7 The Role of BEHAB/Brevican in the Tumor Microenvironment: Mediating Glioma Cell Invasion and Motility

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Abstract

Malignant gliomas are the most common tumors in the central nervous system (CNS) and, unfortunately, are also the most deadly. The lethal nature of malignant gliomas is due in large part to their unique and distinctive ability to invade the surrounding neural tissue. The invasive and dispersive nature of these tumors makes them particularly challenging to treat, and currently there are no effective therapies for malignant gliomas. The brain tumor microenvironment plays a particularly important role in mediating the invasiveness of gliomas, and, therefore, understanding its function is key to developing novel therapies to treat these deadly tumors. A defining aspect of the tumor microenvironment of gliomas is the unique composition of the extracellular matrix that enables tumors to overcome the typically inhibitory environment found in the CNS. One conspicuous component of the glioma tumor microenvironment is the neural-specific ECM molecule, brain-enriched hyaluronan binding (BEHAB)/brevican (B/b). B/b is highly overexpressed in gliomas, and its expression in these tumors contributes importantly to the tumor invasiveness and aggressiveness. However, B/b is a complicated protein with multiple splice variants, cleavage products, and glycoforms that contribute to its complex functions in these tumors and provide unique targets for tumor therapy. Here we review the role of B/b in glioma tumor microenvironment and explore targeting of this protein for glioma therapy.

Keywords

Proteoglycan · Glioma dispersion · Glycosylation · ADAMTS4 · MMP · Lectican · Chondroitin sulfate · Fibronectin · Glioma-initiating cells · EGF receptor · Hyaluronan · ECM · TME · BEHAB · Glycoform

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7.1 Introduction

The tumor microenvironment is comprised of the cells that directly make up the tumor, neighboring normal/non-transformed cells, the extracellular matrix (ECM), and secreted molecules

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found within this space [5, 52, 140]. Importantly, interactions between these constituents define the molecular properties of the specific tumor [52]. High-grade gliomas are the most frequently detected and virulent form of intracranial tumors, but they are also commonly impervious to currently available treatments, including surgery as well as chemotherapy and radiation [125]. One of the primary reasons as to why gliomas are insusceptible to these therapies is due to the fact that gliomas are able to infiltrate surrounding tissues and, thus, are considered to be highly invasive [109, 126].

Many of the components within the tumor microenvironment support the dispersion and heightened motility of glioma cells. Specifically, gliomas exhibit aberrant expression patterns of cell adhesion molecules, ECM molecules, proteolytic enzymes that remodel the ECM, and growth factors [5, 140]. One such ECM molecule that is overexpressed in gliomas is the chondroitin sulfate proteoglycan (CSPG): brain-enriched hyaluronan-binding protein (BEHAB)/brevican (B/b) [42, 67]. This enhanced expression of B/b leads to an increase in the aggressiveness of the resulting tumors [35, 62, 83, 98, 134, 154]. It is important to note that that there are a number of B/b isoforms that are upregulated in glioma samples [133].

Mechanistically, B/b is cleaved by a disintegrin and metalloproteainase with thrombospondin motifs (ADAMTS)-4 [86], and this causes the abnormal adhesion of glioma cells to fibronectin, and the overall motility of the glioma cells is enhanced [62], thereby leading to tumor invasion. Moreover, as a result of an increase in the presence of B/b, fibronectin secretion is increased, as is the expression of a number of cell adhesion molecules [62]. Taken together, these molecular and structural changes to the tumor microenvironment favor glioma cell invasiveness and movement, which contributes to the resistance of gliomas to current therapeutics [109].

In this chapter we first focus on the function of B/b in normal/non-transformed cells and then delve into the molecular composition of the tumor microenvironment. Next, we dissect the specific role that B/b plays in promoting glioma cell invasion and motility. Additionally, the mechanisms underlying these processes will be discussed and will shed further light onto why the current treatments are not more effective at targeting gliomas and why targeting B/b could be a new therapeutic strategy.

