

## Molecular Pathways: Dysregulated Glutamatergic Signaling Pathways in Cancer

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### Abstract

The neurotransmitter glutamate interacts with glutamate receptor proteins, leading to the activation of multiple signaling pathways. Dysfunction in the glutamatergic signaling pathway is well established as a frequent player in diseases such as schizophrenia, Alzheimer disease, and brain tumors (gliomas). Recently, aberrant functioning of this pathway has also been shown in melanoma. In both glioma and melanoma, glutamate secretion stimulates tumor growth, proliferation, and survival through activation of the mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt pathways. In the future, extracellular glutamate levels and glutamatergic signaling may serve as biological markers for tumorigenicity and facilitate targeted therapy for melanoma. *Clin Cancer Res*; 18(16); 4240–6. ©2012 AACR.

### Background

Glutamate is a major excitatory neurotransmitter of the central nervous system (CNS) that potentiates processes such as neuronal precursor-cell proliferation, cellular homeostasis, synaptic transmission, and cell growth, migration, invasion, survival, and death (1–3). Extracellular glutamate binds to and activates multiple glutamate receptors, which can be divided into 2 major types: the ionotropic- (iGluR) and metabotropic-glutamate receptor (mGluR) families. The iGluR family consists of *N*-methyl-D-aspartate receptors (NMDAR),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoazolepropionic acid receptors (AMPA), and kainate receptors (KAR). The mGluR family consists of 8 different subtypes (mGluR1–8 or GRM1–8) that are subdivided into 3 different groups based on their sequence similarity and coupling to small G-proteins. Recent findings have shown expression of functional glutamate receptors in nonneuronal peripheral cells such as skin, placenta, and colon (4, 5). Furthermore, genomic and proteomic studies have revealed glutamate signaling to be the target of dysregulation in several different cancer types including melanoma thus implicating it in cancer tumorigenesis (5–8). These findings make glutamate receptors and their downstream effector molecules compelling candidates for further investigation with regard to their role in melanoma genesis and their potential as therapeutic targets. It must be noted that developmentally, cells of the central nervous system (CNS), such as glia, astrocytes, neurons, and melanocytes,

are derived from neural crest stem cells (9, 10). On the basis of this developmental lineage, we propose that glial and melanocytes share similar signaling pathways that are important for homeostasis, growth, proliferation, and survival, and thus may be subject to similar patterns of deregulation leading to disease.

The iGluRs consist of large complexes of multiprotein subunits that create ion channels in the cell plasma membranes, allowing for influx or efflux of mono- or divalent cations [e.g.,  $\text{Ca}^{2+}$  (11)]. Upon binding of glutamate, these ligand-gated channels change their conformation, giving rise to ion permeability and oscillations that in many neuronal cells (e.g., cerebellar granule cells, neurons, astrocytes, and glial cells) are important for synaptic transmissions and cellular migration and survival (1, 12, 13).

The NMDARs play a major role in many neuronal processes (e.g., learning and memory) and neurodegenerative diseases [e.g., schizophrenia (14)]. The NMDARs are heterotetrameric complexes that consist of 2 NR1 (GRIN1) and 2 NR2 (GRIN2A–D) subunits, or a mixture of GRIN2 and GRIN3 subunits predominantly expressed in neuronal cells. Upon binding of its cognate ligands, NMDAR permits  $\text{Ca}^{2+}$  influx, resulting in increased intracellular calcium levels and hence activation of calcium-dependent signal transduction (12). Moderate levels of intracellular  $\text{Ca}^{2+}$  influx lead to calmodulin (CaM) and CaM-dependent kinase (CaMK) activation, triggering increased protein kinase A (PKA) activity. However, hyperactivation of NMDAR by glutamate leads to calcium-dependent cell death (excitotoxicity), a phenomenon observed in neurodegenerative diseases and brain tumors (15, 16). Recent studies have shown the importance of functional NMDARs, as GRIN2A and GRIN2B were shown to be mutated with decreased channel permeation in patients with schizophrenia (17). Furthermore, a whole-exome screen of 14 tumor samples revealed that GRIN2A was highly mutated in patients with malignant melanoma (18).

