Chapter 11
Proteoglycans in Glioma Stem Cells

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Abstract Proteoglycans are important biomolecules in development, injury, and disease. They are highly prevalent in the central nervous system, where they are components of the extracellular matrix or expressed on the cell surface to contribute to the regulation of cell signaling, cell adhesion, and cell–matrix interactions. Expression of proteoglycan core proteins and key synthetic or degradation enzymes is aberrant in brain cancers, including gliomas. Some proteoglycans, such as CD44 or CSPG4/NG2, have been implicated as cell-surface markers of stem/progenitor cells in the brain. Signaling through these proteoglycans is also promoting glioma progression and cancer stem cell maintenance. This book chapter will review the known functions of proteoglycans in glioma cancer stem cells.

11.1 Introduction

Proteoglycans are present in virtually all mammalian tissues. They are important components of the extracellular matrix (ECM) as well as co-regulators of signaling pathways, cell adhesion, and interactions between cells and their environment. Proteoglycans consist of a protein backbone, referred to as core protein, which is post-translationally modified by the addition of glycosaminoglycan (GAG) chains of varying lengths that contain further chemical modifications (e.g., phosphorylation, sulfation). The different structures of core proteins and modification of GAG chains create a highly diverse family of proteoglycans fine-tuned to fulfill a wide range of biological functions.

Proteoglycans are abundant in the central nervous system (CNS) and serve crucial functions during development, injury, and disease. Deletion or mutation of several proteoglycans result in neurodevelopmental disorders, underlining their importance for the development and function of the CNS (Conway et al. 2011a, b; Mclaughlin

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et al. 2003; Silver and Silver 2014). The chief functions of proteoglycans are to act as an extracellular reservoir for growth factors (when secreted) or as co-receptors for growth factor signaling pathways (when membrane-associated). These functions enable the formation of morphogen gradients, e.g., during development, which may also affect cancer progression and invasion. Additionally, proteoglycans contribute to interactions between a cell and its microenvironment. For instance, CSPGs provide repulsive signals for axon growth cones, generating boundaries between developing components of the brain, but also prevent axonal regrowth after spinal cord injury as part of the resulting glial scar (Tran et al. 2018; Silver and Miller 2004).

Proteoglycan biology is highly complex due to the great structural and functional diversity of these molecules. While the molecular functions of several proteoglycans and specific residues in their GAG motifs have been elucidated, many questions remain unanswered. Particularly in brain cancers, their contributions to tumor progression and malignancy are not fully understood. This chapter will highlight currently known functions of proteoglycans in gliomas, with a specific focus on their relevance for brain cancer stem cells.

11.2 Proteoglycan Family Members and Protein Structure

Proteoglycans consist of a core protein that is decorated with GAG side chains. Depending on the GAG chains, proteoglycans are classified as heparan sulfate (HSPGs), chondroitin sulfate (CSPGs), or keratan/dermatan sulfate proteoglycans. This chapter will focus on HSPGs and CSPGs, which are best characterized. Their core proteins can be used to subclassify HSPGs and CSPGs (Table 11.1). The proteoglycan core protein structure influences proteoglycan functions, such as whether they are membrane-associated or secreted into the extracellular space.

Proteoglycan core proteins can contain a transmembrane domain, a glycophosphatidylinositol (GPI)-anchoring site, or no membrane-associated domains. Thus, some proteoglycans contain both extra- and intracellular domains, while others are tethered to the extracellular membrane or secreted into the extracellular matrix. The core protein structure is, therefore, key to understanding the function of the proteoglycan. For instance, the transmembrane proteoglycan syndecan-1 (SDC1) modulates integrin signaling via its intracellular domain (Beauvais and Rapraeger 2010).

