Published in final edited form as:

Cell Mol Life Sci. 2014 May ; 71(10): 1839–1854. doi:10.1007/s00018-013-1521-z.

Glutamate Transporters in the Biology of Malignant Gliomas

Stephanie M. Robert and

Department of Neurobiology, The University of Alabama at Birmingham, Civitan International Research Center, CIRC 425, 1719 6th Ave S, Birmingham, AL 35294 USA, Telephone: 205-934-4455, Fax: 205-975-6320

Harald Sontheimer

Professor of Neurobiology, Director of the Center for Glial Biology in Medicine, The University of Alabama at Birmingham, Civitan International Research Center, CIRC 410, 1719 6th Ave S, Birmingham, AL 35294 USA, Telephone: 205-975-5805, Fax: 205-975-6320

Stephanie M. Robert: srobe25@uab.edu; Harald Sontheimer: Sontheimer@uab.edu

Abstract

Malignant gliomas are relentless tumors that offer a dismal clinical prognosis. They develop many biological advantages that allow them to grow and survive in the unique environment of the brain. The glutamate transporters System x_c^- and Excitatory Amino Acid Transporters (EAAT) are emerging as key players in the biology and malignancy of these tumors. Gliomas manipulate glutamate transporter expression and function to alter glutamate homeostasis in the brain, which supports their own growth, invasion, and survival. As a consequence, malignant cells are able to quickly destroy and invade surrounding normal brain. Recent findings are painting a larger picture of these transporters in glioma biology, and as such are providing opportunities for clinical intervention for patients. This review will detail the current understanding of glutamate transporters in the biology of malignant gliomas and highlight some of the unique aspects of these tumors that make them so devastating and difficult to treat.

Keywords

Glioma; Glutamate; Glutamate Transporter; System x_c^- (SXC); Excitatory Amino Acid Transporter (EAAT); Glutamate Receptors

Introduction

As intrinsic or primary tumors of the brain, malignant gliomas are believed to derive from non-neuronal glial cells or their progenitors. Astrocytic gliomas account for 80% of all malignant brain tumors [1], with a yearly incidence of 6 per 100,000 people [2]. The most deadly of all brain tumors, glioblastoma multiforme (GBM), makes up more than half of the malignant gliomas diagnosed each year [1], and despite decades of concerted research effort and advancements in treatment, the 5 year survival remains less than 5% [3].

Over the last few decades our understanding of the biology of primary brain tumors has increased significantly. Research is focused not only on learning how these tumors grow and spread, but also how they interact with the surrounding brain to produce symptoms of this disease, which range from headaches and vision changes to memory loss, personality changes, and tumor-associated epilepsy [3–5]. Recent research has uncovered an important role of the excitatory amino acid glutamate in the growth and malignancy of gliomas [6–10], and as the cause of tumor-associated seizures [11–13]. In this Review, we discuss the current understanding of the role of glutamate transporters in glioma biology.

Gliomas: Brief review of classification and grading

Gliomas are thought to originate from stem cells, progenitor cells, and/or de-differentiated glial cells that have regained immature and progenitor properties [14]; however, the true origin of the tumor cells is still controversial and heavily investigated. Clinically, gliomas are classified based on the normal stromal (glial) cells of the brain they most closely resemble [15] and are graded based on tumor histopathology. The major glioma classifications include Ependymoma, Oligodendroglioma, Astrocytoma, and Glioblastoma [16]. Tumor grading is defined by the World Health Organization (WHO) on a scale of I–IV based on presence and degree of atypical morphology of cells, nuclear atypia, mitotic activity, necrotic tissue, and newly formed blood vessels [17]. Tumor grade determines treatment strategy and prognosis. Malignancy increases and prognosis decreases with higher grading; grade I astrocytoma has a 90% 5-year survival rate compared to a grade IV astrocytoma, which has <5% 5-year survival rate.

Astrocytomas and GBMs make up over 75% of malignant gliomas [1], and therefore this review will focus mainly on these two tumor classifications. The diagnosis of GBM is given to grade IV astrocytomas, which are the deadliest and most common glioma. Tumors may form as a glioblastoma (primary GBM) or may develop from previously diagnosed lower grade tumors (secondary GBM). The current standard of care for malignant glioma is surgical resection combined with radiation and Temozolomide chemotherapy [18]. Surgery is beneficial in debulking the tumor mass; however, complete elimination of the tumor is usually impossible. The majority of tumors typically reoccur, and when they do, they are more treatment resistant than the original tumor [5]. As a result, median patient survival after initial diagnosis is 12–15 months for GBM and 2–5 years for other malignant astrocytomas [19].

Glutamate Release and Uptake in the Non-Pathological Brain

Glutamate

The amino acid L-glutamate (L-glu or glutamate) has several important roles in the brain, both as a metabolite and as a neurotransmitter. Glutamate is essential for proper cellular metabolism, and is important for developmental synaptogenesis, cellular migration, differentiation and death. It is also involved in normal brain functions such as memory and learning. As a neurotransmitter, glutamate is the major amino acid mediating excitatory synaptic transmission in the central nervous system (CNS). In glutamatergic synapses, glutamate is vesicularly released by pre-synaptic neurons into the synaptic cleft and binds

glutamate receptors located on post-synaptic neurons (for review see [20,21]). These post-synaptic receptors are classified into three different families of glutamate receptors that mediate specific biological responses.

Glutamate Receptors

Glutamate receptors are located in the plasma membrane of most neurons and glial cells [22,23]. In order for glutamate to exert its signaling role in the brain, it must bind to and activate specific post-synaptic receptors. Primarily found in the CNS, glutamate receptors have been well studied in neuronal physiology and recent evidence suggests that they play an important role in glial cell development as well as function [24–30].

Glutamate receptors are divided into three families: *N*-methyl-D-aspartate (NMDA) receptors, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors, and metabotropic receptors (mGluR) [31]. NMDA and AMPA/kainate receptors are ligand-gated ion channels classified as ionotropic receptors. Glutamate binding to NMDA and AMPA/kainate receptors triggers the opening of cation-specific ion channels that permit influx of mono- or divalent cations [32]. These glutamate-gated ion channels are tetrameric assemblies of different subunits that are Na^+ and/or Ca^{2+} permeable [33]. The third family of glutamate receptors (mGluR) is classified as metabotropic receptors that respond to glutamate binding by activating intracellular signal transduction via G proteins. Ionotropic receptors respond more quickly to glutamate compared to metabotropic receptors [34,35].

NMDA receptor (NMDAR) activity is responsible for synaptic transmission involved with the synaptic plasticity that underlies learning and memory [32,36,37]. The NMDA receptor family consists of five receptor subunits (GluN1, GluN2A-D) [38]. The hetero-tetrameric channels formed by these subunits have specific properties based on their subunit composition. The NR1 subunit is responsible for glycine binding, and is essential to create a functional receptor. The NR2s are glutamate-binding subunits, which allow for the variety of NMDA receptors present in the brain. Upon glutamate binding, NMDA receptors become permeable to both Na^+ and Ca^{2+} ; however, it is Ca^{2+} that mediates most of the physiological effects attributed to the NMDAR [37]. Increased intracellular Ca^{2+} activates Ca^{2+} -dependent signal transduction pathways such as protein kinase A (PKA) [32] or CaMKII [39].

Due to their ability to activate and desensitize more rapidly after glutamate binding than NMDA receptors, AMPA receptors (AMPA) mediate fast excitatory synaptic transmission and play an important role in shaping synaptic plasticity [36,33,38]. The AMPA receptor family consists of four AMPA receptor subunits (GluR1-4) [38]. The subunits, in various combinations, form a functional channel. The channel's cation permeability is governed by the subunits present, most importantly GluR2. If a channel lacks the GluR2 subunit, then it will be permeable to both Ca^{2+} and Na^+ , however, if GluR2 is present, then it will almost always be calcium-impermeable [40,41]. Most AMPA receptors in the mature brain contain GluR2, and therefore are not Ca^{2+} -permeable, which is important because regulation of calcium permeability is key to normal brain functioning [41].

Unlike the ionotropic glutamate receptors, G-protein coupled mGluRs mediate slower glutamate responses. As these receptors are found both pre- and post-synaptically, they primarily serve a regulatory role in synaptic transmission by modulating the release of glutamate from presynaptic neurons and the response to glutamate on postsynaptic neurons [34]. In addition, they modulate NMDA and AMPA receptors, further controlling the postsynaptic neuronal response to glutamate, and as such, have been found to influence synaptic plasticity [38,42–45]. Metabotropic glutamate receptors include eight subtypes, mGluR1-8. As G-protein coupled receptors (GPCR), the mGluRs are coupled to either the phospholipase C/inositol triphosphate/diacylglycerol pathway or negatively coupled to the adenylate cyclase pathway [33,34].

Glutamate Transporters

Excitatory Amino Acid Transporters (EAAT)—Glutamate is an important molecule in the CNS and therefore is abundant in the brain; however, its role as a potent signaling molecule necessitates that the vast majority of CNS glutamate be contained intracellularly [33]. Maintaining low levels of extracellular glutamate is critically important for normal brain function and an imbalance in glutamate homeostasis can have detrimental consequences (discussed in next section). Glutamate transporters found on both neurons and glial cells remove most of the extracellular glutamate [46] to maintain low extracellular concentrations [33] and prevent aberrant glutamate signaling. It is generally accepted that most glutamatergic synapses are associated with astrocytic processes that abundantly express glutamate transporters, allowing rapid removal of glutamate from the synaptic cleft [36,46–48]. Additionally, glutamate transporters are located presynaptically as well as extra-synaptically and on glial cells to further remove excess glutamate that diffuses out of the synaptic cleft during signal transduction [46,49].

Sodium-dependent excitatory Amino Acid Transporters (EAAT), are the principle glutamate transporters responsible for extracellular glutamate homeostasis in the brain. There are five cloned 'high-affinity' Na⁺-dependent EAATs (for Review and References see [33]): EAAT1/GLAST, EAAT2/GLT1, EAAT3/EAAC1, EAAT4, and EAAT5. These glutamate transporters are Na⁺- and K⁺-dependent and transport L- and D-aspartate along with L-glutamate (Fig. 1). EAAT3 [50] and EAAT4 are neuronal glutamate transporters, with EAAT4 predominately expressed in cerebellar Purkinje cells, although small amounts have been found in a subpopulation of neurons in the forebrain [51–53]. EAAT5 appears to be specific to retinal cells [54,55] and expressed in other peripheral tissues [56]. EAAT1 and EAAT2 are primarily astrocytic glutamate transporters [57–59], although some studies have found some EAAT2 expression in axon-terminals of glutamatergic neurons [60,61,49]. EAAT2/GLT1 is the most well studied and abundant glutamate transporter that accounts for nearly 1% of the brain's protein [62] and nearly 95% of total forebrain glutamate uptake activity [63,64].

System x_c⁻ (SXC)—In addition to glutamate uptake through EAAT glutamate transporters, a Na⁺-independent cystine/glutamate exchanger, System x_c⁻ (SXC), also influences extracellular glutamate levels. Although not as robustly expressed as EAAT in the normal brain, SXC plays an important role in cellular protection. Studies conducted *in*

vitro indicate that SXC is predominantly expressed by astrocytes [65], but is also expressed by oligodendrocytes [66], microglia [67], immature cortical neurons [68], and in cell types outside of the CNS. Less is known about xCT localization *in vivo* due to lack of effective antibodies; however, xCT expression in mouse and human brain has been confirmed by both Western blot and immunohistochemistry [[66,69]]. Studies using antisera developed in-house have found that both human and mouse astrocytes express SXC, but there is conflicting evidence for the presence and function of neuronal SXC [[66,70]]. Clearly, better antibodies and more *in vivo* studies are needed to fully characterize the expression of xCT in the brain [71].

