (VLA-4) integrin and vascular endothelial growth factor receptor 2 (VEGFR2). When Sdc1 binds and couples these receptors, VEGFR2 becomes activated and stimulates invasion in tumor cells [49]. The same mechanism potentiates endothelial tube formation and angiogenesis. Synstatin peptides based on either the VEGFR2 or VLA-4 binding site in Sdc1 can prevent invasion of tumor cells and endothelial tube formation [49]. Interestingly, the heparanase inhibitor Ronaparstat, a chemically modified nonanticoagulant heparin, can decrease tumor invasion and angiogenesis by preventing Sdc1 shedding [49] (see also Nosedá and Barbieri, Chap. 21 in this volume).

HSPGs are also involved in the uptake and nuclear translocation of growth factors and cytokines [91]. The vast majority of growth factors with nuclear translocation

bind to HSPGs, which can efficiently deliver molecules to their intracellular targets [92, 93]. Apart from growth factors, a myriad of ligands, viruses, nucleic acids, peptides, lipoproteins, and exosomes enter the cells via HSPG-mediated endocytosis [94]. The juxtamembrane MKKK motif is the required peptide sequence for the efficient raft dependent endocytosis, and this sequence is also part of the same RMKKK motif necessary for Sdc1 nuclear translocation, being crucial for both endocytosis and nuclear transport of Sdc1 and any associated molecule [95].

Both heparanase and Sdc1 can regulate HGF function. In myeloma cells, heparanase enhances HGF expression [96]. This growth factor binds strongly to Sdc1 in the membrane, augmenting the interaction with the c-met receptor and facilitating tumor growth [97, 98]. Shed Sdc1 can also bind to HGF and some evidence suggests that c-met signaling in osteoblasts is stimulated by shed Sdc1/HGF complexes [99]. Heparanase also stimulates VEGF secretion in tumor cells [31, 50]. Secreted VEGF can form a complex with shed Sdc1 that positively regulates VEGF receptor by activating the extracellular signal-regulating kinase (ERK) signaling pathway, leading to augmented endothelial invasion and angiogenesis [50]. Immuno-depletion of the VEGF/Sdc1 complex or treatment with heparinase III, a HS degrading enzyme derived from bacteria, blocks the augmented phosphorylation of ERK. It is interesting to notice that Sdc1 also activates  $\alpha\beta3$  integrin in endothelial cells, which is a key regulator of endothelial activation and angiogenesis [50, 100, 101]. In that manner, Sdc1 promotes endothelial cell activation, angiogenesis, and tube formation. This is mediated not only by  $\alpha\beta3$  integrin activation but also by binding to VEGF presenting it to its high-affinity receptor as a tertiary complex described above. Heparanase plays a central role in this process by up-regulating Sdc1 shedding. Heparanase also inhibits FGF2 signaling in melanoma cells by degrading membrane-bound HS [102]. Modification of these chains is required for effective binding of FGF2 to the cell surface and subsequent stimulation of ERK and FAK phosphorylation [102]. FGF2 high-affinity binding requires HS chains of a minimum size and some specific structural features. Upon cleavage of HS by heparanase, specific sequences in the HS chains that bind to FGF2 could be either removed or revealed [103, 104]. In addition, interplay between heparanase and Sdc1 is required for renal tubular cells to undergo epithelial to mesenchymal transition induced by FGF2 [105] (see also Masola et al.; van der Vlag and Buijsers, Chap. 26 and 27 in this volume).

### 4.3 Functional Cooperation of Syndecan-1 and Heparanase in Inflammation

Inflammation is a complex process that involves interactions of various cell types, most notably leukocytes and endothelial cells, which exchange signals via cytokines and chemokines and their respective transmembrane receptors. Physical interactions between these cells are mediated through cell surface receptors of the selectin, integrin, and the immunoglobulin superfamily of cell adhesion molecules

(CAMs) such as ICAM and VCAM [106–108]. In order to fight pathogens and toxins, leukocytes need to be recruited from the circulation to inflammatory sites via a controlled hierarchy of low- and high-affinity interactions with the endothelium, ultimately resulting in leukocyte transmigration, or diapedesis [108]. A role for HS in leukocyte recruitment has been documented in numerous studies and involves all key steps of this process (reviewed in [54, 108]). At early stages of leukocyte recruitment, HS promotes proinflammatory signaling processes by providing binding sites for growth factors and chemokines, resulting in the formation and stabilization of ternary complexes with their receptors [109, 110], and the establishment of chemokine gradients that are a crucial element in the leukocyte recruitment cascade [55, 111, 112]. Moreover, HS itself shows a distinct basolateral gradient pattern across blood vessels [113]. HS is also needed to ensure directional transendothelial chemokine transport, thus allowing for their presentation at the luminal surface endothelium [114]. Activated endothelial cells express adhesion molecules of the selectin family, which mediate low-affinity interactions with leukocytes that result in leukocyte rolling along the vessel wall [107]. Indeed, HS was shown to interact with L- and P-selectin, and L-selectin-HS complex formation appears to play an important role in enhancing leukocyte rolling mediated by P-selectin-PSGL-1 interactions [108, 114]. As will be pointed out in detail in the following section, Sdc1 plays a crucial role in modulating the next step of leukocyte adhesion, which is mediated by interactions of leukocyte  $\beta 2$  integrins with ICAM-1. Moreover, HPSE-mediated degradation of HS is a means of regulating intraluminal crawling of leukocytes within blood vessels, a process that involves interactions between the leukocyte  $\beta 2$  integrin Mac-1 and endothelial ICAM-1 [115]. Finally, HS modulates leukocyte diapedesis not only by facilitating chemokine gradient formation, but also via interactions with the leukocyte integrins LFA-1 and VLA4, and their binding partners ICAM-1 and VCAM-1 (reviewed in [55, 108]). Notably, several of the HS binding molecular and cellular mediators of inflammation are regulated by Sdc1 and heparanase, respectively, as will be detailed in the following section.

