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TITLE: Optimization of Autophagy Inhibition as a Clinical Target for Brain Tumors

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Introduction:

Autophagy is a multi-stage process. Drugs targeting both early (initiation) and late (fusion) stages of this process are available. The specific stage of autophagy targeted may influence cancer treatment outcomes. CNS tumors with the $BRAF^{V600E}$ mutation are autophagy dependent, and late stage autophagy inhibition improves response to targeted BRAF inhibitors (BRAFi). We investigated early stage inhibition for autophagy dependent CNS tumors. BRAFi-sensitive and resistant AM38 and MAF794 cell lines were evaluated for response to pharmacologic and genetic inhibition of ULK1 and VPS34, two crucial subunits of the autophagy initiation complexes. Changes in autophagy were monitored by western blot and flow cytometry. Short and long-term assays were evaluated. Tumor cells exhibited reduced autophagic flux with pharmacologic and genetic inhibition of ULK1 or VPS34. Pharmacologic inhibition reduced cell survival in a dose dependent manner for both targets. Genetic inhibition reduced cell survival and confirmed it was an autophagy specific effect. Pharmacologic and genetic inhibition were also synergistic with BRAFi, irrespective of RAFi sensitivity. Inhibition of ULK1 and VPS34 are potentially viable clinical targets in autophagy dependent CNS tumors. Further evaluation is needed to determine if early and late stage autophagy inhibition are equally efficacious to determine the optimal clinical target for patients.

Keywords:

Autophagy BRAF Brain tumor Pediatric Resistance

Accomplishments:

The major aims of this project were: (1) Determine the optimal target for autophagy inhibition in BRAF mutated CNS tumors. (2) Determine if the V600E mutation is required for autophagy dependence in CNS tumor cells, or if any cause of dysregulated RAS/RAF/MEK pathway is sufficient to identify autophagy dependence. (3) Determine the mechanism by which autophagy inhibition overcomes multiple BRAF inhibitor resistance mechanisms in CNS tumors.

The major goals of the project as outlined in the SOW and accomplishments are as follows:

Specific Aim 1: Determine the optimal target for autophagy inhibition in BRAF mutated CNS tumors.

Major Task 1: Evaluate genetic inhibition of VPS34 and ULK1 for autophagy inhibition in BRAF^{V600E} mutated CNS Tumor cells.

We have successfully completed the genetic evaluation of inhibition of these targets in MAF794 and AM38 cells. Flow data analysis and survival analysis are below and portrayed in figure form for an upcoming manuscript submission.

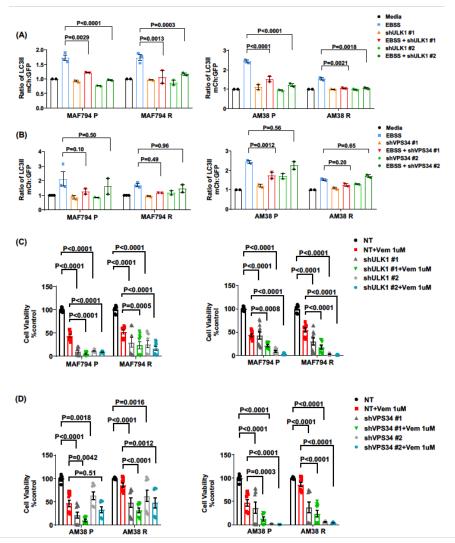


Figure 1. Genetic early stage autophagy inhibition improves sensitivity towards BRAFi. (A-B) Quantifications of basal and induced autophagy in MAF794 and AM38 parental and resistant cells following autophagy inhibition through RNAi against ULK1 (A) or VPS34 (B) compared to non-targeting (NT) RNAi. Autophagic flux was determined as previously described. (C-D) Percent cell viability demonstrating the effectiveness of autophagy inhibition through RNAi against ULK1 (C) or VPS34 (D) compared to NT RNAi in the presence or absence of BRAFi. Percent cell viability was measured by CellTiter Glo assay following 5-day exposure to vemurafenib with or without RNAi against ULK1 or VPS34. Dunnett's multiple comparisons; mean \pm s.e.m (n=2). *p<0.05.

Major Task 2: Evaluate pharmacologic inhibition of VPS34 and ULK1 for autophagy inhibition in BRAF^{V600E} mutated CNS Tumor cells.

We have also successfully completed the pharmacologic evaluation of inhibition of these targets in MAF794 and AM38 cells. Flow data analysis and survival analysis are below and portrayed in figure form for a manuscript submission.

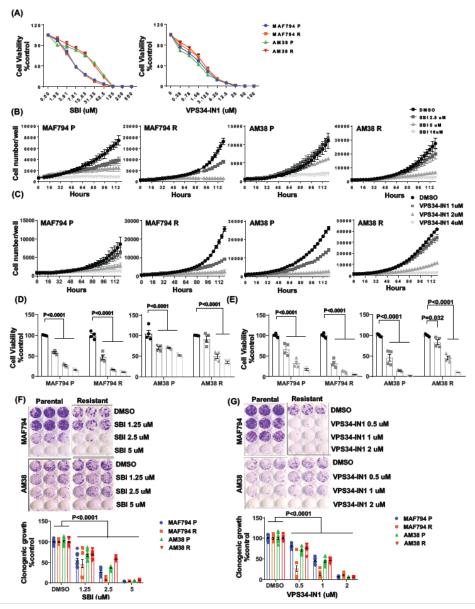
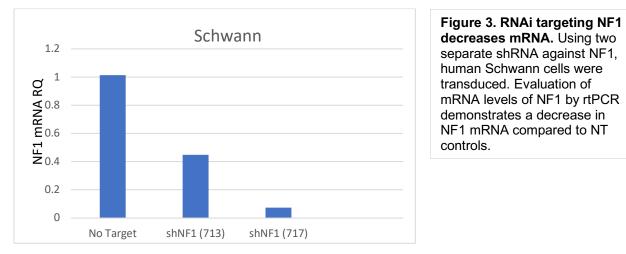


