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Reactive Astrocytes in Glioblastoma Multiforme

Xiudong Guan [1,2,3] · Md Nabiul Hasan [2] · Shelly Maniar [4] · Wang Jia [1,3,5] · Dandan Sun [2,6] (ID)

Abstract

Despite the multidisciplinary integration in the therapeutic management of glioblastoma multiforme (GBM), the prognosis of GBM patients is poor. There is growing recognition that the cells in the tumor microenvironment play a vital role in regulating the progression of glioma. Astrocytes are an important component of the blood-brain barrier (BBB) as well as the tripartite synapse neural network to promote bidirectional communication with neurons under physiological conditions. Emerging evidence shows that tumor-associated reactive astrocytes interact with glioma cells and facilitate the progression, aggression, and survival of tumors by releasing different cytokines. Communication between reactive astrocytes and glioma cells is further promoted through ion channels and ion transporters, which augment the migratory capacity and invasiveness of tumor cells by modifying H^+ and Ca^{2+} concentrations and stimulating volume changes in the cell. This in part contributes to the loss of epithelial polarization, initiating epithelial-mesenchymal transition. Therefore, this review will summarize the recent findings on the role of reactive astrocytes in the progression of GBM and in the development of treatment-resistant glioma. In addition, the involvement of ion channels and transporters in bridging the interactions between tumor cells and astrocytes and their potential as new therapeutic anti-tumor targets will be discussed.

Introduction

Glioblastoma multiforme (GBM) is a WHO grade IV glioma and characterized as the most aggressive, primary malignant tumor [1]. GBM has an incidence of three per 100,000 adults annually, and patients with GBM survive approximately 12 months [2]. Common presenting symptoms include headache, progressive neurological disorder, increased intracranial pressure, and secondary epilepsy. These clinical symptoms not only reduce the patients' quality of life but also shorten patients' survival. Current treatment is surgical resection with adjuvant chemotherapy and/or radiotherapy; such multimodal treatment is necessary given the highly aggressive and progressive capacities of GBM [3]. Even so, therapy often fails to prevent tumor recurrence and patients still show significant mortality with a median survival of less than 15 months [4]. Therefore, there is an urgency to identify novel targets for more effective therapeutic development for GBM treatment.

The past effort has been centered on studying roles of genetic mutations as drivers in tumorigenesis in GBM [5]. Recently, the role of the tumor microenvironment has drawn considerable attention in GBM research [6]. Several studies suggest that the peritumoral tissue may participate in regulating the tumorigenesis, progression, invasion, angiogenesis, and treatment resistance of GBM [7, 8]. Microglia account for 10–15% of the cells in the brain and occupy about 30% of GBM tumor masses [9]. They promote the proliferation and

✉ Wang Jia
jwttyy@sina.com

✉ Dandan Sun
sund@upmc.edu

1 Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University, No.6 Tiantan Xili, Dongcheng District, Beijing 100050, China

2 Department of Neurology, University of Pittsburgh, S-598 South Biomedical Science Tower, 3500 Terrace St., Pittsburgh, PA 15213, USA

3 Chinese National Clinical Research Center for Neurological Diseases, Beijing, China

4 Lake Erie College of Osteopathic Medicine—Seton Hill, Greensburg, PA, USA

5 Beijing Neurosurgical Institute, Beijing, China

6 Veterans Affairs Pittsburgh Health Care System, Geriatric Research, Educational and Clinical Center, Pittsburgh, PA, USA

invasion of GBM by releasing cytokines and immune adaptive factors [10, 11]. Another prominent species in the tumor microenvironment, constituting 40–50% of brain cells, are astrocytes and mature glial cells that are capable of mitosis. While they had initially been thought not to participate in tumor development, new evidence emerged to suggest that astrocytes could be activated by brain tumor cells and play a vital role in the progression, aggression, and angiogenesis of the tumor mass [12].

GBM is further propagated by the interaction between astrocytes and tumor cells via ion channels and ion transporters. They play an important role in maintaining the ionic homeostasis, establishing the membrane potential, regulating cell volume, osmotic pressure, and acid-base balance under physiological conditions. Recent studies have shown that expression and activation of Ca^{2+} and K^+ channels regulate ion influx or efflux and are involved in proliferation, migration, and survival of glioma [13, 14]. This review will cover the role of ion channels in communication between astrocytes and glioma cells. Furthermore, the impact of ion channels and ion transporters in glioblastoma cell proliferation, migration, invasion, and apoptosis will be discussed. These new findings illustrate the potential of these ion channel and ion transporter proteins as therapeutic targets for GBM.

