



## Glutamate in cancers: from metabolism to signaling

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### Abstract

Glutamine and glutamate are major bioenergy substrates for normal and cancer cell growth. Cancer cells need more biofuel than normal tissues for energy supply, anti-oxidation activity and biomass production. Genes related to metabolic chains in many cancers are somehow mutated, which makes cancer cells more glutamate dependent. Meanwhile, glutamate is an excitatory neurotransmitter for conducting signals through binding with different types of receptors in central neuron system. Interestingly, increasing evidences have shown involvement of glutamate signaling, guided through their receptors, in human malignancy. Dysregulation of glutamate transporters, such as excitatory amino acid transporter and cystine/glutamate antiporter system, also generates excessive extracellular glutamate, which in turn, activates glutamate receptors on cancer cells and results in malignant growth. These features make glutamate an attractive target for anti-cancer drug development with some glutamate targeted but blood brain barrier impermeable anti-psychosis drugs under consideration. We discussed the relevant progressions and drawbacks in this field herein.

**Keywords:** glutamate, cancers, metabolism, glutamate receptors, glutamate transporters

### Introduction

Fast growing cancer cells have a high demand for catabolites to produce ATP, maintain a reduction-oxidation balance and generate biomass. After the early finding of a metabolic difference between cancer and normal tissue by Warburg and colleagues, people started to notice that metabolism of cancer is not only different from that of normal tissue but also among different type of tumorous cells<sup>[1-2]</sup>. Rather a common feature of cancer metabolism is that it needs a nutrient that is not only a decent donor of carbon and nitrogen for production of energy sugar, lipids, nucleotide and

amine but also a precursor of an anti-oxidation by-product that can protect cancer cells from oxidative stress. Thus, glutamine appears to be a candidate. Glutamine is the most abundant amino acid precursor in circulation and the main vehicle for circulating ammonia in a nontoxic form<sup>[3]</sup>. In normal tissues and cancer cells, glutamine donates its amide ( $\gamma$  nitrogen) group firstly to become glutamic acid. Then carbon skeleton of glutamate can be incorporated into  $\alpha$ -ketoacids for making ATP and fatty acids, while the nitrogen is used in the synthesis of purines and pyrimidines<sup>[4-5]</sup>. The glutamic acid is a precursor of ornithine, arginine and proline, and its skeleton can be

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used for synthesis of another non-essential amino acids through the Krebs's circle<sup>[4-5]</sup>. In addition, glutamate is a precursor of glutathione that is an antioxidant acting as a free radical scavenger and a detoxifying agent in cells<sup>[6-7]</sup>. It is known that reactive oxygen species (ROS like H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>•-</sup>, OH<sup>•</sup>, etc.) are significantly increased in cancer cells because of mitochondrial dysfunction and altered metabolism, which results in an accumulation of large amounts of oxidized protein, DNA, and lipids<sup>[6-7]</sup>. Therefore, as an adaptive response, cancer cells must harbor elevated levels of ROS-scavengers such as glutathione<sup>[6-7]</sup>.

### Glutamate receptors and transporters

Apart from the roles that glutamine plays in cancer cell metabolism, glutamic acid or its salt glutamate has been found to modulate cancer cell development, proliferation and metastasis through regulating cell signaling pathways<sup>[8-9]</sup>. Until recent decades, people have focused on studies of its function in the central nervous system (CNS) since glutamate has been found to be a key mediator of excitatory signals in the mammalian CNS<sup>[10-11]</sup>. It is known that neurons and non-neuron brain cells express different kinds of glutamate receptors that are classified into ionotropic (iGluR) and metabotropic glutamate receptors (mGluR) in the CNS<sup>[12]</sup>. The iGluRs are quaternary ligand-gate ion channels that allow cation influx upon glutamate binding, which are divided into 3 subfamilies: N-methyl-D-aspartate (NMDA) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and kainate (KA) receptor<sup>[13]</sup>. The mGluRs belong to the superfamily of G-protein coupled receptors, which are classified into three subgroups: group I, II and III. The mGluR1 and mGluR5 belong to group I mGluRs, and they are coupled to G<sub>q/11</sub> G-protein. Upon glutamate binding, G protein activates PLCβ to cause hydrolytic cleavage of phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) and then formation of inositol 1,4,5-triphosphate (IP<sub>3</sub>) and diacylglycerol. As a consequence, the secondary messenger leads to an increment of calcium release from endoplasmic reticulum stores and activation of protein kinase C, resulting in a downstream effector targeted stimulation. Group II receptor consists of mGluR2 and mGluR3, and group III comprises mGluR4, mGluR6, mGluR7 and mGluR8, all of which are coupled with G<sub>i/o</sub> G-protein that negatively regulates adenylate cyclase<sup>[12]</sup>.

