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Unique biology of gliomas: challenges and opportunities

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Abstract

Gliomas are terrifying primary brain tumors for which patient outlook remains bleak. Recent research provides novel insights into the unique biology of gliomas. For example, these tumors exhibit an unexpected pluripotency that enables them to grow their own vasculature. They have an unusual ability to navigate tortuous extracellular pathways as they invade, and they use neurotransmitters to inflict damage and create room for growth. Here, we review studies that illustrate the importance of considering interactions of gliomas with their native brain environment. Such studies suggest that gliomas constitute a neurodegenerative disease caused by the malignant growth of brain support cells. The chosen examples illustrate how targeted research into the biology of gliomas is yielding new and much needed therapeutic approaches to this challenging nervous system disease.

Keywords

glioma; clinical trial; glutamate; MGMT; bradykinin; ion channel

Introduction

Astrocytic gliomas are the most common primary brain cancer [1], with a yearly incidence of 3–5 per 100 000 people [2]. These gliomas occur in all age groups but are most prevalent in adults over 45 years of age [3]. Typical presentation includes headaches, seizures, focal neurological deficits, memory loss, and sometimes personality changes or confusion [2,4,5]. Cellular features of malignant gliomas include unrestricted proliferation, extensive invasion, neovascularization, necrosis, and resistance to apoptosis [1,6]. Gliomas have the unique ability to diffusely invade and infiltrate surrounding brain tissue, intertwining themselves among native cells and so making complete surgical resection typically impossible [7]. The only life-prolonging measures that are somewhat effective are surgical resection followed by radiation and chemotherapy with temozolomide (TMZ) [8,9]. Tumors typically recur after an average of only 6.9 months [8], resulting in a median patient survival of just 12–15 months following diagnosis [10].

Gliomas are often heterogeneous, both phenotypically and with regards to gene expression [11]. Numerous genetic mutations have been identified [11], yet it has been impossible to define a single disease-initiating event. The diversity of mutations has been an obstacle in developing animal models for laboratory investigations that fully replicate the human disease and has hampered the adoption of a universal clinical treatment plan. Recent research on the cell of origin for these tumors suggests that gliomas originate from stem or neural precursor cells as opposed to differentiated glial cells or glial progenitor cells, as

previously believed [12,13]. Indeed, stem and stem-like cells isolated from human brain tumors can give rise to glioma cells, as well as to neurons, astrocytes, and oligodendrocytes [14–16]. Stem-like cells isolated from patient biopsies can generate gliomas in animals that harbor cytogenic and molecular characteristics of the original tumor [16]. However, astrocytes are capable of undergoing symmetric division and can amplify significantly in the postnatal brain [17], raising the possibility that gene mutations in this astrocytic pool give rise to astrocytic gliomas. We refer readers interested in the origin of gliomas to excellent recent reviews on this topic [18–22].

Molecular analysis has identified several specific genetic alterations in gliomas. This in turn has led to the development of new drugs or the use of already US Food and Drug Administration (FDA)-approved compounds that target the affected proteins in these tumors. In this review, we highlight important findings regarding the unique biology of this cancer from a neuroscience perspective, illustrating how these insights have shaped new clinical trials that are providing hope for much needed and more efficacious treatment options. The featured examples suggest that gliomas share many biological traits with nonmalignant brain cells and that their biology can only be understood in the context of their host tissue. We suggest that gliomas represent a rapidly progressive neurodegenerative disorder that causes a loss of brain function by exploiting signaling mechanisms that are unique to the brain and not typically found in other cancers.

The glioma–vascular relationship and interdependence

Most solid cancers, including gliomas, induce new blood vessel growth to satisfy their energy demands, a process known as neovascularization. This typically involves sprouting of existing blood vessels and is stimulated by tumor-released angiogenic factors, such as vascular endothelial growth factor (VEGF) [23–25]. Disruption of angiogenic signals is widely pursued as a therapeutic strategy to slow tumor growth [26–28]. Malignant gliomas are also highly vascularized, often forming glomeruloid bodies, which are capillary buds lined with endothelial cells undergoing VEGF-mediated hyperplasia [23,29], initially suggesting that the same anti-angiogenic strategies pursued elsewhere would be promising in treating gliomas.

