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Proteoglycans in Chronic Liver Disease and Hepatocellular Carcinoma: An Update

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1. Introduction

The last four decades witnessed a brilliant career of proteoglycans (PGs). Once regarded as mere space-fillers or passive structural components of matrices and charge-selective barriers, these fascinating molecules have been increasingly acknowledged as key players in cell-cell and cell-matrix communication, and have become recognized as modulators of most, if not all, aspects of cell behavior including survival, proliferation, and migration. Simultaneously, the range of disease processes with known involvement of PGs has steadily expanded, now covering areas as diverse as host-pathogen interactions, regulation of pathologic fibrogenesis, and tumor progression. Characteristic alterations of PGs in various human malignant tumors, including HCC, were first described more than 20 years ago (for an early review, see Tímár & Kovalszky, 1995).

PGs, glycanated proteins with extensive posttranslational modifications, consist of a protein core and one or more long, linear, sulfated polysaccharide chains, called glycosaminoglycans (GAGs). GAGs are ligated to the protein core at specific serine, threonine, or asparagine residues, although the exact signal sequences that designate the position of attachment are mostly unknown. The multifunctionality of PGs arises from their inherently complex structure: some functions are assigned to the core protein, while others are fulfilled by the GAG chains.

The synthesis of each GAG chain (recently reviewed by Ly et al., 2010) is introduced by the attachment of a short linkage region to Ser in the case of heparan sulfate (HS)/heparin and chondroitin sulfate/dermatan sulfate (CS/DS), and either Asn or Ser/Thr in the case of keratan sulfate (KS) type I and type II, respectively. During the elongation phase of GAG synthesis, acetylated hexosamine and hexuronic acid or galactose residues are added in an alternating fashion to the growing polysaccharide chain. GAGs are classified by their disaccharide composition: the dimeric building block is N-acetyl-glucosamine / glucoronic acid in HS and heparin; N-acetyl-galactosamine / glucoronic acid in CS and DS; and N-acetlyl-glucosamine / galactose in KS. Completed GAG chains then undergo various chemical modifications including *N*-deacetylation, *N*- and *O*-sulfation, and epimerization of the hexuronic acid. Heparin, for example, differs from HS in the extent of sulfation (heparin is sulfated uniformly and nearly exhaustively, whereas HS is sulfated only partially and in a

patterned manner); and DS differs from CS in the degree of uronic acid epimerization (0% in CS vs. 1-100% in DS.) The extent and pattern of modifications not only vary between different GAGs and PGs, but also depend on the type and actual state of a cell, which contributes a great deal to the biological diversity of PGs. If this were still not enough of versatility, PGs may undergo further editing once they are in place: in the matrix or on the cell surface, they may be subject to the action of endoglycosidases that cleave the GAG chain, proteases that cut the protein core, and endosulfatases capable of removing sulfate groups from internal sugar residues.

Historically, PGs were sorted by the type of their GAG chain into one of the categories HSPG, CS/DSPG, or KSPG. Later, however, the discovery that several PGs carry more than one type of GAG (i.e., syndecans and betaglycan carry both HS and CS; aggrecan carries both CS and KS II) prompted a new classification based on structure and tissue localization. In this revised system, each PG belongs to one of three major families: 1) small leucine-rich proteoglycans or SLRPs; 2) modular PGs, further divided into a) hyalectans or hyaluronan-binding PGs and b) non-hyaluronan-binding PGs of the basement membrane; and 3) cell surface PGs. Nevertheless, both the old and new classifications fall short of being perfect; neither is free of overlaps, and neither can properly accommodate, for example, serglycin or endocan. In this review, we shall follow a sort of "hybrid" classification that fits best for our purposes.

A complete listing of all currently known PGs seems unnecessary here (for a comprehensive review, the Reader is referred to Esko et al., 2009); this paper is restricted in scope to PGs present in the healthy or diseased liver, and will concentrate on those involved in, or affected by, chronic liver disease and hepatocarcinogenesis. Also, with a focus on human disease, PGs reported to be present in the liver of experimental animals but not of humans will be omitted.

2. Proteoglycans in the liver

The healthy liver is a dominantly parenchymatous organ with relatively scarce stroma. Consequently, cell surface PGs expressed by hepatocytes are considerably more abundant than matrix PGs, either small or modular. Chronic liver diseases, on the other hand, are hallmarked by the accumulation of connective tissue, and PGs, along with other matrix constituents, become massively deposited as fibrosis progresses. Hepatocarcinogenesis is accompanied by further alterations in liver PG profile. These disease-associated changes are reflected in gene expression levels (i.e., of PG core proteins and GAG synthesis / modification enzymes), in the abundance and/or localization of PGs, and in the quantity and structure of GAGs in the tissue. Specific PGs present in the healthy or diseased liver, their pathology-related changes, as well as known or proposed functions in liver physiology or disease, are listed concisely in **Table 1** (on pages 3-4), and discussed in detail in the following sections.

2.1 SLRPs

The family of SLRPs, extracellular PGs characterized by relatively small (approx. 30-70 kDa) core proteins with leucine-rich repeats and conserved cysteine-containing motifs, currently counts 18 members divided into 5 classes (Schaefer & Iozzo, 2008; Schaefer & Schaefer,

	Healthy liver		Chronic liver disease			HCC			
	Localization	Main physiological roles	Localization	Rel. expr.	Proposed role	Localization	Rel. expr.	Proposed role	Prognostic significance
SLRPs									
Decorin	CV, PT CT, sin w	regulation of collagen fibrillogenesis and TGFβ signaling	fibrotic CT, sin w	↑	antifibrotic	stroma	↓	antitumor	
Biglycan		regulation of TGFβ signaling; endogenous TLR ligand	fibrotic CT, sin w	↑					
Modular HSPGs									
Perlecan	all BMs, sin w, sin ECs, PT MCs, BV w	migration, proliferation, differentiation, angiogenesis	all BMs, sin w, BV w, fibrotic CT	↑		vascular w	↑	pro-angiogenic	
Agrin	BD BM, PT BV w	postsynaptic differentiation, immune cell communication, cytoskeletal organization	BD BM, PT BV w	↑		vascular w	↑	pro-angiogenic?	
Collagen type XVIII / endostatin	all BMs, sin w, BV w	BM organization, angiogenesis	hep, activated HSCs; all BMs, sin w↑, BV w	↑		tu hep?, vascular w	↑↓	anti-angiogenic?	debated - high or low expr. ~ adverse progn.?
Cell membrane HSPGs									
Syndecan-1	hep	Common roles of syndecans: cytoskeletal and ECM organization; cell-cell and cell-ECM interactions; co-receptor function	hep	↑	HBV, HCV coreceptor ???	tu hep	↑↓	enhances migration; inhibits EMT	reduced expr. ~ high metastatic potential
Syndecan-2	PT MCs					stromal MCs	↑		
Syndecan-3	PT BV ECs, HSCs		activated HSCs, MΦs	↑		BV w, ECs			
Syndecan-4	hep					tu hep	↑	enhances migration	

