Cells expressing BRAF^{V600E} have a unique lipid profile

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Background

BRAF is a serine/threonine kinase in the MAPK pathway that is mutated in many human cancers[1].

Conventional therapies targeting BRAF^{V600E}, the most common BRAF mutation, have short-lived benefit due to treatment resistance[2].

Aerobic glycolysis (The Warburg Effect[3]) is the classic paradigm of cancer metabolism, but recent evidence suggests cancers may also oxidize polyunsaturated fatty acids (PUFAs)[4].

Understanding the metabolism of cells expressing BRAF^{V600E} may identify targets to overcome resistance to BRAF inhibitor (BRAFi) therapy.

Question: *How does BRAF^{V600E} status affect metabolism?*

Hypothesis: ***BRAF^{V600E} promotes utilization of PUFAs as an energy source.***

Aims: 1) To understand the metabolic profile of *BRAF^{V600E}* mutated cells and 2) to uncover targets to overcome BRAFi therapy resistance.

Methods

To study lipid metabolism in cells with and without the *BRAF^{V600E}* mutation, we used four stable overexpression models in NIH3T3 cells.

To characterize the lipid profiles of BRAF^{V600E} cells, we performed tandem mass spectrometry (MS^2), immunofluorescence, qRT-PCR, and Agilent Seahorse metabolic flux Assay.

To characterize the lipid profiles of patients who responded vs. did not respond to BRAFi therapy, we performed MS^2 lipidomics analysis of patient serum samples.

Results

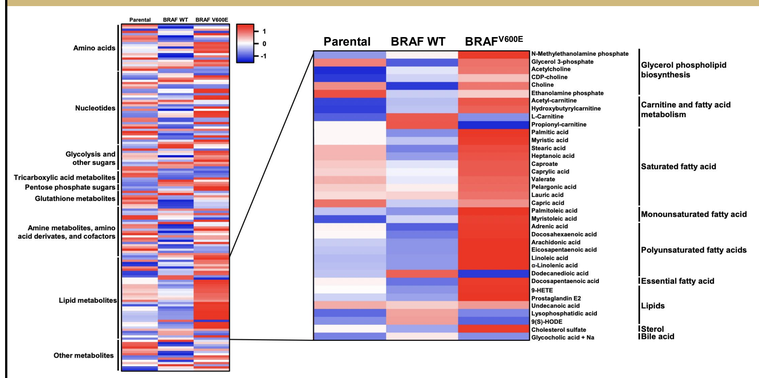

Figure 1. BRAF status and expression modulates the metabolic profile. Heatmap representing relative metabolite abundancies with averaged triplicates normalized to parental control. Red = increased relative abundance, blue = decreased.

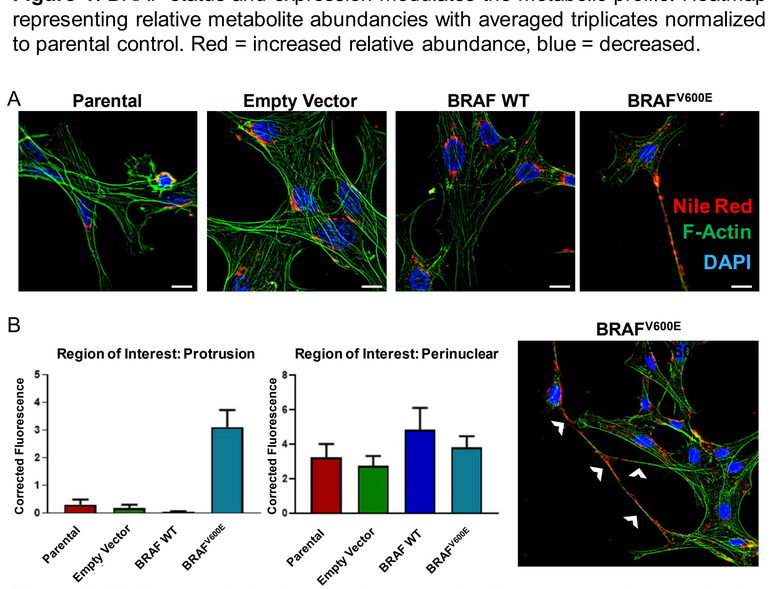

Figure 2. Lipid accumulation in tunneling nanotube-like structures is an exclusive characteristic of cells expressing BRAF^{V600E}. **(A)** Immunofluorescence staining for lipid droplets in red using Nile Red, F-actin using phalloidin in green, and nuclei in blue using DAPI. Scale bars represent 20 μm. **(B)** Regions of interest were quantified using fixed areas to measure fluorescence in the red channel.

