Adhering towards tumorigenicity: altered adhesion mechanisms in glioblastoma cancer stem cells

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Practice points

- Cell adhesion molecules (CAMs) regulate diverse functions in tumor cells that include migration, invasion, proliferation and survival.
- Crosstalk between CAMs on cancer stem cells (CSCs) and the surrounding tumor microenvironment is vital for many aspects of tumor progression.
- A specific set of CAMs are preferentially expressed in glioblastoma CSCs as compared with other neural cells.
- The identification of CSC-specific CAMs in glioblastoma has made them attractive targets for cancer therapy and are potential prognostic biomarkers.

Glioblastoma (GBM) is the most aggressive malignant primary brain tumor in adults with a high recurrence and mortality rate. GBM tumors contain a high degree of cellular heterogeneity, with cells exhibiting stem-like properties (cancer stem cells; CSCs) that are highly efficient at tumor initiation and are resistant to conventional therapies. CSCs interact with their tumor microenvironment by a large group of diverse cell adhesion molecules (CAMs) that participate in intercellular, intracellular and cell-extracellular matrix interactions. Despite the initial description of CAMs as tumor suppressors, recent work has highlighted specific CAMs that are essential for CSC maintenance and tumor progression. This review will highlight recent findings that provide support for a context-specific role of CAMs in CSC function and GBM progression.

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Background

Glioblastoma (GBM, WHO grade IV glioma) is the most malignant astrocytic tumor and is associated by a poor median and overall survival that has remained unchanged over the past several decades. GBM treatments are often aggressive and typically include tumor resection, followed by concomitant radiotherapy and chemotherapy, generally including temozolomide (TMZ) and then 6–12 months of adjuvant TMZ [1,2]. GBMs are characterized by a propensity to migrate from primary site of tumor initiation to distant sites within the brain and form tumors despite local and systematic

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treatment [3,4]. GBMs are highly infiltrative and exhibit a great degree of recurrence, which is attributed to a self-renewing population of stem-like cells, termed cancer stem cells (CSCs) that are the top of a cellular hierarchy and are highly chemo and radioresistant.

CSCs are highly efficient at forming tumor xenografts, indicating their tumor initiation capacity and are resistant to most of the current therapies [5–8]. The CSCs reside in niches that are hypoxic and perivascular in nature. These niches are protective to the CSCs and facilitates stem cell maintenance and tumor propagation [40]. The structural framework of the niche is composed of non-CSCs such as endothelial cells (EC), microglia, pericytes and astrocytes. The EC and microglia secrete specific factors that contribute to CSC cell invasion and accelerate tumor growth [64,65]. The interaction of a stem cell with its niche components via cell adhesion molecules (CAMs) that act as signal transducers facilitate important signaling programs that maintain CSCs within a physical location and are essential for their maintenance [9–12]. The microenvironment also maintains cells in a dormant state preserving their potential to proliferate and differentiate, thus protecting them from the effects of chemo and radiotherapy [41].

Many current molecular markers of CSCs cannot be effectively targeted with drugs since they share expression with normal cells, hence making it challenging to identify the molecules that are uniquely expressed by the CSCs [13]. Recent reports indicate that some CAMs have a preferential expression in CSCs compared with normal cells, making them interesting targets to study and exploit their potential as novel therapeutic mechanisms in treating GBM [14]. In this review, we will focus on the major adhesion mechanisms likely driving CSC maintenance in the niche and highlight CSC-specific mechanisms that may be leveraged for targeting and diagnostic purposes.

Cell adhesion molecules

Adhesion of cells to a substrate and one another is initiated by CAMs that represent a diverse set of molecules. CAMs are known to regulate several different functions in the cell including cell growth, cell motility, signal transduction and inflammation apart from their role in development and tumor progression [15]. Although CAMs facilitate cell–cell adhesion or communication, they have additional roles that

require interactions with a wide range of binding partners that provide additional specificity.

The existing paradigm regarding CAMs suggests that loss of the adhesion molecules is necessary for tumor cell migration and invasion. This is exemplified by several classical epithelial to mesenchymal transition (EMT) markers like E-cadherin whose expression is reduced during tumor migration indicating their role as tumor suppressors [16]. However, this role of CAMs seems to be altered in some cases in GBM, where instead adhesion is a hallmark of the CSC state. CAMs are necessary for CSC interaction with their surrounding tumor microenvironment to facilitate cellular signaling and communication, the result of which is an increase in tumor growth [17–20].