7.2 Brevican: Structure and Function

7.2.1 Structure

B/b is a member of the lectican family of CSPGs along with versican, neurocan, and aggrecan [7, 113, 147]. In terms of structure, members of the lectican family are quite homologous. More specifically, these CSPGs contain a N-terminus, which is known as the hyaluronan (HA)-binding domain and link protein-like region, that mediates interactions between the lectican family members and HA (see Fig. 7.1, [104]). The binding of these CSPGs to HA is a key step in overall organization of the ECM. Within the C-terminus, there is an epidermal growth factor (EGF)-like domain that is characteristic of proteins found within the ECM, a lectin-like domain, and a complement regulatory protein-like domain [120]. It is through this lectin domain that the lecticans can bind tenascin-R, a glycoprotein present within the ECM. Furthermore, this region of CSPGs can also bind to glycolipids found on the cell surface that have been sulfated, which promotes cell adhesion [89].

CSPGs consist of a core protein that is decorated with chondroitin sulfate (CS) sugar chains, which bind to the protein in the CS attachment region (Fig. 7.1). The amount of sugar units that can be added to the protein varies widely between members of the lectican family with B/b having 1–3 CS chains that adorn the protein core [7]. The core protein has been shown to hinder neurite outgrowth in cultured neuroblastoma cells [64], and the CS region of the CSPG has also been reported to inhibit overall growth and regeneration in the central nervous system (CNS) [21, 70]. A substantial body of work utilizing the bacterial enzyme, chondroitinase ABC (chABC), to

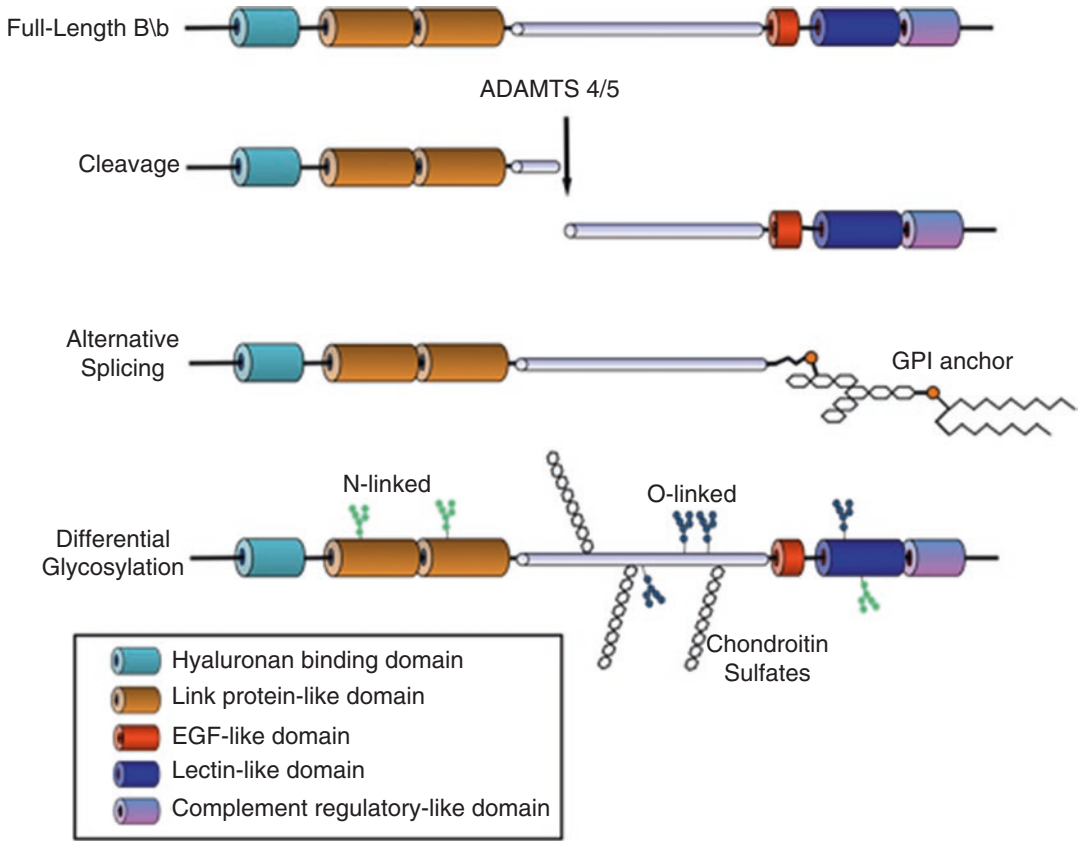

Fig. 7.1 BEHAB/brevican structure diversity, glycosylation, and cleavage. B/b is made as a both a secreted and GPI-linked isoform. It is routinely cleaved at a defined ADAMTS4/5 cleavage site leading to N-terminal and C-terminal fragments. In addition, work has shown that there is a lot of microheterogeneity in the glycosylation of this protein. In gliomas all forms of B/b are upregulated with the N-terminal cleavage fragment perhaps being the most critical functionally. In addition there are glioma-specific glycoforms that are generated that may provide ideal targets for glioma therapy

