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TITLE: Repurpose Phenformin for the Treatment of NF1-Mutant MPNST

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Loss of NF1 tumor suppressor drives hyperactivation of the RAS-RAF-MEK-ERK signaling cascade in MPNST. However, inhibition of this pathway by MEK inhibitor (MEKi) exhibits limited efficacy, probably due to incomplete ERK suppression and adaptive resistance responses. In this project, we aim to systematically characterize MEKi-induced metabolic adaption responses in NF1-mutant MPNST cells and assess the anti-tumor effects of phenformin, a mitochondrial complex I inhibitor, in combination with MEK inhibitor in MPNST. Unfortunately, we fail to detect any effects of phenformin on the response of NF1-mutant MPNST cells to MEK inhibitor, unlike in NF1-mutant melanoma cells as we previously reported. However, our metabolomics analyses reveal that MPNST cells undergo significant metabolic adaption in the pentose phosphate pathway(PPP), and suggest targeting PPP could represent a novel strategy for the treatment of NF1 mutant MPNST.

15. SUBJECT TERMS
None listed.

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1. Introduction

Loss of NF1 tumor suppressor drives hyperactivation of the RAS-RAF-MEK-ERK signaling cascade in MPNST. However, inhibition of this pathway by MEKi exhibits limited efficacy, probably due to incomplete ERK suppression and adaptive resistance responses. We recently demonstrated that phenformin, a mitochondrial complex I inhibitor in the biguanide class of drug that was used to treat type 2 diabetes, enhances the efficacy of ERK/MEK inhibition in NF1-mutant melanoma. In this project, we aim to evaluate the potential utility of phenformin in NF1-associated MPNST. We test the hypothesis that phenformin treatment would overcome MEKi-induced metabolic adaptation in NF1-mutant MPNST cells, enhance the efficacy of MEKi, and delay the development of resistance to MEKi in NF1-associated MPNST.

2. Keywords

Neurofibromatosis type 1 (NF1), Malignant peripheral nerve sheath tumor (MPNST), MEK inhibitor, Phenformin, Cancer cell Metabolism, Drug resistance.

3. Accomplishments

Specific Aim 1: To systematically characterize MEKi-induced metabolic adaption responses in NF1-mutant MPNST cells

- **What were the major goals of the project?**

The major task of this aim is to examine the effects of MEKi and phenformin combination on cellular metabolism in NF1-mutant MPNST cells. We have two subtasks: 1) to assess the metabolic adaptation responses induced by MEKi; 2) to assess the effects of phenformin on MEKi-induced metabolic adaptation. During the project period, we have completed subtask 1. We did not carry out experiments in subtask 2, because we failed to detected any significant effects of phenformin on the responses of MPNST cell lines to MEKi (see results from specific aim 2).

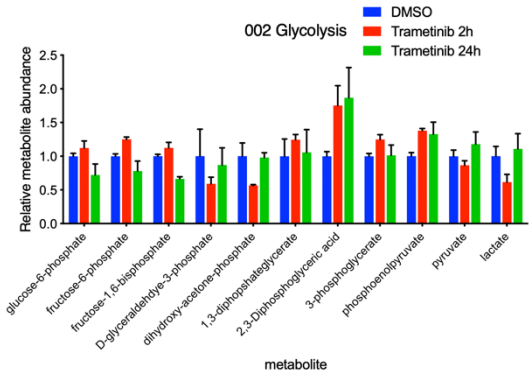

Figure 1. Changes of metabolites in the glycolysis pathway in JH-2-002 cells upon MEKi treatment.

- **What was accomplished under these goals?**

In subtask 1, we carried out LC-MS based metabolomic analysis of MPNST cell lines JH-2-002 and JH-2-031 treated with trametinib MEKi for 2 or 24 hours. We did not include JH-2-023 cells in these analyses, as we found out that these cells grew poorly in our hands. We have analyzed levels of metabolites in central carbon metabolism pathways, including glycolysis, pentose phosphate pathway, TCA cycle, glutamine metabolism, hexosamine biosynthesis pathway, pyrimidine/purine, and one- carbon metabolism.