Astrocytes and Reactive Astrocytes in GBM

Astrocytes comprise nearly half of the total number of cells in mammalian brains [15] and play an important physiological role in the central nervous system (CNS). First, astrocytes support the neuronal network by dividing gray matter into distinct domains and augment the myelinating capacity of white matter [16, 17]. Growing evidence indicates that astrocytes also participate in the formation, development, and maintenance of neural circuits [18]. The membrane processes of astrocytes that contact other neural cells promote cell-cell communication, allowing the recycling of neurotransmitters and meeting the metabolic demands of the cells [19–21]. In addition, astrocyte endfeet are a component of the brain-blood barrier (BBB), which separates circulating blood from extracellular fluid by its highly selective permeability. By detecting the level of glutamatergic activity in the synapse, astrocytes can correspondingly adjust the calcium (Ca^{2+}) concentration in their endfeet and release gliotransmitters that act on vascular smooth muscle to regulate brain blood flow [22].

In certain CNS pathologies, astrocytes are transformed into reactive astrocytes, characterized by hypertrophy, upregulation of intermediate filaments composed of nestin, vimentin, and glial fibrillary acidic protein (GFAP), as well as activation of cell proliferation [23]. Reactive astrocytes, in acute brain injuries like trauma, spinal cord injury, or stroke, form a functional barrier, termed as "glial scar," which serves to restrict and regulate inflammation, isolate the lesion, regulate and repair the BBB, enhance synaptic plasticity, and initiate neuronal circuit reorganization [24]. Glial scar formation may interfere with axonal regeneration [23]. Furthermore, reactive astrocytes release cytokines, chemokines, interleukins, nitric oxide (NO), and other molecules, thereby exacerbating the neuroinflammatory responses [25]. Another pathology that has noted reactive astrocyte development includes the brain tumor microenvironment [6] which is further discussed below.

Glioma and Astrocyte Crosstalk

The tumor microenvironment is recognized to be crucial to the progression of tumors, with specifically the parenchymal, non-malignant cells adjacent to the mass thought to aid its development and growth [6]. The invasiveness of GBM is reflected by a significant amount of non-transformed cells adjacent to the tumor mass during examination of the surgically resected tissue, indicating close interaction between the two cell populations. Particularly, astrocytes display a reactive phenotype when they contact tumor cells, expressing a high concentration of GFAP [26]. These reactive astrocytes aid the parenchymal infiltrative capacity of glioma cells by expressing matrix metalloproteinase-2 (MMP2) and further stimulate uncontrolled proliferation of glioma cells by secreting stromal cell-derived factor-1 (SDF1) [27]. Thus, the reactive astrocytes in the tumor microenvironment augment GBM malignancy by causing aberrant cell proliferation and enhancing migration [28].

Reactive astrocyte formation in GBM can be regulated by the NF-κB signaling pathway [29]. Upon being treated with recombinant RANKL (rRANKL), a well-known activator of NF-κB signaling, astrocytes display increased GFAP, predisposing them to astrogliosis [30]. Lipopolysaccharide (LPS), another activator of NF-κB signaling, has also been shown to promote NF-κB activity by decreasing the protein levels of IκBα, a negative regulator of NF-κB [29, 30]. Inhibition of NF-κB reduced induction of GFAP expression in astrocytes by rRANKL and LPS, thus preventing their transformation into the reactive phenotype [30].

Another important player in the crosstalk between GBM and astrocytes is the tumor suppressor gene *p53*, found mutated in 87% of GBM cases [31]. When healthy cells suffer DNA damage, *p53* is activated in the nucleus initiating cell death or growth arrest. However, the apoptosis mechanism is completely inhibited in cancerous *p53*$^{-/-}$ cells or reduced in heterozygous *p53*$^{+/-}$ cells. In addition to its autonomous function, *p53* has been shown to regulate expression of proteins that are secreted to stimulate adjacent cells [32]. The extracellular matrix (ECM) of *p53*$^{+/-}$ astrocytes had a greater presence of laminin and fibronectin than the ECM of *p53*$^{+/+}$ astrocytes. This richer tumor microenvironment formed by the neighboring *p53*$^{+/-}$ astrocytes aids the survival of GBM cells.

In addition, evidence suggests that conditioned medium from GBM cells can inhibit the function of *p53* in healthy $p53^{+/+}$ astrocytes. Thus, there is a synergistic relation formed between GBM cells and the cells in the tumor microenvironment: GBM cells hinder p53 expression in astrocytes to predispose cells to aberrant cell proliferation, while dysfunctional p53 induces a nutritious environment in which GBM cells can thrive [28].

Reactive astrocytes can communicate with GBM cells directly or indirectly. One direct form of communication involves gap junction channel protein connexin43 (Cx43) in glioma-associated astrocytes [33]. Direct interaction is also facilitated by tunneling nanotubes (TNT), long and thin tubular structures established between GBM cells and astrocytes, participating in cell communication and influencing GBM proliferation [34]. In addition to this direct contact with glioma cells, reactive astrocytes secrete chemokines or cytokines, such as interleukin-6 (IL-6), transforming growth factor- β (TGF-β), insulin-like growth factor-1 (IGF-1), monocyte chemotactic protein-4 (MCP-4), interleukin-19 (IL-19), vascular endothelial growth factor (VEGF), and leukemia inhibitory factor (LIF), among others, to promote tumor cell invasion and migration, proliferation, and growth [35]. These mechanisms will be discussed in detail in the following sections.