There are four main classes of CAMs: integrins, the immunoglobulin (Ig) superfamily, selectins and cadherins. The CAMs mediate adhesion by forming either homophilic interactions with similar cell types or heterophilic interactions with different cell types [21]. These clusters of cell-adhesion molecules form specialized regions in the cell membrane known as cell junctions. There are three principal classes of these junctions: adherens junction, tight junction and gap junction (Figure 1). Some of these CAMs are expressed in GBM and have characterized functions in tumor progression (Table 1).

Integrins

Integrins are heterodimeric CAMs that participate in the regulation of complex processes, such as invasion, tumor growth and angiogenesis by interacting with the extracellular matrix (ECM), cytoplasmic proteins and immunoglobulin superfamily molecules. These are heterodimers formed by the combination of 18 α -subunits and 8 β -subunits. This combination of subunits determines the extent of its adhesion to another cell and its migration capacity [47].

Highly vascularized brain tumors express integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ that are necessary for GBM angiogenesis, hence targeting these with an antiangiogenic antagonist like cilengitide, which is a selective inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins was evaluated in clinical trials. However, a recent large-scale clinical trial CENTRIC, employing cilengitide did not demonstrate improved outcomes. While this approach may not be further developed, integrins remain a potential treatment target for GBM, as they may be essential for tumor cell function [48].

Figure 1. A schematic representing cancer stem cell interaction with adjacent cancer stem cells and non cancer stem cells via cell adhesion molecules localized at different cell junctions in glioblastoma microenvironment.

CAM: Cell adhesion molecules; CSC: Cancer stem cell.

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CSCs have elevated levels of integrin α_3 and can promote glioma cell invasion via the ERK1/2 pathway. Overexpression of integrin α_3 increases glioma migration and invasion associated with a change in the phosphorylation level of the erk 1/2 when tested in human astrocytoma and GBM patient-derived xenograft (PDX) models [49].

Recent studies on integrin α_6 reveal its association with N-cadherin to promote invasiveness of the GSCs through upregulation of ERK signaling pathway in PDX models. When the interaction between N-cadherin and integrin α_6 was minimized upon co-culturing with human umbilical cord blood stem cells, a reduction in the levels of ERK and invasive properties was observed, indicating the potential of human umbilical cord blood stem cells in treating brain tumor invasion by targeting molecular pathways [30]. Integrin α_6 could also be used as a maker to enrich for CSCs using PDX models [50].

$\alpha_5\beta_1$ integrin is highly expressed in high grade glioma and a negative crosstalk was reported between $\alpha_5\beta_1$ integrin and p53 pathways. It

was observed that inhibition of $\alpha_5\beta_1$ integrin oncogenic pathways sensitized the glioma cells to p53-reactivation and modulates the levels of PEA-15 and survivin, which are key antiapoptotic proteins. These observations were made in glioma stem cells and PDX models [51].

Integrins are required by neural cells to adhere, migrate and form processes with ECM molecules. Their interactions with ECM and focal adhesion complex including cytoskeletal proteins and other intracellular signaling molecules are crucial for synaptic transmission, neuroplasticity and other important neuronal functions [52]. Integrins are perhaps the most widely explored CAMs for their therapeutic potential in GBM and other cancers. The complexity and diversity in the structure of the integrins might be a major challenge for drug development and harnessing their therapeutic potential.

Immunoglobulin superfamily of cell adhesion molecules

These are a large group of cell surface glycoproteins with an immunoglobulin like extracellular

Table 1. Cell adhesion molecules and cell junctions in glioblastoma.

