Glutamate receptors: emerging players in melanomagenesis

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Large-scale genetic screens of tumor samples have provided an ideal mechanism for the identification of genes that contain driver mutations in various tumor types, including melanoma, and offer a plethora of information with regard to potential novel drug candidates. Recent reports on exome sequencing and large-scale mutational screening of G protein-coupled receptors (GPCRs) in melanoma tumor samples have identified a variety of genes that can serve this purpose. GPCRs have provided therapeutic targets for multiple diseases and malignancies, and as such, it is not surprising that more GPCRs are being identified as having roles in melanomagenesis and metastasis when mutated or aberrantly expressed. Importantly, such approaches underscore the need to integrate these targets in the design of melanoma therapies.

In the coverage paper, Prickett et al., performed mutational analysis on 734 GPCRs in 11 melanoma samples during which they identified 755 non-synonymous mutations. Matching of genomic DNA of both normal and tumor samples identified 106 somatic mutations in 94 genes. Further analyses were performed on genes that were found to harbor at least two somatic mutations in an additional cohort of 80 melanoma samples. The sequencing of this cohort identified an additional 115 non-synonymous somatic mutations in over 50% of these samples. These mutations are enriched for C>T transitions which is significant as it confirms earlier observations of melanoma mutation signatures. Of the 11 genes identified in the second screen, GRM3 and GPR98 showed a higher rate of mutations than the rest. In particular, GPR98 was mutated in 27.5% of the tumors, while GRM3 was mutated in 16.3% of the tumor samples. The identification of GRM3 mutations in melanoma is noteworthy as previously, another member of the metabotropic glutamate receptor family, GRM1, had been shown to be involved in melanoma development (Pollock et al., 2003). Additional assessment of GRM3 mutations on an independent panel consisting of 57 melanoma samples also found a mutation rate of 15.7% in this gene thus confirming the prior observation. Among the mutations detected in GRM3, the Glu870Lys was identified in one sample in the first cohort and three samples in the second cohort denoting that this is a likely mutational hotspot in the gene and that this mutation likely confers a selective growth advantage to tumors harboring it. The authors set out to examine the functional roles of this mutation and three others; Gly561Glu, Ser610Leu, and Glu767Lys, by creating stable clones expressing GRM3 mutant proteins. These stable clones exhibited faster growth properties in reduced serum media as well as anchorage independence. Treatment of these cells with an agonist of GRM3, DCG-IV, resulted in enhanced levels of activated phosphorylated MEK1/2, a kinase in the MAPK signaling cascade. The MAPK pathway has an integral role in melanoma biology owing to the presence of the activating BRAFV600E mutation in a high percentage of tumors and benign nevi. MEK is known to have a critical role in cell migration, and it was proposed that the presence of GRM3 mutations serves to boost MEK activation and its cell migration functions. Indeed, it was confirmed that cells expressing mutant GRM3 proteins displayed greater migratory abilities than cells with wild-type GRM3. This property may have an impact on their ability to migrate and proliferate to specific metastatic sites. To validate the effects of these mutations in vivo tumor growth, xenograft studies were performed with the results showing that mice inoculated with cells harboring the Ser610Glu, Glu767Lys, or Glu870Lys mutation exhibited a significantly higher rate of pulmonary metastases than wild-type GRM3 cells indicating that these mutations have the ability to modulate tumor metastasis in vivo. siRNA knockdown of GRM3 in cells expressing the mutant proteins led to a reduction in cell migration and proliferation in vitro and in vivo. Considering that GRM3 mutations were shown to enhance MEK1/2 kinase phosphorylation of ERK1/2, AZD-6244, a MEK inhibitor currently being assessed in various clinical trials was used to treat cells harboring these GRM3 mutations. AZD-6244 was able to inhibit MEK activity and enhance cellular apoptosis with greater efficacy in mutant GRM3 cells than in wild-type GRM3 cells. Another interesting observation from these studies was that BRAFV600E melanoma cells harboring the GRM3 activating mutations showed increased sensitivity to AZD-6244 than cells with wild-type GRM3. The presence of these two activating mutations is likely to position these cells’ dependence on MEK signaling for growth and is thus more likely to respond to MEK inhibitor(s) than cells harboring just one activating mutation. It is also likely that the presence of GRM3 activating mutations in BRAFV600E populations could serve as predictors of MEK inhibitor response.