Glioma-derived endothelial cells

Recent studies, however, suggest that the neovascularization occurs quite differently in gliomas compared with other cancers and involves differentiation of tumor cells into blood vessel endothelial cells [30–32] (Figure 1a). This indicates that the growing tumor furnishes cells for the *de novo* growth of blood vessels. This conclusion is based on implantation studies whereby human gliomas were xenografted into the cerebrum of mice. New blood vessels found in the tumor were composed of tumor-derived cells harboring identical mutations in oncogenes and tumor suppressor genes found in the implanted gliomas, rather than the host endothelial cells (Figure 1b). This suggests a remarkable pluripotency, likening glioma cells to stem cells [31,32]. The initial differentiation of tumor stem cells into pluripotent progenitor cells is mediated by neurogenic locus notch homolog protein 1 (Notch1) signaling, whereas VEGF promotes further differentiation into blood vessel endothelial cells [31]. Ablation of glioma-derived progenitor cells results in significantly smaller tumors, highlighting an important role for tumor-derived progenitor cells supporting tumor growth *in vivo* [32]. Although these studies suggest the remarkable versatility of these tumors, these findings are exclusively based on xenograft studies in rodents. Hence, the relative contributions of neovascularization arising from the sprouting of pre-existing vasculature versus glioma cell differentiation into vascular endothelial cells in the human disease require further study.

Glioma chemotaxis

Gliomas are frequently found in close association with blood vessels and appear to have a dynamic relationship with the vasculature (Figure 1c). As alluded to above, gliomas can manipulate native brain cells to initiate microvessel proliferation or grow vessels by differentiation into endothelial cells. In addition, gliomas utilize the vasculature as a pathway for invasion and formation of satellite tumors within the brain [33]. Individual glioma cells can be visualized invading along the abluminal surface of blood vessels (Figure 1c), where they remodel blood vessels and induce angiogenesis at the site of glioma–vasculature contact [33–35]. Perivascular glioma cells may gain biological advantages, such as access to blood-born nutrients and a more favorable space and extracellular matrix environment through which to invade [36].

The peptide bradykinin has recently been shown to have an important role in the interaction of glioma cells with the vasculature [37]. Bradykinin is an endothelial cell-derived member of the kinin signaling molecule family binding to bradykinin receptors, B1 receptor or B2 receptor. Gliomas primarily express the B2 receptor and expression levels increase with tumor grade [38]. Activation of B2 receptors induces Ca^{2+} signals in glioma cells [39] and the expression of matrix metalloproteinases, which are matrix-degrading enzymes used by gliomas to facilitate tissue remodeling and migration in the brain [40,41]. Bradykinin promotes chemotactic invasion of gliomas toward blood vessels *in situ*, which is lost when the B2 receptor is eliminated through short hairpin RNA (shRNA) or pharmacologic inhibition with HOE 140, a bradykinin receptor antagonist [37] (Figure 1d). Preclinical studies in mice are currently examining the antitumor effects of HOE 140. If successful, a B2 receptor inhibitor, icatibant, which is already being clinically tested to treat hereditary angioedema, may serve as a lead candidate for the treatment of gliomas in the future [42].

Epigenetics: regulation of gene expression that alters the response to treatment

Epigenetics refers to the regulation of gene expression that is independent of changes in the DNA sequence of cells. Genes can be epigenetically silenced by the selective addition of methyl groups to promoter regions within the DNA. Commonly, methylation occurs in CpG-enriched areas known as CpG islands, which decreases the amount of gene transcription. Methyl groups interfere with the binding not only of transcriptional factors, but also of methyl-CpG-binding domain proteins that recruit other proteins to decrease transcription. Cancer cells frequently have a hypomethylated genome with gene-specific hypermethylation [43]. Epigenetic modifications have been identified in several solid tumors, including breast, colon, lung, bladder, kidney, prostate, esophageal, and gliomas [43]. Hypermethylated genes in gliomas include epithelial membrane protein 3 (*EMP3*), thrombospondin 1 (*THBD1*), excitatory amino-acid transporter 2 (*EAAT2*), and O6 methylguanine methyl transferase (*MGMT*) [44,45,48]. The latter is one of the best understood glioma genes silenced by methylation. The *MGMT* gene encodes an important DNA repair protein, O6-alkylguanine DNA alkyltransferase (AGT), which is capable of removing alkyl groups found at the O⁶ position of guanine. Failure to remove this alkyl group induces apoptosis [46]. Increased activity of the *MGMT* gene inhibits the effects of TMZ, the standard alkylating chemotherapeutic agent used to treat gliomas [44] and explains why some patients fail to benefit from this treatment. In individuals resistant to TMZ treatment, the *MGMT* promoter [44,47] is unmethylated, and transcription of the gene provides sufficient protein to counteract the effects of TMZ. Conversely, patients with a methylated *MGMT* promoter do not generate the protein and, therefore, are more sensitive to TMZ therapy, particularly if combined with radiation [44].