Glypican-3	not expressed	regulation of growth factor signaling (Wnt, Hh, IGF)	not expressed			tu hep	↑	enhances tu cell growth & migration	high expr. ~ adverse progn.
Other cell membrane PGs									
Betaglycan		co-receptor for TGFβ family members	activated HSCs	↓			↓	tumor suppressor	high expr. ~ lower grade
CD44(v3)	hep (weak)	receptor for hyaluronan and other ECM components	activated progenitors	↑	regeneration	tu hep (cancer stem cells?)	↑	enhances metastasis	high expr. ~ vascular invasion
Neuropilin-1	sin ECs, PT BV Ecs	axonal guidance; co-receptor for VEGFs	sin ECs; activated HSCs	↑	pro-fibrotic	tu BV ECs, tu hep	↑	pro-angiogenic?	
Hyalectans									
Versican			activated HSCs?						
Secreted PGs									
Endocan	not expressed	enhances HGF signaling	not expressed			tu BV ECs, tu hep	↑	pro-angiogenic, enhances tu invasion & growth	high tu expr. & serum levels ~ adverse progn.
PG-100	weakly expressed		reactive ductules	↑					

Abbreviations: BD, bile duct; BM, basement membrane; BV, blood vessel; CT, connective tissue; CV, central vein; EC, endothelial cell; EMT, epithelial-to-mesenchymal transition; HBV/HCV, hepatitis B/C virus; HSC, hepatic stellate cell; HSPG, heparan sulfate PG; MC, mesenchymal cell; MΦ, macrophage; PT, portal tract; rel. expr., expression relative to normal liver tissue; sin, sinusoid(al); SLRP, small leucine-rich PG; tu, tumor(al); w, wall

Table 1.

2010). While most SLRPs carry CS/DS or KS chains, some of them are non-classical PGs lacking GAG chains altogether, and can thus be considered as "honorary" members of the PG superfamily that have been grouped together with SLRPs on the basis of structural and functional homology. So far, only three SLRPs were found in the liver: decorin, the prototypical member of the family; biglycan; and asporin, a non-canonical, GAG-less relative of the former two. Whereas reports leave ambiguity regarding the mere presence of asporin (its mRNA was abundant in the human liver but undetectable in mice) (Lorenzo et al., 2001; Henry et al., 2001), decorin has been widely implicated in liver fibrogenesis, and may also play a role in the regulation of hepatocellular carcinoma (HCC) growth. Knowledge on biglycan in the liver is much more limited; nevertheless, mentioning will be made of it.

Decorin, the archetypal SLRP, is glycanated with a single CS/DS chain, and was originally described as a regulator of collagen fibrillogenesis. Later, an increasingly complex picture has emerged: decorin was found to modulate the signaling of transforming growth factor-β1 (TGF-β1), a key stimulator of fibrogenesis, and it also became evident that decorin establishes contacts with multiple receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR), insulin-like growth factor-I receptor (IGF-IR), and Met (Iozzo & Schaefer, 2010). Decorin has a broad tissue distribution, being most abundant in the skin, connective tissues, muscles, and the kidney (Kalamajski & Oldberg, 2010).

In the healthy liver, strong decorin immunoreaction is solely seen in the Glisson's capsule, with some positive labeling around central veins, and only delicate, spot-like extracellular staining in the periportal connective tissue and, occasionally, in sinusoidal walls. In the course of chronic liver injury, however, decorin accumulates in the areas of periportal and bridging fibrosis, and becomes increasingly deposited in sinusoidal walls as capillarization ensues. Co-localization studies suggest association of decorin with collagen fibers, and its interaction with TGF-β1 (Dudás et al., 2001).

Decorin can directly bind and sequester TGF-β1, or indirectly influence its effect via association with the LRP-1 receptor (Cabello-Verrugio & Brandan, 2007). While the net outcome of these interactions depends on the cell type, decorin seemed to inhibit TGF-β1-dependent fibroblast proliferation and matrix production in the context of experimental renal fibrogenesis (Isaka et al., 1996). Importantly, decorin exerted a similar inhibitory effect on a human hepatic stellate cell (HSC) line *in vitro* (Shi et al., 2006). Since activated hepatic stellate cells are the major culprits in liver fibrosis, decorin might limit worsening of the condition, and the use of decorin as a TGF-β1 blocking agent for the treatment of chronic liver disease has been repeatedly proposed (Breitkopf et al., 2005). A recent report provides indirect support to this approach, demonstrating increased susceptibility to thioacetamide-induced fibrogenesis, and impaired recovery from established fibrosis in decorin-null mice (Baghy et al., 2011). The absence of decorin, with consequently higher activity of TGF-β1, not only increased fibrogenesis but also impeded resolution of fibrosis by interfering with matrix metalloprotease action. However, the transferability of these results to human liver fibrosis is unclear. At least, massive accumulation of decorin in the course of chronic liver disease indicates that, even if interpreted as a defense mechanism, overexpression of decorin is largely inefficient in preventing fibrosis. Being itself upregulated by TGF-β1 in HSCs (Baghy et al., 2011), the deposition of decorin in the connective tissue is more likely a by-product of fibrogenesis rather than a protective reaction against it.

While little is known about the involvement of decorin in hepatic carcinogenesis, available studies unequivocally assign an antitumor role to decorin in HCC. Decorin has been reported to be downregulated in HCC tissue relative to the normal liver (Miyasaka et al., 2001; Chung et al., 2002), and it became re-expressed in the HCC cell line SMMC-7721 upon knockdown of DNA methyltransferase 1 (Fan et al., 2007), indicating active repression by the tumor cells. Moreover, decorin was found to inhibit proliferation of HuH7 cells in a concentration-dependent manner (Shangguan et al., 2009), and killed xenografted HCC cells via adenoviral gene transfer (Tralhão et al., 2003). These findings are in good agreement with the general view that decorin, via its suppressive interactions with TGF-β1 and RTKs, and upregulation of p21, attenuates cell proliferation, possibly curbs angiogenesis, and thus inhibits both primary growth and metastatic spread of tumors (Goldoni & Iozzo, 2008). However, an expected inverse correlation between decorin expression and aggressive behavior of HCC still waits to be demonstrated.

Biglycan, another class I member SLRP, was found to be produced by activated HSCs during rat and human fibrogenesis (Gressner et al., 1994), and it is strongly deposited in fibrotic areas along with decorin (Högemann et al., 1997). Biglycan, like decorin, is also known to interact with members of the TGFβ/BMP family (Schaefer & Iozzo, 2008); however, up to now, no mechanistic details of its contribution to liver disease have been revealed.

2.2 Modular and cell surface HSPGs

Just like SLRPs span multiple classes of the old PG nomenclature, HSPGs cross the borders of the new classification, with some members belonging to the non-hyaluronan-binding modular group and others to the cell surface group. However, the many common features they share owing to their HS chains speak for the conservation of the traditional HSPG category.