### 4.3.1 *Lessons from Mouse Models*

The relevance of Sdc1 and HPSE as regulators of inflammatory processes *in vivo* has been explored with the help of transgenic and knockout mouse models in a variety of experimental models of inflammation and repair [32, 54, 55, 108]. Early studies in Sdc1 knockout mice revealed that these mice show increased recruitment of leukocytes to the endothelium of the ocular vasculature [116]. Bone marrow transplantation experiments demonstrated that the increased adhesion was due to the lack of Sdc1 on leukocytes rather than the endothelium. Notably, intravital microscopy of TNF $\alpha$ -stimulated mesentery venules demonstrated that loss of Sdc1 was associated not only with a massively increased adhesion of leukocytes to blood vessels, but also with a substantial increase in leukocyte diapedesis [116]. Further mechanistic studies revealed that increased adhesion of Sdc1-deficient

polymorphonuclear cells (PMNs) and monocytes to human umbilical vein endothelial cells (HUVEC) *in vitro* could be inhibited by heparin if the endothelium was not activated. The increased adhesion was not altered by heparin when Sdc1-deficient leukocytes were allowed to adhere to TNF $\alpha$ -stimulated HUVECs, suggesting that the Sdc1-dependent adhesion phenotype involves HS/heparin-sensitive and insensitive steps, that depend on the activation state of the endothelium [117]. Further studies employing Sdc1-deficient and WT leukocytes *in vitro* revealed that increased adhesion of Sdc1-deficient PMNs to ICAM-1 could be inhibited by heparin, suggesting a role for HS in this process [118]. Moreover, increased adhesion of Sdc1 KO PMNs to ICAM-1 could be efficiently blocked with antibodies directed against the leukocyte integrin CD18 [119]. In summary, these data suggest that the lack of Sdc1 on leukocytes may allow them to interact with non-activated endothelium, and enhances leukocyte-endothelial interactions in HS-dependent manner at the level of ICAM-1-CD18 interactions.

In addition to Sdc1, the role of heparanase in leukocyte recruitment has been studied *in vitro* and *in vivo*. *In vivo* studies in rats demonstrated that intraperitoneal injection of heparanase resulted in increased recruitment of inflammatory cells to the peritoneal cavity, and an increase in leukocyte rolling and adhesion in postcapillary venules, as evidenced by intravital microscopy of mesentery microvessels [120]. Moreover, *in vitro* adhesion assays showed that heparanase treatment increased neutrophil and mononuclear cell adhesion to HUVEC cells [120]. Surprisingly, in contrast, a study utilizing heparanase knockout and overexpressing mice found that monocyte, but not neutrophil, recruitment into peritoneal cavities inflamed by zymosan treatment depended on heparanase [121]. Moreover, although heparanase was upregulated in effector T-cells, it was not required for extravasation inside inflamed lymph nodes or skin in adoptive transfer experiments. However, in an experimental mouse model of sepsis-associated acute lung injury, inhibition of heparanase prevented endotoxemia-associated glycocalyx loss and neutrophil adhesion [122]. Moreover, in an experimental model of inflammation that utilized heparanase overexpressing vs WT mice, heparanase was shown to interfere with the process of intraluminal crawling of leukocytes within blood vessels [115]. Cleavage of HS by heparanase perturbed an experimentally applied gradient of the chemokine CXCL2 in the cremaster muscle, resulting in a loss of directionality of intraluminal leukocyte crawling. Finally, a study on transendothelial migration of hepatocellular carcinoma cells revealed that cell lines expressing high levels of heparanase show a higher transendothelial migration rate compared to cells with lower expression and that heparanase inhibition or downregulation suppressed this process both *in vitro* and *in vivo* [123]. In conclusion, in spite of context- and cell-type-specific effects, the aforementioned studies suggest that heparanase has a similar effect on leukocyte recruitment as the knockout of Sdc1.

As mentioned above, increased recruitment of Sdc1-deficient leukocytes to sites of inflammation was observed in a variety of experimental models of inflammation [55]. Interestingly, a range of similar or even identical disease models has been used to study the function of heparanase in inflammation employing transgenic and knockout mice with altered heparanase expression. In the following section, we will



discuss the effect of Sdc1 and heparanase on four selected experimental models of inflammation (contact allergy, colitis, kidney inflammation, and experimental autoimmune encephalomyelitis), as they will allow us to compare the individual functions of Sdc1 and heparanase in different inflammatory diseases.