In addition to these receptors, there are a group of glutamate transporters, termed as excitatory amino acid transporters (EAATs), which transport L- and D-

aspartate along with glutamate from extracellular environment to the cells and maintain low level of extracellular glutamate. EAATs are a family of high-affinity Na<sup>+</sup>/K<sup>+</sup>-dependent transporters for glutamate and aspartate with five EAATs, the EAAT1–5 in this family, which are encoded by genes *SLC1A3*, *SLC1A2*, *SLC1A1*, *SLC1A*, and *SLC1A7*, respectively ([https://en.wikipedia.org/wiki/Glutamate\\_transporter](https://en.wikipedia.org/wiki/Glutamate_transporter)). EAAT1 and EAAT2 are predominantly expressed in astrocytes, especially within their processes surrounding glutamatergic synapses, where they are responsible for the immediate uptake of synaptic released glutamate. EAAT3 is expressed in neurons, but in the kidney and the intestinal mucosa as well<sup>[14-15]</sup>. EAAT4 is predominantly expressed in cerebellar Purkinje cells, and EAAT5 is expressed in rod photoreceptor and bipolar cells of the retina. Another transporter, termed as antiporter, is Na<sup>+</sup>-independent cystine/glutamate exchanger, e.g. system Xc<sup>-</sup> (SXC), which uptakes extracellular cysteine and exports intracellular glutamate outside of the cells. However in recent decades, increasing evidences have unveiled that specific glutamate receptors and/or glutamate transporters are also expressed in non-neurological tissues and even in immune system, which suggests that glutamate plays an important role in the regulation of physiological function in various peripheral organs<sup>[16]</sup>. Further, a large amount of data have shown that glutamine and glutamate has been extensively involved in development and transformation of tumors in both CNS and peripheral tissues<sup>[9,17]</sup>. This is the subject of our current review including the involvement of glutamine and glutamate in cancer development and their potential therapeutic significance.

### Glutamine and glutamate in cancer metabolism

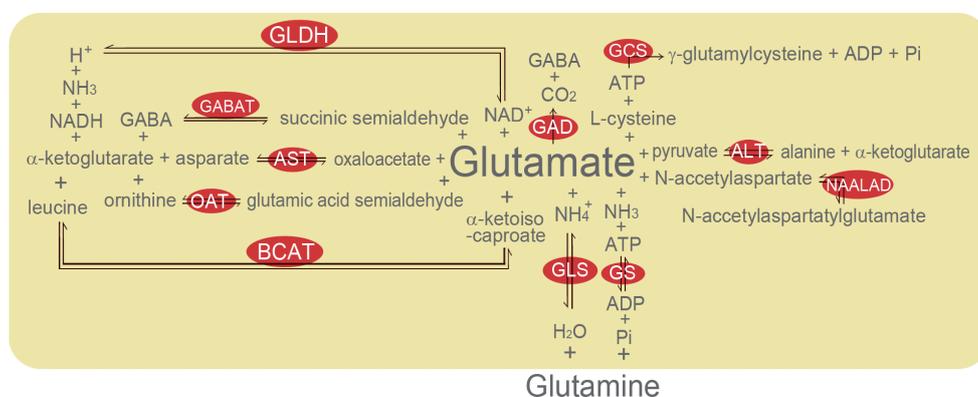
Altered energy metabolism is acknowledged as one of the emerging hallmarks of cancer. Initially, Warburg and co-workers showed that cancer tissues metabolize approximately 10-fold more glucose to lactate in a given time than normal tissues which led them to conclude that cellular respiration chain was damaged in cancer cells<sup>[1,18]</sup>. This proposal was argued for decades<sup>[1,18]</sup> and has been well accepted until recent years. Recently, people identified a number of mutated genes that encode enzymes functioning in cellular bioenergetics and biosynthesis metabolism in different cancer cells<sup>[19-21]</sup>. The fact that proliferating tumor cells are highly dependent on glutamine was firstly highlighted by Eagle in 1955<sup>[22]</sup>, who

