Comparing ERK signaling and Tumor Microenvironment in BRAF-altered Gliomas

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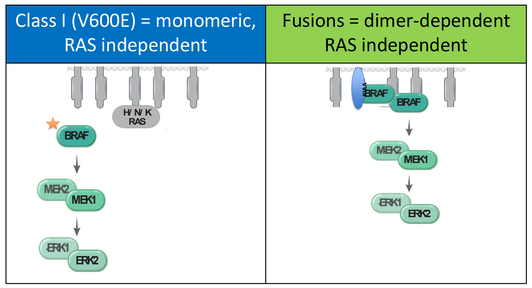
Introduction

- BRAF Class I and fusion alterations are two common oncogenic drivers in glioma[1]:



- The FDA has approved two regimens for the treatment of BRAF-altered glioma: a BRAF-V600E monomer inhibitor (dabrafenib) in combination with a MEK inhibitor (trametinib) or a pan-RAF inhibitor (tovorafenib)
- However, the efficacy of BRAF inhibitors (BRAFi) is limited by intrinsic and acquired resistance
- In other solid tumors, MAPK/ERK-dependence signatures have been identified that predict overall survival and response to BRAFi [2,3,4]
- The tumor immune microenvironment (TME) also mediates response to BRAFi and predicts survival in melanoma[5,6,7, 8, 9]
- However, it is unknown whether MAPK/ERK-dependence and TME signatures can predict survival and resistance to BRAFi in glioma

Objectives

1) Assess MAPK/ERK activation and the TME in pediatric, young adult, and adult low-grade glioma (LGG) and high-grade glioma (HGG) with differing BRAF alterations
2) Correlate MAPK/ERK activation and TME to survival and resistance to BRAFi

Materials and Methods

Study Design	Retrospective cohort study
Setting	Samples from Caris Life Sciences (Phoenix, AZ) that had undergone next-generation sequencing and whole transcriptome sequencing.
Sample/Population	All HGG and LGG with a BRAF Class I (V600) or fusion alterations. Controls were BRAF-wt, IDH1/2-wt, and NF1-wt.
Predictors	The MAPK Pathway Activity Score (MPAS) and two MEK inhibitor sensitivity signatures were calculated from RNA-seq data. The TME was assessed using immune deconvolution (quanTIseq) and the Tumor Inflammation Signature (TIS).
Outcomes	Overall survival
Statistical Analysis	Pairwise comparisons between BRAF alterations were performed using Mann-Whiney U tests at α = 0.05. Survival analysis was performed using Kaplan-Meier analysis and the log-rank test. Pathway analysis was performed using GSEA 4.3.2 (pre-ranked).

Table 1: Patient demographics by grade and BRAF alteration class

	High Grade Glioma			Low Grade Glioma		
	Class I	Fusions	WT	Class I	Fusions	WT
n	124	28	4959	54	70	349
Age						
<40	57	11	308	44	48	141
>=40	67	17	4651	10	22	208
Gender						
Male	71	18	2926	33	41	190
Female	53	10	2033	21	29	159

Results

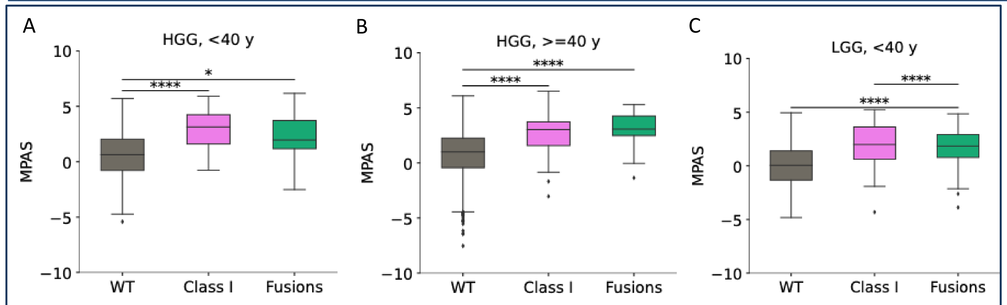


Figure 1 – BRAF-altered glioma exhibit greater MAPK/ERK dependence regardless of grade or age category. Comparison of MAPK Activation Signature (MPAS)[2] scores between glioma samples from patients (A) ≥ 40 years old with HGG, and (B) <40 years old with HGG, and (C) <40 years old with LGG. Boxes show the median and interquartile range (IQR), with whiskers extending to ± 1.5 IQR.*p<0.05, **p<0.01, **p < 0.001, ****p<0.0001.