### **Role of Sdc1 and Heparanase in Delayed-Type Hypersensitivity**

Delayed-type hypersensitivity (DTH) is a mouse model for allergic contact dermatitis, which consists of a sensitization phase involving covalent modification of surface proteins with haptens such as oxazolone or TNCB (2,4,6-trinitro-1-chlorobenzene), which are subsequently taken up and processed by dendritic and Langerhans cells. These cells migrate to lymph nodes and prime hapten-specific T cell populations, which are recruited and activated during the elicitation phase, resulting in cytokine and chemokine release, mast cell degranulation and massive leukocyte infiltration of the skin [124, 125]. Consistent with the previously described increase in leukocyte recruitment in Sdc1-KO mice, elicitation of an oxazolone-mediated DTH response resulted in increased leukocyte recruitment, and increased and prolonged edema formation. Compared to wild-type mice, expression of ICAM-1, cytokines (i.e., TNF $\alpha$  and IL-6), and chemokines (i.e., CCL5/RANTES, CCL-3/MIP-1 $\alpha$ ) was increased in Sdc1-deficient animals [119]. Interestingly, loss of Sdc1 can compensate for the loss of another proteoglycan, decorin, in this model: decorin-deficient mice show reduced DTH responses, associated with attenuation of leukocyte infiltration, which is overcome in the absence of Sdc1 [125]. However, while the *in vivo* data suggest that loss of Sdc1 releases the block in diapedesis observed in decorin-deficient mice, the detailed mechanisms underlying this observation still need to be elucidated. In the TNBS model of DTH, Sdc1-deficient dendritic cells migrated at a higher rate and faster to draining lymph nodes, resulting in an increased DTH response both in Sdc1 KO mice and in WT mice subjected to adoptive transfer of Sdc1 KO dendritic cells [126]. Moreover, upregulation of CCL2, CCL3, VCAM1 and talin, and a prolonged presence of CCR7 at the cell surface was observed on Sdc1 KO vs WT cells during dendritic cell maturation. Notably, Sdc1-KO dendritic cells showed an increased migration towards CCL21 and CCL19 compared to WT cells [126]. In addition to Sdc1, the role of heparanase during DTH has been studied *in vivo*. While Sdc1 expression is downregulated prominently in the epithelium of inflamed skin during oxazolone-induced DTH response [119], heparanase is upregulated particularly by the endothelium at the site of DTH-induced inflammation [127]. Moreover, heparanase-overexpressing mice showed a substantially increased DTH response compared to WT animals, whereas heparanase inhibition in WT mice resulted in a reduced DTH response and less vascular leakage [127]. In summary, these data suggest that upregulation of heparanase and absence of Sdc1 generate a similar, pro-inflammatory phenotype during DTH in mice. However, while Sdc1 negatively regulates endothelial leukocyte recruitment and dendritic cell migration during DTH responses, heparanase appears to primarily act at the level of the endothelium, where it regulates vascular permeability.



## **Role of Sdc1 and Heparanase in Anti-Glomerular Basement Membrane Glomerulonephritis**

Experimental anti-glomerular basement membrane (anti-GBM) nephritis is a model of inflammation that mimics aspects of the autoimmune disease Goodpasture syndrome [128]. This experimental animal model is based on the injection of antibodies directed against antigenic material from the GBM of mice into recipient mice. An initial heterologous phase, where leukocyte influx peaks within hours and albuminuria become apparent can be observed within 24 h. This phase is followed by an autologous phase during which endogenous anti-GBM IgG is produced, leading to persistent albuminuria [128]. In Wild-type (WT) mice, induction of anti-GBM glomerulonephritis resulted in an upregulation of Sdc1, and Sdc4 protein 2 and 18 h after induction of the disease, which normalized after 4 days [129]. In contrast, heparanase is upregulated both in the heterologous and autologous phase of the disease [130]. 4 and 8 days after administration of rabbit anti-GBM IgG, glomerular deposition of mouse anti-rabbit IgG was higher in Sdc1 KO compared to WT mice [129]. Notably, the numbers of PMNs and macrophages were significantly higher in inflamed glomeruli of Sdc1 KO mice in the heterologous phase, whereas the numbers of CD4+ and CD8+ T-cells were higher in Sdc1 KO mice in the autologous phase compared to WT. As a result, Sdc1 KO mice developed more severe albuminuria and showed worsened kidney function compared to WT mice. These changes were accompanied by significant increases of ICAM-1, L-selectin, IL-1 $\beta$ , MCP-1, IL-6 and IL-10 expression in Sdc1 KO mice vs WT during the heterologous phase, and in numerous ECM proteins (fibronectin, collagen IV, collagen XVIII, laminins, MMP7 and MMP9) along with L-selectin and MCP-1 during the autologous phase of the disease. Overall, Sdc1 KO mice showed a shift of the Th1/Th2 balance towards a Th2 response [129]. Compared to WT mice, heparanase-deficient mice showed better kidney function in this experimental model, which was accompanied by a reduced influx of PMNs and macrophages, reduced glomerular damage, and a reduction of the expression of numerous inflammatory factors along with reduced Sdc1 levels [130]. Heparanase KO mice showed reduced expression of both Th1 and Th2 cytokines. Reduced degradation of basement membrane HS was identified as a mechanistic aspect of the beneficial effects of heparanase deficiency, as shown *in vitro* using heparanase-silenced mouse glomerular endothelial cells, which displayed a lower transendothelial albumin passage compared to controls. It appears that Sdc1 and heparanase may play distinct mechanistic roles in this experimental model, although it is conceivable that heparanase deficiency may result in reduced Sdc1 ectodomain shedding, which may dampen the inflammatory response by reducing the influx of leukocytes [129, 130].

