Washington University in St. Louis

Measuring Metabolic Fluxes Spatially in Tissues by Using Mass Spectrometry Imaging

PATTI LAB

Kevin Cho[1,2,3], Michaela Schwaiger-Haber[1,2,3], Ethan Stancliffe[4], Dhanalakshmi S. Anbukumar[1,2,3], Kayla Adkins-Travis[1,2,3], Leah P. Shriver[1,2,3], and Gary J. Patti[1,2,3]

Departments of Chemistry[1], Medicine[2], and Center for Mass Spectrometry and Metabolic Tracing[3], Washington University in St. Louis, St. Louis, MO, USA ; Panome Bio[4], St. Louis, MO, USA

Overview

- Mass spectrometry imaging (MSI) offers insights into the metabolic landscape of many diseases, such as tumors.
- Downstream analysis and biological interpretation might be confounded
  - Potential interferences due to lack of chromatographic separation
  - Potential differences in ionization efficiencies between different tissue regions
- Introducing stable isotopes into animals can overcome some of the limitations despite introducing more complexity[1]

Introduction

- Advances in spatial transcriptomics have increased interest in complementary techniques for characterizing the multi-dimensional architecture of tissues
- Different ionization strategies are available. Two were used in this study:
  - Matrix Assisted Laser Desorption/Ionization (MALDI; Bruker timsTOF fleX)
  - Desorption Electrospray Ionization (DESI; Waters Synapt XS)
- Stable isotopes provide a deeper view into metabolism, e.g., via flux analysis
- Interferences can arise from labeled or unlabeled isotopologues of other compounds of interest or any other ions, such as contaminants
- Potential interferences have to be identified to avoid confounding downstream analysis
- Including unlabeled control samples is crucial[2]
- Validation with LC/MS can be helpful

Methods

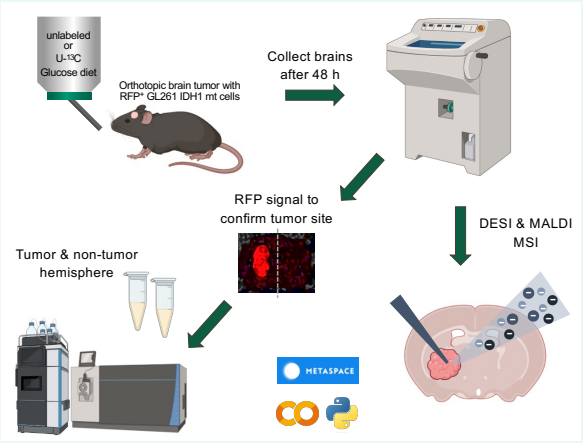


Figure 1: Sample preparation and mass spectrometry imaging (MSI) analysis. C57BL mice bearing GL261 IDH1 (RFP+) mutant orthotopic tumors were fed unlabeled or U-^{13}C glucose via a liquid diet for 48 h based on a previous protocol[3]. Brains were harvested and 10 or 20 μm thick sections were analyzed with MALDI and DESI MSI, respectively. Additionally, tumor and non-tumor hemispheres were extracted and analyzed via LC/MS.

Results

Different ionization techniques can lead to opposite results in pool size data

- Palmitate and stearate were analyzed in tumor brain samples
- When using DESI, the tumor region shows an elevated palmitate and stearate signal, whereas MALDI data seem to indicate a decreased signal
  - If only one of those techniques was used, the results would seem clear and would be used for biological interpretation.
  - Which one is true? Or is there any difference at all?
  - LC/MS data of tumor vs non-tumor hemisphere did not show any difference
- LC/MS data can provide complementary information

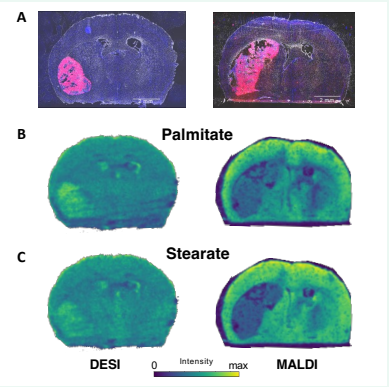


Figure 2: Unlabeled mouse brains harboring GL261 tumors. A) Microscopy images showing overlays of brightfield (grey), DAPI (blue), and RFP (red) images. The tumor region (pink) can be easily identified based on the RFP expressed by the implanted GL261 cells. Palmitate (B) and stearate (C) signals from DESI (left) and MALDI (right) MSI.

