MALDI Imaging of $^{13}C_6$-Glucose Uptake Measured in TCA Metabolites from Glioblastoma Mouse Brain

Joshua L. Fischer[1], Kari Wilder-Romans[2], Ethan Yang[1], Savannah R. Snyder[1], Katherine A. Stumpo[1], Angelica Lin[2], Zitong Zhao[2], Jie Xu[2], Daniel R. Wahl[2]

[1] Bruker Scientific LLC, Billerica, MA 01821, USA

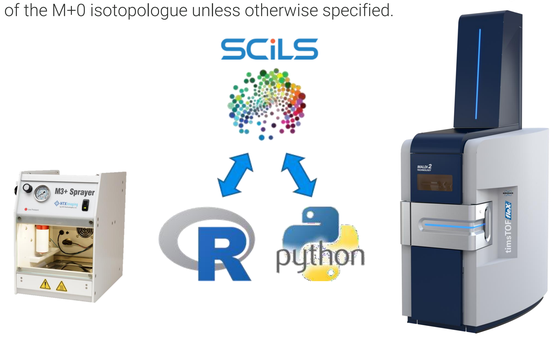
[2] University of Michigan, 1500 E Medical Center Dr, Ann Arbor, MI 48109

Introduction

Human brain cancer models have shown increased metabolism of glucose to lactate in the presence of oxygen, known as the Warburg Effect. However, the capacity of human brain cancers to metabolize glucose to lactate through the citric acid (TCA) cycle is still unknown, creating a demand to monitor this process. Various monitoring methods exist, including magnetic resonance spectroscopy and positron emission topography, which indirectly measure metabolism through these pathways using substitute tracer molecules. Here, we show the spatial distribution of isotopically labeled TCA metabolites via MALDI Imaging of a glioblastoma (GBM) mouse dosed interperitoneally with $^{13}C_6$-glucose, revealing downregulation of TCA cycle activity in the GBM tissue relative to the healthy tissue.

Methods

Mice with patient derived xenografts (PDX) were dosed interperitoneally with $^{13}C_6$-labeled glucose at time *t* = 0 min and *t* = 30 min before being sacrificed at *t* = 60 min and their organs harvested. Sections of glioblastoma afflicted mouse brains were mounted on standard glass slides and sprayed with N-(1-naphthyl) ethylenediamine dihydrochloride (NEDC) using an HTX M3+ sprayer. MALDI Imaging data were acquired on a timsTOF fleX MALDI-2 (MALDI, negative polarity) at a lateral spatial resolution of 20 µm/pixel. Ion images were generated using SCiLS™ Lab 2023a and annotations were performed in MetaboScape® 2023a. Relative isotopic abundance statistics were generated with an R-script utilizing the SCiLS REST API to measure intensities of the parent and all carbon isotopes within a 15 ppm mass range of isotope M+n. All ion images are of the M+0 isotopologue unless otherwise specified.



Results

- SCiLS Lab was used to perform a segmentation analysis based on the mass spectra from each pixel and clearly differentiates GBM from healthy tissue, as well as dosed and control tissue
- Ion images clearly show uptake of $^{13}C_6$-glucose through modulation of TCA cycle metabolites
- Healthy and cancerous tissue, as well as dosed and control tissue readily distinguished using multiple statistical analysis performed in SCiLS Lab
- Extracted isotopic ratios show differences in flux of labeled glucose between the cancerous and healthy tissue
- MALDI Imaging performed on a timsTOF fleX allows glucose flux to be measured and distinguished between healthy and cancerous tissue

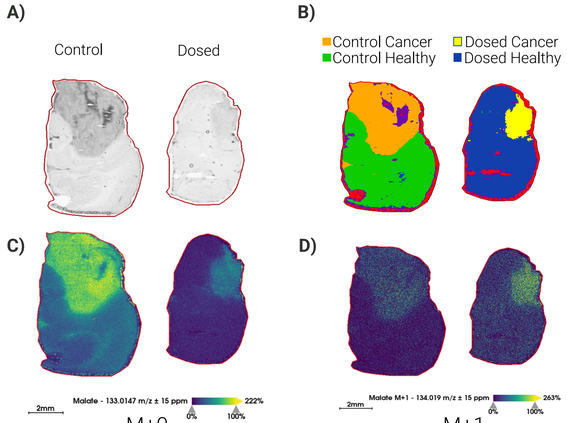


Figure 1. A) Optical image showing the control and dosed tissues. The darkened region is the GBM. B) Segmentation analysis used to create regions used to extract normalized isotope abundances. C) Ion image of the malate M+0 isotope, showing the M+0 isotope is in higher relative abundance in the control. D) Ion image of the malate M+1 isotope, showing the M+1 isotope is in higher abundance in the dosed tissue. E) PCA of the extracted regions determined in B.