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AMPA and KARs are involved in synaptic transmissions in the mammalian CNS and are important for short-term and long-term synaptic plasticity. Both types of receptors have been implicated in neuronal diseases such as seizures, epilepsy, and Alzheimer disease (19). AMPARs and KARs form hetero- or homomeric complexes that are found primarily in the pre- and/or postsynaptic regions of neuronal cells (12). Glutamate-mediated receptor activation allows for increased membrane permeability, resulting in  $\text{Ca}^{2+}$  influx and hence differential neuronal plasticity (short-term vs. long-term) and signaling mechanisms, culminating in variable pathway activation (13). Dysfunction of AMPA and KAR has been implicated as a possible candidate in certain tumorigenic models (20), but to date very little research has been done on the role of these receptors in melanoma genesis. Therefore, in the interest of time and space, this issue will not be discussed any further in detail.

The mGluR family is composed of 8 different subtypes called GRMs or mGluRs. The mGluR subtypes are subdivided into 3 groups (groups I, II, and III) based on sequence homology and intracellular effects potentiated by variable G-protein coupling (21). mGluRs are members of the G-protein coupled receptor (GPCR) superfamily. GPCRs are membrane-bound proteins that mediate signal transduction upon ligand binding and subsequent activation of their cognate trimeric G-protein complex ( $\alpha$ ,  $\beta$ , and  $\gamma$  subunits). Activation of the secondary messenger proteins, the GTP-bound  $G_\alpha$  subunit and  $G_{\beta\gamma}$  dimer, allows for transit stimulation of signaling pathways that are important for cellular proliferation, growth, migration, and survival, and calcium-mediated cellular homeostasis (21–23). Recently, investigators have implicated some of these receptors in both glioma and melanoma tumorigenesis through pharmacological inhibition studies, gain-of-function mutations, and overexpression experiments using transgenic mice (24–28).

Group I consists of mGluR1 (GRM1) and mGluR5 (GRM5). Group I mGluRs are coupled to  $G_{\alpha q}/G_{\alpha 11}$  G-proteins that upon glutamate-mediated activation result in stimulation of  $\text{PLC}_\beta$ , thus causing hydrolytic cleavage of phosphatidylinositol-4,5-bisphosphate ( $\text{PIP}_2$ ) and formation of inositol 1,4,5-triphosphate ( $\text{IP}_3$ ) and diacylglycerol (DAG). Release of these secondary messengers leads to increased calcium release from endoplasmic reticulum stores and activation of protein kinase C (PKC), resulting in downstream effector target stimulation.

Group II consists of mGluR2 (GRM2) and mGluR3 (GRM3), which are coupled to  $G_{\alpha i/o}$ . Activation of the  $G_{\alpha i/o}$  proteins causes  $G_{\alpha i/o}$ -mediated inhibition of adenylyl cyclase, leading to reduced production of cyclic adenosine monophosphate. The release of their cognate  $G_{\beta\gamma}$  subunit reduces certain ion channel properties and other downstream effector molecules (21). Group III contains the rest of the mGluRs (mGluR4 and mGluR6–8). These receptors are also coupled to the  $G_{\alpha i/o}$  proteins and potentiate or attenuate some of the same pathways that are targeted by group II mGluRs, including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt signaling pathways (29, 30).

### Glutamate Receptors as Novel Drivers in Cancer

Recent studies have shown the role played by glutamate receptors in neurodegenerative diseases and tumorigenesis (see Table 1). The concept that NMDAR functions as a conveyor of cellular signals that are important for cognitive learning and apoptosis has been well established in studies of neuronal diseases and brain tumors (13, 31, 32). A recent genetic screen of patients with schizophrenia by Ende and colleagues (17) revealed novel somatic mutations in the genes encoding either GRIN2A or GRIN2B. They showed that mutation of these NMDAR channel proteins resulted in suppression of ion permeability or channel conductance. They proposed that reduced  $\text{Ca}^{2+}$  influx may lead to reduced activation of  $\text{Ca}^{2+}$ -dependent signaling mechanisms, which may be the underlying cause of the disease. However, the causative effect seen in tumors is not the lack of  $\text{Ca}^{2+}$  or inhibition of influx, but rather an uncontrolled ion permeability in neuronal cells that leads to cell death and hence dramatic changes in the architecture of the surrounding tissue within the brain (15, 16).