SXC acts by exchanging intracellular glutamate for extracellular cystine in a 1:1, electroneutral, Cl⁻-dependent, Na⁺-independent manner [72,73] (Fig. 1). The uptake of cystine, which is reduced intracellularly to cysteine, becomes the rate-limiting step for glutathione (GSH) synthesis. GSH, a non-protein thiol composed of glutamate, cysteine, and glycine, is a critical intracellular antioxidant in the CNS [74–76] that neutralizes reactive oxygen species (ROS) that are continuously generated as a result of oxidative metabolism [77,73]. In most living cells, it maintains intracellular redox homeostasis and prevents cell damage from the toxic effects of ROS. Cells can tolerate low amounts of ROS; however, high levels incur rapid and irreversible damage, thus 0.1–10mM of GSH is typically present in most cells. To maintain this intracellular redox control, cells ramp up SXC expression and activity in order to provide more intracellular cystine to replenish GSH [76].

SXC belongs to the heteromeric amino acid transporter (HAT) family and is composed of a heavy subunit, CD98 (or 4F2hc), and a light subunit, xCT, coupled by a disulfide bridge. The two subunits must be coupled for the transporter to function. xCT confers activity of the transporter and CD98 regulates its trafficking to the cell membrane [77,73]. Since CD98 is constitutively expressed on the membrane and interacts with other subunits, the expression of xCT is used as an indicator of SXC activity.

Expression of xCT is induced through an Antioxidant Response Element (ARE) or Electrophile Response Element (EpRE) promoter region that becomes activated by oxidative stress or electrophiles [77]. As a result, SXC is an inducible transporter that can be upregulated when cells need to increase production of GSH [78–80]. In addition to its role as an intracellular antioxidant, GSH is essential to maintain cellular redox state, metabolism, protein and nucleic acid synthesis, and as a form of cysteine transport and storage [81,82]. Refer to Figure 2a for an overview of glutamate transporters and receptors in the non-pathological brain.

Sodium-coupled Neutral Amino Acid Transporters (SNAT)—Astrocytic glutamate uptake limits the accumulation of extracellular glutamate, which is important to prevent glutamate toxicity and aberrant synaptic transmission. However, upon release of glutamate from neuronal synaptic vesicles, neurons must replenish glutamate for continued glutamatergic synaptic transmission. Neurons depend on astrocytic derived glutamine to resynthesize glutamate, due to their lack of pyruvate carboxylase. This enzyme, which is found in astrocytes but not neurons, is required to synthesize glutamate from glucose or lactate [83]. Astrocytes can recycle glutamate enzymatically after uptake by converting it to

glutamine using the cytoplasmic enzyme glutamine synthetase[84]. Glutamine is then released into the extracellular space through system N transporters (SN), also known as sodium-coupled neutral amino acid transporters (SNAT), taken up by neuronal system A transporters (SAT) [85,86], and used to resynthesize glutamate through the action of the mitochondrial enzyme phosphate-activated glutaminase [87,88]. Once converted back to glutamate, it can be packaged into synaptic vesicles by vesicular glutamate transporter (VGLUT) molecules [89,90], and is again ready for release into the synaptic cleft during neuronal excitatory signaling. This glutamine-glutamate cycle pathway is critical for the replenishment of neuronal glutamate while maintaining low extracellular glutamate levels.

Glial glutamate transporters, specifically SNAT3 and SNAT5 (also known as SN1 and SN2, respectively), function in the bidirectional electroneutral movement of glutamine across astrocytic membranes [91–95]. These transporters, enriched in the processes of glial cells, are coupled to sodium co-transport and proton exchange. SNAT5 also transports the amino acids alanine, serine, histidine, asparagine, and glycine, and its distribution closely correlate with that of VGLUT molecules [92]. Interestingly, the membrane trafficking of SNAT3 resembles that of EAAT1 and EAAT2. Recent findings suggest a functional and physical coupling between EAAT1 and SNAT3 in astrocytes [96]. Astrocytes seem to respond to neuronal glutamate release and astrocytic glutamate uptake by upregulating SNAT-mediated transport activity [97–99]. Therefore, these glutamine transporters are inherently important in maintaining glutamate homeostasis and proper metabolic functioning in the non-pathological brain. In addition, gamma-aminobutyric acid (GABA), the inhibitory neurotransmitter that regulates neuronal excitability, is also synthesized through the glutamine-glutamate cycle. GABA synthesis and function is beyond the scope of this review, but see [100,98,101] for review of GABA and the glutamine-glutamate cycle.

Maintenance of Glutamate Homeostasis in the Brain

Importance of maintaining glutamate homeostasis in the CNS

Glutamate transporters are critical to maintain normal excitatory signaling in the brain (Fig. 1). In the non-pathological brain, extracellular glutamate concentrations are tightly maintained at $\sim 1\mu\text{M}$ [102,103], but can be as low as 25 nM [104]. The synaptic cleft, where the majority of glutamate movement occurs, is designed so that glutamate transporters are in close proximity to rapidly remove glutamate after release (Fig 2a). This rapid re-uptake prevents prolonged glutamatergic signaling and damaging excitotoxicity.

Prolonged exposure to high concentrations of extracellular glutamate can kill neurons. *In vitro* experiments using cultured brain slices have shown that EAAT inhibition causes a rapid increase in extracellular glutamate within seconds [105]. When glutamate concentrations build up, excessive and unregulated binding to glutamate receptors results in Ca^{2+} -mediated neuronal excitotoxicity and rapid and/or delayed neuronal death. Due to its high Ca^{2+} permeability and incomplete desensitization, NMDA receptors are thought to be the main mediators of glutamate-induced excitotoxicity and death, although AMPAR and mGluR also play a role [106].

Overstimulation of NMDARs causes constitutive activation of the receptor that result in excessive Ca^{2+} influx into neurons. Increased intracellular Ca^{2+} triggers many intracellular events, which ultimately cause neuronal death due to bioenergetic failure and apoptotic factor release. Mitochondrial dysfunction and generation of ROS seem to be the most important mediators of cell death. These events result from membrane depolarization that occurs upon mitochondrial Ca^{2+} uptake. Mitochondrial membrane depolarization leads to inhibition of ATP production, depletion of cytosolic ATP, and increased formation of ROS, all of which then trigger further mitochondrial damage and ultimately cell death. In addition, increased intracellular Ca^{2+} activates a host of other signaling pathways and events that contribute to neuronal cell death [37,107,108]. Glutamate binding activates both AMPARs and mGluRs as well. AMPAR activation increases Na^+ influx into neurons, depolarizes the membrane, and activates both voltage-dependent Ca^{2+} channels and NDMARS; mGluRs, as GPCRs, are coupled to multiple signaling pathways that also increase cytosolic calcium, all of which contribute to additional Ca^{2+} influx. Furthermore, changes in membrane excitability due to excessive glutamate binding affects normal glutamatergic signaling [108], creating a hyper-excitabile network that is more susceptible to seizure-type activity.

Glutamate homeostasis and neurological diseases

Glutamate homeostasis is disrupted in numerous neurological diseases; both increased and decreased glutamate levels have been implicated in the pathology of various diseases. Dysfunction and/or loss of expression of glutamate transporters is commonly seen, which may explain in part why excitotoxicity is a common death pathway in neurological pathologies such as ischemia, trauma, stroke, and neurodegeneration [see [33] for more in depth discussion of the role of glutamate transporters in disease].

Ischemia occurs when localized blood supply is insufficient or absent, resulting in reduced levels of oxygen and metabolic substrates (i.e. glucose) and a loss of detoxification mechanisms in the presence of damaging metabolites (i.e. CO_2). Reduced blood flow depletes the energy supply of the brain, making brain cells highly vulnerable to damage [33]. In addition, ischemic conditions result in the buildup of extracellular glutamate [109–111] and neurons already deprived of energy become further stressed by the excitotoxic environment [110]. Glutamate is released in high concentrations during ischemia in part due to neuronal energy failure that collapses ion gradients and reverses glutamate transporters [112]. Ischemia mediates much of the pathology associated with traumatic brain injury and stroke, and therefore these conditions show similar types of excitotoxic damage.

Neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's, Huntington's, and Parkinson's, demonstrate excitotoxicity as part of their pathology [108,102]. Neurodegeneration results from the loss of structure and function of neurons and in many of these neurodegenerative diseases this loss can be attributed to glutamate-mediated neuronal dysfunction and death [113]. (For more information on glutamate's role in these diseases, see [33] and [108]). Interestingly, malignant glioma share many features of neurodegenerative diseases, including glutamate-mediated neuronal excitotoxicity, and as such, it has been suggested that glioma should also be considered a neurodegenerative disease [114].

Loss of glutamate homeostasis in glioma

Glioma cells do not display the same expression of glutamate transporters as their non-malignant counterparts. To support their rapid growth and metabolism, they increase cystine uptake through SXC to enhance proliferation and ROS detoxification [115,76]. Consequently, glioma cells release large amounts of glutamate into the extracellular space (Fig. 2b).

In the non-pathological brain, high extracellular glutamate concentrations are easily managed through EAAT-mediated glutamate uptake. However, glioma cells appear to have lost the ability to take up glutamate. Even after being challenged with 100 μ M of glutamate in culture conditions, glioma cells continue to release excess glutamate into the medium, whereas astrocytes quickly remove excess glutamate within a few hours. Further investigations discovered that glioma cells lack EAAT2-mediated glutamate transport [116–121,12]. These findings were corroborated by a microarray analysis of human glioma biopsies demonstrating the absence of EAAT2 expression in patient glioma tissue [116]. The absence of EAAT2 may result from DNA methylation [122], aberrant splicing of EAAT2 mRNA [121], and/or nuclear mis-localization of EAAT2 protein [119] in these tumors. As EAAT2 is responsible for most of the glutamate uptake in the normal mature brain, its absence in glioma cells that are quickly overtaking normal brain drastically alters localized glutamate homeostasis. In addition to EAAT2, most commonly used glioma cell lines have no functional activity of any EAAT. A few of the astrocytoma cell lines do have detectable transcripts for EAAT1 and/or EAAT3; however, actual transporter function has been detected in a minority of glioma cell lines, and interestingly, in these cases the primary transporter that is mediating uptake is neuronal EAAT3 [118,123].

The release of glutamate by gliomas has been well studied in *in vitro*, *in vivo*, and in the patient population [124,13,6,12,125,10,117]. Most striking are clinical studies that show that cerebrospinal fluid from ambulatory glioma patients contain up to 400 μ M glutamate, 400-fold the normal glutamate concentration and well above the threshold for excitotoxicity. This finding, as well as those in animal models, questions what happens to peritumoral astrocytes that normally maintain glutamate homeostasis. In the non-pathological brain, astrocytic EAAT2 is responsible for the majority of glutamate uptake, and astrocytes can quickly reduce large extracellular glutamate concentrations back to non-lethal levels [117]. Therefore, it serves to reason that if glutamate levels are elevated peritumorally, then the tumor must be influencing astrocytic glutamate uptake as well, otherwise peritumoral astrocytes would control glutamate levels. We hypothesize that gliomas influence astrocytic down-regulation of EAAT2, leading to the elevated peritumoral levels seen in glioma patients and in animal models. In support of our hypothesis, a recent study investigating elevated glutamate levels in neuroinflammation found that microglial-mediated glutamate release downregulated astrocytic EAAT expression [126]. Therefore, it is possible that glutamate release by gliomas (and most likely other glioma-released factors) induces the loss of astrocytic glutamate uptake, allowing for perturbation of glutamate homeostasis.