The recognition that this specific epigenetic modification dramatically alters the potency of a commonly used chemotherapeutic was an important scientific discovery, as it allows for patient-specific treatment. Unfortunately, the lack of an effective alternative treatment leaves patients who are TMZ resistant with few options. It is possible that DNA methylation has a much broader role in the biology of gliomas, affecting other proteins involved in differentiation or normal cell–cell signaling. At least one gene, encoding the astrocyte-specific EAAT2 glutamate transporter, appears to be silenced [48] as discussed further below.

Glioma and glutamate: toxin and multifunctional growth factor

Unlike other cancers that utilize the vasculature and lymphatics to spread to other tissues and organs, gliomas migrate and invade only within brain tissue. Furthermore, as the tumor expands, the cranium provides spatial constraints to the growing tumor, which in turn begins to destroy native brain cells to create room for expansion. Thus, glioma cell invasion and growth require unique mechanisms to deal with constraints not found in any other cancer; these mechanisms have now been linked to aberrant glutamate signaling.

In the normal brain, glutamate is the main excitatory neurotransmitter. It binds and activates postsynaptic glutamate receptors (GluRs), namely NMDA receptors (NMDARs) and AMPA receptors (AMPA). Glutamate is quickly removed from the synaptic cleft by surrounding astrocytes through Na^+ -dependent excitatory amino-acid transporters, particularly by EAAT1 and EAAT2, the principle glutamate transporters of astrocytes [49]. This astrocyte-mediated clearance is important in maintaining low extracellular glutamate levels necessary to assure normal glutamatergic transmission [50]. In addition, it prevents glutamate from accumulating in perisynaptic regions, where sustained elevations in extracellular glutamate, as a consequence of impaired glial reuptake, can cause Ca^{2+} -dependent excitotoxicity [51,52].

Unlike their non-malignant counterparts, gliomas release glutamate. Gliomas also lack functional EAAT transporters, due to epigenetic silencing of the gene (*EAAT2*) [48], active repression of protein expression (EAAT2) [53], or mislocalization of the protein (EAAT1) [54,55]. Moreover, gliomas highly express a cystine-glutamate antiporter, system x_c^- (SXC), a solute transporter, importing cystine in exchange for glutamate [56]. Cystine is used as a precursor for the cellular synthesis of the antioxidant glutathione (GSH), which creates a reducing environment and protects the cell from endogenously and exogenously generated free radicals. SXC expression and function is upregulated in gliomas under stress conditions [56–59], endowing the tumor with enhanced protection, for example, against radiation-induced damage. However, as SXC expression increases, so does the release of glutamate, and consequently glioma cells create a microenvironment characterized by excess extracellular glutamate [60] (Figure 2a).

In patients with glioma where glutamate was sampled by microdialysis, peritumoral glutamate concentrations reached 100–400 μM , over 100-fold higher than that found in normal brain and far in excess of the threshold for neurotoxicity [61]. Rats implanted with C6 glioma cells that assiduously release glutamate grow large, aggressive tumors, whereas animals implanted with glioma cells not secreting glutamate failed to establish a tumor mass [62]. Inhibition of glutamate release, either by genetic knockdown of SXC [60] or pharmacological inhibition with the SXC inhibitor sulfasalazine (SAS) [63], impaired tumor growth and/or promoted animal survival. These experiments support a pivotal role for SXC-mediated glutamate release in providing a growth advantage for gliomas. This probably occurs by glutamate killing peritumoral neurons, thereby vacating space for the tumor to expand.

Glutamate and seizures

Glutamate release by gliomas may also contribute to peritumoral seizures, a very common co-morbidity and often persisting symptom in patients with glioma [64,65]. Increases in glutamatergic transmission are widely implicated in seizure disorders and, hence, the excess glutamate generated by gliomas may directly contribute to tumor-associated epilepsy [66] (Figure 2b). This notion is supported by a recent study where implantation of human gliomas into severe combined immunodeficiency (*SCID*) mice generated spontaneous recurring epileptiform neuronal activity that increased in frequency as the disease progressed [67]. Tissue from these animals showed an increase in extracellular glutamate, measured by high-performance liquid chromatography (HPLC), as well as an increase in epileptic events in animals determined by whole-cell patch clamp slice electrophysiology experiments [67]. These tumor-mediated phenomena were effectively suppressed when SXC was pharmacologically inhibited by SAS [67]. It remains to be seen whether a sustained inhibition of glutamate release from gliomas is achievable by pharmacological inhibition of SXC, as cancer cells frequently escape such intervention through upregulation of compensatory proteins.

Glutamate and glioma invasiveness

In addition to its seizure-promoting and neurotoxic effects, glutamate appears to also have pro-invasive properties. GluR stimulation results in activation of focal adhesion kinase (FAK), which is crucial to the regulation of growth factor and integrin-mediated cell motility and invasion [68]. Invasion of glioma cells requires glutamate activation of Ca^{2+} -permeable AMPARs, whereby Ca^{2+} influx is essential to allow glioma cells to invade the brain parenchyma [41,54,68]. Glutamate released by the tumor via SXC may be the ligand for these receptors, essentially establishing an autocrine- or paracrine-signaling loop that generates Ca^{2+} signals and so promoting invasion [54].