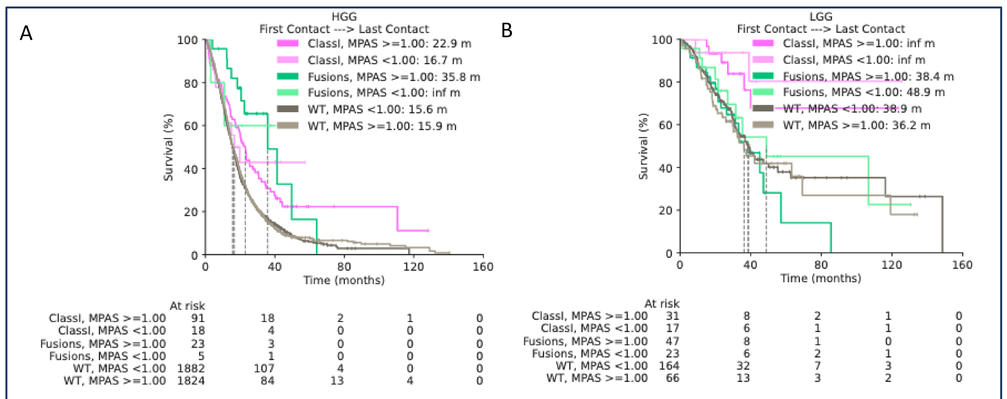


Figure 2 – MAPK/ERK-dependence signatures do not predict survival in BRAF-altered glioma
Kaplan-Meier curves for high vs. low MPAS scores in (A) HGG and (B) LGG, as determined relative to the median MPAS score across all samples. Samples are stratified by BRAF alteration class. Log-rank test p < 0.05 for all comparisons within BRAF classes.

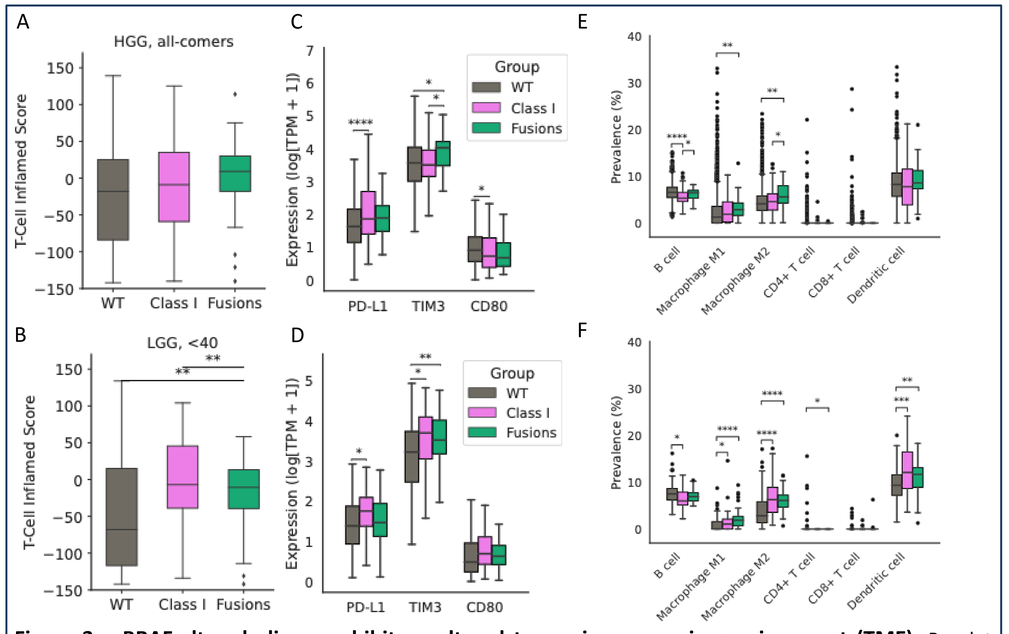


Figure 3 – BRAF-altered glioma exhibit an altered tumor immune microenvironment (TME). Boxplots comparing (A-B) the tumor immune microenvironment (TIS) signature[10], (C-D) the expression of immuno-oncology genes, and (D-E) immune cell infiltration between BRAF-alteration classes in HGG of all ages (top) and LGG < 40 years old (bottom). Only immune cell populations with differential infiltration are displayed. All boxes show the median and interquartile range (IQR), with whiskers extending to ± 1.5 IQR.*p<0.05, **p<0.01, **p < 0.001, ****p<0.0001.