The sodium-coupled neutral amino acid transporters may also play a role in the biology of glioma. The astrocytic transporters SNAT3 and SNAT5 are able to bidirectionally transport

glutamine. Glutamine is an important metabolite in tumor cells, one which gliomas cannot synthesize themselves [127,128]. One study found that SNAT3 expression is increased in GBM tissues compared to lower grade glioma and normal brain, suggesting that SNAT3 expression may be a marker of highly malignant glioma [129]. Therefore, it is conceivable that the upregulation of glutamine transporters may enhance tumor cell metabolism. In addition, glioma cells require glutamine uptake for growth [130] and as they express glutaminase [131], the enzyme that mediates the reaction of glutamine to glutamate, glutamine transporters may enhance intracellular glutamate synthesis by providing substrate for release through SXC. Clearly, more work is needed to elucidate what, if any, role these transporters play in glioma biology.

Additionally, glioma cells express NMDA, AMPA, and mGluRs [132–135]. Interestingly, electrophysiological studies suggest that the electrophysiological properties of glioma cells are more functionally similar to neurons than glial cells [136–138] due to the function of these receptors. When glutamate receptors are activated in tumor cells, action potentials reminiscent of neuronal activity can be elicited. Increases in intracellular cytosolic Ca^{2+} concentrations are also detected [138,137]. What the role of these action potentials might be, and if they are of any biological significance, remains unclear.

While a loss of glutamate homeostasis is clearly detrimental to the brain tissue surrounding tumors, it is advantageous for glioma growth and survival. The next section will discuss the specific advantages glutamate provides for glioma cells and the devastating effects it has on the brain.

Glioma Glutamate Transporters Confer a Biological Advantage

Gliomas alter glutamate transporter and receptor expression to enhance their own growth and survival ability (Fig. 2). In the non-pathological brain, SXC and EAAT work in concert to protect the brain from too much extracellular glutamate and too little intracellular cystine and GSH (Fig. 1). Recent research has uncovered the ability of glioma cells to upregulate SXC and downregulate EAAT to alter glutamate homeostasis for their own advantage.

Enhanced Survival

Glutathione is an important antioxidant present in most cells, and its levels are known to be increased in a number of human cancers, including brain tumors [82,139]. The ability of tumor cells to increase intracellular GSH synthesis provides growth and survival advantages against both intrinsic and extrinsic factors. Non-malignant cells continuously produce ROS as a byproduct of normal oxidative metabolism [77,140]. Glioma cells undergoing rapid proliferation increase their metabolic rate, and as a result produce ROS much more quickly than normal brain cells. In response to oxidative stress and ROS, glioma cells upregulate SXC activity to provide intracellular cystine for enhanced GSH synthesis. This increased GSH production prevents ROS-mediated cell death from increased proliferation [82].

As tumor cells proliferate and the tumor mass expands, it outgrows its blood supply and oxygen becomes limited, creating chronically hypoxic areas within the tumor. Hypoxia induces production of ROS, which threatens cell survival. Glioma cells prevent hypoxia-

mediated death by increasing cystine uptake through SXC for the production of GSH. Increased GSH neutralizes ROS, preventing cell destruction from hypoxia [80]. GSH has also been linked to control of cellular apoptosis cascades; higher levels of GSH inhibit apoptosis signaling, further protecting cells from degradation [82].

In addition to protection from these intrinsic metabolic factors, GSH protects cells from extrinsic factors introduced during treatment. Cancer cells commonly develop resistance to chemotherapeutic agents, and GSH plays an important role in the development of this resistance. In glioma and other cancers, higher GSH levels often correlate with greater treatment resistance [141,142]. Ionizing radiation, both environmentally and therapeutically delivered, kills cells either by directly or indirectly damaging DNA. Indirect damage is accomplished through creation of hydroxyl free radicals ($\text{OH}\cdot$) from water that then interact with DNA and cause cell death [76,142]. GSH acts to neutralize these intracellular free radicals and prevent the cytotoxic effects of radiation [143]. When astrocytes in culture are irradiated, intracellular GSH increases in a dose-dependent manner [144], suggesting that the cellular response to radiation-induced free radicals is to increase GSH production and utilization.

GSH also protects cells from drug-induced oxidative stress by stimulating the efflux of xenobiotics [77]. When chemotherapeutic drugs enter cancer cells they are recognized as foreign and can be conjugated to GSH and excreted out of the cell via a family of GSH transporters called Multidrug Resistance Proteins (MRP). This mechanism essentially allows the cells to reject the offending drug, protecting itself from chemotherapeutic toxicity [77,82].

In cancer cells, GSH activity has been linked to better survival, enhanced growth, and increased drug and radiation resistance [82]. The ability of SXC to enhance tumor growth and survival by providing cystine for GSH synthesis [78] seems essential for many tumors, attested to by the fact that most studies of glioma cells have found increased SXC expression and/or activity [117,119,145,115].

Enhanced Migration & Invasion

SXC plays an important role in glioma cell migration and invasion. Experimental SXC inhibition in animal models, either pharmacologically with the drug sulfasalazine (SAS), or by siRNA knockdown of xCT, results in better cell-cell adhesion, less cell-extracellular matrix adhesion, and a decrease in cancer cell metastasis. These effects result from the GSH depletion that occurs after SXC inhibition, leading to ROS accumulation and oxidative stress. ROS-mediated p38 MAPK activation causes upregulation of caveolin-1. Caveolin-1 recruits β -catenin to the cell membrane, enhancing cell-cell adhesion and preventing cell invasion [8]. These findings suggest that SXC directly influences glioma cell migration and invasion.

SXC-mediated glutamate release works in an autocrine/paracrine manner to promote Ca^{2+} -mediated glioma metastasis. GBM cells express GluR2-lacking AMPA receptors [146], possibly due to an under-editing of GluR2 RNA [147], creating AMPARs that are highly Ca^{2+} -permeable. Experimental conversion of Ca^{2+} -permeable AMPARs into Ca^{2+} -

impermeable receptors using adenoviral vector-mediated transfer of GluR2 DNA inhibits cell migration and invasion, as well as induces apoptosis [134]. SXC activity can be detected by the presence of intracellular Ca^{2+} oscillations. Blocking glioma AMPARs inhibits these oscillations and, in turn, glioma cell migration. SXC inhibitors SAS and S-(4)-CPG also inhibit glioma cell migration *in vivo* [10].

Further, AMPAR expression correlates with an increase in surface expression of $\beta 1$ integrin, a molecule implicated in glioma invasion, and AMPAR stimulation increases glioma cell de-adhesion from surrounding cells [9]. Glial AMPA receptors activate the focal adhesion kinase (FAK), which influences cytoskeletal rearrangement and focal adhesion contact formation [148,149]. In glioma cells, inhibition of FAK activity leads to cell detachment, apoptosis, and inhibition of cell invasion and motility [150–152]. $\beta 1$ integrin activates FAK, and in patient tumor tissue overexpression of either $\beta 1$ integrin or FAK correlates with decreased survival. Taken together, these findings support that view that Ca^{2+} -permeable AMPARs serve to support tumor invasion *in vivo*.

Enhanced Growth

Stimulation for Growth—Glutamate also acts in an autocrine/paracrine manner to directly promote glioma growth, specifically through AMPARs and mGluRs. Glioma AMPAR inhibition decreases cell proliferation, increases cell death, and enhances susceptibility of cells to chemotherapeutics [132]. Through Ca^{2+} -dependent and Ca^{2+} -independent mechanisms, AMPARs are important mediators of glioma cell growth. Ca^{2+} influx into glioma cells through AMPARs activates Akt, which has anti-apoptotic activity. Blocking AMPAR activity inhibits Akt activation and induces apoptosis. AMPA receptor activity also activates the MAPK pathway, which is independent of Ca^{2+} influx, providing another mechanism for glioma cell proliferation [153]. In line with these findings, in tissue microarray analysis of human glioma tumor samples and GBM tumor stem cells (or Brain Tumor Initiating Cells (BTIC)), high-grade gliomas overexpress GluR1 and Ca^{2+} -permeable AMPA receptors [154]. Additionally, glioma cell proliferation is reduced following exposure to an mGlu2/3 inhibitor, LY341495 [133]. Inhibition of mGlu2/3 reduces ERK1/2 phosphorylation, further implicating the MAPK pathway in glioma cell proliferation. These findings contrast previous data showing that mGlu2/3 receptor activation decreases astrocytic proliferation [155], suggesting mGluRs may undergo a malignant transformation in glioma cells.

Regulation of EAAT2 expression and function may be another mechanism tumor cells use to promote their own growth and malignancy. One study looking at the relationship between the Astrocyte Elevated Gene-1 (AEG-1), an oncogene that is overexpressed in 90% of brain tumors, suggests that there is a strong negative correlation between expression of AEG-1 and EAAT2 in glioma patient tissue samples. Transcriptionally, AEG-1 was found to negatively regulate EAAT2 expression [156]. Interestingly, EAAT2 expression seems to be inversely correlated with tumor grade, increasing with decreasing tumor grade. Grade IV astrocytomas (GBMs) express little to no EAAT2 while Grade II express the highest levels [157], suggesting loss of EAAT2 expression enhances tumor malignancy.

Space for Growth (Peritumoral Excitotoxicity)—Due to their location in the brain, gliomas are faced with a unique and challenging environment in which to grow. Unlike most systemic tumors, which have unrestricted growth space in soft tissue, brain tumors are physically limited to the constraints of the skull. The brain is composed of 85% tissue and 15% CSF, and therefore, in the early stages of growth the tumor mass can push into the fluid spaces of the brain. For continued growth, however, the tumor must begin integrating into normal brain tissue. Gliomas have developed the ability to vacate space for their own growth and expansion by destroying surrounding brain [158].

Recently, unopposed glutamate release from glioma cells has been implicated as the mechanism allowing these tumors to create space for growth. Studies have found that gliomas release glutamate in sufficient quantities to cause neuronal cell death [159,115], which is mediated by prolonged activation of neuronal NMDA receptors resulting in Ca^{2+} influx and Ca^{2+} -mediated cell death [102]. Interestingly, administration of the competitive NMDA receptor antagonist MK801 or uncompetitive NMDA receptor antagonist memantine to glioma-bearing mice attenuates tumor growth but does not reduce cellular proliferation [160]. These findings suggest that gliomas need to create space in order to grow as a large mass in the brain.

This hypothesis is further supported by results from experiments on glioma cell lines engineered to release glutamate in different amounts [160]. Following intracranial implantation of these cloned cell lines into rodents, cells that release the most glutamate form the largest tumors, while clones unable to secrete glutamate fail to establish tumors. Peritumorally, a greater number of degenerating neurons are seen surrounding glutamate-releasing tumor in mice, and neuronal death is attenuated by administration of MK801. The inability of non-glutamate releasing tumors to kill surrounding neurons restricts tumor growth, supporting the idea that gliomas have the innate ability to destroy normal brain and occupy the vacated space. SXC is the only transporter that has been found in glioma cells that has the ability to release large amounts of glutamate [117]. SXC upregulation combined with EAAT downregulation provides glioma cells with the ability to grow without space limitations [119].