digest CS chains supports these findings. In these studies, application of chABC resulted in recovery after spinal cord injury [14, 75, 136]. Additionally, administration of chABC has been reported to enhance axonal regeneration within the CNS in undamaged animals [31, 90]. Pizzorusso and colleagues used this enzyme to reopen the critical period and, thus, restore plasticity within the visual system of adult rats [105]. Taken together, both the core protein and the CS region of CSPGs inhibit growth and regeneration within the CNS and thus play a key role in restricting overall brain plasticity.

Specifically, B/b can exist in a number of different ways: a glycosylphosphatidylinositol form that is anchored to the plasma membrane [119, 120] and a form that is secreted right into the ECM [119], and additionally, B/b can be present as a glycosylated proteoglycan or as a core protein that is not glycosylated (Fig. 7.1, [146]). Interestingly, the form that is anchored to the plasma membrane was detected primarily in white matter tracts where axons are located as well glial cells that were classified as diffusely distributed throughout the brain. The form that is secreted into the ECM was highly expressed in the gray matter within the cerebral cortex, hippocampus, cerebellum, and particular thalamic nuclei [119]. Through Western blot analysis, in the adult rat brain, the full-length B/b protein

runs at 145 kDa, but cleavage products have been described at 90 kDa and 50 kDa [145, 154].

Temporally, B/b expression is first detected on embryonic day 15 (E15). In all assayed regions of the CNS, the onset of B/b expression occurred after neurogenesis and instead was consistent with the generation of glial cells [66]. It has also been demonstrated that B/b expression is upregulated in response to injuries within the brain [41]. Following a stab wound to the adult rat brain, B/b was detected in regions of active gliosis [65], and, similarly, B/b expression within astrocytes was increased in response to lesions introduced into the entorhinal cortex in rats [127]. In that same vein, B/b mRNA was dramatically increased within the glial scar following cryo-injury in mice [64].

7.2.2 Function

B/b is one of the molecular constituents of the perineuronal net (PNN) that is found within the CNS. PNNs surround the cell body and proximal neurites of particular populations of neurons within the CNS. Typically, these cells are GABAergic interneurons, but they also can be found around excitatory cells. This structure serves to restrict plasticity by closing the critical period [9, 17, 23, 24, 48, 53, 58, 59, 124, 153]. Other work postulates that PNNs provide a buffering mechanism to preserve the balance of cationic charges within the extracellular milieu [17, 18, 54, 55]. Using B/b deficient mice, Bekku and colleagues revealed that B/b regulates the assembly of the proteoglycan, phosphacan, and tenascin-R at Nodes of Ranvier within the CNS [8], which is likely critical in action potential propagation. Proper B/b expression is also needed to maintain normal speeds of synaptic transmission at the calyx of Held in the medial nucleus of the trapezoid body within the brainstem, where PNNs are found in abundance [13]. Studies performed using B/b knockout mice highlight a potential role for B/b in modulating long-term potentiation in the CA1 region of the hippocampus. Of particular interest, the mice lacking B/b displayed less prominent PNNs, meaning that they were less condensed and focused at the cellular surface and instead exhibited a more diffuse expression pattern [15].

7.3 Gliomas

7.3.1 Invasion and the Tumor Microenvironment

The tumor microenvironment describes the environment around a particular tumor and consists of both cellular and non-cellular components [5, 52, 140]. It is important to note that the tumor cells and the other constituents of the microenvironment interact with one another, and this can influence the growth and spread of the tumor [52]. In gliomas their most conspicuous ability is their invasive properties within the central nervous system, which are typically very inhibitory to cellular movement.