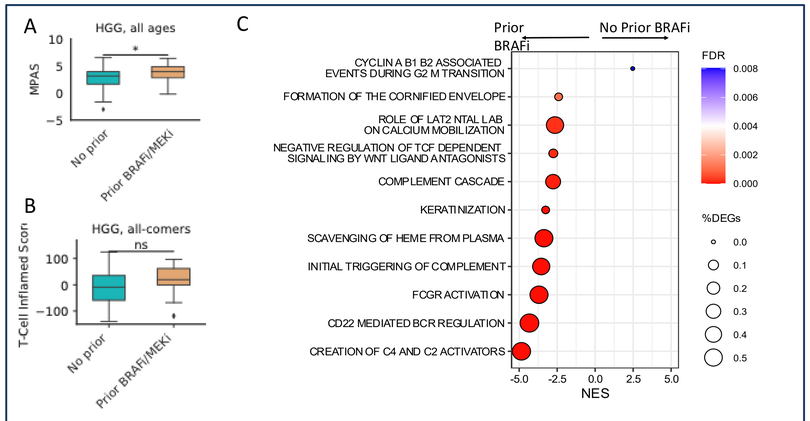


Figure 4 – MAPK/ERK-dependence, complement, and humoral immunity signatures are altered in HGG with Class I BRAF alterations after progression on BRAFi. Comparison of (A) MPAS scores and (B) TIS scores from HGG samples with BRAF Class I alterations from patients who had received no prior vs. prior treatment with BRAFi. Boxes show the median and interquartile range (IQR), with whiskers extending to ± 1.5 IQR.*p<0.05, **p<0.01, **p < 0.001, ****p<0.0001. (C) Dot plot displaying normalized enrichment scores (NES) and false discovery rate from GSEA comparing samples from patients with no prior vs. prior BRAFi. All Reactome pathways with an FDR < 0.01 are displayed.

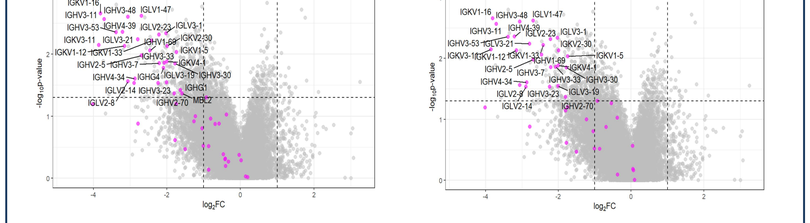


Figure 5 – Immunoglobin genes are upregulated in HGG with Class I BRAF alterations after progression on BRAFi. Volcano plots highlighting differentially expressed genes (DEGs) in the (A) the Reactome Creation of C4 and C2 Activators and (B) Reactome CD22-mediated BCR Regulation pathways. Thresholds for DEGs defined as Log2-Fold-change ≥ 1 OR ≤ -1 (vertical dashed lines) and Benjamini-Hochberg corrected p-values ≤ 0.05 (horizontal dashed line). Magenta: genes in pathway. Gray: all other measured genes.

Conclusion

- MAPK/ERK signaling is increased in BRAF-altered glioma regardless of age and grade, with BRAF Class I exhibiting the greatest ERK-dependence.
- MAPK/ERK-dependence does not predict survival in either HGG or LGG within BRAF alteration classes
- BRAF-altered LGG but not BRAF-altered HGG exhibits increased tumor inflammation/T-cell inflammation, with BRAF Class I demonstrating the highest inflammation
- BRAF-altered HGG and LGG exhibit increased expression of PD-L1 and HAVCR2
- BRAF-altered HGG and LGG glioma exhibit decreased B-cell infiltration and increased M1/M2 macrophage infiltration
- Multiple transcriptional changes occur during the acquisition of resistance to BRAFi, including downregulation of MAPK/ERK signaling and changes to the TME
- Changes to the TME in acquired resistance to BRAFi are largely due to the upregulation of immunoglobulin genes

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