Abstract Book

2024 SUMMER WORKSHOP

MADISON, WISCONSIN

Table of Contents

Anderton, Christopher

Development of Methods for Untargeted Spatial Metabolomics and Untargeted Spatial Proteomics on the Same Tissue Section

Marija Velickovic, Gregory W. Vandergrift, Dusan Velickovic, Le Z. Day, Sarah M. Williams, Paul D. Piehowski, Christopher R. Anderton

Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, WA, USA

Introduction

Spatially-resolved omics approaches that can measure biomolecules within tissue anatomical regions are powerful new tools for correlation of protein expression with metabolite abundances, and they are able to detect small changes within discrete tissue functional units masked in bulk measurements. There are promising new developments to map the proteome and metabolome within the same sample. Nevertheless, many methods only visualize targeted proteins (e.g., immunohistochemistry), and those that can measure both proteins and metabolites in an untargeted fashion are limited by sample preparation constraints, which restrict comprehensive detection to one class of biomolecule at a time. We present unique approaches combining mass spectrometry imaging (MSI)-based spatial metabolomics with informed microscale proteomics interrogation for deep profiling of specific cell populations and tissue substructures.

Methods

Rat brain sections (12 µm) were imaged by desorption electrospray ionization (DESI) using a Waters Select Series Multi Reflecting Time-of-Flight (MRT) MS and by matrix-assisted laser desorption/ionization (MALDI) using a Bruker solariX 12T Fourier transform ion cyclotron resonance (FTICR)-MS. Human kidney tissue sections (12 µm) were also measured by MALDI-FTICR-MS. Resultant MSI datasets were exported to .imzml for annotation via METASPACE. Regions of interest from the same tissue sections, informed by the MSI-based spatial metabolomics results, were dissected and collected into microwell chips using a PALM MicroBeam laser capture microdissection system. Sample preparation was carried out on-chip following our microscale Processing in One pot for Trace Samples (microPOTS) preparation approach and proteomic analysis by liquid chromatography tandem MS.

Results

Using non-sample consuming or sample destructive MSI approaches enabled detailed spatial metabolite imaging first, which served as a map for subsequent laser capture microdissection (LCM)-based proteomics analyses on the same tissue sample. The DESI-MSI (35 µm spatial resolution) analysis resulted in >150 annotated lipid ion images in METASPACE, directly from rat brain tissue mounted on polyethylene naphthalate (PEN) membrane slides, which are ideal for LCM-based proteomics. Moreover, the DESI-MSI concomitantly preserved the integrity of the sample tissue itself. Guided by spatial metabolomics data, cell populations/tissue substructures were isolated using LCM, including those that weren't immediately obvious via optical or histological imaging. A total of 5153 unique proteins were identified from the collected tissue voxels (200 µm x 200 µm voxel size), with around 4,000 proteins per voxel being detected. We also developed a strategy to mount and analyze tissue sections onto PEN membrane slides for high lateral resolution MALDI-MSI analyses. This approach permitted us to obtain ~90% of the same lipid annotations in METASPACE as control samples mounted on ITO slides imaged simultaneously (>350 annotations total). We explored the number of proteins we could detect from different voxel sizes (e.g., 50 µm x 50 µm, etc.) using our LCM-microPOTS proteomics assay post-MALDI-MSI analyses. These results scale with our post-DESI-MSI data, with ~2100 proteins being detected using 100 µm x 100 µm voxel sizes. We are in the process of improving this assay, however, our results combined provide increased coverage of metabolites and proteins from the same sample when compared with existing approaches. We are currently developing and validating a workflow to look at other tissue types (i.e., human kidney tissue). For Post-DESI-MSI spatial metabolomics analysis, principal component analysis revealed clear segregation across the ROIs in our spatial proteomics analysis, which also exhibited distinct spatial metabolomics features. Additional visualization strategies (e.g., Lipid Mini-On) are currently being implemented to link the spatial metabolomics and proteomics datasets.

Novel Aspect

Sensitive untargeted spatial proteomics informed by upstream non-destructive mass spectrometry imaging-based spatial metabolomics on the same sample.

Bayram, M. Furkan
Single Cell Recognition and Semi-automated Sample Segmentation

M. Furkan Bayram*1, James Dressman*1, Anand S. Mehta*1

1. Medical University of South Carolina, Cell & Molecular Pharmacology

Introduction
The rapid evolution of digital imaging and analysis in scientific research demands tools that not only increase precision but also reduce the operational time involved in data processing. In fields such as cellular biology and digital pathology, where detailed and accurate imaging is crucial, the ability to efficiently process large volumes of data is essential. The development of SoloCell and SmartROI addresses these needs by providing automated solutions for cell recognition and sample segmentation, respectively. These tools are designed to support the complex requirements of modern scientific investigations by enhancing both the efficiency and accuracy of image analysis.

Methods
SoloCell automates cell coordinate determination on MALDI-MSI grid-patterned slides. Users select an image, define the analysis area, and mark reference cells. The software calculates coordinates using a self-adjustable algorithm, optimizing measurement sequences by adjusting for grid imperfections with contour detection. This process also sorts coordinates to efficiently solve the traveling salesman problem. A convolutional neural network evaluates the likelihood of cells being solo, aiding further analysis. Concurrently, SmartROI preprocesses the color space of the image for enhanced ROI selection. Users dynamically adjust values via trackbars, refining selections with advanced boundary identification. These methods collectively streamline the process, enabling precise and efficient analysis suitable for large-scale scientific research.