Taken together, these studies suggest that gliomas utilize the neurotransmitter glutamate in several ways to gain a growth advantage. The observed glutamate excitotoxicity is well documented for other neurological diseases, including many neurodegenerative processes [69–71]. The fact that gliomas use a neurotransmitter to promote the progressive destruction of the brain challenges researchers to consider gliomas as a neurodegenerative disease as opposed to merely a cancer growing in the brain (Box 1). Given that glutamate is released by a pharmacologically tractable transporter (i.e., SXC) and acts on several GluRs for which selective drugs are available, clinical trials have either started (or are under development) to exploit a disruption of the glutamatergic signaling axes (see below).

Ion channels: regulators of growth and invasion

Research on ion channels is largely focused on understanding neuronal excitability. However, interesting roles for channels in basic biological functions, such as growth control, cell differentiation, and cell migration, have emerged in studies of normal [72–74] and malignant cells [75,76].

The mechanism(s) by which channels affect the biology of a given cell has been largely elusive, yet some of the best mechanistic insights have been gained from studies of human glioma cells. These have been shown to use ion channels to flux ions, notably K^+ and Cl^- , to generate an osmotic gradient for cytoplasmic water flux, thereby altering cellular volume [36,77,78]. Profound volume changes precede each cell division as glioma cells retract their processes, condense and round up to a sphere of approximately 5500 fL volume [79] (Figure 3a,b). Cell rounding requires the release of cytoplasmic water, accomplished by the coordinated secretion of potassium chloride (KCl) with osmotically obligated water (Figure 3c). Outward directed gradients for K^+ and Cl^- are established by the activity of the Na^+/K^+ -

ATPase and the sodium-potassium-chloride co-transporter isoform-1 (NKCC1), respectively. Intracellular Cl^- reaches concentrations of 100–140 mM, approximately tenfold higher than that of neurons and other cells [77]. The condensation engages chloride channel-3 (ClC-3)-type channels, which are activated through Ca^{2+} -dependent phosphorylation by Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) [80,81]. K^+ efflux occurs via Ca^{2+} -activated K^+ channels [82,83], and water leaves through aquaporins [84]. This highly orchestrated interplay of ion transporters and ion channels allows a cell to condense its cell volume rapidly within approximately 40 minutes, facilitating the pre-mitotic rounding of the cell [79].

Similar volume changes occur as gliomas invade the brain through narrow extracellular passageways along white matter tracts or blood vessels. To navigate the tortuous extracellular brain spaces, glioma cells dynamically adjust their cellular volume. On encountering barriers too narrow to pass, cells quickly reduce their cellular volume by approximately 33%, regardless of the size of the barrier [36]. Cell shrinkage is achieved by the efflux of Cl^- ions along with essentially all unbound cytoplasmic water and maximizes the chance of the cell to advance past a barrier [36]. This volume reduction utilizes the same Cl^- and K^+ channels described above [36], and these channels are activated downstream of intracellular Ca^{2+} increases originating from the activation of AMPARs, bradykinin receptors [37,54], or transient receptor potential channels (TRPC) [85] (Figure 3c).

The above-described cell volume changes associated with cell migration suggest several new possible pharmacological targets that interfere with glioma invasion. For example, the diuretic bumetanide (Bumax) inhibits intracellular Cl^- accumulation in glioma cells [77] and, in a mouse model, reduced the ability of gliomas to invade and generate satellite tumors [86]. In preclinical studies, the pharmacological inhibition of Cl^- channels or genetic downregulation of ClC-3 reduced the ability of gliomas to invade [87–89]. Furthermore, the Cl^- channel inhibitor, chlorotoxin, has been used in a phase I/II clinical study in patients with glioma [90]. Additionally, the regulators of channels and transporters, such as CaMKII, which regulates ClC-3 [88], or the with-no-lysine kinase-3 (WNK3), which regulates NKCC1 [91], may be promising additional targets. It must be emphasized that the hydrodynamic model of glioma invasion described here assumes that ion channels participate in a facilitatory role. Given that cell invasion constitutes a complex and highly coordinated biology, the disruption of only one pathway may not be sufficient to derail the process.

