Imaging the Metabolic Evolution of Glioblastoma Throughout Tumor Regression Following Radiotherapy to the Point of Relapse with Hyperpolarized Magnetic Resonance Imaging

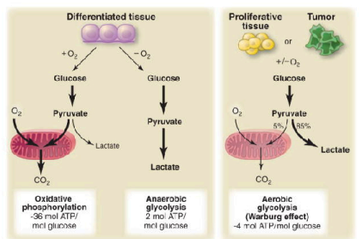
Travis Salzillo[1,2], Joy Gumin[3], Jaehyuk Lee[1], Niki Zacharias Millward[1], Frederick F. Lang[3], Pratip Bhattacharya[1]

[1]Department of Cancer Systems Imaging, The University of Texas MD Anderson Cancer Center, Houston, TX
[2]MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX
[3]Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction

Glioblastoma (GBM) is the most aggressive form of brain cancer with a median survival of merely 15 months and is almost always recurrent. Thus, minimizing time to diagnose these tumors, to determine the efficacy of therapy, and to predict relapse can greatly impact patient survival. With regards to these three clinical goals, the objective of this research is to determine whether pyruvate-to-lactate conversion measured with hyperpolarized MRI can detect changes in tumor progression prior to changes in tumor volume measured with conventional MRI.

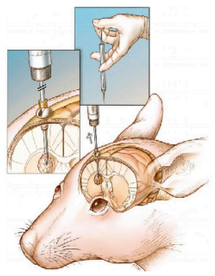
Cellular metabolism is directly linked with proliferation. The Warburg effect states that, unlike in healthy cells, highly proliferative cells, such as cancer, convert the majority of their pyruvate to lactate, regardless of oxygenation status. This pathway is a quick (but inefficient) way to generate ATP, increase glycolysis, and produce biomass for proliferation. By imaging the conversion of hyperpolarized [1-^{13}C]pyruvate to lactate with MRI, this effect can be observed *in vivo*.



Vander Heiden et al. *Science* (2009)

Methods and Materials

Tumor tissue was collected from a cohort of consenting patients in accordance with the Institutional Review Board of The University of Texas MD Anderson Cancer Center. Glioma sphere-forming cells (GSC) were cultured from these tumors and intracranially injected into mice as PDXs. Control mice were intracranially injected with PBS. Median survival of the mice was 34 days.



Lal et al. *J Neurosurg* (2000)

Anatomical growth was measured every 3 days using T1-weighted, T2-weighted, and fluid-attenuated MRI pulse sequences. The tumors were carefully segmented, and volume was calculated from these voxels.

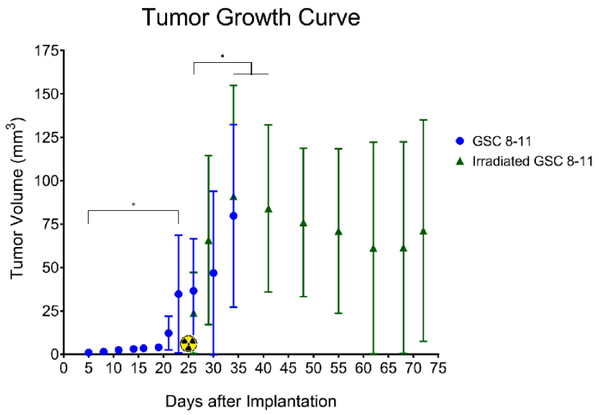
Every 7 days, the conversion of pyruvate to lactate was measured *in vivo* with hyperpolarized MRI. With this technique, the signal of ^{13}C-enriched pyruvate was amplified by ~10,000-fold, dissolved into solution, and injected through the tail vein of the mouse. An ROI over the tumor measured the relative concentration of pyruvate and it's biochemical product, lactate, every 2 seconds with pulse-acquired spectroscopy. The conversion was quantified using the metric, nLac, which is the time-integrated ratio of lactate:lactate+pyruvate.

After each hyperpolarization experiment, tumors were excised and flash-frozen. ^{1}H NMR spectroscopy was performed on these samples to measure steady-state concentrations of several metabolites which were then correlated to tumor growth and hyperpolarization results.

On Days 25 and 27 following GSC implantation, half of the mice received 5 Gy of whole brain irradiation to treat the tumor. Tumor volume and nLac were measured weekly throughout tumor regression to the early stages of tumor relapse.

