Published in final edited form as:
Wiley Interdiscip Rev Membr Transp Signal. 2012 ; 1(2): 211–220. doi:10.1002/wmts.21.

mGlu Receptors and Cancerous Growth

Jessica Teh and **Suzie Chen**
Department of Chemical Biology, Ernest Mario School of Pharmacy, Susan Lehman Cullman Laboratory for Cancer Research, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020

Suzie Chen: suziec@rci.rutgers.edu

Abstract

G-protein coupled receptors (GPCR) represent a class of therapeutic targets that have been widely exploited for drug designs and development. Metabotropic glutamate receptors (mGluRs) belong to Class C GPCRs and are predominantly involved in maintaining cellular homeostasis in the central nervous system (CNS). The surprising accumulating evidence suggesting other functional roles of mGluRs in human malignancies in addition to synaptic transmission has presented intriguing possibilities to make mGluRs putative novel targets for human cancers. Since our group first described the aberrant expression of mGluR1 as the driving force in melanomagenesis in transgenic mouse models, other subtypes of mGluRs have been implicated in the pathogenesis of various cancer types such as malignant gliomas and medulloblastomas. As such, increased efforts have been generated to elucidate the mechanisms by which mGluRs confer oncogenic potentials. Current knowledge on the participation of various mGluRs in several human cancers suggests that mGluRs are “druggable” members of the GPCR superfamily and their oncogenic implications in cancer, so further understanding on anti-mGluR strategies will be beneficial.

The amino acid L-glutamate is a predominant excitatory neurotransmitter responsible for regulating signaling in normal brain function [1]. While most studies on glutamate signaling have been restricted to the central nervous system (CNS), emerging investigations in the past decade have highlighted their functional role in peripheral tissues[2-9]. The notion that glutamate can have dual functions in maintaining cellular homeostasis in the CNS and as an external stimulatory signal in non-neuronal peripheral cells has given rise to a host of studies implicating the glutamatergic system in the pathophysiology of human diseases.

Glutamate exerts its signaling abilities by acting on glutamate receptors, which are located on the cell surface. Glutamate receptors exist as either ionotropic receptors (iGluRs) or metabotropic glutamate receptors (mGluRs). Members of the iGluR family were the first components of the glutamatergic system to be identified[10]. iGluRs are classical ligand-gated ion channels, which include N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (iGluR1-4) and kainite (KA) subfamilies (iGluR5-7, KA1 and KA2)[11]. Unlike their iGluR counterparts, mGluRs are seven transmembrane domain receptors that mediate their signal by coupling to GTP-binding proteins (G-proteins) and stimulate second messengers such as inositol 1,4,5-triphosphate (IP3), diacylglycerol (DAG) and cyclic adenosine monophosphate (cAMP) [12-14].

In contrast to iGluRs, which only mediate synaptic transmission, mGluRs are considered better drug targets due to their ability to modulate cellular signaling cascades. mGluR1 was the first mGluR discovered over twenty years ago by the formation of inositol phosphate in cultured striatal neurons upon glutamate stimulation and was subsequently cloned from rat brain [15,16]. mGluR1 was shown not to have sequence similarity with other known G-protein coupled receptors (GPCRs) at the time and hence gave rise to a brand new subfamily of

GPCRs. Since then, eight mGluR subtypes have been identified and grouped according to their sequence homology, pharmacologic response, and intracellular second messengers[17]. Upon binding of the ligand, Group I receptors, which is comprised of mGluR1 and mGluR5, couple via G_q to phospholipase C (PLC) leading to the formation of IP3 and DAG. Group II, comprises mGluR2 and mGluR3 and Group III comprises mGluR4, mGluR6, mGluR7 and mGluR8. Both Group II and III are negatively coupled via $G_{i/o}$ to adenyl cyclase leading to cAMP formation.

The functional roles of mGluRs have been extensively characterized in the CNS. The distribution of mGluRs expression is widespread in neurons with certain groups also located in astrocytes[17]. At the synapse, Group I mGluRs are generally found in postsynaptic locations hence, possessing excitatory properties whereas Group II and Group III mGluRs are localized mostly in presynaptic locations lending to their inhibitory effect. Early efforts described the role of mGluR1 in synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LDP), mechanisms that underlie long-term memory formation and learning[18,19]. In addition, mGluR2 activation has been linked to cognitive impairment while mGluR3 may confer neuroprotective properties[20,21]. In the mid-1980s, Yoneda and colleagues reported a significant activity of L-[3H] glutamic acid binding in rat adrenal glands and pituitary suggesting the functional expression of glutamate receptors in peripheral cells [22,23]. More than a decade after this initial report, other investigations describing the expression of mGluRs in non-neuronal cell types emerged within a few years of each other. Gill and colleagues showed that all three subtypes, mGluR1, mGluR2/3 and mGluR5 were expressed in rat heart but with a specific distribution within the cardiac structures [5]. Around the same time, the sporadic expression of mGluR1 and mGluR2/3 in basal keratinocytes with both subtypes displaying distinct patterning was published[4]. This was followed by several reports on mGluR1 expression in primary osteoblasts and mGluR5 expression in melanocytes and hepatocytes[3,9,24]. The preferential localization of different mGluR subclasses in specific cell types strongly suggests that each of them may participate in a particular physiological role beyond just the regulation of synaptic transmission.

mGluRs in human malignancies

Increasing evidence points to the pathophysiology of mGluRs in cancer (Table 1). Recently, an evaluation of various glutamate receptor subunit expressions was carried out in cancer cell lines from both neuronal and non-neuronal origin[25]. In this report, expression of mGluRs was demonstrated at the mRNA level in a variety of human cancer cell lines. The same research group also probed for mGluR expression in pediatric tumors of the CNS such as glioblastomas and medulloblastomas[26]. mGluR1 and mGluR4-6 were found to be over-expressed in higher grade tumors. Furthermore, Seidlitz and colleagues examined several cancer cell lines with a propensity for bone metastasis and found them to secrete excessive glutamate into the extracellular environment through the cysteine/glutamate antiporter[27,28]. Since glutamate signaling is involved in bone homeostasis the authors postulate that metastatic cancer cells may utilize this to their advantage[29]. In this review, we will summarize the evidence for roles of functional mGluRs in cancer cell growth and progression also highlighting the potential therapeutic applications of these preclinical findings.

mGluR1

Our group was the first to report that the aberrant expression of mGluR1 is the driving force of melanomagenesis in transgenic mouse models. Melanoma is the most deadly form of skin cancer. With early detection, melanoma patients have a very good chance for survival when

treated by surgical resection. However, advanced melanoma is a fatal disease with poor prognosis and chemoresistance to most current therapeutic modalities.