Glioma & Seizures

Most recently glutamate has been implicated in the peritumoral seizure activity that is commonly seen in brain tumor patients. As many as 80% of patients with gliomas experience at least one seizure during the course of their illness [161,162], and for many, it is the presenting symptom of the tumor. Numerous theories have been proposed as to the underlying mechanism behind tumor-associated seizures, such as peritumoral amino acid dysregulation and dysfunction of NMDA glutamate receptors on peritumoral neurons [163], however little evidence as to the true pathophysiology has been uncovered until recently.

Glutamate is involved in many seizure disorders and clinical studies have found that extracellular glutamate concentrations are increased prior to seizure occurrence, suggesting that glutamate may precipitate seizure activity [164]. Animal studies in mice and clinical studies in awake, ambulatory glioma patients, have found that glutamate is highly elevated

in peritumoral brain [160,165,124,12]. Additional studies in glioma-implanted mice have found that the epileptic focus is often located within 2 mm from the tumor mass [166]. The finding that glioma cells, through an upregulation of SXC and downregulation of EAAT [119], release concentrations of glutamate that is excitotoxic to neurons [117], spurred interest in SXC as a contributor to tumor-associated seizures. A recent paper showed that glutamate release from glioma cells via SXC induces epileptic activity in human glioma-bearing mice [13]. In this study electroencephalogram (EEG) recordings detected spontaneous and recurring abnormal brain activity that is significantly diminished following treatment with SAS.

An electrophysiological study of peritumoral neuronal networks further supports the idea that SXC-mediated glutamate release induces epileptic activity. Peritumoral neurons display spontaneous epileptiform activity, as well as alterations in synaptic transmission and hyper-excitability. Additionally, SAS application abolishes Mg^{2+} -free induced ictal-like network activity in acute brain slices from tumor-bearing animals [167]. These findings indicate that SXC is a major source of glutamate that contributes to the hyper-excitable peritumoral environment.

Clinical studies of SXC and EAAT in patients have seen similar correlations between expression of these glutamate transporters and seizure activity. In a recent study looking at 288 glioma patients, investigators found that 42% of the patients had tumor-associated seizures. Hallmarks associated with an increased risk of these seizures are increased glutamate concentrations, reduced EAAT2 expression, and increased SXC expression [12].

Putting it all Together: Gliomas Exploiting an Endogenous Neurotransmitter to Enhance their Biology and Gain a Survival Advantage in the Brain

Brain tumors are a unique type of cancer. Their location in the brain presents challenges to which gliomas have adapted well. Glutamate, an excitatory neurotransmitter critical to normal brain function, is used to ensure the growth and survival of tumor cells at the expense of normal brain tissue. The upregulation of SXC provides both protection and growth advantages through uptake of cystine and release of glutamate. Intracellular cystine is used to synthesize GSH, which protects against cell death due to intrinsically generated oxidative stress and radio- and chemo- therapy generated free radicals. As a consequence of cystine uptake, glutamate is released in large amounts into the extracellular space, perturbing normal glutamate homeostasis. The loss of EAAT expression in glioma cells, combined with the likely dysfunction of glutamate transporters on astrocytes, prevents re-establishment of glutamate homeostasis. Elevated extracellular glutamate binds to NMDARs, AMPARs, and mGluRs that are overexpressed on glioma cells, therefore enhancing their survival, growth, migration, and invasion in the brain. Despite these growth and survival adaptations, gliomas could not continue to grow if they did not have space. Excessive glutamate overactivates neuronal NMDA receptors, ultimately killing neurons to clear space for their continual growth. See Fig. 2 and 3 for an overview of these biological advantages glutamate transporters provide to malignant glioma.

Translating Glioma Biology to Patient Management and Treatment: Clinical Potential & Outlook

The role of glutamate transporters in glioma biology is supported clinically and offers unique treatment challenges and opportunities. A clinical study of 40 patients with GBM determined that xCT expression is elevated in 50% of patient tumors. Increased xCT expression correlates more strongly with an infiltrative phenotype on MRI and is significantly associated with shorter periods of remission and more rapid mortality [11]. In addition, patient tumor samples measured thus far have not shown EAAT2 immunoreactivity, attesting to the loss of this important glutamate transporter in patient tumors.

In addition to SXC and EAAT, one may also argue that seizures are a precursor to, or biomarker for, tumor growth and progression. Evidence from *in vitro*, *in vivo*, and clinical studies strongly supports the idea that increased SXC and decreased EAAT activity leads to faster tumor progression, higher extracellular glutamate levels, and a greater incidence of tumor-associated seizures. Therefore, it is conceivable that seizures could be used clinically to indicate tumor growth, as they are used as an indicator of disease on a patient's first presentation.

Numerous pre-clinical studies and clinical trials are currently ongoing for glioma. Many promising treatments target glutamate-related aspects of tumor biology [114]. Currently prescribed drugs such as valproic acid [168,169] and beta-lactam antibiotics [170] increase EAAT expression and may prove to be beneficial in regulating peritumoral glutamate levels and decreasing tumor growth. Propentofylline, a drug which increases EAAT1 and EAAT2 expression, increases astrocytic glutamate uptake *in vitro*, and causes increased glioma cell apoptosis as a result of decreased extracellular glutamate concentration [171]. These and other *in vitro* studies suggest pharmacologically increasing EAAT expression in the tumor or in peritumoral tissues may be neuroprotective and an effective adjuvant therapy to decrease tumor growth [172,12,173,174,116]. Talampanel, an allosteric inhibitor of AMPAR, is currently in a phase II clinical trial, and may provide a survival advantage when used in combination with current therapy [175,176]. NMDAR and AMPAR antagonists have shown promise by enhancing the effects of cytostatic drugs in culture [132] and they may be a useful adjuvant treatment to sensitize glioma cells to chemotherapy. In preclinical studies, SAS, a potent SXC inhibitor, is effective in slowing tumor growth and decreasing SXC-mediated tumor glutamate release and resulting seizure activity [7,77,13]. A clinical trial is currently underway to determine the effectiveness of SAS on lowering peritumoral glutamate levels and to assess the efficacy of SAS treatment in reducing tumor size and seizure burden.

Gliomas are one of the most challenging cancers to treat, which is apparent by the lack of treatment advances that have led to improvements in patient survival. Their location, combined with their unique biology, makes them unlike the majority of solid tumors found in the body. As a result, these tumors warrant a new and innovative treatment strategy. As we learn more about the biology of malignant gliomas, we hope to be able to offer more efficacious treatments and eventually a cure for these tumors.

Conclusions

The interaction of glutamate, glutamate transporters, and glutamate receptors has a significant role in the malignancy of gliomas. Research over the past couple of decades has implicated glutamate as an important player in tumor growth, survival, and peritumoral seizure activity. More recently research has linked the upregulation of SXC and subsequent downregulation of EAAT with excessive tumor glutamate release. Although chances are the story is much bigger than glutamate and its transporters and receptors, the evidence reviewed here presents at least part of the picture of how and why malignant gliomas are such devastating tumors, cause such serious side effects, and prove to be almost impossible to cure.

Acknowledgments

This work was supported by US National Institutes of Health grants R01NS052634, 2T32NS048039, and T32GM008361. The authors would also like to thank Dr. Sue Buckingham for her help in editing this manuscript.

References

1. Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. *Neuro-oncology*. 2012; 14(Suppl 5):v1–49.10.1093/neuonc/nos218 [PubMed: 23095881]
2. Porter KR, McCarthy BJ, Freels S, Kim Y, Davis FG. Prevalence estimates for primary brain tumors in the United States by age, gender, behavior, and histology. *Neuro-oncology*. 2010; 12 (6):520–527.10.1093/neuonc/nop066 [PubMed: 20511189]
3. Preusser M, de Ribaupierre S, Wohrer A, Erridge SC, Hegi M, Weller M, Stupp R. Current concepts and management of glioblastoma. *Annals of neurology*. 2011; 70 (1):9–21.10.1002/ana.22425 [PubMed: 21786296]
4. Adamson C, Kanu OO, Mehta AI, Di C, Lin N, Mattox AK, Bigner DD. Glioblastoma multiforme: a review of where we have been and where we are going. Expert opinion on investigational drugs. 2009; 18 (8):1061–1083.10.1517/13543780903052764 [PubMed: 19555299]
5. Chamberlain MC, Kormanik PA. Practical guidelines for the treatment of malignant gliomas. *The Western journal of medicine*. 1998; 168 (2):114–120. [PubMed: 9499745]
6. Ruban A, Berkutzki T, Cooper I, Mohar B, Teichberg. Blood glutamate scavengers prolong the survival of rats and mice with brain-implanted gliomas. *Investigational new drugs*. 2012.10.1007/s10637-012-9794-x
7. Chung WJ, Sontheimer H. Sulfasalazine inhibits the growth of primary brain tumors independent of nuclear factor-kappaB. *Journal of neurochemistry*. 2009; 110 (1):182–193.10.1111/j.1471-4159.2009.06129.x [PubMed: 19457125]
8. Chen RS, Song YM, Zhou ZY, Tong T, Li Y, Fu M, Guo XL, Dong LJ, He X, Qiao HX, Zhan QM, Li W. Disruption of xCT inhibits cancer cell metastasis via the caveolin-1/beta-catenin pathway. *Oncogene*. 2009; 28 (4):599–609.10.1038/onc.2008.414 [PubMed: 19015640]
9. Piao Y, Lu L, de Groot J. AMPA receptors promote perivascular glioma invasion via beta1 integrin-dependent adhesion to the extracellular matrix. *Neuro-oncology*. 2009; 11 (3):260–273.10.1215/15228517-2008-094 [PubMed: 18957620]
10. Lyons SA, Chung WJ, Weaver AK, Ogunrinu T, Sontheimer H. Autocrine glutamate signaling promotes glioma cell invasion. *Cancer research*. 2007; 67 (19):9463–9471.10.1158/0008-5472.CAN-07-2034 [PubMed: 17909056]
11. Takeuchi S, Wada K, Toyooka T, Shinomiya N, Shimazaki H, Nakanishi K, Nagatani K, Otani N, Osada H, Uozumi Y, Matsuo H, Nawashiro H. Increased xCT expression correlates with tumor invasion and outcome in patients with glioblastomas. *Neurosurgery*. 2013; 72 (1):33–41. discussion 41. 10.1227/NEU.0b013e318276b2de [PubMed: 23096413]