Reactive Astrocytes Are Involved in Proliferation and Invasion of GBM

Tumor cells interact with one another and with astrocytes via gap junctions, integrins, and by releasing signaling molecules like growth factors and cytokines to enhance their proliferative and invasive capacity. It is thought that reactive astrocytes upregulate expression of Cx43 to confer chemotherapy resistance in addition to enhancing glioma cell proliferation and migration. In response to temozolomide (TMZ) exposure, Cx43 modulates the levels of Bcl-2 and Bax2 in LN229 human glioma cells, inhibiting the mitochondrial apoptotic response. Cx43 expression further blocks cytochrome C release from the mitochondria, which also prevents the malignant cell from undergoing apoptosis [36]. Besides resistance to TMZ treatment, Cx43 could augment the invasiveness of glioma cells. Substituting the wild-type Cx43 protein with a C-terminal truncated mutant in astrocytes reduced its communication with glioma, and the invasiveness of the glioma cells into the parenchyma was decreased [37]. Glioma cells have the capability to deliver miRNAs to astrocytes via gap junctions for intracellular communication. Thus, elimination of miRNAs in astrocytes decreases glioma invasiveness [38].

L-Glutamine (Gln) is involved in balancing the carbon and nitrogen requirements of neural tissue. In GBM, such metabolic dysfunction as Gln starvation could hinder glioma proliferation [39]. Although GBM cells can produce Gln by themselves, it is not sufficient to meet the metabolic need of the cell. To facilitate this requirement, astrocytes synthesize and secrete Gln, which was subsequently taken up by GBM cells, as seen in the astrocyte-glioma co-culture system [39]. Thus, there is an interdependence created in the tumor microenvironment between the glioma cells and the astrocytes, where the two work synergistically to promote GBM proliferation and invasiveness.

Indirectly, astrocytes secrete chemokines and cytokines to influence GBM proliferation and invasion. One such cytokine, IL-6, triggers several oncogenic factors, to create a suitable tumor microenvironment that promotes tumor proliferation, invasion, and angiogenesis [40]. Through the activation of the IL-6/p-STAT3 mechanistic pathway, IL-6 increases MMP2 expression, a transmembrane proteinase critical for the degradation of ECM to enhance the invasiveness of GBM [41]. Recent findings suggest that tumor-associated astrocytes secrete IL-6 to promote the progression of GBM in the astrocyte-glioma co-culture system, increasing its malignancy [35, 41]. IL-6 secreted by astrocytes also induces upregulation of MMP-14 and activates the Akt/p38MAPK/ERK(1/2) signaling pathway, further enhancing the invasive capacity of GBM [35]. The interaction between glioma cells and the surrounding astrocytes also activates MMP-2 via uPA-plasmin cascade [42]. In addition, reactive astrocytes secrete glial cell line-derived neurotrophic factor (GDNF), which binds to the RET/GFR1 receptor and activates the PI3K/Akt pathway to induce GBM migration. One study found that reducing GDNF concentration can decrease the size of the GBM tumor [43].

Initially identified in fetal astrocytes, astrocyte elevated gene-1 (AEG-1) presence is associated with poor survival in GBM [44] because of its involvement in the progression and migration of glioma cells [6]. Specifically, AEG-1 enhances the proliferation and the invasiveness of GBM by modulating the PI3K/Akt, NF-κB signaling pathway and upregulating MMP-2 and MMP-9 expression [6, 44–46]. Inhibition of AEG-1 with DYT-40, a novel synthetic 2-styryl-5-nitroimidazole derivative, was shown to reduce tumor volume and induce cell apoptosis in a rat model. Its mechanism of action involves downregulating the MAPK and PI3K/Akt signaling cascades, blocking the translocation of NF-κB, and ultimately inhibiting expression of AEG-1 [47].

Reactive Astrocytes and Resistance of Radiochemotherapy Treatment

Given the predisposition of GBM cells for aberrant proliferation and their extensive invasive capacity, the prognosis of patients is poor. Traditional clinical treatment includes surgery, radiation therapy (RT), and chemotherapy. With the advent of new medical technology, treatment became multidisciplinary with the integration of neuroimaging, surgery, neuropathology, chemotherapy, and palliative care [48].

Unfortunately, these are not sufficient to prevent tumor recurrence, and median survival of GBM remains 12–15 months. With recent evidence, suggesting the interaction between tumor cells and their microenvironment contributes to the resistance of tumor cells to therapy, therapeutic research has pivoted to find ways to combat such resistance [12].

