Inhibition of RAS Pathways as a Novel Therapeutic Approach to Pediatric Brain Cancers

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INTRODUCTION

Neuroblastoma and Medulloblastoma are subsets of pediatric brain cancers that are currently lacking in effective and or non-cytotoxic treatments. Neuroblastoma is a pediatric tumor of the sympathetic nervous system that shows highly unpredictable behavior. Survival rates for high-risk neuroblastoma are below 50%. Current therapies produce an initial response in most patients but up to 60% of these patients will relapse with therapy resistant tumors. Medulloblastoma is a pediatric tumor of the hindbrain and the most common malignant tumor for children under 4 years old. Current treatments for medulloblastoma can cure a majority of patients but these therapies cause a number of cytotoxicities and there are few effective treatments available for those patients with high-risk disease or recurrent tumors. Overexpression of the downstream components of RAS signaling pathways, such as AKT and ERK, has been demonstrated in medulloblastoma. And while this overexpression is not seen widely in non-relapsed neuroblastomas, studies indicate that nearly 80% of relapsed tumors have mutations that overactivate the RAS-MAPK pathway. We hypothesize, therefore, that medulloblastoma and relapsed neuroblastoma should be sensitive to RAS inhibition through small molecule inhibitors. We have developed such a RAS inhibitor molecule this molecule may have potential as a novel therapeutic approach for pediatric brain cancers.

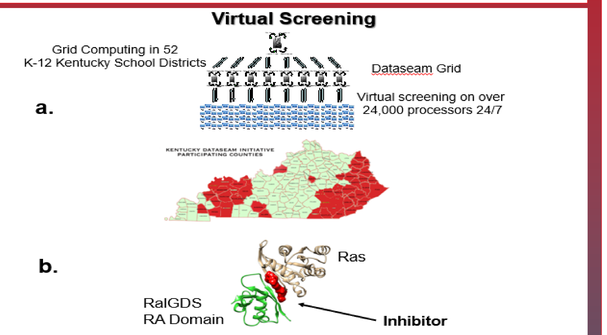

Figure 1. a. Virtual Screening for RAS Inhibitors. In collaboration with the Kentucky Dataseam Initiative and Dr. John Trent of the UofL Molecular Modeling Core, 24,000 computers have been placed in 52 K-12 Kentucky school districts for educational enhancement. This also allows for a continuous background screening of chemical libraries to identify potential RAS binding agents. This led to the identification of ***F3***. **b. Binding affinity site for compound *F3*.**

RESULTS

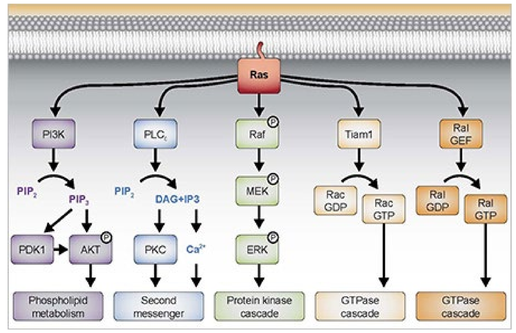

Figure 2. RAS Signaling Pathways. RAS can bind at least 9 distinct effector proteins to promote distinct cellular responses. Of these, the MAPK and PI3K/AKT effector pathways have been found to be hyperactivated in numerous cancer types driven by RAS activation, leading to increased cell proliferation and tumor growth. RAS inhibition should suppress these pathways which can be measured by examining the activation status of key components downstream components.

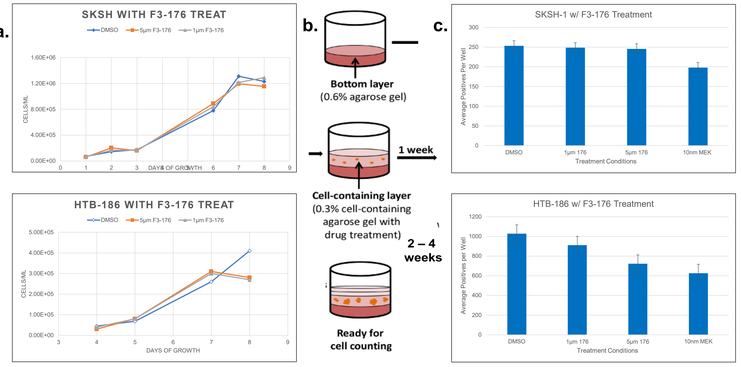

Figure 3. a. 2D Growth Curves. SKSH-1 (NB) and HTB-186 (MB) cell lines were grown in 2D with an enhanced version of F3, ***F3-176***, to assess the cytotoxicity of the drugs on non-specific targets. The findings from this assay indicate that both the 5µm and 1µm F3-176 treatments treatment were non-cytotoxic. **b. 3D Soft Agar Assays**. 3D soft agar assays mimic the 3D cellular environments seen in-vivo, allowing for the testing of effects of drug treatment on tumor growth. The two cell lines were plated in agar with differing drug concentrations and the colonies were counted after 2-4 weeks **c. Treatment with F3-176 slightly inhibits the growth of HTB-186 in Agar but has no effect of the growth of SKSH-1 cells.**