A transgenic mouse line, TG-3 was constructed with a 2kb genomic DNA clone (clone B) that was shown to induce adipocyte differentiation in somatic cells *in vitro*[30]. However, none of the five transgenic founder mice attained the expected obese phenotype but one of them, transgenic mouse number 3 (TG-3), at 8 month of age, showed heavily pigmented lesions in the ear (pinnae), eyelid, perianal region and snout. Histological analysis determined the pigmented lesions as melanoma. Subsequent progeny of TG-3 displayed identical melanoma-prone phenotype[31,32]. Molecular studies identified that the 2kb transgene DNA fragment was inserted in intron 3 of the gene encoding for mGluR1 with concomitant deletion of about 70kb of host sequence[33]. Comparison of mGluR1 expression between normal and tumor pinnae revealed that mGluR1 expression was only detectable in tumor but not normal samples. To distinguish between cause and consequence in ectopic mGluR1 expression in tumor samples a second transgenic line was made using mouse mGluR1 cDNA under the regulation of a melanocyte specific promoter, dopachrome tautomerase (Dct). This second transgenic line, Tg(Grm1)EPv (E), developed pigmented tumors with very similar onset and progression as TG-3. These results verified that ectopic expression of mGluR1 in melanocytes was indeed sufficient to induce spontaneous melanoma development *in vivo*.

mGluR5 is the other member of the Group I mGluRs and its expression is normally detected in melanocytes. Possible involvement of mGluR5 in the development of spontaneous melanoma was investigated using an mGluR5 null transgenic line[34]. Progeny that were derived from crosses between TG-3 and mGluR5 null mice exhibit indistinguishable melanoma prone phenotype as the original TG-3. Taken together, these results demonstrated that aberrant mGluR1 expression in melanocytes is sufficient to generate melanoma prone mice with 100% penetrance in the absence of other tumor types. Recently, Ohtani and colleagues described a melanoma-prone mGluR1 transgenic model driven by a conditional neuron-specific enolase (NSE) promoter in melanocytes. These results were consistent with our findings that ectopic expression of mGluR1 induced melanoma formation[35]. Earlier studies by others have shown that GPCRs can become tumorigenic when exposed to an excess of locally produced or circulating ligands and agonists [36,37] while other GPCRs harboring mutations in key conserved residues can have transforming activity even in the absence of their ligands [13,37-42]. It has also been found that the level of expression of GPCRs is not as important to oncogenesis as the fact that the receptor is expressed[42] which supposes our speculation that ectopically expressed mGluR1 in melanocytes is sufficient to yield melanoma-prone phenotype in vivo.

Our finding that mGluR1 played a role in melanomagenesis in mice prompted us to examine human melanoma for expression of the human form of mGluR1. We first examined human melanoma cell lines and found that 23 of 25 lines tested expressed hGRM1[33,43]. We next examined 175 melanoma biopsies from primary to metastatic lesions and found approximately 60% of these samples express hGRM1 mRNA and protein, which was further confirmed by a melanoma tissue array[33,43] In addition, a recent meeting report by Nishigori and colleagues showed mGluR1 expression in 80% (49/61) of melanoma tissue samples tested including superficial spreading, nodular, lentigo, maligna, acral lentiginous, and metastatic melanomas. mGluR1 expression was also detected in 33% (6/18) of common, blue and Spitz nevi. Positive mGluR1 was observed in 75% (6/8) of human melanoma cell lines and 50% (2/4) of nevus lines while normal melanocytes were negative for mGluR1 expression[44]. These additional data strongly point toward a role for mGluR1 deregulation in human melanoma. A better understanding of the regulation of mGluR1 expression in melanocytes at the molecular level will likely identify the functional role of mGluR1 and the

mGluR1 pathway, which will be important for the development of new therapeutic strategies.

To study the underlying mechanisms in mGluR1-mediated melanocyte transformation, we generated several stable mGluR1-mouse melanocytic clones (MASS clones) when introduced into the non-tumorigeneic immortalized C57BL6-derived Melan-a melanocytes [45]. These clones exhibited common transformed characteristics and were found to be aggressively tumorigenic when allografted into both nude and syngeneic mice with short latency. Furthermore, it was demonstrated that persistent mGluR1 expression was required to maintain the tumorigenic phenotypes *in vivo* as evidenced by an inducible siRNA construct to mGluR1[45]. Ohtani and co-workers confirmed these results where inhibition of mGluR1 alone was sufficient to suppress tumor growth and ERK signaling *in vivo* [35].

Through previous studies using TG-3 tumor-derived cells we demonstrated the activation of two major signaling cascades that have been shown by many investigators to be critical in melanoma pathogenesis. These two signaling pathways are Mitogen-Activated Protein Kinase (MAPK) and phosphatidylinositol 3-kinase/Protein Kinase B (PI3K/AKT). The specificity of mGluR1-mediated activation of MAPK and PI3K/AKT in our system was provided by the responsiveness of cells to stimuli and inhibitors of mGluR1 using the components of these signaling pathways as read-outs. Furthermore, we identified AKT2 as the predominant AKT isoform that is involved in mGluR1-mediated oncogenesis[46]. While earlier studies by others established the role of deregulated AKT3 in melanoma development[47], we have since demonstrated that AKT2 but not AKT3 is the predominant isoform activated in human melanoma biopsies in addition to mGluR1 mouse melanocytic tumor allografts. Moreover, Nogueira and colleagues also highlighted AKT2 in melanoma metastasis when they showed that loss of PTEN promoted invasion and migration via activation of AKT2[48].