Surgerical resection remains essential to the management of all grade gliomas, including GBM. Gross total resection is usually the frontline therapy considered because of its better clinical outcome. However, such maximal excision should only be performed if most of the patient's neurological function can be protected [48]. Given the extent of GBM cells' invasiveness and uncontrolled proliferation, it is difficult to remove the tumor totally [49]. Furthermore, the tumor microenvironment has a crucial role in regulating the progression, metastasis, and angiogenesis of GBM, which makes total tumor resection more difficult [12]. The tumor-associated astrocytes are a significant component of the tumor microenvironment and promote the increased proliferation of glioma cells, even after resection of tumor [50].

After surgery, patients are generally treated with combinational chemotherapy. TMZ is thought to be the most effective chemotherapeutic agent because of its high penetrance past the BBB [51]. TMZ's mechanism of action involves increasing DNA strand breaks and triggering apoptosis in glioma cells [52]. Nevertheless, some patients exhibit resistance to TMZ treatment, in part, due to expression of O6-methylguanine-DNA methyltransference (MGMT). MGMT causes a decrease in TMZ-mediated methylation of DNA, reducing toxicity in the cell, and thus, lessens apoptotic activity [53].

Gap junction communication (GJC) between glioma cells and astrocytes has also been suggested to decrease sensitivity to TMZ chemotherapy [54]. These gap junctions allow the transfer of small molecules and microRNAs between cells that confer therapy-resistive properties [55]. Gap junction Cx43 in glioma cells can account for TMZ resistance [36]. Upon being exposed to TMZ, epidermal growth factor receptor (EGFR) is upregulated on the surface of tumor cells, which activates the JNK-ERK-AP-1 axis. This serves to increase Cx43 expression to ultimately reduce the sensitivity of glioma cells to TMZ [55]. Meanwhile, it was observed that knockdown of Cx43 in astrocytes increased TMZ-induced apoptosis of glioma cells [56]. It was noted above that upregulation of Cx43 in astrocytes promoted the proliferative and migratory capacities of glioma cells, so by experimentally downregulating this gap junction protein, TMZ was able to increase its effectiveness in combating GBM.

Another option for treatment of GBM involves radiotherapy. One model, to better understand the impact of the tumor microenvironment in treatment resistance, co-cultured human glioblastoma stem-like cells (GSCs) with astrocytes and discovered that the presence of astrocytes diminished the sensitivity of glioma cells to radiotherapy. Astrocytes were proposed to act in a paracrine mechanism to induce repair of DNA strand breaks. Chemokine profiling identified the activation of STAT3 in GSCs grown among astrocytes, which was not present in GSCs grown alone. The study proposed treating glioma cells with WP1066, a JAK/STAT3 inhibitor, and discovered that the radiosensitivity of the cancer cells increased, making them more vulnerable to therapy [57]. Therefore, studying the tumor microenvironment is crucial in understanding additional targets for therapy optimization.

Ion Channels and Ion Transporters as a Bridge Between Reactive Astrocytes and Glioma

The interaction between glioma cells and astrocytes in the tumor microenvironment is facilitated further via ion channels and ion transporters. Ion channels, considered pore-forming and multimeric proteins, are located in the plasma membrane of cells and serve to establish an electrical potential across the cell membrane. This prompts the flow of ions in response to chemical or mechanical stimuli, which regulates the cell volume. Recently, emerging new evidences suggest the role of ion channels in the progression, migration, and angiogenesis of tumor cells [58–60]. A multitude of ion channels are expressed in glial cells, including Na^+ channels, K^+ channels, Ca^{2+} channels, and Cl^- channels [61]. This section discusses the role of ion channels and ion transporters in the communication between astrocytes and glioma cells.

Ion channels are involved in augmenting cell development, passing them through the necessary cell cycle checkpoints, and thus, playing a role in proliferation. The likely mechanism of action is by regulating the cell's membrane potential; there is a distinct increase in K^+ channel activity during the G1/S checkpoint, and similar Cl^- channel activity is involved in the G2/M checkpoint [62]. As mentioned previously, GBM cells are highly invasive, and one feature that aids migration is the unregulated activity of ion channels. Water and ion movement is modulated by ion channel activity, having a great influence on the morphology of glioma cells. In addition to aiding invasiveness, ion channels contribute to the proliferative capacity of glioma cells [60].