Results
This study introduces two software tools aimed at enhancing digital imaging efficiency and precision in scientific analysis. The first, SoloCell, is a user-friendly program that automates the process of determining the coordinates of solo cells arranged in a grid pattern on a slide for MALDI-MSI analysis. Users select a slide image, define the area for analysis, and mark a few reference cells. SoloCell employs a self-adjustable algorithm to calculate cell coordinates, adapting frame positions using contour detection to account for imperfect grid geometries. This system not only identifies coordinates but also optimizes their sequence to address the traveling salesman problem, thereby enhancing the efficiency of the measurement process. After the coordinates are established, SoloCell evaluates each cell's likelihood of being solo using a convolutional neural network and outputs a log file, an image with framed single cells, a histogram of confidence levels, and a text file with sorted single cell coordinates. The second tool, SmartROI, utilizes a color space to allow precise and dynamic selection of regions of interest within images. Through user-operated trackbars and advanced boundary identification, SmartROI provides a highly adjustable and accurate method for sample segmentation in fields such as digital pathology. Together, SoloCell and SmartROI offer innovative solutions that significantly improve the accuracy and efficiency of complex image analyses in scientific research.

Novel Aspect
SoloCell and SmartROI integrate adaptive algorithms and dynamic segmentation techniques to enhance precision and efficiency in scientific digital imaging tasks.

Bell, Jonathan

MALDI-ISH Transcriptomic and Multiomic Spatial Imaging of Alzheimer's Disease Mouse Brain Tissue

Jonathan M. Bell*1, Mark J. Lim1, Gargey B. Yagnik1 and Kenneth J. Rothschild1,2

1. AmberGen, Inc., 44 Manning Road, Billerica, MA 01821, 2. Department of Physics and Photonics Center, Boston University, Boston, MA 02215

Introduction

Alzheimer's Disease (AD) currently affects over 6.7 million persons in the U.S. with a 10.3% incidence over the age of 65. We previously introduced a whole-slide, high-plex, multiomic MALDI mass spectrometric imaging (MSI) method and associated workflows for tissues termed MALDI-IHC. Based on novel photocleavable mass-tags (PCMTs) linked to antibodies, over 100-plex targeted intact protein imaging has recently been achieved in tissues. We now introduce a similar approach for transcriptomic imaging based on PCMTs linked to in situ hybridization probes termed MALDI-ISH. This approach was used to image selected mRNA transcripts in Alzheimer's transgenic mouse brain tissue. Combined multiomic workflows were evaluated which allow spatial imaging of both targeted intact proteins and mRNA biomarkers.

Methods

To create the MALDI-ISH probes, custom PCMTs that have an azide reactive group were linked to 5'-thiol modified oligonucleotides available from ACD Bio. This was achieved with a 2-step DBCO click chemistry reaction followed by purification using size exclusion chromatography. FFPE tissue slices (3 µm) from hAbetaSAA mouse brain blocks (JAX) were deposited on microscope slides for the MALDI-ISH experiments. Amplification and imaging of selected mRNA transcripts was achieved with a modified RNAscope HiPlex Flex assay (ACD Bio). Slides were then pre-UV-illuminated to photocleave the PCMTs, sprayed with CHCA matrix and imaged on a Bruker timsTOF fleX instrument at 20 µm resolution. High-plex, multiomic imaging of targeted transcripts and protein biomarkers was achieved by combining workflows for MALDI-IHC and MALDI-ISH.

Results

Whole hAbetaSAA mouse brain FFPE tissue sections processed with up to 12 PCMT-oligo probes were imaged by MALDI-MSI in ~3hrs at 20 µm spatial resolution. The spatial distribution of targeted transcripts agreed well with the expected distribution based on both the Mouse Whole Brain Atlas and MALDI-IHC imaging from adjacent slices using corresponding PCMT-conjugated antibody probes. For example, both transcripts and the cognate expressed parvalbumin protein were found concentrated in the cerebellum, whereas GFAP transcript and protein were found concentrated in the periventricular regions. Individual amyloid plaques imaged using the PCMT-antibody probes for amyloid β42 appeared colocalized with the GFAP transcripts characteristic of astrocytic immune inflammatory response. Different workflows were evaluated for the same-slide detection of both RNA transcripts and expressed proteins using a combination of MALDI-IHC and MALDI-ISH protocols. These workflows offer a high-plex, multiomic capability to rapidly image both transcripts and expressed proteins from whole mouse brain FFPE tissue samples. Autofluorescence as well as the need for reiterative cycling is eliminated compared to conventional fluorescence-based approaches. Cellular resolution (5 µm) can be achieved using the Bruker microGRID accessory. Importantly, MALDI-ISH can be performed using conventional MALDI-MSI instruments instead of specialized instruments needed for cyclic fluorescence imaging or Imaging Mass Cytometry (IMC). It can also be combined initially with MALDI-MSI imaging of metabolites such as lipids and drugs from fresh frozen tissue. We anticipate that the PCMT-oligo probes and MALDI-ISH can be adapted to various amplified FISH approaches such as branched DNA (bDNA) or SABER. In general, MALDI-ISH combined with MALDI-IHC provides a promising new high-plex, multiomic whole-slide spatial imaging approach which can rapidly profile a variety of different biomarkers from tissues and cells.

Novel Aspect

The combination of MALDI-IHC and MALDI-ISH promises a new high-plex, multiomic approach to rapid spatial imaging of tissues and cells.

Bemis, Kylie
Scalable, reproducible MSI analysis with Cardinal v3

Kylie Bemis*1, Melanie Christine Föll2,3, Dan Guo1, Sai Srikanth Lakkimsetty1, Olga Vitek1

1 Northeastern University, Khoury College of Computer Sciences, Boston, MA, USA, 2 Institute of Surgical Pathology, Medical Center, University of Freiburg, Faculty of Medicine, Freiburg, Germany, 3 German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany

Introduction
Cardinal v3 is an open-source software package for scalable and reproducible analysis of mass spectrometry imaging (MSI) experiments. Over the past decade, the increasing size and complexity of MSI datasets have posed challenges for computational analysis. In the next decade, new practices such as integration with ion mobility and other imaging modalities such as magnetic resonance imaging (MRI) and microscopy introduce new levels of complexity. In this major update, Cardinal v3 streamlines and simplifies existing workflows, while establishing new infrastructure for analyzing the next generation of MSI experiments.

