

Heparan sulfate proteoglycans and their modification as promising anticancer targets in hepatocellular carcinoma (Review)

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Received July 20, 2020; Accepted December 8, 2020

DOI: 10.3892/ol.2021.12434

Abstract. Hepatocellular carcinoma (HCC) is one of the most common types of primary liver cancer. Despite advancements in the treatment strategies of HCC, there is an urgent requirement to identify and develop novel therapeutic drugs that do not lead to resistance. These novel agents should have the potential to influence the primary mechanisms participating in the pathogenesis of HCC. Heparan sulfate proteoglycans (HSPGs) are major elements of the extracellular matrix that perform structural and signaling functions. HSPGs protect against invasion of tumor cells by preventing cell infiltration and intercellular adhesion. Several enzymes, such as heparanase, matrix metalloproteinase-9 and sulfatase-2, have been reported to affect HSPGs, leading to their degradation and thus enhancing tumor invasion. In addition, some compounds that are produced from the degradation of HSPGs, including glypican-3 and syndecan-1, enhance tumor progression. Thus, the identification of enzymes that affect HSPGs or their degradation products in HCC may lead to the development of novel therapeutic targets. The present review discusses the main enzymes and compounds associated with HSPGs, and their involvement with the pathogenicity of HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) accounts for 85-90% of primary liver cancer cases. HCC is an aggressive cancer, which has a marked clinical and epidemiological impact, with 600,000 mortalities and ~1,000,000 new cases of HCC being reported annually, worldwide (1,2). Following a diagnosis of HCC, patients survive <1 year. In addition, the mortality rates are high, with an overall survival rate of <12% (3). HCC arises from the aggregation of normal cells following accumulation of several genetic changes that activates oncogenes and deactivates tumor suppressor genes, nuclear factors, growth factors and cytokines (4). The functions of the liver, as well as the liver reserve, are altered and damaged during the course of disease. Despite advancements in the treatment strategies of HCC, the prognosis of patients remains poor due to metastasis and development of drug resistance. Currently, hepatic resection or transplantation are considered the only curative therapies (5). Even with surgery, ~30% of patients with HCC undergo hepatectomy as they receive a diagnosis at an advanced tumor stage. Furthermore, radiofrequency ablation is used to treat patients with early-stage HCC. Several factors lead to the poor prognosis of patients with HCC, including history of cirrhosis, poverty and limited medical resources. The rapid onset and fast-growing characteristics of HCC results in limited treatment options for patients. In addition, HCC is characterized by a high angiogenic capacity due to portal vein obstruction (6). Thus, there is an urgent requirement to identify and develop novel therapeutic drugs for the treatment of HCC.

2. Heparan sulfate proteoglycans (HSPGs)

Structure. HSPGs are highly anionic carbohydrate compounds. HSPGs are composed of a limited quantity of a core protein that is covalently linked to ≥ 1 sugar chain, representing HS side chains. These chains are considered linear polysaccharides, which are built of ≤ 200 units of a repeated disaccharide formed by N-acetyl glucosamine with uronic acid, glucuronic or iduronic acids (7).

The structure of HSPGs is modified in the Golgi apparatus following the addition of sulfate groups instead of acetyl groups, or by sulfation of the hydroxyl groups at C-6 and C-3

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Key words: heparanase, matrix metalloproteinase-9, sulfatase-2, glypican-3, syndecan-1, fascic

in the N-acetylglucosamine moiety, or by sulfation of the hydroxyl group at C-2 in the uronic acid moiety (8). There are several families of cell surface HSPGs, such as syndecan and glypican. The presence of several carboxyl and sulfate groups in HS is similar to the polyanionic nature of mammalian cells compared with the neighboring cells and extracellular matrix (ECM) (9). Thus, cell surface HSPGs produce multiple structural and signaling functions due to their capability to interact with several protein ligands, including growth factors and their receptors, proteases, cytokines, chemokines, adhesion molecules and ECM proteins, including fibronectin, collagen and fibrin (10).