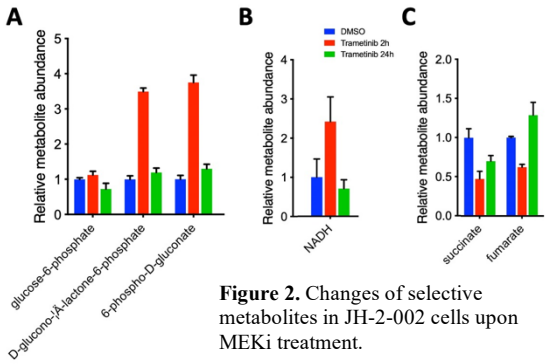

Figure 2. Changes of selective metabolites in JH-2-002 cells upon MEKi treatment.

For JH-2-002 cells, our analysis indicated that there were a significant decrease in the levels of lactate and another glycolytic intermediate Dihydroxyacetone phosphate DHAP, after 2 h of MEKi treatment, but it came back up after 24 h (**Fig. 1**), indicative of a metabolic adaption. In addition, we observed there was transient increases of several metabolites in the pentose phosphate

pathway(PPP), including 6-phosphogluconolactone and 6-phosphogluconate (**Fig. 2A**). Other metabolites from JH-002 cells that show significant adaptative responses included NADPH and TCA cycle intermediates succinate and fumarate (**Fig. 2B-C**).

For JH-2-031 cells, we also observed adaptative responses for metabolites in the PPP pathway and NADPH (**Fig. 3A-B**). Although we observed significant decrease of lactate level upon 2 hr of MEKi treatment, but we did not observed a bounce back at 24 hr, and we did not detect significant adaptive responses for succinate or fumarate either (**data not shown**). However, we found that several intermediates in the serine biosynthesis pathway went up upon 2hr of MEKi treatment but came down at 24 hr (**Fig. 4**), which is unique to JH-2-031 cells, but not in JH-2-002 cells.

In summary, both JH-2-002 and JH-2-031 showed remarkable metabolic adaptation upon MEKi treatment, but with heterogenous responses. Although MEKi treatment caused significant decreases in lactate levels in both cell lines, most of the changes in the branching pathways of the glucose flux are quite different, except the PPP pathway. Different MPNST tumors may have different adaptation mechanism in response to inhibition of the RAS-RAF-MEK pathway, and the PPP pathway could be a common adaptive response mechanism.

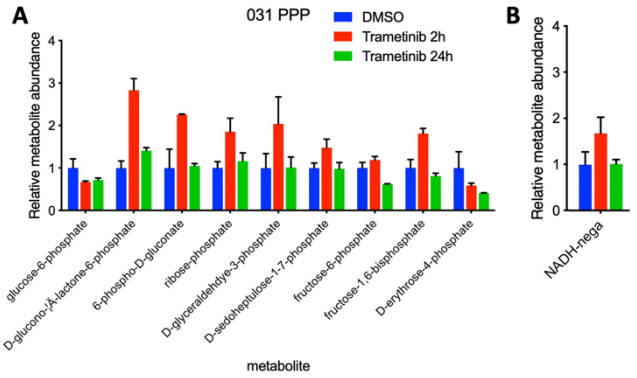

Figure 3. Changes of metabolites in the PPP pathway and NADH in JH-2-031 cells upon MEKi treatment.

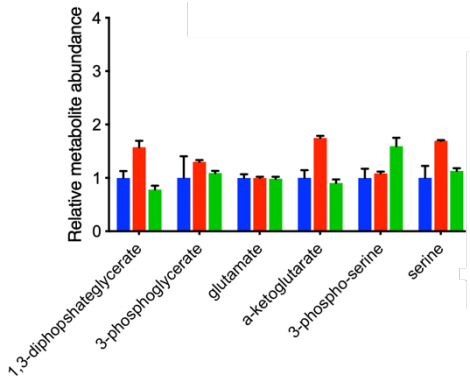

Figure 4. Changes of metabolites in the serine biosynthesis pathway in JH-2-031 cells upon MEKi treatment.

- **What opportunities for training and professional development has the project provided?**

  Nothing to Report

- **How were the results disseminated to communities of interest?**

  Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**

  Nothing to Report

Specific Aim 2: To assess the anti-tumor effects of phenformin in combination with MEK inhibitor in MPNST

- **What were the major goals of the project?**

The major task of this aim is to explore the effects of phenformin-MEKi combination in MPNST cell lines and mouse models. We have two subtasks: 1) to assess the effects of phenformin-MEKi combination on the viability of MPNST cell lines; 2) to assess the effects of phenformin-MEKi combination on MPNST tumor growth in mice.