HSPGs are by far the most thoroughly studied representatives of the PG superfamily. This distinctive attention is all but unmerited: due to the incredibly broad spectrum of their functions, HSPGs permeate all fields of mammalian physiology (for a comprehensive review, see Bishop et al., 2007). The paradigm that GAGs can specifically bind extracellular mediators such as growth factors and cytokines, regulate their availability and activity, and assist their binding to primary receptors, has been derived from the study of HS, and knowledge on other GAGs in this respect is still lagging behind. The interaction of HS with fibroblast growth factors (FGFs) and their receptors has been characterized in greatest detail (Pellegrini, 2001), but a host of other heparin-/HS-binding growth factors and mediators have been identified (Dreyfuss et al., 2009). HS synthesis, structure, function, and modifications have been extensively reviewed elsewhere (for references, see Esko et al., 2009); hence, only a brief outline is provided here.

Following synthesis of the *N*-acetyl-glucosamine/glucuronic acid (GlcNAc/GlcUA) copolymer, the prospective heparin/HS molecule undergoes extensive modifications. GlcUA residues may be epimerized into iduronic acid (IdoUA); *N*-acetyl groups may be removed and replaced by N-sulfates; and further sulfates may be transferred to the 6-*O* and 3-*O* positions on GlcN, as well as to the 2-*O* position of IdoUA. These modifications are carried out by epimerase, *N*-deacetylase-sulfotransferases (NDSTs), and position-specific *O*-sulfotransferases (OSTs), respectively; some of these enzymes have several isoforms.

Whereas epimerized, *N*-, 2-*O*- and 6-*O*-sulfated disaccharides are predominant and uniformly distributed in heparin, HS is characterized by the alternation of unmodified (*N*-acetylated) and modified (*N*-sulfated) regions, the latter being flanked by partially modified transition zones. The rarest modification in both heparin and HS is 3-*O*-sulfation which, however, is indispensable for important biological functions such as antithrombin binding (Chen & Liu, 2005). Unlike heparin, native HS has little anticoagulant activity; on the other hand, it possesses a delicate fine structure that is pivotal in determining its specificity and affinity towards potential binding partners. Consequently, even subtle alterations in the sulfation pattern of HS may substantially affect its biological properties.

HS may be further shaped post-synthetically by the action of heparanase, a secreted endoglucuronidase capable of cleaving the polysaccharide in a limited fashion, and the SULFs, endosulfatases removing 6-*O*-sulfate groups from specific disaccharide motifs. Since these enzymes also modulate the growth factor binding properties of HS, their activities have broad implications for tumor growth, angiogenesis, and metastasis.

Of course, HSPGs possess a protein core, too, which may participate in a number of interactions. The complex modular structure of matrix HSPGs warrants their involvement in intricate protein networks within the matrix as well as between matrix and cells; thus, they often occupy a bridging position between the matrix and cell surface. Transmembrane HSPGs may similarly establish contacts with the matrix, but may also associate with other cell surface receptors or recruit cytoskeletal and signaling proteins via their cytoplasmic domain (Mythreye & Blobe, 2009).

2.2.1 Modular HSPGs of the basement membrane: Perlecan, agrin, and collagen type XVIII

Perlecan. This large HSPG, consisting of numerous modules grouped into five major domains, is secreted into the pericellular space. Perlecan, ubiquitously found in basement membranes (BMs) and other extracellular matrices, is strategically positioned to mediate signaling events related to cell migration, proliferation, and differentiation. Most of its functions are assigned to the HS chains capable of binding growth factors, but the modular protein core also participates in numerous cell-matrix and matrix-matrix interactions (Whitelock et al., 2008).

Perlecan was detected in the healthy liver in all BMs, including those of bile ducts and blood vessels; even in the poorly organized BM of sinusoids. Endothelial cells, portal mesenchymal cells, and arterial walls were also immunopositive for perlecan (Roskams et al., 1995). Accumulation of perlecan in chronic liver disease is mainly attributable to ductular reaction and sinusoidal capillarization, although perlecan appears not only in BMs but diffusely in connective tissue septa (Roskams et al., 1996; Kovalszky et al. 1998). In HCC, robust perlecan immunostaining labels the vasculature of the tumor (Roskams et al., 1998).

The role of perlecan in the pathomechanism of chronic liver diseases and HCC has not been investigated. Data on the role of perlecan in fibrogenesis are missing altogether, and inferences regarding HCC can be drawn from studies on other cancer types only. Perlecan plays a central role in both developmental and pathologic angiogenesis (Iozzo & Sanderson, 2011). Intriguingly, perlecan can either stimulate or inhibit angiogenesis by two entirely distinct mechanisms. Its stimulatory role is primarily explained by the high capacity of its

HS chains to bind vascular endothelial growth factor (VEGF) and FGF-2, two major proangiogenic factors. Deposited around tumor cells and in capillary basement membranes, perlecan is ideally situated to support neovessel growth. Perlecan is supposed to act in concert with heparanase that liberates bound growth factors from its HS chains, or alone by cross-linking VEGF receptor with integrins on the surface of endothelial cells. The antiangiogenic effect, on the other hand, is exerted by a C-terminal proteolytic fragment of perlecan, termed endorepellin. Binding of endorepellin to $\alpha_2\beta_1$-integrin, a master matrix receptor of endothelial cells, triggers disruption of the cytoskeleton and blocks endothelial migration and survival. This dual nature of perlecan notwithstanding, both experimental and clinical data indicate that the proangiogenic role dominates in human malignancies, and perlecan promotes progression of various tumor types including carcinomas of the breast, prostate, and colon, as well as metastatic melanoma (Bix & Iozzo, 2008). Although the role of perlecan in HCC progression has not yet been specifically addressed, it is tempting to speculate that it may favor neovessel growth in this hypervascular tumor type.

Agrin. Another large HSPG with complex domain structure, agrin shares some homology with perlecan, but possesses many unique features and is probably less ubiquitous in the body. One peculiarity of agrin is the existence of both secreted and membrane-bound isoforms. Agrin was first described as organizer of the postsynaptic receptor apparatus in neuromuscular junctions, and was subsequently shown to play a similar role in the central nervous system. It was later found in the renal glomerular basement membrane, on the surface of immune cells, and in the blood-brain barrier (Bezakova & Ruegg, 2003).

In the healthy liver, agrin is so scarce that it was understandably missed on the first survey (Gesemann et al., 1998). No sooner than its accumulation in cirrhosis and HCC raised attention could the purposeful quest find some agrin in the walls of portal blood vessels and in the BM of bile ducts (Tátrai et al., 2006). Similar to perlecan, increased deposition of agrin during chronic liver injury is associated with ductular reaction and neovessel formation in the connective tissue septa; however, in contrast with perlecan, no diffuse agrin immunostaining is seen in fibrotic areas, and agrin is virtually absent from all sinusoids, either healthy or capillarized. In HCC, agrin is deposited in a pattern similar to that of perlecan, i.e. in the wall of tumoral blood vessels. Since agrin, unlike perlecan, is missing from normal and cirrhotic sinusoids, the appearance of agrin in microvascular walls is a useful immunohistochemical marker of malignant hepatocellular transformation (Tátrai et al., 2009). To date, no mechanistic or clinical data are available on the role of agrin in HCC, or in tumor biology as a whole. With only *a priori* knowledge at hand, a proangiogenic role can be hypothesized, based on agrin's structural and functional similarities with perlecan, including the ability of its HS chains to bind growth factors and the possible interactions of its core protein with integrin receptors (Burgess et al., 2002).

