Combining Drug and Targeted Protein Imaging to Uncover Molecular Changes Associated with Cancer Treatment

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Overview

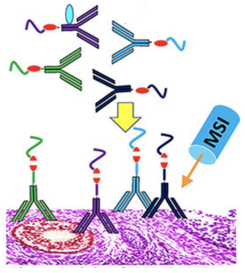
- Mass spectrometry imaging enables molecular specificity and spatial localization of molecules in tissue sections
- With proper experimental planning multiple MSI modalities (small molecule and MALDI-IHC) can be carried out sequentially on the same tissue section
- Source pressure as well as the mass analyzer can influence molecules observed in imaging experiments
- Similarities, as well as subtle differences, observed on the 3 different MSI platforms

Introduction

Mass spectrometry imaging (MSI) is well-established for the localization and quantification of drugs in tissue after different routes of dosing. Typically, drugs and other small molecule metabolites are detected simultaneously. However, a means of correlating the effects of drug treatment with protein changes is lacking. The MALDI-IHC approach using novel photocleavable mass-tags (PC-MTs) allows for targeted imaging of intact proteins and can be performed on the same tissue section and the same instrument as other MSI experiments.[1] Other imaging modalities such as fluorescence can also be combined with MALDI-IHC. Here, using rat liver tumors, we present the integration of drug and targeted protein imaging on the same tissue section. We also compare these results across 3 different MSI platforms.

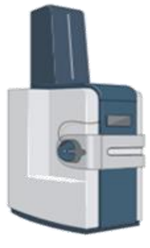
Methods

Orthotopic rat liver tumors were treated via transarterial intratumoral delivery of a PLGA-based microsphere that elutes imiquimod, a synthetic TLR7 agonist. Additional tumors were saline (control) or treated with blank microsphere (PLGA).Tissues were sectioned at 12 µm thickness and coated with 40 mg/mL DHB in 50% ACN, 0.1% TFA for drug and metabolite imaging in positive ion mode. After small molecule imaging, matrix was removed, and the sections were stained with antibodies conjugated to photocleavable mass-tags for MALDI-IHC imaging of the same section.[1]

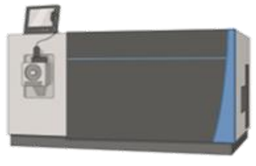


A cross-platform comparison was carried out where imaging of the drug and mass-tags was performed at 50 µm resolution on 3 different MSI instruments; a Bruker rapifleX, a Bruker timsTOF fleX, and a Thermo Fusion Lumos with a MassTech AP/MALDI source.

Bruker rapifleX
High vacuum
Axial TOF (R)



Bruker timsTOF fleX
Intermediate vacuum
QTOF (T)



Thermo Fusion Lumos
Atmospheric Pressure (MassTech)
Orbitrap (L)

Serial sections of each sample were collected for H&E staining. Data were visualized using SCiLS Lab MVS Version 2024b Pro and putative IDs generated using MetaboScape.