It is well established that glioma cells secrete glutamate to the extracellular milieu where glioma cells and neurons reside both *in vitro* and *in vivo* (15). High concentrations of glutamate cause neurons to become permeable to  $\text{Ca}^{2+}$  ions. Overstimulation of glutamate receptors causes increased intracellular levels of  $\text{Ca}^{2+}$ , which leads to apoptosis and gives glioma cells room to grow and spread (16). The limitations for growth that gliomas face because of the nonexpandable nature of the cranium necessitate damage to and elimination of normal brain tissue. Recent research has shown that at the periphery of glioma tumor growth, one observes the highest levels of glutamate [found at micromolar levels, which is 10 times higher than the normal extracellular concentration seen in synapses (33)]. It is this prolonged activation or hyperactivation of glutamate receptors (especially NMDARs) on neuron cells via this excess extracellular glutamate that leads to the phenomenon called excitotoxicity (15, 16). Furthermore, the normal protective mechanism that helps maintain excess levels of glutamate in the extracellular space surrounding cells, which uses a  $\text{Na}^{2+}$ -dependent transport system, is diminished (34). The transporters responsible for this are collectively referred to as excitatory amino acid transporters 1–4 (EAAT1–4) and are typically found on astrocytic cells. Recent studies using samples from patients with glioma, and knockout studies in mice have clearly shown the role these transporters play in neurodegenerative diseases and glioma (35, 36). Collectively, these findings illustrate the major role glutamate receptors and transporters play in cellular homeostasis of neurons, glial cells, and astrocytes.

Recently, through the use of whole-exome sequencing, our laboratory revealed for the first time (to our knowledge) the high prevalence of somatic mutations in GRIN2A in malignant melanoma (18). Whole-exome sequencing of 14 tumor and normal matched samples from treatment-naïve patients with melanoma resulted in the unexpected discovery that the gene encoding for NMDAR subunit  $\epsilon$ -1 or

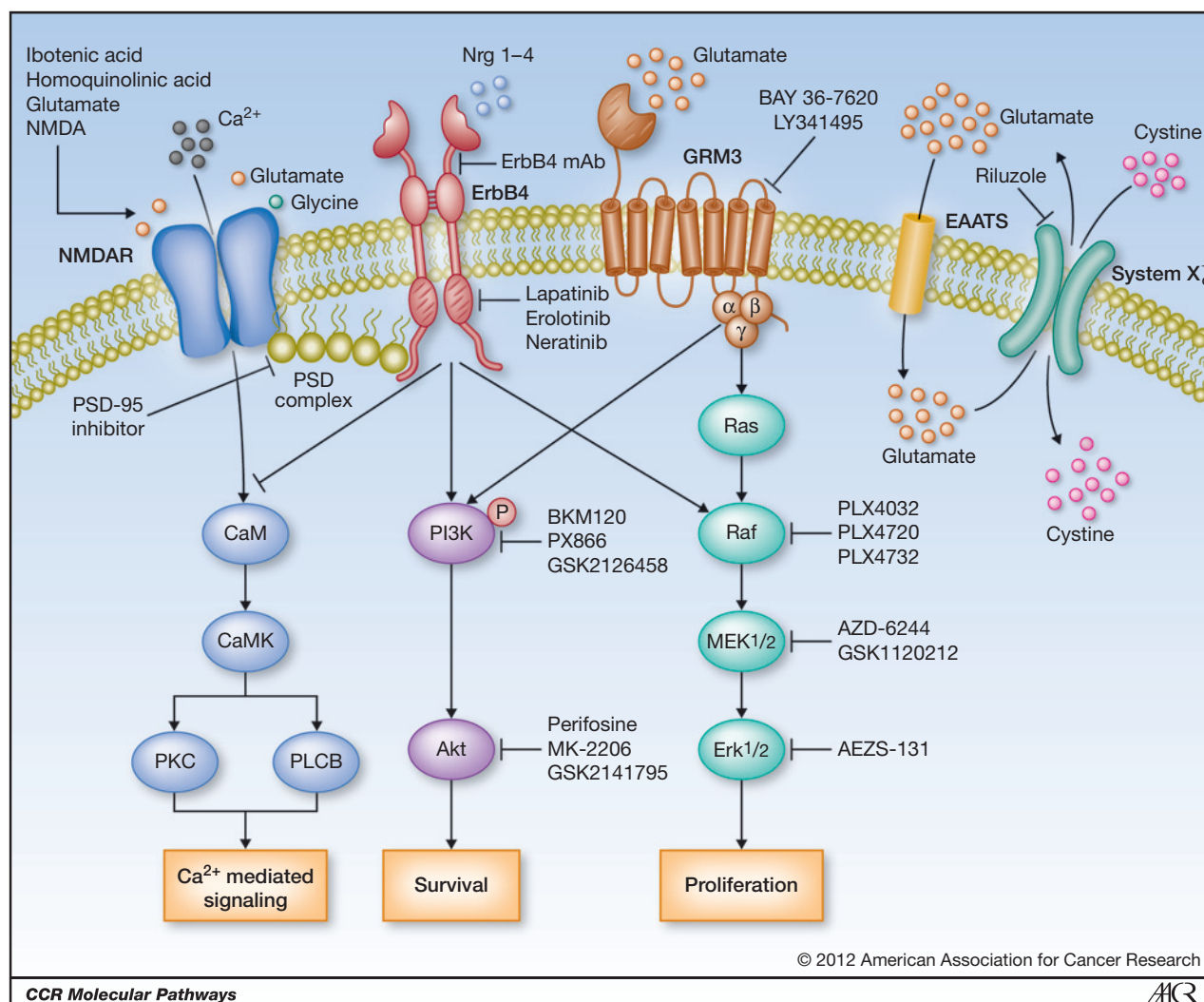
**Table 1.** Glutamate receptors in various diseases and malignancies