CAMs	Members	Expressed in GBM	Functions
Integrins	$\alpha\beta$ integrin dimers	$\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, α_3 , α_6	Enrichment of CSCs (α_6) [50], role in invasion ($\alpha_v\beta_3$, $\alpha_v\beta_5$, α_3) [48,49], correlates with poor prognosis ($\alpha_5\beta_1$) [51]
Selectins	E, P and L selectins	E-selectin	Angiogenic role [60]
Immunoglobulin superfamily	ICAM, NCAM, VCAM, PECAM, CAECAM etc.	L1CAM, VCAM-1, PSA-NCAM, CAECAM-1L	Invasion (L1CAM, VCAM-1) [57,54], biomarker function (PSA-NCAM) [56], CSC maintenance (CAECAM-1L) [55]
Cadherins	Classical (CDH), desmosomal (DSG) and protocadherins (PCDH)	Cdh19, Cdh11, Cdh5, Cdh1 (E-cadherin), Cdh2 (N-cadherin)	Invasion (Cdh1, 2) [17,30], migration (Cdh 11) [25], transition of CSCs to ECs (Cdh9, Cdh1) [28,13], marker for minimally infiltrative CSCs (Cdh19) [24]
Tight junction CAMs	JAMs, claudins, occludins	JAM-A, Cldn1, 5	CSC maintenance (JAM-A) [11,18]
Gap junctions CAMs	Connexins	Cx43, Cx46, Cx30	Cx43 – tumor suppressor [45], intracellular motor proteins (Cx30) [44]. CSC maintenance factor (Cx46) [42]
Adherens junction CAMs	Cadherins, adherens junction associated proteins	AJAP1	Loss of AJAP1 is associated with migration [31,32,62]

CAECAM: Carcinoembryonic antigen-related cell adhesion molecule; CAM: Cell adhesion molecule; CSC: Cancer stem cell; ICAM: Intercellular cell adhesion molecule; JAM: Junctional adhesion molecule; NCAM: Neural cell adhesion molecule; PECAM: Platelet-endothelial cell adhesion molecule; VCAM: Vascular cell adhesion molecule.

domain. Members of this family include neural cell adhesion molecules (NCAM), vascular cell adhesion molecules (VCAM), carcinoembryonic antigen-related cell adhesion molecule (CAECAM), intercellular cell adhesion molecule (ICAM), platelet-endothelial cell adhesion molecule (PECAM) etc., are known to interact with several other adhesion molecules such as integrins [53].

VCAM-1 was upregulated upon EGFR activation in human GBM cell lines. It was shown to mediate macrophage and GBM cell interactions, enhancing EGFR activated tumor cell invasion [54]. Hence indicating the importance of CAMs for immune cell–GBM interaction, and could be a potential immunotherapeutic target.

CAECAM-1L was identified to be expressed in CSCs and acts as a substrate of the insulin growth factor receptor and EGFR, both of which are frequently activated in malignant gliomas and is important for CSC maintenance and tumorigenesis via STAT3 signaling. Cell lines studied include mouse and human patient-derived CSCs [55].

PSA-NCAM is polysialic acid NCAM upregulated in GBM. It has a role in cell migration as observed in rat glioma and human GBM cell lines and is a novel biomarker for GBM [56].

L1CAM is a NCAM enriched in CSCs and targeting of it suppressed CSC growth as studied in GBM PDX models and impaired DNA damage repair and sensitized CSCs to radiation and chemotherapy [57–59].

The members of the immunoglobulin superfamily of CAMs have been reported to be important for CSC maintenance, as cell

surface markers for CSC enrichment and their possible roles as biomarkers. There are many other members of this group that have been well characterized in other carcinomas and need to be studied in the context of CSCs in GBM.

Selectins

Selectins are transmembrane proteins and include E, L and P selectins [53]. Although E-selectin expression was seen in high grade glioma blood vessels, its role in GBM CSCs needs to be further investigated [60].

Adherens junction proteins

Cadherins at adherens junctions are cell surface glycoproteins responsible for calcium-dependent homophilic cell–cell adhesion and play important roles in embryogenesis and tissue homeostasis [22]. Its cytoplasmic domain is required for binding to several cytoplasmic proteins including β -catenin and p120 [23]. Cadherins are most widely studied molecules in cancer in terms of EMT and cadherin switching, when they switch from epithelial state to a metastatic mesenchymal state. The differential expression of some of these cadherins in GBM makes it all the more challenging to understand their function. Several cadherins were found to be upregulated in GBM and a novel angiogenic role of cadherins has been reported [24–28].

CDH19 is preferentially upregulated only in a set of minimally infiltrative CSCs and has a potential angiogenic role during tumorigenesis. CDH19 has also been reported to be responsible for myelination during development [24]. Hence,

it might be worth following up on its restricted expression patterns in CSCs.

