Multiplex and multiomic MS imaging of drugs, metabolites, and immunolabeled pathogenic protein markers within a single tissue section

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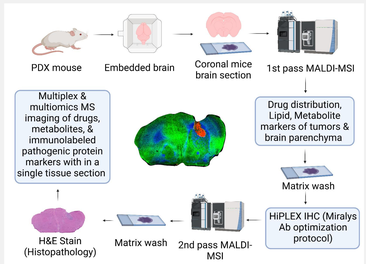
Overview

- MALDI mass-spectrometry imaging (MALDI-MSI) is a technique capable of the label-free identification and visualization of analytes in tissue sections.
- The combination of unlabeled small molecule drug, metabolite, and lipid imaging with HiPLEX immunohistochemistry by MALDI-MSI has great potential application for drug discovery, drug efficacy, and toxicology studies.
- Performing all these analyses within a single tissue section enables precise co-localization of multiplex/multiomic MSI datasets and drug accumulation within specific cell populations to be determined.
- Information regarding the ability of the drug to cross the blood-brain barrier and reach all regions of the tumor is currently lacking and is crucial for optimization of drug delivery systems and potentially extending patient survival.
- Here, we apply this approach to evaluate the distribution of temozolomide (TMZ), the main chemotherapy drug targeting glioblastoma multiforme (GBM), into patient-derived xenograft (PDX) tumors in mouse brain tissues.
- We optimized and applied a MALDI-MSI immunohistochemistry multiplex approach that used MS imaging to visualize peptide labelled antibodies as a high throughput alternative to fluorescence labeling and microscopy to visualize proteins and cell markers in tumor and healthy brain region of GBM that could be detected in same tissue as the drug.

Methods

- PDX mice were dosed at 50 mg/kg orally and necropsy was performed at 0.5h or 1.5h post-dose to collect brains.
- Coronal brain sections (10µm) were prepared using cryostat (Leica Biosystems) on ITO slides and sprayed with DHAP (20 mg/ml) (using HTX™ sprayer).
- Initial MSI was performed in positive ionization mode to visualize drug and lipid and metabolite markers of tumor and brain parenchyma.
- Following initial analyses, the matrices were removed by washing and HiPLEX IHC was performed using Miralys™ antibodies following optimized protocols.
- The 2nd pass MSI was performed in positive mode using DHAP matrix to visualize the photocleaved peptide mass tags (PC-MTs).
- Further tissue washing and H&E staining were performed. The workflow (HiPLEX IHC MALDI MSI) is shown in the adjacent panel.
- MALDI-MSI was performed at 25µm lateral resolution using a Q-Exactive HF Hybrid Quadrupole Orbitrap equipped with a Spectroglyph MALDI/ESI injector source.
- MS image processing, alignment, and ROI signal quantitation were conducted using SCiLS software. Lipids and metabolites were identified using Lipidmaps and Human Metabolome Database (HMDB) with an accurate mass tolerance of 2 ppm.



Conclusions

- Overall, we successfully demonstrated the ability of the multiplexed MSI technique to detect TMZ, metabolic tumor biomarkers, protein tumor markers (such as Ki67 and PDPN), and H&E histology all in a single tissue section.
- Current work is focused on applying the Multiplex MSI workflow to human pulmonary TB studies and visualizing drug penetration and distribution into specific immune cell populations using markers including CD4, CD8, and CD68.
- Developed method will be a promising tool for use in the field of tissue pathology, disease diagnosis, therapeutics development, and precision medicine.

Novelty

- Multiplex imaging of drugs, metabolites, lipids, and immunolabeled proteins within a single tissue section for PK and disease pathogenesis studies.

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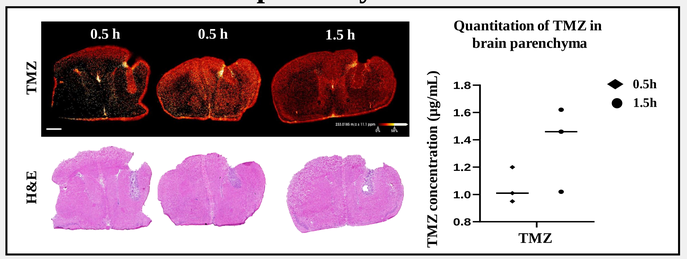
Conflict of interest disclosure

- Authors declare no competing financial interest.

Results

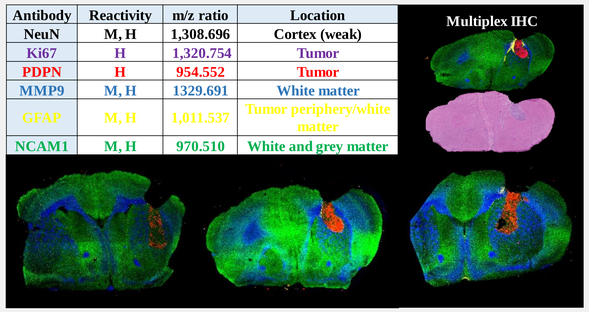
» Temozolomide distribution in GBM tumor and parenchyma«



Temozolomide distribution in tumor and brain: TMZ ([M+K]$^+$ *m/z* 233.0185) drug penetration and distribution in tumor and parenchyma at 0.5h and 1.5h post oral dose. MSI of brain sections from TMZ-dosed GBM xenograft mice at 0.5h and 1.5h post-dose timepoint revealed drug distribution throughout the brain including cortex, myelinated regions, and tumor. Quantitation of the TMZ drug (1µg/ml) done at the 0.5h and 1.5h. Drug quantitation in heathy brain by LCMS/MS is shown in the 2nd panel.