12. Yuen TI, Morokoff AP, Bjorksten A, D'Abaco G, Paradiso L, Finch S, Wong D, Reid CA, Powell KL, Drummond KJ, Rosenthal MA, Kaye AH, O'Brien TJ. Glutamate is associated with a higher risk of seizures in patients with gliomas. *Neurology*. 2012; 79 (9):883–889.10.1212/WNL.0b013e318266fa89 [PubMed: 22843268]
13. Buckingham SC, Campbell SL, Haas BR, Montana V, Robel S, Ogunrinu T, Sontheimer H. Glutamate release by primary brain tumors induces epileptic activity. *Nature medicine*. 2011; 17 (10):1269–1274.10.1038/nm.2453
14. Chen J, McKay RM, Parada LF. Malignant glioma: lessons from genomics, mouse models, and stem cells. *Cell*. 2012; 149 (1):36–47.10.1016/j.cell.2012.03.009 [PubMed: 22464322]
15. Westphal M, Lamszus K. The neurobiology of gliomas: from cell biology to the development of therapeutic approaches. *Nature reviews Neuroscience*. 2011; 12 (9):495–508.10.1038/nrn3060
16. Ernest, NJ.; Sontheimer, H. Glioma. In: Larry, RS., Editor-in-Chief, editor. *Encyclopedia of Neuroscience*. Vol. 4. Academic Press; Oxford: 2009. p. 877-884.<http://dx.doi.org/10.1016/B978-008045046-9.09007-0>
17. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. *Acta neuropathologica*. 2007; 114 (2):97–109.10.1007/s00401-007-0243-4 [PubMed: 17618441]
18. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England journal of medicine*. 2005; 352 (10):987–996.10.1056/NEJMoa043330 [PubMed: 15758009]
19. Wen PY, Kesari S. Malignant gliomas in adults. *The New England journal of medicine*. 2008; 359 (5):492–507.10.1056/NEJMra0708126 [PubMed: 18669428]
20. Sudhof TC. The synaptic vesicle cycle: a cascade of protein-protein interactions. *Nature*. 1995; 375 (6533):645–653.10.1038/375645a0 [PubMed: 7791897]
21. Augustine GJ, Burns ME, DeBello WM, Pettit DL, Schweizer FE. Exocytosis: proteins and perturbations. *Annual review of pharmacology and toxicology*. 1996; 36:659–701.10.1146/annurev.pa.36.040196.003303
22. Bergles DE, Roberts JD, Somogyi P, Jahr CE. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature*. 2000; 405 (6783):187–191.10.1038/35012083 [PubMed: 10821275]
23. Seifert G, Steinhauser C. Ionotropic glutamate receptors in astrocytes. *Progress in brain research*. 2001; 132:287–299.10.1016/s0079-6123(01)32083-6 [PubMed: 11544996]
24. Teichberg VI. Glial glutamate receptors: likely actors in brain signaling. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 1991; 5 (15):3086–3091. [PubMed: 1660422]
25. Steinhauser C, Gallo V. News on glutamate receptors in glial cells. *Trends in neurosciences*. 1996; 19 (8):339–345. [PubMed: 8843603]
26. Lee MC, Ting KK, Adams S, Brew BJ, Chung R, Guillemin GJ. Characterisation of the expression of NMDA receptors in human astrocytes. *PloS one*. 2010; 5 (11):e14123.10.1371/journal.pone.0014123 [PubMed: 21152063]
27. Murugan M, Ling EA, Kaur C. Glutamate receptors in microglia. *CNS & neurological disorders drug targets*. 2013; 12 (6):773–784. [PubMed: 24047523]
28. Flores-Mendez MA, Martinez-Lozada Z, Monroy HC, Hernandez-Kelly LC, Barrera I, Ortega A. Glutamate-dependent translational control in cultured Bergmann glia cells: eIF2alpha phosphorylation. *Neurochemical research*. 2013; 38 (7):1324–1332.10.1007/s11064-013-1024-1 [PubMed: 23536022]
29. Jansson LC, Louhivuori L, Wigren HK, Nordstrom T, Louhivuori V, Castren ML, Akerman KE. Effect of glutamate receptor antagonists on migrating neural progenitor cells. *The European journal of neuroscience*. 2013; 37 (9):1369–1382.10.1111/ejn.12152 [PubMed: 23383979]
30. Renzel R, Sadek AR, Chang CH, Gray WP, Seifert G, Steinhauser C. Polarized distribution of AMPA, but not GABAA, receptors in radial glia-like cells of the adult dentate gyrus. *Glia*. 2013; 61 (7):1146–1154.10.1002/glia.22505 [PubMed: 23633386]

31. Hollmann M, Heinemann S. Cloned glutamate receptors. Annual review of neuroscience. 1994; 17:31–108.10.1146/annurev.ne.17.030194.000335
32. Prickett TD, Samuels Y. Molecular pathways: dysregulated glutamatergic signaling pathways in cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012; 18 (16):4240–4246.10.1158/1078-0432.CCR-11-1217 [PubMed: 22648273]
33. Danbolt NC. Glutamate uptake. Progress in neurobiology. 2001; 65 (1):1–105. [PubMed: 11369436]
34. Nakanishi S. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. Neuron. 1994; 13 (5):1031–1037. [PubMed: 7946343]
35. Tang CM, Dichter M, Morad M. Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. Science. 1989; 243 (4897):1474–1477. [PubMed: 2467378]
36. de Groot J, Sontheimer H. Glutamate and the biology of gliomas. Glia. 2011; 59 (8):1181–1189.10.1002/glia.21113 [PubMed: 21192095]
37. Hardingham GE. Coupling of the NMDA receptor to neuroprotective and neurodestructive events. Biochemical Society transactions. 2009; 37 (Pt 6):1147–1160.10.1042/BST0371147 [PubMed: 19909238]
38. Rojas A, Dingledine R. Ionotropic glutamate receptors: regulation by G-protein-coupled receptors. Molecular pharmacology. 2013; 83 (4):746–752.10.1124/mol.112.083352 [PubMed: 23348498]
39. Leonard AS, Lim IA, Hemsworth DE, Horne MC, Hell JW. Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96 (6):3239–3244. [PubMed: 10077668]
40. Kim DY, Kim SH, Choi HB, Min C, Gwag BJ. High abundance of GluR1 mRNA and reduced Q/R editing of GluR2 mRNA in individual NADPH-diaphorase neurons. Molecular and cellular neurosciences. 2001; 17 (6):1025–1033.10.1006/mcne.2001.0988 [PubMed: 11414791]
41. Isaac JT, Ashby MC, McBain CJ. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron. 2007; 54 (6):859–871.10.1016/j.neuron.2007.06.001 [PubMed: 17582328]
42. Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience. 2001; 106 (3):579–587. [PubMed: 11591458]
43. Gubellini P, Pisani A, Centonze D, Bernardi G, Calabresi P. Metabotropic glutamate receptors and striatal synaptic plasticity: implications for neurological diseases. Progress in neurobiology. 2004; 74 (5):271–300.10.1016/j.pneurobio.2004.09.005 [PubMed: 15582223]
44. Pisani A, Calabresi P, Centonze D, Bernardi G. Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. British journal of pharmacology. 1997; 120 (6):1007–1014.10.1038/sj.bjp.0700999 [PubMed: 9134210]
45. Bonsi P, Cuomo D, De Persis C, Centonze D, Bernardi G, Calabresi P, Pisani A. Modulatory action of metabotropic glutamate receptor (mGluR) 5 on mGluR1 function in striatal cholinergic interneurons. Neuropharmacology. 2005; 49(Suppl 1):104–113.10.1016/j.neuropharm.2005.05.012 [PubMed: 16005029]
46. Bergles DE, Jahr CE. Synaptic activation of glutamate transporters in hippocampal astrocytes. Neuron. 1997; 19 (6):1297–1308. [PubMed: 9427252]
47. Berger T, Schnitzer J, Kettenmann H. Developmental changes in the membrane current pattern, K⁺ buffer capacity, and morphology of glial cells in the corpus callosum slice. J Neurosci. 1991; 11:3008–3024. [PubMed: 1941072]
48. Berger UV, DeSilva TM, Chen W, Rosenberg PA. Cellular and subcellular mRNA localization of glutamate transporter isoforms GLT1a and GLT1b in rat brain by in situ hybridization. J Comp Neurol. 2005; 492 (1):78–89.10.1002/cne.20737 [PubMed: 16175560]
49. Chen W, Mahadomrongkul V, Berger UV, Bassan M, DeSilva T, Tanaka K, Irwin N, Aoki C, Rosenberg PA. The glutamate transporter GLT1a is expressed in excitatory axon terminals of mature hippocampal neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004; 24 (5):1136–1148.10.1523/jneurosci.1586-03.2004 [PubMed: 14762132]

50. Holmseth S, Dehnes Y, Huang YH, Follin-Arbelet VV, Grutle NJ, Mylonakou MN, Plachez C, Zhou Y, Furness DN, Bergles DE, Lehre KP, Danbolt NC. The density of EAAC1 (EAAT3) glutamate transporters expressed by neurons in the mammalian CNS. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012; 32 (17):6000–6013.10.1523/jneurosci.5347-11.2012 [PubMed: 22539860]
51. Dehnes Y, Chaudhry FA, Ullensvang K, Lehre KP, Storm-Mathisen J, Danbolt NC. The glutamate transporter EAAT4 in rat cerebellar Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1998; 18 (10):3606–3619. [PubMed: 9570792]
52. Massie A, Vandesande F, Arckens L. Expression of the high-affinity glutamate transporter EAAT4 in mammalian cerebral cortex. *Neuroreport*. 2001; 12 (2):393–397. [PubMed: 11209956]
53. de Vivo L, Melone M, Bucci G, Rothstein JD, Conti F. Quantitative analysis of EAAT4 promoter activity in neurons and astrocytes of mouse somatic sensory cortex. *Neuroscience letters*. 2010; 474 (1):42–45.10.1016/j.neulet.2010.03.003 [PubMed: 20211693]
54. Lee A, Anderson AR, Barnett NL, Stevens MG, Pow DV. Alternate splicing and expression of the glutamate transporter EAAT5 in the rat retina. *Gene*. 2012; 506 (2):283–288.10.1016/j.gene.2012.07.010 [PubMed: 22820393]
55. Arriza JL, Eliasof S, Kavanaugh MP, Amara SG. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94 (8):4155–4160. [PubMed: 9108121]
56. Lee A, Anderson AR, Stevens M, Beasley S, Barnett NL, Pow DV. Excitatory amino acid transporter 5 is widely expressed in peripheral tissues. *European journal of histochemistry : EJH*. 2013; 57 (1):e11.10.4081/ejh.2013.e11 [PubMed: 23549460]
57. Lehre KP, Levy LM, Ottersen OP, Storm-Mathisen J, Danbolt NC. Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1995; 15 (3 Pt 1):1835–1853. [PubMed: 7891138]
58. Danbolt NC, Storm-Mathisen J, Kanner BI. An [Na⁺ + K⁺]coupled L-glutamate transporter purified from rat brain is located in glial cell processes. *Neuroscience*. 1992; 51 (2):295–310. [PubMed: 1465194]
59. Levy LM, Lehre KP, Rolstad B, Danbolt NC. A monoclonal antibody raised against an [Na⁺(+)+K⁺]coupled L-glutamate transporter purified from rat brain confirms glial cell localization. *FEBS Lett*. 1993; 317 (1–2):79–84. [PubMed: 7679083]
60. Furness DN, Dehnes Y, Akhtar AQ, Rossi DJ, Hamann M, Grutle NJ, Gundersen V, Holmseth S, Lehre KP, Ullensvang K, Wojewodzic M, Zhou Y, Attwell D, Danbolt NC. A quantitative assessment of glutamate uptake into hippocampal synaptic terminals and astrocytes: new insights into a neuronal role for excitatory amino acid transporter 2 (EAAT2). *Neuroscience*. 2008; 157 (1):80–94.10.1016/j.neuroscience.2008.08.043 [PubMed: 18805467]
61. Aoyama K, Suh SW, Hamby AM, Liu J, Chan WY, Chen Y, Swanson RA. Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. *Nature neuroscience*. 2006; 9 (1):119–126.10.1038/nn1609
62. Danbolt NC, Pines G, Kanner BI. Purification and reconstitution of the sodium- and potassium-coupled glutamate transport glycoprotein from rat brain. *Biochemistry*. 1990; 29 (28):6734–6740. [PubMed: 1697765]
63. Haugeto O, Ullensvang K, Levy LM, Chaudhry FA, Honore T, Nielsen M, Lehre KP, Danbolt NC. Brain glutamate transporter proteins form homomultimers. *The Journal of biological chemistry*. 1996; 271 (44):27715–27722. [PubMed: 8910364]
64. Tanaka K, Watake K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science*. 1997; 276 (5319):1699–1702. [PubMed: 9180080]
65. Gochenauer GE, Robinson MB. Dibutyryl-cAMP (dbcAMP) up-regulates astrocytic chloride-dependent L-[3H]glutamate transport and expression of both system xc(–) subunits. *Journal of neurochemistry*. 2001; 78 (2):276–286. [PubMed: 11461963]