Translating the biology to therapy

The typical treatment of a patient with glioma involves a team of physicians from different disciplines, including neurology, neurosurgery, neuropathology, neuroradiology, and oncology [2]. Surgical debulking removes as much tumor as possible. Subsequent radiation therapy aims to further reduce tumor burden adjacent to the resection cavity. Lastly, TMZ is administered followed by a period of maintenance TMZ therapy [2] to contain further growth. Unfortunately, recurrence is inevitable and numerous clinical trials are investigating additional therapies to alter the course of this disease (Table 1). Promising targets include epidermal growth factor (EGF) receptor [92] and platelet-derived growth factor (PDGF) receptor [93]. The examples below illustrate trials directly resulting from research described above exploiting a better understanding of the unique glioma biology:

Anti-angiogenesis trials

Given that angiogenesis is a fundamental trait shared by most solid cancers, considerable hope is placed on drugs that target tumor angiogenesis, such as bevacizumab, a humanized monoclonal antibody directed against VEGF-A. Approved in 2008 for the treatment of

metastatic breast cancer, the drug was used in two independent studies as monotherapy for recurrent gliomas. These studies reported favorable 6-month progression-free survival rates of 42.6% [94] and 29% [95], significantly greater than the 18% utilizing TMZ [96]. These studies also showed a reduction in peritumoral edema, allowing a lowering in the corticosteroid dose used in the management of these patients [95]. These successes promoted further trials looking at the benefits of adding bevacizumab to radiation and TMZ [97–99]. At present, there are 114 registered clinical trials using bevacizumab in patients with glioma, typically in combination with other treatment modalities (<http://www.clinicaltrials.gov>; accessed April 22, 2012).

Unfortunately, the benefit of anti-angiogenic therapies so far remains limited [100]. This may in part be due to the above-described way in which the growing tumor also furnishes its own vasculature. Moreover, anti-angiogenic therapy has been shown to result in a more invasive and aggressive tumor cell phenotype, including increased expression of other proangiogenic factors [101], upregulation of pro-invasion proteins such as matrix metalloproteinases [101], activation of pro-invasive signaling pathways, such as the phosphatidylinositol 3-kinase (PI3K)- and Wnt-signaling pathways [102], and an increase in tumor hypoxia by upregulation of hypoxia-inducible factor 1 α (HIF1 α) [102].

Trials capitalizing on epigenetic modifications

The identification of two distinct populations of patients with glioblastoma unveiled the methylation status of the *MGMT* gene as a predictor for the therapeutic efficacy of radiation and TMZ chemotherapy. Patients with *MGMT* promoter methylation who received TMZ and radiation therapy showed a median survival time of 21.7 months compared with 12.7 months for those who had unmethylated *MGMT* [44]. Two-year survival was 46% with *MGMT* methylation, compared with 13.8% without *MGMT* methylation [44]. These data show the importance of patient stratification based on a known biology to enhance treatment efficacy. Currently, efforts are being undertaken to assess whether patients who lack *MGMT* methylation should receive alternative dosing schedules of TMZ to outcompete their body's innate DNA repair mechanisms, increasing the effectiveness of the drug in a TMZ-resistant patient population [103].

Trials targeting glutamate signaling

Preclinical studies disrupting glutamate release from the tumor and/or overactivation of GluRs on peritumoral neurons are promising. SAS is an effective SXC inhibitor [67] that is in clinical use for Crohn's disease and other chronic inflammatory conditions. A small and underpowered clinical study reported a lack of effectiveness of SAS in nine patients with glioblastoma [104]. A larger clinical trial is now investigating the effectiveness of SAS on peritumoral glutamate in patients with recurrent disease (NCT01577966). This study also assesses the efficacy of SAS treatment in reducing seizure burden after drug administration.

Attempts to exploit therapies that protect neurons against glutamate-mediated excitotoxicity are also ongoing. The AMPAR antagonist, talampanel, penetrates the central nervous system (CNS) effectively [105]. A phase II study of 30 adults with recurrent glioblastoma or anaplastic glioma reported only limited alterations in the disease when used as a monotherapy [106]. However, a second phase II study, in which patients newly diagnosed with glioblastoma received talampanel in conjunction with radiation and TMZ, reported that this combination showed a promising survival advantage [107]. Such a finding was confirmed in a second single cohort study [108]. Taken together, the possible therapeutic advantage of talampanel appears to exist when using this agent on patients who are newly diagnosed in combination with the standard therapy.

Trials targeting other ion channels

The identification of a role of ion channels, specifically Cl^- channels, in glioma migration and invasion promoted the investigation and identification of drugs and small molecule inhibitors that target the flux of Cl^- . One example is chlorotoxin, a scorpion peptide that acts on Cl^- channels to inhibit glioma invasion *in vitro* [109,110] and inhibit glioma growth *in vivo* [111]. Clinical studies of patients with recurrent glioma showed tumor-specific binding of chlorotoxin when conjugated to ^{131}I [90], and phase II data showed a survival benefit in 59 patients (NCT00040573).