The high mortality rate of patients with high-grade gliomas is explained by the fact that gliomas uniquely invade the central nervous system [109, 126]. The neural ECM is usually thought of as an inhibitory environment, one that is not conducive to large-scale reorganization or remodeling; this has been attributed to the high presence of CSPGs [107, 113]. Gliomas are able to circumvent this inhibitory barrier. One of the main ways gliomas are able to do this is through the secretion of molecules that facilitate cell adhesion and movement, which include fibronectin and collagen [12, 22, 30, 44, 45, 99, 106]. Other ECM molecules have been demonstrated to regulate the phenotypic characteristics of gliomas including laminin, vitronectin, and tenascin-C. Using in vitro assays, it has been reported that glioma cells produce and secrete laminin [87, 103]. Expression of the glycoprotein, vitronectin, is correlated with the glioma grade, and its expression has been linked to increased cell survival of glioma cells [131]. Similarly, tenascin-C expression is also linked to glioma grade, and its expression is thought to be involved in mediating cell adhesion, migration, and cell dispersion [57,

152]. Gliomas have also been shown to contain high levels of other ECM components like osteopontin, secreted protein acidic and rich in cysteine (SPARC), and thrombospondin [10]. Expressions of B/b, neurocan, and versican are also increased in glioma samples [100, 132, 134].

Enhanced expression of MMPs is characteristic of many tumor types, including gliomas. These enzymes degrade parts of the ECM, which then allows for the glioma cells to move through the ECM and infiltrate surrounding tissues. The MMPs that are upregulated in glioma cells include MMP-2, 3, 7, 9, 12, 13, 14, 16, 19, and 26 [33, 46, 63, 71, 73, 76, 85, 92, 106, 111, 115, 117, 118, 137, 138, 141, 148–150]. Other enzymes that are also responsible for the invasive properties of glioma cells are cathepsin B and urokinase-type plasminogen activator [11, 46, 71, 106, 115, 118, 141, 149] and heparanases and sulfatases [82]. In human gliomas, the overexpression of the forkhead box m1b (Foxm1b) factor leads to the enhanced invasion of glioma cells through an increase in transcription of the MMP-2 gene [32, 80].

Growth factors like epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and transforming growth factor-β (TGF-β) have been reported to mediate glioma cell invasion [28]. Glioma cells commonly display mutations or amplifications in the EGF receptor (EGFR) gene, and there is an increased presence of this receptor on the surface of the tumorigenic cells [81, 96]. Interestingly, the activation of EGFR and extracellular signal-regulated kinase (ERK) is thought to result in an increase in the expression of fibronectin [38, 130, 155], which likely underlies the increase in migration exhibited by glioma cells. Hepatocyte growth factor (HGF) is commonly overexpressed in glioma cells, and, as a result, cell migration pathways are activated, which leads to the enhanced movement of these cells [47]. Similarly, insulin-like growth factor (IGF) is also overexpressed in these tumorigenic cells, and, in specific, an increase in expression of IGFBP2 leads to an upregulation of genes that are involved in cancer cell invasion, including MMP-2 [139]. High levels of the angiogenic factor, angiopoietin-2 (Ang2), are detected in more invasive areas of gliomas, and this enhanced expression induces upregulation of MMP-2 both in vivo and in culture assays [50, 61, 69, 71]. The cell surface chemokine receptor, CXCR4, is also highly expressed in invasive glioma cells [36], and when the receptor interacts with a specific ligand, the Akt and ERK1/2 signaling pathways are activated, which affords glioma cells an increase in survival and cell division. This results in a more invasive phenotype [112, 142].

The canonical hyaluronan receptor, CD44, activates Rac1, which leads to a dramatic restructuring of the actin cytoskeleton within glioma cells. This receptor can be cleaved by ADAMTS10, and the product increases the invasive properties of glioma cells [3, 10, 91]. Rac not only facilitates the rearrangement of the actin cytoskeleton but is also known to increase cell motility [16, 110]. To demonstrate this, investigators inhibited Rac expression and found that glioma cell invasion was decreased [26, 29]. Rac does not work independently to mediate such important events; it has been reported that Rac works with the polypeptide P311 [84, 88]. The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is detected at high levels in glioma cells and has been hypothesized to afford these glioma cells an enhanced cell survival rate [114].