Methods
As a free and open-source R package, Cardinal v3 provides a powerful yet accessible platform for the full pipeline of MSI analysis, including data import, pre-processing, visualization, machine learning, and statistical inference. Cardinal v3 is hosted by the Bioconductor project for open-source bioinformatics software, enabling easy installation on all major systems (Windows, macOS, and Linux) with minimal external dependencies. Out-of-memory computation enables Cardinal v3 to process and analyze datasets much larger than computer memory using efficient native (C/C++) code, with the option to use parallel processing if the user's system supports it. We provide a collection of case studies and tutorials to show the user how to perform common workflows.

Results
We will begin by outlining the major improvements in Cardinal v3 compared to previous versions as well as existing alternatives. These improvements include a new companion package dedicated to reading and writing imzML, more advanced spectral processing methods to improve reliability when pre-processing MSI datasets, new data structures providing greater control when working with raw MSI data, and efficiency and usability improvements for machine learning and statistical inference methods. We will demonstrate these improvements on three common analytic goals: (1) segmentation, (2) classification, and (3) class comparison.

For segmentation, where the goal is to model the spatial structure of an unlabeled sample and the associations between molecules and tissue morphology, we will present improvements in the speed and usability of our novel spatial shrunken centroids (SSC) segmentation method.

For classification, where the goal is to learn class labels (e.g., disease versus healthy) from training data and correctly label subregions of new data, we will present our framework for multiple instance learning. This is a technique for semi-supervised machine learning newly available in Cardinal v3. This strategy can improve classification accuracy when the training data is labeled at a coarser level than the predictions, which is often the case in MSI where classes are commonly labeled tissue-by-tissue rather than pixel-by-pixel.

For class comparison, where the goal is to test molecules for differential abundance between conditions, we will show how our novel spatial Dirichlet Gaussian mixture model (sDGMM) segmentation method can enable inference on MSI using traditional statistical models.

Finally, we will provide a roadmap for future Cardinal updates. We will discuss our current work developing support for ion mobility and novel methods for co-registering MSI with supplemental imaging modalities such as MRI and microscopy.

Novel Aspect
Cardinal v3 is a major update to a versatile open-source software for MSI analysis that streamlines existing workflows and sets groundwork for supporting new technologies.

Bergamin de Castro, Tialfi

Alterations in the lipid profile caused by Bordetella pertussis infection and pertussis toxin in a non-human primate infection model

Tialfi Bergamin de Castro*1, Kelsey Gregg2, Todd Merkel2, Alison J Scott1

1. Microbial Pathogenesis, School of Dentistry – University of Maryland Baltimore – Baltimore, Maryland; 2. Center for Biologics Evaluation and Research (CBER); Food and Drug Administration (FDA) - Silver Spring, Maryland

Introduction

Bordetella pertussis (BP), the bacterium causing whooping cough, remains a global health concern. With an estimated 16 million cases annually worldwide, including more than 160,000 deaths of children younger than 5 years old, its transmission occurs through respiratory droplets. Despite available vaccines, BP persists due to reduced immunity, gaps in immunization coverage and incomplete vaccination, especially in infants, leading to potential complications including pneumonia and death. Pertussis toxin (PT), a unique virulence factor secreted by BP, plays a critical role in the pathogenesis by disrupting cellular signaling and causing leukocytosis with lymphocytosis, leading to characteristic symptoms like severe coughing. Although PT is part of all BP vaccines, the contribution of the toxin to pathology and protection is still incomplete.

Methods

Two Olive baboons, 6 months of age, were infected with BP D420 strain, wild type (WT), and D420 ΔPT, a genetically modified strain with reduced virulence. Both animals were inoculated with 1mL of BP strains directly in the trachea via intubation followed by inoculation of 0.5mL in each nostril. Both animals were euthanized after 7 days of infection. One lung of each animal was inflated with 2% gelatin, snap frozen and stored in -80°C. The lungs were cut in half (coronal plane) and the lobes were separated. Cryosections of 12µm were placed in large format Superfrost glass slides (3x2in). Norharmane 7.5mg/mL matrix was used and a timsTOF Flex was used for negative ion mode phospholipids acquisition at 30µm spatial resolution.

Results

SCiLS software was used for MSI analysis. A feature list was created with the 50 most intense peaks, with medium filtering and the list was used for segmentation and creation of specific regions with only on-tissue peaks. The mean spectra of the on-tissue regions were extracted, and the intensities were used to compare between the groups. Alex123 was used for identification of the m/z values. An increased intensity of several phosphatidylinositol (PI) was found in the WT group. The intensities of PI 34:2, PI 36:1, PI 36:2 and PI 34:1, including LPI 18:0 and Hex2Cer 32:1;4, were found between 2.1 and 3.6 times higher than the ΔPT group. Interestingly, the opposite was found for phosphatidylserine (PS) in the ΔPT group, showing intensities of PS 36:1 and PS 27:0, 2 and 3.2 times higher, respectively, than the WT group. Although the PS in higher intensity, they are well spread over the parenchyma of the ΔPT sample group. Inversely, the PIs observed in the WT samples are organized around the airways, suggesting a specific alteration linked to the immune response and infiltration of not only neutrophils, but also microcolonies of bacteria. During the first days of the infection, PT can suppress the recruitment of neutrophils generated by the BP lipooligosaccharide (LOS) signaling, however, later in infection, the combination of PT, LOS and adenylate cyclase toxin (ACT), another BP toxin, induce IL-17 response and promote a large-scale neutrophil recruitment. The increased number of neutrophils can cause significant tissue damage, leading to acute disease and death. We concluded that the ΔPT group, with attenuated PT, showed less phospholipid alterations and less PI accumulation due to reduced neutrophil response.

Novel Aspect

Large format MSI for analysis of tissues from higher order pathogenesis model systems.