Will investigator-initiated trials provide answers?

Glioma, although a devastating disease, does not affect a large population of individuals, making it a financially unattractive disease for pharmaceutical companies to pursue. Surprisingly, however, there are significantly more active clinical trials for gliomas than for neurological diseases that affect much larger patient populations, such as multiple sclerosis (MS), Parkinson's disease (PD) or Alzheimer's disease (AD) [a search of <http://www.clinicaltrials.gov> (on April 20, 2012) showed 369 active glioma trials that are recruiting patients compared with 240 for MS, 273 for PD, and 267 for AD]. This is probably because the paucity of effective intervention for gliomas greatly reduces the barriers imposed by the FDA for clinical investigation of new drugs or treatment approaches. This permits rapid implementation of investigator-initiated trials, which often occur at single institutions. In support of this conclusion, 317/369 (86%) of currently enrolling glioma trials are early exploratory phase I or phase II studies, few of which will ever progress to phase III. Hopefully, despite a lack of interest from big pharmaceutical companies to pursue this disease, these investigator-driven approaches will eventually yield a much-needed breakthrough.

Concluding remarks and future directions

Despite the vast growth in understanding, important questions remain regarding glioma biology and clinical management (Box 2). Gliomas remain one of the most challenging cancers to treat, as demonstrated by the lack of improvements in patient survival. This may be partially attributed to the fact that current treatment approaches largely mirror those used for other solid-tumors, often ignoring the unique neurobiology of gliomas described above. Gliomas exhibit many of the hallmarks of other neurological disorders, including altered vasculature, seizures, and excitotoxicity. Targeting the unique nervous system biology and the interaction of gliomas with normal brain tissue may yield more promising treatments in the future.

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Box 1. Malignant glioma: a neurodegenerative disorder?

Neurodegenerative diseases are defined as hereditary or sporadic conditions characterized by progressive nervous system dysfunction. Although most typically used to describe disorders such as Alzheimer's disease and Parkinson's disease, we suggest that gliomas should also be considered in this group. They share a progressive neuronal loss along with many of the hallmarks of other neurodegenerative disorders listed below. Ultimately, it is this loss of nervous system function, as opposed to the cancer growth per se, that kills the patient.

Features of gliomas shared by other neurodegenerative diseases

1. Progressive neuronal loss [53,113–115]
2. Abnormal vasculature [23,29,116–119]
3. Seizures [2,120,121]
4. Edema [2,117,122,123]
5. Glutamate-mediated neuronal excitotoxicity [69–71,124–126]
6. Pathogenesis mediated by genomic changes [11,127,128]
7. Increased oxidative stress [129,130]
8. Reactive gliosis [131–133]

Box 2. Outstanding questions

- **How important are epigenetic changes?**

Past glioma research has largely focused on genetic alterations, searching for the disease-causing mutation. As exemplified by the epigenetic silencing of the MGMT gene, disease-altering changes may occur independently of, or in addition to, genetic mutation. Silencing of genes that would otherwise promote cell differentiation and drive cell proliferation, and invasion may be affected. Many proteins normally expressed in non-malignant astrocytes are not functional in gliomas, such as the weakly inwardly rectifying K⁺ channel subunit Kir4.1 [134] and the EAAT2 glutamate transporter [135]. For the latter, epigenetic silencing has been reported [48]. Mutant isocitrate dehydrogenase 1 (IDH1) interferes with cell differentiation and has been linked to a glioma hypermethylator phenotype that characterizes a subset of gliomas exhibiting extensive epigenomic aberrations [136]. Hence, researchers are just beginning to appreciate fully the importance of epigenetic gene regulation in gliomas.

- **Are glioma-derived vascular cells the exception or the norm?**

The finding that gliomas grow their own vasculature by differentiating cells into endothelial cells is potentially a ‘game changer’ regarding anti-angiogenic therapy. The degree to which newly formed, tumor-derived vessels contribute functionally to the tumor vasculature remains to be determined. Moreover, whether this process can be disrupted by anti-angiogenic therapies needs to be investigated. The limited success of bevacizumab therapy [100] may suggest that a better understanding of the multimodal vascularization will lead to a refined anti-angiogenic strategy in the future.