Collagen type XVIII, a ubiquitous BM-HSPG (reviewed by Iozzo et al., 2009; Seppinen & Pihlajaniemi, 2011) combines features of collagens and modular PGs. Three variant forms of collagen type XVIII result from alternative transcription initiation sites and splicing. The shortest one is found in most vascular, muscle fiber and epithelial BMs as well as in various ocular structures, whereas the two long variants are expressed predominantly in the liver. Despite its widespread presence in vascular BMs, collagen XVIII does not seem to be essential for blood vessel development; rather, its mutations lead to malformations of the eye and the central nervous system. Main functions of collagen XVIII are linked to its role in

maintaining the structural integrity of BMs, especially those in the eye, as well as to the anti-angiogenic effect of its C-terminal proteolytic fragment termed endostatin. Endostatin (O'Reilly et al., 1997), a potent endogenous inhibitor of angiogenesis, can be liberated from full-length collagen XVIII by matrix proteases, and interferes with virtually every step of the angiogenic process. It curbs proliferation and migration of endothelial cells and promotes their apoptosis; impedes the recruitment of pericytes; and reduces mobilization of endothelial progenitor cells into the circulation. The mechanism of action of endostatin is complex, involving multiple pathways such as $\alpha_5\beta_1$-integrin-, VEGFR-, and Wnt/β-catenin signaling (Seppinen & Pihlajaniemi, 2011). External administration of endostatin, either as recombinant protein or in the form of gene therapy, has been shown to suppress growth of numerous animal and xenografted human tumor types including HCC (Folkman, 2006).

As mentioned above, collagen XVIII is abundant in the healthy liver, where it is deposited both perisinusoidally and in BM zones of bile ducts, blood vessels, and peripheral nerves (Musso et al., 1998). Unlike most other extracellular matrix (ECM) proteins which are produced primarily by HSCs, collagen XVIII in the normal liver mostly originates from hepatocytes. Liver parenchymal cells produce both long variants under liver-specific transcriptional control; interestingly, variant #2 is secreted into the plasma rather than retained in the sinusoidal BM. Non-parenchymal cells such as bile duct epithelial, endothelial, and vascular smooth muscle cells express the short, ubiquitous variant (Musso et al., 2001a). Activated HSCs step on the stage in active fibrosis, and short collagen XVIII becomes a major component of remodeled BM in capillarized sinusoids; then, in quiescent cirrhosis, hepatocytes once again take over the primacy in collagen XVIII synthesis.

It seems proven that HCC cells initially maintain or even increase their expression of long collagen XVIII, and stromal cells continue to produce short collagen XVIII in HCC (Musso et al., 2001a, 2001b). Controversy exists, on the other hand, as to whether collagen XVIII levels in tumor hepatocytes increase or decrease with HCC progression. While some authors demonstrate that high tumoral expression of collagen XVIII correlates with increased VEGF activity and poor prognosis (Hu et al., 2005), others argue that, as it can be expected of an angiogenesis suppressor, collagen XVIII becomes downregulated by HCC cells in parallel with increasing tumor size, microvessel density, and clinical aggressiveness (Musso et al., 2001b). Since long collagen XVIII variants are regulated by liver-specific transcription factors, it has been suggested that their downregulation may reflect the loss of hepatocytic phenotype. It has also been proposed that decreased tumoral expression of the longest (#3) variant may favor progression by allowing higher activation of the Wnt/β-catenin pathway (Quélard et al., 2008). Variant #3 possesses a domain homologous to the Wnt-receptor frizzled which, when cleaved off proteolytically, localizes to the cell surface and blocks Wnt/β-catenin activation by sequestering Wnt3a. Thus, downregulation of variant #3 may relieve this block and allow enhanced tumor growth. Moreover, in further support of decreased tumoral expression of collagen XVIII, higher levels of endostatin were found in adjacent liver tissue relative to HCC in a tissue array-based immunohistochemical study (Yu et al., 2010).

2.2.2 Cell surface HSPGs: Syndecans and glypicans

Syndecans. All four members of this transmembrane cell surface HSPG family (reviewed by Xian et al., 2010) share highly conserved membrane-spanning domains and cytoplasmic regions, while their extracellular domains are divergent. Syndecans play prominent roles in

cell-cell and cell-ECM interactions including cell adhesion, as well as in matrix organization and assembly. Via their intracellular domain they can communicate with actin-associated and signaling molecules; some of their intracellular partners are PDZ domain-containing proteins. Unlike giant multimodular HSPGs, the core protein of syndecans is rather small, ranging between 20-40 kDa. Besides HS chains, syndecan-1 and -3 may also bear CS/DS GAGs. The extracellular domain of syndecans may be cleaved off proteolytically and solubilized in a process referred to as ectodomain shedding, which is effected by matrix metalloproteinases (Couchman, 2010).

Of the four family members, syndecan-1, a ubiquitous epithelial membrane HSPG, is the most abundant in the healthy liver. Syndecan-1 is robustly expressed on hepatocytes, resulting in a primarily sinusoidal and, to a lesser extent, lateral membrane-associated immunostaining pattern. Syndecan-1 is also present on biliary epithelial cells, with basolateral accentuation, and on sinusoidal but not on portal vessel endothelial cells (Roskams et al., 1995).

Endocytic clearance of triglyceride-rich lipoprotein remnants, a major metabolic task of hepatocytes, is mediated by HS as a receptor, and syndecan-1 has been identified as the primary HSPG involved in this process (Stanford et al., 2009; Williams & Chen, 2010). Cell surface HS is also a main clue for pathogens in the recognition of their host cells and in endocytic entry (Y. Chen et al., 2008). *Plasmodium* sporozoites dock on hepatocytes using HS receptors (Pinzon-Ortiz et al., 2001); it has been suggested that blood-borne sporozoites are literally "filtered out" by liver-specific, highly sulfated HS structures (Pradel et al., 2002; Coppi et al., 2007). Furthermore, HSPGs act as receptors or co-receptors for obligate and facultative hepatotropic viruses including dengue, hepatitis B, C, and E viruses (Hilgard & Stockert, 2000; Barth et al., 2003; Schulze et al., 2007; Kalia et al., 2009). The previously established role of HS in adenoviral infection of the liver is currently a matter of debate (Di Paolo et al., 2007; Bradshaw et al., 2010; Corjon et al., 2011). Being the major liver cell membrane HSPG, syndecan-1 may plausibly turn out to be the key mediator in most hepatocyte-pathogen interactions, although at least one study has shown that syndecan-1 is dispensable for murine liver infection by *Plasmodium yoelii* (Bhanot & Nussenzweig, 2002). Theoretically, as being also expressed by hepatocytes, syndecan-4 might overtake some of the roles of syndecan-1, although it is present in minor amounts only and in a distinct, bile canalicular localization (Roskams et al., 1995).