Glioma cells also exhibit changes in expression of cell adhesion molecules. For example, glioma cells express focal adhesion kinase (FAK) at higher levels than non-tumor cells [51, 56, 135], which has been linked to increases in cell proliferation [79, 151]. On the other hand, some cell adhesion molecules may exhibit decreased levels of expression in glioma cells. Expression of neural cell adhesion molecule (NCAM), for example, is reduced in glioma cells, which allows them to separate from neighboring cells and disperse into surrounding tissues [101, 116]. Cell surface integrin receptors that help join cells to one another are upregulated in glioma cells; specifically, this includes integrin α3β1, αvβ1, αvβ3, and αvβ5 [74]. Other cell adhesion molecules that display abnormal expression patterns in gli-

oma cells include adhesion molecule on glia/β2 subunit of Na, K-ATPase (AMOG/β2), ephrin receptor tyrosine kinases (EphB2–B3), fibroblast growth factor inducible 14 receptor (Fn14), and protein tyrosine phosphatases zeta/beta [37, 94, 95, 121, 129]. Cadherin molecules also work by joining adjacent cells to one another to form structures like adherens junctions. Any changes to the structure and stability of these junctions result in an increase in the movement and invasiveness of glioma cells [4]. Glioma cells that express high levels of E-cadherin are phenotypically highly invasive [77], while cells that contain high levels of N-cadherin demonstrate the opposite, in that they are less invasive [19, 93].

7.3.2 Glioma-Initiating Cells (GICs)

Gliomas are highly resistant to current therapeutic interventions, and, as a result, patient mortality rates are still quite high [126]. The invasive properties of gliomas are key in their therapeutic resistance; however, also key is the existence of glioma-initiating cells (GICs) within these tumors. In terms of the cellular composition, GICs that are present within the tumor are molecularly distinct from other cells found within the glioma. More specifically, the GICs are capable of self-renewing and exhibit multipotency, which means that they can differentiate into any subpopulation of cells found within the CNS as well as within the tumor itself. After orthotopic transplantation, these stem cells possess the ability to form a tumor that physically matches the parental tumor [25, 39, 123].

The GICs express many of the same proteins as those that are detected within normal stem cell niches [34] including laminin [122], tenascin-C [2, 40], members of the lectican family of CSPGs [68], and phosphacan [1, 2] as well as members of the integrin family [122]. Furthermore the GICs tend to be localized near vasculature within the tumors [43, 122]. It is important to note that the vasculature may develop due to the tumor presence itself, or the GICs might possess the ability to differentiate into endothelial cells, thus forming new blood vessels [108].

7.3.3 A Key Role for B/b in the Glioma Microenvironment

As noted above the interactions with tumors are complex within the tumor microenvironment; however, B/b presents as a uniquely intriguing target within this complex environment, and its specific roles are detailed below.

7.4 The Role of B/b in Gliomas

7.4.1 B/b Expression in Gliomas

Enhanced levels of B/b expression have been detected in human glioma samples, including oligodendrogliomas, all examined grades of astrocytomas, and gliosarcomas, relative to normal brain tissue and tissues derived from non-glioma tumors [42, 67]. More specifically, this increase in B/b expression was detected within the ECM as well as the cytoplasm of glioma cells. Importantly, within higher grades of astrocytomas (grades III and IV), B/b staining was more dispersed, indicative of an increase in infiltration, compared to lower grades [83]. Glioma cell lines (e.g., 9L, CNS-1, and C6) that are propagated under normal culture conditions do not express B/b, but if they are grown as intracranial grafts, then they express B/b. This phenomenon was not noted in cells taken from noninvasive tumors [67].

In rodent and human glioma samples, B/b is cleaved, and the resulting products are a N-terminal fragment that includes the hyaluronan-binding portion of the protein core (~50–60 kDa) and a C-terminal fragment (~90–100 kDa). Full-length B/b runs at ~160 kDa [133, 154].

Gary and colleagues set forth the hypothesis that B/b modulates the invasiveness of gliomas [41]. To examine the properties and behaviors of gliomas further, investigators introduced B/b into CNS-1 cells in vitro through transfection. These cells were transfected either with a green fluorescent protein (GFP) control, full-length B/b, the C-terminus cleavage fragment of B/b, or the N-terminus cleavage fragment of B/b. These cells

were then injected into rats to assess the resulting tumors. The rats that were injected with the CNS-1 cells transfected with the various forms of B/b, exhibited a lower survival rate than those rats that received the control cells. Furthermore, the B/b-derived tumors were more invasive and were highly vascular compared to the control tumors. This led to the conclusion that B/b enhances the aggressive properties of gliomas [98].