- **Is glioma cell invasion a regulated or a stochastic process?**

Important aspects of glioma invasion have been discovered, including identification of signaling pathways [137], interactions of invading cells with the extracellular matrix and neighboring cells [137], cell volume changes [36], and AMPAR signaling [41]. Whether invasion is purposeful, whereby cells follow delineated signals, or whether this is a random, stochastic process, remains unknown. The chemotactic role of bradykinin [37] suggests that at least the attraction of gliomas to blood vessels appears to be a guided form of migration, but what encourages cells to move along blood vessels and choose a direction at branch points? What, if any, are the stop signals? How do stationary cells decide to develop satellite tumor masses? It has been proposed that satellite tumors preferentially form at vessel branch points [33]. Being the major impediment to surgical treatment, glioma cell invasion requires a more refined understanding. Such knowledge will hopefully advance the development of future anti-invasive adjuvant treatments (e.g., [138]).

- **Do glioma cells co-opt the stromal tumor environment?**

Gliomas must destroy normal brain to vacate room for their growth. Neuronal death occurs via glutamate excitotoxicity, yet what happens to the surrounding ‘stromal’ cells (i.e., astrocytes, oligodendrocytes, microglia, and endothelial cells) is unknown. Astrocytes are not susceptible to glutamate toxicity and can withstand millimolar concentrations of glutamate [139]. In fact, unless they have lost functional glutamate transport, they should be able to contain any spillage of glutamate from the tumor into the brain. Gliomas appear to also co-opt microglial function, because tumor-infiltrating microglial cells promote tumor

growth by suppressing the innate immune response [140]. Therefore, changes to stromal cells, inflicted by the tumor, may significantly contribute to the growth and invasion of gliomas.

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Figure 1.

Glioma cells establish a clear relationship and interdependence with the cerebral vasculature. **(a)** Gliomas comprise a heterogeneous population of tumor cells, including glioma stem cells, which are capable of propagating the tumor itself and differentiating into glioma endothelial progenitor cells [30–32]. These endothelial progenitor cells differentiate into glioma-derived endothelial cells (purple cells) and facilitate tumor-mediated neovascularization. **(b)** Confocal microscopy images of vasculature demonstrating the differentiation of human glioma cells into vascular endothelial cells when implanted into a mouse [31]. Vasculature identified with cluster of differentiation 31 (CD31) and lectin

(green). Human origin confirmed via human-specific endothelial markers, neural cell adhesion molecule (NCAM) and CD105 (red). DAPI staining (blue) was used to indicate cell nuclei. Scale bar = 10 μm . **(c)** Glioma cells preferentially migrate and invade along the abluminal surface of the vasculature [36]. *In vivo* demonstration of the intimate association of human glioma cells (green) with the vasculature (red) in a xenograft model as viewed through a cranial window in a live mouse. 600X magnification. Scale bar = 50 μm . **(d)** Schematic of bradykinin receptor (B2R) signaling that mediates glioma attraction to the vasculature. Glioma cells express the B2R, which binds vascular endothelial cell-released bradykinin (BK) [37]. The initiation of this signaling cascade facilitates glioma association with blood vessels. Reproduced, with permission, from [31] (b) and [36] (c).

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Figure 2.

Glioma cells extrude high concentrations of glutamate (Glu) via the system X_c^- transporter (SXC), supporting tumor growth and expansion [54,60,62]. (a) Glu is released from glioma cells in exchange for cystine, which is converted into the antioxidant glutathione, protecting glioma cells from free radical damage. Simultaneously, overwhelming amounts of extracellular Glu cannot be sufficiently removed by astrocytes through normal uptake mechanisms involving the excitatory amino acid transporters excitatory amino-acid transporter 1 (EAAT1) and EAAT2. This leads to prolonged neuronal exposure to Glu, resulting in neuronal hyperexcitability and death [51,52,56,60]. (b) Glu release from gliomas

by the system X_c^- transporter (SXC) incites epileptic activity exhibited by an electroencephalography (EEG) recording from a tumor-implanted mouse [67]. Reproduced, with permission, from [67] (EEG recording) and [86] (MRI of xenograft tumor).

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Figure 3.

The movement of ions and water facilitates the cellular volume changes needed for glioma cell proliferation, invasion, and migration [36,77–79]. **(a)** Glioma cells undergo significant shape and volume changes during the cell cycle, including a dramatic decrease in volume just before M phase. Cell volumes were determined after three-dimensional rendering of up to 400 individual optical sections, (400 μ m z-spacing), as shown in 1–5 taken by confocal imaging [79]. **(b)** Volume of glioma cells at M phase, the last time point measured before M phase (Pre M Phase), and the maximum volume (Peak) measured for each glioma cell [79]. **(c)** Schematic of the ion channels and co-transporters used to establish ionic gradients to

promote water flux across the cell membrane allowing for volume changes needed. Abbreviations: ATPase, sodium-potassium ATPase; ClC-3, voltage-gated chloride channel 3; K_{Ca}^{2+} , calcium-activated potassium channels; NKCC1, sodium-potassium-chloride co-transporter isoform-1. Reproduced, with permission, from [79] (a,b).

