Eur. Phys. J. Plus (2016) **131**: 85
DOI 10.1140/epjp/i2016-16085-7

THE EUROPEAN
PHYSICAL JOURNAL PLUS

Review

The involvement of heparan sulfate proteoglycans in stem cell differentiation and in malignant glioma*

Soumi Kundu, Anqi Xiong, and Karin Forsberg-Nilsson[a]

Department of Immunology, Genetics and Pathology, and Science for Life Laboratory, Rudbeck Laboratory,
Uppsala University, 75185 Uppsala, Sweden

Received: 20 December 2015
Published online: 8 April 2016 – © Società Italiana di Fisica / Springer-Verlag 2016

Abstract. Heparan sulfate (HS) proteoglycans (HSPG) are major components of the extracellular matrix. They interact with a plethora of macromolecules that are of physiological importance. The pattern of sulfation of the HS chain determines the specificity of these interactions. The enzymes that synthesize and degrade HS are thus key regulators of processes ranging from embryonic development to tissue homeostasis and tumor development. Formation of the nervous system is also critically dependent on appropriate HSPGs as shown by several studies on the role of HS in neural induction from embryonic stem cells. High-grade glioma is the most common primary malignant brain tumor among adults, and the prognosis is poor. Neural and glioma stem cells share several traits, including sustained proliferation and highly efficient migration in the brain. There are also similarities between the neurogenic niche where adult neural stem cells reside and the tumorigenic niche, including their interactions with components of the extracellular matrix (ECM). The levels of many of these components, for example HSPGs and enzymes involved in the biosynthesis and modification of HS are attenuated in gliomas. In this paper, HS regulation of pathways involved in neural differentiation and how these may be of importance for brain development are discussed. The literature suggesting that modifications of HS could regulate glioma growth and invasion is reviewed. Targeting the invasiveness of glioma cells by modulating HS may improve upon present therapeutic options, which only marginally enhance the survival of glioma patients.

Introduction to heparan sulfate proteoglycans, stem cell differentiation and brain tumors

Heparan sulfate proteoglycans (HSPGs), macromolecules ubiquitously present in mammalian tissues, are involved in a multitude of developmental and pathological processes, including those involving the nervous system. HSPGs are synthesized via a complex series of stepwise enzymatic modifications, which is regulated at several levels and allows fine-tuning of the structure of the heparan sulfate (HS) for specific interactions with growth factors. If the regulation is perturbed, severe developmental defects may result, as shown with mouse strains in which many of the components involved have been knocked out. Furthermore, studies on embryonic stem cells (ESC) deficient in the enzymes EXT1 and NDST1/2 differentiated in vitro indicate that HS plays a critical role in neural differentiation [1–4]. Improved understanding of the interactions between HSPGs and factors regulating neural differentiation will be important for developing more sophisticated protocols for generating neural cells from human ESCs for potential therapeutic applications.

The involvement of HSPG in the development of brain tumors is becoming increasingly clear, as will be outlined in this paper. HSPGs are major constituents of the ECM in the brain and alteration of their composition may facilitate the growth and spread of glioma. By regulating the responses of tumor cells to growth factors, HSPGs augment their proliferation and enhance tumor size.

Accordingly, the biosynthesis and modification of these macromolecules are presumed to play a key role in the aggressive glioma invasion, a main reason these tumors are so deadly. In addition, the involvement of HS and the enzymes that modify HS could be important for tumor angiogenesis, another key aspect of the tumor microenvironment. Since HS interacts with a wide variety of molecules involved in the initiation and progression of tumors this substance, as well as the enzymes that synthesize and modify it are potential therapeutic targets. In this context,

* Contribution to the Focus Point on "The Physics of Cancer" edited by M. Ben Amar.

[a] e-mail: karin.nilsson@igp.uu.se

 Eur. Phys. J. Plus (2016) **131**: 85

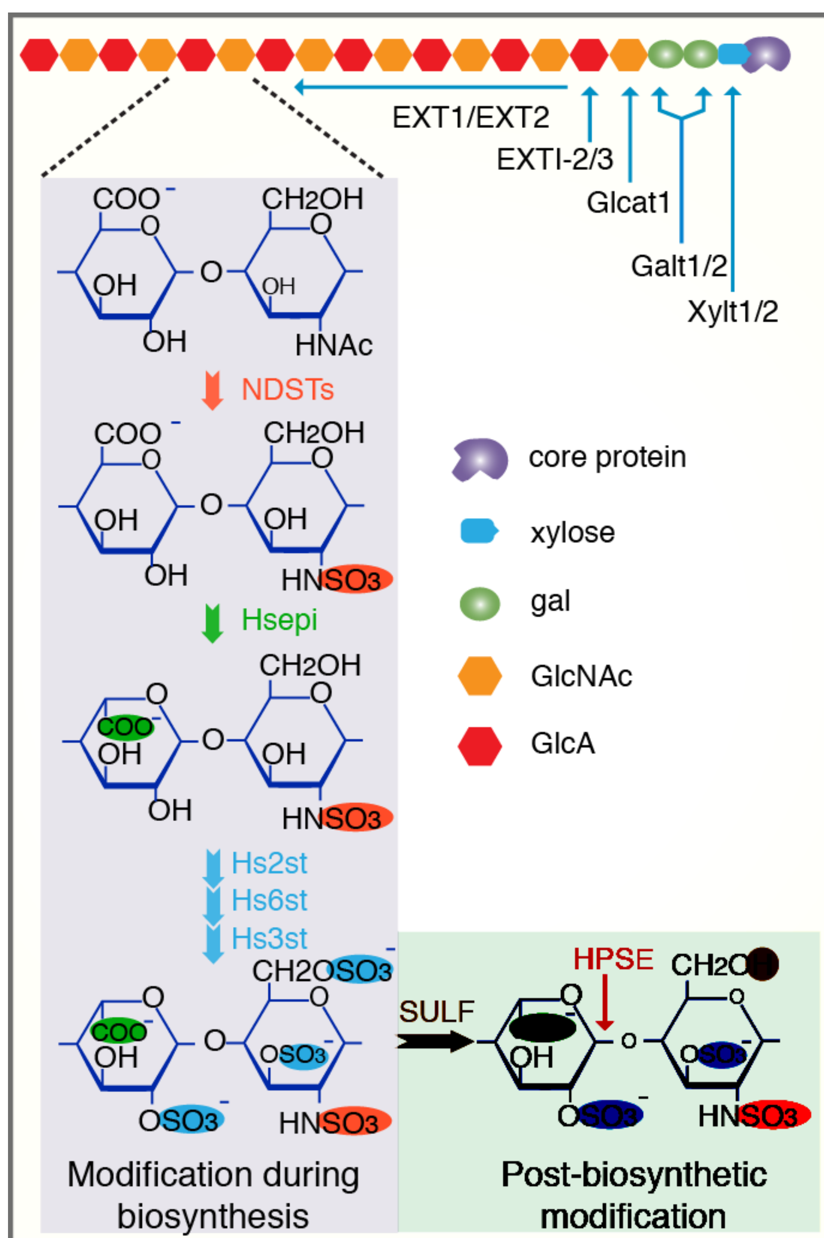

Fig. 1. Schematic representation of the structure of heparan sulfate and of the different steps and enzymes involved in its biosynthesis and modification.

the development of mimetics designed to block HS-mediated stimulation of both tumor growth and angiogenesis are highly interesting [5].