While it was established that full-length B/b is overexpressed in human glioma samples [42, 67], it was then identified that two novel isoforms of B/b were present in tumor tissues [133]. The two new isoforms were denoted as $B/b_{\Delta g}$ with a molecular mass of 150 kDa that was found within the membrane fraction and B/b_{sia}, which had a molecular weight higher than 150 kDa and was located in both the membrane and soluble fractions. It is important to note that the benign tumors that were assayed did not express these B/b isoforms, thereby indicating that perhaps these identified forms of B/b could be used as indicators of tumor grade. Furthermore, these isoforms were specifically identified in gliomas and were not present in tissues derived from patients with epilepsy or Alzheimer's disease. Neither isoform of B/b was found within individuals over 1 year of age. Only the $B/b_{\Delta g}$ form was faintly detected in samples harvested from embryos at 16 weeks of gestation to infants aged 19 days [133].

Through biochemical analyses, it was determined that these new isoforms of B/b were not cleavage fragments, and the peptide sequences of these forms were identical to the full-length protein, thereby indicating that these forms were derived from the same mRNA transcript. Attention was then focused on determining how these isoforms were molecularly distinct from the full-length protein. Deglycosylating enzymes were used to remove N-linked and O-linked sugars from the protein core as well as CS chains (through the use of chondroitinase), and this revealed that the $B/b_{\Delta g}$ form was underglycosylated. Further work determined that this particular isoform associates with the cell membrane in a manner distinct from other B/b forms. Importantly, this does not require calcium, interaction with the CS chains, nor the N-terminal domain of B/b, affirming that the molecular association with the membrane is unique for this specific isoform. The B/b_{sia} form is an over-sialylated version of the protein and is generated when there is an increase in the amount of sialic acid added to O-linked carbohydrates [133].

Having established that B/b is expressed at high levels in gliomas, the next step was to ascertain which specific cellular population contained the highest amounts of B/b. Human glioblastoma tumor sections were analyzed, and B/b was found around cells that expressed Olig2 and CD133 markers, both of which are indicative of highly tumorigenic cells [20, 49]. Interestingly, these markers are also detected within GICs. To examine B/b expression in these cells, researchers utilized two GIC lines: 0627 and 0913. They determined that both B/b protein and mRNA were present in these cell lines, although the 80–90 kDa C-terminal cleavage fragment was only expressed in the 0627 cells. Interestingly, B/b knockdown did not alter any of the assayed physical properties of the glioma-initiating cells including proliferation rate, viability, adhesive properties, migration, and invasion. Based on these results, it does not appear that B/b is needed for the GICs to behave normally nor for the maintenance of their characteristic physical properties, so likely B/b works in this cell population during the later stages of glioma pathogenesis [35].

7.4.2 Cleavage of B/b in Gliomas Leads to Increased Invasiveness

To address the ability of B/b to promote invasiveness, cultured 9L cells were transfected with either the full-length protein or the N-terminal fragment described above. It is important to note that the 9L cell line is characterized as a noninvasive cell line. 9L cells that expressed either the full-length form of B/b or the N-terminal fragment displayed a higher degree of motility and invasion as compared to cells that were trans-

fected with GFP. Of particular importance, when these cells were injected into rats, only the tumors that expressed the N-terminal cleavage fragment were able to invade surrounding brain tissue. This was not noted in tumors that expressed the full-length form of B/b, thereby suggesting that the cleavage of B/b is a key event that mediates glioma cell invasiveness in rat models [154].

This finding prompted the investigation into which molecule cleaves the lectican family member. This was addressed through the generation of an antibody against the putative cleavage site at Glu^{395}-Ser^{396} within B/b [86]. The cleavage site is homologous to the well-characterized site in another CSPG, aggrecan [156]. Cleavage of aggrecan at that particular site is regulated at least partially by ADAMTS4 [128].

The resulting antibody exclusively recognizes the N-terminal fragment of B/b and is referred to as B50. Through use of the invasive CNS-1 cell line, cleavage activity was detected in culture, and most of the resulting product was detected in the media and, thus, was soluble. Investigators then aimed to determine the proper conditions for B/b cleavage by altering calcium, zinc, and sodium chloride levels in addition to pH and temperature. Administration of calcium chelators, and metalloproteinase inhibitors to the cultures, diminished the cleavage of B/b. From this work, Matthews and colleagues examined the potential role of ADAMTS4 in mediating the cleavage of B/b. They concluded that ADAMTS4 not only was expressed in CNS-1 cells but was also capable of cleaving B/b. This work pinpoints a critical role for ADAMTS4 to regulate B/b cleavage and, by extension, the invasive behavior displayed by glioma cells [86].