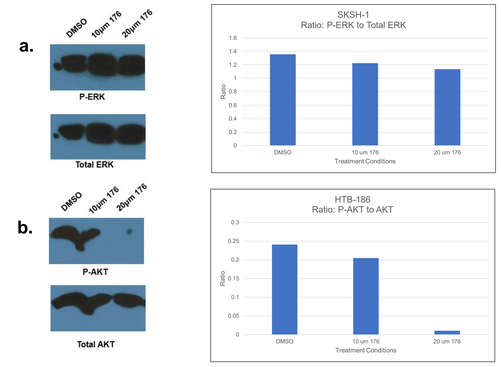

Figure 4. a. F3-176 has little effect on ERK signaling in SKSH-1 cells. Both cell lines were plated out, grown to confluence, and transiently treated with F3-176 or DMSO for 4 hours. The cells were then lysed, and the lysates were probed for ERK and AKT signaling to assess RAS inhibition. Phosphorylation serves as an activator for ERK and AKT. **b. F3-176 inhibits AKT activation in HTB-186 cells**

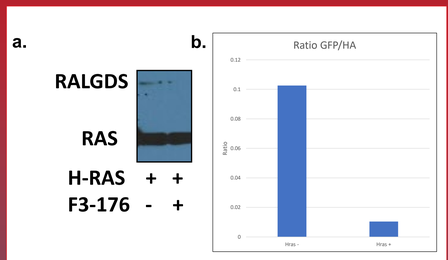

Figure 5. a. RAS/RALGDS co-IP. HA tagged H-RAS12v and GFP-tagged RALGDS were transiently transfected into COS-7 cells using lipofectamine 2000. After 24 hours, the cells were lysed and the clarified supernatant split into equal halves. One half was treated with DMSO, one half with F3-176 derivative F3-8-60. Lysates were incubated with drug or carrier and anti-HA beads over night. The beads were then washed and Western blotted to measure levels of RAS (HA) and RALGDS (GFP). **b. The drug reduced RAS/effector complex formation.**

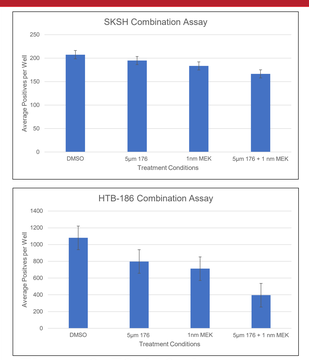

Figure 6. F3-176 shows a cooperative treatment effect on HTB-186 when paired with a MEK inhibitor in 3D soft agar assays. Cells were grown in agar with a combination of F3-176 and MEK to assess possible cooperativity. This effect was not seen in the SKSH-1 line.

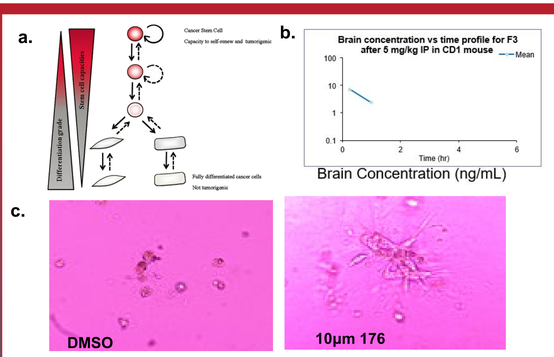

Figure 7. a. Cancer stem cells self-renew and differentiate The balance in NB cells is controlled by the PI-3K pathway**.** We have shown that F3-176 inhibits this pathway **b. F3 can cross the blood brain barrier.** Therefore, this compound has the potential to enter the brain and inhibit the pathway. **c. SKSH-1 cells grown in Matrigel differentiate in the presence of F3-176 but not DMSO.** We hypothesize that this is due to repression of the PI-3 kinase pathway.

DISCUSSION

The objective of this work was to determine if F3-176 could be a novel therapeutic drug for neuroblastoma and medulloblastoma. We have shown that treatments with F3-176 alone slightly inhibits growth of tumor colonies in medulloblastoma without showing cytotoxic effects in 2D. We have also shown that the combination of F3-176 and a MEK inhibitor shows greater inhibition of tumor growth in medulloblastoma. In addition to this, we have shown that F3-176 inhibits the PI3K/AKT signaling pathway in medulloblastoma. The SKSH-1 neuroblastoma line appears to have been resistant to these treatments. However, it did show differentiation in Matrigel, compatible with inhibition of the PI3K pathway. These results suggest that F3-176 merits further study as a possible therapeutic approach for pediatric brain cancers.

ACKNOWLEDGEMENTS

I would like to thank the members of the Clark Lab for their support and guidance throughout the summer. I would also like to thank the NCI R25 University of Louisville Cancer Education Program (R25-CA134283) for sponsoring this research.