**Background**

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

Conventional therapies targeting BRAF\(^{V600E}\) (the most common BRAF mutation) have short-lived benefit due to treatment resistance. Aerobic glycolysis (The Warburg Effect) is the classic paradigm of cancer metabolism, but recent evidence suggests cancers may also oxidize polyunsaturated fatty acids (PUFAs).

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\(^2\) lipodomics 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 phallacidin 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.

**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 PUFAs 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.

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