To directly assess if this cleavage event is necessary for the pro-invasive properties seen in glioma cells, a mutant construct in which B/b was not cleaved was introduced into CNS-1 cells. Tumor spheroids were created and then applied to organotypic slice cultures, and migration of the cells was examined. The spheroids containing the wild-type form of B/b migrated across the slice cultures more than those that expressed the mutant form of B/b that could not be cleaved. The CNS-1 cells that were transfected with wild-type B/b were implanted into rats intracranially, and the resulting tumors were more invasive, dispersed, and larger compared to those tumors that formed when the CNS-1 cells transfected with the mutant form of B/b were injected. Rats that had tumors that were more invasive exhibited a decreased survival rate compared to their counterparts [134].

7.4.3 Molecular Mechanisms: How B/b Cleavage Promotes Invasiveness

Having determined that the cleavage of B/b promotes glioma cell invasion, the mechanisms underlying this were next explored. B/b was introduced into glioma cells (U87MG, U373MG, and CNS-1 cells) through transduction in culture. Expression of B/b enhanced glioma cell adhesion to specific substrates: fibronectin, collagen, and hyaluronic acid, but this was not noted when laminin and poly-L-lysine were used. Moreover, investigators probed glioma cell motility and reported that glioma cells expressing B/b were more mobile in response to hyaluronic acid and fibronectin substrates, in comparison with control cells that did not express B/b. B/b cleavage was required for the adhesion between B/b and fibronectin and hyaluronic acid. To provide further support, the glioma cells were added to organotypic slice cultures to measure the amount of cell dispersion. Glioma cells that expressed either the full-length form of B/b or the N-terminal cleavage fragment of B/b exhibited a significant increase in cell movement compared to those cells that expressed the form of B/b that was resistant to cleavage [62].

The expression of a number of cell adhesion molecules is altered in glioma samples [4, 19, 37, 51, 56, 74, 77, 79, 93, 94, 95, 101, 121, 129, 135, 151, 157], and Hu and colleagues then focused on identifying which cell adhesion molecules might be involved in modulating glioma cell invasion. Glioma cells that expressed B/b and were plated on fibronectin displayed an increase in protein expression of β-3 integrin, a phosphorylated form of the β-3 integrin, and NCAM, in comparison

with control cells that did not express B/b [62]. These results are in accordance with reports that β-3 integrin expression induces cell dispersion and spreading [143, 144]. It is known that both B/b and fibronectin are upregulated in gliomas [99, 133] compared to normal brain tissue [97, 102] and tumors that spread to the brain, but did not originate in the brain [67]. Specifically, fibronectin was found at the cell surface on glioma cells that possessed either the full-length form of B/b or the N-terminal cleavage fragment of B/b. The expressed fibronectin was organized in microfibrillar structures, which is thought to facilitate rearrangement of the ECM and promote movement of tumor cells [60, 72].

When U87MG glioma cells were incubated in conditioned media that contained either secreted full-length B/b or the N-terminal cleavage fragment of B/b, there was an increase in the amount of phosphorylated EGFR and phosphorylated ERK1/2 compared to control cells. If the glioma cells were treated with an EGFR inhibitor, then phosphorylation was inhibited, and, correspondingly, fibronectin mRNA levels decreased. Importantly, as a result of the treatment with this inhibitor, the B/b-expressing glioma cells did not adhere as well to fibronectin relative to cells that were treated with a control empty vector [62]. This work is consistent with reports that the expression of EGFR is increased in glioma cells [81, 96]. Additionally, the results presented by Hu et al. [62] corroborate previous demonstrations that the activation of EGFR and ERK induces an increase in fibronectin expression [38, 130, 155]. Precisely how fibronectin and B/b might associate with one another was directly addressed through co-immunoprecipitation and dot blot assays, in which it was shown that fibronectin binds to the N-terminal cleavage fragment of B/b, but not the full-length form of the protein. This clearly shows that the cleavage of B/b is an important event that is required for binding to fibronectin, which results in the enhancement of glioma cell movement [62].