discovered that the glutamine consumption rate in many cell lines exceeded the consumption of any other amino acid by 10-fold. Afterwards, Kovacevic and colleagues observed glutamine carbons was released by cells at the form of carbon dioxide, providing evidence that glutamine could serve as a combustible fuel<sup>[23]</sup>. Then, Reitzer and co-workers reported that Hela (derived from cervical cancer) cells predominantly consume glutamine as a biofuel<sup>[24]</sup>. These studies represented the earlier findings on substitution of glucose by glutamine in cancer bioenergetics metabolism. Of note, blood glutamine levels were increased in patients with advanced-stage cancers when serum compounds were being traced during cancer development<sup>[25]</sup>. Serum glutamate levels in prostate cancer patients were also correlated with Gleason score, which inferred the extent of progression of the prostate cancers<sup>[26]</sup>. An analysis of freshly frozen human samples also demonstrated that glutamate level is strikingly increased in chronic pancreatitis and pancreatic ductal adenocarcinoma tissues compared to that in normal pancreatic tissue<sup>[27]</sup>. Glutamate release was also found to be high in breast cancers, which makes it a new biomarker for breast cancer diagnosis<sup>[28]</sup>.

For better understanding the glutamate associated metabolism, the major reactions involved are listed as the following<sup>[29]</sup>: Aspartate aminotransferase reaction: Glutamate + oxaloacetate  $\leftrightarrow$   $\alpha$ -ketoglutarate + aspartate; Glutamate dehydrogenase reaction:  $\alpha$ -ketoglutarate + NH<sub>3</sub> + NADH + H<sup>+</sup>  $\leftrightarrow$  glutamate + NAD<sup>+</sup>; Glutamic acid decarboxylase reaction: Glutamate  $\rightarrow$  GABA + CO<sub>2</sub>; Glutaminase reaction: Glutamine + H<sub>2</sub>O  $\rightarrow$  glutamate + NH<sub>4</sub><sup>+</sup>; GABA transaminase reaction: GABA +  $\alpha$ -ketoglutarate  $\leftrightarrow$  glutamate + succinic semialdehyde; Glutamine

synthetase reaction: Glutamate + NH<sub>3</sub> + ATP  $\leftrightarrow$  glutamine + ADP + Pi;  $\gamma$ -glutamylcysteine synthetase reaction: Glutamate + L-cysteine + ATP  $\rightarrow$   $\gamma$ -glutamylcysteine + ADP + Pi; N-acetylated- $\alpha$ -linked-amino dipeptidase reaction: N-acetylaspartatyl-glutamate  $\leftrightarrow$  N-acetylaspartate + glutamate; Alanine aminotransferase reaction: Glutamate + pyruvate  $\leftrightarrow$  alanine +  $\alpha$ -ketoglutarate; Ornithine aminotransferase reaction: Ornithine +  $\alpha$ -ketoglutarate  $\leftrightarrow$  glutamate + glutamic acid semialdehyde; Branched-chain amino acid aminotransferase reaction:  $\alpha$ -ketoisocaproate + glutamate  $\leftrightarrow$  leucine +  $\alpha$ -ketoglutarate. Those are combined in **Fig. 1**.

The dependence of cancer cells on glutamine metabolism has made it an attractive anticancer therapeutic target<sup>[2,30-31]</sup>. Many glutamine mimetic antimetabolites (including acivicin, DON and azaserine) were developed and intended to become anti-cancer medicine before 1990s<sup>[5]</sup>. However, these substances deprive glutamine from both tumor and normal cells, which made them more toxic than therapeutic<sup>[31]</sup>. The pre-clinic trial for those substances generally failed, and then clinical development stopped. One exception was phenylacetate that can conjugate with glutamine in the blood plasma and form phenylacetylglutamine<sup>[32]</sup>. Long-term administration of phenylacetate was shown to reduce plasma glutamine levels and was well-tolerated<sup>[31-33]</sup>. Development of phenylacetate to be an anti-cancer reagent was stepped into phase I and II clinical trials in 2000s<sup>[34]</sup>. However, no promising report followed. Meanwhile, the mechanism on "glutamine-addiction" has been studied in efforts to find any key steps in mutant cancer metabolic pathways that differ from that of normal cells in host<sup>[2,31]</sup>. The findings from those works enlightened some new interest in the



**Fig. 1** Glutamine and glutamate metabolic reactions and key enzymes in cells. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BCAT: branched-chain amino acid aminotransferase; GABAT: GABA transaminase; GAD: glutamic acid decarboxylase; GCS:  $\gamma$ -glutamylcysteine synthetase; GLDH: glutamate dehydrogenase; GLS: glutaminase; GS: glutamine synthetase; NAALAD: N-acetylated- $\alpha$ -linked-amino dipeptidase; OAT: ornithine aminotransferase.

development of glutamine/glutamate associated anti-cancer therapeutic approaches. Among them, allosteric inhibitors of (kidney-type) glutaminase<sup>[35]</sup> have shown promise in preclinical models of cancer, such as CB839 that has shown efficacy against triple-negative breast cancer and haematological malignancies in preclinical studies<sup>[36]</sup>, and bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide that has been shown to block the growth of cancer cells *in vitro* and xenografts *in vivo*<sup>[37–38]</sup>.