The CSPGs expressed in the CNS belong to the lectican subfamily and contain a conserved N-terminal and C-terminal globular domain which are linked by a backbone of varying length. CSPG core proteins range in length from 294 to 3396 amino acids (Silbert and Sugumaran 2002). CSPGs can be grouped as ECM or membrane-bound proteoglycans (Table 11.1). Extracellular CSPGs are an essential part of the brain parenchymal ECM, together with hyaluronic acid and other linker proteins. Examples of membrane-bound CSPGs are CD44 and NG2, which act as signaling co-receptors. In the CNS, CSPGs are well known for their repulsive functions on

Table 11.1 Representative members of HSPG and CSPG families. Shown are the size of core protein (amino acids), number and type of GAGs, as well as their localization as extracellular matrix (ECM), membrane-bound, or secreted proteoglycans

Family	Proteoglycan	Gene symbol	Core protein (aa)	GAGs	Localization
HSPG	Perlecan	HSPG2	3588, 4346	1–3 HS	ECM
	Agrin	AGRN	2045	1–3 HS	ECM
	Collagen XVIII	COL18A1	1336, 1516, 1751	2–3 HS	ECM
	Syndecan 1-4	SDC1-4	198, 201, 310, 443	1–3 HS/ 1–2 CS	Membrane-bound
	Glypican 1-6	GPC1-6	555–580	1–3 HS	Membrane-bound
	Serglycin	SRGN	158	10–15 heparin/CS	Secreted
CSPG	Aggrecan/ CSPG1	ACAN	2431	~100 CS	ECM
	Versican/ CSPG2	VCAN	655, 1642, 2409, 3396	12–15 CS	ECM
	Neurocan/ CSPG3	NCAN	1321	1–2 CS	ECM
	NG2/CSPG4	CSPG4	2322	2–3 CS	Membrane-bound
	Brevican/ CSPG7	BCAN	911	0–4 CS	ECM
	Phosphacan	PTPRZ1	1448, 1455, 2308, 2315	2–5 CS	Membrane-bound / ECM
	Decorin	DCN	359	1 CS	ECM
	Biglycan	BGN	368	1–2 CS	ECM
	CD44	CD44	294–493	1–4 CS	Membrane-bound

Modified from Masu (2016); Bulow and Hobert (2006)

axon growth during development and injury. In development, this guides the formation of axonal projections across the brain, whereas deposition of CSPGs in injury contributes to the formation of a glial scar that is prohibitory to axonal regeneration (Silver and Silver 2014).

HSPG core proteins range in length from 158 to 4346 amino acids and consist of three groups: membrane-associated, ECM, and secretory vesicle types (Table 11.1) (Bulow and Hobert 2006). The membrane-associated HSPGs include syndecans (SDCs; transmembrane proteins) and glypicans (GPCs; GPI-anchored), and act as co-receptors for growth factor signaling, protease receptors, and receptors for cell attachment (Esko et al. 2009). ECM HSPGs include perlecan, aggrin, and collagen XVIII, which provide an extracellular substrate for cell attachment and migration, as well as axon guidance molecules. In the CNS, ECM HSPGs are mostly part of the basal membrane around blood vessels and/or the pial surface. Lastly, serglycin comprises the secretory vesicle type (Esko et al. 2009).

The GAG chains decorating the core proteins are added and elongated through a complex biosynthetic pathway involving a large number of enzymes. GAGs form a

long, unbranched chain that is attached to serine residues in the core protein and always starts with a conserved sequence called tetrasaccharide linker (Silbert and Sugumaran 2002). The number of GAG chains on core proteins varies widely, ranging from 1 to >100 (Reviewed in (Masu 2016)). Some HS and CS sequence motifs have been well characterized structurally and biochemically, such as those binding to fibroblast growth factors (FGFs) (Xu and Esko 2014; Meneghetti et al. 2015, Mizumoto et al. 2015). We will discuss interactions between proteoglycans and FGFs in a later section.

GAG chain biosynthesis is initiated in the endoplasmic reticulum (ER) and happens simultaneous with the synthesis of the core proteins. The tetrasaccharide linker is added in the ER, whereas chain elongation is catalyzed in the Golgi apparatus. Chain elongation of GAGs is accomplished by alternate addition of monosaccharide residues through the cooperative and coordinated action of several different enzymes. While the tetrasaccharide linker is synthesized in the same way, chain elongation is notably different for CSPGs and HSPGs. CS-GAG backbone chains consist of *N*-acetylgalactosamine (GalNac) and glucuronic acid (GlcA), whereas HS-GAGs consist of *N*-acetylglucosamine (GlcNac) and GlcA (Masu 2016). CS-GAG chains are initiated by GalNAc transferase I and elongated by chondroitin synthases, chondroitin polymerizing factor, and CS *N*-acetylgalactosaminyltransferases (Masu 2016; Kwok et al. 2012). CS-GAGs are then modified, e.g., by phosphorylation and sulfation.