Specifically modified heparan sulfate chains regulate a variety of growth-factor–related processes

HSPGs are present on the surface of cells (syndecans and glypicans), in the ECM (perlecans, agrins and collagen type XVIII) and in the secretory granules of mast cells (serglycin). Each HSPG is composed of a core protein to which one or several heparan sulfate (HS) glycosaminoglycan (GAG) chains are covalently *O*-linked. It is these chains that interact with various biomolecules, including growth factors, chemokines, and enzymes, as well as other ECM and plasma proteins to protect the integrity of the ECM, and mediate cell-cell and cell-ECM interactions.

Synthesis of HSPGs in the Golgi compartment is initiated by transfer of a xylose molecule to the core protein by xylosyltransferase-1/2 (XylT1/2), followed by attachment of two Gal residues by galactosyltransferases-1/2 (Galt1/2) and GlcA by glucuronyltransferase-1 (GlcAT-1) to form the tetrasaccharide linkage [6] (fig. 1). Thereafter, elongation

starts by attachment of an initial GlcNAc residue to this tetrasaccharide by exostosin-like-2/3 (EXTl-2/3), followed by alternating GlcA and GlcNAc residues [7] catalyzed by the EXT1/EXT2 complex.

As this process proceeds, the HS chains undergo a series of modifications. The first, which is a prerequisite for most of the subsequent modifications, is *N*-deacetylation/*N*-sulfation of GlcNAc residues to form GlcNS catalyzed by *N*-deacetylase/*N*-sulfotransferases (NDSTs). Four such enzymes have been identified and most mammalian cells express mRNA coding for NDST-1 and NDST-2 [8]. The next modification is epimerization of GlcA in N-sulfated regions of the chain to IdoA by glucuronyl C5-epimerase (Hsepi). Afterwards, the HS chains undergo *O*-sulfation at different sites by 2-*O*- (HS2ST), 6-*O*- (HS6ST), and 3-*O*-sulfotransferase (HS3ST) [9]. In addition to such modifications, endo-6-*O*-sulfatases (SULFs) can release 6-*O*-sulfates from HS chains. Heparanase (HPSE) is primarily responsible for the degradation of HS, cleaving the ß-1, 4-glycosidic bond between a *D*-glucuronate and a *D*-glucosamine residue [10], thereby also altering HS composition.

Certain regions of the HS chain can escape modification, which results in different patterns of sulfation and/or acetylation, *i.e.*, unmodified N-acetylated regions (NA domains), *N*-sulfation-rich regions (NS domains) and NA/NS junctions between these two. It is generally thought that such variation in HS sequence and the pattern of sulfation confers specificity in the binding of HS to signaling molecules. Moreover, organ-specific differences have been observed [11] along with "compensatory" mechanisms, *e.g.*, up-regulation of *N*- and 6-*O*-sulfation in HS2ST-deficient mice [12].

One major function of HS is as co-receptors for growth factors on the cell surface, facilitating ligand-receptor binding, lowering the threshold for activation of signaling pathways and altering the duration of the response. Extensive investigations have revealed that HS chains serve as essential templates for binding of FGFs to their cell surface receptor [13–15]. Similar models have been proposed for many other signaling pathways, including those involving BMP [16], WNT [17] SHH [18,19], PDGF [20,21] and VEGF [22]. In addition to such regulation of cell signaling, HS chains cooperate with integrins and adhesion receptors to facilitate cell-ECM interactions and cell motility, and act as receptors for endocytic clearance of the ligands they bind. Thus, HS can potentially regulate most growth factor-related processes in the body, which has important implications for both normal development and carcinogenesis.

Heparan sulfate proteoglycans participate in neural stem cell commitment and differentiation

Neural stem cells (NSCs) are multipotent cells that form neurons, astrocytes and oligodendrocytes. The multipotency of NSCs in culture is governed by the mitogens FGF2 and EGF, and upon mitogen withdrawal NSCs differentiate into all three mature cell types. The proliferation, differentiation and commitment of these cells are tightly regulated by various growth factors and several of these signaling pathways depend on HS. FGF2 and Notch promote NSCs proliferation and inhibit differentiation [23,24], while activation of FGF4 leads to differentiation. Activation of WNT signaling promotes the proliferation [25] and determines the neuronal fate of NSCs [26], while PDGF is involved in the expansion of neural progenitors [27] and oligodendrocyte differentiation [28].

The affinity of a growth factor for HS chains depends largely on the pattern of sulfation, and expression of the enzymes involved in the modification of HS is spatially and temporally orchestrated. For instance, as neuroepithelial cells start to differentiate into neurons, both the length and pattern of 6-*O*-sulfation of their HS chain length change, switching from potentiation of FGF2 to FGF1 signaling [29]. Moreover, alterations in *N*-, 3-*O*- and 6-*O*-sulfation all occur during stem cell differentiation [30,31].

The fact that so many HSPGs and their modifying enzymes are indispensible for embryogenesis [20–22,32–35] often makes it impossible to examine their developmental functions in the intact mouse nervous system. Instead, their involvement in stem cell commitment and maturation can be readily assessed employing ESC *in vitro* differentiation. These cells are derived from the inner cell mass (ICM) of embryos at the blastocyst stage and defined as pluripotent, since they are capable of differentiating into all the different types of cells in the body [36,37]. While ESCs can differentiate spontaneously into a heterogeneous population of various cell types when deprived of the factors that maintain their stem cell properties [38] from the culture, numerous culture conditions direct differentiation to specific cell lineages [39–42] providing systems highly suited to the characterization of early development and commitment. In addition to offering new insights into the role of HS in neural differentiation, such studies may also help us to modulate cell fate in order to achieve even more homogenous differentiation.

For differentiation of ESCs, *in vitro*, either aggregates known as embryoid bodies (EB), formed in suspension are allowed to adhere for differentiation, or alternatively, monolayers are exposed to specific components to direct the cell fate. These two approaches are exemplified by protocols for generating neural stem cells (NSCs) from mouse ESCs. The first approach generates large numbers of neural progenitors that differentiate efficiently into neurons and glia [43], but is rather time-consuming, while improvement of directed monolayer cultures has produced highly enriched neural progenitors [41].