Calcium Ion Channels in GBM

Ca^{2+} concentration in glioma cells helps regulate cell proliferation, migration, and apoptosis [63, 64]. GBM cells are proposed to use Ca^{2+} as a second messenger to aid in migration, the fluctuations in concentration regulated by ion channels. These changes in Ca^{2+} concentration induce Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to activate ClC-3 chloride channels, which begins GBM invasion [62]. The release of intercellular Ca^{2+} stores through inositol 1,4,5-

trisphosphate receptor (IP$_3$R) [65] and triggers Ca^{2+} entry into glioma cells, mediated by stromal interacting molecular 1 (STIM1) and Ca^{2+} channel Orai 1 [66, 67]. One study found that knockdown of STIM1 and Orai l, which decreases the intercellular Ca^{2+} concentration reduces GBM proliferation and invasion and induces tumor apoptosis [67, 68]. Furthermore, inhibition of IP$_3$R reduces GBM migration and increases survival in a mouse xenograft model [65].

Cytosolic Ca^{2+} concentration helps modulate cell proliferation. There are several Ca^{2+}-permeable channels observed in glioma cells, including T-type low voltage-gated calcium channels (VGCC) and transient receptor potential (TRP) channels, as well as purinergic receptors, which modulate ion flux [69]. Through managing Ca^{2+} influx and changing intercellular Ca^{2+} concentration, T-type VGCC promoted proliferation of one GSC cell line while inhibition of T-type VGCC by endostatin suppressed the tumor growth and increased tumor cell apoptosis [70]. The α1 subunit of T-type VGCC may play a key role in abnormal proliferation of GBM; siRNA-mediated knockdown of this subunit was demonstrated to decrease proliferation of GBM cells [71]. Furthermore, inhibition of T-type VGCC disrupted Akt signaling, a pathway involved in the proliferation of GBM cells [72]. In brief, T-type VGCC regulates glioma cell proliferation and apoptosis by controlling Ca^{2+} entry, but also via mediating Akt signaling.

Another subtype of Ca^{2+} channels is TRP channels, located in the plasma membrane of almost every cell type, where they act as the driving force for ion entry [73]. Subtypes of TRP channels were significantly overexpressed in GBM cells as compared to low-grade gliomas; these channels include transient receptor potential melastatin subfamily member-2 (TRPM2), TRPM3, TRPM7, TRPM8, transient receptor potential canonical channel protein-1 (TRPC1), TRPC6, transient receptor potential vanilloid subfamily member-1 (TRPV1), and TRPV2 [13, 74–76]. The TRPM channels are involved in the regulation of Ca^{2+} entry and the development of proliferation in tumor cells [73]. Studies show that TRPM7, when stimulated by hepatocyte growth factor/scatter factor (HGF/SF), induced influx of Ca^{2+} to increase the migration of glioma cells [77]. This mechanism involves TRPM7 modulating the PI3K/Akt and MEK/ERK signaling pathways to ultimately influence GBM cell proliferation and migration [78]. TRPM7 also works by activating the JAK/STAT3 and Notch signaling pathways to increase progression and aggression of GBM [79].

The TRPC family, activated directly by diacylglycerol (DAG) or indirectly through IP$_3$-mediated Ca^{2+} release, serves to increase cytosolic Ca^{2+} concentration and plays a role in regulating cell growth [74]. TRPC1, a subtype of TRPC, was detected as one of the leading causes of cell migration, when stimulated by epidermal growth factor (EGF), which activates the p38MAPK/JNK signaling pathway [80]. Another subtype

of TRPC, TRPC6, has been shown to be overexpressed in glioma cells [62]. It activates the Notch signaling pathway, increasing the aggression of glioma cells [81]. Inhibition of TRPC6 suppresses intracellular Ca^{2+} concentration, leading to inhibition of the cell cycle at the G$_2$/M checkpoint. This response was utilized in mouse models with xenografted tumors, where blocking TRPC6 demonstrated reduced tumor volume [62].

However, not all subfamilies of TRP promote the proliferation and migration of glioma cells. Stock et al. demonstrated that neural precursor cells (NPCs) release endovanilloids that activate TRPV1 on glioma cells [76]. Activation of TRPV1 triggers glioma cell death [82], specifically via activating transcription factor-3 (ATF3), which mediates an endoplasmic reticulum stress pathway [76]. This innate defense mechanism is weakened with age, but TRPV1 agonists illustrate potential as therapeutic agents against GBM.

Potassium Ion Channels in GBM

Physiologically healthy glial cells have a very negative resting membrane potential (between -80 and -90 mV) and prefer K$^+$ uptake, caused by inwardly rectifying currents. This contrasts from GBM cells, with more positive resting membrane potentials, between -20 and -40 mV. They tend to expel K$^+$, meaning they express outwardly rectifying currents. Given their predisposition to expel K$^+$, glioma cells are thought to express fewer inwardly rectifying K$^+$ (K$_{ir}$) channels than healthy glial cells [62].