Glutamate receptor type	Subclass	Disease type	Genetic alteration	Possible mechanism	References
iGluR	GRIN1	malignant melanoma	somatic mutations	not reported	(18)
	GRIN2A/2B	schizophrenia	germline and <i>de novo</i> translocations	lack of proper ion conductance	(17)
	GRIN2A	malignant melanoma	somatic mutations (mini hotspots)	not reported	(18)
	GRIN3	malignant melanoma	somatic mutations	not reported	(18)
	GluR2	schizophrenia, Alzheimer disease, glioblastoma multiforme	downregulation of the GluR2 subunit, which is less permissive to Ca <sup>2+</sup> ion influx	deregulated AMPAR function	(21)
mGluR	mGluR1	malignant melanoma	possible amplification	deregulated overexpression	(26)
		breast cancer	not reported	inhibition with antagonists resulted in decreased breast cancer cell growth in a xenograft model	(47)
	mGluR2	glioma	not reported	mGluR2 activity important for glioma growth <i>in vitro/in vivo</i>	(24, 25)
	mGluR3	glioma	not reported	mGluR3 activity important for glioma growth <i>in vitro/in vivo</i>	(24, 25)
		malignant melanoma	somatic mutations (mini hotspots)	activating mutations leading to hypersensitivity to agonist stimulation of the MEK-MAPK pathway	(27)
	mGluR4	colorectal carcinoma	overexpression in tumor tissue	not reported	(46)
	mGluR5	malignant melanoma	overexpression	increased activation of MAPK pathway	(28)
	mGluR8	malignant melanoma	somatic mutations	not reported	(28)

*GRIN2A* harbored 34 somatic mutations in 125 melanoma samples (25.2%). The mutations were distributed throughout the gene, with clustering of mutations at amino acids 371, 372, and 373, or amino acids 1073, 1074, and 1076 within important functional domains. We also observed 3 recurrent alterations at S278F, E371K, and E1175K, as well as 5 nonsense mutations. Furthermore, another group recently published a whole-exome screen of 8 melanoma samples and found 2 additional somatic mutations in *GRIN2A*, suggesting that genetic alteration of this gene is important (37). We hypothesize that *GRIN2A* is a novel tumor suppressor in melanoma. The high percentage of somatic mutations that introduce a premature stop codon (in 15% of cases) would result in a truncated protein that could affect ion channel assembly and function. A preliminary functional assessment of mutant *GRIN2A* corroborates this hypothesis. This report by our group was the first to show that the glutamate signaling pathway is significantly altered in melanoma (Fig. 1). We have since identified additional genes within the glutamate pathway to be somatically mutated in melanoma, creating a broader picture of the genetic landscape that is important for deregulating this pathway. Our laboratory found *GRIN1*, *GRIN3*, *PLCβ4*, *GRM3*, *PYK2*, and *ERBB4* to be highly mutated in melanoma. Of interest, genetic findings in patients with schizophrenia implicated alterations in some of the same genes that were found to be mutated in melanoma (14, 17, 38).