The involvement of HS in ESC differentiation has been studied primarily using EXT1 knockout and NDST1/2 double knockout cells. The former cannot synthesize any HS chains and grow in phenotypically normal cell colonies with high levels of pluripotency markers [31,1]. However, complete loss of EXT1 prevents differentiation in monolayer

culture [31,1] and although they could form embryonic bodies, these cells were unable to differentiate terminally into lineages [2]. Differentiation of EXT1-null cells into mature neurons can be partially rescued by heparin [31]. Furthermore, a soluble GAG induces neural differentiation of shRNAi- EXT1 ESCs via various tyrosine kinase receptors [3].

N-sulfation by NDST is a prerequisite for most additional sulfation and ESC in which both NDST1 and NDST2 are knocked out are completely devoid of *N*-sulfation and demonstrate very little 6-*O*-sulfation [44]. As with the EXT1 knockout, these cells maintain their pluripotency in culture [44] but generally fail to differentiate upon formation of EB formation [45]. Furthermore, angiogenesis is severely delayed and adhesion of pericytes to nascent sprouting vessels reduced in these embryoid bodies [46].

Somewhat surprisingly, these NDST1/2-null cells can generate osteoblasts, at least one kind of mesodermal cell, although with a less efficacy than wild-type ESCs but they could not differentiate into adipocytes [4]. Under conditions that induce neural differentiation, these cells cannot proceed to neural progenitors, but appear to remain arrested as primitive ectoderm-like cells expressing the early ectodermal marker FGF5. Similarly, these cells cannot form an endoderm and although they express the primitive endodermal marker GATA4, no later markers of endodermal differentiation can be detected. The capacity of NDST1/2-null cells to continue neural differentiation can, however, be restored by heparin in combination with FGF2 or FGF4, but only within a very narrow range of concentrations [4].

These deficiencies associated with deletion of EXT1 or NDST1/2 are consistent with the observation that the overall level of HS sulfation tends to increase as ESCs differentiate [30]. However, so far, no ESCs deficient in other HS synthesizing or editing enzymes have been described. The findings that ESCs exhibit a low level of *N*-sulfation and express increasing levels of NDST4, 6*O*- and 3*O*-sulfotransferases during neural differentiation suggest that HS with different patterns of sulfation participates in stem cell differentiation [31].

Heparan sulfates in cancer

GAGs and proteoglycans have long been implicated in one way or another at all stages of carcinogenesis, including cancer cell proliferation, tumor invasion and metastasis, as well as angiogenesis. Many types of cancer have been associated with alterations in either the core proteins and/or composition of HS and HSPG as well as the relevant biosynthetic modifying enzymes.

As demonstrated in the case of metastatic melanoma [47] and colon carcinoma cells [19], specific structural changes in the HS chain can either promote or inhibit tumor growth. Germline mutations in the glycosyltransferase EXT1, responsible for polymer inititation causes benign osteochondromas, while elevating the risk of developing malignancies such as chondrosarcomas and osteosarcomas [48]. Such observations suggest that HS synthesis in cancer cells is disturbed and implies that the gene encoding EXT1 is a tumor suppressor gene.

The EXT1 promoter is hyper-methylated in, *e.g.*, leukemia, which lowers expression, and moreover, when expression is re-induced, tumor growth is attenuated [49]. Furthermore, loss of heterozygosity of EXT1 is often observed in connection with hepatocellular carcinoma [50] and EXT2 is mutated in breast carcinomas [51]. In the case of multiple myeloma, expression of nine enzymes that synthesize or modify HS including EXT2, HS3ST2 and HS2ST1 and HPSE and SULF2 was reported to be altered in comparison to normal plasma cells [52]. In this same investigation, EXT expression by multiple myeloma was found to be associated with poor prognosis [52], in contrast to the observations described above.

The sulfatases SULF1 and SULF2 selectively remove the 6-*O* sulfate moieties, thereby affecting the binding of FGF-2 [53], EGF, HGF, WNT and GDNF [54] to HS. Accordingly, the levels of these activities can exert a pronounced impact on many cancers. For instance, desulfation of HSPGs, catalyzed primarily by SULF1 and SULF2, has been associated with pulmonary carcinogenesis in humans [55]. Moreover, SULF2 is especially pro-angiogenic and up-regulated in breast cancer [56]. HPSE, which is secreted and cleaves extracellular HS into small bioactive fragments appears to influence the tumor proliferation, angiogenesis, invasion and metastasis of many different types of cancer [57].

Glioma, a malignant brain tumor with poor prognosis

Gliomas, a diverse group of brain tumors, have traditionally been categorized as astrocytoma, oligodendroglioma and oligoastrocytoma on the basis of histology. These tumors are classified into malignancy grades I-IV [58], with grades III-IV being most malignant and having a very poor prognosis. Patients with the most aggressive form, glioblastoma (GBM), have a medium survival of only 15 months despite combined treatment with surgery, radiation and chemotherapy [59]. Although GBM are extremely heterogeneous, emerging diagnostic techniques allow them to be divided into subtypes on the basis of their molecular profiles. For example, distinct subgroups of GBM demonstrate different mutation frequencies and mRNA profiles [60,61], that allow them to be divided into Proneural, Neural, Classical and Mesenchymal subtypes.

In Proneural GBMs, the 4q12 locus, harboring the PDGFRA gene is amplified, and PDGFRA is expressed at a high level, and there are numerous point mutations in IDH1. Classical GBMs exhibit high rates mutations in EGFR,

together with focal loss of 9p21.3, which affects CDKN2A. In the case of Mesenchymal GBM, deletions of the NF1 gene are common and genes encoding members of the superfamily of tumor necrosis factor is elevated. The Neural subtype, with an expression profile similar to the normal brain is more elusive but does demonstrate increased expression of neuronal markers such as NFL and SYT1.

An extended classification [61] that takes the global pattern of DNA methylation into account and includes pediatric GBM arrived at six subgroups. The Classical (designated RTKII) and Mesenchymal subtypes were corroborated, while the Proneural group (in adults) was sub-divided into tumors in which RTKI/PDGFRA is amplified and those in which IDH1 is mutated along with CpG island promoter methylation. Two additional classes of GBM with high incidences in children and young adults were also identified.

Gliomas of high-grade malignancy are invasive and infiltrate the healthy brain parenchyma, which is the main reason they remain fatal despite resection, and cells that have already migrated away lead to rapid regrowth of the tumor growth. Moreover, gliomas that appear less aggressive at the time of diagnosis can eventually become more malignant. Recently recurrent gliomas originating from a low-grade brain tumor (oligodendroglioma or astrocytoma) were shown to have evolved into a more malignant form by accumulating multiple mutations in response to temozolamide [62].

Changes in the extracellular matrix of glioma include alterations in core proteins of proteoglycans, as well as in the levels of enzymes that synthesize and modify it

The major components of the unique ECM of the brain are hyaluronan and proteoglycans. Whereas other ECM is rich in collagen, in the normal brain, collagen is only present in the basement membranes in the meninges and around blood vessels. The basement membrane of these vessels also contains fibronectin, laminin, vitronectin and HSPG. From the blood vessels, extensions of the basal lamina, referred to as fractones, protrude into the subventricular zone [63]. These fractones are rich in HSPG and participate in, *e.g.*, the FGF signaling that supports the maintenance and self-renewal of neural stem cells [64].