## **Sdc1 and Heparanase in Experimental Autoimmune Encephalitis (EAE)**

Experimental autoimmune encephalitis (EAE) is an experimental T-cell dependent *in vivo* model of multiple sclerosis. In EAE, antigen-specific CD4+ Th1 cells cause inflammatory damage in the central nervous system, thus mimicking the

demyelination, axonal loss and progressive paralysis caused by autoreactive T-cells in human multiple sclerosis [131, 132]. In the EAE model, Sdc1-deficient mice showed a higher severity of the disease compared to their WT counterparts and recovered more slowly [133]. Mechanistically, Sdc1 is upregulated at the transcriptional level, but shed from epithelial cells of the choroid plexus to the cerebrospinal fluid in WT mice, resulting in a loss of cell-surface bound CCL20 chemokine, which showed a partial co-localization with Sdc1 in naïve WT mouse brain. Notably, in Sdc1 KO mice, early recruitment of leukocytes, and levels of IL-6 were enhanced, resulting in recruitment of Th17 cells and aggravation of the inflammatory reaction. Moreover, enhanced plasma cell levels and higher levels of myelin oligodendrocyte glycoprotein-specific antibodies – a driving factor of the disease – in Sdc1 KO mice may be the underlying cause for delayed recovery from EAE [133]. The role of heparanase in EAE was studied applying recombinant heparanase via daily intraperitoneal administration starting from the day of immunization with proteolipid protein until day +7 or day +17 [134]. In this study, heparanase ameliorated the clinical signs of the disease. Moreover, the formation of clusters of inflammatory cells, as seen in WT mice in the white matter zone of the spinal cord, was not observed in heparanase-treated mice. Mechanistic *in vitro* and *in vivo* experiments revealed that heparanase treatment caused a shift of the cytokine spectrum toward Th2 cytokines (IL-4, IL-6, IL-10), resulting in an inhibition of a mixed lymphocyte reaction and mitogen-induced splenocyte proliferation [134]. While these data clearly demonstrate a role for both Sdc1 and heparanase in EAE, both molecules acted via different mechanisms in the two studies presented, and the focus on different mechanistic aspects and application of different analytical assays impedes a direct comparison. Further investigations may help to clarify the possible interplay of Sdc1 and heparanase in EAE. For example, it could be envisaged that heparanase is involved in the shedding of Sdc1, however, it is not clear if it is expressed in the epithelium of the choroid plexus during the EAE reaction. Moreover, while upregulation of IL-6 in Sdc1 KO mice was seen as a factor promoting disease progression in the study by Zhang et al. [133], it was presented as part of an anti-inflammatory Th2 signature in the study by Bitan et al. [134]. Differences in the time-courses of IL-6 expression, and in the tissues the cytokine is derived from, may account for the deviating interpretations of the respective phenotype.

### **Role of Sdc1 and Heparanase in Inflammatory Bowel Disease and Colitis-Associated Colon Cancer**

Inflammatory bowel diseases are complex diseases that constitute a major health burden, which is characterized by an aberrant immune response in the gastrointestinal tract [135]. An important experimental model of inflammatory bowel diseases like Crohn's disease and ulcerative colitis is the dextran sodium sulfate (DSS) colitis murine model, which is based on DSS-induced epithelial cell injury, which is followed by the entry of luminal bacteria and associated antigens into the mucosa

and a subsequent inflammatory reaction [136]. Of note, reduced expression of Sdc1 has been observed in patients with ulcerative colitis, which has been linked to disrupt healing of colonic ulcers [137].

When the mechanistic role of Sdc1 in colitis was studied by inducing the disease with 3% DSS in Sdc1 KO mice, a substantial increase in mortality was observed compared to WT animals (61% versus 5%) [118]. Sdc1 KO mice showed prolonged recruitment of leukocytes and impaired mucosal healing, which were accompanied by an upregulation of TNF $\alpha$ , CCL3/MCP1, and VCAM-1 in the inflamed tissue. Notably, treatment with enoxaparin improved mucosal wound repair and reduced lethality of Sdc1 KO mice, suggesting that heparin may be able to compensate for the loss of the heparan sulfate chains of Sdc1 and the associated poor outcome in this disease model [118]. Of particular clinical relevance, chronic inflammatory bowel disease increases the risk of colon cancer [138], a disease that is also associated with a downregulation of Sdc1 expression in the colonic epithelium [139, 140]. Colitis-associated cancer can be experimentally modeled in mice by application of the carcinogen azoxymethane (AOM) and subsequent induction of chronic colitis with DSS [141]. Sdc1 KO mice developed more severe inflammation during chronic DSS colitis, associated with increased recruitment of inflammatory cells, increased crypt damage, and increased weight loss compared to WT mice [142]. IL-6 expression and activation of STAT3 were increased in the inflamed colon tissue of Sdc1 KO vs. Wt mice. Notably, Sdc1 KO mice formed larger tumors than their WT controls in the AOM-DSS model, which was attributed to increased activation of STAT3, and an upregulation of cyclin D1, CCL2, and c-Myc in the tumor tissue [142]. Overall, these data suggest that the increased inflammation and tissue damage in the absence of Sdc1 drive colon cancer progression via enhanced signaling through the IL-6/STAT pathway. While Sdc1 is downregulated in IBD and colon cancer, heparanase is upregulated in the inflamed and tumor tissue in these diseases [143, 144], and in the experimental AOM / DSS and the acute and chronic DSS animal models [145]. Notably, when mice that transgenically overexpress heparanase were subjected to DSS colitis, high heparanase expression preserved the inflammatory conditions, along with increased expression of TNF $\alpha$  and Cyclin D1 (thus showing a resemblance to Sdc1 KO mice in this model) [142], and a substantially increased recruitment of TNF $\alpha$ -expressing macrophages [145]. Similar to Sdc1 KO mice, tumors were larger in heparanase transgenic mice [142, 145]. Moreover, the number of tumors was higher compared to WT mice, and tumor angiogenesis was enhanced. Overall, these data suggest that heparanase drives a vicious cycle that promotes colitis and chronic-inflammation-related tumorigenesis. The phenotype shows undeniable similarities to the Sdc1 KO mouse in this experimental model, however, the interrelation between these molecules still awaits experimental investigation. Table 4.2 summarizes the phenotypes and molecular mechanisms of the mouse models that were presented in this section, revealing similarities and disparities in the mechanisms by which Sdc1 and heparanase modulate inflammatory processes *in vivo*.