HS-GAG synthesis is initiated by exostosin-like-3, which adds GlcNac to the tetrasaccharide linker. Chains are elongated by the HS polymerase complex consisting of exostosins 1 and 2, adding alternating GlcA and GlcNac units (Xiong et al. 2014). The HS-GAG chain is then modified by a series of enzymes, starting with *N*-deacetylase/*N*-sulfotransferases 1-4, which deacetylate some GlcNac residues and convert them to glucosamine-*N*-sulfate. Then, glucuronyl C-5 epimerase converts some GlcA to Iduronic Acid (IdoA) residues. This is followed by several O-sulfation steps catalyzed by HS-2-*O*-sulfotransferase, HS-3-*O*-sulfotransferase, and HS-6-*O*-sulfotransferase. Finally, extracellular, postsynthetic modification (e.g., by endo-6-*O*-sulfatases or by heparanase, HPSE) can further increase the structural diversity of HSPGs (Xiong et al. 2014; Masu 2016).

The complex synthesis pathways enable the generation of highly diverse families of CSPGs and HSPGs that is mirrored in their functional diversity. The different functions of proteoglycans in gliomas and glioma stem cells will be discussed after a brief introduction to brain cancers in the next section.

11.3 Gliomas

The overwhelming majority of CNS tumors are glial in nature and are termed gliomas. Based on their histopathological appearance and molecular characteristics, gliomas can be divided into astrocytic (astrocytomas) or oligodendroglial (oligodendrogliomas) tumors (Deangelis 2001; Capper et al. 2018). Gliomas can

be either high-grade or low-grade. Low-grade astrocytomas can be divided into benign (e.g., pilocytic astrocytoma) and malignant lesions, whereas all oligodendrogliomas are malignant (Louis et al. 2016). Glioblastoma (GBM) is the only brain tumor of WHO grade IV and is the most frequent type of brain cancer in adults. GBM is the most malignant astrocytoma and can occur de novo (primary GBM) or arise from pre-existing lower-grade tumors (secondary GBM). Mutations in the *IDH1* gene (Toedt et al. 2011) distinguish primary (where they are absent) from secondary GBM (where they are present) and hint at a different origin and evolution of these entities.

Tumor grading is based on histopathological hallmarks, such as nuclear atypia, mitosis, vascular proliferation, and necrosis, and increasingly also considers molecular characteristics, such as loss of chromosomes 1p/19q and *IDH1* mutation status (Louis et al. 2016). Historically, gliomas have been classified based on their histopathological appearance, e.g., as astrocytomas or oligodendrogliomas, but more recent classification schemes also incorporate next-gen sequencing data and DNA methylation profiling (Capper et al. 2018). While the general glioma types are upheld in classification methods based on DNA methylation profiling and next-gen sequencing, these have revealed a much more diverse and heterogeneous nature of gliomas on the molecular level (Capper et al. 2018).

The glioma type has important implications for the prognosis of the tumor. For instance, oligodendrogliomas tend to respond much better to therapy, and the average survival of patients suffering from these tumors is longer than in astrocytomas (Van Den Bent et al. 2017). GBM, the only WHO grade IV brain cancer, is the most malignant brain tumor and also the most common in adults. GBM is incurable with a median survival of only 15–20 months with treatment. This is because GBMs initially respond to therapies, but over time become resistant and recur. Molecular profiling has revealed the rich heterogeneity of GBM, which may contribute to their therapeutic resistance. Gene expression profiling has demonstrated that GBM can be subclassified into multiple molecular subtypes, which frequently co-exist within the same tumor (Verhaak et al. 2010; Sottoriva et al. 2013; Neftel et al. 2019). GBM pathobiology is characterized by its diffusely invasive nature, high proliferation index, and propensity for neo-angiogenesis (Deangelis 2001). The histological appearance of GBM can vary widely, with high vascularization in some areas and others showing characteristic patterns of necrosis (Alexander and Cloughesy 2017). Necrotic areas within GBM are zones of hypoxia and associated with specific pathobiological processes (Colwell et al. 2017). Thus, the tumor microenvironment (TME) in GBM can be vastly different, and separate niches have been defined that impact tumor biology. These niches include the hypoxic, vascular, and invasive niche (Lathia et al. 2011).