## Glutamate receptors and cancers

### Association of iGluRs with cancers

The iGluRs family includes NMDA, AMPA, and KA receptors in the CNS<sup>[13]</sup>. The NMDA receptors are composed of two GluN1 subunits and two GluN2 subunits or a combination of a GluN2 and a GluN3 subunit<sup>[13]</sup> and activated upon binding to the glutamate and glycine on the cell membrane<sup>[39]</sup>. Activation of NMDA receptors allows calcium flux into the cells, which is thought to be critical in synaptic plasticity but not engaged in depolarizing membrane potential and initiation of action potential<sup>[40]</sup>. In recent years, both iGluRs and mGluRs have been found in the peripheral tissues outside of CNS, such as the kidney, lung, liver, heart, stomach, bone, and skin<sup>[9,16,41]</sup>. The roles they might play in tumor or cancer development are also broadly studied<sup>[9,42]</sup>. For example, NMDA receptor activation affects some cell-proliferation signaling activity, such as mTOR pathway that controls cell growth<sup>[43–44]</sup>, either through a Ca<sup>2+</sup> dependent regulation of p-ERK, which in turn modulates mTOR, or *via* inhibition of membrane cationic amino acid transporters<sup>[43–44]</sup>. This role of NMDA receptor in peripheral tissues may be an important contributor for human malignancies<sup>[45]</sup>.

Reasonably in the CNS, most of glutamate receptors and transporters including all EAATs are involved in the development of different types of glioma or neuroblastoma. The presence or lack of GluR2 subunits of the AMPA receptor appears to be crucial for glioma cell invasive potential. Previous studies have shown that the majority of invasive gliomas are either lack of GluR2 expression<sup>[46]</sup> or significantly lower level of its expression. Clinically, it was observed that GluR2 expression was lacking in highly malignant glioblastomas like ependymomas or medulloblastomas (all WHO III or IV), and a higher level of GluR2 was expressed in low-grade astrocytomas (WHO I or II)<sup>[47]</sup>. In addition, an RNA intervention study on a low-grade glioma cell line

revealed that down-regulation of GluR2 expression induced a significant increment of cell proliferation<sup>[48]</sup>. Nonetheless, absence of GluR2 in highly malignant pediatric glioblastomas had been verified clinically<sup>[47]</sup>. It is known that in neurons, GluR2 negative AMPA receptor is calcium permeable but a GluR2 composed one with a positive charged amino acid facing the channel is not permeable to divalent cation<sup>[49]</sup>. This is consistent with an observation that intracellular calcium oscillation plays a crucial role in growth and motility of a patient derived glioma cell line probably due to disassembly of focal adhesions triggered by this oscillation<sup>[50]</sup>; meanwhile, both PCR and Western blot test exhibited those cells lack of GluR2 in their AMPA receptors<sup>[46]</sup>.

In recent decades, iGluRs have been identified in many types of cancers outside the CNS where glutamate signaling stimulates tumor cell proliferation *via* iGluR activation<sup>[42,51]</sup>. The mRNA of iGluR subunits has been detected upregulated in thyroid, lung and breast carcinomas, multiple myeloma, colon adenocarcinoma, and T cell leukemia<sup>[52]</sup>, in larynx<sup>[53]</sup> and gastric cancer cells<sup>[54]</sup>, as well as in osteosarcoma<sup>[55]</sup>. The iGluR subunit proteins are also found elevated in prostate<sup>[56]</sup>, breast<sup>[57]</sup>, and lung<sup>[58]</sup> cancers, as well as in melanoma<sup>[59]</sup> and hepatocellular carcinoma cell lines<sup>[60]</sup>. Accordingly, iGluRs antagonists are applied in treatment of many kinds of cancers. For example, an NMDA receptor antagonist GYKI52466, showed anti-tumor effect in colon adenocarcinoma, astrocytoma, and breast and lung carcinoma cells<sup>[51]</sup>. NMDA receptor antagonists, memantine and MK-801, can reduce viability of the cells derived from a recurrent and drug-resistant small cell lung cancer<sup>[58]</sup>. An AMPA receptor antagonist CFM-2 is able to inhibit cancer cell growth by down-regulating the expression of survivin<sup>[61]</sup>. Talampanel is a newly developed non-competitive antagonist of the AMPA receptor that has shown some promising results for clinical use in cancer therapy<sup>[62–63]</sup>; encouragingly, a phase II clinic trial study of talampanel combined with radiation therapy plus the DNA methylation agent temozolomide has been performed for treatment of newly diagnosed glioblastoma patients<sup>[62–63]</sup>.