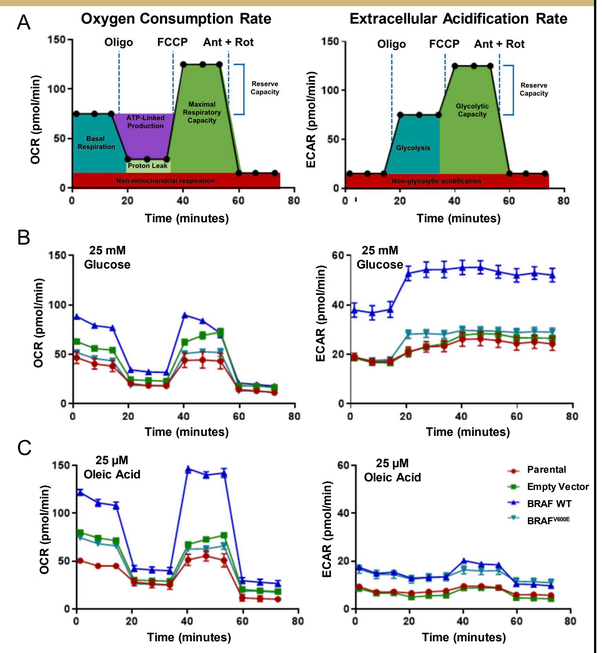

Figure 3. WT BRAF cells have a more flexible metabolism than BRAF^{V600E} cells. **(A)** Schematics of Seahorse metabolic flux assay for both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) with metabolic poisons. **(B,C)** OCR and ECAR in 25 mM glucose and 25 μM oleic acid conditions.

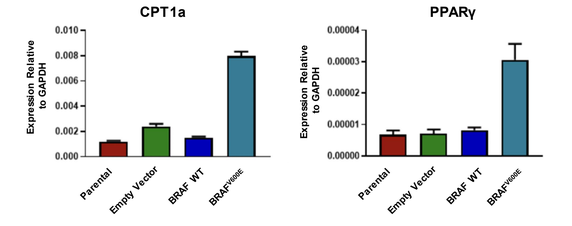

Figure 4. BRAF^{V600E} cells have a unique transcriptomic signature. Quantified mRNA expression of CPT1a and PPARγ by qRT-PCR analysis. Expression was normalized to GAPDH. SEM of triplicates are represented by error bars.

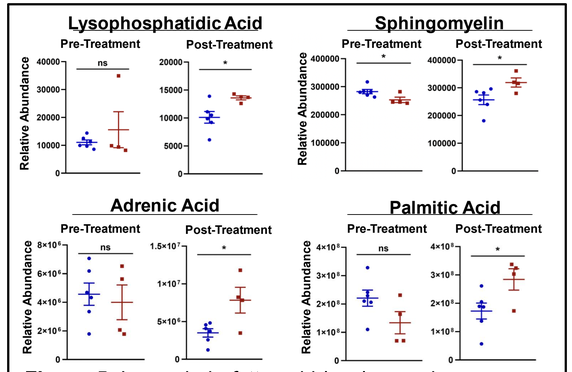

Figure 5. Long chain fatty acid levels vary in response BRAFi therapy in advanced stage melanoma patients. Blue = responders; red = non-responders.

Conclusions and Limitations

BRAF^{V600E} expression plays a critical role in determining lipid use and accumulation.

Lipid droplets aggregate in tunneling nanotube-like projections in BRAF^{V600E} cells.

The immunomodulatory and long-chain PUFA profile of patients who do not respond to BRAFi therapy is distinct from patients who do respond.

While these findings represent a potential target to improve response to BRAFi therapy, patient data were limited due to sample size. Cancer cell lines should also be analyzed in future studies.

Acknowledgements

This work was supported by the University of Colorado Cancer Center, the American Cancer Society Research Scholar Grant 16-256-01-TBE, and the Hertz Foundation.

A special thanks to Jackie Turner!

References: **1.** Davies, H. et al. Mutations of the BRAF gene in human cancer. Nature 417, 949-954 (2002) **2.** Long, G. et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. NEJM 371, 1877–1888 (2014) **3.** Warburg, O. et al. The Metabolism of Tumors in the Body. J Gen Physiol 8, 519-530 (1927). **4.** Nagarajan, S. et al. The diversity and breadth of cancer cell fatty acid metabolism. Cancer Metab 9, 2. (2021). **Conflicts of Interest:** The authors of this poster have no COIs to disclose.