Thus, glial cells experience more K$^+$ influx and actively distribute K$^+$ according to concentration, which involves diffusing the ions through gap junctions between cells. This K$^+$ uptake by glial cells serves a crucial purpose: increased extracellular K$^+$ in neural tissue would cause neurons to remain depolarized and unable to fire action potentials, needed for signaling [60]. Glioma cells have a diminished number of K$_{ir}$ channels and are unable to perform the function carried out by healthy glial cells to remove extracellular K$^+$. This accrual of K$^+$ in the extracellular space causes aberrant neuron signaling and may explain the prevalence of epileptic seizures seen in GBM patients, through an indirect mechanism of action. The presence of extracellular K$^+$ causes the less negative resting potential seen in glioma cells (from -40 to -20 mV), which also weakens the Na$^+$ gradient across the plasma membrane. Subsequently, the Na$^+$-dependent glutamate transporters are downregulated, causing a higher glutamate presence extracellularly [62]. Excess glutamate concentration causes excitotoxicity, a condition correlated with epilepsy [83].

One type of voltage-dependent K$^+$ channel, the human ether a-go-go-related gene potassium channel (hERG), is overexpressed in endometrial adenocarcinoma, myeloid leukemia, and colorectal cancer [84], a finding also observed in

GBM cells [85]. hERG promotes VEGF secretion to initiate angiogenesis and increases the capacity for GBM proliferation [84]. When hERG was inhibited, either by siRNA knockdown or by treatment with doxazosin, apoptosis of glioma cells was observed [86]. Recent evidence indicates the involvement of microRNA in regulating hERG activity, to mediate proliferation and chemotherapy resistance in GBM cells. MiRNA-133b/miRNA-34a activates hERG, so upregulation of MiRNA-133b/miRNA-34a induces increased expression of hERG in glioma cells, leading to apoptosis resistance [87]. However, upregulation of another species of microRNA, miRNA296-3p, reduces the expression level of hERG, promoting anti-proliferation and reducing resistance to treatment [88]. Abnormal expression of other voltage-gated K^+ channels, Kv1.5, Kv10.1, and Kv11.1, were also detected in GBM. Studies found that expression of these channels seemed to be associated with prognosis of GBM patients: for example, high Kv1.5 expression was correlated with a longer survival rate, while high Kv10.1 level predicted a shorter lifespan [89, 90].

Another key type of K^+ channel is intermediate-conductance Ca^{2+}-activated K^+ (IK) channels. IK channels play a crucial role in maintaining the membrane potential, specifically by favoring outwardly rectifying K^+ currents. Because IK channels are activated by Ca^{2+}, increases in concentration make the potential of cells more negative [62]. Recently, overexpression of IK was detected to be correlated with the malignancy of the tumor, and thus indicative of poor patient survival [91]. Several studies indicate that chemokines like CXC-motif chemokine ligand 12 (CXCL12) can modulate GBM cell migration by activating IK channels [92, 93]. However, when cells were treated with TRAM-34, an inhibitor of IK channels, the migratory capacity of GBM cells was reduced, diminishing its invasiveness [94]. In addition, IK channels function by regulating the ERK pathway to promote tumor aggression [95]. A new study shows that ionizing radiation can activate IK channels, which subsequently enhances glioma cell proliferation and induces therapy resistance [96]. This suggests that IK channel antagonists may prove a viable therapeutic target to circumvent therapy-resistant cells and induce apoptosis.

Another subtype of Ca^{2+}-activated K^+ channels are big conductance Ca^{2+}-activated K^+ (BK) channels, which present with several isoforms because of the alternative splicing seen in their α-subunits. GBM cells express the isoform glioma BK (gBK) channels, formed as a result of a novel splice of hSlo, the gene that codes for the α-subunits [62]. gBK channels can be activated by TRPM8-mediated increases in intercellular Ca^{2+} concentration. Upon activation by changes in Ca^{2+} concentration, gBK channels stimulate changes in cell volume to promote migration and invasiveness of glioma cells [97]. Its role in migration was further demonstrated when gBK channel inhibitors like tetraethylammonium and paxilline actually impaired the migration of glioma cells [62]. gBK channels may also contribute to conferring therapy resistance to glioma cells; when cells were treated with TMZ, gBK channels were demonstrated to be activated, and the glioma cells' apoptotic response was arrested [98]. Therefore, gBK channels are not only involved with the progression and invasion of GBM but can also enhance the glioma cells' resistance to treatment.