To date, eight metabotropic glutamate receptors have been identified and have been classified into three groups based on agonist pharmacology, sequence homology, and signal transduction mechanisms via coupling to second messenger systems. GRM1 and GRM5 are Group I GRMs and are coupled to Gα proteins to increase phosphoinositide hydrolysis via activation of phospholipase C, which results in intracellular calcium release and protein kinase C (PKC) activation (Choi et al., 2011; Pollock et al., 2003). GRM2 and GRM3 are Group II, while GRM4, GRM6, GRM7, and GRM8 are Group III which are both coupled to Gα/γ proteins and mediate downstream signaling through adenylyl cyclase inhibition systems (Stepulak et al., 2009). It has now been shown that several GRMs, similar to other oncogenic GPCRs, when overexpres-
sed, aberrantly expressed or mutated can also be oncogenic in melanoma. GRM1 was the first metabotropic glutamate receptor to demonstrate its ability to transform melanocytes in vitro and induce tumorigenesis in vivo in the absence of mutation(s) (Pollock et al., 2003). Unlike GRM1, the identification of GRM3 activating mutations in melanoma populations and their activities in promoting melanoma cell migration and metastatic ability suggest that there are various paths for a ‘normal’ protein to become ‘oncogenic’. In this same report, Prickett et al., also detected mutations in another metabotropic glutamate receptor, GRM8, in 8% of tumors analyzed. Although some of them were inactivating mutations, others were predicted to have a deleterious effect on protein function by SIFT analysis. It remains to be seen what roles these GRM8 mutations play. Earlier, the same group also described mutations in GRIN2A, an ionotropic NMDA glutamate receptor, which was found mutated in 25% of melanoma samples (Wei et al., 2011). The majority of these mutations were somatic C>T substitutions commonly found in melanoma. However, the underlying mechanisms of these GRIN2A mutations in melanoma pathogenesis remain unknown.

Additionally, Choi et al. (2011), have shown that overexpression of GRM5, a gene that has previously been detected in normal melanocytes and is thought to have a role in pigmentation, can induce melanoma in transgenic mice. The overexpression of GRM5 under a melanocyte specific promoter, TRP1, led to the development of hyperpigmented melanoma lesions with 100% penetrance as early as 4 days on the tails, ears, snout, perianal regions, meninges, and feet. These lesions were also invasive with muscle, bone, and lymph node involvement. GRM5 is normally expressed in human melanocytes, but the evaluation of metastatic melanoma cell lines and tissue samples showed higher expression in the tumor samples than in the cell lines. Similar to ectopic expression of GRM1 in melanocytes, GRM5 overexpression also activates ERK1/2, a kinase in the MAPK signaling pathway. This activation of MAPK signaling by GRM1 and GRM5 is concordant with the MEK activation observed with GRM3 activating mutations. Thus, targeting the MAPK pathway in cells with aberrant glutamate receptor signaling might be a viable option as recently reported by Lee et al. (2011).

Taken together, results from these studies put forward the notion that altered functions of metabotropic glutamate receptors by aberrant expression, overexpression, or activating mutations have deleterious consequences, tumorigenesis. The growing list of glutamate signaling with involvement in cancer biology may shift the paradigm in the design of therapies not only for melanoma but also for other malignancies. The expression of AMPA, NMDA, kainate, and metabotropic glutamate receptors has been reported in multiple tumor types (Stepulak et al., 2009), and given that glutamate receptor antagonists have been reported to have antitumor properties, a different class of cancer therapies is emerging and warrants investigation.

References