**Table 4.2** Phenotype of Sdc1- and heparanase-manipulated mice in experimental models of inflammation

Disease model	Gene manipulated	Phenotype compared to WT	Molecular changes
Contact allergy (DTH) [119, 126, 127]	Sdc1 knockout	Increased leukocyte recruitment, increased and prolonged edema formation, increased dendritic cell migration and chemotaxis in vivo and in vitro	Sdc1 downregulated in WT; increased expression of ICAM-1, TNF $\alpha$ , IL-6, CCL5 and CCL-3 in inflamed KO tissue; increased expression of CCL2, CCL3, VCAM1 during KO dendritic cell maturation
	Heparanase overexpression and inhibition	HPSE overexpression leads to increased DTH response, whereas HPSE inhibition in WT mice resulted in a reduced DTH response and less vascular leakage.	HPSE upregulated in WT endothelium; IFN $\gamma$ induces HPSE in vitro.
Goodpasture syndrome (anti-GBM nephritis) [129, 130]	Sdc1 knockout	Increased glomerular deposition of mouse anti-rabbit IgG, and of early infiltration with PMNs and macrophages and late infiltration with CD4+ and CD8+ T-cells. More severe albuminuria and worsened kidney function. Shift of the Th1/Th2 balance towards a Th2 response.	Increased ICAM-1, L-selectin, IL-1 $\beta$ , CCL2, IL-6 and IL-10 during early phase, and increased fibronectin, collagen IV, collagen XVIII, laminin, MMP7, MMP9, L-selectin and CCL2 during late phase.
	Heparanase knockout	Better kidney function, reduced influx of PMNs and macrophages, reduced glomerular damage. Lower transendothelial albumin passage in vitro.	Reduced expression of numerous inflammatory factors (both Th1 and Th2) and of Sdc1. Reduced degradation of basement membrane heparan sulfate.
Multiple sclerosis (EAE) [133, 134]	Sdc1 knockout	Increased early recruitment of leukocytes, recruitment of Th17 cells aggravates inflammatory reaction. Enhanced plasma cell levels.	Increased Sdc1 shedding from choroid plexus of WT mice leads to loss of cell-surface bound CCL20. Higher IL-6 levels in KO mice. Higher levels of myelin oligodendrocyte glycoprotein-specific antibodies in KO mice.
	Heparanase enzyme therapy	Heparanase ameliorates clinical signs of EAE. Reduced formation of inflammatory cell clusters. Inhibition of a mixed lymphocyte reaction and of mitogen-induced splenocyte proliferation ex vivo.	Heparanase treatment causes shift toward Th2 cytokines (IL-4, IL-6, IL-10).

(continued)

**Table 4.2** (continued)

Disease model	Gene manipulated	Phenotype compared to WT	Molecular changes
Ulcerative colitis (DSS colitis)[218, 142, 145]	Sdc1 knockout	Substantial increase in mortality, prolonged recruitment of leukocytes and impaired mucosal healing.	Upregulation of TNF $\alpha$ , CCL3 and VCAM-1 in KO mice.
	Heparanase overexpression	High heparanase preserves the inflammatory conditions, substantially increased recruitment of TNF $\alpha$ -expressing macrophages.	Increased expression of TNF $\alpha$ and Cyclin D1 in heparanase overexpressing mice.
Colitis-associated colon cancer (AOM-DSS) [142, 145]	Sdc1 knockout	More severe inflammation during chronic DSS colitis (increased recruitment of inflammatory cells, increased crypt damage, and increased weight loss) compared to WT mice. Sdc1 KO mice form larger tumors.	IL-6 expression and activation of STAT3 are increased in inflamed Sdc1 KO colon tissue. Increased activation of STAT3, and upregulation of cyclin D1, CCL2, and c-Myc in Sdc1 KO tumor tissue.
	Heparanase overexpression	Larger size and higher incidence of tumors, enhanced tumor angiogenesis.	Increased expression of TNF $\alpha$ and Cyclin D1.

