Assessing the Response of Mixtures of EGFR Amplified and Non-amplified Glioma Stem Cell Lines in Response to MEK Inhibitor Treatment
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Glioblastoma’s ability to resist treatment arises from the heterogeneity of glioma stem cells and the heterogeneity of the composition of tumor microenvironments. EGFR amplification (EGFRAmp) is the most common genetic alteration in GBM and can lead to oncogenic activation of the ERK pathway. Although microenvironmental signals can impact gene expression in GSCs, how signaling between EGFRAmp and non-amplified cells in response to treatment alters the cells’ phenotype and sensitivity to treatment remains unexplored. Using an in-vitro model, we assessed the MEK inhibitor (MEKi) sensitivity in EGFR amplified (MEKi sensitive) or non-amplified (MEKi resistant) GSCs collected from patient samples. To differentiate cell lines, cells were engineered to express either GFP or RFP. We treated various proportions of mixtures of sensitive and resistant GSC lines with one of several MEK inhibitors, inactivating ERK signaling. We then assessed the cell viability, phenotype, and expression of ERK pathway components of both cell lines in the mixtures via Cell Titer-Glo, flow cytometry, and qPCR analysis. We also assessed if overexpression of ETV5, a product of the ERK pathway, could alter GSC proliferation and MEKi sensitivity. In the mixing experiments, there is a sharp increase in MEKi resistance with proportions composed of more than 50% resistant cells. In addition, current work is inconclusive as to whether ETV5 overexpression in MEKi-sensitive cell lines can increase MEKi resistance, but it does not appear to increase proliferation rates of cells. Future could possibly elucidate the mechanisms through which MEKi resistance is conferred between EGFRAmp and non-amplified cells.

Methods

Cell lines: All GSC lines were derived from patients. Cell lines had RFP and GFP plasmids transfected into appropriate clones, and negative clones were separated out via flow cytometry.

Cell Mixing: For mixing experiments, sensitive and resistant tagged cell lines were mixed in the following 5 proportions:

- Sensitive cell lines: RFP Tagged
- Resistant cell lines: GFP Tagged

Cell Viability: GSCs were assayed for viability after 72 h treatment of 0, 1, 10, 100, 500, 1000, 5000, and 10000 nM MEKi via CellTiter-Glo® Luminescent Cell Viability Assay.

Flow Cytometry Analysis: Cells were assayed for cell viability after 72 h treatment of 0, 100, and 1000 nM MEKi treatment via flow cytometry apoptosis analysis with DAPI staining. Individual cell lines in mixtures were identified by GFP and RFP expression.

ETV5 Overexpression: GSC 11 was transfected with dCas9-VPR plasmids and one of two sgRNA plasmids designed for ETV5: positive clones were selected with appropriate antibiotics.

Results

Initial MEKi Sensitivity Screening

![Figure 1](Image 0x1516 to 208x741)

Figure 1. Percentage of viable cells relative to untreated controls after 72 h of treatment with various concentrations of MEKis as determined by Cell-Titer Glo. IC50 values were calculated from corresponding best fit curves of cell viability. 1a-1e represent different cell line/MEKi combinations.

Resistant/Sensitive Cell Mixture MEKi Sensitivity

![Figure 2](Image 0x610x845 to 1075x1094)

Figure 2. MEKi sensitivity of GSC 8-11-GFP (MEKi-resistant) and 11-RFP (MEKi-sensitive) mixtures. a. Percentage of viable cells relative to untreated controls of same mixture after 72 h of treatment with various concentrations of trametinib as determined by Cell-Titer Glo. IC50 values were calculated from corresponding best fit curves of cell viability. b. Percentage of GSC 8-11-GFP cells in mixtures and corresponding Log(IC50) values of mixture.

MEKi Sensitivity in GSC 11 ETV5 OE Cell lines

![Figure 3](Image 1085x1046 to 1130x1094)

Figure 3: Phenotypic differences in GSC 11 (MEKi-sensitive) cells with ETV-5 OE a and b. Percentage of viable cells relative to untreated controls after 72 h of treatment with various concentrations of trametinib as determined by Cell-Titer Glo. IC50 values were calculated from corresponding best fit curves of cell viability. GSC 11 ETV5 OE 0011 and 0012 lines were derived from two separate guide RNA sequences to induce ETV5 overexpression.

Conclusions

- ETV5 overexpression may affect trametinib resistance, but current results are inconclusive. MEKi sensitivity may be related to ERK expression or expression of other components of the ERK pathway.
- The proportion of EGFRAmpl cells in mixtures and the Log(IC50) of the mixture have a relatively linear relationship
- MEKi sensitivity is highest in non-amplified cells, but the degree of sensitivity varies by inhibitor

Ongoing Work

- Complete flow cytometry analysis of mixed cell lines to determine cell cycle/viability of treated cells for each cell line in mixture
- Perform qPCR analysis of mixed cell lines and ETV5 OE lines to determine expression of glioma subtype markers/ERK pathway components

References