Bruce, Emily

Evaluating Quantification of Glutathione with Incorporation of DOE Optical Train for IR-MALDESI MSI

Emily R. Bruce*1, Russel R. Kibbe1, Emily C. Hector2, David C. Muddiman1

1. FTMS Laboratory for Human Health Research, Department of Chemistry, North Carolina State University, Raleigh, North Carolina, USA 2. Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA

Introduction

Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) uses an infrared laser to desorb biological molecules for mass spectrometry imaging (MSI) applications. The Gaussian profile of the laser results in adjacent tissue heating and non-ablated tissue due to the circular ablation spots. A diffractive optical element (DOE) has been incorporated into the optical path to correct for these disadvantages. The DOE produced a Top-hat profile which has a uniform energy distribution and produces square ablation spots. While beneficial to MSI, little is known about how this optic affects the ability to perform quantitative MSI (qMSI). In this work, we evaluate the performance of the DOE optical train against our conventional optics to determine the quantitative capacity of the new optical train.

Methods

Absolute quantification of glutathione (GSH) was achieved by spotting a dilution series of standard isotope labeled glutathione (SIL-GSH) directly on the liver section to account for matrix effects. The spotting of SIL-GSH acts as a calibration curve to calculate the absolute concentration GSH in tissue. Once the liver sections were spotted with the dilution series, they were analyzed using IR-MALDESI MSI with either the conventional optical train or with the DOE incorporated. The resulting data was visualized and analyzed using MSiReader Pro. The MSI quantification tool was used to extract tissue volumes, construct calibration curves, and return the concentration of endogenous GSH in each liver section.

Results

Initial data acquired on the mouse liver visually indicated that both optical trains were capable of producing high quality ion images with good detection. Additionally, absolute quantification of glutathione was achievable by using the DOE optical train. To determine the performance of the DOE optical train against conventional optics, a two-sample t-test was administered on the calculated concentration of glutathione between the conventional and DOE optical trains. The average concentrations of GSH in investigated mouse liver sections were 1612.24 and 1680.06 micrograms of GSH per gram of tissue for the DOE and conventional optical trains, respectively. From this test, the p-value was larger than the significance level of 0.05 ($p=0.084$) which indicates that there was no statistically significant difference between the optical trains. Additionally, the confidence intervals for both optical trains overlapped, also indicating no significant differences between average abundances. Furthermore, since it was known that the quantified concentration did not differ, the calibration curves were compared by analyzing the confidence intervals of the slope and intercept to determine if there were significant differences. This test indicated that the calibration curves obtained from the different optical configurations were not statistically significantly different from one another. With these results, there was evidence that both optical trains can be used for quantification without loss in signal. Therefore, we can now confidently utilize the additional benefits of the DOE without being concerned about the qMSI capabilities for this optical configuration.

Novel Aspect

This presents the first application of qMSI by IR-MALDESI using the DOE and its comparison to the conventional optical train.

Buckman, Raven

Machine Learning for Mass Spectrometry Imaging of in vivo Isotope Labeled Duckweed

Raven L. Buckman*1, Vy T. Tat1, Young Jin Lee1

Department of Chemistry, Iowa State University

Introduction

Spatial monitoring of in vivo isotope labeled metabolites with mass spectrometry imaging (MSI), referred as MSIi, can provide insights on metabolic pathways. We have recently demonstrated the utility of MSIi in an aquatic plant Lemna minor by monitoring spatiotemporal changes in galactolipids biosynthesis with in vivo isotope labeling with D2O and 13CO2. Cardinal is an open-source, R-based software package that provides a platform for machine learning analysis of MSI data. Here, we utilize Cardinal's spatial shrunken centroids (SSC) algorithm to segment unlabeled and labeled L. minor samples and parse our isotopologue distributions.

Methods

L. minor was propagated in 0.5x Schenk and Hildebrandt (SH) media with a 16/8-hr light cycle. To label metabolites with D, healthy fronds were grown in Petri dishes containing 50% D2O, H2O:D2O (50:50 mol:mol) for five days. For 13C labeling, fronds were grown in an Erlenmeyer flask system that allowed for the purging of 12CO2 and replenishment of 13CO2 by reacting Ba13CO3 with lactic acid. Imaging was performed with an orbitrap mass spectrometer Q Exactive HF (Thermo Scientific, San Jose, CA, USA) equipped with a medium pressure MALDI source (Spectroglyph, Kennewick, WA, USA). Computations were performed using Iowa State University's Pronto cluster and imaging analysis was conducted in RStudio (ver. 2024.04.1; R ver. 4.4.0) with Cardinal MSI (ver. 3.6).

Results

In this study, we present the use of spatial shrunken centroid (SSC) clustering for the unsupervised segmentation of both unlabeled and labeled L. minor samples. SSC is an integrated Cardinal framework that takes imzML formatted MSI datasets and conveys spatial and multivariate information through a probabilistic approach. Informative spectral features that are unique to a spatial segment and t-statistics are used to express whether the centroids are higher or lower in abundance for the segment, on average, than the global mean spectrum. With SSC, downstream statistical analysis can readily pick out the isotopologue distributions at various stages of duckweed growth. While L. minor is morphologically simple, SSC segmentation of the unlabeled images reveals parsed out the image background and the duckweed sample. Additionally, spectral differences between the parent and daughter fronds could be observed. Segmentation of the isotopically labeled samples highlighted the spectral differences between various stages of growth. Older regions of growth were identified by systematically higher intensities of monoisotopic peaks. In contrast, newer growth regions showed systematically lower, monoisotopic peak abundances and various isotopologue distributions. Similarly, intermediate growth regions exhibited evidence of both monoisotopic and isotopologue signatures. Our results demonstrate that unsupervised segmentation by SSC with Cardinal is a promising avenue for advanced analysis of isotopically labeled MSI data.

Novel Aspect

We present spatial segmentation with unsupervised machine learning and statistical analysis of isotopologue distributions correlating to various stages of growth in isotope labeled duckweed samples.