Syndecan-2, also called fibroglycan, is a mesenchymal-type syndecan. As such, it is produced by mesenchymal cells of the portal tract, but not by quiescent HSCs (Roskams et al., 1995). Syndecan-3, despite its general reputation as a neuronal syndecan, was also detected immunohistochemically in the normal liver, where it was localized to the endothelial lining of portal blood vessels, as well as to HSCs in the sinusoids (Roskams et al., 1995).

The quantity and distribution of syndecans is affected in several ways by chronic liver disease. Regenerative hepatocytes in chronic cholestatic disease show increased syndecan-1 expression which, accompanied by a relative gain in lateral membrane localization, results in an almost honeycomb-like immunostaining pattern (Roskams et al., 1996). Similar alterations occur in cirrhosis (Tátrai et al., 2010). Additionally, reactive ductules also exhibit strong syndecan-1 immunoreaction. Syndecan-3 is intensely seen in activated HSCs and macrophages. Disturbed polarity of hepatocytes is indicated not only by a less restricted

localization of syndecan-1, but also by the dispersion of granular syndecan-4 immunostaining originally concentrated around the bile canalicular pole (Roskams et al., 1996).

In most HCCs, the honeycomb pattern of syndecan-1 immunostaining is preserved, and the overall intensity is increased relative to the normal liver (Roskams et al., 1998). However, syndecan-1 may be gradually silenced in parallel with tumor progression, and reduced expression of syndecan-1 has been shown to correlate with high metastatic potential (Matsumoto et al., 1997). Downregulation of syndecan-1 during the progression of epithelial cancers is a common phenomenon which, especially when accompanied by simultaneous loss of E-cadherin, is thought to indicate epithelial-to-mesenchymal transition (Iozzo & Sanderson, 2011). Loss of tumor cell syndecan-1 expression in carcinomas, occasionally combined with aberrant stromal expression of the same protein, is typically considered as a predictor of poor prognosis (for references, see Máthé et al., 2006). Stromal syndecan-1 in HCC has not been reported, but abnormal cytoplasmic, or even nuclear, staining in tumor cells was observed. Unlike syndecan-1, syndecan-2 in HCC appears on stromal mesenchymal cells, and syndecan-3 in vessel walls and on endothelial cells. Syndecan-4 is strikingly enhanced in HCC, with some tumor cells showing intense and diffuse cytoplasmic immunostaining.

The functions of syndecans in chronic liver disease and HCC are largely unknown. Upregulation and increased shedding of syndecan-1 is characteristic of wound healing (Manon-Jensen et al., 2010), and fibrosis is a process analogous to wound healing in many aspects. Indeed, the amount of syndecan-1 ectodomains shed into the serum has been reported to reflect the severity of fibrosis (Zvibel at al., 2009). Additionally, it can be speculated that enhanced and broadened expression of syndecan-1 on the surface of hepatocytes may facilitate entry of hepatitis viruses, and thus create a positive feedback loop that may contribute to the perpetuation of infection in the fibrotic/cirrhotic liver (András Kiss, personal communication).

The role of syndecan-1 in tumorigenesis and tumor progression is contradictory, and varies with tumor stage and type: it is downregulated in some carcinomas (e.g., certain breast cancers) but overexpressed by other tumors (e.g., pancreatic cancers, myelomas) (Manon-Jensen et al., 2010). Based on an *in vitro* study performed with multiple HCC cell lines, it has been proposed that syndecan-1 and -4 may assist in the binding of the chemokine CCL5/RANTES, and thus promote migration and invasion of tumor cells (Charni et al., 2009).

Glypicans. The glypican family (reviewed by Filmus et al., 2008) consists of six glycosyl-phosphatidylinositol-anchored HSPGs with relatively small (555-580 amino acid) core proteins, and HS chains located close to the cell surface. Glypicans may also shed from the cell surface (and hence appear in the serum), and may undergo proteolytic cleavage. The main function of glypicans lies in regulating the signaling of Wnts, Hedgehogs (Hh's), FGFs, and bone morphogenetic proteins (BMPs). Research has mostly been focused on glypican-3, luckily for us, since this is the only member of the family with true relevance to the liver. Glypican-3 exerts opposite effects on Wnt and Hh pathways: it facilitates binding of Wnts to frizzled and increases signaling, whereas it competes with patched for Hh binding, and directs Hh toward endocytic breakdown, leaving smoothened and its signal cascade inactive (Filmus et al., 2008).

In the liver, glypican-3 behaves as an oncofetal antigen: it is expressed in the fetal but not in the adult liver, and becomes re-expressed in hepatocytes upon malignant

transformation only. Quite intriguingly, although glypican-3 appears to be a negative regulator of growth during development and regeneration of the liver (Liu et al., 2009, 2010), and its forced expression suppresses hepatocyte proliferation in mice (Lin et al., 2011), glypican-3 is nearly uniformly overexpressed in human liver cancers, and glypican-3-positive HCCs have significantly worse prognosis when compared to the relatively few glypican-3-negative cases (Shirakawa et al., 2009). In fact, glypican-3 has recently emerged as one of the most promising immunocytochemical, immunohistochemical and serum markers of HCC. Glypican-3, in combination with other markers, has been shown to facilitate detection of early HCC both in biopsies and from the serum (Roskams & Kojiro, 2010; Malaguarnera et al., 2010).

Glypican-3 is thought to promote HCC progression through multiple mechanisms. Overexpression of glypican-3 in HCC was not only found to correlate with enhanced nuclear localization of β-catenin (indicating its role as a stimulator of Wnt signaling), but also with increased expression of matrix metalloproteinases, members of the FGF signaling pathway, and SULF2 (Akutsu et al., 2010). Oncogenic potential of GPC-3 may be related to its ability to stimulate IGF-II / IGF-1R interaction, too (Cheng et al., 2008). Activation of all the above-mentioned molecules and pathways had been observed previously in HCC, and hypotheses can now be formulated as to whether and how they are mechanistically related to the upregulation of glypican-3. Yet it is difficult to foresee at the moment how the supposed anti-proliferative and pro-oncogenic effects of glypican-3 can be consolidated into a single self-consistent theory. Also, while some important details of the transcriptional regulation of glypican-3 have been elucidated (Morford et al., 2007), the basis for its re-activation in HCC remains to be investigated.

2.2.3 Enzymes involved in HS synthesis and modification

As chronic liver diseases and HCC bring about profound alterations in HSPG synthesis, it is logical to expect accompanying changes in the levels and/or activities of enzymes involved in HS synthesis and modification. Moreover, it has been demonstrated that besides a rise in its quantity, the fine structure (and, consequently, the biological activity) of HS also becomes altered in HCC (Dudás et al., 2000). So far, however, very little attention has been directed toward the expression and activity of NDSTs and OSTs (the enzymes transferring sulfates on the nascent HS chain) in the healthy and diseased liver. The role of heparanase and SULFs in HCC progression has apparently attracted more interest.

