Glycosaminoglycans and Glioma Invasion

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Glioblastoma

Gliomas are classified according to WHO criteria in grades I–IV where glioblastoma is the most aggressive and also the most common malignant primary brain tumor among adults. Every year, approximately 3 per 100,000 individuals are diagnosed with glioblastoma [1] for which the standard treatment is surgery followed by radiation and chemotherapy. Even though the introduction of temozolomide has somewhat prolonged life expectancy, the outcome is still poor with a median survival of only ~15 months [2].

In addition to the classical WHO classification, glioblastoma can be further divided into subtypes, using a molecular-based taxonomy. This aims at better describing the heterogeneous nature of these tumors. The term glioblastoma multiforme (GBM) is used synonymously with glioblastoma to emphasize its efficient migration, eg, along white-matter tracts of several centimeters from the original tumor site often occurs [9]. The high motility of glioma cells is underscored by their ability to spread even to the contralateral hemisphere via the corpus callosum. However, in contrast to its efficient invasion within the brain, GBM rarely metastasizes via the cerebrospinal fluid [10].

Therapies to target GBM invasion are highly warranted because of its efficient migration, eg, along white-matter tracts [11]. For a tumor to spread away from the original neoplasm, cancer cells have to detach and migrate through the parenchyma, which includes breakdown of several extracellular matrix molecules. It is well-known that glioma cells express cell adhesion molecules that facilitate the invasion process [12]. Tumour cell migration needs to be accompanied by matrix degradation, which is carried out by a variety of enzymatic processes [13]. This leads to ECM remodelling since the migrating tumour cells lay down de novo ECM of their own, composed primarily of ECM molecules supporting migration [14].

As of today, novel therapeutic approaches mainly target the cancer cells per se and focus less on the surrounding, non-tumor environment. In fact, most clinical trials have been directed against the bulk of cancer cells and few drugs have been tested that target the invasive mechanisms. Attempts at reducing matrix metalloproteinase (MMP) levels in glioma with marimastat and temozolomide was approximately equivalent to temozolomide alone and also caused toxic effects [15, 16]. Another example are clinical trials with cilenitide, an inhibitor of integrin αvβ3 and integrin αvβ5 [17] that may affect both growth and spread of tumor cells as well as angiogenesis.

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Abstract: There is a great need for novel therapies to target malignant glioma, a disease with an often dismal prognosis. One of the hallmarks of malignant glioma is its efficient invasion of the healthy brain parenchyma, which leads to rapid recurrence upon surgical removal of the original tumor. To be able to establish new tumors at a distance from the original neoplasm, glioma cells must detach, migrate through the microenvironment, settle, and proliferate in their new location. This includes changing adhesive characteristics, breaking down extracellular matrix molecules (ECM), and perturbed growth factor signaling. Investigations of the glioma-specific ECM composition may therefore provide new insights into glioma infiltration. In this review, we focus on glycosaminoglycans, important components of the ECM that are long unbranched polysaccharides composed of repeating disaccharide units. We discuss the roles for hyaluronan, one of the major brain ECM molecules, and that of the proteoglycans, heparan sulphate proteoglycans (HSPG) and chondroitin sulphate proteoglycans (CSPG), in glioma biology. Heparan sulphate (HS) and chondroitin sulphate (CS) chains act together with a wide variety of bioactive molecules, and these interactions depend on the HS and CS sulphation patterns. HS and CS chain modifications are implicated not only in normal development and homeostasis but they also play important roles in pathological conditions including cancer. Dysregulated glycosaminoglycans, their biosynthetic and degradation enzymes as well as the proteoglycan core proteins are known to affect several stages of tumor progression, angiogenesis, and metastasis. Finding the specific characteristics of tumour cells that confer this infiltrative capacity of glioma may offer new avenues for drug development.

Key words: heparan sulphate, chondroitin sulphate, extracellular matrix, tumour invasion

Glioblastoma Invasion Is a Major Clinical Problem

A typical feature of GBM is the extensive infiltration of tumor cells into the healthy parenchyma that makes complete resection impossible. This feature is common for all diffuse astrocytic tumours, but it is a particular problem in GBM [8]. After surgical removal, tumour recurrence within a distance of several centimetres from the original tumour site often occurs [9]. The high motility of glioma cells is underscored by their ability to spread even to the contralateral hemisphere via the corpus callosum. However, in contrast to its efficient invasion within the brain, GBM rarely metastasizes via the cerebrospinal fluid [10].


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However, in February 2013, it was announced that the phase-III clinical trial of cilengitide did not increase overall survival over standard treatment comprising radiation and temozolomide. In light of these failures, more targets specific for tumour cell invasion are needed, based on mechanisms responsible for tumour cell infiltration. In this review, we focus on the role of glycosaminoglycans because they are major components of the extracellular matrix and important players in the invasive processes.