Function. HSPGs are one of the most important elements of the ECM, and are located on the surface of the cell membrane of most animal cells, such as hepatocytes and leukocytes (11). HSPGs are involved in several interactions between adjacent cells or between cells and the ECM. HSPGs regulate several signaling pathways and receptor trafficking, and control ligand secretion. The variability of HS generated by its modifying enzymes led to the hypothesis of 'sugar code', which is characterized by specific HS alterations observed in the embryo to orchestrate development through modification of certain signaling pathways. It depends on the regulation of special areas of HS-modifying enzymes to regulate their activity or even change their functions. The sugar code is considered a dynamic process as HS chains can be hydrolyzed by heparanase or sulfatase enzymes (12). HSPGs participate in an extensive range of biological processes, such as development (13), homeostasis control (11) and enhancement of inflammatory and malignant diseases (14). In addition, HSPGs control cell adhesion, motility, proliferation, differentiation and apoptosis (8).

HSPGs and cancer. HSPGs act as anchors for the lipoprotein lipase located on the outer surface of capillary endothelial cells. They protect against invasion of tumor cells by preventing both cell infiltration and intercellular adhesion (15). The activities of HSPG-degrading enzymes are elevated in highly invasive cancer cells compared with less invasive cells (16). When the basal membrane is ruptured by hematogenous metastatic cancer cells, HSPGs located inside the tumor microenvironment are attacked by several enzymes, such as heparanase, matrix metalloproteinase-9, sulfatase-2, which are capable of modifying the proteoglycan structure, which alters transportation of inflammatory cells from vessels into the surrounding tissues (17). Consequently, cytokines, proteases, growth factors and angiogenic factors, which bind to HSPGs, are released and promote the infiltration and metastasis of cancer cells (15).

3. Enzymes hydrolyzing HSPGs

Matrix metalloproteinase (MMP)-9. MMPs constitute a family of transmembrane zinc-dependent endopeptidases that have the ability to digest the ECM and basement membrane. The MMP family consists of 25 members in vertebrates and 22 in humans (18). Previously, MMP-9 was called type IV collagenase or gelatinase B. MMP-9 is capable of degrading type IV collagen, a major constituent of the basement membrane (19,20). The active zone of MMP-9 consists of

two zinc ions and five calcium ions. The proteolytic activity of MMP-9 is maintained by the two zinc ions and cysteine switch motif of the pro-domain (21). MMP-9 also contains a fibronectin-like domain, which is strongly O-glycosylated and is important for binding to collagen or gelatin (18).

MMPs play an important role in proliferation, invasion and metastasis of tumor cells (22). Deryugina and Quigley (23) demonstrated an association between ECM degradation by MMPs and the invasion of cancer cells. MMP-9 releases fibroblast growth factor (FGF)-1 and FGF-2 from their stores, producing potent angiogenic effects (24). In addition, MMP-9 attacks HSPGs inside the tumor microenvironment to enhance the proteolytic release of syndecan-1 and to potentiate tumor growth and metastasis (17,25). Thus, tumors that express MMP-9 at high levels are more likely to exhibit relapse or metastasis compared with tumors that express low levels of MMP-9.

Previous studies have demonstrated the role of certain MMP-9 inhibitors in the treatment of HCC both *in vivo* and *in vitro* (Table I). Although, several synthetic MMP inhibitors have been developed, none of them have reached phase III clinical trials due to either lack of efficacy or serious side effects.

Heparanase. Heparanase is an endo- β -glucuronidase that belongs to the glycoside hydrolase 79 family. Heparanase hydrolyses HS at specific intrachain positions with low sulfation and participates in the degradation and remodeling of the ECM (26).

Heparanase is upregulated in several types of human tumor, such as HCC, myeloma and breast cancer, and it strongly enhances the invasiveness of tumors in experimental animals (27). Heparanase releases HS fragments associated with angiogenic factors from the tumor microenvironment to produce an angiogenic response. In addition, heparanase facilitates vascularization, accelerates primary tumor growth and provides a gate for invading metastatic cells, thus leading to cancer progression (28).