» MALDI-multiplexed IHC for protein marker of the tumor «

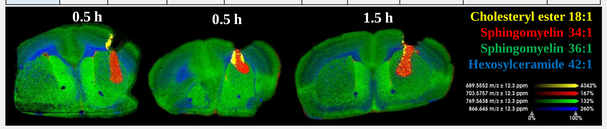
Antibody	Reactivity	m/z ratio	Location
NeuN	M, H	1,308.696	Cortex (weak)
Ki67	H	1,320.754	Tumor
PDPN	H	954.552	Tumor
MMP9	M, H	1329.691	White matter
GFAP	M, H	1,011.537	Tumor periphery/white matter
NCAM1	M, H	970.510	White and grey matter



MALDI-multiplexed IHC for protein biomarkers of the tumor: Second-pass MSI using Miralys™ PC-MTs mass tagged antibodies revealed the presence of cell proliferation marker Ki67 (PURPLE) and oncogenic invasion, metastasis, and inflammation marker Podoplanin (PDPN, RED) within the tumor region. GFAP, glial fibrillary acidic protein-strong biomarker for GBM, (YELLOW) positive astrocytes were localized at the tumor border and co-localized with cholesteryl ester distribution. NCAM 1, neural cell adhesion molecule 1-immunoglobulin-like neuronal surface glycoprotein which mediate adhesion, guidance, and differentiation during neuronal growth and neurogenesis, (GREEN) and MMP, matrix metalloproteinase9-helps in invasion and neurovascularization of GBM, (BLUE) was abundantly present throughout the brain parenchyma but could not observed within the tumor as expected. This distribution may be due to antibody cross-reactivity with myelin proteins

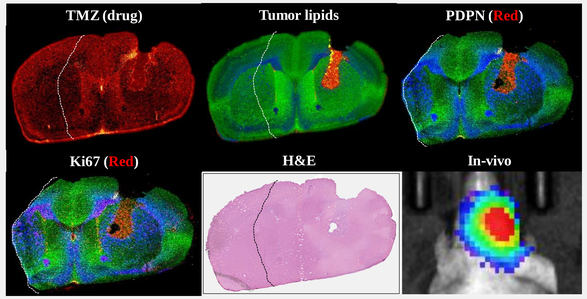
» MALDI-1 MSI to visualize lipid biomarkers and metabolites of the tumor«

Lipid/ Metabolite	Carnitine	Acylcarnitine	Indoleacrylic acid	Phosphorylcholine	Ceramide d18/16:0	Cholesteryl ester 18:1	Sphingomyelin 34:1	Phosphatidylcholine 34:1	Triglyceride 54:5
Location of comparison ROI	Tumor (cellular)	Tumor (cellular)	Tumor (cellular)	Tumor (cellular)	Tumor (cellular)	Tumor (necrotic)	Tumor (cellular)	Tumor (cellular)	Tumor (necrotic)
Ion	$[M+H]^+$	$[M+H]^+$	$[M+H]^+$	$[M+K]^+$	$[M+H-H_2O]^+$	$[M+K]^+$	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$
m/z ratio	162.1125	204.1229	188.0708	222.0295	520.509	689.564	703.579	798.5411	919.716



MSI of lipid and metabolite tumor biomarker. TMZ penetration and distribution throughout brain parenchyma and tumor. From the same acquisition, clear metabolic profiles of the tumor, tumor border, and parenchyma could be determined. Lipid markers and metabolites were all specifically localized to the tumor. Several cholesteryl esters (YELLOW) were localized to periphery of the necrotic tumor region. Phosphatidylcholine (34:1), Sphingomyelin (34:1), Ceramide (d18:16:0), Carnitine, Acylcarnitine, and Indoleacrylic acid were all specifically localized to the tumor. Phosphatidylcholine (***marker for gray matter***) and Hexosylceramide (***markers for white matter***) were clearly visualized.

» Multiplex MSI technique to detect drug, tumor lipids, and MALDI-IHC on single tissue section«



Multiomic MSI of drugs, metabolites, and immunolabeled biomarkers: Multiomic MSI was successfully demonstrated to detect drug (TMZ) distribution into tumor, metabolite lipid biomarkers, protein biomarker for the GBM (such as Ki67 and PDPN), and H&E histology all in a single tissue (10µm) section. Thus, developed approach may perform both label-free untargeted small molecule MSI and multiplex PC-MT-based targeted MSI of macromolecular biomarkers on the same tissue section. Label free untargeted small-molecule MSI can directly analyze lipids, drugs, and metabolites, which is not possible using standard IHC.