#### 4.4 Syndecan-1 and Heparanase as Pathogenesis Factors and Therapeutic Targets in Malignant Disease

The investigation of biopsies and blood from cancer patients has revealed that Syndecan-1 and heparanase are mis-expressed in a large number of cancers, underscoring their clinicopathological relevance (see Table 4.1). As of August 2019, close to 1000 publications for each molecule have described a role for Syndecan-1 or heparanase in different types of cancer in the PubMed database, generating a need to present selected examples of their molecular functions in malignant disease. The molecular mechanisms that govern tumorigenesis and cancer progression have been conceptually summarized by Hanahan and Weinberg in their landmark article ‘The Hallmarks of Cancer’, which originally included sustained proliferation, evasion of growth suppression, death resistance, replicative immortality, induced angiogenesis, and initiation of invasion [146]. Additional hallmarks were defined 10 years later in an updated version of the article and included the aspect of chronic inflammation (see Sect. 4.3) and avoidance of immune destruction [147] (see reference [148] for a recent review on the role of cell surface proteoglycans in immunotherapy). Notably, proteoglycans such as Sdc1 and enzymes like heparanase, which utilize HS proteoglycans as substrates have been shown to modulate most, if not all of these Hallmarks [148, 149]. For example, regarding the cancer hallmark of *sustained proliferation*, Syndecan-1 acts as a co-receptor for receptor tyrosine kinases,

thus contributing to proliferative signaling and tumor growth, as demonstrated for example in breast cancer, colon cancer, and multiple myeloma [22, 97, 108]. Notably, it has been shown that the soluble ectodomain of Syndecan-1 can competitively inhibit mitogenicity of the cytokine FGF2, whereas platelet heparanase is able to convert the HS chains of soluble Syndecan-1 from an inhibitor into heparin-like HS fragments that substantially activate FGF-2 mitogenicity [150]. Moreover, both latent heparanase and its mature active form promote signaling through multiple pathways with relevance to tumor progression, including the Src, MAPK, HGF-, IGF-, and EGF-receptor pathways [151]. The *insensitivity to antigrowth signals* has been defined as an additional hallmark of cancer. An important growth-inhibitory cytokine and mediator of epithelial-to-mesenchymal transition is TGF $\beta$  which has been shown to be negatively regulated by Syndecan-1 during liver fibrogenesis [152]. Interestingly, heparanase-mediated shedding of Syndecan-1 has been shown to result in an upregulation of TGF $\beta$  in hepatocellular carcinoma, providing a link between heparanase and Syndecan-1 in the regulation of growth-inhibitory signals [153]. The *enabling of replicative immortality* adds to the six original hallmarks of cancer [146]. Replicative immortality is a shared feature of cancer stem cells and is partially linked to the activity of telomerase, which prevents the shortening of chromosome ends [154]. Syndecan-1 has emerged as an important regulator of the cancer stem cell phenotype: The reduction of a wnt-responsive precursor cell population has been identified as a molecular mechanism underlying the resistance of juvenile Sdc1 KO mice to breast cancer and additional forms of experimentally induced cancer [154–156]. Moreover, siRNA mediated knockdown of Sdc1 in human breast cancer cell lines representative of different molecular classifications resulted in a reduction of stem cell properties, including the expression of typical cancer stem cell markers (CD44<sup>+</sup>/CD24<sup>low</sup>, side population, ALDH) as well as colony and mammosphere formation [157, 158]. Modulation of the stemness-related notch, wnt, and IL-6/STAT signaling pathways by Syndecan-1 was identified as the mechanistic basis for this finding. Notably siRNA knockdown of Syndecan-1 also resulted in an upregulation of heparanase expression in this model system [159]. Indeed, modulation of stem cell properties in the context of malignant disease has not only been ascribed to Syndecan-1, but also to heparanase, which acts in a context-dependent manner. In breast cancer, nuclear heparanase has been shown to induce tumor cell differentiation [160]. Moreover, heparanase-mediated modifications in the bone marrow microenvironment regulate the retention and proliferation of hematopoietic progenitor cells [161], modulate clonogenicity, proliferative potential and migration of mesenchymal stem cells in the bone marrow [162], and shift the differentiation potential of osteoblast progenitors within the myeloma bone microenvironment from osteoblastogenesis to adipogenesis [163]. Moreover, embryonic stem cells overexpressing heparanase proliferated faster than wild-type controls in culture and formed larger teratomas in vivo [164], indicating an important role for heparanase in (cancer) stem cells and the modulation of replicative immortality. Closely linked to the previous hallmarks of cancer is the *resistance to cell death*, as it allows for the proliferation of tumor cells which carrying mutations, ultimately leading to another enabling characteristic, genome instability.