The Extracellular Matrix of the Healthy and Malignant Brain

Several aspects of ECM biology are different between the brain and non-CNS tissues. Collagen-containing basement membranes that are typical for the extra-CNS tissue are only present in the meninges and around blood vessels in the normal brain. Basal lamina extensions from the vessels in the subventricular zone form structures called fractones [18] that have been suggested to enrich growth factors and other HS-binding signalling molecules in the neurovascular niche, where adult neural stem cells reside. Cancer stem cells (CSC) in glioblastoma share many properties with normal neural stem cells, such as their ability for self-renewal, and they are highly motile [19]. The perivascular niche in glioma is believed to harbour CSCs that are supported by trophic factors from the vasculature [20]. Sequestration of growth factors by HSPG in the tumourigenic niche could thus maintain cancer stem cells in a tumourigenic niche with similarities to the normal neurogenic niche (reviewed in [21]). In contrast to the normal brain glioblastoma has greatly increased levels of collagen [22] and the importance of the collagen structure for glioblastoma neovascularization and tumour growth has recently been reported [23].

In the adult brain, the extracellular matrix regulates structural and functional plasticity, partly through the brain-specific dense ECM structures called perineuronal nets. These were first described by Golgi (reviewed in [24]) and are composed mainly of chondroitin sulphate proteoglycans, hyaluronan, Tenascin-R, and Sema3A [26]. The perineuronal nets restrict reorganization of process formation mainly through inhibitory CSPG and Sema3A. CSPGs are highly up-regulated in glioma [27] but their role in glioma invasiveness is not fully elucidated [28, 29], as will be discussed below.

Glycosaminoglycans Are Major Components of the Brain ECM

Glycosaminoglycan (GAG) is the common term for linear polysaccharides that are composed of repetitively appearing disaccharides consisting of an N-acetylated or N-sulphated hexosamine together with either uronic acid (glucuronic acid or iduronic acid) or galactose. These molecules are highly conserved during evolution, which points at their ubiquitous functions in many biological processes [30]. Hyaluronan, a major constituent of the extracellular matrix (ECM), is a large, unbranched glycosaminoglycan that lacks sulphate groups. The glycosaminoglycans heparan sulphate (HS), chondroitin sulphate (CS), dermanan sulphate (DS), and keratan sulphate (KS) make up the proteoglycans, consisting of core proteins covalently linked to one or more glycosaminoglycan chains. The glucosaminoglycan components of proteoglycans are sulphated in various positions, with a differing disaccharide composition. Dermatan sulphates (DS) have iduronic acid, while chondroitin sulphates (CS) lack these residues. Keratan sulphates (KS) have no uronic acid and are instead made up by N-acetylglucosamine and galactose units. Proteoglycans are either attached to the plasma membrane, found in vesicles inside cells, or exported to the extracellular space [31]. Because of the high abundance of hyaluronan, heparan sulphate, and chondroitin sulphate in the brain, relative to dermatan sulphate and keratan sulphates, this review will focus on the first 3 types of molecules.

Hyaluronan

Hyaluronan (HA) is the principal component of the brain ECM with a unique composition, and it does not undergo sulphation and epimerization [32, 33]. HA content is elevated in primary brain tumours [34]. In contrast to other GAGs, HA is not synthesized in the Golgi apparatus. Hyaluronan synthase is the primary biosynthetic enzyme in mammals, which occurs in 3 isoforms synthesizing HA polymers of different lengths. The enzyme is located in the plasma membrane and catalyzes polymerization as well as translocation of HA out of the cell [35, 36]. In most tissues, HA is rapidly degraded by hyaluronidase (Hyal). Mammalian Hyal has overlapping substrate specificity and is known to degrade HA and CS and, to some extent, DS. Hyaluronidase acts in concert with 2 other exoglycosidases (removing sugars from the non-reducing end [37]). Human glioblastoma expressed levels of hyaluronan synthases above that of normal cells [38, 39] and over-expression of hyaluronan synthase-2 reduced growth of murine glioma, but only if hyaluronidase was concomitantly present [38]. Furthermore, hyaluronan synthase has been associated with increased receptor tyrosine kinase activity [40] and hyaluronan synthase-2 mRNA levels were higher in GBM than in normal brain [41]. This implies an important balance between synthesis and breakdown of HA in brain tumour biology.