Interactions between neural stem cells and the collagen-containing ECM are modulated as the niche for these cells develops [65] and this niche is also altered in brain tumors. In the case of GBM, the levels of collagen are increased [66] and contents of hyaluronan and several proteoglycans are increased. Calabrese and colleauges have described a specialized perivascular niche that harbors glioma stem cells in GBM [67]. It appears likely that sequestration of growth factors by HSPG in this niche can promote the growth of cancer stem cells in a manner similar to, but more pronounced than the normal neural stem cell niche. Thus, detailed understanding of the niche architecture with special regard to HSPG may open new avenues for treatment of these fatal brain cancers.

Various biochemical and histological analyses revealed that glioma cells in culture synthesize HS, express GAG chains on their surface and release these chains into the culture medium to a greater extent than normal cell [68]. Furthermore, in contrast to the distinctive punctate staining for HSPGs in normal brain or low-grade astrocytomas, the corresponding high-grade glioma staining is intense, diffuse and localized to the cell surface [68]. Another early study demonstrated various amounts of GAGs in glioma depending on type, but with no clear correlation to WHO grade [69]. In low-grade glioma, the content of GAGs was higher than in normal brain, the levels in anaplastic astrocytoma were more similar to normal tissue; and GBM, again, exhibited more abundant GAGs.

The core proteins of several HSPGs of which glypican-1 [70] and syndecan-1 [71] have been most studied, are altered in glioma (fig. 2). While syndecans 2; 3 and 4 are all ubiquitously expressed in both malignant glioma and normal brain tissue, syndecan-1 is not detected in the non-neoplastic brain [71]. These authors also suggested that syndecan-1 was up-regulated via NFkB activation.

The more extensive analysis by Xu and colleagues [72] on more than 100 gliomas confirmed their elevated expression of syndecan-1 as well as an association with WHO grade. Highest expression was found in grade IV and patients with higher expression survived for a shorter time. Moreover, a survey of the Cancer Genome Atlas Data Portal revealed enhanced expression of glypican-6, agrin, serglycin and perlecan/HSPG2 by these tumors [73]. In addition, perlecan/HSPG2 recently appeared in a set of genes predictive of poor prognosis for patients with high-grade (III and IV) gliomas [74], extending this list of HSPG molecules for which alterations in the core proteins can influence glioma growth.

In addition, enzymes involved in editing the HS chain, are differentially expressed in glioma, for instance SULF2, which removes 6-*O*-sulfate moieties and thereby activates multiple signaling pathways, is up-regulated in glioma [73]; see further below. Exploring the TCGA database containing 424 human GBM samples, Wade and co-workers concluded that, in general, the HS biosynthetic enzymes are down-regulated, with the exception of HS6ST1-3, which is up-regulated, suggesting elevated 3-*O*-sulfation in combination with reduced 6-*O*-sulfation [75].

Another HS-modifying enzyme overexpressed by glioma is HPSE (fig. 2) [73], the main catalytic enzyme that degrades HS at specific sites and implicated in metastasis and invasion in various cancers [57]. However, reports in this area are somewhat contradictory. Although over-expression of HPSE in the U87 glioma cell line enhanced invasion in vitro and tumor size after xenografting, the highest levels of this enzyme were associated with attenuated tumor

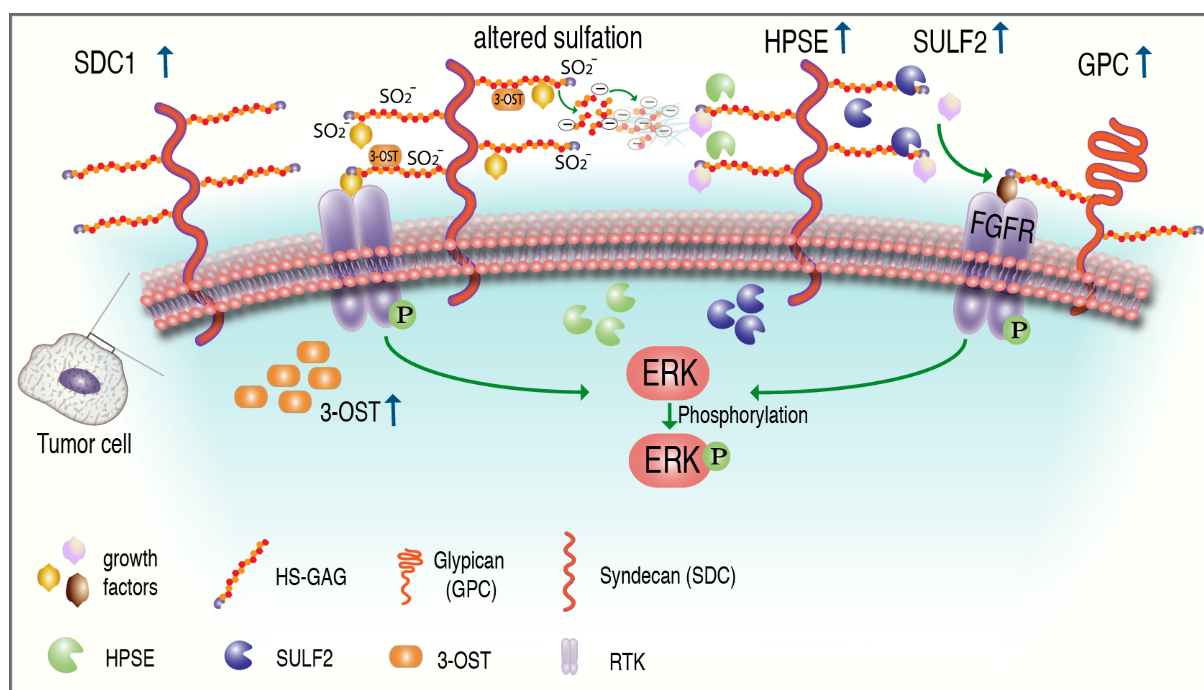

Fig. 2. Differing levels of core proteins (in particular syndecan and glypican) and altered sulfation (in particular, in the enzymes SULF2 and HSPE) of HSPG are involved in several tumor-promoting processes in glioma.

growth [76]. In another report, HPSE could not be detected in human GBM and following injection of U87 cells into the brain of immunologically compromised mice, HPSE expression in these cells disappeared [77]. When comparing the HPSE expression across oligodendroglioma, anaplastic astrocytoma and GBM, the authors did not find a correlation between HPSE and WHO grade, although the expression level was higher than normal brain [78]. In U251 glioma cells, HPSE overexpression led to increased invasion, colony formation and AKT phosphorylation [79].

Proliferative signaling pathways dependent on heparan sulfate are mutated in glioma

Abnormal activation of tyrosine kinases receptors is commonly seen in glioma and HSPGs influence the signaling mediated by these receptors thereby contributing to the progression of such tumors. The pronounced negative charge of HS-GAGs enables them to bind many growth factors and thus play a key role in RTK activation (fig. 2). One prime example of this is PDGFRA signaling.