NDSTs and OSTs. NDSTs substitute the acetyl group with sulfate on glucosamine, while OSTs place sulfates on selected disaccharide units at the 6-*O*- and 3-*O*-positions of glucosamine, as well as at the 2-*O*-position of the uronic acid. *N*-sulfation, uronic acid epimerization, and *O*-sulfation are not independent steps; rather, they follow a hierarchy suggested by the above order (Murphy et al., 2004). Both NDST isoforms NDST-1 and -2 have been reported to be present and enzymatically active in the healthy liver, although NDST-2 does not seem to contribute to HS sulfation (Ledin et al., 2006). Out of the many 2-, 3- and 6-OSTs, only a few isoforms have been detected in the normal liver (Shworak et al., 1999). The relative neglect of these enzymes in research is probably unjust, as they make key contributions to the synthesis of functional liver HS. Specifically, *N*- and 2-*O*-sulfated (and probably also 6-*O*-sulfated) HS is necessary for remnant lipoprotein uptake (Stanford et al., 2010; K. Chen et al., 2010), and various highly sulfated HS motifs are required for the

interaction of parenchymal cells with pathogens such as *Plasmodium* sporozoites or hepatitis B and C viruses (Barth et al., 2006; Coppi et al., 2007; Schulze et al., 2007). Suppressed NDST expression in diabetes has been suggested to impair lipoprotein uptake and thus worsen dyslipidemia (Williams et al., 2005).

Both NDST isoforms, as well as 3-OST-1 and 6-OST-1 have been found to be overexpressed in fibrotic liver diseases and HCC. The enzymes 2-OST-1 and 3-OST-3B, on the other hand, were highly expressed in the normal liver but not significantly upregulated in disease (Tátrai et al., 2010). Although the clinicopathologic significance of these alterations are unknown, it can be speculated that overexpression of 3-OST-1, the 3-OST isoform most potent in the synthesis of anticoagulant HS (Girardin et al., 2005), coupled with release of liver HS into the bloodstream by heparanase, may contribute to the coagulopathy observed in some cirrhotic patients. Altered HS structure created by hyperactive 3-OSTs may also influence signaling pathways involved in oncogenesis.

Heparanase (reviewed by Levy-Adam et al., 2010) is an endo-β-D-glucuronidase capable of cleaving HS chains in a limited fashion. Fragmentation of HS by heparanase (HPSE) breaks the integrity of the ECM and BMs, and mobilizes bound growth factors. Such remodeling of the ECM is generally thought to promote multiple steps of tumor progression including angiogenesis, invasion, and metastasis. Hence, it is not surprising that high expression of HPSE is an unfavorable prognostic factor in most tumor types (Barash et al, 2010). In fact, the mechanism of action of HPSE in tumors is much more complex and goes beyond growth factor mobilization. E.g., increased HPSE activity enhances syndecan-1 shedding, either directly or via induction of MMP-9; moreover, it influences clustering, PKCα-mediated signaling, and internalization of syndecan-1 (Yang et al., 2007; Fux et al., 2009). HPSE also stimulates the production of hepatocyte growth factor (HGF) in an enzymatic activity-independent fashion. Shed syndecan-1 may form active complexes with HGF, and further potentiate tumor growth (Ramani et al., 2011). Additional non-enzymatic effects of HPSE include enhanced Erk phosphorylation, as well as activation of the AKT and EGFR pathways (Fux et al., 2009).

HPSE is detected in the developing liver, but not in the healthy adult organ. It becomes induced, however, during liver regeneration (Goldshmidt et al., 2004), and elevated HPSE mRNA levels were measured in fibrogenic liver diseases (Tátrai et al., 2010), although other studies have found no difference in the amount of HPSE protein between normal liver tissue and cirrhosis (Xiao et al., 2003; G. Chen et al., 2008). Despite its overall pro-oncogenic profile, literature data are equivocal on the role of HPSE in HCC progression (for a recent review, see Dong & Wu, 2010). The majority of papers report on the elevation of HPSE mRNA and/or protein in HCC relative to both adjacent non-tumorous and normal liver tissue, and most investigations have found significant positive correlation between tumoral HPSE levels, HCC progression, and adverse prognosis. In some studies, however, the levels of HPSE mRNA were found to be decreased in HCC when compared to adjacent non-cancerous tissue (Ikeguchi et al., 2002, 2003), and mean HPSE mRNA expression was shown to be elevated in HCC relative to the healthy liver but lower than in fibrogenic diseases (Tátrai et al., 2010). In explanation of a supposed tumor-inhibitory effect it has been proposed that, as opposed to the stimulatory effect of moderate HPSE activity, excessive HS fragmentation by HPSE may be detrimental to FGF-2 signaling and may therefore lead to increased apoptosis rates (Ikeguchi et al., 2003; Dong & Wu, 2010).

SULFs (reviewed by Bret et al., 2011) are secreted endosulfatases that remove specific 6-*O*-sulfates from HS. Both SULF1 and SULF2 have broad tissue distribution in the healthy human organism (Morimoto-Tomita et al., 2002). Although they catalyze the same reaction and possess similar substrate specificities, the two enzymes appear to exert distinct effects. In mice, Sulf2 can complement the lack of Sulf1 during embryonic development, whereas Sulf2-knockout animals are born with severe central nervous system defects and die by day 14 postnatally (Kalus et al., 2009). Such differences in regulation and mechanism of action may explain the surprising observation that SULF1 and SULF2 act oppositely in the process of tumorigenesis: unlike SULF1 that is generally regarded as a tumor suppressor, SULF2 is a pro-oncogenic endosulfatase overexpressed in several tumor types including HCC (Bret et al., 2011).

In the healthy liver, SULF1 mRNA is expressed at low levels; SULF2 mRNA is approx. 10 times more abundant. On the other hand, while average SULF2 mRNA levels are only slightly increased during chronic liver disease and HCC, SULF1 mRNA is robustly overexpressed in both conditions (Tátrai et al., 2010). Nevertheless, SULF1 has been proven to behave as a suppressor of HCC growth both *in vitro* and *in vivo*. By desulfating HS, it inhibits the co-receptor function of HSPGs in multiple heparin-binding growth factor – tyrosine kinase receptor pathways, and forced overexpression of SULF1 in HCC cell lines results in delayed xenograft growth (Lai et al., 2008a). While SULF1 is downregulated in 30% of resected HCCs only, it was found to be silenced in 82% of established HCC cell lines (Lai et al., 2008b). This discrepancy between primary tumors and cell lines can possibly be explained by selection bias associated with *in vitro* culturing.