66. Burdo J, Dargusch R, Schubert D. Distribution of the cystine/glutamate antiporter system xc⁻ in the brain, kidney, and duodenum. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 2006; 54 (5):549–557.10.1369/jhc.5A6840.2006 [PubMed: 16399997]
67. Piani D, Fontana A. Involvement of the cystine transport system xc⁻ in the macrophage-induced glutamate-dependent cytotoxicity to neurons. *Journal of immunology (Baltimore, Md : 1950)*. 1994; 152 (7):3578–3585.
68. Murphy TH, Schnaar RL, Coyle JT. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 1990; 4 (6):1624–1633. [PubMed: 2180770]
69. Massie A, Schallier A, Mertens B, Vermoesen K, Bannai S, Sato H, Smolders I, Michotte Y. Time-dependent changes in striatal xCT protein expression in hemi-Parkinson rats. *Neuroreport*. 2008; 19 (16):1589–1592.10.1097/WNR.0b013e328312181c [PubMed: 18806690]
70. Pow DV. Visualising the activity of the cystine-glutamate antiporter in glial cells using antibodies to aminoadipic acid, a selectively transported substrate. *Glia*. 2001; 34 (1):27–38. [PubMed: 11284017]
71. Lewerenz J, Hewett SJ, Huang Y, Lambros M, Gout PW, Kalivas PW, Massie A, Smolders I, Methner A, Pergande M, Smith SB, Ganapathy V, Maher P. The cystine/glutamate antiporter system x(c)⁻ in health and disease: from molecular mechanisms to novel therapeutic opportunities. *Antioxidants & redox signaling*. 2013; 18 (5):522–555.10.1089/ars.2011.4391 [PubMed: 22667998]
72. Bannai S. Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *The Journal of biological chemistry*. 1986; 261 (5):2256–2263. [PubMed: 2868011]
73. Sato H, Tamba M, Ishii T, Bannai S. Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *The Journal of biological chemistry*. 1999; 274 (17):11455–11458. [PubMed: 10206947]
74. Sagara JI, Miura K, Bannai S. Maintenance of neuronal glutathione by glial cells. *Journal of neurochemistry*. 1993; 61 (5):1672–1676. [PubMed: 8228986]
75. Watanabe H, Bannai S. Induction of cystine transport activity in mouse peritoneal macrophages. *The Journal of experimental medicine*. 1987; 165 (3):628–640. [PubMed: 2880923]
76. Deneke SM, Fanburg BL. Regulation of cellular glutathione. *The American journal of physiology*. 1989; 257 (4 Pt 1):L163–173. [PubMed: 2572174]
77. Lo M, Wang YZ, Gout PW. The x(c)⁻ cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases. *Journal of cellular physiology*. 2008; 215 (3):593–602.10.1002/jcp.21366 [PubMed: 18181196]
78. Shih AY, Erb H, Sun X, Toda S, Kalivas PW, Murphy TH. Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006; 26 (41):10514–10523.10.1523/jneurosci.3178-06.2006 [PubMed: 17035536]
79. Sato H, Shiya A, Kimata M, Maebara K, Tamba M, Sakakura Y, Makino N, Sugiyama F, Yagami K, Moriguchi T, Takahashi S, Bannai S. Redox imbalance in cystine/glutamate transporter-deficient mice. *The Journal of biological chemistry*. 2005; 280 (45):37423–37429.10.1074/jbc.M506439200 [PubMed: 16144837]
80. Ogunrinu TA, Sontheimer H. Hypoxia increases the dependence of glioma cells on glutathione. *The Journal of biological chemistry*. 2010; 285 (48):37716–37724.10.1074/jbc.M110.161190 [PubMed: 20858898]
81. Meister A. Glutathione metabolism and its selective modification. *The Journal of biological chemistry*. 1988; 263 (33):17205–17208. [PubMed: 3053703]
82. Singh S, Khan AR, Gupta AK. Role of glutathione in cancer pathophysiology and therapeutic interventions. *Journal of experimental therapeutics & oncology*. 2012; 9 (4):303–316. [PubMed: 22545423]
83. Benjamin AM, Quastel JH. Metabolism of amino acids and ammonia in rat brain cortex slices in vitro: a possible role of ammonia in brain function. *Journal of neurochemistry*. 1975; 25 (3):197–206. [PubMed: 1159417]

84. Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. *Science*. 1977; 195 (4284):1356–1358. [PubMed: 14400]
85. Chaudhry FA, Reimer RJ, Edwards RH. The glutamine commute: take the N line and transfer to the A. *The Journal of cell biology*. 2002; 157 (3):349–355.10.1083/jcb.200201070 [PubMed: 11980913]
86. Jenstad M, Quazi AZ, Zilberter M, Haglerod C, Berghuis P, Saddique N, Gojny M, Buntup D, Davanger S, FMSH, Barnes CA, McNaughton BL, Ottersen OP, Storm-Mathisen J, Harkany T, Chaudhry FA. System A transporter SAT2 mediates replenishment of dendritic glutamate pools controlling retrograde signaling by glutamate. *Cerebral cortex* (New York, NY : 1991). 2009; 19 (5):1092–1106.10.1093/cercor/bhn151
87. Kvamme E, Torgner IA, Roberg B. Kinetics and localization of brain phosphate activated glutaminase. *J Neurosci Res*. 2001; 66 (5):951–958. [PubMed: 11746423]
88. Svenneby G. Pig brain glutaminase: purification and identification of different enzyme forms. *Journal of neurochemistry*. 1970; 17 (11):1591–1599. [PubMed: 5474660]
89. Bellocchio EE, Hu H, Pohorille A, Chan J, Pickel VM, Edwards RH. The localization of the brain-specific inorganic phosphate transporter suggests a specific presynaptic role in glutamatergic transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1998; 18 (21):8648–8659. [PubMed: 9786972]
90. Fremeau RT Jr, Troyer MD, Pahner I, Nygaard GO, Tran CH, Reimer RJ, Bellocchio EE, Fortin D, Storm-Mathisen J, Edwards RH. The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron*. 2001; 31 (2):247–260. [PubMed: 11502256]
91. Nakanishi T, Sugawara M, Huang W, Martindale RG, Leibach FH, Ganapathy ME, Prasad PD, Ganapathy V. Structure, function, and tissue expression pattern of human SN2, a subtype of the amino acid transport system N. *Biochemical and biophysical research communications*. 2001; 281 (5):1343–1348.10.1006/bbrc.2001.4504 [PubMed: 11243884]
92. Cubelos B, Gonzalez-Gonzalez IM, Gimenez C, Zafra F. Amino acid transporter SNAT5 localizes to glial cells in the rat brain. *Glia*. 2005; 49 (2):230–244.10.1002/glia.20106 [PubMed: 15390093]
93. Chaudhry FA, Reimer RJ, Krizaj D, Barber D, Storm-Mathisen J, Copenhagen DR, Edwards RH. Molecular analysis of system N suggests novel physiological roles in nitrogen metabolism and synaptic transmission. *Cell*. 1999; 99 (7):769–780. [PubMed: 10619430]
94. Boulland JL, Osen KK, Levy LM, Danbolt NC, Edwards RH, Storm-Mathisen J, Chaudhry FA. Cell-specific expression of the glutamine transporter SN1 suggests differences in dependence on the glutamine cycle. *The European journal of neuroscience*. 2002; 15 (10):1615–1631. [PubMed: 12059969]
95. Boulland JL, Rafiki A, Levy LM, Storm-Mathisen J, Chaudhry FA. Highly differential expression of SN1, a bidirectional glutamine transporter, in astroglia and endothelium in the developing rat brain. *Glia*. 2003; 41 (3):260–275.10.1002/glia.10188 [PubMed: 12528181]
96. Martinez-Lozada Z, Guillem AM, Flores-Mendez M, Hernandez-Kelly LC, Vela C, Meza E, Zepeda RC, Caba M, Rodriguez A, Ortega A. GLAST/EAAT1-induced glutamine release via SNAT3 in Bergmann glial cells: evidence of a functional and physical coupling. *Journal of neurochemistry*. 2013; 125 (4):545–554.10.1111/jnc.12211 [PubMed: 23418736]
97. Nissen-Meyer LS, Popescu MC, Hamdani el H, Chaudhry FA. Protein kinase C-mediated phosphorylation of a single serine residue on the rat glial glutamine transporter SN1 governs its membrane trafficking. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011; 31 (17):6565–6575.10.1523/jneurosci.3694-10.2011 [PubMed: 21525297]
98. Nissen-Meyer LS, Chaudhry FA. Protein Kinase C Phosphorylates the System N Glutamine Transporter SN1 (Slc38a3) and Regulates Its Membrane Trafficking and Degradation. *Frontiers in endocrinology*. 2013; 4:138.10.3389/fendo.2013.00138 [PubMed: 24106489]
99. Broer A, Deitmer JW, Broer S. Astroglial glutamine transport by system N is upregulated by glutamate. *Glia*. 2004; 48 (4):298–310.10.1002/glia.20081 [PubMed: 15390112]
100. Coulter DA, Eid T. Astrocytic regulation of glutamate homeostasis in epilepsy. *Glia*. 2012; 60 (8):1215–1226.10.1002/glia.22341 [PubMed: 22592998]