1. Yagnik et al. *JASMS* **2021**, 32, 977-988.

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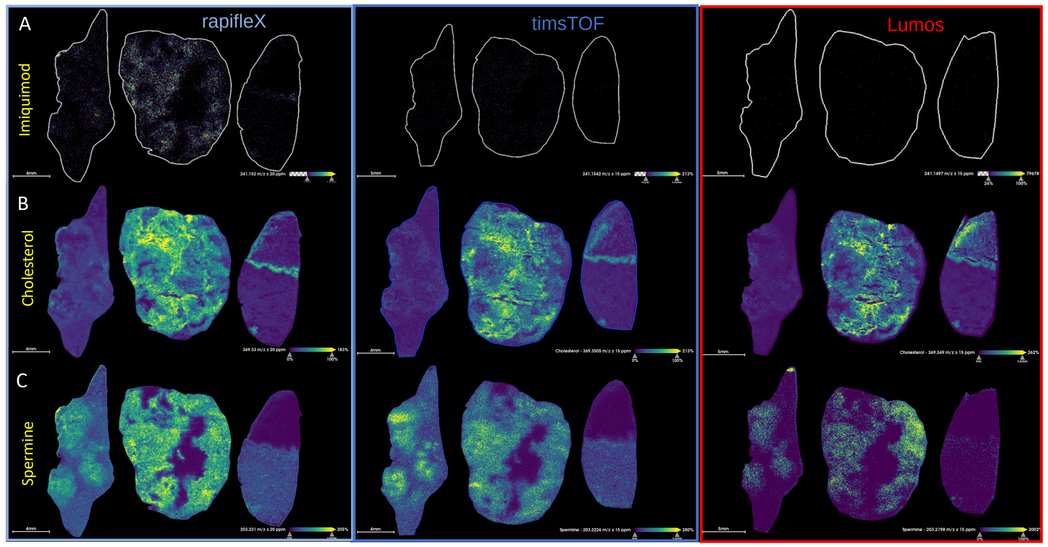
Imiquimod/Metabolite Imaging



Imiquimod

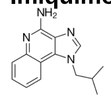


Figure 1. Imiquimod and metabolite imaging. A) imiquimod in rat liver tumors, best signal achieved with the rapifleX. B) Cholesterol showed highest signal in necrotic areas of treated tumors. C) Spermine was detected in areas of viable tumor. D) Average spectra from the 3 different instruments. The Lumos with the AP/MALDI source showed the most even distribution of peak intensity across the *m/z* range, while the timsTOF spectrum was heavily dominated by matrix peaks. Light blue – rapifleX, medium blue – timsTOF, red – Lumos.