CDH11 is upregulated in GBM, and is known to mediate TGF- β induced migration/motility in CSCs. Co-culture experiments of primary GBM cells with EC increased CDH11 protein levels, indicating that its expression is regulated by ECs in GBM cells [25]. Recently its novel role in cell survival is reported [26].

Cdh5/VE-cadherin is the main constituent of endothelial adherens junctions [27]. It is specifically activated in CSCs but not in normal neural stem cells and non-GSCs, and its high expression predicts poor prognosis. It is an important target for angiogenesis in GBM, required for vascular integrity and permeability and controls EC assembly into tubular structures. Recently, a novel role of CDH5 in the potential transition from CSCs to an EC-like state has been reported, suggesting the possibility that CSCs enriched in vascular and perinecrotic niches in which hypoxia is a critical component have the potential to transition to EC-like state through hypoxia inducible factors. This was observed across all four grades of astrocytomas [28]. These findings provides further understanding into the mechanisms of hypoxia-induced angiogenesis in GBM.

E-Cadherin is a known tumor suppressor, whose expression is lost in many carcinomas and is associated with a less favorable prognosis. During EMT, the expression of the epithelial E-cadherin is reduced in exchange for increased expression of mesenchymal cadherins such as N-cadherin. But E-cadherin expression in GBM exhibiting an epithelial/pseudo-epithelial morphology correlates with a worse prognosis compared with patients with no expression of E-cadherin. E-cadherin expression also correlates with increased invasiveness of PDX cells in orthotopic mouse models of invasion. Hence its expression in GBM appears to be an exception to the EMT rule [17]. E-Cadherin expressing CSCs may also transition into EC according to another study [13].

N-cadherin expression remains upregulated in GBM and is known to be associated with ERK and integrin α_6 to promote CSC invasion [29,30].

• AJAP1

AJAP1 was recently identified as tumor suppressor-like gene, deleted and epigenetically silenced in some GBMs. Loss of its expression leads to increased migration of tumors and also predicts

poor clinical outcomes in GBM patients [31,62]. AJAP1 gene promoter hypermethylation and gene body hypomethylation was observed in GBM cell lines [32]. AJAP1 is known to be translocated to the nucleus through its intracellular interactions with β -catenin complexes, and regulates transcription of genes effecting cell cycle and induction of apoptosis. It mediates potentiation of apoptosis induced by TMZ in glioma cells through the transcriptional downregulation of MAGEA2 [33].

Since β -catenin is a positive regulator of the Wnt signaling pathway that is critical for glioma tumorigenesis, studying AJAP1 in the context of Wnt/ β -catenin pathway might provide further insights into the signaling mechanisms [34].

Tight junction CAMs

The cell adhesion transmembrane proteins at tight junctions include claudins, junctional adhesion molecules (JAMs) and occludins.

Claudins are tetraspan proteins capable of forming tight junction strands and thus the backbone of the tight junction. The PDZ-binding motif at its C-terminal cytoplasmic end is required for binding to PDZ domain-containing peripheral membrane proteins [35]. CLDN1 and CLDN5 were downregulated when tested in several GBM cell lines [36]. But their expression levels in CSCs remains to be explored.

JAMs are type I transmembrane proteins and members of the immunoglobulin superfamily, consisting of two extracellular Ig-like domains, cytoplasmic tail with a PDZ-binding motif. JAMs form homophilic and heterophilic interactions among their family members and bind to several scaffold proteins intracellularly and to CAMs like integrins [37,38]. Among several other family members, JAM-A has been well studied in GBM. JAM-A was found to be elevated in CSCs, and its high levels correlated with poor patient prognosis and has an important role as a CSC maintenance factor [11]. Recently it was shown that a microRNA, miR-145 regulates the expression of JAM-A and its expression inversely correlated to that of JAM-A levels in CSCs when tested in PDX models [18]. A monoclonal antibody 6F4 specifically targeting JAM-A decreased cell proliferation both *in vitro* and *in vivo* and exerted significant antitumor properties in different xenograft models of human tumors [39].

Therefore, targeting JAM-A might be a feasible option, due to its elevated expression in CSCs and its function as a CSC maintenance factor.