All three niches have been found to provide a fertile environment for cancer stem cells, and it is likely that different factors in each niche promote cancer stem cell self-renewal through separate pathways. For instance, it was found that hypoxia induces cancer stemness through HIF1a and HIF2a (Li et al. 2009; Seidel et al. 2010), while VEGF signaling is active in the vascular niche (Gilbertson and Rich 2007). The invasive niche is less understood, but recent work has identified Notch signaling as

key pathway for enabling cancer stem cells to migrate along white matter tracts (Wang et al. 2019).

Cancer stem cells are capable of self-renewal, extensive proliferation, and initiation of tumor growth, whereas non-stem cancer cells are not (Lathia et al. 2015). Since their identification in GBM (Ignatova et al. 2002; Singh et al. 2003), the so-called cancer stem cell hypothesis has caused a major paradigm shift in cancer research and in our understanding of tumor development and progression. It is thought that cancer stem cells are at the apex of a hierarchy of tumor cells and are uniquely capable of initiating and promoting tumor growth (Vescovi et al. 2006). Akin to their normal counterparts, cancer stem cells can self-renew, producing another cancer stem cell and a non-stem cancer cell that lacks the ability to form new tumors. Their ability for extensive proliferation enables cancer stem cells to generate sufficient progenies to fuel the growth of a tumor. Cancer stem cells also have a much greater capacity to resist therapies, and therefore are likely culprits for treatment-refractive recurrence of GBM that results in their high lethality. Several transcriptional regulators have been identified that function to maintain the stem cell identity of cancer stem cells, including MYC (Wurdak et al. 2010; Chan et al. 2012), SOX2 (Gangemi et al. 2009), OLIG2 (Ligon et al. 2007), ZEB1 (Siebzehnrubl et al. 2013; Singh et al. 2017), STAT3 (Sherry et al. 2009), GLI1 (Clement et al. 2007), and others. These are activated by niche signaling pathways (Rheinbay et al. 2013; Day et al. 2013; Fan et al. 2010). Additionally, growth factor signaling, e.g., from EGF, PDGF, IL-6, TGF-b, and FGF2, maintains stemness in GBM (Kim et al. 2012, Wang et al. 2009, Jun et al. 2014, Ikushima et al. 2009, Jimenez-Pascual and Siebzehnrubl 2019; Jimenez-Pascual et al. 2019, Gargiulo et al. 2013). As described below, proteoglycans are important reservoirs for growth factor ligands in the extracellular space and co-regulators for the activation of their cognate receptors in brain cancer.

11.4 Proteoglycan Functions in Glioma

In glioma, proteoglycans form part of the ECM and the tumor microenvironment (TME). As in normal tissue, proteoglycans act as reservoir for growth factors, receptors for proteases, and co-receptors for signaling pathways. Thus, glioma cells may gain access to important pro-survival and pro-mitogenic factors through their release of proteolytic enzymes that cleave proteoglycans and release trophic factors into the TME (Kundu et al. 2016). Additionally, cleavage of GAG chains from HSPGs by the action of HPSE has been associated with increased angiogenesis and inflammation in other cancers (Vlodavsky et al. 2012). Aberrant cell-surface expression of proteoglycans may result in abnormal pathway activation that promotes survival and growth of glioma cells. For instance, upregulation of GPCs increases the sensitivity of cancer cells to growth factors, such as FGF2 (Su et al. 2006). Wade et al. (Wade et al. 2013) analyzed the prevalence of different HSPG and CSPG core proteins in GBM using publicly available gene expression datasets (i.e.,

from The Cancer Genome Atlas Project) and found differential regulation of several core proteins compared to normal brain tissue. For instance, GPC5 is downregulated in GBM, whereas GPC1 is upregulated. Among the CSPGs, CSPG4/NG2, PTPRZ1, CD44, as well as VCAN were upregulated. The specific functions of GPC1/5, PTPRZ1, and VCAN in glioma remain unclear. Some functions of CSPG4/NG2 and CD44 have been elucidated and will be discussed in the next section.