Unlabeled control samples are crucial

- Unlabeled samples should only show M0 isotopologue after natural isotope abundance correction
- Help detect potential interferences in labeled samples

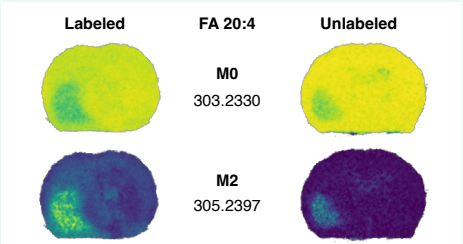


Figure 3: DESI data of fatty acid 20:4 in an unlabeled and labeled mouse brain sample. After natural abundance correction, even the unlabeled brain shows an M2 isotopologue, indicating an interfering *m/z*.

Stable isotope labeling shows different labeling patterns in adenosine monophosphate

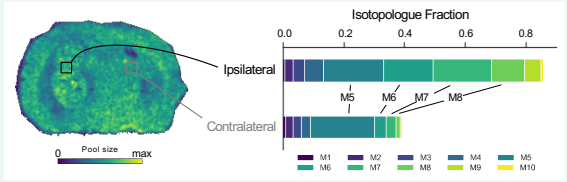


Figure 4: Adenosine monophosphate (AMP) isotopologue imaging using MALDI. AMP pool size from a labeled brain sample generated by summing of all isotopologues (M0-M10). Labeling patterns can be extracted from any region of interest.

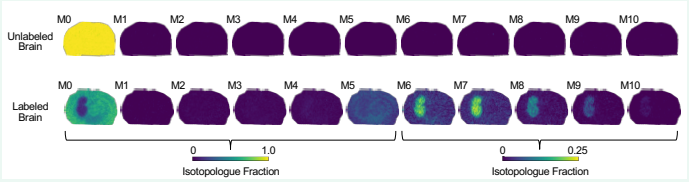
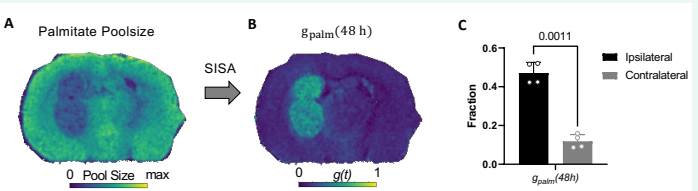


Figure 5: Adenosine monophosphate (AMP) isotopologue imaging using MALDI. Individual AMP isotopologue images show consistent M5 labeling across the whole brain, but increased labeling of higher isotopologues in the tumor region indicating increased usage of glucose for purine synthesis.

Spatial Isotopologue Spectral Analysis (SISA) to assess fractional fluxes

Figure 6: Palmitate isotopologue imaging using MALDI. A) Palmitate pool size from labeled brain by summing all isotopologues. B) Flux image after applying SISA to the labeled tumor brain shows the palmitate fractional turnover, $g_{palm}(48\ h)$, is higher in the tumor region. Consistent results between MALDI (shown here) and DESI. A limitation is that background contamination can lead to a higher unlabeled fraction. C) Comparison of $g_{palm}(48\ h)$ for four mice.



Potential improvements

- Improving ionization techniques to decrease tissue-specific effects
- Optimizing matrix application
- Internal standards
- Higher mass resolution
- Ion mobility spectrometry

Conclusions

- MSI is a very powerful technique, especially when combined with stable isotope labeling
- Unlabeled control samples are crucial to detect potential interferences
- Complementary LC/MS data can be helpful
- Data must be analyzed carefully
- De novo lipogenesis flux and fatty acid elongation flux are elevated in tumors relative to surrounding healthy brain tissue

Acknowledgements & References

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[1] Schwaiger-Haber et al., *Nat. Comm. 14, 2876* (2023)
[2] Schwaiger-Haber et al., *Anal Bioanal. Chem* 411, 3103–3113 (2019)
[3] Sun et al., *Nat Comm.* 8, 1646 (2017)

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