Sodium Channels in GBM

Na^+ ions contribute to maintaining osmotic pressure and electrolyte balance in cell. Patients with Na^+ channel mutations, according to a univariate analysis, undergo a more aggressive disease course and suffer shorter survival rate than patients without Na^+ channel mutations [99]. Deg/epithelial Na^+ channels (ENaC) are responsible for Na^+ uptake and water homeostasis under physiological conditions [100]. However, an abnormally high expression of ENaC was observed in GBM tissue [101, 102]. ENaC was shown to enhance the capacity of GBM cell migration [103]. One possible therapeutic option is the administration of psalmotoxin-1 (PcTX-1), an inhibitor of cation currents. In human GBM samples and GBM cell lines, PcTX-1 did block Na^+ currents, as detected by electrophysiological properties. However, it had little effect on human astrocytes [62]. In addition, ENaC is also involved in the regulation of GBM proliferation by affecting the cell cycle. Treatment with PcTX-1 or benzamil could arrest cell growth in G_0/G_1 phase and reduce cell accumulation in S and G_2/M phase [103]. Other antiproliferative treatments include ouabain and digoxin, which demonstrated reduced cell division when applied to GBM cell lines [62]. Additionally, acid-sensing ion channel-1 (ASIC1), a proton-gated cation channel when interacting with the ENaC family, may modulate the invasiveness of GBM [104]. ASIC1 interacts with α-ENaC and γ-ENaC, a process mediated by CaMKII [105]. Knockdown of either ASIC1, α-ENaC, or γ-ENaC reduces the glioma cell migration [102].

Chlorine Ion Channels in GBM

Cl^- is essential to maintaining ion homeostasis and helps regulate cell volume and morphology [106]. Some studies discovered that upregulation of certain chloride intracellular channel proteins (CLICs) was also detected in glioma cells, such as CLIC-1, CLIC-3, and CLIC-7 [107]. CLICs are involved in the progression of GBM by promoting glioma cell proliferation and self-renewal. For instance, CLIC-1, a subtype of CLIC, takes part in regulating the cell cycle when activated during the G_1/S phase, and inhibition of CLIC-1 was illustrated to cause G_1 arrest [108]. In addition, CLIC-1 is involved in secreting extracellular vesicles, which play an important role in cell-cell communications, and further regulate cell growth [109]. Given their involvement in cell

proliferation, high expression of CLIC1 correlate with shorter survival time for GBM patients [110].

Another subtype of CLICs, CLIC-3, is involved in regulation of glioma cell migration. Inhibition of CLIC-3 by chlorotoxin notably suppressed the invasiveness of tumor cells in vitro, although not completely. Similar results were found when CLIC-3 was blocked by siRNA-mediated knockdown [111]. CLIC-3 can also be phosphorylated by CAMKII, which serves to regulate channel activity. CAMKII inhibition in CLIC-3-expressing GBM cells reduced migration the same degree as when CLIC-3 was directly inhibited with chlorotoxin [62]. Thus, CAMKII proves to be an important player in ion transfers, which is crucial for the invasive cell migration seen in GBM.

As a part of neuron-glial network, astrocytes set up bidirectional communication with other cells by receiving and sending neurotransmitters, such as glutamate and γ-aminobutyric acid (GABA). Various mechanisms have been proposed for gliotransmission, including Ca^{2+}-regulated vesicular exocytosis and release via plasma membrane ion channels under physiological condition [112]. In recent years, it was discovered that cell-cell communication via secreted extracellular vesicles is more common than initially thought. Exosomes are 30–100 nm in diameter and transport miRNAs and proteins that promote the progression of GBM [113]. Some ion channel proteins are circulating proteins secreted by other cell lines. In addition to CLIC-1-mediated GBM proliferation and self-renewal, CLIC-1 proteins are secreted via extracellular vesicles to promote glioma cell growth, as seen in vitro and in vivo. CLIC-1 silencing or overexpression can respectively diminish or enhance the effects of the secreted exosome [109].

Na-Dependent Ion Transporters in Glioma

Chemotherapy triggers a significant reduction in glioma cell volume resulting from loss of K^+ and Cl^-, which is a hallmark of apoptosis and is referred to as apoptotic volume decrease (AVD) [114]. In responding to cell volume loss triggered by apoptosis, cells will compensate by activating regulatory volume increase (RVI) pathways. RVI could be mediated by the gain of active solute such as Na^+, K^+, and Cl^- or changes in osmotic pressure. Na-K-Cl cotransporter (NKCC1) is the main regulator of RVI, functioning by bringing Na^+, K^+, and Cl^- into cells to maintain cell volume and counteract cell shrinkage [115]. It has been shown that bumetanide, an inhibitor of NKCC1, impairs RVI following TMZ treatment and thus enhances TMZ-mediated apoptosis in glioma cells [116].