We hypothesize that mutations in *GRIN1* and *GRIN3* result in loss of function, as proposed earlier for the somatic mutations observed in *GRIN2A*. In contrast, select mutations in *GRM3* (an mGluR) in melanoma resulted in activation of GPCR signaling to mitogen-activated protein–extracellular signal-regulated kinase (MEK)1/2, causing increased melanoma cell migration and anchorage-independent growth (27). *PLCβ* is directly downstream of both the NMDARs and GRM receptors, and is stimulated in a calcium-dependent manner via CaMKs. Somatic mutations in *PLCβ* may thus result in aberrant hydrolysis of PIP<sub>2</sub>. Lastly, we showed that mutation of *ErbB4* in melanoma cells causes constitutive kinase activity resulting in increased proliferation, transformation, and migration (39).

Pathway analysis and statistical tests of our melanoma exomes allowed us to identify the glutamate signaling pathway as being significantly deregulated in melanoma (18), thus integrating the genes described above as somatically mutated into one single pathway. A similar scenario was described by Hahn and colleagues (32), who showed a direct link between the glutamate receptor (NMDAR) and the receptor tyrosine kinase (RTK) *ErbB4* in patients with schizophrenia. Increased NRG1-induced *ErbB4* activity, causing attenuation of the NMDAR function, was observed in postmortem tissue samples from these patients. In agreement with these results, several recent reports have further shown a link between glutamate receptor signaling via the



**Figure 1.** Glutamate signaling pathways in melanoma. The genes that function in glutamate signaling are specified. Potential therapeutic intervention points are indicated by arrows or inhibition bars followed by a list of small-molecule inhibitors or proposed mAbs. System X<sub>c</sub><sup>-</sup>, cystine-glutamate cotransporter protein.

NMDAR and the cellular components of the postsynaptic density (PSD) complex, which consists of PSD-95, PSD-93, SAP-102/97, Pyk2, and Fyn (40–44). The PSD complex, through direct binding with the C-terminal tails in NMDAR2A (GRIN2A) and ErbB4, helps to localize this signalosome at the cellular/synaptic surface. To date, most research on the PSD complex has been done in neuronal-derived samples. However, we hypothesize that NMDAR and ErbB4 mediate downstream signal transduction via a cross-talk mechanism using a form of the PSD complex that is present in melanoma cells important for tumorigenesis (Fig. 1). In addition to the role of iGluR signaling in disease, support for a role in signaling through mGluRs in both neuronal and peripheral cells has also been established (1–5). Activation of mGluR1, mGluR3, mGluR4, and mGluR5 supports proliferation and survival of neural stem cells in fetal brain and neural regions of the adult brain, gliomas, breast cancer, colorectal carcinoma, and melanoma tumors through stimulation of the MAPK and PI3K/Akt signaling

pathways (16, 23–28, 30, 31). Studies by Pollock and colleagues (26) on glutamate signaling and melanoma have helped bring to light the important role played by mGluRs in the development of this disease. They first established the link using an insertional mutant mouse (TG3) that developed spontaneous melanoma in hairless regions. They showed that the insertion fell within intron 3 of the *grm1* gene in these mice, resulting in ectopic expression of mGluR1. To confirm that the effects of ectopic expression were directly responsible for melanoma development, Pollock and colleagues used a melanocyte-specific promoter to drive the expression of mGluR1 in transgenic mice, and they observed the same phenotype. A similar effect was observed in the hyperproliferation of melanocytes leading to transformation and malignant melanoma. Furthermore, Shin and colleagues (45) and Namkoong and colleagues (46) found that mGluR1-positive human melanoma and stably expressing mGluR1 melan-a cells secreted more extracellular glutamate compared with normal melanocytes or

control melan-a cells. They hypothesized that aberrant secretion of glutamate leads to activation of the mGluR1-dependent signaling pathways responsible for the aggressive tumorigenic phenotypes observed *in vitro* and *in vivo*.