Campbell, Melanie (MJ)

Spatial Mapping of Ether Lipids in Tissue via MALDI TIMS Imaging Mass Spectrometry: Characterization of Biomarkers of Peroxisomal Disorders

Melanie J. Campbell*1; Erin H. Seeley1; Wei Cui2; Ann B. Moser5,6; Nancy E. Braverman2,3,4; Jennifer S. Brodbelt1

1. Department of Chemistry, The University of Texas at Austin, Austin, TX, USA; 2. Research Institute of the McGill University Health Center; 3. Department of Human Genetics; 4. Department of Pediatrics, McGill University, Montreal, Quebec, Canada; 5. Kennedy Krieger Institute; 6. School of Medicine, Johns Hopkins University, Baltimore, MD, USA

Introduction

Rhizomelic chondrodysplasia punctata (RCDP) is a rare peroxisomal disorder that dramatically shortens the lifespan of children and is characterized by a severe deficiency of vinyl ether glycerophospholipids, also known as plasmalogens. Previous reports demonstrate that myelination is abnormal in RCDP patients and is correlated to plasmalogen dysregulation. We aim to understand the spatial distribution and composition of plasmalogens in both brain and lung tissue from mice afflicted with RCDP by employing matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS). Additionally, trapped ion mobility spectrometry (TIMS) is used to determine the collisional-cross sections (CCS) for ether lipids that are under-characterized by ion mobility.

Methods

Brain and lung samples from Pex7 null/null and Pex7 WT/null mice were provided by the Research Institute of McGill University Health Centre. Brain samples were cryo-sectioned at a 12 µm thickness, thaw-mounted onto glass slides, and washed three times with cold ammonium formate (50 mM). The slides were heated to 50°C and coated with 10 mg/mL of 1,5-diaminonaphthalene in 70% acetonitrile using an HTX M5 sprayer. All experiments were performed in negative mode using a Bruker timsTOF FleX mass spectrometer. Tissue imaging data was acquired at 50 µm resolution with 200 shots per pixel. TIMS mode was operated with a ramp time of 650 ms and a reduced mobility (1/Ko) range of 1.25-1.55 (V·s)/cm2.

Results

Previously, we utilized LC-MS with 213 nm ultraviolet photodissociation (UVPD) to identify and relatively quantify phosphatidylethanolamine plasmalogen (PE-P) lipids in isolated regions of peroxisomal disorder mouse brain tissue (Pex7 model). In the cerebellum and cortex of both control and Pex7 model mice, 17 PE-P lipids were identified, showing a downregulation of PE-P lipids in the Pex7 model. We used these findings to guide our MALDI-TIMS IMS analysis.

MALDI-TIMS IMS was performed on control and Pex7 brain tissue, revealing the distribution of 9 PE plasmalogens. Plasmalogens are absent in the Pex7 tissue, but insight into their spatial distribution in healthy tissue can be discerned. For example, m/z 722.51, identified as PE(P-16:0/20:4) via LC-MS/MS analysis of the cortex, is the most abundant in the frontal lobe. LC-MS with 213 nm UVPD analysis identified isomers PE(P-18:2/20:4) and PE(P-16:0/22:6) (m/z 746.51) in the cerebellum; IMS localized these isomers to the grey matter, providing further insight into their role in the brain.

By combining LC-MS with 193 nm UVPD and TIMS analysis, PE plasmalogens can be comprehensively characterized. An ion abundance map was generated for m/z 774.54 showing localization throughout the cortex and in the grey matter of the cerebellum. MetaboScape identified the sum composition of the species as PE(O-40:7) with 10 ppm mass accuracy. The LC-MS with 193 nm UVPD method was employed to not only determined the lipids retention time (29.05 min), but resolve the lipid's identity down to the double bond position: PE(P-18:0/22:6(Δ4, Δ7, Δ10, Δ13. Δ16, Δ19). Finally, an extracted ion mobilogram was obtained from the TIMS data to establish the CCS value for this under-reported species as 281.7 ± 0.6 Å2. This data analysis workflow was performed for all PE-P lipids identified by the LC-MS with 193 nm UVPD method to enhance our biological understanding of RCDP.

Novel Aspect

Combine MALDI-TIMS IMS and LC-MS with 193 nm ultraviolet photodissociation to spatially localize and structurally characterize phosphatidylethanolamine plasmalogens in peroxisomal disorder tissue

Chan, Yat Ho (Horace)

Gel-assisted mass spectrometry imaging enables sub-micrometer spatial lipidomics

Yat Ho Chan1, Koralege C. Pathmasiri1, Dominick Pierre-Jacques1, Nannan Tao2, Joshua L. Fischer2, Ethan Yang2, Stephanie M. Cologna1,3, Ruixuan Gao1,3,4*

1University of Illinois Chicago, Chicago, IL; 2Bruker Daltonics, Billerica, MA; 3Laboratory for Integrative Neuroscience, University of Illinois Chicago, Chicago, IL; 4Department of Biological Sciences, University of Illinois Chicago, Chicago, IL

Introduction

MALDI mass spectrometry imaging (MALDI-MSI) is a powerful tool for biomarker discovery and drug screening as it enables label-free investigation of a broad range of biomolecules in intact specimens. The ~5-50 μm raster distance (or pixel size) of existing MALDI-MSI instrument, however, has limited its application to single-cell and subcellular studies. In particular, the current spatial resolution limit of off-the-shelf MALDI-TOF mass spectrometers has made spatial omics investigation challenging to perform at (sub)cellular level. Herein, we report a sample preparation and imaging method named gel-assisted mass spectrometry (GAMSI), in which intact specimens are embedded in a swellable hydrogel and physically expanded. Using GAMSI, we show that the spatial resolution of existing MALDI-MSI can be enhanced ~4-6-fold to the sub-micrometer scale.

Methods

Fresh-frozen mouse brain sections (~25 μm in thickness) are first lightly fixed, treated with a small-molecule linker, and polymerized to form a swellable hydrogel composite. The gelled tissue sections are then digested with trypsin to (bio)chemically break down its structural integrity while preserving its original spatial and molecular information. Next, the sample-hydrogel composite is swelled in water and subsequently dried and mounted onto an ITO-coated slide or MALDI plate. After matrix application, MALDI imaging experiments are performed on the mounted samples using off-the-shelf MALDI-TOF mass spectrometers. During imaging, the target analytes can get desorbed and ionized through interactions with the matrix and organic solvents as they are tethered to the hydrogel polymer network reversibly.