- **What was accomplished under these goals?**

In subtask 1, we carried out MTS cell viability assays in both JH-2-002 and JH-2-031 cells treated with various doses of phenformin in combination with MEK inhibitor treminitib or ERK inhibitor SCH772984. Unfortunately, we did not observe

significant effects of phenformin on enhancing the response of JH-2-002 and JH-2-031 cells to either MEK (**Fig. 5**) or ERK inhibitors (**data not shown**).

In subtask 2, at the beginning we failed to established xenografted tumors of JH-2-002 and JH-2-031 in immune deficient mice in multiple attempts. Eventually, after modifying the xenograft protocol to mix tumor cells with matrigel, we were able to establish tumors from both these cell lines in NSG mice (**Fig. 6**). Since we did not observe any effects of phenformin *in vitro*, we have not carried out the combination treatment experiment of phenformin and MEKi in these mouse models.

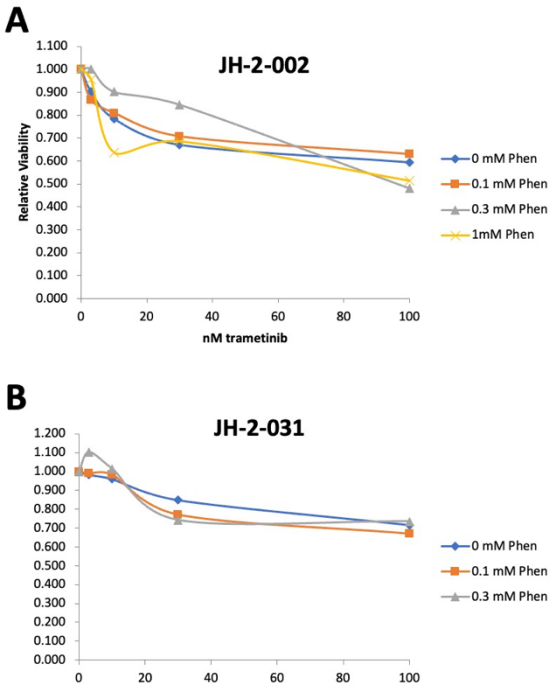

Figure 5. Effects of phenformin and MEK inhibitor combination on cell viability of JH-2-002 and JH-2-031 cells as measured by the MTS assay.

- **What opportunities for training and professional development has the project provided?**

  Nothing to Report

- **How were the results disseminated to communities of interest?**

  Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**

  Nothing to Report

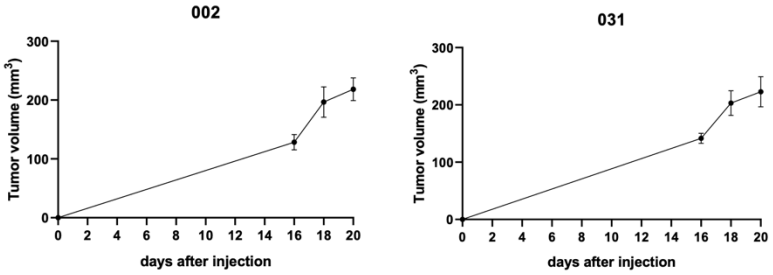

Figure 6. Growth of xenograft tumors of JH-2-002 and JH-2-031 cells in 7 week old NSG male mice.

4. Impact

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

Our findings indicate that NF1 mutant MPNST cells undergo significant metabolic adaptation upon treatment with MEK inhibitor. Although our data failed to reveal any effects of phenformin on the response of NF1 MPNST cells to MEK inhibitor, our metabolomics study suggest that the pentose phosphate pathway could represent a common metabolic adaptation response among MPNST cells, and pharmacological or genetic manipulation of the PPP could be tested in the future to see whether it would enhance the response of MPNST cells to MEK inhibition.

- **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

5. Changes/Problems

Nothing to Report

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name:	*Da Teng*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	12
Contribution to Project:	*Dr. Teng has performed work in both aims 1 and 2*
Funding Support:	*N/A*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

- **What other organizations were involved as partners?**

Nothing to Report

8. Special Reporting Requirements

Nothing to Report

9. Appendices

N/A