While an mGluR1 specific antagonist is not yet available for therapeutic purposes, our group has translated our findings into the clinic with Riluzole (2-amino-6-trifluoro-methobenzothiazole), an FDA approved drug for the treatment of amyotrophic lateral sclerosis (ALS). Riluzole has been reported to indirectly antagonize glutamate receptors by inhibiting glutamate release as well as the inactivation of voltage-gated Na+ channels [49]. Previously, we have shown that human melanoma cells release elevated levels of glutamate suggesting the existence of an autocrine loop in these cells[43]. We hypothesize that the presence of Riluzole should decrease the availability of the ligand, glutamate, for the mGluR1 receptor thus leading to a reduction in mGluR1 stimulation. This notion was tested in several human melanoma cell lines. We showed that mGluR1-expressing human melanoma cells were much more sensitive to Riluzole than mGluR1-negative human melanoma cells[43]. mGluR1-expressing human melanoma cells accumulated in the G2-M phase at 24 hrs post-Riluzole treatment and proceeded to the subG1 phase of the cell cycle by 48 hrs. Several well-known apoptosis markers confirmed the apoptotic responses of these cells[43]. These preclinical studies led to a Phase 0 trial of Riluzole in patients with stage III and IV melanoma, in which about one third of the patients exhibited remarkable clinical and metabolic responses. Comparisons using biochemical markers between pre- and post-treatment samples showed suppression of components of two of the major signaling pathways important in melanoma pathogenesis, MAPK and PI3K/AKT, and an increase in the number of apoptotic cells in post-treatment tumor samples[50]. Currently, a Phase I/II trial of escalating single agent Riluzole is underway. A recent report by Biechele and colleagues described the enhancement of the Wnt/β catenin pathway by Riluzole in melanoma cells[51]. The activation of Wnt/β catenin signaling has been shown to correlate with better prognosis in melanoma patients and forced expression of Wnt3A has led to decreased cell

proliferation[52]. Depletion of the ligand, glutamate, by Riluzole or suppression of mGluR1 signaling by genetic means led to Wnt mediated activation of β catenin. These results present an intriguing inverse relationship between the glutamatergic and Wnt/β catenin pathways.

As most human cancers are carcinomas and derive from epithelial origins, we were interested to know if mGluR1 also plays a role in the pathogenesis of tumors other than melanoma. In an ongoing study, our laboratory has shown that ectopic expression of mGluR1 can indeed transform immortalized baby mouse kidney epithelial cells (iBMK). These iBMK cells are immortalized through E1A and dominant negative p53 (p53DD)[53]. When allografted into immunodeficient mice, the stable mGluR1 clones were found to be tumorigenic with a shorter latency as compared to the parental cells. Downregulation of mGluR1 with a tetracycline-inducible siRNA was sufficient to suppress tumor formation, similar findings as to what we have shown previously in the melanocytic system[45]. A recent report from Gorski and colleagues confirmed our observation that the oncogenic properties of mGluR1 also extend to epithelial cancers. MDA-231, a triple-negative metastatic breast cancer cell line when treated with either mGluR1 specific antagonist, Bay 36-7620 or a decrease in the levels of available ligand, glutamate by Riluzole or a reduction in mGluR1 expression by shRNA led to decline in the number of viable cells *in vitro* and tumor progression *in vivo*. Taken together, these results strongly suggest mGluR1 may contribute to epithelial tumorigenesis including mammary tissue and the potential of mGluR1 as a novel therapeutic target in the treatment of triple-negative subtype of breast cancer.

mGluR2/3

Malignant gliomas derived from glial cells are a type of tumor that starts within the brain or CNS. Glioblastoma multiform (GBM) is an example of high-grade (III & IV) glioma representing a disease with extremely poor prognosis and only a 12 month survival rate after diagnosis [54]. One of the hallmarks of glioma cells is their ability to invade surrounding tissues in promoting their own growth. This is where glutamate mediates its effects. The excessive secretion of glutamate by glioma cells leads to the death of surrounding brain cells hence creating more area for the malignant tumor cells to grow in addition to an autocrine/paracrine glutamate signaling loop [55]. D'onofrio and colleagues have shown that mGluR3 has a functional role in supporting the growth of U87MG human glioma cells both *in vitro* and *in vivo*. Using an mGluR2/3 antagonist, LY341495, they showed that it was effective in reducing the tumor growth of U87MG cells that were inoculated subcutaneously or intracranially in nude mice[56,57]. In their most recent study, mGluR3 (not mGluR2)-expressing glioma initiating cells (GICs) were isolated from human GBMs[58]. GICs share the same surface markers, self-renewal and anti-differentiation properties with neural stem cells (NSCs) and are thought to fuel therapy resistance in GBM [59]. LY341495 was successful in limiting the growth of GIC xenografts and promoted an astroglial differentiation phenotype mediated by type IV-bone morphogenic protein receptor (BMP4)[58]. There is substantial evidence for a crucial role of BMP4 receptors in promoting astrocytic differentiation in NSCs and GICs[58,60]. These results highlight a novel crosstalk network between mGluR3 and BMP4. Our group has done some preliminary *in vitro* and *in vivo* studies addressing the effect of Riluzole on human glioma cells. We found that Riluzole was able to suppress the *in vivo* growth of U87MG xenografts. However, more studies are warranted to determine Riluzole's potential use in malignant glioma therapy.