### Association of mGluRs with cancers

Similarly, ample evidences have shown the involvement of mGluRs in cancer development. Group I mGluRs are coupled to G<sub>q/11</sub> G-protein, and group II and III mGluRs are coupled to G<sub>i/o</sub> G-protein. All mGluRs share a basic structure that includes a large extracellular N-terminus, a cysteine-rich domain,

a seven-alpha-helical transmembrane domain and an intracellular C-terminal. The mGluRs function when two glutamate molecules bind the N-terminus and the cysteine-rich domains are dimerized<sup>[64]</sup>. In recent years, high-throughput cancer genome deep sequencing analyses have revealed that approximately 20% of all cancers harbor mutated G-protein coupled receptors, with mGluRs highly mutated in many types of cancers, and that specific mutations in *Gas*, *Gaq* and *Gα<sub>11</sub>* sites lead to constitutive and persistent aberrant signaling that may drive tumor progression<sup>[65]</sup>.

The function of mGluRs in human malignancy was first discovered in CNS tumors. In normal brain glial cells, mGlu1, mGlu3, and mGlu5 receptors are found in astrocytes, mGlu2, mGlu3, and mGlu5 receptors are expressed in microglial cells, and mGlu1 and mGlu4 are highly expressed in oligodendrocytes<sup>[66-67]</sup>. In brain tumors, over expression of mGluR1, mGluR2, and mGluR6 are observed in malignant medulloblastomas, ependymomas, and glioblastomas<sup>[47]</sup>. Expression or predominant expression of different groups and/or subunits of the mGluRs in different glioma cell lines and patient derived tumor cells has been reviewed in super detail by a large research group recently<sup>[68]</sup>. Noteworthy, a comprehensive study of pediatric brain tumors showed mGluR1, mGluR2 and mGluR6 expression levels are elevated in malignant medulloblastomas, ependymomas and glioblastomas, compared to those in low-grade astrocytomas<sup>[47]</sup>. This over expression pattern is similar among tumor cells of different histological origins, indicating that a common glutamatergic signaling cascade plays a key role in these cancers and could have potentials to be a therapeutic target in pediatric brain tumors<sup>[47]</sup>. On the other hand, pharmacological blockade of mGlu3 receptors by its antagonist LY341495 guided differentiation of highly malignant cultured glioblastoma cells into astrocyte lineage<sup>[69]</sup>. Consistently, clinical data suggest that transcript levels of mGluR3 may be a potential predictor for survival of glioblastoma patients and that mGluR3 antagonists could be recommended as adjuvant drugs for treatment of glioblastoma<sup>[70]</sup>. In a clinical study of 87 glioblastoma cases, the group of patients with low tumoral mGluR3 mRNA levels showed a significantly higher survival rate than high tumoral mGluR3 mRNA cases. Five patients who survived longer than 36 months had tumoral mGluR3 mRNA expression far below normal range<sup>[70]</sup>.

In addition to CNS neoplasms, mGluRs are expressed broadly in peripheral organs, such as the gastrointestinal tract, kidney, liver and immune cells,

and they play a role of commanding cell proliferation, differentiation and transformation<sup>[64]</sup>. In non-neuronal cancers, mGluRs are found elevated in melanoma, breast, prostate, renal and colorectal cancers, and osteosarcoma as well<sup>[71-73]</sup>; and over expression or activation of them promotes these cancers' growth. In contrast, activation of group III mGluR4 in bladder cancer has been reported to promote cell apoptosis and inhibit proliferation<sup>[74]</sup>. Notably, expression of mGluR1 in breast cancer has been broadly explored and mGluR1 is up-regulated in five triple-negative breast cancer cell lines compared with that in normal mammary epithelial cells<sup>[75]</sup>. Silencing mGluR1 results in reduction of tumor cell proliferation and progression, and induction of tumor cell apoptosis *in vitro* and *in vivo*<sup>[75]</sup>. Furthermore, over expression of mGluR1 in another breast cancer cell line, *e.g.*, MCF10AT1 cells, was reported to significantly increase the proliferation and facilitate cell migration and invasion<sup>[76]</sup>. Expression of mGluR1 is a specific feature of melanoma since approximately 80% of melanoma tissue samples express mGluR1, whereas normal melanocytes are mGluR1 negative<sup>[77]</sup>. Using an inducible mouse model, the authors found that induction of mGluR1 expression led melanocytes growth into melanoma, while silencing mGluR1 resulted in tumor abrogation<sup>[77]</sup>. Another group also showed downregulation of mGluR1 by shRNA decreased cell viability of human melanoma cells *in vitro* and tumor growth *in vivo* in a xenograft model<sup>[78]</sup>. Contrarily, stimulation of mGluR1 resulted in activation of MAPK and PI3K-AKT pathways, which enhanced cell proliferation but prevented apoptosis<sup>[79]</sup>. Accordingly, mGluR1 has been targeted for development of anti-cancer agents especially in treatment of breast cancer and melanoma<sup>[75,80-81]</sup>. However, clinical mGluR antagonist is still lacking, although mGluR1 antagonist LY367385 and BAY36-7620 or a potential antagonist riluzole have exhibited some anti-cancer effects *in vitro* and in mice xenograft<sup>[75,80,82]</sup>.