The role of mGluR3 in glioma tumorigenesis has been well delineated in functional studies and studies using pharmacological antagonists specific for individual receptors (24, 25). mGluR3 expression is ubiquitous in glioma cells and glioblastomas. Treatment of glioma cells using a specific antagonist for group II mGluRs, LY341495, caused reduced cellular growth *in vitro* and *in vivo* via suppression of MAPK and Akt pathways. Recently, we revealed, through exon-capture sequencing of the GPCR superfamily in malignant melanoma, that *GRM3* is highly mutated (27). *GRM3* (or mGluR3) is a group II mGluR that is a 7-transmembrane (7-TM) receptor protein that binds glutamate as a ligand, resulting in a shift from an inactive to an active state. As described above, ligand binding induces activation of the cognate small G-protein via a conformational change, thus signaling to downstream effector molecules. The identified mutations were located throughout the coding region of the gene and affected the extracellular domains as well as the 7-TM domain, with 2 mini-hotspots (M547K and E807K) located proximal to the TM domains. We discovered that 4 of the nonsynonymous changes in the 7-TM domain (G561E, S610L, E767K, and E870K) resulted in increased activation of the MEK1/2 kinase, increased migration, and anchorage independence of melanoma cells. Furthermore, melanoma cells harboring mutant forms of *GRM3* exhibited reduced survival in the presence of the MEK1/2 inhibitor AZD-6244, and were sensitive to shRNA-mediated depletion compared with wild-type (WT) expressing cells in *in vitro* and *in vivo* assays. Expression of mGluRs is restricted to certain tissues and cell types in the CNS, highlighting the essential role they play in neurological processes (21). Hoogduijn and colleagues (4) showed that human melanocytes lack expression of most forms of mGluRs but express certain forms of NMDAR and AMPAR subunits, indicating a possible role for glutamate signaling in melanocyte homeostasis. The absence of mGluR1, mGluR2/3, mGluR4, and mGluR6–8 on melanocytes has been corroborated by others; however, Nicolletti and colleagues (47) reported the expression of functional mGluR5 receptors in cultured human melanocytes. They observed mGluR5-dependent cell proliferation and survival using specific group I mGluR agonists and antagonists. Of interest, a recent report by Choi and colleagues (28) implicated ectopic expression of mGluR5 in the development of melanoma in mice. The authors generated transgenic mice using 2 forms of mGluR5 (WT and S901A) to delineate the effects of PKC-dependent phosphorylation on mGluR5, and the role it plays in CaM binding and subsequent receptor function in Ca<sup>2+</sup> oscillations in cells. PKC phosphorylation on S901 in mGluR5 inhibits CaM binding and mGluR5-dependent Ca<sup>2+</sup> oscillations. They discovered that one founder mouse (mGluR5 S901A) exhibited pronounced formation of melanoma tumors and increased muscle and bone metastases. Using a melanocyte-specific

promoter to drive mGluR5 expression, they observed an increased incidence in mice with melanoma, with a 100% penetrance in the progeny for both WT and S901A mice. MAPK was activated in mice expressing mGluR5 under the TRP1 promoter, suggesting an increased presence of cellular signaling important for proliferation and growth.

### Clinical-Translational Advances

One of the most important advances for the melanoma field was the validation of c-KIT and BRAF as successful therapeutic targets in patients with melanoma (48–54). However, it is still important to search for additional melanoma drug targets, because not all patients harbor c-KIT or BRAF mutations, and even patients who harbor these mutations and show response to the inhibitors can develop resistance to the drug (55–58).