Ion transporters also play a key role in the progression of GBM. The sodium/hydrogen exchanger (NHE) promotes migration and survival of GBM by inducing H^+ extrusion, which maintains an alkaline intracellular pH and an acidic interstitial/extracellular pH [117]. Glioma cells secrete factors into the tumor microenvironment, such as IL-6, IL-10, STAT, TGF-β, bFGF, EGF, and MMP [12]. The production of
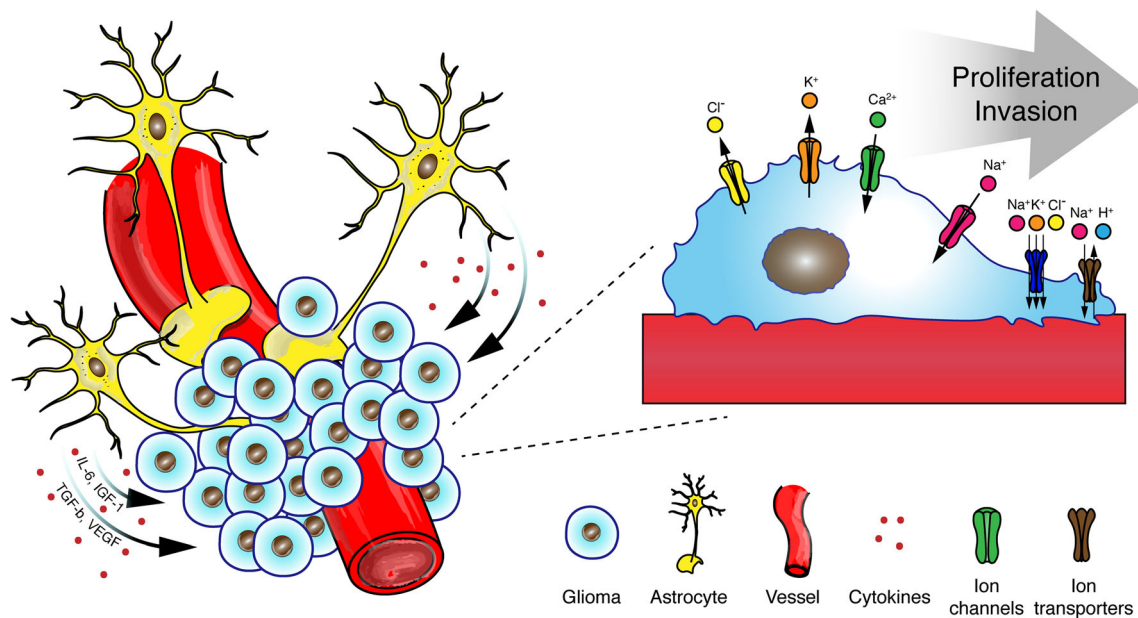

Fig. 1 Schematic illustration of glioma and tumor associated astrocytes. Tumor-associated astrocytes interact in a complex way with glioma cells, endothelium, and the tumor microenvironment to promote the proliferation, invasion, and treatment resistance of GBM. In addition to gap junctions, ion channels and ion transporters play a key role in the progression, metastasis, and tumorigenesis of GBM. They serve as a bridge of communication between tumor cells and the microenvironment. Blockage of ion channels and/or ion transporters have been shown to reduce the intercellular connections and decrease the proliferative and invasive nature of GBM in experimental studies. Collectively, further investigation of the intercellular interactive mechanisms and combined treatment with ion channel and ion transporter inhibitors may enhance the efficacy of GBM therapies

proinflammatory cytokines stimulate astrocyte reactivity and disrupt ion homeostasis [118]. Cytokines secreted by glioma cells also regulate expression of ion channels in glioma-associated cells [119]. Meanwhile, reactive astrocytes release abundant chemokines into the tumor microenvironment, which further promote the progression, aggression, and tumorigenesis of GBM. Ion channels in the plasma membrane of these astrocytes are involved in regulating the release of secretion. For instance, activation of Ca^{2+} channels induces endothelin-1 secretion by astrocytes [120]. Blockade of Ca^{2+} channels prompts reduced astrocytic release of IL-6 and interleukin 1β (IL-1β) which may stimulate glioma cells [121]. Therefore, ion transporters play equally important roles in intercellular communications in glioma and glioma progression.

Conclusion

For several decades, studies of glioblastoma biology have generally focused on the tumor's own genetic and molecular changes. However, tumor cells are not isolated, but grow in a particular environment by communicating with other cells, which influences progression, aggression, and survival of cancer [122]. Newly emerged evidence indicates that tumor-associated astrocytes can be activated by neighboring glioma cells and interact in a complex way with the tumor microenvironment to promote the proliferation, invasion, and treatment resistance of GBM. However, there are many unresolved questions and the mechanisms of interaction between tumors and astrocytes need to be explored further. In addition to gap junctions, ion channels and ion transporters play a key role in the progression, metastasis, and tumorigenesis of GBM (Fig. 1). They serve as a bridge of communication between tumor cells and the microenvironment. Blockage of ion channels and/or ion transporters have been shown to reduce the intercellular connections and decrease the proliferative and invasive nature of GBM (Fig. 1). In addition, combined treatment of TMZ with ion channel or ion transporter inhibitors enhanced apoptosis of tumor cells in vitro and/or in animal models. Collectively, further investigation of the intercellular interactive mechanisms and combined treatment of ion channel and ion transporter inhibitors may bring researchers one step closer to understanding GBM and creating a therapy that effectively combats its various defenses.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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