The work presented by Hu and colleagues was supported by another set of experiments where U251 and U87 glioma cells were induced to express B/b, which resulted in an increase in the adhesion of glioma cells to fibronectin and an overall increase in motility of the glioma cells [83].

7.4.4 Impact of B/b Knockdown on Glioma Cells

To further pinpoint the critical role that fibronectin plays in mediating glioma cell motility, siRNA constructs were made to knockdown fibronectin expression. As a result, glioma cells adhered less to hyaluronan and fibronectin and additionally were less motile when plated on these substrates. Phenotypically, these glioma cells now presented the same as the control cells in terms of adhesion and motility [62].

To more thoroughly analyze how B/b is involved in regulating glioma cell behavior, U251 cells were first transduced to express B/b, and then the protein was knocked down using shDNA. Due to the knockdown of B/b, these glioma cells displayed a decrease in the rate of division and reductions in the following properties: invasiveness, migration, and dispersion or spreading distance, in direct comparison to the shDNA and mock controls. To examine resulting tumor growth in vivo, the transduced cells were introduced into nude mice. The mice that received the B/b knockdown cells developed tumors that were less infiltrative and less dispersed relative to the mice that received the control cells. This work further defined the role of B/b role in regulating glioma cell migration and invasion [83].

Dwyer and colleagues then aimed to elucidate what occurs when B/b is knocked down in intracranial gliomas. To this end, investigators transfected CNS-1 cells with B/b expression constructs at the same time as B/b knockdown constructs. After determining the knockdown efficiency, the generated CNS-1 cells were injected in the thalamus of rats. The tumors that formed after CNS-1 cells exposed to the shRNA construct specific to B/b displayed a reduction in overall volume and were less invasive compared to the tumors that developed when a control shRNA construct was introduced into the CNS-1 cells. In light of this, the survival rates of the rats injected with the B/b

knockdown cells were higher than those rats that received the control cells [35]. This body of evidence suggests that B/b expression in gliomas results in increased motility and invasion [35, 83].

As stated above, B/b expression was detected in GICs [20, 35, 49], but the question as to how B/b functions in this cell population was next addressed. shRNA constructs were introduced into GICs and then were injected into the striatum of nude mice to examine the properties of the tumors that were generated as a result. The GICs that expressed the knockdown constructs to reduce B/b expression formed a tumor that was smaller in volume and was less invasive relative to the cells that contained control constructs [35]. Therefore, it does appear that B/b mechanistically functions in the same capacity in the glioma-initiating cells as in glioma cells to promote invasion, spread, and migration.

7.5 Future Directions

B/b is a key molecule present within the tumor microenvironment of gliomas that works to promote cell invasion and movement [35, 41, 62, 83, 86, 98, 133, 134, 154]. Due to the fact that glioma cells possess the ability to infiltrate the normally inhibitory ECM, patient prognosis and response to current treatment options are quite poor [126]. In addition, work suggests that B/b contributes to tumor vascularization, but the mechanism by which it does this is completely unknown [98]. Future work investigating the interaction between B/b and other cells in the tumor microenvironment such as pericytes and vascular endothelial cells is clearly necessary. In addition, future studies need to be aimed at creating treatments that specifically target the GIC population. These cells are capable of self-renewal and also are able to form all of the cells within a glioma [39, 123]. Importantly, these cells create and maintain an environment that fuels tumor development, which not only is likely responsible for driving the initial establishment of the tumor but also explains why relapses might occur [6, 27, 78]. Therefore, treatments tailored to targeting the GICs might provide promising new avenues that could lead to better patient survival rates. B/b is an intriguing target in this regard as it seems to be an important component of the stem cell niche.

A complicating factor in treating individuals with gliomas is the fact that there is a great degree of molecular heterogeneity in these types of tumors. More specifically, cell adhesion molecules, ECM constituents, enzymes, and growth factors are just some examples of molecules that may underlie glioma pathogenesis. Importantly, these molecules work together to create an intricate and complex tumor microenvironment. In light of this, the best way to devise treatments is to precisely pinpoint how these molecules work together to contribute to the development and maintenance of gliomas in addition to defining the specific role of each of these molecules. This will give us a more complete picture as to how these tumors develop, thereby, providing us with the information necessary to generate more effective treatments.

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