Alteration of proteoglycan core proteins and/or GAG chains may create a permissive environment for tumor growth and invasion, as well as silence the host response against the tumor. Silver et al. have shown that modification of CSPGs in malignant glioma affects the host response to the tumor and promotes tumor invasion (Silver et al. 2013). Crucially, this function is dependent on the depletion of CS-GAGs on the core proteins. If the core protein is glycosylated, host cells respond to the tumor with reactive gliosis and immune activation. This results in the formation of a glial scar that presents a boundary to infiltrating tumor cells, thus curtailing tumor invasion. Notably, this occurs only in the most benign gliomas. Conversely, de-glycosylation of the core proteins causes host cells to remain silent to the tumor and results in an absence of glial reactivity. This enables tumor cells to freely move throughout the neuropil and fosters invasion. De-glycosylated CSPGs seem to be a typical part of all invasive gliomas. Whether this is due to an active de-glycosylation of the core proteins or whether the biosynthesis pathways of CS-GAGs are compromised in these tumors remains to be determined. Wade et al. (2013) have found that the expression of several biosynthetic enzymes for both HSPGs and CSPGs is reduced in GBM, indicating that GAG chain structure and/or sulfation may differ crucially from healthy tissues. This implies that proteoglycan biosynthesis is compromised in glioma, but many other studies also indicate that proteolytic enzyme secretion is increased in malignant gliomas (Kundu et al. 2016; Jimenez-Pascual et al. 2019; Markovic et al. 2009). Aside from proteolytic digestion of the core proteins, extracellular enzymes that cleave GAG chains from HSPGs (e.g., HPSE) or change sulfation patterns (e.g., SULFs) are upregulated in gliomas, and this is correlated with patient survival (Wade et al. 2013; Kundu et al. 2016).

Proteolytic cleavage of proteoglycans and/or expression of structurally different proteoglycans by glioma cells may result in increased levels of trophic factors in the TME that create a permissive environment for the growth of cancer stem cells. The specific roles of proteoglycans in promoting glioma stemness are discussed in the next section.

11.5 Proteoglycan Functions in Glioma Stem Cells

Some CSPGs have been directly associated with stem/progenitor cells in development and cancer. The most notable examples include CD44 and NG2/CSPG4 (Pietras et al. 2014; Yadavilli et al. 2016). CD44 has been identified as a stem cell antigen in several tissues, including the brain (Zoller 2011). CD44 has also been identified as a marker on cancer stem cells in GBM (Pietras et al. 2014; Anido et al.

2010; Fu et al. 2013). CD44 is a receptor for hyaluronic acid, an ECM GAG that lacks a core protein and that is a key component of the ECM framework and thereby mediates cell adhesion to the ECM. Importantly, CD44 can signal to the nucleus and enhance HIF2a activity (Pietras et al. 2014). CD44 is therefore functionally involved in cancer stem cell maintenance by activating stemness-associated signaling pathways.

CSPG4 (more commonly known as neuron glia-antigen 2, NG2) was first identified to label a population of glial precursor cells that are capable of proliferating and generating oligodendrocytes and are therefore referred to as oligodendrocyte precursor cells (OPCs). Of note, OPCs are considered as a potential cell of origin for brain cancers (Liu et al. 2011). NG2 expression is increased in malignant glioma, including GBM, where it is associated with poor survival (Yadavilli et al. 2016; Svendsen et al. 2011). Whether NG2 expression is associated with a genuine cancer stem cell population is not fully resolved. It has been shown that NG2 knockdown results in slower glioma growth and reduced angiogenesis in vivo (Wang et al. 2011). A recent study reported NG2 expression in putative GBM cancer stem cells (Lama et al. 2016), but did not rigorously test whether isolating NG2 expressing GBM cells enriches for a cell population with higher tumorigenicity upon limiting-dilution orthotopic transplantation, which is the gold standard (Lathia et al. 2015). Nevertheless, this work showed that NG2 is also expressed on pericytes within the GBM core and at the invasion front, indicating that NG2 may also have crucial functions within the TME (Lama et al. 2016). NG2 can act as a signaling molecule, with its intracellular domain capable of binding extracellular regulated kinases (ERK1/2) and protein kinase C-alpha (PKC-a) (Ampofo et al. 2017). NG2 can thus activate cell migration, survival, and angiogenesis signaling pathways, all of which are relevant to tumor progression in malignant glioma.