Table 1Examples of currently active clinical trials for primary and recurrent malignant gliomas^a

Type of treatment	Target ^b	Investigational agents	Clinical trial identifier ^c
Anti-angiogenic inhibitors	VEGF	Bevacizumab	NCT00884741, NCT00943826, NCT01149850, NCT00612339
	VEGF/PIGF	Aflibercept	NCT00650923
	VEGF receptor	Vatalanib, CT-322, cediranib	NCT01310855, NCT00458731, NCT00768911
	VEGF receptor, EGF receptor	Vandetanib	NCT00293566, NCT00441142, NCT00821080
	VEGF receptor, Raf, PDGF receptor	Sorafenib	NCT00734526, NCT00884416, NCT00093613
	VEGF receptor, PDGF receptor	Sunitinib	NCT00606008, NCT00923117, NCT00499473
	Others	Thalidomide, lenalidomide	NCT00671801, NCT01183663
Growth factor receptor inhibitors	EGF receptor	Gefitinib, erlotinib, cetuximab, AEE788	NCT00086879, NCT01238237, NCT00301418
	EGF receptor, Her2	Lapatinib, afatinib	NCT00977431
	PDGF receptor	Imatinib	NCT00039364, NCT00354068, NCT00613132
	PDGF receptor, Src, ephrin receptors	Dasatinib	NCT00895960
	PDGF receptor, FLT-3, c-kit	Tandutinib	NCT00379080
Small molecule inhibitors	Ras (FTI)	Tipifarnib, lonafarnib	NCT00049387, NCT00335764, NCT00102648
	mTOR	Sirolimus, temsirolimus, everolimus	NCT00821080, NCT00329719, NCT01062399
	PKC	Enzastaurin	NCT00509821, NCT00295815, NCT00586508
	PKC/PI3K/AKT	Enzastaurin	NCT00586508, NCT00295815, NCT00438997
	PI3K	XL147	NCT01240460
	PI3K, mTOR	XL765	NCT01240460
	Proteasome	Bortezomib	NCT01435395, NCT00998010, NCT00611325
	AKT	Perifosine	NCT01051557
	Microtubule disruption	mpc-6827, epothilones	NCT00397072, NCT00424060, NCT00635557
	PARP	Iniparib, veliparib	NCT00687765, NCT00770471, NCT01514201
	MGMT	O ⁶ -benzylguanine	NCT01269424, NCT00017147, NCT00362921
	HDAC I/II	Vorisinostat	NCT00731731, NCT00268385, NCT01378481
	α _v integrins	Cilengitide	NCT00813943, NCT00689221, NCT00085254
	c-MET	Rilotumumab, cabozantinib	NCT01113398, NCT00704288, NCT00960492
	CD95	APG101	NCT01071837
		¹³¹ I-TM601 (scorpion venom peptide), isotretinoin	NCT00114309, NCT00591058, NCT00555399, NCT00112502, NCT01103375
Topoisomerase inhibitors		Irinotecan, etoposide	NCT00613223, NCT00762255, NCT00921167, NCT00735436, NCT00979017
Alkylating agents		Temozolomide, carmustine, carboplatin, fotemustine, lomustine	NCT00995007, NCT00295815, NCT00438997, NCT01120639, NCT01310868, NCT01474239
Immunotherapies		PRX321, NBI-3001, aldesleukin, rindopepimut, basiliximab, vitespen, IMA950, survivin vaccine	NCT00797940, NCT00003842, NCT00002572, NCT01498328, NCT01480479, NCT00458601, NCT00626015, NCT00905060, NCT01250470,

Type of treatment	Target ^b	Investigational agents	Clinical trial identifier ^c
			NCT01403285, NCT01222221
Gene therapies		AdV-tk, recombinant adenovirus-hIFN-beta	NCT00589875, NCT00751270, NCT00036725,
Miscellaneous		Talampanel, sulfasalazine, hydroxyurea	NCT00064363, NCT00613132, NCT01577966

^a The standard of care for primary glioma has been surgical removal followed by a period of radiation and chemotherapy with TMZ. Although not an exhaustive list, this table highlights the possible therapies that could benefit patients with glioma. Examples of current clinical trials are noted for each category of drugs under investigation. Some data modified after [4,112].

^b Abbreviations: CD95, cluster of differentiation 95; FLT-3, fms-like tyrosine kinase receptor-3; FTI, farnesyl transferase inhibitor; HDAC, histone deacetylase inhibitor; Her2, human epidermal growth factor receptor 2; mTOR, mammalian target of rapamycin; PARP, poly-(ADP ribose)-polymerase; PIGF, placental-derived growth factor; PKC, protein kinase C.

^c <http://www.clinicaltrials.gov>.