HA can reach high molecular mass, consisting of 25,000–30,000 disaccharide repeats under normal conditions, and it attains complex secondary and tertiary structures regulating physiological processes. As a major element of the brain ECM, HA is abundant in white-matter tracts [42]. These are the preferred migration routes for neural stem cells, eg, after transplantation into the injured rodent brain [43–45], and similarly these tracts constitute dissemination paths for tumour cells in glioma [11, 14, 46]. HA is also present in the neurogenic niche and important for stem cell maintenance [47]. Under pathological conditions, such as injury, inflammation, and repair, it undergoes regulations. The HA polymer becomes degraded by a series of enzyme reactions, thus generating HA with a range of molecular weights that are involved in various biological functions [37]. Low and intermediate molecular-weight HA has distinct biological functions as compared to native high molecular-weight HA, for example stimulating gene expression in macrophages, endothelial cells, and certain epithelial cells as well as scar formation [48]. In glioma, low molecular-weight HS oligomers have been shown to act
as an inhibitor of hyaluronan-dependent release of putative effector molecules from tumour cells [49].

HA interacts with several receptors [50] out of which CD44 and RAHM (receptor for hyaluronic acid-mediated motility) affect cell growth and motility, and thereby has the ability to mediate primary tumour cell invasion and migration [51, 52]. CD44 is the principal cell surface receptor for HA, and HA-CD44 interactions play a crucial role in eg development and inflammation, tumour growth, and metastasis [53, 54]. Signalling through the intracellular part of CD44 was shown to affect cell adhesion and motility through interactions with cytoskeletal proteins [55, 56]. In addition, CD44 interacts with a large number of signalling molecules that promotes matrix degradation and the spread of tumour cells (reviewed in [57]). HA-independent roles for CD44 have also been proposed in cell adhesion/migration because CD44 can act as a cell-surface anchor for the ECM-degrading enzyme MMP-9 [58].

Heparan Sulphate

Heparan sulphate proteoglycans (HSPG) are found either at the cell surface (syndecans and glypcicans) or secreted (eg, perlecan and agrin) [59]. HSPGs are main components of the ECM where they interact with a large number of physiologically important macromolecules, thereby influencing biological processes [60]. HSPGs modulate growth factor activities, regulating interactions between ligand and receptor [61], and during CNS development, morphogen gradients can be maintained by HSPGs [62] (Figure 1). Its unique molecular design is composed of clusters of N- and O-sulphated sugar residues, separated by regions of low sulphation, which determines specific protein-binding properties [59]. In the adult brain, HS is associated with neural stem cell niches [63], where it may be involved in regulating neural stem cell maintenance. Recently, levels of 6O-sulphation of HS have also been linked to injury response as increased sulphation promoted glial scarring [64].

Mouse gene knockout experiments have shown the vital role of HSPGs in development and homeostasis [65]. Studies on neural differentiation have, to a large extent, been performed on in vitro differentiated ES cells to neural progenitors due to early embryonic lethal phenotypes of mice with deletions in crucial HS chain modification enzymes [66]. Our recent publication showed that N-sulphation of HS biosynthesis is indispensable for neural differentiation and that the ratio between HS and FGF was the crucial factor determining neural differentiation [67].

It has long been known that HSPGs are involved in the progression of various cancers [68] including glioma, where the levels of HSPGs are higher than in the normal brain [69]. Syndecan-1 [70] and glypcican-1 [71] are examples of HSPG core proteins with increased expression in glioma cells compared to non-neoplastic cells. Su et al [71] found that glioma cell-associated HSPG was more capable of stimulating FGF-2 signalling than HSPG from normal cells, further supporting the role for HS-mediated growth factor signalling in glioma. There are several receptor tyrosine kinase pathways that can be modulated by altered HS amounts and composition. The extracellular sulphatase Sulf-2, which controls 6O-sulphation, thereby regulating HSPG interactions with eg PDGF receptors, has recently been shown to be highly expressed in glioblastoma and demonstrated to increase tumour size [72]. Increased Sulf-2 expression was most prominent in the proneural subclass that is associated with increased PDGF receptor activity, and the PDGF alpha receptor pathway was particularly influenced by altered Sulf-2 levels although IGF1 receptor and EPHA2 stimulation were affected as well.

A systematic analysis of proteoglycan expression of glioblastomas included in The Cancer Genome Atlas [73] showed that several of the proteoglycans as well as their biosynthetic and degradation enzymes were differently regulated in GBM compared with the normal brain [41]. The authors found by means of database mining that biosynthetic enzymes were mostly down-regulated, except for HS3ST3a1, as previously reported [71]. Sulphation of HS is critical for its function as a co-receptor for various growth factor receptors, and therefore the increase in 3O-sulphation (by HS3ST3a1) and decreased 6O-sulphation (by increased Sulf2 [72]) may be specific for GBM [41].

Heparanase, the major HS-degrading enzyme, was found to be up-regulated in TCGA GBM patients [41]. There are some reports implicating heparanase in glioma. In a study using human glioma cell line U87, heparanase over-expression led to increased motility and invasion. Larger tumours were found after xenografting of glioma cells with elevated heparanase, whereas at further increased levels, tumour growth was inhib-
Concluding Remarks

Conflict of Interest

The authors declare that they have no conflicts of interest.