The PDGFRA gene is frequently amplified and PDGF over-expressed of PDGF in glioma. Extracellular SULF2 is expressed by primary human GBM tumors and cell lines and the corresponding gene has been identified as a candidate for insertional mutagenesis in connection with retrovirus-driven mouse glioma induced by PDGF [80]. Recently SULF2 was reported to increase tumor size by regulating HSPG-dependent signaling by RTKs (fig. 2) [81]. This is most prominent in the case of the Proneural subclass, which is characterized by augmented PDGFRA activity and although SULF2 levels affected primarily PDGFRA signaling, IGFR and EPHA2 are also influenced. Thus, the HS-SULF2-RTK axis enhances glioma development by increasing the bioavailability of growth factors in the microenvironment.

Glioma growth can also be stimulated as a result of enhanced FGF2 signaling due to elevated levels of core HSPG proteins (*e.g.*, glypican-1) and/or structural alterations of the HS chain. Glioma cells and GBM show alterations in their expression of sulfotransferases, in particular up-regulation of HS3ST3a1 [70,75]. An early discovery that glypican-1 is involved in regulating cell division and survival, resulted from the observation of its overexpression in C6 glioma [82].

Glypican-1 is highly expressed in human glioma cell lines where it forms ternary complexes that promote FGF-2-dependent signaling via FGFR1c, thereby conferring a growth advantage [70]. Elevated levels of glypican-1 have also been detected in astrocytoma and oligodendroglioma, without any correlation to WHO grade [70]. Glypican-1 has been shown to exert a direct effect on regulation of glioma cell cycle through inactivation of the G1/S checkpoint and stimulation of the MAPK and PI3K pathways [83]. Several additional tumor-promoting signaling pathways can be affected by modifications of HS, *e.g.* removal of moieties by SULF1 and SULF2 can cause release of VEGF, SDF-1 and Wnts [54].

HSPG and vascularization in glioma

Many of the growth factors with which HS interact also have important effects on angiogenesis, in so that HS can even promote the angiogenesis required for solid cancers to grow beyond a few millimeters in diameter. The interactions between, *e.g.*, syndecan or glypican and the angiogenic factors FGF and VEGF have been extensively characterized but it is also possible for HSPG to affect tumor angiogenesis by several other means [84]. Syndecan-2, the major syndecan expressed by microvascular endothelial cells has proven to be a regulator of glioma angiogenesis and on the basis of findings with the mouse GL261 tumor model it has been proposed that MMP-dependent shedding of syndecan-2 from endothelial cells induced by growth factors is pro-angiogenic in this context [85]. Glypican-1 is overexpressed in the endothelial cells of glioma vessels and activates mitogenic signaling through its ternary complex with FGF2/FGFR [83]. A recent novel mechanism proposed for stimulation of glioma angiogenesis involves activation by HPSE of TGLI1, a splice variant of the transcription factor GLI1, with consequent up-regulation of VEGF-A [86]. Although absent from normal brain tissue, TGLI1 is widely expressed in GBM and enhances the growth and vascularity of GBM xenografts. The integrity of the blood-brain barrier (BBB) is compromised in GBM as reflected in morphological alterations such as vessel fenestrations and pericyte detachment. In this context, agrin was shown to be an important regulator of BBB composition [87] and its loss from GBM vessels is associated with their typically elevated permeability [88].

Glioma infiltration remains a clinical challenge

Extensive infiltration into the normal parenchyma is a hallmark of glioma, rendering total resection impossible and leading to tumors recurrence [89]. Although this is the main reason why these tumors are fatal, most therapies focus on the cancer cells themselves, rather than the surrounding environment. The few attempts to target invasion, *e.g.*, with inhibitors of matrix metalloproteinases have been unsuccessful. The phase II clinical trial involving treatments of high grade recurrent GBM with marimastat, a MMP inhibitor in combination with Temozolomide (TMZ) did not show any improvement in disease-free survival in comparison to treatment with TMZ alone, and there was therapy-related toxicity [90].

How do the changes in ECM composition described above favor the growth and invasiveness of glioma cells? One interesting possibility is that glioma cells prefer a stiffer ECM, as reflected in the observation that they prefer to move along the boundaries between white matter and blood vessels rather than over the softer regions of the parenchyma [91]. Several studies have addressed the involvement of glycosaminoglycan in glioma invasion, focusing primarily on the non-sulfated GAG hyaluronan, the major constituent of brain ECM [92]. The elevated levels of hyaluronan in glioma are involved in cell adhesion and motility via interaction with its CD44 receptor [93].

Thrombospondin-1 (TSP-1), which binds to HSPG and integrin is secreted by malignant glioma but not normal brain cells, and plays important roles in cell-matrix interactions and glioma motility [94]. A subsequent study by this same group suggested that syndecan-1 is a potential receptor for TSP-1 [71] and that the integrins $\alpha v\beta 3$ and $\alpha 3\beta 1$ are part of the functional complex needed for the migration of glioma cells.

As mentioned above, migration of the U87 glioma cell line in vitro is stimulated by HPSE, but the role of this enzyme in glioma infiltration remains to be elucidated [76]. A recent concept in the area of inter-cellular signaling involves exosomes, *i.e.* membrane-bound vesicles that contain RNA and various signaling molecules. Upon fusion with the plasma membrane, these vesicles are released and can then be taken up by other cells. Exosomes are dependent on HSPG for attachment to and uptake by glioma cells and hve been reported to stimulate the migration of these cells [95]. Moreover, HPSE enhances exosome secretion and alters their composition [96]. Disruption of exosomes and their macromolecular cargo provide a new approach to target tumor invasion.

Final remarks

Heparan sulfate proteoglycans, which are involved in numerous biological processes, can bind to many growth factors and other regulatory proteins and thus sequester growth factors in the pericellular space. Here, we have summarized published information concerning involvement of the HSPG in neural differentiation, as well as the roles for these molecules in the self-renewal and fate choice of stem cells.

The data available are indicative of a correlation between more extensive sulfation and a mature phenotype, *i.e.*, neural differentiation cannot proceed without the appropriate modifications that increase the complexity of the HS structure. In addition to providing a basic insight into the role of HS in neural differentiation, future investigations in this area should suggest new approaches to modulating the fate of cells, and even how to obtain more homogenous populations of specific types of cells. The latter could aid in the development of therapy for neurodegenerative disorders.

Furthermore, we have reviewed the involvement of HSPG in carcinogenesis of high-grade glioma, tumors with a very poor prognosis. HSPGs are major constituents of both the normal brain and the complex microenvironment of glioma. In GBM, several core proteins are up- regulated while the biosynthetic enzymes are down-regulated, with few exceptions. Deregulation of HSPGs is thus associated with growth and progression of brain tumors and novel biomarkers and therapeutic targets are likely to be found among these molecules. In conclusion, the HS in immature and cancer cells is orchestrated by a low degree of sulfation whereas highly sulfated HS is found in terminally differentiated and normal adult cells.