Our genomic screening and functional studies have shown the dysregulation of the glutamate receptor signaling pathway consisting of iGluR and mGluR in melanoma (18, 27). Figure 1 depicts the glutamate receptor signaling pathways and genes known to be affected by genetic alteration in melanoma. There are several nodes in the glutamate receptor pathway that could be utilized for clinical applications, as highlighted in Fig. 1 and described below: (i) Targeted activation of NMDAR using specific agonists, such as ibotenic acid, homoquinolinic acid, or glutamate (glutamate analogs-NMDA), would increase the intracellular levels of Ca<sup>2+</sup> and potentially induce calcium-mediated cell death. (ii) Inhibition of ErbB4 signaling in melanoma cells that harbor mutant forms of ErbB4 using small-molecule inhibitors such as lapatinib, erlotinib, and neratinib, or monoclonal antibodies (mAb) to the ErbB4 ectodomain could inhibit ligand-induced dimerization and transactivation. This approach is supported by a study of melanoma cells expressing mutant forms of ErbB4, which showed sensitivity to low doses of lapatinib, leading to suppression of Akt activation and increased apoptosis (39). (iii) The use of mGluR antagonists or mGluR3-specific antagonists (e.g., BAY 36-7620 and LY341495) could inhibit glutamate signaling via the GPCR. In a study of glioma cells, the use of LY341495 resulted in reduced cellular growth via suppression of the MAPK and Akt pathways (24). The only caveat to using this approach is that prolonged suppression of mGluR3 causes reduced expression of glutamate transporters and potentially reduced proliferation of glioma stem cells (59, 60). (iv) Targeting downstream effector molecules that are nodal points in cellular signaling for malignant melanoma will allow for better combinatorial therapeutic approaches. Melanoma cells with aberrant glutamate receptor signaling exhibit hyperactive PI3K/Akt and MEK/MAPK pathways (27, 39). Thus, using inhibitors to PI3K (e.g., BKM120, PX866, and GSK2126458), Akt (perifosine, MK-2206, and GSK2141795), Raf (PLX4032, PLX4720, and PLX4732), MEK (AZD-6244 and GSK1120212), or MAPK (AEZS-131) in combination with one of the above-mentioned therapies (ErbB4 inhibitor or *GRM3* antagonists) to attenuate proliferative and survival signals, resulting in increased and specific tumor cell apoptosis, may be

suggested. (v) Secretion suppression of excess glutamate to the extracellular milieu. The use of riluzole (a U.S. Food and Drug Administration–approved drug for treatment of amyotrophic lateral sclerosis) to treat mGluR1-expressing cells showed reduced secreted glutamate levels, resulting in decreased melanoma cell proliferation compared with normal melanocytes lacking mGluR1 expression (46, 61). Furthermore, Yip and colleagues (62) recently completed a phase 0 clinical trial in which patients with late-stage malignant melanoma were treated with riluzole, and 33% of the patients exhibited a significant clinical response. Of importance, patient samples exhibited reduced MAPK and Akt activation in the presence of riluzole, highlighting the efficacy and importance of targeting glutamate availability to tumors.

The notion of glutamate addiction in melanoma is corroborated by findings that human melanoma cells secrete elevated levels of glutamate and express reduced levels of the glutamate transporter proteins EAAT2/EAAT4 (our unpublished data, and refs. 45 and 46). The EAAT2/4 proteins are normally expressed on neuronal cells to help reduce the excitotoxicity effect of excess glutamate released by glial cells and astrocytes in the CNS (36). Evidence suggests that high-grade level glioblastomas have reduced expression of EAAT2 and potentially other EAATs (63). Overexpression of EAAT2 in glioblastomas resulted in decreased growth and tumorigenesis *in vitro* and *in vivo*. In our laboratory, we observed that a panel of malignant melanoma cell lines exhibited reduced transcript levels of both EAAT2 and EAAT4, but found no difference in the expression levels of EAAT1, EAAT2, or EAAT5 (unpublished data). Lastly, another potential target pertaining to the

glutamate pathway is the (vi) PSD complex. Cook and colleagues (64) recently reported that uncoupling the PSD complex in neuronal cells using a PSD-95 inhibitor prevented stroke damage due to neurotoxicity. This approach may be promising for melanoma because both ErbB4 and GRM3 may use PSD complexes for proximal localization and regulation of downstream molecules.

## Conclusions

The evidence presented in this review suggests that the glutamatergic signaling pathways play key roles in melanoma tumorigenesis. Targeting these pathways directly in conjunction with their downstream effector molecules may prove effective for treating melanoma patients.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** T.D. Prickett, Y. Samuels

**Development of methodology:** T.D. Prickett, Y. Samuels

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** T.D. Prickett

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** T.D. Prickett, Y. Samuels

**Writing, review, and/or revision of the manuscript:** T.D. Prickett, Y. Samuels

**Study supervision:** Y. Samuels

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