Results

We first characterized the retention rate and chemical profile of lipids in the GAMSI sample using fluorescence microscopy and mass spectrometry imaging. Our fluorescence microscopy data indicated that approximately 90% of phospholipids were retained in the expanded mouse cerebellum compared to the fresh-frozen sample. Additionally, the mass spectrometry data revealed a ~60% overlap in the detected lipid peaks between the expanded and fresh-frozen samples. As expected, when imaged on a commercial MALDI-TOF mass spectrometer, the GAMSI sample showed a drastic improvement in the spatial resolution (compared to its fresh-frozen counterpart) that corresponded to its linear expansion factor. To demonstrate the generality of GAMSI, we further performed multiplexed lipid and protein imaging by introducing an additional step of HiPLEX-IHC probe modification. As result, we were able to concurrently map endogenous lipids and targeted proteins with the enhanced spatial resolution. Lastly, we performed lipid GAMSI using MALDI-TOF mass spectrometers with higher sensitivity and smaller pixel size. With ~4-fold expansion, we observed local enrichment of specific lipids in a cellular pattern between the granular cell layer and the molecular layer in the mouse cerebellum. After cross-validation using immunostaining against a protein marker for Purkinje cells, we observed clear colocalization between the local lipid enrichment and Purkinje cell. With ~6-fold expansion, we were able to push the spatial resolution down to 0.8 μm and resolve subcellular features such as the glial and neuronal nuclei in the mouse cerebellum white matter. These results showed that GAMSI allows multiplexed lipid and protein imaging at single cell and subcellular resolution using existing MALDI-MSI instruments. (CHAN Y.H., GAO. R. et al. Nat Commun 15, 5036 (2024))

Novel Aspect

GAMSI enables sub-micrometer MALDI-MSI without modifying the hardware or the analysis workflow of an off-the-shelf MALDI mass spectrometer.

Charkoftaki, Georgia

A novel MALDI IMS approach for improved spatial visualization of small molecules in formalin-fixed paraffin-embedded samples

Georgia Charkoftaki*1, Athina Lisgara1, Alvaro Santos-Neto1,2, Reza Aalizadeh1, Michael Becker3, Nina Gonella4, Vladimir Papov4, Vasilis Vasiliou1

1Department of Environmental Health Sciences, Yale School of Public Health, Yale University, New Haven, CT, USA., 2São Carlos Institute of Chemistry, University of São Paulo, São Carlos 13566-590, SP, Brazil, 3Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riss, Germany, 4Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA

Introduction

In the clinical field, numerous archived tissue samples are fixed in formalin and embedded in paraffin (formalin-fixed paraffin-embedded; FFPE). This process maintains excellent tissue morphology and allows indefinite sample storage at room temperature. As FFPE tissue specimens are routinely acquired in health care and it is imperative that tissue-based diagnostic techniques can analyze such samples. MALDI IMS is routinely applied to fresh frozen tissues, however there are challenges to achieve similar performance for FFPE samples. FFPE sample preparation traditionally relies on washing procedures which, however, may cause the delocalization and wash out the molecules of interest. Here we investigated a novel a novel blotting approach to dewax the samples, thus expanding the samples available for imaging mass spectrometry.

Methods

One liver lobe was fixed in 10% formalin and embedded in paraffin (FFPE), while the adjacent lobe was fresh frozen in 10% w/v gelatin. Intensity and spatial localization of small molecules (m/z<1000) were investigated using: (i) traditional washing steps (xylene, ammonium formate and water), and (ii) a novel approach, in which a filter was pressed against the tissue to absorb the wax, avoiding spatial delocalization (blotting). Two filters were used: nitrocellulose (less hydrophilic) and cellulose: pressed on top of the tissue, placed a slide on top, used binder clips to stabilize it, and placed in the oven (700C, 1 h). 1,5- diaminonaphthalene was sprayed; acquisition and data analysis: timsTOF fleX in negative mode, 50-µm step size and SCiLS Lab software.

Results

The blotting approach was faster than the traditional washing method since the washing steps were omitted. As expected, the total number of features was higher in the fresh frozen sample (4,913) compared to the samples prepared with washes (1,685) and nitrocellulose (1,498) or cellulose (1,553) filter. Average intensity of all spectra in the FFPE samples was similar. Only 25.2% of features were common amongst all samples (features matched with 7 ppm accuracy) and 61.8% were unique to the fresh frozen tissue. The embedding process involves steps immersing the tissue in solvents and many small molecules are dissolved. For example, we were not able to detect any of the bile acids in the FFPE samples, while detected in the fresh frozen sample (e.g. taurocholic acid and chenodeoxycholic acid). In the FFPE samples, respectively for washing, nitrocellulose, and cellulose methods, 33.7%, 30.0% and 31.3% features were in common compared to the ones detected in fresh frozen tissue; also similar in intensity and variety. However, when we investigated the spatial localization of the common most intense features in the FFPE samples we found that the samples with the blotting method had improved spatial resolution. For example, the distribution for m/z 298.135, 313.146 and 515.1829, was spatially delocalized and exhibited ‘drop-like’ formations at the edge of the tissue in the washing method. The center of the tissue consistently was showing decreased signal while, increased intensity was found at the bottom edge of the tissue. This was likely due to the washes of the tissue in the solvents during dewaxing. These formations were not observed for the same features in the samples prepared using the blotting approaches: we were able to discriminate discrete histological features in the liver and enabled us to generate images of similar quality to the fresh frozen for the same feature.

Novel Aspect

The blotting approach provides a novel method for imaging small molecules in FFPE samples with improved spatial resolution.