mGluR4

So far, we have discussed the advantages of antagonizing two separate groups of mGluRs to suppress tumor cell growth. In the case of medulloblastomas, the activation of mGluR4 may

actually prove beneficial. Medulloblastomas are a common pediatric malignant brain tumor and similar to gliomas, current views support the presence of stem-like progenitor cells within these tumors[61]. It is thought that transformation of cerebellar granule cell neuroprogenitors (GNPs) give rise to medulloblastomas. These GNPs exist in the external granular layer of the cerebellar cortex and migrate inward to the granular layer where they finally differentiate into mature granule cells[6]. It was hypothesized that the tendency towards a differentiated phenotype is supported by glutamate that is released by surrounding cells. Interestingly, mGluR4 receptor expression was detected in cultured cerebellar granule cells enriched with GNPs[62]. Canudas and colleagues took advantage of a specific mGluR4 enhancer, PHCCC (N-phenyl-7-(hydroxyimino)/cyclopropa[b]chromen-1a-carboxamide), which interacts with a site within the seven transmembrane region in mGluR4. They found that amplified activity of mGluR4 receptors with PHCCC could effectively reduce GNP proliferation and promote them to differentiate into mature granule cells. These results suggested that mGluR4 is crucial for maintaining a mature differentiated phenotype. These observations were reinforced when it was found that the over-expression of mGluR4 in cultured cells also promoted neuritogenesis. Therefore, it is not surprising that these findings prompted the same group to investigate the role of mGluR4 in medulloblastoma, a clinically relevant malignant disease. Approximately 75% of the biopsied human medulloblastoma samples stained positive for mGluR4 and was found to correlate with improved prognosis [63]. PHCCC treatment of mGluR4-expressing medulloblastoma cell lines resulted in decreased PI3K signaling, DNA synthesis and cell proliferation. These observations were found to be independent of other signaling pathways such as Insulin-like Growth Factor (IGF-1), canonical Wnt and Sonic hedgehog (SHH) all of which are known to contribute to GNP proliferation and play crucial roles in the development of medulloblastomas [64-67]. Further evidence for the benefit of enhancing mGluR4 expression/function in medulloblastoma was shown with an in vivo irradiated transgenic mouse model lacking Shh receptor Patched 1 (Ptc neo67/+). When Ptc neo67/+ mice are irradiated one day after birth they are predisposed to developing medulloblastomas with an incidence of >80% [68]. However, PHCCC treatment during the first eight days of life of irradiated mice led to inhibition of medulloblastoma development[63]. Taken together, results from these studies suggest an inverse correlation of mGluR4 with the progression of the disease. While the data is exciting, further investigation is warranted to examine whether later stages of medulloblastoma are sensitive to mGluR4 activation especially in metastatic stage, which was shown to have lower expression levels of the receptor. In addition to this promising new approach for medulloblastoma, stimulation of mGluR4 expression is also a potential treatment for Parkinson's disease [69].

On the opposite end of the spectrum, Chang and colleagues have found a link between mGluR4 and the pathogenesis of colorectal cancer. 5-fluorouracil (5-FU) is a standard chemotherapeutic agent for patients with colorectal cancer and resistance toward this drug is a major hurdle. Chang and co-workers first generated a 5-FU resistant colorectal cancer cell line and identified over-expression mGluR4 mediated the 5-FU resistance phenotype in these cells[70]. In a follow up study, expression of mGluR4 was found in 54% of the malignant tissues of colorectal carcinomas, which correlated with poor prognosis [71]. In addition, the same group also showed that several mGluR4-expressing colon cancer cell lines treated with mGluR4 antagonist resulted in suppression of cell proliferation.

These studies suggest that mGluR4 may be involved in neuronal as well as non-neuronal neoplasia in humans.

mGluR5

It has been shown by others that this particular subclass of mGluRs is expressed in a variety of non-neuronal cells such as melanocytes, hepatocytes, myocytes and embryonic stem cells [72]. In embryonic stem cells, pharmacological blockade of mGluR5 with MPEP (methylphenylethynylpyridine) was able to drive the cells toward differentiation even though the cells were grown in vitro with leukaemia inhibitory factor (LIF), a cytokine known to sustain ES cells in their self-renewal stage[73]. It was later found that mGluR5 synergizes with LIF to sustain the increase of c-Myc expression in ES[74]. Over-expression of c-Myc is the hallmark of many human cancers. It will be interesting to determine how inhibition of mGluR5 activity/function by its antagonist, MPEP, promote ES cells towards differentiation even in the presence of LIF and break down the synergy between LIF and mGluR5 to relinquish the enhanced expression of c-Myc.

A recent study also implicated mGluR5 in oral squamous cell carcinoma (SCC). Of the 131 cases of oral cancer tissues probed for mGluR5 expression, 72% were either weakly or strongly positive[75]. Most importantly, mGluR5 expression correlated with the advancement of the disease. In cultured cell studies, group I agonist, DHPG (3,5-dihydroxyphenylglycine) was found to support the migration, invasion and adhesion of HSC3 oral cancer cells whereas mGluR5 specific antagonist, MPEP abolished this effect. Further experiments need to be done to establish if mGluR5 can be used as a prognostic marker of oral SCC.

Finally, there is evidence of the regulation of the glutamatergic system in MG63, an osteoblast-like osteosarcoma cell line[76]. Dexamethasone, a synthetic glucocorticoid was found to upregulate mGluR5 and a few other glutamate signaling components in MG63, suggesting possible roles of the glutamatergic system in bone pathophysiology[77].

mGluR6-8

The implication of mGluR6-8 in tumor formation has yet to be characterized. However, mGluR6 expression was recently shown to correlate with higher-grade pediatric CNS tumors[26]. Considerable attention has been given to the role of glutamatergic signaling in a host of homeostatic functions in the bone. In particular, mGluR8 has been suggested to play a role in bone homeostasis due to their expression in osteoclasts, cells responsible for bone resorption[7]. Previous studies have also identified other mGluR subtypes expression in osteoblasts, which are bone-forming cells[78]. Morimoto and colleagues showed that stimulation of mGluR8 with specific agonist inhibits glutamate release and the secretion of bone degradation products whereas treatment with specific antagonist promoted bone resorption. Given the recent report that highly metastatic cancer cells are capable of secreting extracellular glutamate into the environment, it is conceivable to investigate whether tumor-released glutamate can impact bone homeostasis mediated by mGluR8 or other mGluRs in bone metastasis.