Several studies have documented a role for Syndecan-1 as a regulator of apoptosis in an oncological setting. For example, Syndecan-1 suppresses apoptosis in multiple myeloma by activating the IGF1 receptor [88], and in endometrial cancer by enhancing Erk and Akt activation [165]. In contrast, Syndecan-1-dependent MAPK-signaling was shown to be of importance for the pro-apoptotic effect of the n-3 polyunsaturated fatty acid docosahexaenoic acid in prostate and breast cancer cells. Likewise, heparanase modulates cancer cell apoptosis in a context-dependent manner [166, 167]. Early studies indicated a positive correlation between heparanase expression and spontaneous apoptosis in hepatocellular carcinoma [168]. Along these lines, an apoptosis-enhancing function of heparanase was revealed in growth-hormone secreting pituitary tumor cells [169]. In contrast, orthotopic xenograft experiments utilizing heparanase overexpressing breast cancer cells identified heparanase as a survival factor for breast cancer cells in vivo [170]. Similarly, overexpression of heparanase inhibited apoptosis in cervical cancer cells [171]. Since Syndecan-1 and heparanase modulate similar signal transduction pathways, it can be envisaged that they cooperate in the regulation of cell death, however, more studies are needed to confirm the mechanistic involvement in more detail. The *induction of angiogenesis* is another hallmark of cancer that is modulated both by Syndecan-1 and heparanase. The sprouting and growth of blood vessels from existing blood vessels is a carefully orchestrated physiological process, that is hijacked by tumor cells via secretion of angiogenic factors and by causing an imbalance between pro- and anti-angiogenic factors [89, 172]. Tumor angiogenesis is a prerequisite for supplying the tumor with nutrients and oxygen, and for promoting metastatic spread via the circulation. Of note, syndecan-1 expression has been identified as part of a molecular signature marking an angiogenic switch in early stages of breast cancer [173] and stromal syndecan-1 expression was shown to correlate with microvessel density and blood vessel area both in human breast cancer specimens and in xenograft models [174]. In vivo studies have demonstrated that absence and overexpression of syndecan-1 modulate angiogenesis via enhanced leukocyte recruitment and by promoting proteolysis, respectively [42, 116]. Moreover, syndecan-1 promotes angiogenesis as a classical co-receptor for angiogenic factors such as FGF-2, VEGF, and c-Met [97, 173, 175]. In addition, MMP9-induced syndecan-1 is part of a mechanism that is responsible for radiation-induced angiogenesis in medulloblastoma [176]. Moreover, syndecan-1 regulates tumor angiogenesis via lateral association of its extracellular core protein domain with proangiogenic integrins [86, 89]. Notably, in multiple myeloma, heparanase stimulates shedding of syndecan-1, which leads to a deposition of VEGF that is bound to the syndecan-1 HS chains in the ECM, where it stimulates endothelial invasion [50]. Heparanase-induced syndecan-1 shedding also promotes hepatocarcinoma lymphangiogenesis via the VEGF-C/ERK pathway [175]. However, heparanase promotes tumor angiogenesis not only via induction of syndecan-1 shedding but also via the release of proangiogenic factors from heparan sulfate on cell surfaces and the ECM, through Src-dependent upregulation of VEGF expression, enhancement of Akt signaling and stimulation of PI3K- and p38-dependent endothelial cell migration and invasion [177].



Finally, the *activation of invasion and metastasis* has been defined as a hallmark of cancer, which is of utmost importance as cancer-associated metastasis to vital organs is the leading cause of cancer-related mortality [178]. The misexpression of Syndecan-1 that is observed in numerous tumor entities (Table 4.1) contributes to metastatic behavior in several ways. While the membrane-bound form of Syndecan-1 had an invasion-inhibiting effect on the human breast cancer cell line MCF-7, overexpression of the soluble ectodomain and of the intact proteoglycan resulted in a substantial increase in Matrigel invasion chamber assays [22, 159]. The increase in invasiveness could be attributed to a downregulation of the MMP inhibitor TIMP-1 and upregulation of urokinase-type plasminogen activator receptor (uPAR) in cells overexpressing soluble Syndecan-1. Moreover, a downregulation of the anti-invasive homotypic cell adhesion molecule was observed both in MCF-7 breast cancer cells overexpressing soluble Syndecan-1, and in the highly invasive breast cancer cell line MDA-MB-231 upon Syndecan-1 depletion [22, 159]. While this observation may suggest a potential involvement of syndecan-1 in regulating the pro-metastatic process of epithelial-to-mesenchymal transition (EMT) in breast cancer, no consistent upregulation of mesenchymal markers was observed upon Syndecan-1 depletion in the same experimental system [157]. However, an indirect regulatory impact on EMT of prostate cancer cells was recently proposed, involving alterations in EMT-regulating microRNA processing that was modulated by syndecan-1-dependent changes in expression of the miRNA processing enzyme, Dicer [179]; Syndecan-1 promotes EMT in hepatocellular carcinoma by enhancing TGFbeta function [180]. In breast cancer, time-lapse microscopy analysis revealed that silencing of Syndecan-1 in human MDA-MB-231 breast cancer cells results in a substantial increase in cell motility [159] and migration through fibronectin-coated filter membranes [181]. Inhibitor studies and signal transduction analysis revealed that this increase could be attributed to increased activation of focal adhesion kinase and dysregulation of Rho-GTPases. In lung cancer, Syndecan-1 was shown to inhibit metastasis in vivo and invasiveness in vitro via a mechanism that relies on the cleavage of Syndecan-1 core protein by ADAM17 and gamma-secretase, and the generation of a cytoplasmic fragment with biological activity [48]. Moreover, similar to its function during inflammation, Syndecan-1 promotes chemotaxis of breast and lung cancer cells as a co-receptor for chemokines such as MIP1 [182]. Of note, heparanase-mediated processing of Syndecan-1 has an important role in the regulation of metastasis. Heparanase promotes shedding of syndecan-1 in myeloma and breast cancer cells, whereas syndecan-1 promotes processing of heparanase [45, 183]. Moreover, heparanase regulates the secretion of tumor exosomes, including their Syndecan-1 content, thus driving metastatic behaviour [81] (Fig. 4.3). Finally, Syndecan-1 can inhibit heparanase-mediated in vitro invasion of melanoma cells in an HS-dependent manner, indicating complex regulatory circuits between heparanase and Syndecan-1 [184]. Apart from the mechanisms involving Syndecan-1 processing, heparanase has been shown to promote invasive growth and metastasis of a wide range of tumor entities [185] (Table 4.1). Indeed, heparanase promotes metastasis via several mechanisms, as demonstrated