Heparanase inhibitors notably decrease the incidence of metastasis in experimental animals (29). Suramin was subsequently assessed in rats with HCC, where it was demonstrated to elevate the percentage of survival rate of rats with HCC, and decrease the level of serum α -fetoprotein. Furthermore, suramin has been demonstrated to ameliorate fibrosis, thus producing an hepatoprotective effect (15). Table II summarizes several studies that assessed heparanase inhibitors in the treatment of HCC.

Sulfatase-2. Sulfatase-2 is an extracellular enzyme that enhances the removal of 6-O-sulfate from HS disaccharides, and controls the interactions between HSPGs and extracellular factors. Sulfatase-1 and -2 are expressed in malignant tumors, including highly invasive brain cancer (30). Sulfatase-1 acts as a tumor suppressor gene, which downregulates the phosphorylation and activation of tyrosine kinase receptors (31). Conversely, sulfatase-2 decreases the affinity of HSPGs for several signaling molecules, such as glypican-3 and syndecan-1, detaching them from HSPGs and preparing the transition of different signaling pathways, particularly the insulin-like growth factor (IGF) pathway (32).

Upregulation of sulfatase-2 is considered oncogenic, and is associated with HCC in human, animal and tissue culture

Table I. Summary of studies that assessed MMP-9 inhibitors in the treatment of HCC.

Model	Summary	Cell type	(Refs.)
Human	Diosmetin downregulates MMP-9, leading to the inhibition of migration and invasion of HCC cells	SK-HEP-1	(69)
	Niclosamide downregulates MMP-9, leading to the suppression of HCC cell migration	HLF and PLC/PRF/5	(70)
	Retinoic acid-induced protein I inhibits the migration and invasion of HCC cells by decreasing MMP-9 levels	Liver cells	(71)
	MicroRNA-133a targets MMP-9, and prevents HCC cell proliferation, migration and invasion	HepG2 and SMMC-7721	(72)
	The antibiotic salinomycin blocks the invasion and migration of HCC cells through JNK/JunD-mediated MMP-9 expression	HCCLM3	(73)
	Glabridin inhibits the invasion of human HCC cells, and may act as a chemopreventive agent against liver cancer metastasis	Huh7 and Sk-Hep-1	(74)
	Tanshinone II-A prevents the invasion and metastasis of HCC cells by blocking the activity of MMP-9	Liver cells	(75)
Rat	Epigallocatechin-gallate blocks MMP-9 activity, leading to hepatoprotective and chemoprotective effects	Liver cells	(76)
	Doxycycline produces hepatoprotective and antitumor activity by blocking MMP-9	Liver cells	(77)

MMP, matrix metalloproteinase; HCC, hepatocellular carcinoma.

Table II. Summary of studies that used heparanase inhibitors for the treatment of HCC.

Model	Summary	Cell type	(Refs.)
Human	Downregulation of heparanase enhances the suppression of invasion, migration and adhesion in HCC	HepG2, BEL-7402, and HCCLM3	(77,78)
	Administering 160 mg/day PI-88 produces significant clinical advantages for patients with HCC	Liver cells	(79,80)
Rats	Suramin exerts antitumor activity in HCC through the deactivation of heparanase	Liver cells	(15)

HCC, hepatocellular carcinoma.

models (33,34). Sulfatase-2 enhances the expression of growth factors available to cell surface receptors, thus promoting the proliferation and migration of tumor cells. In addition, it enhances the activity of glypican-3, activates FGF signaling, potentiates the phosphorylation of both Erk and Akt, and induces Wnt/ β -catenin signaling (35).

Some studies have focused on the use of sulfatase-2 inhibitors for treating HCC. Adiponectin, a suppressor of the synthesis of sulfatase-2 protein, has been reported to exhibit antitumor activity both *in vivo* and *in vitro* (35). In addition, OKN-007, an inhibitor of sulfatase-2, significantly decreases solid tumor growth (36). Table III summarizes the results of previous studies that used sulfatase-2 inhibitors for the treatment of HCC.