Concluding remarks

Since the etiologic role of mGluR1 in melanomagenesis was identified and demonstrated, we have made significant progress not only in characterizing the oncogenic properties of mGluR1in other cell types but also by translating our laboratory findings into the clinic. Riluzole is an FDA approved drug for ALS and other neuronal disease patients. Although the precise mode of action of Riluzole has been somewhat controversial, it is clear that Riluzole indirectly antagonizes the stimulation of glutamate receptors through its suppressive effect on the release of glutamate, the ligand for the mGluRs. So far our findings support mGluR1 as a valid therapeutic target in melanoma patients as shown by the results of the Phase 0 clinical trial of Riluzole[50]. Notably, a recent study involving whole-exome

sequencing of 14 matched normal and metastatic melanoma tumor DNA has uncovered a previously unidentified gene, GRIN2A. GRIN2A encodes for ionotropic glutamate (NMDA) receptor subunit ε-1[79] and was mutated in 33% of the melanoma samples. While the mechanism by which GRIN2A exerts its tumorigenic effect is not yet known, the frequency of its mutation combined with the establishment of mGluR1 in melanomagenesis has clear implications for the benefit of targeting the glutamate pathway. With the realization that most human tumors are heterogeneous, we have also initiated several combinatorial studies of Riluzole and other known inhibitors to the downsteam pathways in melanoma. Clearly, more work will be required to define the value of Riluzole in other cancers with dysregulated glutamate signaling such as gliomas.

It is becoming apparent that mGluRs participate in the tumor progression of more cancer types than we anticipated. However, the involvement of mGluRs is not always a causal relationship indicating that mGluR ligands or positive allosteric modulators may also have clinical applications. For example, we described the advantage of mGluR4 activation in early stage of medulloblastoma development to prevent tumor progression. Thus, it will be imperative to investigate the fine line between the time period where the tumor is most susceptible to mGluR4 enhancers and the significance of mGluR antagonists for malignant stages of medulloblastoma. On the flipside, we now know that expression of other mGluRs such as mGluR1, mGluR5, mGluR6 and even mGluR4 are correlated with higher-grade tumors of the same disease. Taken together, it is clear that glutamatergic signaling plays critical roles in a diverse group of human disorders.

Acknowledgments

The authors would like to thank Brian A. Wall for helpful discussion. This work is supported by New Jersey Commission for Cancer Research 09-1143-CCR-E0 and NIH R01CA73077.

References

1. Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci. 1994; 17:31–108. [PubMed: 8210177]
2. Skerry TM, Genever PG. Glutamate signalling in non-neuronal tissues. Trends Pharmacol Sci. Apr; 2001 22(4):174–181. [PubMed: 11282417]
3. Frati C, Marchese C, Fisichella G, et al. Expression of functional mGlu5 metabotropic glutamate receptors in human melanocytes. J Cell Physiol. Jun; 2000 183(3):364–372. [PubMed: 10797311]
4. Genever PG, Maxfield SJ, Kennovin GD, et al. Evidence for a novel glutamate-mediated signaling pathway in keratinocytes. J Invest Dermatol. Mar; 1999 112(3):337–342. [PubMed: 10084312]
5. Gill SS, Pulido OM, Mueller RW, McGuire PF. Immunochemical localization of the metabotropic glutamate receptors in the rat heart. Brain Res Bull. Jan 15; 1999 48(2):143–146. [PubMed: 10230705]
6. Melchiorri D, Cappuccio I, Ciceroni C, et al. Metabotropic glutamate receptors in stem/progenitor cells. Neuropharmacology. Sep; 2007 53(4):473–480. [PubMed: 17675103]
7. Morimoto R, Uehara S, Yatsushiro S, et al. Secretion of L-glutamate from osteoclasts through transcytosis. EMBO J. Sep 20; 2006 25(18):4175–4186. [PubMed: 16957773]
8. Storto M, Capobianco L, Battaglia G, et al. Insulin secretion is controlled by mGlu5 metabotropic glutamate receptors. Mol Pharmacol. Apr; 2006 69(4):1234–1241. [PubMed: 16424079]
9. Storto M, de Grazia U, Knopfel T, et al. Selective blockade of mGlu5 metabotropic glutamate receptors protects rat hepatocytes against hypoxic damage. Hepatology. Mar; 2000 31(3):649–655. [PubMed: 10706555]
10. Hollmann M, O'Shea-Greenfield A, Rogers SW, Heinemann S. Cloning by functional expression of a member of the glutamate receptor family. Nature. Dec 7; 1989 342(6250):643–648. [PubMed: 2480522]