in transgenic mouse models overexpressing heparanase and in tumor cell lines subjected to heparanase silencing or inhibition. These mechanisms including a degradation of basement membrane HS, thus removing a physical barrier of metastasis in epithelia and endothelia [186, 187], through the release of stored growth factors and chemokines from the ECM and cell surfaces, thus promoting chemotaxis and pro-invasive signaling [103, 188, 189], and through the impact on exosome secretion and function [190], as mentioned above (Fig. 4.3).

Given the importance of Syndecan-1 and heparanase as prognostic markers in various tumor entities, and as mechanistically relevant factors in driving cancer progression, it is no surprise that these molecules have been identified as therapeutic targets in malignant disease [188, 191]. Regarding a therapeutic targeting of Syndecan-1, it has to be considered that it is highly expressed on several healthy epithelia, including the gut, skin, and lung [8, 41], thus increasing the danger of an unfavorable side effects profile upon therapeutic targeting. Nevertheless, promising results have been achieved upon Syndecan-1-targeting in preclinical models. One approach includes the interference of Syndecan-1 integrin interactions with the Syndecan-1-derived peptide synstatin, which successfully disrupted tumor angiogenesis *in vivo* [86, 89]. Moreover, Syndecan-1-conjugates with cytotoxic drugs (i.e., indatuximab and ravtansine) have shown efficacy in preclinical *in vivo* models of triple-negative breast cancer [192]. In patients with relapsed or refractory multiple myeloma, this antibody-drug conjugate entered phase I/IIa clinical trials and showed efficiency with respect to clinical activity, although side effects on epithelial tissues were also observed [148, 193]. Finally, CAR-T cell therapy has been employed to target Syndecan-1, showing efficacy in a preclinical model of multiple myeloma, and promising results in patients, including stable disease for over 3 months, and a reduction of myeloma cells in the peripheral blood, respectively [148, 194, 195]. Compared to Syndecan-1, heparanase represents an even more attractive drug target, as it is only expressed in a few cell types in healthy adults (mainly leukocytes), thus limiting potential therapy-associated side effects [189]. A recent comprehensive review provided an overview of different heparanase inhibitors that have been explored in preclinical studies and partially also in clinical trials [196]. These inhibitors include heparin and its derivatives (e.g., roneparstat/SST0001 and mupofastat/PI-88), nucleic acid-based inhibitors (e.g. defibrotide), synthetic inhibitors (e.g., suramin) and heparanase-neutralizing antibodies. Apart from targeting tumor cell-derived heparanase, these inhibitors can also have an impact on the tumor stroma and inhibit processes that support tumor progression, e.g. tumor-promoting inflammation and angiogenesis. Indeed, several of these inhibitors have demonstrated anti-tumor, anti-angiogenic and anti-metastatic efficacy in preclinical animal models of the disease, including e.g. myeloma [51], lymphoma [197], and sarcoma models [198]. Moreover, heparanase inhibitors have shown to be largely well-tolerated in phase I and phase II clinical trials [196, 199–201], providing a positive outlook for progress in targeted cancer therapy in the near future (see Nosedá and Barbieri; Hammond and Dredge; Chhabra and Ferro, Chaps. 19, 21 and 22 in this volume).

## 4.5 Concluding Remarks

Syndecan-1 and heparanase fulfill important and pleiotropic physiological functions during development and tissue homeostasis. Notably, several processes regulated by Syndecan-1 and heparanase are also relevant in the context of inflammatory and malignant disease. These processes include the regulation of cell proliferation and survival, cell motility and cell invasion, leukocyte recruitment and angiogenesis, which are achieved by modulation of growth factor-, chemokine- and morphogen-mediated signaling processes and via regulation of the composition and functional properties of the ECM. While Syndecan-1 and heparanase retain some autonomous functions, many of their properties are functionally linked. Syndecan-1 can act as a substrate for heparanase, and heparanase promotes Syndecan-1 shedding as an important mechanism of conversion of this membrane-bound molecule into a soluble paracrine effector. In turn, Syndecan-1-dependent signaling mechanisms regulate heparanase expression, and the cytoplasmic domain of Syndecan-1 plays an important role in heparanase processing. Moreover, ligands bound to the HS chains of Syndecan-1 can be released by the action of heparanase, resulting in an altered functionality. In contrast, the HS chains of Syndecan-1 can have an inhibitory impact on heparanase activity, depending on their fine structure. In most cases, synergistic effects of Syndecan-1 and heparanase are observed, which contribute to the pathogenesis of inflammatory diseases and tumor progression. Therefore, Syndecan-1 and heparanase have emerged as important targets for therapeutic approaches. Due to their pleiotropic functions and their mechanistic involvement in inflammatory and malignant diseases, their targeting represents a highly promising approach, as it can be expected that therapeutics simultaneously inhibit multiple processes related to disease progression. Substantial evidence from preclinical models and promising results from phase I/II clinical trials provide a promising perspective regarding the translation into a clinical setting.

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