101. Albrecht J, Sidoryk-Wegrzynowicz M, Zielinska M, Aschner M. Roles of glutamine in neurotransmission. *Neuron glia biology*. 2010; 6 (4):263–276.10.1017/s1740925x11000093 [PubMed: 22018046]
102. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron*. 1988; 1 (8):623–634. [PubMed: 2908446]
103. Nicholls D, Attwell D. The release and uptake of excitatory amino acids. *Trends in pharmacological sciences*. 1990; 11 (11):462–468. [PubMed: 1980041]
104. Herman MA, Jahr CE. Extracellular glutamate concentration in hippocampal slice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27 (36):9736–9741.10.1523/jneurosci.3009-07.2007 [PubMed: 17804634]
105. Jabaudon D, Shimamoto K, Yasuda-Kamatani Y, Scanziani M, Gahwiler BH, Gerber U. Inhibition of uptake unmasks rapid extracellular turnover of glutamate of nonvesicular origin. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96 (15):8733–8738. [PubMed: 10411944]
106. Choi DW. Excitotoxic cell death. *Journal of neurobiology*. 1992; 23 (9):1261–1276.10.1002/neu.480230915 [PubMed: 1361523]
107. Fujikawa DG. Prolonged seizures and cellular injury: understanding the connection. *Epilepsy & behavior : E&B*. 2005; 7(Suppl 3):S3–11.10.1016/j.yebeh.2005.08.003
108. Mattson MP. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Annals of the New York Academy of Sciences*. 2008; 1144:97–112.10.1196/annals.1418.005 [PubMed: 19076369]
109. Torp R, Arvin B, Le Peillet E, Chapman AG, Ottersen OP, Meldrum BS. Effect of ischaemia and reperfusion on the extra- and intracellular distribution of glutamate, glutamine, aspartate and GABA in the rat hippocampus, with a note on the effect of the sodium channel blocker BW1003C87. *Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale*. 1993; 96 (3):365–376.
110. Lipton P. Ischemic cell death in brain neurons. *Physiological reviews*. 1999; 79 (4):1431–1568. [PubMed: 10508238]
111. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *Journal of neurochemistry*. 1984; 43 (5):1369–1374. [PubMed: 6149259]
112. Rossi DJ, Oshima T, Attwell D. Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature*. 2000; 403 (6767):316–321.10.1038/35002090 [PubMed: 10659851]
113. Kim K, Lee SG, Kegelmann TP, Su ZZ, Das SK, Dash R, Dasgupta S, Barral PM, Hedvat M, Diaz P, Reed JC, Stebbins JL, Pellicchia M, Sarkar D, Fisher PB. Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: opportunities for developing novel therapeutics. *Journal of cellular physiology*. 2011; 226 (10):2484–2493.10.1002/jcp.22609 [PubMed: 21792905]
114. Watkins S, Sontheimer H. Unique biology of gliomas: challenges and opportunities. *Trends in neurosciences*. 2012; 35 (9):546–556.10.1016/j.tins.2012.05.001 [PubMed: 22683220]
115. Chung WJ, Lyons SA, Nelson GM, Hamza H, Gladson CL, Gillespie GY, Sontheimer H. Inhibition of cystine uptake disrupts the growth of primary brain tumors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2005; 25 (31):7101–7110.10.1523/JNEUROSCI.5258-04.2005 [PubMed: 16079392]
116. de Groot JF, Liu TJ, Fuller G, Yung WK. The excitatory amino acid transporter-2 induces apoptosis and decreases glioma growth in vitro and in vivo. *Cancer research*. 2005; 65 (5):1934–1940.10.1158/0008-5472.CAN-04-3626 [PubMed: 15753393]
117. Ye ZC, Sontheimer H. Glioma cells release excitotoxic concentrations of glutamate. *Cancer research*. 1999; 59 (17):4383–4391. [PubMed: 10485487]
118. Dunlop J, Lou Z, McIlvain HB. Properties of excitatory amino acid transport in the human U373 astrocytoma cell line. *Brain research*. 1999; 839 (2):235–242. [PubMed: 10519046]
119. Ye ZC, Rothstein JD, Sontheimer H. Compromised glutamate transport in human glioma cells: reduction-mislocalization of sodium-dependent glutamate transporters and enhanced activity of

- cystine-glutamate exchange. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1999; 19 (24):10767–10777. [PubMed: 10594060]
120. Jimenez AL, Chou AH, Khadadadi O, Palos TP, Howard BD. Wnt-1 has multiple effects on the expression of glutamate transporters. *Neurochemistry international*. 2003; 42 (4):345–351. [PubMed: 12470708]
 121. Guo H. Human Glioma Cells and Undifferentiated Primary Astrocytes That Express Aberrant EAAT2 mRNA Inhibit Normal EAAT2 Protein Expression and Prevent Cell Death. *Molecular and Cellular Neuroscience*. 2002; 21 (4):546–560.10.1006/mcne.2002.1198 [PubMed: 12504589]
 122. Zschocke J, Allritz C, Engele J, Rein T. DNA methylation dependent silencing of the human glutamate transporter EAAT2 gene in glial cells. *Glia*. 2007; 55 (7):663–674.10.1002/glia.20497 [PubMed: 17311293]
 123. Palos TP, Ramachandran B, Boado R, Howard BD. Rat C6 and human astrocytic tumor cells express a neuronal type of glutamate transporter. *Brain research Molecular brain research*. 1996; 37 (1–2):297–303. [PubMed: 8738164]
 124. Marcus HJ, Carpenter KL, Price SJ, Hutchinson PJ. In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. *Journal of neuro-oncology*. 2010; 97 (1):11–23.10.1007/s11060-009-9990-5 [PubMed: 19714445]
 125. Savaskan NE, Seufert S, Hauke J, Trankle C, Eyupoglu IY, Hahnen E. Dissection of mitogenic and neurodegenerative actions of cystine and glutamate in malignant gliomas. *Oncogene*. 2011; 30 (1):43–53.10.1038/ncr.2010.391 [PubMed: 20802520]
 126. Takaki J, Fujimori K, Miura M, Suzuki T, Sekino Y, Sato K. L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the ‘collusion’ hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. *Journal of neuroinflammation*. 2012; 9:275.10.1186/1742-2094-9-275 [PubMed: 23259598]
 127. Medina MA, Sanchez-Jimenez F, Marquez J, Rodriguez Quesada A, Nunez de Castro I. Relevance of glutamine metabolism to tumor cell growth. *Molecular and cellular biochemistry*. 1992; 113 (1):1–15. [PubMed: 1640933]
 128. Portais JC, Martin M, Canioni P, Merle M. Glutathione, but not glutamine, is detected in ¹³C-NMR spectra of perchloric acid extracts from C6 glioma cells. *FEBS Lett*. 1993; 327 (3):301–306. [PubMed: 8394244]
 129. Sidoryk M, Matyja E, Dybel A, Zielinska M, Bogucki J, Jaskolski DJ, Liberski PP, Kowalczyk P, Albrecht J. Increased expression of a glutamine transporter SNAT3 is a marker of malignant gliomas. *Neuroreport*. 2004; 15 (4):575–578. [PubMed: 15094455]
 130. Dranoff G, Elion GB, Friedman HS, Campbell GL, Bigner DD. Influence of glutamine on the growth of human glioma and medulloblastoma in culture. *Cancer research*. 1985; 45 (9):4077–4081. [PubMed: 2862994]
 131. Seltzer MJ, Bennett BD, Joshi AD, Gao P, Thomas AG, Ferraris DV, Tsukamoto T, Rojas CJ, Slusher BS, Rabinowitz JD, Dang CV, Riggins GJ. Inhibition of glutaminase preferentially slows growth of glioma cells with mutant IDH1. *Cancer research*. 2010; 70 (22):8981–8987.10.1158/0008-5472.can-10-1666 [PubMed: 21045145]
 132. Rzeski W, Turski L, Ikonomidou C. Glutamate antagonists limit tumor growth. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98 (11):6372–6377.10.1073/pnas.091113598 [PubMed: 11331750]
 133. D’Onofrio M, Arcella A, Bruno V, Ngomba RT, Battaglia G, Lombardi V, Ragona G, Calogero A, Nicoletti F. Pharmacological blockade of mGlu2/3 metabotropic glutamate receptors reduces cell proliferation in cultured human glioma cells. *Journal of neurochemistry*. 2003; 84 (6):1288–1295. [PubMed: 12614329]
 134. Ishiuchi S, Tsuzuki K, Yoshida Y, Yamada N, Hagimura N, Okado H, Miwa A, Kurihara H, Nakazato Y, Tamura M, Sasaki T, Ozawa S. Blockage of Ca(2+)-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. *Nature medicine*. 2002; 8 (9):971–978.10.1038/nm746

135. Matute C, Arellano RO, Conde-Guerri B, Miledi R. mRNA coding for neurotransmitter receptors in a human astrocytoma. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; 89 (8):3399–3403. [PubMed: 1348861]
136. Patt S, Labrakakis C, Bernstein M, Weydt P, Cervos-Navarro J, Nisch G, Kettenmann H. Neuron-like physiological properties of cells from human oligodendroglial tumors. *Neuroscience*. 1996; 71 (2):601–611. [PubMed: 9053811]
137. Labrakakis C, Patt S, Weydt P, Cervos-Navarro J, Meyer R, Kettenmann H. Action potential-generating cells in human glioblastomas. *Journal of neuropathology and experimental neurology*. 1997; 56 (3):243–254. [PubMed: 9056538]
138. Labrakakis C, Patt S, Hartmann J, Kettenmann H. Glutamate receptor activation can trigger electrical activity in human glioma cells. *The European journal of neuroscience*. 1998; 10 (6): 2153–2162. [PubMed: 9753101]
139. Frithz G, Ronquist G, Hugosson R. Perspectives of adenylate kinase activity and glutathione concentration in cerebrospinal fluid of patients with ischemic and neoplastic brain lesions. *European neurology*. 1982; 21 (1):41–47. [PubMed: 6284509]
140. Boveris A, Oshino N, Chance B. The cellular production of hydrogen peroxide. *The Biochemical journal*. 1972; 128 (3):617–630. [PubMed: 4404507]
141. Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. *Critical reviews in clinical laboratory sciences*. 2006; 43 (2):143–181.10.1080/10408360500523878 [PubMed: 16517421]
142. Feun LG, Savaraj N, Landy HJ. Drug resistance in brain tumors. *Journal of neuro-oncology*. 1994; 20 (2):165–176. [PubMed: 7807193]
143. Bump EA, Brown JM. Role of glutathione in the radiation response of mammalian cells in vitro and in vivo. *Pharmacology & therapeutics*. 1990; 47 (1):117–136. [PubMed: 2195553]
144. Brouazin-Jousseau V, Guitton N, Legue F, Chenal C. GSH level and IL-6 production increased in Sertoli cells and astrocytes after gamma irradiation. *Anticancer research*. 2002; 22 (1A):257–262. [PubMed: 12017299]
145. Kim JY, Kanai Y, Chairoungdua A, Cha SH, Matsuo H, Kim DK, Inatomi J, Sawa H, Ida Y, Endou H. Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochimica et biophysica acta*. 2001; 1512 (2):335–344. [PubMed: 11406111]
146. Oh MC, Kim JM, Safaei M, Kaur G, Sun MZ, Kaur R, Celli A, Mauro TM, Parsa AT. Overexpression of calcium-permeable glutamate receptors in glioblastoma derived brain tumor initiating cells. *PloS one*. 2012; 7 (10):e47846.10.1371/journal.pone.0047846 [PubMed: 23110111]
147. Maas S, Patt S, Schrey M, Rich A. Underediting of glutamate receptor GluR-B mRNA in malignant gliomas. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98 (25):14687–14692.10.1073/pnas.251531398 [PubMed: 11717408]
148. Millan A, Arias-Montano JA, Mendez JA, Hernandez-Kelly LC, Ortega A. Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors signaling complexes in Bergmann glia. *J Neurosci Res*. 2004; 78 (1):56–63.10.1002/jnr.20237 [PubMed: 15372493]
149. Millan A, Aguilar P, Mendez JA, Arias-Montano JA, Ortega A. Glutamate activates PP125(FAK) through AMPA/kainate receptors in Bergmann glia. *J Neurosci Res*. 2001; 66 (4):723–729. [PubMed: 11746393]
150. Golubovskaya VM, Huang G, Ho B, Yemma M, Morrison CD, Lee J, Eliceiri BP, Cance WG. Pharmacologic blockade of FAK autophosphorylation decreases human glioblastoma tumor growth and synergizes with temozolomide. *Molecular cancer therapeutics*. 2013; 12 (2):162–172.10.1158/1535-7163.mct-12-0701 [PubMed: 23243059]
151. Hecker TP, Grammer JR, Gillespie GY, Stewart J Jr, Gladson CL. Focal adhesion kinase enhances signaling through the Shc/extracellular signal-regulated kinase pathway in anaplastic astrocytoma tumor biopsy samples. *Cancer research*. 2002; 62 (9):2699–2707. [PubMed: 11980671]
152. Lindemann C, Hackmann O, Delic S, Schmidt N, Reifenberger G, Riemenschneider MJ. SOCS3 promoter methylation is mutually exclusive to EGFR amplification in gliomas and promotes