References

1. D.C. Kraushaar, Y. Yamaguchi, L. Wang, J. Biol. Chem. **285**, 5907 (2010).
2. R.J. Holley, C.E. Pickford, G. Rushton, G. Lacaud, J.T. Gallagher, V. Kouskoff, C.L. Merry, J. Biol. Chem. **286**, 6241 (2011).
3. C.E. Pickford, R.J. Holley, G. Rushton, M.P. Stavridis, C.M. Ward, C.L. Merry, Stem Cells **29**, 629 (2011).
4. M. Forsberg, K. Holmborn, S. Kundu, A. Dagalv, L. Kjellen, K. Forsberg-Nilsson, J. Biol. Chem. **287**, 10853 (2012).
5. J. Rong, H. Habuchi, K. Kimata, U. Lindahl, M. Kusche-Gullberg, Biochemistry **40**, 5548 (2001).
6. J.D. Esko, L. Zhang, Curr. Opin. Struct. Biol. **6**, 663 (1996).
7. M. Busse, M. Kusche-Gullberg, J. Biol. Chem. **278**, 41333 (2003).
8. K. Grobe, J. Ledin, M. Ringvall, K. Holmborn, E. Forsberg, J.D. Esko, L. Kjellen, Biochim. Biophys. Acta **1573**, 209 (2002).
9. M. Kusche-Gullberg, L. Kjellen, Curr. Opin. Struct. Biol. **13**, 605 (2003).
10. I. Vlodavsky, Y. Friedmann, J. Clin. Invest. **108**, 341 (2001).
11. X. Shi, J. Zaia, J. Biol. Chem. **284**, 11806 (2009).
12. C.L. Merry, S.L. Bullock, D.C. Swan, A.C. Backen, M. Lyon, R.S. Beddington, V.A. Wilson, J.T. Gallagher, J. Biol. Chem. **276**, 35429 (2001).
13. A.C. Rapraeger, A. Krufka, B.B. Olwin, Science **252**, 1705 (1991).
14. A. Yayon, M. Klagsbrun, J.D. Esko, P. Leder, D.M. Ornitz, Cell **64**, 841 (1991).
15. K. Forsten-Williams, C.C. Chua, M.A. Nugent, J. Theor. Biol. **233**, 483 (2005).
16. R. Ruppert, E. Hoffmann, W. Sebald, Eur. J. Biochem. **237**, 295 (1996).
17. F. Reichsman, L. Smith, S. Cumberledge, J. Cell. Biol. **135**, 819 (1996).
18. I. The, Y. Bellaiche, N. Perrimon, Mol. Cell **4**, 633 (1999).
19. A. Molist, M. Romaris, U. Lindahl, J. Villena, M. Touab, A. Bassols, Eur. J. Biochem. **254**, 371 (1998).
20. G. Fager, G. Camejo, U. Olsson, G. Ostergren-Lunden, G. Bondjers, In Vitro Cell Dev. Biol. **28A**, 176 (1992).
21. A. Abramsson, S. Kurup, M. Busse, S. Yamada, P. Lindblom, E. Schallmeiner, D. Stenzel, D. Sauvaget, J. Ledin, M. Ringvall, U. Landegren, L. Kjellen, G. Bondjers, J.P. Li, U. Lindahl, D. Spillmann, C. Betsholtz, H. Gerhardt, Genes. Dev. **21**, 316 (2007).
22. S. Gengrinovitch, B. Berman, G. David, L. Witte, G. Neufeld, D. Ron, J. Biol. Chem. **274**, 10816 (1999).
23. H.G. Kuhn, J. Winkler, G. Kempermann, L.J. Thal, F.H. Gage, J. Neurosci. **17**, 5820 (1997).
24. A. Androutsellis-Theotokis, R.R. Leker, F. Soldner, D.J. Hoeppner, R. Ravin, S.W. Poser, M.A. Rueger, S.K. Bae, R. Kittappa, R.D. McKay, Nature **442**, 823 (2006).
25. Q. Qu, G. Sun, W. Li, S. Yang, P. Ye, C. Zhao, R.T. Yu, F.H. Gage, R.M. Evans, Y. Shi, Nat. Cell Biol. **12**, 31 (2010).
26. D.C. Lie, S.A. Colamarino, H.J. Song, L. Desire, H. Mira, A. Consiglio, E.S. Lein, S. Jessberger, H. Lansford, A.R. Dearie, F.H. Gage, Nature **437**, 1370 (2005).
27. A. Erlandsson, K. Brannvall, S. Gustafsdottir, B. Westermark, K. Forsberg-Nilsson, Cancer Res. **66**, 8042 (2006).
28. R.C. Rao, J. Boyd, R. Padmanabhan, J.G. Chenoweth, R.D. McKay, Stem Cells **27**, 116 (2009).
29. Y.G. Brickman, M.D. Ford, J.T. Gallagher, V. Nurcombe, P.F. Bartlett, J.E. Turnbull, J. Biol. Chem. **273**, 4350 (1998).
30. A.V. Nairn, A. Kinoshita-Toyoda, H. Toyoda, J. Xie, K. Harris, S. Dalton, M. Kulik, J.M. Pierce, T. Toida, K.W. Moremen, R.J. Linhardt, J. Proteome Res. **6**, 4374 (2007).
31. C.E. Johnson, B.E. Crawford, M. Stavridis, G. Ten Dam, A.L. Wat, G. Rushton, C.M. Ward, V. Wilson, T.H. van Kuppevelt, J.D. Esko, A. Smith, J.T. Gallagher, C.L. Merry, Stem Cells **25**, 1913 (2007).
32. T. Izumikawa, N. Kanagawa, Y. Watamoto, M. Okada, M. Saeki, M. Sakano, K. Sugahara, K. Sugihara, M. Asano, H. Kitagawa, J. Biol. Chem. **285**, 12190 (2010).
33. X. Lin, G. Wei, Z. Shi, L. Dryer, J.D. Esko, D.E. Wells, M.M. Matzuk, Dev. Biol. **224**, 299 (2000).
34. M. Ringvall, J. Ledin, K. Holmborn, T. van Kuppevelt, F. Ellin, I. Eriksson, A.M. Olofsson, L. Kjellen, E. Forsberg, J. Biol. Chem. **275**, 25926 (2000).
35. E. Forsberg, G. Pejler, M. Ringvall, C. Lunderius, B. Tomasini-Johansson, M. Kusche-Gullberg, I. Eriksson, J. Ledin, L. Hellman, L. Kjellen, Nature **400**, 773 (1999).
36. G.R. Martin, Proc. Natl. Acad. Sci. U.S.A. **78**, 7634 (1981).
37. J.A. Thomson, J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, J.M. Jones, Science **282**, 1145 (1998).
38. N.M. Gough, R.L. Williams, D.J. Hilton, S. Pease, T.A. Willson, J. Stahl, D.P. Gearing, N.A. Nicola, D. Metcalf, Reprod. Fertil. Dev. **1**, 281 (1989).