Colley, Madeline

Advancing Lipid Annotation in Human Kidney Tissues: Integrating Imaging Mass Spectrometry with Multi-Platform Characterization

Madeline E. Colley*1,2, Martin Dufresne*1,2, Lukasz G. Migas*3, Katerina V. Djambazova*2,4, Jamie L. Allen*2,4, Alina Theisen*5, Christopher Wootton*5, Raf van de Plas*1,3, Jeffrey M. Spraggins*1,2,4,6"

1. Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, 2. Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, 3. Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands, 4. Department of Cell & Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 5. Bruker Daltonik GmbH, Fahrenheitstraße 4, 28359 Bremen, Germany, 6. Department of Chemistry, Vanderbilt University, Nashville, TN

Introduction

The complexity of human tissues requires both high spatial and spectral resolution imaging mass spectrometry (IMS) to confidently associate specific molecules to cell types and their neighborhoods. Elucidating the structure of these specific molecules is critical to understanding the biological pathways associated with the structures. Here, we outline our strategies to annotate lipids and metabolites from human tissues using a variety of techniques and collaborations.

Methods

Human kidney tissues were sectioned into ITO slides at 10 um thickness for all IMS analyses. In-house developed matrices were utilized with both an HTX M5 sprayer and HTX Sublimate. Serial sections, including those from an 84 patient cohort, were homogenized and analyzed by 4D-PASEF LC-MS/MS. A Bruker timsTOF FleX MS was used for all PASEF experiments and a prototype MALDI TIMS FT-ICR was used for high spectral resolution experiments.

Results

One tool we employ is untargeted lipid and metabolomics by LC-MS/MS which gives a comprehensive view of both species and structure in four dimensions: 1) mass accuracy, 2) fragmentation, 3) collision cross section (ion mobility), and 4) retention time. Our methods for untargeted lipidomics take advantage of PASEF on the timsTOF mass spectrometers as well as MS/MS stepping to increase the mass range of detectable species. This technology provides a tissue-specific database of species present within the sample though the spatial context is lost. A second tool we employ is prm-PASEF IMS which enables highly multiplexed in situ fragmentation to confirm up to 100 lipid species within a 30 x 30 um pixel. This analytical technique can be used to mine the LC-MS/MS database for both tandem MS spectral comparisons and collisional cross sections. A third technology we are developing in collaboration with Bruker Daltonics is the use of a custom developed hybrid ion mobility Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer which offers ultra-high spectral resolution that enables highly confident molecular annotation on tissue through sub-ppm mass accuracy and fine isotope analysis. We utilize this system to study the human kidney at 10 um spatial resolution and with a resolving power of 1.1 million at m/z 400. The result is highly confident chemical formulae which are given deeper molecular annotation by comparison with the custom LC-MS/MS database. The combination of technologies with custom sample preparation strategies and in-house developed software provide a unique matrix of tools to confirm the identity of species we visualize with IMS.

Novel Aspect

A three-part structural characterization platform for lipids using a variety of MS technologies

Croslow, Seth

Profiling of Cruentaren A-Treated Human-Derived Breast Cancer Organoids Using High Spatial Resolution MALDI-2 TIMS Mass Spectrometry

Seth W. Croslow*1, Chitra Subramanian2, Bhargav A. Patel3, Brian S. J. Blagg3, Mark S. Cohen2, Jonathan V. Sweedler1

1. Department of Chemistry and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, USA., 2.Biomedical and Translational Sciences, Carle Illinois College of Medicine at the University of Illinois at Urbana-Champaign, Urbana, IL, USA., 3. Department of Chemistry and Biochemistry, The University of Notre Dame, Notre Dame, IN, USA

Introduction

Patient-derived breast tumor organoids (BTUs) provide a more accurate representation of in vivo tumors compared to traditional cell lines, as they preserve the three-dimensional structure and complex phenotypic and genetic features. Because of this, BTUs are a valuable model system for therapeutic studies that enable testing of clinical responses and the evaluation of treatment efficacy in a patient-specific context. BTUs were treated with Cruentaren A, a natural product, that targets F0F1 ATP synthase activity to understand the metabolic and lipidomic changes that occur during drug treatment.

Methods

BTUs were grown in Matrigel in appropriate growth medium. The organoids were dissociated and washed with 1xDPBS twice and then embedded in 15% gelatin in dry ice ethanol bath. The embedded organoids were then cryosectioned at 10 µm thickness and thaw mounted onto conductive ITO glass slides. These slides were then sublimated with one of three matrices (1,5-diaminonapthlene, α-Cyano-4-Hydroxycinnamic Acid, or 9-aminoacridine) and analyzed in either positive or negative mode on a Bruker timsTOF FleX MALDI-2. Organoids were imaged using both MALDI and MALDI-2 with a 10 µs delay time and additional runs were performed with TIMS for separation of isobaric species. Organoids were imaged at 10 µm lateral resolution with a mass range of m/z 100 – 1,600.

Results

Here, we utilize matrix-assisted laser desorption ionization with laser post-ionization trapped ion mobility spectroscopy mass spectrometry imaging (MALDI-2 TIMS MSI) to profile the lipidomic and metabolomic response of patient-derived breast tumor organoids (BTUs) treated with Cruentaren A. MALDI-2 was shown to enhance detection of a wide variety of lipid species in positive mode—including monoacylglycerols, triacylglycerols, and phosphatidylinositols—and enabled more accurate putative assignments based on MALDI-2 response. MALDI-2 MSI enables the spatial distribution of various lipids to be determined, and several species were shown to be specifically localized to a single zone within the organoid 3D structure including sphingomyelin (necrotic), phosphatidylinositol (quiescent), and phosphatidylserine (proliferative), while other features were more ubiquitous and present evenly throughout the organoid structure (di- and triacylglycerol species). Treatment with CA lead to a downregulation of numerous phosphatidylcholine species as well as upregulation of phosphatidic acid species. Overall, this work leverages entire power of the timsTOF FleX MALDI-2 providing high spatial resolution measurements covering metabolites and lipids in both positive and negative mode with isobaric separation via TIMS, enabling effective MALDI MS profiling of organoids.

Novel Aspect

Using MALDI-2 TIMS MS at 10 µm resolution, we show the lipidomic and metabolomic alterations in Cruentaren A-treated, patient-derived breast cancer organoids.