In Vivo Tumor Volume Results

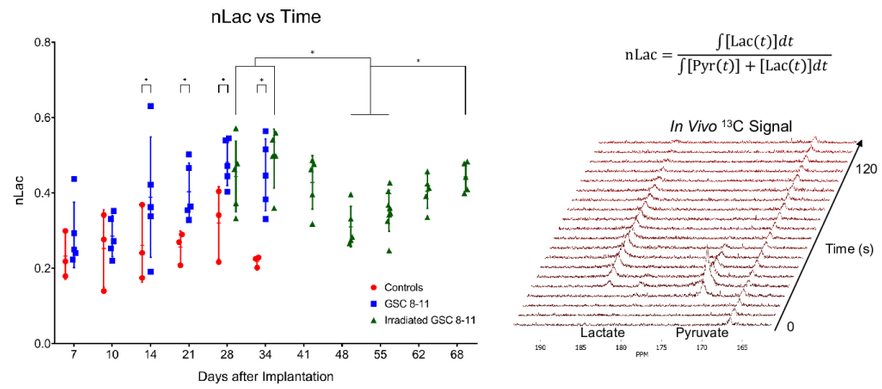
N = 5 tumor-bearing mice were scanned with T1-w, T2-w, and FLAIR sequences to measure tumor volume every 3 days. Baseline volume was determined on Day 5. During tumor development, significant increases in volume compared to baseline were observed starting on Day 23.



Following irradiation, tumor volume appeared to shrink, reaching a minimum by Day 62, before beginning to regrow, but statistical significance was not achieved compared to pre-treatment volumes. Statistical analysis was conducted with one-way ANOVA and significance was attributed to p-values < 0.05.

In Vivo Pyruvate-to-Lactate Results

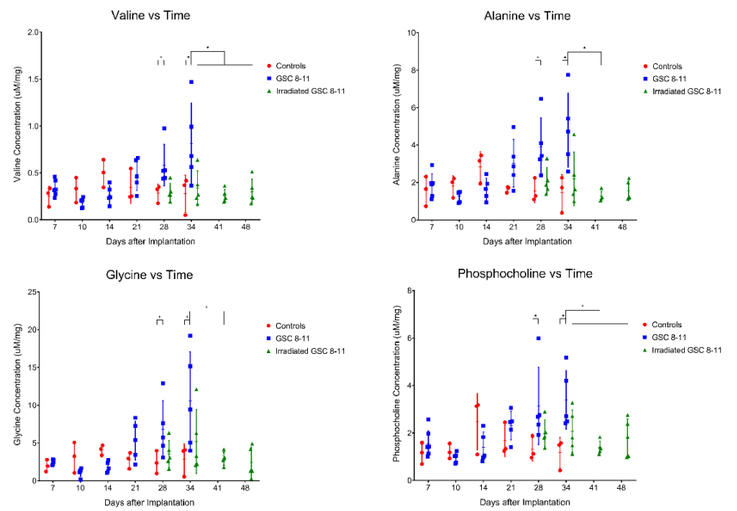
N = 5 tumor-bearing mice and N = 3 control mice underwent hyperpolarization experiments every 7 days (with an extra time-point on Day 10 to capture early tumor dynamics). [1-^{13}C]pyruvate was polarized and injected, and it's conversion to lactate in the tumor was measured and quantified with nLac. Significant increases in nLac were observed in tumor-bearing mice compared to controls starting on Day 14 and persisted throughout tumor development.



During tumor regression following radiotherapy, nLac in treated tumors began to decrease and was significantly reduced on Days 48 and 55 compared to Days 28 and 34. Then as the tumors began to recur, nLac once again rose and was significantly increased in treated mice by Day 68 compared to Days 48 and 55. Statistical analysis was conducted with one-way ANOVA and significance was attributed to p-values < 0.05.

Ex Vivo Global Metabolomics Results

Following each of the hyperpolarization experiments, the tumors were excised and flash-frozen. The samples were lysed and their metabolites extracted and purified. The relative concentrations of these metabolites were measured with ^{1}H NMR spectroscopy.



Thirty metabolites were identified and quantified. The 4 shown above were found to be significantly increased in tumor-bearing mice compared to control mice during tumor development and treated mice during regression. Statistical analysis was conducted using two-way ANOVA and significance was attributed to adjusted p-values < 0.05.

Conclusions

These experiments reveal a detailed anatomic and metabolic evolution of GBM over time. *In vivo* pyruvate-to-lactate conversion measured with hyperpolarized MRI was significantly altered during tumor growth, regression, and early stages of relapse prior to significant changes in tumor volume. This has significant clinical impact as it gives physicians more time to adapt to tumor progression than merely waiting for the tumor to grow or shrink.

In addition to elevated pyruvate-to-lactate ratio, the increase in amino acid and cell membrane metabolites, measured *ex vivo* with ^{1}H NMR spectroscopy, support the notion that these tumors have adapted their metabolism for increased proliferation as described by the Warburg effect. This type of global metabolomic analysis can help identify metabolic vulnerabilities which could be targeted with novel therapies. Further inter-experimental correlations are currently being investigated.

Acknowledgements

The research has been funded in part by MD Anderson Institutional Research Grants, MD Anderson Institutional Startup, Brain SPORE Developmental Research Award and Koch Foundation. We also thank the CCSG-funded NMR facility core facility and small animal imaging facility (SAIF) (CA016672). TS thanks CPRIT Research Training Award (RP170067) for graduate stipend support.