39. E.D. Rosen, P. Sarraf, A.E. Troy, G. Bradwin, K. Moore, D.S. Milstone, B.M. Spiegelman, R.M. Mortensen, Mol. Cell **4**, 611 (1999).
40. B.W. Phillips, N. Belmonte, C. Vernochet, G. Ailhaud, C. Dani, Biochem. Biophys. Res. Commun. **284**, 478 (2001).
41. L. Conti, S.M. Pollard, T. Gorba, E. Reitano, M. Toselli, G. Biella, Y. Sun, S. Sanzone, Q.L. Ying, E. Cattaneo, A. Smith, PLoS Biol. **3**, e283 (2005).
42. G. Keller, Genes. Dev. **19**, 1129 (2005).
43. S. Okabe, K. Forsberg-Nilsson, A.C. Spiro, M. Segal, R.D. McKay, Mech. Dev. **59**, 89 (1996).
44. K. Holmborn, J. Ledin, E. Smeds, I. Eriksson, M. Kusche-Gullberg, L. Kjellen, J. Biol. Chem. **279**, 42355 (2004).
45. F. Lanner, K.L. Lee, M. Sohl, K. Holmborn, H. Yang, J. Wilbertz, L. Poellinger, J. Rossant, F. Farnebo, Stem Cells **28**, 191 (2010).
46. S. Le Jan, M. Hayashi, Z. Kasza, I. Eriksson, J.R. Bishop, I. Weibrecht, J. Heldin, K. Holmborn, L. Jakobsson, O. Soderberg, D. Spillmann, J.D. Esko, L. Claesson-Welsh, L. Kjellen, J. Kreuger, Arterioscler. Thromb. Vasc. Biol. **32**, 1255 (2012).
47. D. Liu, Z. Shriver, G. Venkataraman, Y. El Shabrawi, R. Sasisekharan, Proc. Natl. Acad. Sci. U.S.A. **99**, 568 (2002).
48. G.A. Schmale, E.U. Conrad 3rd, W.H. Raskind, J. Bone Joint Surg. Am. **76**, 986 (1994).
49. S. Ropero, F. Setien, J. Espada, M.F. Fraga, M. Herranz, J. Asp, M.S. Benassi, A. Franchi, A. Patino, L.S. Ward, J. Bovee, J.C. Cigudosa, W. Wim, M. Esteller, Hum. Mol. Genet. **13**, 2753 (2004).
50. Z. Piao, H. Kim, B.K. Jeon, W.J. Lee, C. Park, Cancer **80**, 865 (1997).
51. A. Yoneda, M.E. Lendorf, J.R. Couchman, H.A. Multhaupt, J. Histochem. Cytochem. **60**, 9 (2012).
52. C. Bret, D. Hose, T. Reme, A.C. Sprynski, K. Mahtouk, J.F. Schved, P. Quittet, J.F. Rossi, H. Goldschmidt, B. Klein, Br. J. Haematol. **145**, 350 (2009).
53. W.C. Lamanna, M.A. Frese, M. Balleininger, T. Dierks, J. Biol. Chem. **283**, 27724 (2008).
54. K. Uchimura, M. Morimoto-Tomita, A. Bistrup, J. Li, M. Lyon, J. Gallagher, Z. Werb, S.D. Rosen, BMC Biochem. **7**, 2 (2006).
55. H. Lemjabbar-Alaoui, A. van Zante, M.S. Singer, Q. Xue, Y.Q. Wang, D. Tsay, B. He, D.M. Jablons, S.D. Rosen, Oncogene **29**, 635 (2010).
56. M. Morimoto-Tomita, K. Uchimura, A. Bistrup, D.H. Lum, M. Egeblad, N. Boudreau, Z. Werb, S.D. Rosen, Neoplasia **7**, 1001 (2005).
57. I. Vlodavsky, P. Beckhove, I. Lerner, C. Pisano, A. Meirovitz, N. Ilan, M. Elkin, Cancer Microenviron. **5**, 115 (2012).
58. D.N. Louis, H. Ohgaki, O.D. Wiestler, W.K. Cavenee, P.C. Burger, A. Jouvet, B.W. Scheithauer, P. Kleihues, Acta Neuropathol. **114**, 97 (2007).
59. R. Stupp, W.P. Mason, M.J. van den Bent, M. Weller, B. Fisher, M.J. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R.C. Janzer, S.K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J.G. Cairncross, E. Eisenhauer, R.O. Mirimanoff, N. Engl. J. Med. **352**, 987 (2005).
60. R.G. Verhaak, K.A. Hoadley, E. Purdom, V. Wang, Y. Qi, M.D. Wilkerson, C.R. Miller, L. Ding, T. Golub, J.P. Mesirov, G. Alexe, M. Lawrence, M. O'Kelly, P. Tamayo, B.A. Weir, S. Gabriel, W. Winckler, S. Gupta, L. Jakkula, H.S. Feiler, J.G. Hodgson, C.D. James, J.N. Sarkaria, C. Brennan, A. Kahn, P.T. Spellman, R.K. Wilson, T.P. Speed, J.W. Gray, M. Meyerson, G. Getz, C.M. Perou, D.N. Hayes, Cancer Cell **17**, 98 (2010).
61. D. Sturm, H. Witt, V. Hovestadt, D.A. Khuong-Quang, D.T. Jones, C. Konermann, E. Pfaff, M. Tonjes, M. Sill, S. Bender, M. Kool, M. Zapatka, N. Becker, M. Zucknick, T. Hielscher, X.Y. Liu, A.M. Fontebasso, M. Ryzhova, S. Albrecht, K. Jacob, M. Wolter, M. Ebinger, M.U. Schuhmann, T. van Meter, M.C. Fruhwald, H. Hauch, A. Pekrun, B. Radlwimmer, T. Niehues, G. von Komorowski, M. Durken, A.E. Kulozik, J. Madden, A. Donson, N.K. Foreman, R. Drissi, M. Fouladi, W. Scheurlen, A. von Deimling, C. Monoranu, W. Roggendorf, C. Herold-Mende, A. Unterberg, C.M. Kramm, J. Felsberg, C. Hartmann, B. Wiestler, W. Wick, T. Milde, O. Witt, A.M. Lindroth, J. Schwartzentruber, D. Faury, A. Fleming, M. Zakrzewska, P.P. Liberski, K. Zakrzewski, P. Hauser, M. Garami, A. Klekner, L. Bognar, S. Morrissy, F. Cavalli, M.D. Taylor, P. van Sluis, J. Koster, R. Versteeg, R. Volckmann, T. Mikkelsen, K. Aldape, G. Reifenberger, V.P. Collins, J. Majewski, A. Korshunov, P. Lichter, C. Plass, N. Jabado, S.M. Pfister, Cancer Cell **22**, 425 (2012).
62. B.E. Johnson, T. Mazor, C. Hong, M. Barnes, K. Aihara, C.Y. McLean, S.D. Fouse, S. Yamamoto, H. Ueda, K. Tatsuno, S. Asthana, L.E. Jalbert, S.J. Nelson, A.W. Bollen, W.C. Gustafson, E. Charron, W.A. Weiss, I.V. Smirnov, J.S. Song, A.B. Olshen, S. Cha, Y. Zhao, R.A. Moore, A.J. Mungall, S.J. Jones, M. Hirst, M.A. Marra, N. Saito, H. Aburatani, A. Mukasa, M.S. Berger, S.M. Chang, B.S. Taylor, J.F. Costello, Science **343**, 189 (2014).
63. F. Mercier, J.T. Kitasako, G.I. Hatton, J. Comp. Neurol. **451**, 170 (2002).
64. A. Kerever, F. Mercier, R. Nonaka, S. de Vega, Y. Oda, B. Zalc, Y. Okada, N. Hattori, Y. Yamada, E. Arikawa-Hirasawa, Stem Cell Res. **12**, 492 (2014).
65. T. Bergstrom, K. Holmqvist, T. Tararuk, S. Johansson, K. Forsberg-Nilsson, Biochim. Biophys. Acta **1840**, 2526 (2014).
66. T. Mammoto, A. Jiang, E. Jiang, D. Panigrahy, M.W. Kieran, A. Mammoto, Am. J. Pathol. **183**, 1293 (2013).
67. C. Calabrese, H. Poppleton, M. Kocak, T.L. Hogg, C. Fuller, B. Hamner, E.Y. Oh, M.W. Gaber, D. Finklestein, M. Allen, A. Frank, I.T. Bayazitov, S.S. Zakharenko, A. Gajjar, A. Davidoff, R.J. Gilbertson, Cancer Cell **11**, 69 (2007).
68. P.A. Steck, R.P. Moser, J.M. Bruner, L. Liang, A.N. Freidman, T.L. Hwang, W.K. Yung, Cancer Res. **49**, 2096 (1989).
69. A. Bertolotto, M.L. Magrassi, L. Orsi, C. Sitia, D. Schiffer, J. Neurooncol. **4**, 43 (1986).
70. G. Su, K. Meyer, C.D. Nandini, D. Qiao, S. Salamat, A. Friedl, Am. J. Pathol. **168**, 2014 (2006).
71. A. Watanabe, T. Mabuchi, E. Satoh, K. Furuya, L. Zhang, S. Maeda, H. Naganuma, J. Neurooncol. **77**, 25 (2006).
72. Y. Xu, J. Yuan, Z. Zhang, L. Lin, S. Xu, Mol. Biol. Rep. **39**, 8979 (2012).
73. J.J. Phillips, Oncotarget **3**, 568 (2012).