11. Traynelis SF, Wollmuth LP, McBain CJ, et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev. Sep; 2010 62(3):405–496. [PubMed: 20716669]
12. Hermans E, Challiss RA. Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. Biochem J. Nov 1; 2001 359(Pt 3):465–484. [PubMed: 11672421]
13. Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: emerging paradigms. Trends Pharmacol Sci. Jul; 2001 22(7):368–376. [PubMed: 11431032]
14. Dorsam RT, Gutkind JS. G-protein-coupled receptors and cancer. Nat Rev Cancer. Feb; 2007 7(2): 79–94. [PubMed: 17251915]
15. Sladeczek F, Pin JP, Recasens M, Bockaert J, Weiss S. Glutamate stimulates inositol phosphate formation in striatal neurones. Nature. Oct 24-30; 1985 317(6039):717–719. [PubMed: 2865680]
16. Masu M, Tanabe Y, Tsuchida K, Shigemoto R, Nakanishi S. Sequence and expression of a metabotropic glutamate receptor. Nature. Feb 28; 1991 349(6312):760–765. [PubMed: 1847995]
17. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol. 2010; 50:295–322. [PubMed: 20055706]
18. Aiba A, Kano M, Chen C, et al. Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell. Oct 21; 1994 79(2):377–388. [PubMed: 7954803]
19. Levenes C, Daniel H, Jaillard D, Conquet F, Crepel F. Incomplete regression of multiple climbing fibre innervation of cerebellar Purkinje cells in mGLuR1 mutant mice. Neuroreport. Jan 20; 1997 8(2):571–574. [PubMed: 9080450]
20. Higgins GA, Ballard TM, Kew JN, et al. Pharmacological manipulation of mGlu2 receptors influences cognitive performance in the rodent. Neuropharmacology. Jun; 2004 46(7):907–917. [PubMed: 15081787]
21. Corti C, Battaglia G, Molinaro G, et al. The use of knock-out mice unravels distinct roles for mGlu2 and mGlu3 metabotropic glutamate receptors in mechanisms of neurodegeneration/ neuroprotection. J Neurosci. Aug 1; 2007 27(31):8297–8308. [PubMed: 17670976]
22. Yoneda Y, Ogita K. [3H]glutamate binding sites in the rat pituitary. Neurosci Res. Jul; 1986 3(5): 430–435. [PubMed: 3018640]
23. Yoneda Y, Ogita K. Localization of [3H]glutamate binding sites in rat adrenal medulla. Brain Res. Sep 24; 1986 383(1-2):387–391. [PubMed: 2876754]
24. Gu Y, Publicover SJ. Expression of functional metabotropic glutamate receptors in primary cultured rat osteoblasts. Cross-talk with N-methyl-D-aspartate receptors. J Biol Chem. Nov 3; 2000 275(44):34252–34259. [PubMed: 10950953]
25. Stepulak A, Luksch H, Gebhardt C, et al. Expression of glutamate receptor subunits in human cancers. Histochem Cell Biol. Oct; 2009 132(4):435–445. [PubMed: 19526364]
26. Brocke KS, Staufner C, Luksch H, et al. Glutamate receptors in pediatric tumors of the central nervous system. Cancer Biol Ther. Mar 15; 2010 9(6):455–468. [PubMed: 20061814]
27. Seidlitz EP, Sharma MK, Saikali Z, Ghert M, Singh G. Cancer cell lines release glutamate into the extracellular environment. Clin Exp Metastasis. 2009; 26(7):781–787. [PubMed: 19526315]
28. Sharma MK, Seidlitz EP, Singh G. Cancer cells release glutamate via the cystine/glutamate antiporter. Biochem Biophys Res Commun. Jan 1; 2010 391(1):91–95. [PubMed: 19896463]
29. Seidlitz EP, Sharma MK, Singh G. Extracellular glutamate alters mature osteoclast and osteoblast functions. Can J Physiol Pharmacol. Sep; 2010 88(9):929–936. [PubMed: 20921979]
30. Chen S, Teicher LC, Kazim D, Pollack RE, Wise LS. Commitment of mouse fibroblasts to adipocyte differentiation by DNA transfection. Science. May 5; 1989 244(4904):582–585. [PubMed: 2470149]
31. Chen S, Zhu H, Wetzel WJ, Philbert MA. Spontaneous melanocytosis in transgenic mice. J Invest Dermatol. May; 1996 106(5):1145–1151. [PubMed: 8618055]
32. Zhu H, Reuhl K, Zhang X, et al. Development of heritable melanoma in transgenic mice. J Invest Dermatol. Mar; 1998 110(3):247–252. [PubMed: 9506443]
33. Pollock PM, Cohen-Solal K, Sood R, et al. Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. Nat Genet. May; 2003 34(1):108–112. [PubMed: 12704387]