To date, no HSPGs that are exclusive to, or enriched on, glioma stem cells have been identified, despite several HPSGs being upregulated in malignant glioma. Several studies have shown that FGFR1 is upregulated on GBM stem cells (Gouaze-Andersson et al. 2016; Jimenez-Pascual et al. 2019; Kowalski-Chauvel et al. 2019), and HSPGs act as co-receptors for FGF2-FGFR1 signaling. It is therefore possible that GBM stem cell-specific HSPGs or HPSG isoforms exist and will be identified in the future. Both CSPGs and HSPGs can bind FGFs and modulate FGF signaling (Guimond and Turnbull 1999; Allen and Rapraeger 2003; Djerbal et al. 2017). They may therefore be important co-regulators of cancer stem cell maintenance pathways, but these functions remain to be investigated.

As mentioned in the previous section, proteolytic cleavage of ECM proteoglycans may increase levels of bioavailable FGF2, e.g., to invasive glioma cells. Increased expression of membrane-bound CSPGs or HSPGs has been found in GBM (see above) (Wade et al. 2013). In glioma cells and glioma-associated blood vessels, aberrant expression of the HSPG GPC1 increases FGF2 sensitivity (Qiao et al. 2003; Su et al. 2006). Whether similar mechanisms exist on GBM cancer stem cells remains to be shown, but GPC1 is among the most prominently upregulated HSPGs in GBM (Wade et al. 2013), where it is predictive of invasion and poor prognosis (Saito et al. 2017). It is therefore conceivable that altered expression

and/or structure of CSPGs and/or HSPGs are key contributors to cancer stemness in glioma, but this remains to be shown.

11.6 Proteoglycans as Therapeutic Targets in Glioma

Because CSPGs and HSPGs are important co-regulators of receptor-tyrosine kinase signaling pathways dysregulated in glioma, they are candidate targets for anti-cancer therapies. Indeed, genetic depletion of CSPG4/NG2 was shown to reduce tumor growth in experimental models of glioma (Wang et al. 2011). The membrane-bound CSPG, CD44, has been used as a cell-surface marker for cancer stem cells, and it was shown that CD44 is functionally transducing signaling pathways on GBM cancer stem cells (Anido et al. 2010; Pietras et al. 2014).

As discussed above, posttranslational modification of proteoglycans is an important mechanism to generate their structural and functional diversity. It may therefore not be surprising that changes in posttranslational modification of HSPGs and CSPGs reflect on glioma malignancy. For instance, changes in HSPG sulfation are associated with more aggressive tumor growth, and knockdown studies of SULF2 resulted in decreased PDGFRa signaling and in vivo tumor growth (Phillips et al. 2012). Increased expression of the HSPG degrading enzyme, HPSE, is associated with reduced survival in GBM patients (Kundu et al. 2016). In experimental gliomas, it was found using syngeneic glioma transplants into HPSE-transgenic mice that host-derived HPSE contributes to tumor growth, immune evasion, and angiogenesis (Kundu et al. 2016). Knockdown approaches demonstrated that blocking expression of HPSE in pediatric glioma cells also decreases proliferation and invasion of these cells upon transplantation in vivo (Spyrou et al. 2017). The growth-promoting actions of HPSE with increased CD24 expression on GBM cells (Barash et al. 2019). For CSPGs, it was shown that de-glycosylation of CS core proteins is associated with increased invasion in GBM, whereas artificially increasing glycosylated CSPG levels in the ECM potently blocked tumor invasion (Silver et al. 2013).