- glioma cell invasion through STAT3 and FAK activation. *Acta neuropathologica*. 2011; 122 (2): 241–251.10.1007/s00401-011-0832-0 [PubMed: 21590492]
153. Yoshida Y, Tsuzuki K, Ishiuchi S, Ozawa S. Serum-dependence of AMPA receptor-mediated proliferation in glioma cells. *Pathology international*. 2006; 56 (5):262–271.10.1111/j.1440-1827.2006.01954.x [PubMed: 16669875]
 154. de Groot JF, Piao Y, Lu L, Fuller GN, Yung WK. Knockdown of GluR1 expression by RNA interference inhibits glioma proliferation. *Journal of neuro-oncology*. 2008; 88 (2):121–133.10.1007/s11060-008-9552-2 [PubMed: 18317690]
 155. Ciccarelli R, Sureda FX, Casabona G, Di Iorio P, Caruso A, Spinella F, Condorelli DF, Nicoletti F, Caciagli F. Opposite influence of the metabotropic glutamate receptor subtypes mGlu3 and -5 on astrocyte proliferation in culture. *Glia*. 1997; 21 (4):390–398. [PubMed: 9419014]
 156. Lee SG, Kim K, Kegelman TP, Dash R, Das SK, Choi JK, Emdad L, Howlett EL, Jeon HY, Su ZZ, Yoo BK, Sarkar D, Kim SH, Kang DC, Fisher PB. Oncogene AEG-1 promotes glioma-induced neurodegeneration by increasing glutamate excitotoxicity. *Cancer research*. 2011; 71 (20):6514–6523.10.1158/0008-5472.CAN-11-0782 [PubMed: 21852380]
 157. Gegelashvili G, Dehnes Y, Danbolt NC, Schousboe A. The high-affinity glutamate transporters GLT1, GLAST, and EAAT4 are regulated via different signalling mechanisms. *Neurochemistry international*. 2000; 37 (2–3):163–170. [PubMed: 10812201]
 158. Sontheimer H. A role for glutamate in growth and invasion of primary brain tumors. *Journal of neurochemistry*. 2008; 105 (2):287–295.10.1111/j.1471-4159.2008.05301.x [PubMed: 18284616]
 159. Behrens PF, Langemann H, Strohschein R, Draeger J, Hennig J. Extracellular glutamate and other metabolites in and around RG2 rat glioma: an intracerebral microdialysis study. *Journal of neuro-oncology*. 2000; 47 (1):11–22. [PubMed: 10930095]
 160. Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M. Glutamate release promotes growth of malignant gliomas. *Nature medicine*. 2001; 7 (9):1010–1015.10.1038/nm0901-1010
 161. van Breemen MS, Rijsman RM, Taphoorn MJ, Walchenbach R, Zwinkels H, Vecht CJ. Efficacy of anti-epileptic drugs in patients with gliomas and seizures. *Journal of neurology*. 2009; 256 (9): 1519–1526.10.1007/s00415-009-5156-9 [PubMed: 19434440]
 162. Matthew E, Sherwin AL, Welner SA, Odusote K, Stratford JG. Seizures following intracranial surgery: incidence in the first post-operative week. *The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques*. 1980; 7 (4):285–290. [PubMed: 7214242]
 163. Beaumont A, Whittle IR. The pathogenesis of tumour associated epilepsy. *Acta neurochirurgica*. 2000; 142 (1):1–15. [PubMed: 10664370]
 164. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*. 1993; 341 (8861):1607–1610. [PubMed: 8099987]
 165. Fan G, Sun B, Wu Z, Guo Q, Guo Y. In vivo single-voxel proton MR spectroscopy in the differentiation of high-grade gliomas and solitary metastases. *Clinical Radiology*. 2004; 59 (1): 77–85.10.1016/j.crad.2003.08.006 [PubMed: 14697379]
 166. Senner V, Kohling R, Puttmann-Cyrus S, Straub H, Paulus W, Speckmann EJ. A new neurophysiological/neuropathological ex vivo model localizes the origin of glioma-associated epileptogenesis in the invasion area. *Acta neuropathologica*. 2004; 107 (1):1–7.10.1007/s00401-003-0771-5 [PubMed: 13680280]
 167. Campbell SL, Buckingham SC, Sontheimer H. Human glioma cells induce hyperexcitability in cortical networks. *Epilepsia*. 2012; 53 (8):1360–1370.10.1111/j.1528-1167.2012.03557.x [PubMed: 22709330]
 168. Bianchi MG, Franchi-Gazzola R, Reia L, Allegri M, Uggeri J, Chiu M, Sala R, Bussolati O. Valproic acid induces the glutamate transporter excitatory amino acid transporter-3 in human oligodendroglioma cells. *Neuroscience*. 2012; 227:260–270.10.1016/j.neuroscience.2012.09.055 [PubMed: 23041758]
 169. Aguirre G, Rosas S, Lopez-Bayghen E, Ortega A. Valproate-dependent transcriptional regulation of GLAST/EAAT1 expression: involvement of Ying-Yang 1. *Neurochemistry international*. 2008; 52 (7):1322–1331.10.1016/j.neuint.2008.01.015 [PubMed: 18336953]

170. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*. 2005; 433 (7021): 73–77.10.1038/nature03180 [PubMed: 15635412]
171. Jacobs VL, De Leo JA. Increased glutamate uptake in astrocytes via propentofylline results in increased tumor cell apoptosis using the CNS-1 glioma model. *J Neurooncol*. 2013; 114 (1):33–42.10.1007/s11060-013-1158-7 [PubMed: 23695515]
172. Sattler R, Tyler B, Hoover B, Coddington LT, Recinos V, Hwang L, Brem H, Rothstein JD. Increased expression of glutamate transporter GLT-1 in peritumoral tissue associated with prolonged survival and decreases in tumor growth in a rat model of experimental malignant glioma. *Journal of neurosurgery*. 2013; 119 (4):878–886.10.3171/2013.6.jns122319 [PubMed: 23909244]
173. Vanhoutte N, Abarca-Quinones J, Jordan BF, Gallez B, Maloteaux JM, Hermans E. Enhanced expression of the high affinity glutamate transporter GLT-1 in C6 glioma cells delays tumour progression in rat. *Experimental neurology*. 2009; 218 (1):56–63.10.1016/j.expneurol.2009.04.004 [PubMed: 19374901]
174. Vanhoutte N, Hermans E. Glutamate-induced glioma cell proliferation is prevented by functional expression of the glutamate transporter GLT-1. *FEBS Lett*. 2008; 582 (13):1847–1852.10.1016/j.febslet.2008.04.053 [PubMed: 18474242]
175. Iwamoto FM, Kreisl TN, Kim L, Duic JP, Butman JA, Albert PS, Fine HA. Phase 2 trial of talampanel, a glutamate receptor inhibitor, for adults with recurrent malignant gliomas. *Cancer*. 2010; 116 (7):1776–1782.10.1002/cncr.24957 [PubMed: 20143438]
176. Grossman SA, Ye X, Chamberlain M, Mikkelsen T, Batchelor T, Desideri S, Piantadosi S, Fisher J, Fine HA. Talampanel with standard radiation and temozolomide in patients with newly diagnosed glioblastoma: a multicenter phase II trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009; 27 (25):4155–4161.10.1200/jco.2008.21.6895 [PubMed: 19636006]

Fig. 1.

Glutamate transporters in the non-pathological brain. Two glutamate transporters, System x_c^- (SXC) and the Excitatory Amino Acid Transporters (EAAT) function in concert to support normal brain function and glutamate (Glu) homeostasis. SXC is a Na^+ -independent, Cl^- -dependent, cystine (Cys-Cys)/glutamate exchanger. SXC provides intracellular cysteine (Cys) for synthesis of glutathione (GSH). EAAT removes glutamate released into the extracellular space from SXC and other sources, maintaining extracellular glutamate homeostasis. EAAT also provides intracellular glutamate, which combines with cysteine (from SXC) and glycine (Gly) to form GSH, an important molecule for intracellular redox control and cellular protection. EAAT is a family of Na^+ -dependent transporters that exchange a proton and K^+ along with uptake of glutamate. In the non-pathological brain, SXC and EAAT complement each other by providing a glutamate gradient for SXC-mediated cystine uptake and prevention of glutamate toxicity in the surrounding brain.

Fig. 2.

Glutamate transporter and receptor expression and function in the non-pathological brain and in glioma. **a** In the non-pathological brain, astrocytes are in close proximity to the synaptic cleft of glutamatergic neurons. Glutamate (Glu) is released vesicularly from presynaptic neurons during synaptic transmission; Glu diffuses across the synaptic cleft, binds postsynaptic neuronal glutamate receptors (NMDAR, AMPAR, mGluR), and is then removed from the extracellular space by Excitatory Amino Acid Transporter (EAAT) glutamate transporters located on astrocytes (EAAT 1, 2) and neurons (EAAT 2). EAAT also balance the glutamate released through System x_c^- (SXC) in exchange for cystine (Cys_2) uptake. **b** The replacement of normal brain tissue by glioma cells disrupts the normal synaptic arrangement, which alters glutamate homeostasis. Glioma cells have increased SXC activity but lack EAAT function, greatly increasing peritumoral glutamate concentrations. Increased SXC benefits cells by providing increased cystine uptake, which after intracellular reduction to cysteine (Cys), is used for glutathione (GSH) synthesis. GSH, an intracellular antioxidant, is especially important in malignant cells as they neutralize reactive oxygen species (ROS) produced. Additionally, elevated extracellular glutamate interacts with glioma glutamate receptors (AMPA & mGluR) to stimulate cell proliferation, migration, and invasion. With the loss of glutamate homeostasis, neurons become susceptible to glutamate-mediated excitotoxicity and death as a result of overstimulation of neuronal glutamate receptors, which leads to uncontrolled intracellular Ca^{2+} rises, aberrant neuronal signaling, and ultimately excitotoxic neuronal death. Additionally, we hypothesize that glioma cells alter peritumoral astrocyte EAAT function, preventing them from being able to re-establish glutamate homeostasis, as they would in the non-pathological brain. (Image is not drawn to scale)

Fig. 3.

Glutamate transporters confer biological advantages to malignant gliomas. The upregulation of System x_c^- (SXC) and loss of Excitatory Amino Acid Transporter (EAAT) glutamate transporters in glioma cells alter glutamate homeostasis in the brain. SXC-mediated cystine (Cys) uptake provides substrate for synthesis of glutathione (GSH), an important intracellular antioxidant that promotes cell survival. Glutamate (Glu) released in exchange for cystine uptake increases extracellular glutamate concentrations. Excess glutamate works in an autocrine/paracrine manner by activating glutamate receptors on glioma cells to promote tumor growth and invasion. Similarly, glutamate overstimulates glutamate receptors on surrounding neurons, causing excitotoxic neuronal death, which creates space for further tumor growth. Tumor growth and neuronal excitotoxicity lead to tumor-associated seizures and epilepsy in patients with malignant glioma.