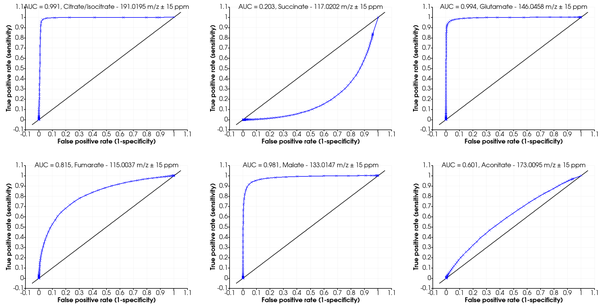


Figure 2. ROC plots generated using the Control Cancer and Dosed Cancer spatial information (generated through the segmentation analysis), showing clear ability to distinguish cancerous tissue from healthy tissue using TCA metabolites.

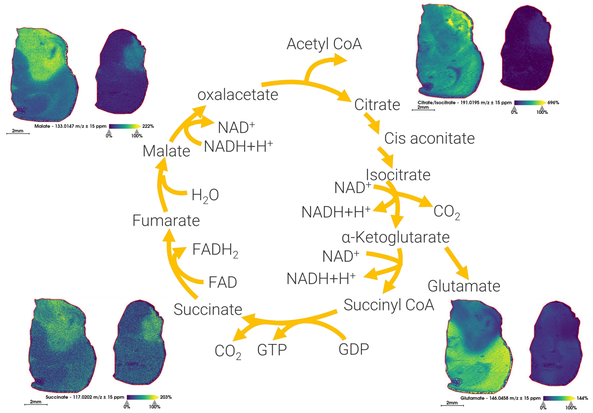


Figure 3. Ion images of selected M+0 TCA metabolites.

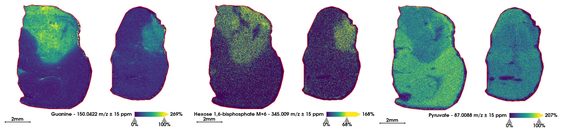


Figure 4. Extracted ion mages of metabolites from pathways of potential interest

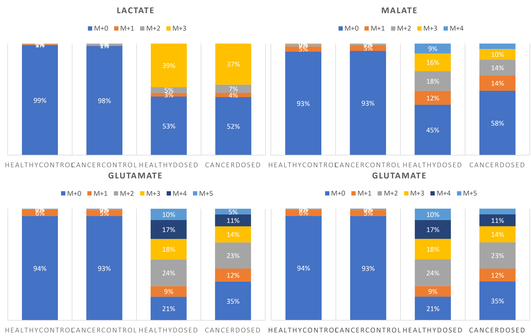


Figure 5. Normalized relative isotope abundances for example TCA metabolites extracted from ion images. The relative abundances were verified by LC-MS measurements (data not shown).

Summary

MALDI Imaging was used to visualize the uptake of $^{13}C_6$-glucose in a glioblastoma PDX-mouse model by tracking the intensities of TCA metabolites. The mass spectral data associated with each pixel was used in segmentation analysis to statistically cluster not only cancerous and healthy tissue, but also dosed and un-dosed tissue. Summed intensities from the segmented regions were used to provide relative isotope ratios for various TCA metabolites, finding results agreeing with LC-MS analysis. The ability to spatially measure metabolomic flux with stable isotope labeling is demonstrated.

Conclusion

- MALDI Imaging can spatially resolve numerous downstream fates of ^{13}C glucose.
- Applying this methodology to heterogeneous tumors such as glioblastoma could yield insight into intratumoral metabolic heterogeneity.
- This workflow also provides a foundation to use MALDI Imaging to measure the spatial flux of metabolites in disease models

Imaging MS: Pharmaceuticals, Metabolites, Lipids, and Glycans