4. Important HSPGs products

Syndecan-1. Syndecan-1 is a transmembrane HSPG that is located on epithelial cells. The syndecan family consists

of four members, syndecan-1, syndecan-2, syndecan-3 and syndecan-4. Among these four members, syndecan-1 has been extensively studied. Its name is derived from the Latin *syndein*, which means binding together, since syndecans are involved in the binding of cells to the ECM (37). Syndecans are composed of three domains forming highly conserved intracellular and transmembrane domains, as well as an extracellular domain, which is uniquely characteristic to each member (38).

Syndecan-1 controls cell-cell and cell-ECM adhesion interactions, as well as their activities through its HS chains. It modulates certain proteolytic enzymes and chemokines *in vivo*, and controls the recruitment of leukocytes and the remodeling of tissues during inflammation (39). In addition, syndecan-1 modulates proteolytic v, thus leading to the regulation of leukocyte recruitment with subsequent remodeling of tissues (40).

The release of syndecan-1 from its membrane-bound form by MMP-9 (syndecan-1 sheddase) to the soluble molecule

Table III. Summary of studies that assessed sulfatase-2 inhibitors in the treatment of HCC.

Model	Summary	Cell type	(Refs.)
Human	2,4-Disulfonylphenyl-tert-butyl nitron (OKN-007) produces antitumor effects against HCC by suppressing TGF- β 1/SMAD2 and Hedgehog/GLI1 signaling	Huh7	(36)
Rats	Adiponectin inhibits sulfatase-2 activity, leading to hepatoprotective and chemoprotective effects	Liver cells	(35)
	Sodium ascorbate produces cytotoxic effects against HCC, which can be explained by the inhibition of sulfatase-2	Liver cells	(81)
Mice	Silencing sulfatase-2 signaling inhibits angiogenesis and tumor growth by inhibiting TGF- β 1/SMAD	Liver cells	(82)

HCC, hepatocellular carcinoma.

inside the circulation represents the transition of the tumor from a proliferative stage to an invasive stage (25). Syndecan-1 binds to both the ECM and FGF family. Overexpression of the MMP-9/syndecan-1/FGF-2 axis potentiates the apoptosis pathway in several tumor models (41,42).

A previous study demonstrated the role of inhibiting syndecan-1 by synstatin, which exhibits promising antitumor activity against rats with HCC (43).

Glypican-3. Glypican-3 is the most commonly studied member of the glypican family of glycosyl-phosphatidylinositol-(GPI) cell-surface HSPGs (44). It consists of six medium-sized HSPGs that are attached to the cell surface via a GPI anchor, with an insertion of 2-4 HS chains. Glypican-3 regulates Wnt, Hedgehog and FGF signaling (38).

Glypican-3 is upregulated in HCC (34); thus, serum glypican-3 may be a promising potential selective marker for HCC (45). Glypican-3 regulates multiple tumor activities through Wnt signaling modulation (46). Glypican-3 enhances both *in vitro* and *in vivo* HCC growth, and interacts with growth factors, such as IGF-II and its receptor leading to activation of its signaling pathway (47).

Glypican-3 is considered an attractive therapeutic target in HCC. Antibodies against glypican-3 exhibit strong antitumor activities in several models of HCC (33,34). Recently, several mouse monoclonal antibodies targeting glypican-3 have been produced (48). One of these antibodies is the humanized GC33 (hGC33), which has been assessed in a phase I clinical trial. hGC33 acts against the carboxyl-terminal region of glypican-3 and is effective in HepG2 xenografts (49). In addition, another human heavy chain variable domain antibody, NH₃, inhibits the proliferation of glypican-3-positive cells and blocks HCC xenograft growth in nude mice by modulating the TGF- β /SMAD pathway (50). Zaghoul *et al* (34) demonstrated that treatment of rats with HCC with monoclonal anti-glypican-3 increased survival rate up to 90% and decreased the level of serum AFP. In addition, anti-glypican-3 was demonstrated to affect the sulfatase-2/IFG-II pathway. Glypican-3 has also been reported to act as a predictive marker of HCC recurrence following radical surgery (51). Table IV represents a summary of studies that have assessed the role of glypican-3 inhibitors in treating HCC.

Fascin. Fascin is an actin-binding protein that controls cell movement under physiological or pathological conditions (52). It regulates cell motility and is considered one of the cytoskeleton-regulatory proteins (53).

