Investigating the Effect of Inhibiting p53 on Glioma Metabolism

Rachel Erin Smith[1], Anders Persson[2], Ken Smith[3], and Tracy Richmond McKnight[3]

[1]Radiology & Biomedical Imaging, University of California at San Francisco, San Francisco, CA, United States, [2]Neurology, University of California at San Francisco, San Francisco, Ca, United States, [3]Radiology & Biomedical Imaging, University of California at San Francisco, San Francisco, Ca, United States

INTRODUCTION: The tumor suppressor protein, p53, is often mutated in many forms of cancer and neurodegenerative disease, including malignant gliomas. It has been shown that p53 inhibition, through the small-molecule inhibitor pifithrin-α, causes an increased sensitivity to chemotherapy[1], however, the mechanisms governing this are unknown. A previous study using high resolution magic angle spinning (HRMAS) magnetic resonance spectroscopy (MRS) on tissue biopsies from normal and abnormal p53 glioma showed that they have different metabolic profiles[2] . The most common metabolic alterations in cancer are changes in phosphocholine (PC) and glycerophosphocholine (GPC), which are involved in phospholipid metabolism (Cheng, 1998). We hypothesize that inhibiting p53 alters the metabolic profile in gliomas, particularly with respect to the choline-containing compounds, resulting in increased sensitivity to chemotherapy.

METHODS: We used HRMAS MRS to compare the concentration of 9 metabolites in a second set of normal and p53-inhibited U87 glioma cells transfected with luciferase. Cells were cultured in T150 flasks until they reached 95% confluency. They were then washed with d20-PBS, centrifuged into pellets, then frozen at 1°C per hour in a -80°C freezer. For metabolic examination with HRMAS analysis, the pellets were spun at 2250Hz at 1°C and spectra was acquired using a 1D presaturation sequence. Concentrations were determined using ERETIC and in house HRQUEST software having units of mmolal. The concentrations of glutamate (Glu), lactate, creatine, alanine, myo-inositol, glutathione, choline, PC, and GPC were quantified. A Student's T-test was used to determine differences between populations ($p<0.05$). To monitor the growth rate, cells were cultured as monolayers in single well dishes and treated with either 10µmol/L of 5mmol cyclic pifithrin-α p-nitro, or 10µL DMSO once a day for three consecutive days. Cells were then treated with 25uL of lucierin and then imaged on a Xenogen IVIS-50 bioluminescence system to determine cell viability. A fluorescence caspase assay was performed on a spectrafluorometer to determine the levels of apoptotic cells after being administered 100µmol/L of 5mmol cyclic pifithrin-α p-nitro.

RESULTS: We observed a significant decrease in GPC ($p=0.04$) and PC ($p=0.02$) and increase in Glu ($p=0.02$) with inhibition of p53. Mean+/- SD of GPC, PC, and Glu were 0.41+/-0.26, 0.38+/-0.27, and 0.11+/-0.02 in functional p53 cells (n=9), and 0.12+/-0.07, 0.00+/-0.00, and 0.38+/-0.22 in inhibited cells (n=7), respectively. Bioluminescence imaging studies shows a significant ($p<0.05$) decrease in growth rate with the inhibited population (n=6), and an apoptotic assay shows no increase in apoptosis with inhibition.

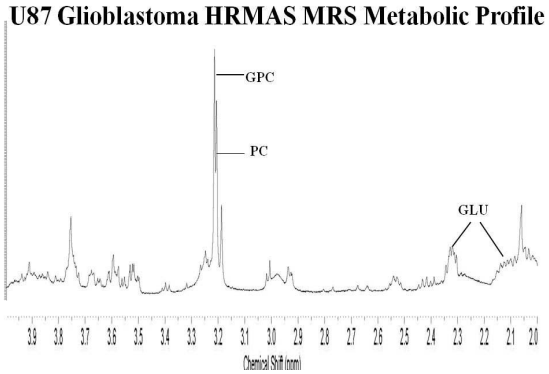

Fig. 1 HRMAS MR spectroscopic profile of U87FL glioblastoma cells treated with DMSO as a control for 3 consecutive days

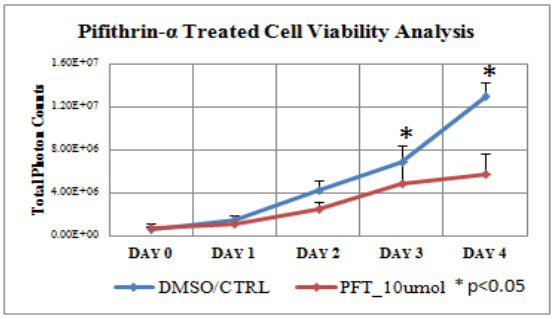

Fig. 2 HRMAS MRS metabolite concentrations of glycerophosphocholine, phosphocholine, and glutamate in U87FL glioblastoma cells treated with either DMSO as a control, or with the p53 inhibitor, 10umol cyclic pifithrin-α p-nitro, for 3 consecutive days.

CONCLUSION: We conclude that p53 inhibition decreases PC and GPC, and increases Glu concentrations. PC and GPC contribute to phospholipid synthesis and, degradation, which accompany cell proliferation. A possible explanation for the decreased concentrations of GPC and PC in p53i cells is attributable to the decrease in phospholipid synthesis and cell proliferation that we observed. Glu has been found to be associated with neurotransmission in neurons at low-levels. However, high levels of this neurotransmitter can be cytotoxic. However, we did not find an increase in apoptosis in the inhibited population. Additional studies using transient knock-down of TP53 in patient-derived glioma cells will be performed to confirm that our findings are indeed the result of p53 inhibition and rather than side effects of pifithrin.

References

1) Dinca EB, Lu KV, Sarkaria JN, *et.al. Cancer Research* 2008; 68: 10034-10039
2) McKnight, T. ISMRM 2010

Acknowledgments

1) R21 CA154049, NIH R01 CA159869

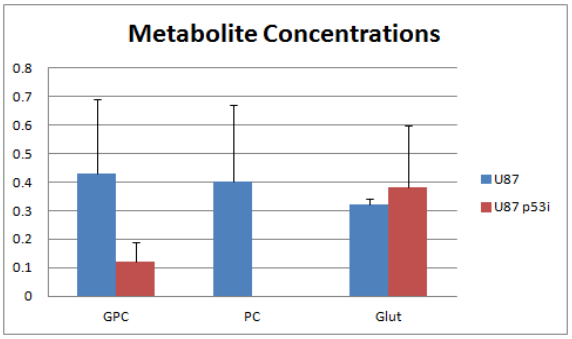

Fig. 3 Cell viability analysis of luciferin treated U87FL cells either given DMSO as a control or 10umoL of cyclic pifithrin-α p-nitro, once a day, for 3 days: days 1, 2 and 3. Images were taken over 5 consecutive days, beginning with day 0 as 24 hours