The mGluR2 and mGluR3 of Group II receptors are coupled to *Gai/o* and linked to the regulation of cyclic adenosine<sup>[64]</sup>. In malignant melanoma, mGluR3 was shown to be highly mutated and mutations in the seven transmembrane domain resulted in increased activation of MEK1/2 kinase with more aggressive migration and anchorage independence of melanoma cells *in vitro* and *in vivo*<sup>[83]</sup>. In a recent study from our lab, we unveiled that the mGluR3 was up-regulated in colon cancer cell lines *in vitro* and colon cancer tissues *in vivo*<sup>[84]</sup>. Further, inhibition of mGluR3 expression by shRNA or with antagonist LY341495

reduced anchorage dependent growth of colon cancer cells, and attenuated tumor growth in a xenograft model<sup>[84]</sup>. In the following study, we attempted to explore the possibility of using different mGluR3 antagonists and/or its signaling pathway inhibitor, combined with other anti-cancer reagents for the treatment of colon cancer and other malignancies<sup>[84]</sup>.

Significantly, in a clinical study on a large cohort of renal cell carcinoma samples with none-cancer controls, the authors detected gene expression of mGluR3, mGluR4 and mGluR5 on cancers of all participated patients<sup>[85]</sup>. Analysis of different risk factors implied associations of mGluR3, mGluR4 and mGluR5 with tumorigenesis and overall survivals<sup>[85]</sup>. Specifically, the mGluR4 and mGluR5 gene expression showed an increasing trend in carcinogenetic tissues, compared to that in normal tissue; whereas mGluR3 gene was down regulated in renal cancer cells<sup>[85]</sup>. The increased expression of mGluR4 gene and decreased expression of mGluR3 gene were associated with poorer survival of patients with renal cell carcinoma. Although there was a slight trend towards higher mGluR5 expression levels in cancer tissues than in adjacent normal tissues, GRM5 expression was not associated with survival of patients.

## EAATs and cancers

In the CNS, different glutamate transporters work together and maintain a low extracellular glutamate concentration at 0.3–1  $\mu\text{mol/L}$ , ten to several ten thousands fold lower than intracellular concentration which, for example, is 3–10  $\text{mmol/L}$  in neurons<sup>[86–87]</sup>. This gradient of glutamate is probably driven by the ionic gradients generated by ion-exchanging pumps such as  $\text{Na}^+/\text{K}^+$ -ATPase<sup>[86–87]</sup>. In general, glutamate is not able to diffuse across the cell membrane<sup>[14]</sup>. EAATs are also found in peripheral tissues; for instance, EAAT3, originally known as EAAC1, was reported to have mRNA expressed in the liver, the intestine, the kidney and the heart of rabbits<sup>[14]</sup>. Lately, a low level of EAAT1 mRNA was identified in the mouse liver, kidney and intestine, with relatively a little higher EAAT2 in the liver, much higher EAAT3 at intestine and highest EAAT3 in the kidney<sup>[88]</sup>. Moreover, broad expression of EAAT5 in the liver, kidney, intestine, heart, lung and skeletal muscle of rats has been identified at both mRNA and protein levels<sup>[89]</sup>. The similar data on humans is still lacking. Meanwhile cystine/glutamate exchanger SXC is known to express widely in many human tissues, including the brain, the pancreatic islets, and the

stromal and immune cells, and involvement of SXC in human carcinogenesis has been documented<sup>[90–91]</sup>. The uptake of cystine is significant for cells to maintain the intracellular levels of glutathione especially for the tissues that generate high levels of oxidative stress as in tumors<sup>[91]</sup>. However, the more cystine that is taken up through SXC, the more intracellular glutamate will be exchanged into extracellular spaces, which may be toxic to excitable cells. Consistently, overexpression and/or over activity of SXC transporter play pivotal roles in the growth and invasion of glioma cells by markedly enhancing extracellular glutamate concentration<sup>[46,90]</sup>.