Fascin expression has been associated with tumor invasion and metastasis, and its expression is low in normal tissues (52). Overexpression of fascin elevates cell membrane processes, such as broken intercellular junctions, and enforces cell movement associated with changes to the cytoskeleton and ECM, thus facilitating tumor metastasis (54). It has been reported that upregulation of fascin in several tumors, including HCC, is associated with tumor invasion and metastasis (55). In addition, fascin is unable to control cell migration alone, unless it is supported by other factors, such as MMP-9. This can be explained by the fact that the ability of fascin to act as a migration factor is only associated with epithelial-to-mesenchymal transition or MMP-9, which facilitate their invasiveness (56).

5. Products of ECM

Glucosaminoglycans. Glucosaminoglycans are linear polysaccharides composed of repeat units, with areas of glucuronic acid and N-acetyl glucosamine. They contain regions of 2-O-sulfated iduronic acid and N-sulfoglucosamine. Between these regions, there are transition zones with both sulfo-glucosamine and acetyl-glucosamine, which are associated with polypeptide core-forming HSPGs (57).

Glucosamine (2-amino-2-deoxy- α -D-glucose). Glucosamine is an amino saccharide that is present in almost all tissues, and abundant in liver, kidney and cartilage (58). It is the predominant building unit in the synthesis of glycolipids, glycoproteins, glycosaminoglycans and proteoglycans (59). Glucosamine induces autocrine TGF- β activity (60) and helps in the O-linked glycosylation of proteins. As an alteration of the structure of proteins with O-linked N-acetylglucosamine, glucosamine has evolved as an important regulator of cellular physiology. This alteration is associated with several diseases, such as cancer, neurodegenerative disorders and cardiovascular diseases (61). Notable elevation in the serum levels of glucosamine has been observed in patients and animals with HCC (15,62,63).

Table IV. Summary of studies that assessed glypican-3 inhibitors in the treatment of HCC.

Model	Summary	Cell type	(Refs.)
Human	Human monoclonal antibody targeting glypican-3 prevents the migration and motility of HCC	Hep3B and HepG2	(83)
	Glypican-3-targeted chimeric antigen receptor T cell provides a promising therapeutic target for glypican-3-positive HCC	HepG2, Hep3B, PLC/PRF/5 and SK-Hep-1	(84)
	Silencing the glypican-3 gene protects against HCC	HepG2	(85-87)
	Interfering glypican-3 gene transcription blocks HCC cell apoptosis and prevents metastasis via the Wnt/ β -catenin signaling pathways	MHCC-97H and Huh7	(88)
	hGC33 protects patients with HCC	Liver cells	(89-91)
	By targeting glypican-3, microRNA-219-5p exerts antitumor effects in HCC	Liver cells	(92)
Rat	Anti-glypican-3 antibody protects against HCC	RH7777	(93)
	Anti-glypican-3 antibody exerts antitumor and hepatoprotective effects against HCC	Liver cells	(34)
Mice	Targeted photoimmunotherapy for glypican-3 combined with nanoparticle albumin-bound paclitaxel is a promising method for treating HCC	Liver cells	(94)
	Glypican-3 cDNA vaccine by using a recombinant plasmid encoding murine glypican-3 cDNA for treatment of HCC produces specific and effective antitumor immunity against HCC	Liver cells	(95)

HCC, hepatocellular carcinoma.