34. Marin YE, Namkoong J, Shin SS, et al. Grm5 expression is not required for the oncogenic role of Grm1 in melanocytes. Neuropharmacology. 2005; 49(Suppl 1):70–79. [PubMed: 16040064]
35. Ohtani Y, Harada T, Funasaka Y, et al. Metabotropic glutamate receptor subtype-1 is essential for in vivo growth of melanoma. Oncogene. Dec 4; 2008 27(57):7162–7170. [PubMed: 18776920]
36. Julius D, Livelli TJ, Jessell TM, Axel R. Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. Science. Jun 2; 1989 244(4908):1057–1062. [PubMed: 2727693]
37. Gutkind JS. Cell growth control by G protein-coupled receptors: from signal transduction to signal integration. Oncogene. Sep 17; 1998 17(11 Reviews):1331–1342. [PubMed: 9779981]
38. Allen LF, Lefkowitz RJ, Caron MG, Cotecchia S. G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the alpha 1B-adrenergic receptor enhances mitogenesis and tumorigenicity. Proc Natl Acad Sci U S A. Dec 15; 1991 88(24):11354–11358. [PubMed: 1662393]
39. Bais C, Santomasso B, Coso O, et al. G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. Nature. Jan 1; 1998 391(6662):86–89. [PubMed: 9422510]
40. Dhanasekaran N, Tsim ST, Dermott JM, Onesime D. Regulation of cell proliferation by G proteins. Oncogene. Sep 17; 1998 17(11 Reviews):1383–1394. [PubMed: 9779986]
41. Hermouet S, Aznavoorian S, Spiegel AM. In vitro and in vivo growth inhibition of murine melanoma K-1735 cell by a dominant negative mutant alpha subunit of the Gi2 protein. Cell Signal. Mar; 1996 8(3):159–166. [PubMed: 8736698]
42. Montaner S, Sodhi A, Molinolo A, et al. Endothelial infection with KSHV genes in vivo reveals that vGPCR initiates Kaposi's sarcomagenesis and can promote the tumorigenic potential of viral latent genes. Cancer Cell. Jan; 2003 3(1):23–36. [PubMed: 12559173]
43. Namkoong J, Shin SS, Lee HJ, et al. Metabotropic glutamate receptor 1 and glutamate signaling in human melanoma. Cancer Res. Mar 1; 2007 67(5):2298–2305. [PubMed: 17332361]
44. Funasaka Y, H T, Aiba A, Nishigori C. Expression of metabotropic glutamate receptor 1 and phosphorylated extracellular signal-regulated kinase 1/2 proteins in human melanocytic lesions. Pigment Cell and Melanoma Res. 2006; 19:656.
45. Shin SS, Namkoong J, Wall BA, Gleason R, Lee HJ, Chen S. Oncogenic activities of metabotropic glutamate receptor 1 (Grm1) in melanocyte transformation. Pigment Cell Melanoma Res. Jun; 2008 21(3):368–378. [PubMed: 18435704]
46. Shin SS, Wall BA, Goydos JS, Chen S. AKT2 is a downstream target of metabotropic glutamate receptor 1 (Grm1). Pigment Cell Melanoma Res. Feb; 2010 23(1):103–111. [PubMed: 19843246]
47. Stahl JM, Sharma A, Cheung M, et al. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res. Oct 1; 2004 64(19):7002–7010. [PubMed: 15466193]
48. Nogueira C, Kim KH, Sung H, et al. Cooperative interactions of PTEN deficiency and RAS activation in melanoma metastasis. Oncogene. Nov 25; 2010 29(47):6222–6232. [PubMed: 20711233]
49. Cheah BC, Vucic S, Krishnan AV, Kiernan MC. Riluzole, neuroprotection and amyotrophic lateral sclerosis. Curr Med Chem. 2010; 17(18):1942–1199. [PubMed: 20377511]
50. Yip D, Le MN, Chan JL, et al. A phase 0 trial of riluzole in patients with resectable stage III and IV melanoma. Clin Cancer Res. Jun 1; 2009 15(11):3896–3902. [PubMed: 19458050]
51. Biechele TL, Camp ND, Fass DM, et al. Chemical-genetic screen identifies riluzole as an enhancer of Wnt/beta-catenin signaling in melanoma. Chem Biol. Nov 24; 2010 17(11):1177–1182. [PubMed: 21095567]
52. Chien AJ, Moore EC, Lonsdorf AS, et al. Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. Proc Natl Acad Sci U S A. Jan 27; 2009 106(4):1193–1198. [PubMed: 19144919]
53. Degenhardt K, White E. A mouse model system to genetically dissect the molecular mechanisms regulating tumorigenesis. Clin Cancer Res. Sep 15; 2006 12(18):5298–5304. [PubMed: 17000662]
54. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. Aug; 2007 114(2):97–109. [PubMed: 17618441]

55. Ye ZC, Sontheimer H. Glioma cells release excitotoxic concentrations of glutamate. Cancer Res. Sep 1; 1999 59(17):4383–4391. [PubMed: 10485487]
56. Arcella A, Carpinelli G, Battaglia G, et al. Pharmacological blockade of group II metabotropic glutamate receptors reduces the growth of glioma cells in vivo. Neuro Oncol. Jul; 2005 7(3):236–245. [PubMed: 16053698]
57. D'Onofrio M, Arcella A, Bruno V, et al. Pharmacological blockade of mGlu2/3 metabotropic glutamate receptors reduces cell proliferation in cultured human glioma cells. J Neurochem. Mar; 2003 84(6):1288–1295. [PubMed: 12614329]
58. Ciceroni C, Arcella A, Mosillo P, et al. Type-3 metabotropic glutamate receptors negatively modulate bone morphogenetic protein receptor signaling and support the tumourigenic potential of glioma-initiating cells. Neuropharmacology. Sep; 2008 55(4):568–576. [PubMed: 18621067]
59. Denysenko T, Gennero L, Roos MA, et al. Glioblastoma cancer stem cells: heterogeneity, microenvironment and related therapeutic strategies. Cell Biochem Funct. Jul; 2010 28(5):343–351. [PubMed: 20535838]
60. Gomes WA, Mehler MF, Kessler JA. Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. Dev Biol. Mar 1; 2003 255(1):164–177. [PubMed: 12618141]
61. Huse JT, Holland EC. Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. Nat Rev Cancer. May; 2010 10(5):319–331. [PubMed: 20414201]
62. Canudas AM, Di Giorgi-Gerevini V, Iacovelli L, et al. PHCCC, a specific enhancer of type 4 metabotropic glutamate receptors, reduces proliferation and promotes differentiation of cerebellar granule cell neuroprecursors. J Neurosci. Nov 17; 2004 24(46):10343–10352. [PubMed: 15548648]
63. Iacovelli L, Arcella A, Battaglia G, et al. Pharmacological activation of mGlu4 metabotropic glutamate receptors inhibits the growth of medulloblastomas. J Neurosci. Aug 9; 2006 26(32): 8388–8397. [PubMed: 16899734]
64. Ye P, Xing Y, Dai Z, D'Ercole AJ. In vivo actions of insulin-like growth factor-I (IGF-I) on cerebellum development in transgenic mice: evidence that IGF-I increases proliferation of granule cell progenitors. Brain Res Dev Brain Res. Aug 20; 1996 95(1):44–54.
65. Dahmane N, Ruiz i Altaba A. Sonic hedgehog regulates the growth and patterning of the cerebellum. Development. Jun; 1999 126(14):3089–3100. [PubMed: 10375501]
66. Yokota N, Nishizawa S, Ohta S, et al. Role of Wnt pathway in medulloblastoma oncogenesis. Int J Cancer. Sep 10; 2002 101(2):198–201. [PubMed: 12209999]
67. Gilbertson RJ. Medulloblastoma: signalling a change in treatment. Lancet Oncol. Apr; 2004 5(4): 209–218. [PubMed: 15050952]
68. Pazzaglia S, Mancuso M, Atkinson MJ, et al. High incidence of medulloblastoma following X-ray-irradiation of newborn Ptc1 heterozygous mice. Oncogene. Oct 24; 2002 21(49):7580–7584. [PubMed: 12386820]
69. Hopkins CR, Lindsley CW, Niswender CM. mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. Future Med Chem. Jun 1; 2009 1(3):501–513. [PubMed: 20161443]
70. Yoo BC, Jeon E, Hong SH, Shin YK, Chang HJ, Park JG. Metabotropic glutamate receptor 4-mediated 5-Fluorouracil resistance in a human colon cancer cell line. Clin Cancer Res. Jun 15; 2004 10(12 Pt 1):4176–4184. [PubMed: 15217955]
71. Chang HJ, Yoo BC, Lim SB, Jeong SY, Kim WH, Park JG. Metabotropic glutamate receptor 4 expression in colorectal carcinoma and its prognostic significance. Clin Cancer Res. May 1; 2005 11(9):3288–3295. [PubMed: 15867225]
72. Shin SS, Martino JJ, Chen S. Metabotropic glutamate receptors (mGlus) and cellular transformation. Neuropharmacology. Sep; 2008 55(4):396–402. [PubMed: 18554669]
73. Cappuccio I, Spinsanti P, Porcellini A, et al. Endogenous activation of mGlu5 metabotropic glutamate receptors supports self-renewal of cultured mouse embryonic stem cells. Neuropharmacology. 2005; 49(Suppl 1):196–205. [PubMed: 16023153]
74. Spinsanti P, De Vita T, Di Castro S, et al. Endogenously activated mGlu5 metabotropic glutamate receptors sustain the increase in c-Myc expression induced by leukaemia inhibitory factor in