Aberrant expression or activity of glutamate transporters is usually attributed to an imbalance of glutamate homeostasis in the CNS<sup>[92]</sup>. In brain tumors, EAAT2 is found down regulated in high-grade glial tumors compared with that in low-grade astrocytoma and normal brain<sup>[93]</sup>. Accordingly, EAAT2 expression is inversely correlated with tumor grade; re-expression of EAAT2 significantly prevents cell proliferation in several glioma cell lines<sup>[93]</sup>. In addition, lacking of EAAT1 is found in rat C6 glioma cell line, which increases releasing of glutamate to the medium<sup>[94]</sup>. In animal models, C6 clone cells release more glutamate and ensure more robust tumorigenesis comparing to cells that do not release glutamate<sup>[94]</sup>. In addition, SXC mutation is also involved in glioma development and migration<sup>[46,90–91]</sup>. Gliomas are aggressive cancers, whose growth is restricted by the bony cavity of the skull or spinal canal. Overexpression of SXC and/or deficiency of EAATs is able to help gliomas to overcome this physical limitation by secreting or gathering more glutamate, which kills neurons and vacates space for the growth of tumor cells<sup>[46,91]</sup>. Meanwhile, cystine uptake through this process sustains the synthesis of glutathione, and promotes glioma cells survival and growth<sup>[91]</sup>. In addition, choroid plexus tumors are rare neoplasms of neuroectodermal origin and their distinction from metastatic or secondary carcinomas is often difficult in adult cases<sup>[95]</sup>. A clinical study comparing patient primary tumor biopsies with metastatic samples found that EAAT1 expression in primary tumors was positively correlated to patient age but EAAT1 immunostaining in metastatic tumors was absent<sup>[96]</sup>. This finding indicates that EAAT1 immunohistochemistry may be useful in distinguishing original choroid plexus tumors from metastatic carcinomas.

Potential roles of EAATs and/or SXC have been suggested in some cancer types outside of the CNS, such as in pancreatic and gastric cancers, leukemias and lymphomas<sup>[15,91]</sup>. In a large clinical study of

primary gastric cancers plus most established cancer cell lines, *CD44-EAAT-2* gene fusions were detected in 1%–2% of primary tumors with zero expression in adjacent matched normal gastric tissues<sup>[97]</sup>. Further, silencing of this fusion gene led to reduction of intracellular glutamate level and sensitized cells to chemotherapy<sup>[97]</sup>. The role of SXC in leukemias and lymphomas seems peculiar since these tumor cells are incapable of synthesizing cysteine, which makes cystine/cysteine taken up from the micro-environment become essential amino acid for the viability and progression of such cancers<sup>[98]</sup>. However, clinically available specific activator or inhibitor for EAATs and/or SXC, especially for those mutated up- or down-regulated expression in cancers is still lacking. The drugs that can block glutamate release, such as riluzole<sup>[99–100]</sup>, has been used in therapeutic approach for many types of cancers.

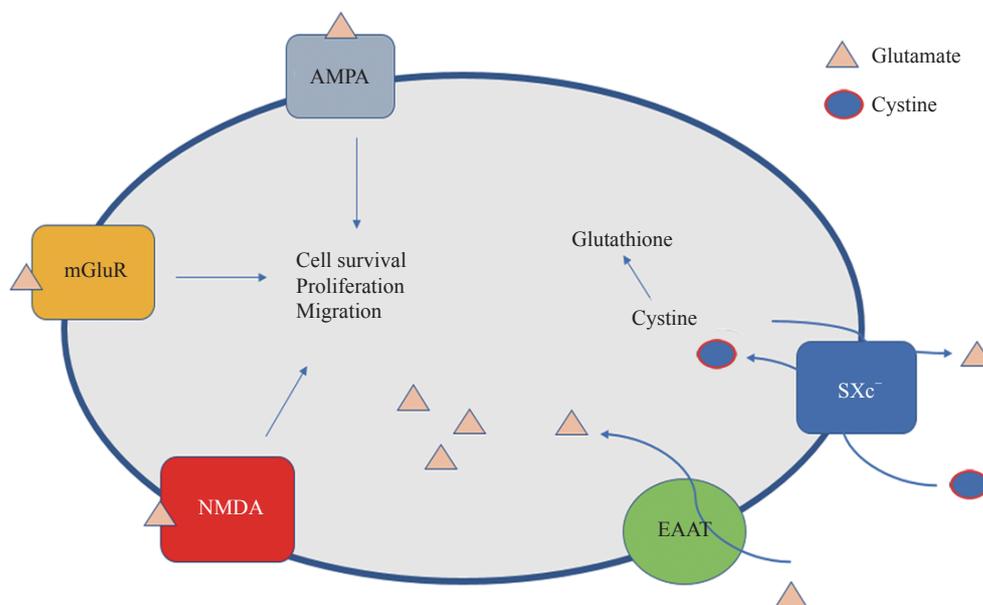
Riluzole is a US FDA approved drug originally for treatment of amyotrophic lateral sclerosis and it has a low toxicity in normal condition. Riluzole has shown promising anti-tumor effect in glioma, melanoma, breast and prostate cancers, and osteosarcoma as well<sup>[72,80–81,101]</sup>. Combination of riluzole with the other glutamate antagonistic drugs, such as memantine or valproate, is reported to have better effects for glioma treatment<sup>[101–102]</sup>.