Figure 2. Parental and resistant BRAF^{V600E} brain tumor cell lines demonstrate sensitivity toward early stage autophagy inhibition. (A) Effect of SBI or VPS34-IN1 on short-term viability in MAF794 and AM38 parental (P) and resistant (R) cells treated with increasing doses of SBI or VPS34-IN1 for 5 days. Viability was determined using the CellTiter Glo assay. (B-C) Growth curves of MAF794 and AM38 P and R cells following SBI (B) or VPS34-IN1 (C) treatment. Cell number per well was obtained overtime using Incucyte Zoom (Essen Bioscience). (D-E) Percent cell viability compared to DMSO control measured by CellTiter Glo assay following a 5-day treatment of SBI (D) or VPS34-IN1 (E). (F-G) Representative long-term clonogenic assays and quantified collated data of cells treated with SBI (F) or VPS34-IN1 (G) as indicated. Dunnett's multiple comparisons; mean ± s.e.m, n=2. *p<0.05.

Specific Aim 2: Determine if the V600E mutation is required for autophagy dependence in CNS tumor cells, or if any cause of dysregulated RAS/RAF/MEK pathway is sufficient to identify autophagy dependent CNS tumor cells.

Major Task 1: Development of MAPK pathway driven tumors

We had a goal of producing most NF1 driven tumors utilizing human Schwann cells with RNAi of NF1 and the establishment of FGFR driven tumor cells. We have successfully produced the NF1 model (rtPCR data below) and are in the process of completing the FGFR-TACC1 and FGFR-TACC3 cells.



Major Task 2: Evaluate the efficacy of autophagy inhibition in MAPK pathway driven tumor models.

Due to the early termination of this award, these tasks are not yet completed.

Specific Aim 3: Determine the mechanism by which autophagy inhibition overcomes multiple BRAF inhibitor resistance mechanisms in CNS tumors.

Due to the early termination of this award, these tasks are not yet completed.

Opportunities for training and professional development:

I maintain a regular co-lab meeting of autophagy focused research labs including that of Dr. Andrew Thorburn. My lab also participates in a weekly meeting of the Pediatric Neuro-oncology Research Program. We present regularly at these meetings as well as participate in the discussion of other lab presentations. These meeting also incorporate journal club reviews of new research related to both autophagy and separately the pediatric neuro-oncology reserch fields.

How were the results disseminated to communities of interest:

Since the beginning of this award, my lab has participated in the Children's Hospital Colorado Pediatric Research Winter Poster Session. We have also prepared a manuscript of the initial data found with AM38 and MAF794 cells for submission and publication. See attached appendices for full draft of article.

Plan for the next reporting period:

Nothing to report (final report).

Impact:

What was the impact on the development of the principal discipline(s) of the project?

We have evaluated the potential of early stage autophagy inhibitors in the treatment of autophagy dependent CNS tumor cells. The work completed over the DOD award period is the natural progression of my previously funded DoD Mentored award. Specifically, I found that inhibition of initiation (early) stages of autophagy are able to successfully improve response of both parental and resistant cell lines to BRAF inhibition. Work to complete the proposed studies continue with the additional support of the NIH: National Institute of Neurological Disorders and Stroke. This new R01 award necessitated the early termination of this funding, but also validates the importance of this work and ensures that the studies proposed here will continue to completion. We have also been able to make contact with Sprint Biosciences to further advance these studies with a new VPS34 inhibitor that has improved potential for in vivo effectiveness and future direct clinical use.

What was the impact on other disciplines? Nothing to report.

What was the impact on technology transfer? Nothing to report.

What was the impact on society beyond science and technology? Nothing to report.

Change/Problems? Nothing to report.

Products: Nothing to report

Publications, conference papers, and presentations

Conference poster presentations reported above.

Manuscript in process for submission/publication included in appendices.

Participants & Other Collaborating Organizations What individuals have worked on the project?

Name:	Jean M. Mulcahy Levy
Project Role:	PI
Nearest person month worked:	0.86
Contribution to Project:	Dr. Mulcahy Levy oversaw all aspects of this project in addition to performing experiments.
Funding Support:	New R01 funding award necessitating early termination of this award per guidelines.

Name: Project Role: Nearest person month worked:	Shadi Zahedi Professional Research Assistant 12
Contribution to Project:	Ms. Zahedi was primarily in charge of performing experiments.
Funding Support:	New R01 funding award necessitating early termination of this award per guidelines.

Since the last reporting period the PI of this project was awarded an NIH/NINDS (1R01NS107313-01A1). The DOD award was relinquished prior to the start of the R01award per granting requirements.

What other organizations were involved as partners? Nothing to report.

Special reporting requirements: None Appendices: None