Other JAM family members need to be further investigated in the context of GBM.

• Occludins

Role of occludins in GBM CSCs seems to be unexplored and potential area to focus future efforts.

Connexins

Connexins are major constituents of gap junction channels, formed by six connexin subunits that assemble at the interface between adjacent cells allowing direct cell–cell communication. This gap junction-dependent role of connexins is shown to be critical for GBM growth and CSC maintenance [42]. Apart from their known role in direct cell–cell communication, they have been involved to mediate migration, cell–cell adhesion by interacting with several signaling molecules [43]. The cell adhesion activity was found to be independent of their channel function and is brought about by the cysteine residues in the extracellular loops, a point mutation in which led to reduced cell–cell adhesion. The C-tail of Cx43 interacts with tight and adherens junction molecules and E-cadherin and Cx30 interacts with actin microfilaments and microtubules [44–46]. Connexins are also differentially expressed in CSCs and non-CSCs. Cx43 had a lower CSC expression and have an antitumorigenic effect [45]. Whereas Cx46 had a higher expression in CSCs and has a protumorigenic role and is essential for CSC maintenance when tested in PDX models [42].

Altogether, this suggests that connexins have a noncanonical function in adhesion and tumor progression, and also differ in their expression among CSCs and non-CSCs. Thus, it is essential to further understand and validate the connexin adhesion mechanisms and how it effects cellular communication and tumor progression in GBM.

• CD antigens (cluster of differentiation)

Apart from the above mentioned CAMs there are also a variety of cell surface proteins known as cluster of differentiation (CD) antigens that have role in adhesion. One such important molecule is CD44 that is widely expressed in the CSCs. CD44 is implicated in cell–cell and cell–matrix adhesion, cell migration and signaling and is a useful prognostic indicator with respect to GBM [63,67]. Among several CD44 isoforms known, variant form 6 (CD44v6) is of interest based on its preferential expression in GBM

CSCs as compared with normal mouse brains or neural progenitor cells [68]. There are many more CD molecules that mediate adhesion in GBM, we regret for not including them due to limited space and scope in this review.

Conclusion & future perspective

CSCs are located in the GBM microenvironment and in order for these cells to drive tumor growth, they require stronger adhesion mechanisms to mediate proliferation, cell signaling, invasion and migration. Experimental evidence supports a model in which CAMs may accelerate tumor progression, not serve as tumor suppressors. Therefore, cell adhesion can be considered as a hallmark of the CSC state, which is in contrast to the classical paradigm of CAMs being tumor suppressors. Since CAMs represent a diverse group of molecules, which in turn bind to a wide variety of binding partners to mediate several cellular functions, it is essential to understand their role in tumor progression and find methods to effectively target them. CAMs mediate adhesion with several other key players in the niche, like EC and microglia. Hence, studying their interaction mechanisms might provide us more insights into the signaling pathways involving these cell populations. Among several antiangiogenic drugs that are been used to target GBM tumor angiogenesis, integrin antagonist cilengitide is the only anti-CAM therapeutic strategy that has been clinically evaluated [48]. These studies demonstrate that CAMs can be developed as anti-GBM therapies and given their role in tumor cell function, may be leveraged to neutralize proliferation and CSC maintenance.

The preferential expression of some CAMs in CSCs as compared with the normal brain makes them exciting targets to pursue, and could also be used as cell surface markers to enrich CSCs within tumors. Their potential as biomarkers to study the disease progression is also untapped. Efforts interrogating the expression patterns of CAMs across low and high grade gliomas should be considered to understand their roles during tumor progression and invasion. By utilizing currently available genetic resources, ECM proteins including laminin alpha 2 and CAMs such as JAM-A have been demonstrated to be elevated in GBM versus lower grade tumors [11,66]. Since several key adhesion phenotypes may be lost during *in vitro* study, the use of PDX models that maintain patient genetic abnormalities and GBM phenotype better are necessary to

understand the complexity of CAMs [61]. Hence, it would be of great importance if better and effective *in vitro* models are used, findings from which when translated *in vivo* for clinical studies would yield promising outputs. Therefore, integrating the concept that adhesion mechanisms are not tumor suppressors but rather hallmarks of the CSC state provides a framework for future studies and is likely to drive the identification of additional GBM targets and biomarkers.

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