SULF2, as a contrast, was found to be upregulated in 8/11 (72%) HCC cell lines and approx. 60% of primary tumors, and patients with the highest SULF2 expression had significantly worse prognosis (Lai et al., 2008c). SULF2 enhances FGF-2 signaling, thereby promoting tumor cell growth and migration. Moreover, SULF2 upregulates both glypican-3 and Wnt3a expression of cancer cells, and facilitates release of Wnt3a from glypican-3 by desulfating its HS chains; the outcome is boosted Wnt/β-catenin signaling (Lai et al., 2010).

2.3 Other transmembrane PGs

Membrane-spanning PGs other than syndecans include betaglycan, melanoma chondroitin sulfate proteoglycan (also known as CSPG4), neuropilin-1, and the variant forms of CD44 (Couchman, 2010). Similar to syndecans, these transmembrane PGs may also interact with a plethora of extracellular partners; further, although their short intracytoplasmic domains lack intrinsic enzymatic activity, they may recruit cytoskeleton-associated and signaling molecules, often via their PDZ binding site. Since CSPG4, despite its discovery in several human tissues and cancer types after melanoma, has not been identified in the liver, it is omitted from our discussion.

Betaglycan, also referred to as type III TGFβ receptor, can be substituted with either HS or CS/DS (Couchman, 2010). As its alternate name indicates, betaglycan acts as a co-receptor for members of the TGFβ family. Like neuropilin-1 (see below), betaglycan also has a close relative, endoglin, which shares sequence homology, some details of domain structure, and related functions with betaglycan, but lacks attached GAG (Bernabeu et al., 2009). Betaglycan seems to be implicated in epithelial-to-mesenchymal transition (EMT), and

betaglycan-null mouse embryos die *in utero* due to multiple malformations involving the heart and the liver (Stenvers et al., 2003). Paradoxically, while betaglycan is required to sustain TGFβ signaling in embryonic cells undergoing EMT, it is silenced by most neoplastic cells embarking on the same process (Bernabeu et al., 2009). On the whole, betaglycan is considered as a suppressor of cancer progression that inhibits tumor cell migration, proliferation, invasion, and tumor angiogenesis; accordingly, it becomes downregulated in many human malignancies (Gatza et al., 2010).

Although betaglycan expression of the healthy human liver tissue has not been investigated, it was shown to be expressed by cultured human and rat HSCs and myofibroblasts, and its mRNA levels decreased during transition from HSC to myofibroblast (Weiner et al., 1996; Meurer et al., 2005). In line with its accepted role as a tumor suppressor, expression of betaglycan mRNA was found to be reduced in 7/10 HCCs relative to the corresponding normal liver tissues, and its levels were inversely correlated with tumor grade (Bae et al., 2009).

CD44, a ubiquitous cell surface receptor of hyaluronan and other ECM components, is expressed in numerous variant forms (reviewed by Sackstein, 2011). Part of its diversity stems from a strikingly complex genomic structure: the human CD44 gene, besides 10 'standard' exons, contains 9 functional 'variant' exons that can be alternatively spliced. Additionally, CD44 may undergo extensive posttranslational modifications, comprising the attachment of CS/DS, KS, and – in the presence of the v3 exon – also HS chains (van der Voort et al., 1999; Sackstein, 2011). The smallest form of CD44, termed CD44s, lacks all variant exons, and it is primarily expressed on cells of hematopoietic origin, including stem and progenitor cells, and mature but naïve lymphocytes. The larger, GAG-bearing variants (CD44v forms) appear on normal and cancerous epithelia, as well as on activated and malignantly transformed hematopoietic cells (Sackstein, 2011). A role for CD44 in tumor progression has long been suggested (Naor et al., 1997). More recently, standard and variant CD44 forms have been recognized as cancer stem cell markers (Keysar & Jimeno, 2010), and CD44 is among the genes that identify liver cancer stem cells (Liu et al., 2011).

In the normal liver, only few hepatocytes were observed to exhibit weak membrane expression of CD44 (Endo & Terada, 2000). Elevated CD44 mRNA levels were measured in chronic liver diseases where the liver progenitor cell population is activated. High CD44 mRNA expression was restricted to progenitor cells and reactive ductules, indicating that CD44 is a 'stemness' marker not only for cancer stem cells, but for non-malignant liver progenitors, too (Spee et al., 2010).

Standard and variant forms of CD44 were upregulated in approx. half of HCCs investigated. High expression of CD44 on tumor cells was found to be associated with vascular invasion, and correlated with poorer disease outcome (Mathew et al., 1996; Endo & Terada, 2000). In an *in vitro* study, v3-containing, HS-decorated CD44 variants, but not the forms lacking the v3 exon, have been demonstrated to confer metastatic phenotype to an otherwise non-metastatic HCC cell line, SKHep1 (Barbour et al., 2003). Therefore, high expression of CD44, and of CD44v3 in particular, might be positively correlated with a dominance of 'stem-like' character and aggressive behavior of HCC cells.

Neuropilin-1 (NRP1) is a single-span transmembrane PG glycosylated with HS or CS/DS (Couchman, 2010). Just like its non-PG relative neuropilin-2, NRP1 has first been described

as a co-receptor for class 3 semaphorins, soluble signal molecules implicated in axonal guidance and vascular patterning (Adams & Eichmann, 2010). Later, neuropilins have also been identified as accessory receptors of VEGFs. NRP1 affects VEGFR signaling in a way that enhances migration and survival of endothelial cells, modulates vascular permeability, and stimulates angiogenesis (Koch et al., 2011).

During development, NRP1 is broadly expressed in the vasculature, preferentially in arteries (Koch et al., 2011). In the adult liver, NRP1 immunostaining positively labels both sinusoidal and portal vessel endothelial cells, but not the hepatocytes (Bergé et al., 2011). When sinusoidal endothelium is subjected to increased shear stress, either *in vitro* or during liver regeneration following partial hepatectomy, NRP1 becomes upregulated in concert with other proangiogenic factors such as VEGF and angiopoetin-1 (Kraizer et al., 2001; Braet et al., 2004). NRP1 is also induced in HSCs upon activation, and has been shown to promote progression of fibrosis by stimulating platelet-derived growth factor- (PDGF-) dependent chemotaxis and TGFβ-mediated matrix deposition of myofibroblasts (Cao et al., 2010).

In HCC, NRP1 expression is seen not only in endothelial cells of tumoral vessels, but – in approx. 50% of cases, and with variable intensity – in tumor hepatocytes, too. The significance of tumor cell NRP1 is unclear, but blocking NRP1-VEGF interaction was shown to inhibit vascular remodeling and growth of primary murine HCC (Bergé et al., 2011).

2.4 Hyalectans

Hyalectans are hyaluronan- and lectin-binding PGs of the ECM. They all share a tridomain structure, with the central domain carrying the majority of GAG chains (the number of which varies from 3 in brevican up to 100 in aggrecan), and the N- and C-terminal domains making contact with hyaluronan and lectins, respectively (Schaefer & Schaefer, 2010). Strategically positioned around hyaluronan, a principal ECM component, hyalectans regulate matrix assembly, and mediate a plethora of cell-ECM interactions. The group currently counts four members: versican, aggrecan, neurocan, and brevican.