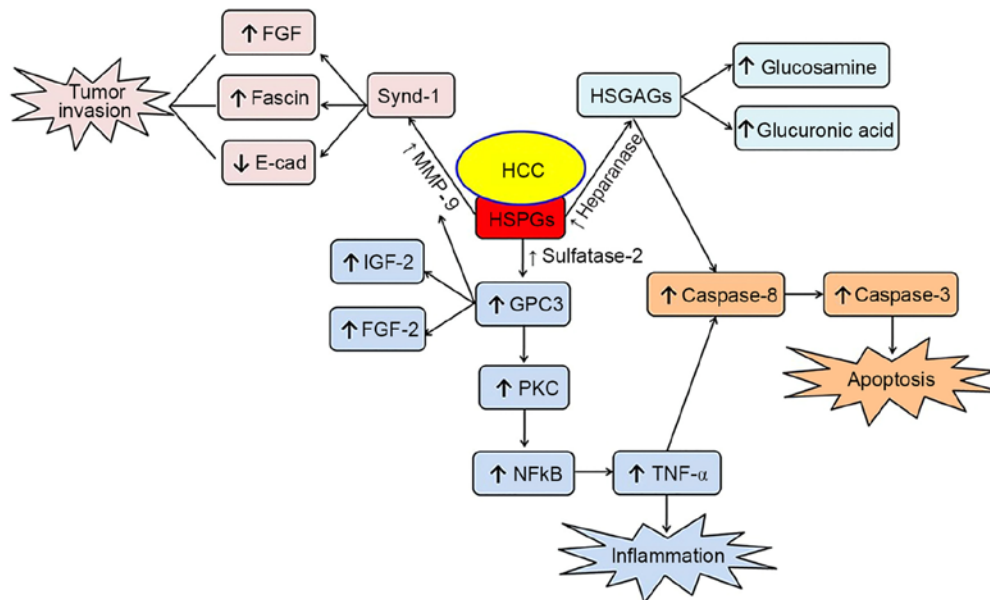


Figure 1. HSPGs pathway changes in HCC. HCC increases the ability of the enzymes, MMP-9, sulfatase-2 and heparanase to attack HSPGs, leading to the formation of several intermediate compounds that enhance inflammation and tumor invasion. HSPGs, heparan sulfate proteoglycans; HCC, hepatocellular carcinoma; MMP, matrix metalloproteinase; FGF, fibroblast growth factor; E-cad, E-cadherin; Synd-1, syndecan-1; IGF, insulin-like growth factor; GPC3, glypican-3; PKC, protein kinase C; NF κ B, nuclear factor κ B; TNF- α , tumor necrosis factor- α .

Glucuronic acid. Glucuronic acid is synthesized from UDP-glucose inside the liver via UDP-glucosedehydrogenase. It participates in several detoxification pathways, such as xenobiotic and bilirubin (64). Elevated levels of hyaluronic acid in liver diseases are the main cause for increased levels of serum glucosamine and glucuronic acid (65). Degradation of hyaluronic acid, which is initiated by its binding to CD44,

notably enhances the activation of cell migration molecules, thus leading to tumor motility (66).

Sialic acid (N-acetyl neuraminic acid). Sialic acid is part of the plasma membrane of mammalian cells. It binds to N-acetyl galactosamine via an O-glycosidic linkage, which is associated with the proteins that form glycoproteins (58).

Sialic acid is a major player in several physiological and pathological processes, such as progression and spread of multiple malignancies, such as neuroblastoma, oral cancer and breast cancer (67). A variation in the sialic acid levels in patients with cirrhosis and HCC is an important diagnostic tool. Elevated sialic acid levels in HCC may be explained by endothelial cell dysfunction or macrovascular disease (68).

6. Conclusions and future directions

HCC triggers metabolic and dynamic modifications that lead to the activation of certain enzymes, such as MMP-9, sulfatase-2 and heparanase, resulting in the degradation of HSPGs. Increasing evidence suggests that some of the HSPG degradation products, such as syndecan-1 and glypican-3, are associated with the activation, migration and apoptosis of tumor cells (Fig. 1). Thus, an improved understanding of the role of HSPGs and their degradation products will aid the identification of novel effective therapeutic targets and strategies for preventing and treating HCC.

Cancer treatment has shifted from single target treatment to multiple target therapies. HSPGs represent a goal for a new trend in multiple target therapies, since they comprise several enzymes and important compounds located in the tumor microenvironment that control multiple biological and pathological processes. Prospective studies will focus on the specific post-translational modifications of these compounds in the HSPG pathway, along with further assessment of the inhibitors and modulators of cell signaling.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

All data and materials are included in the present review.

Authors' contributions

MAA and NNA contributed to the study design. MMA and MAAG acquired the data. MMHAG contributed to the study concept and design. All authors helped draft the initial manuscript, and read and approved the final version.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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