All these studies implicate HSPGs and CSPGs as important contributors to glioma growth and malignancy. Because proteoglycans and certain proteoglycan-modifying enzymes reside in the extracellular domain, these constitute attractive targets for anti-cancer therapy. This has been evaluated in a number of studies using heparan sulfate mimetics, small-molecule inhibitors, and GAG antagonists. Heparan sulfate mimetics are sulfated oligosaccharides that can block HPSE and SULFs. They can scavenge ligands binding to HS side chains and may thus drain the TME of growth factors promoting glioma growth and progression (Johnstone et al. 2010; Dredge et al. 2011). One particular HS mimetic, M402, has shown promising results in experimental models of other solid tissue malignancies (Joyce et al. 2005) and is in clinical trials for pancreatic cancer. Additional inhibitors of HPSE have been developed (e.g., PG545), which block the release of biologically active GAGs from HSPGs in the TME (Hammond et al. 2013). In experimental models of glioma,

PG545 was shown to induce apoptosis in glioma cells, to reduce invasion, and to attenuate tumor growth in vivo (Kundu et al. 2016; Spyrou et al. 2017).

A recent study using a small-molecule sulfated GAG antagonist (Surfen) found that this molecule blocked CSPG receptor expression on glioma cells and decreased tumor invasion (Logun et al. 2019). While no specific inhibitors for the CSPG CD44 exist, this molecule is cleaved by gamma secretase for intracellular signaling, and gamma-secretase inhibitors showed promising results in experimental studies (Tanaka et al. 2015).

11.7 Summary and Conclusions

The functions of proteoglycans in glioma in general and glioma cancer stem cells, in particular, remain incompletely understood. The rich structural diversity of these molecules results in a wide range of functions that are fine-tuned according to biological needs. In brain cancer, it is becoming apparent that proteoglycan core protein expression, GAG synthesis, posttranslational modification, and extracellular structure are dissimilar to the normal brain. This diverse portfolio of potential structural changes of proteoglycans indicates that these molecules are important contributors to glioma growth and cancer stem cell maintenance.

A number of questions remain unanswered. Firstly, spatiotemporal heterogeneity of core protein expression and/or glycation is not fully understood. Many studies have investigated the HSPGs and CSPGs in glioma, but whether expression and/or glycation of these molecules changes in different areas of the tumor, or over time, is unclear. Secondly, the relationship between proteoglycan-modifying enzymes and cancer stem cells has not been properly addressed. HPSE and SULFs are capable of dramatically altering the structure of HSPGs, but the impact of this on cancer stemness is not understood. It is conceivable that extracellular modifications of HSPGs (and/or CSPGs) result in local changes of cytokine levels in the microenvironment that may promote stemness in glioma cells. Expression of HPSE and SULFs, too, may be subject to topological or temporal changes. Thirdly, whether proteoglycans act as signaling co-receptors and glioma cells and/or glioma stem cells has been only partially resolved. Whether certain cell-surface HSPGs or CSPGs are expressed preferentially on glioma cancer stem cells remains unclear, with the exception of CD44, where a firm relationship has been established, and potentially NG2, where expression of GBM cancer stem cells has been suggested. Cell-surface expression of proteoglycans on cancer stem cells likely results in an increased sensitivity of these cells to extracellular mitogens that enable cancer stem cells to thrive and populate tumor-free tissue. It will therefore be interesting to explore GBM cancer stem cell-specific expression of proteoglycans and their functions in the future.

The link of proteoglycans to pro-tumorigenic signaling pathways and tumor invasion also highlights their potential as possible therapeutic targets in glioma. Some studies have tested proteoglycan blocking agents in experimental models of

glioma (Kundu et al. 2016; Phillips et al. 2012), and some of these compounds are even in clinical trials for other solid tissue malignancies. Whether any promising compounds are able to cross the blood-brain-barrier, a major obstacle in drug delivery to brain cancers will need to be evaluated. Nevertheless, there is genuine potential for a new class of anti-cancer therapeutics aimed at the TME and at barring access of glioma cells to essential mitogens.

In summary, much more research needs to be done to unlock the potential functions of proteoglycans in glioma and to exploit these for therapeutic targeting.

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