MALDI-IHC Imaging

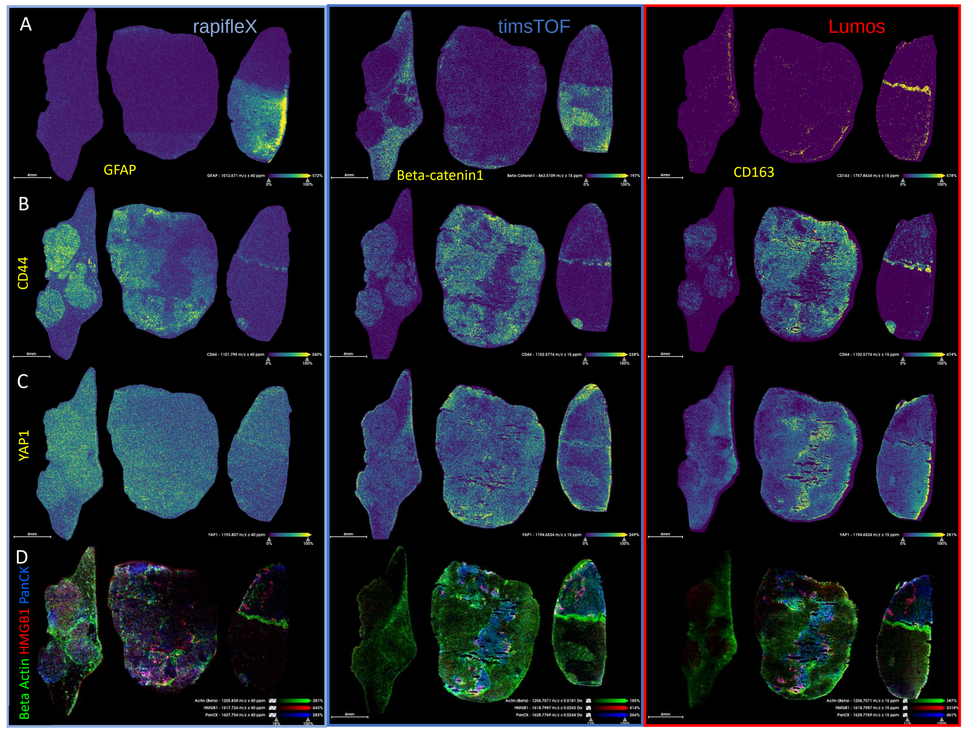


Figure 2. MALDI-IHC imaging of the same sections after imiquimod/metabolite imaging. A) Some variability in detected PC-MTs were observed between instruments. At left GFAP (rapifleX, hepatic stallate cells); center Beta-catenin1 (timsTOF, cholangiocytes); right CD6163 (Lumos, macrophages) B) CD44 PC-MT from all 3 instruments. CD44 is a marker for cell surface adhesion and migration. This PC-MT was well detected on all 3 platforms with the same spatial localization. C) YAP1 PC-MT from all 3 instruments. YAP1 is a marker of proliferation/apoptosis suppression. Better signal for this PC-MT was observed on the timsTOF and the Lumos as compared to the rapifleX. D) Co-registered signals for Beta Actin (green, structural/cell growth), HMGB1 (red, inflammation), and PanCK (blue, tumor marker) on all 3 platforms. Similar patterns were observed from all 3 instruments, though the rapifleX has slightly noisier signal. Light blue – rapifleX, medium blue – timsTOF, red – Lumos.

Histology

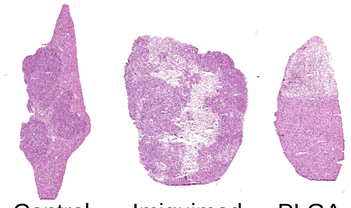


Figure 3. Hematoxylin and eosin staining of serial sections of the liver tumors. Necrotic areas are observed within both treated (imiquimod and PLGA) tumors. Imiquimod was detected in the viable tumor area of the dosed tumor (top panel of **Figure 1).** This section layout is conserved across all figures.

Photocleavable Mass Tags

Protein Target	PC-MT [M+H]⁺	Detected
Beta-Catenin1	863.52	T,L
NFκB	974.53	T,L
GFPA	1011.55	---
HLA-DR	1035.55	R,T,L
CD44	1102.59	R,T,L
CD3ε	1161.65	---
YAP1	1194.66	R,T,L
Actin (Beta)	1206.72	R,T,L
CD11b	1467.81	T,L
HMGB1	1618.81	R,T,L
PanCK	1628.79	R,T,L
CD163	1757.89	R,T,L

Glycogen

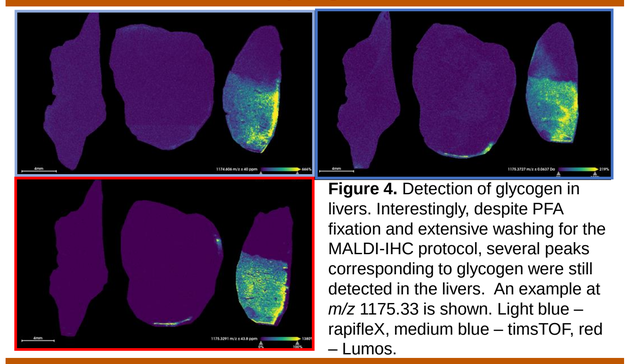


Figure 4. Detection of glycogen in livers. Interestingly, despite PFA fixation and extensive washing for the MALDI-IHC protocol, several peaks corresponding to glycogen were still detected in the livers. An example at *m/z* 1175.33 is shown. Light blue – rapifleX, medium blue – timsTOF, red – Lumos.

Conclusions

- Imiquimod/metabolite imaging followed by MALD-IHC imaging successfully achieved on 3 different imaging platforms
- Best imiquimod signal achieved using the rapifleX – high vacuum, axial TOF instrument
- Most of the applied PC-MTs were detected on all 3 platforms, with a few lost to noise/lower mass resolution on the rapifleX
- Sequential imaging allow different molecular classes to be imaged from the *same* cells

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The authors declare the following competing financial interest(s): Authors CAR, GY, KJR and MJL are employed by the company AmberGen, Inc.