Apart from it, a series of amino acid transporters, belonging to 4 distinct gene families: *SLC1*, *SLC6*,

*SLC7*, and *SLC38* have been reported to be involved in carcinogenesis of many different organs<sup>[103]</sup>. Bhutia and Ganapathy<sup>[103]</sup> has comprehensively reviewed the distribution of these transporters with their links to glutamate metabolism in different tissues, the involvement of their gene modification in carcinogenesis, and the potential roles of their gene and/or proteins therein.

## Conclusions

As aforementioned, the metabolic phenotype of cells within and around cancers is heterogeneous and distinct from their normal counterparts<sup>[2,30]</sup>. In order to target the glutamate metabolic chain in cancer but not severely disturb their normal counterparts, further study to find a tumor specific step in a metabolic chain is indispensable. Various kinds of mutations may occur in the cancer metabolic network<sup>[19–21,104]</sup> and these mutations may create specific therapeutic target for cancer treatment. The involvement of glutamate receptor-guided cell signaling and/or transporters in cancer development are well documented (**Fig. 2**) but clinically available antagonists for treatment of cancer still remain unclear. Actually, a set of glutamatergic signaling inhibitors or glutamate transmembrane traveling blocker, like LY341495, Riluzole and so on, were developed for therapy of neuropsychiatric disorders. Paradoxically, many of those antagonists



**Fig. 2** Glutamate signaling involved in tumorigenesis. Extracellular glutamate binds to glutamate receptors (AMPA, mGluRs and NMDA) and activates signal pathways, such as MAPK, PI3K, to promote cell proliferation, survival and migration. Of note, EAATs transport extracellular glutamate into cells. Contrarily, Xc<sup>-</sup>-glutamate antiporter exporting intracellular glutamate and importing extracellular cystine. Anabolism of cystine, L-glutamic acid and glycine produces glutathione that is a critical anti-oxidative element in cancer cells. AMPA:  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionate; mGluR: metabotropic glutamate receptors; NMDA: N-methyl-D-aspartate; EAAT: excitatory amino acid transporters; SXC<sup>-</sup>: system X<sub>c</sub><sup>-</sup>.

and/or inhibitors lack the ability to penetrate blood brain barriers, which makes them fairly problematic for treatment of neuropsychiatric disorders. However, this defect may make them a promising medicine for peripheral cancer treatment as there is no concern of the effects beyond the blood brain barrier. Notably, a metabolic imaging guided identification for "glutamine addicted" cancer cells has been suggested to distinguish glutamine dependent tumor cells from those less dependent ones. Since not all of cancers or all of cells in a diagnosed cancer are exactly glutamine dependent, more detailed identification for the extent of glutamine addiction is undoubtedly beneficial<sup>[105]</sup>. As <sup>18</sup>fluoro-dexoyglucose could indicate glucose metabolic level, using UDP-linked n-acetylglucosamine as positron could reflect the glutamine metabolic level in cancers or cancer cells<sup>[105]</sup>. As we discussed above, as a consequence of glutamine addition, glutamate metabolism might be elevated in some types of cancers, for example, enzalutamide-resistant prostate cancers and triple negative or heavily treated breast cancers<sup>[105]</sup>. However, there is still lack of evidence to show whether those types of cancers are more dependent on glutamate signaling for survival. But recent discoveries on glutamate signaling in brain as well as peripheral cancers shed light on the importance of glutamate in tumor development. Future research might help to build a clear connection between glutamate addiction and glutamate signaling in cancer. Consequently, anti-glutamate treatment for cancers would reach its maximum efficacy in cancers that are more glutamine dependent.

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