Although the CS/DSPG versican is expressed throughout the body, is known to regulate a multitude of cellular processes, and has been reported to contribute to the progression of several tumor types (Ricciardelli et al., 2009; Theocharis et al., 2010), no specific investigations have been targeted to versican in the liver, except for a single study where versican was detected in activated rat HSCs (Szende et al., 1992). With respect to the liver, even less is known about aggrecan, a CS/KSPG primarily found in cartilage and brain, and the CSPGs brevican and neurocan which have never been observed outside of the central nervous system (Theocharis et al., 2010).

2.5 Secreted PGs: Endocan and PG-100

Secreted PGs are 'odd one outs' in the new classification of PGs, being neither anchored to the cell surface nor immobilized in the ECM. *Endocan* (Béchard et al., 2001), or endothelial cell-specific molecule-1 (ESM-1), is a soluble PG by default which is secreted by endothelial cells directly into the bloodstream. Production of endocan by endothelial cells is boosted by both inflammatory and pro-angiogenic mediators; endocan, in turn, enhances HGF signaling. Endocan has been shown to be overexpressed in several human tumor types, and elevated serum levels in late-stage cancer patients is regarded as an adverse prognostic factor (Abid et al., 2006; Sarrazin et al., 2006).

	Proposed effect	Therapeutic action	Reference	Level of evidence
Decorin	antifibrotic	The protein inhibits matrix production of activated HSCs	Shi et al., 2006	in vitro
	antitumor	The protein inhibits proliferation of HuH7 cells	Shangguan et al., 2009	in vitro
	antitumor	Adenovirus-mediated gene transfer exhibits oncolytic activity on xenografted HCC	Tralhão et al., 2003	in vivo
Collagen type XVIII / endostatin	antitumor	Adenovirus-mediated delivery of endostatin inhibits HCC xenograft growth	Li et al., 2004	in vivo
	antitumor	Adeno-associated virus-mediated delivery of endostatin reduces growth and vascularization of HCC xenograft	Liu et al., 2005	in vivo
	antitumor	Forced expression of endostatin in xenografted HCC cells potentiates the action of doxorubicin	Liu et al., 2007	in vivo
Syndecan-1 and -4	antitumor	Knockdown reduces CCL5/RANTES-dependent migration of HCC cells	Charni et al., 2009	in vitro
Glypican-3	antitumor	Humanized antibodies to GPC3 evoke NK-mediated ADCC of HCC cells (phase I clinical trial recruiting)	Ishiguro et al., 2008	in vivo
	antitumor	A GPC3-derived peptide vaccine sensitizes CTLs against HCC cells	Yoshikawa et al., 2011	phase I clinical trial
SULF1	antitumor	Forced expression inhibits HCC xenograft growth and potentiates the effect of HDAC inhibitors	Lai et al., 2006	in vivo
Neuropilin-1	antifibrotic	Knockout or silencing decreases motility of activated HSCs	Cao et al., 2010	in vitro / in vivo
	antitumor	Blocking inhibits growth and vascular remodeling of primary murine HCC	Bergé et al., 2011	in vivo
Endocan	antitumor	Silencing inhibits growth and migration of HCC cells	Kang et al., 2011	in vitro

Table 2. Proteoglycans in future therapies of chronic liver disease

Endocan is differentially expressed in the endothelium of HCC blood vessels, being absent from the sinusoids of both peritumoral non-malignant and healthy liver tissue; thus, immunohistochemistry for endocan helps visualize HCC vasculature (Huang et al., 2009). Endocan-positive microvessel density (MVD), unlike CD34-positive MVD, was shown to be predictive of poor survival, and high expression of endocan by tumoral endothelial cells correlated with the angiogenic and invasive potential of the tumor (Huang et al., 2009; L. Y. Chen et al., 2010). In a recent study, endocan production by tumoral hepatocytes has also been reported, and silencing of endocan has been shown to inhibit tumor cell growth and migration *in vitro* (Kang et al., 2011).

PG-100 is the PG form of macrophage colony-stimulating factor and, as such, is a 'part-time' PG that may exist in a GAG-less or CS/DS-substituted form, the latter exhibiting less than 1% of cytokine activity compared with the non-PG variant (Schwarz et al., 1990; Partenheimer et al. 1995). PG-100 was first discovered in the conditioned medium of osteosarcoma cells, and later found to be produced by other cell types including endothelia (Nelimarkka et al., 1997). PG-100 was only faintly immunostained in the normal liver, whereas in active fibrosis it was strongly visualized in bile duct epithelia, and thus proposed as a marker of ductular reaction (Högemann et al., 1997). The significance of this elevated expression remains to be clarified.

3. Outlook: PGs in future therapies of chronic liver disease and HCC

PGs are emerging therapeutic targets in inflammatory, fibrogenic and malignant diseases. As a summary to this review, in **Table 2** we have collected some (however, by no means all) current attempts to exploit the multiple actions of PGs for countering the progression of chronic liver disease and HCC. Such experiments may involve delivery of PGs with supposed therapeutic effect, inhibition of those known to promote the pathologic process, modulation of HS structure, or application of HS-mimicking molecules. Some PGs expressed in the liver may be well-studied therapeutic targets in other organs or tumor types (e.g. perlecan, heparanase), yet have not been included in the list because their therapeutic potential has not been addressed specifically in the context of liver disease. The level of evidence (*in vitro*, *in vivo*) is also indicated in the table.

Therapeutic approaches targeting glypican-3 have reached closest to human application; some phase I clinical trials have been completed or are underway. Several other PGs show remarkable promise, but these apparently have a longer way ahead.

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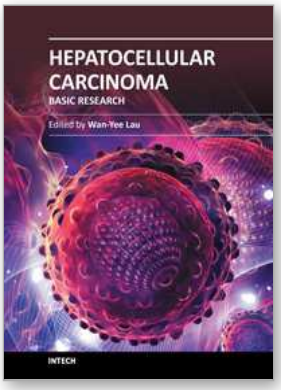

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Hepatocellular Carcinoma represents a leading cause of cancer death and a major health problem in developing countries where hepatitis B infection is prevalent. It has also become increasingly important with the increase in hepatitis C infection in developed countries. Knowledge of hepatocellular carcinoma has progressed rapidly. This book is a compendium of papers written by experts to present the most up-to-date knowledge on hepatocellular carcinoma. This book deals mainly with the basic research aspect of hepatocellular carcinoma. The book is divided into three sections: (I) Biomarkers / Therapeutic Target; (II) Carcinogenesis / Invasion / Metastasis; and (III) Detection / Prevention / Prevalence. There are 18 chapters in this book. This book is an important contribution to the basic research of hepatocellular carcinoma. The intended readers of this book are scientists and clinicians who are interested in research on hepatocellular carcinoma. Epidemiologists, pathologists, hospital administrators and drug manufacturers will also find this book useful.

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