74. T. Reme, J.P. Hugnot, I. Bieche, V. Rigau, F. Burel-Vandenbos, V. Prevot, M. Baroncini, D. Fontaine, H. Chevassus, S. Vacher, R. Lidereau, H. Duffau, L. Bauchet, D. Joubert, PLoS One **8**, e66574 (2013).
75. A. Wade, A.E. Robinson, J.R. Engler, C. Petritsch, C.D. James, J.J. Phillips, FEBS J. **280**, 2399 (2013).
76. A. Zetser, Y. Bashenko, H.Q. Miao, I. Vlodavsky, N. Ilan, Cancer Res. **63**, 7733 (2003).
77. Y. Ueno, M. Yamamoto, I. Vlodavsky, I. Pecker, K. Ohshima, T. Fukushima, J. Neurosurg. **102**, 513 (2005).
78. X. Hong, K.K. Nelson, A.C. deCarvalho, S.N. Kalkanis, J. Neurosurg. **113**, 261 (2010).
79. X. Hong, F. Jiang, S.N. Kalkanis, Z.G. Zhang, X. Zhang, X. Zheng, H. Jiang, T. Mikkelsen, M. Chopp, J. Exp. Clin. Cancer Res. **27**, 23 (2008).
80. F.K. Johansson, J. Brodd, C. Eklof, M. Ferletta, G. Hesselager, C.F. Tiger, L. Uhrbom, B. Westermark, Proc. Natl. Acad. Sci. U.S.A. **101**, 11334 (2004).
81. J.J. Phillips, E. Huillard, A.E. Robinson, A. Ward, D.H. Lum, M.Y. Polley, S.D. Rosen, D.H. Rowitch, Z. Werb, J. Clin. Invest. **122**, 911 (2012).
82. Y. Liang, M. Haring, P.J. Roughley, R.K. Margolis, R.U. Margolis, J. Cell. Biol. **139**, 851 (1997).
83. D. Qiao, K. Meyer, C. Mundhenke, S.A. Drew, A. Friedl, J. Biol. Chem. **278**, 16045 (2003).
84. R.V. Iozzo, R.D. Sanderson, J. Cell Mol. Med. **15**, 1013 (2011).
85. C.Y. Fears, C.L. Gladson, A. Woods, J. Biol. Chem. **281**, 14533 (2006).
86. H. Zhu, R.L. Carpenter, W. Han, H.W. Lo, Cancer Lett. **343**, 51 (2014).
87. G. Rascher, A. Fischmann, S. Kroger, F. Duffner, E.H. Grote, H. Wolburg, Acta Neuropathol. **104**, 85 (2002).
88. A. Warth, S. Kroger, H. Wolburg, Acta Neuropathol. **107**, 311 (2004).
89. M.C. Chamberlain, J. Neurooncol. **101**, 319 (2011).
90. M.D. Groves, V.K. Puduvalli, K.R. Hess, K.A. Jaeckle, P. Peterson, W.K. Yung, V.A. Levin, J. Clin. Oncol. **20**, 1383 (2002).
91. T.A. Ulrich, E.M. de Juan Pardo, S. Kumar, Cancer Res. **69**, 4167 (2009).
92. B. Radotra, D. McCormick, J. Pathol. **181**, 434 (1997).
93. Y. Akiyama, S. Jung, B. Salhia, S. Lee, S. Hubbard, M. Taylor, T. Mainprize, K. Akaishi, W. van Furth, J.T. Rutka, J. Neurooncol. **53**, 115 (2001).
94. H. Naganuma, E. Satoh, T. Asahara, K. Amagasaki, A. Watanabe, H. Satoh, K. Kuroda, L. Zhang, H. Nukui, J. Neurooncol. **70**, 309 (2004).
95. H.C. Christianson, K.J. Svensson, T.H. van Kuppevelt, J.P. Li, M. Belting, Proc. Natl. Acad. Sci. U.S.A. **110**, 17380 (2013).
96. C.A. Thompson, A. Purushothaman, V.C. Ramani, I. Vlodavsky, R.D. Sanderson, J. Biol. Chem. **288**, 10093 (2013).