cultured mouse embryonic stem cells. J Neurochem. Oct; 2006 99(1):299–307. [PubMed: 16987252]

75. Park SY, Lee SA, Han IH, et al. Clinical significance of metabotropic glutamate receptor 5 expression in oral squamous cell carcinoma. Oncol Rep. Jan; 2007 17(1):81–87. [PubMed: 17143482]
76. Kalariti N, Lembessis P, Koutsilieris M. Characterization of the glutametergic system in MG-63 osteoblast-like osteosarcoma cells. Anticancer Res. Nov-Dec;2004 24(6):3923–3929. [PubMed: 15736433]
77. Kalariti N, Lembessis P, Papageorgiou E, Pissimissis N, Koutsilieris M. Regulation of the mGluR5, EAAT1 and GS expression by glucocorticoids in MG-63 osteoblast-like osteosarcoma cells. J Musculoskelet Neuronal Interact. Apr-Jun;2007 7(2):113–118. [PubMed: 17627080]
78. Taylor AF. Osteoblastic glutamate receptor function regulates bone formation and resorption. J Musculoskelet Neuronal Interact. Mar; 2002 2(3):285–290. [PubMed: 15758456]
79. Wei X, Walia V, Lin JC, et al. Exome sequencing identifies GRIN2A as frequently mutated in melanoma. Nat Genet. May; 2011 43(5):442–446. [PubMed: 21499247]
80. Speyer CL, Smith JS, Banda M, Devries JA, Mekani T, Gorski DH. Metabotropic glutamate receptor-1: a potential therapeutic target for the treatment of breast cancer. Breast Cancer Res Treat. Jun 17.2011
81. Pissimissis N, Papageorgiou E, Lembessis P, Armakolas A, Koutsilieris M. The glutamatergic system expression in human PC-3 and LNCaP prostate cancer cells. Anticancer Res. Jan; 2009 29(1):371–377. [PubMed: 19331175]
82. Aleman M, M A, Harsch KM, Detore N, Klein E, Heston WDW. Expression of metabotropic glutamate receptors in human prostate cancers and its potential signaling through prostate specific membrane antigen. Proc Am Assoc Cancer Res. 2004; 45:1196.

Table 1

mGlu receptors: classification and implication in cancers

	Subclass	Membrane-associated signal transmitters	Potential anti-cancer agents*[6,17]	Implications	Reference
Group I	**mGluR1**	$G_{q/11}$	mGluR1 NAM: Bay-36-7620	Melanoma Breast Cancer Glioma Medulloblastoma	[33]Pollock et al., 2003 [80]Speyer et al., 2011 [26]Brocke et al., 2010
	mGluR5	Phospholipase C stimulation		Oral Squamous Cell Carcinoma Osteosarcoma Prostate Cancer Glioma Medulloblastoma	[75]Park et al., 2007 [77]Kalariti et al., 2007 [81]Pissimissis et al., 2009 [26]Brocke et al., 2010
Group II	**mGluR2**	$G_{i/o}$ Inhibition of adenyl cyclase		Prostate Cancer Glioma	[82]Aleman et al., 2004 [56,57]D'Onofrio et al., 2003; Arcella et al., 2005
	mGluR3		mGluR2/3 Pref ant: LY341495	Glioma	[56-58]D'Onofrio et al., 2003; Arcella et al., 2005; Ciceroni et al., 2008
Group III	**mGluR4**	$G_{i/o}$ Inhibition of adenyl cyclase	mGluR4 PAM: PHCCC	Colorectal carcinoma Glioma Medulloblastoma	[71]Chang et al., 2005 [26]Brocke et al., 2010 [26,63]Iacovelli et al., 2006; Brocke et al.,2010
	mGluR6			Glioma Medulloblastoma	[26]Brocke et al., 2010
	mGluR7			?	
	mGluR8			?	

*validated in *in-vivo* xenograft models, NAM; negative allosteric modulators, Pref ant; preferential antagonists, PAM; positive allosteric modulators.