Drake, Richard

Expansion of the N-glycome human atlas of normal and tumor tissues defined by MALDI mass spectrometry imaging: Now with more brains and kidneys!

Richard R Drake*1, Aaron Angerstein1, Caroline Kittrell1, Lyndsay EA Young1, Elizabeth N Wallace1, Grace Grimsley1, Sabine Hombach-Klonisch2, Thomas Klonisch2

1. Medical University of South Carolina, 2. University of Manitoba

Introduction

Our group recently reported an initial human N-glycome tissue atlas for fifteen normal and tumor tissue types, derived from multiple N-glycan MALDI-MSI workflows. N-glycan similarities and differences were determined across all normal and tumor tissues, by structural type, branching, and overall fucosylation and sialylation. There are still many organs to include, especially brain. Characterization of N-glycosylation of specialized cell clusters within different organs were also not included. Herein, we will present a basic N-glycome of cerebral cortex tissues, as well as two brain tumor types, glioblastoma and oligodendroglioma. Also, the specific N-glycome of kidney glomeruli and tubules is presented, contrasted with heterogeneous patterns of kidney tumor N-glycans associated with clear cell renal carcinomas.

Methods

Established MALDI-MSI workflows on a Bruker timsTOF fleX and N-glycan databases were used as described in Wallace et al. Sci. Rep. 2024,14(1):489, for representative full tissues and multi-tumor/nomal tissue microarrays for fifteen organs: bladder, breast, cervix, colon, esophagus, gastric, kidney, liver, lung, skin, pancreas, prostate, sarcoma, thyroid, uterus. New tissues analyzed included frontal cortex from healthy human donors, as well as primary glioblastoma (GBM) and oligodendroglioma (ODG) tumor tissues. A multi-enzymatic approach allowed for further information on structural composition, using EndoF3 to confirm core fucose and AAXL stabilization chemistry to distinguish sialic acid linkages. Multiple normal kidney tissues and biopsies, as well as twenty one clear cell renal carcinoma tissues were also analyzed.

Results

For brain tissues, a panel of 64 unique N-glycans were identified for comparison across the normal, GBM and ODG tissues. Normal brain cortex has multiple abundant high mannose and bisecting fucosylated N-glycans that are distinct from not only brain tumor tissues, but all other organs evaluated. Multi-fucosylated tetra-antennary N-glycans co-localize with synaptic proteins like synaptophysin. Also notable in normal cortex relative to all other tissues evaluated is the paucity of sialylated N-glycans present, except in areas with blood vessels. The most abundant N-glycans in normal tissues are all decreased or absent in the ODG and GBM tumors. Most notable in GBM tissues was an increase in sialylated N-glycans in the tumor regions compared to ODG and normal samples. These sialic acids are primarily in alpha-2,6 linkages. In normal kidneys, four distinct N-glycan classes were identified that distinguish cortex glomeruli and tubules from medulla. Specific multi-antennary N-glycans associate with the medulla and cortex interface region. Glomeruli have distinct tri- and tetra-antennary N-glycans that when sialylated are in alpha2,3 linkages. Tubules have abundant bisecting bi-,tri- and tetra-antennary structures with 1-4 fucoses. It appears that the antennae number progressively increases along the length of the tubule. The medulla N-glycans are representative of the more common abundant N-glycans found in most tissues. Even though RCC arises in the tubules, the N-glycans detected in the tumor regions across the 21 RCC tissues featured different structural themes, some having glomeruli-like structures, others tubule-like, and some mixed. RCC is known to be one of the most heterogenous tumor types.

Novel Aspect

Normal kidney and frontal cortex tissues have two of the most distinct N-glycomes yet identified. We anticipate identifying other unique tissue N-glycomes using similar approaches.

Dunne, Jaclyn

Multimodal, Multiplexed Approaches to Assess the Spatial Regulation of the Collagen Proteome in the Normal Breast at Risk of Cancer and Triple-Negative Breast Cancer

Jaclyn B. Dunne*1; Heather Jensen-Smith2; Laura Spruill1; Taylor Hulahan1; Mark J. Lim3; Gargey B. Yagnik 3; Kenneth J. Rothschild 3,4; George E. Sandusky5; M.A. Hollingsworth2 Anand S. Mehta1; Richard R. Drake1; Jeffrey Marks7; Harikrishna Nakshatri5; Graham Colditz6; Marvella E. Ford1; Peggi M. Angel1

1Medical University of South Carolina, Charleston, SC; 2University of Nebraska Medical Center, Omaha, NE; 3AmberGen Inc, Billerica, MA; 4Boston University, Department of Physics and Photonics Center, Boston, MA; 5Indiana University, Indianapolis, IN; 6Washington University in Saint Louis, St. Louis, MO; 7Duke University, Durham, NC

Introduction

Black women have a 40% higher mortality rate in breast cancer (BC) compared to White women and are diagnosed with triple-negative breast cancer (TNBC), the most aggressive form of BC, at nearly twice the incidence. Within the breast microenvironment emergent data shows that stromal collagen plays a significant role in aggressiveness of breast cancer linked to ancestral traits. However, the molecular composition of collagen stroma has yet to be linked to ancestry and aggressiveness of breast cancer. In this study, we use multiplexed, multiomic approaches to characterize the cellular and extracellular microenvironment that influences cancer stage and survival status using a large cohort of ancestry-defined TNBC lumpectomy tissue samples and investigate ECM in normal breast tissues at risk of BC.

Methods

Formalin-fixed, paraffin-embedded (FFPE) sections of normal breast tissue and triple-negative breast cancer tumors underwent hematoxylin and eosin (H&E) staining and second harmonic generation (SHG) imaging using an Olympus FVMPE-RS microscope. H&E stained TNBC tissues were evaluated by a surgical pathologist for tumor annotations. Collagen fiber characteristics including length, width, curvature, alignment, and orientation were quantified in individual SHG images. Established methods were used to analyze collagen by MALDI-MSI on a serial section. These tissues were dewaxed, deglycosylated with PNGase F, and digested with collagenase type III. For TNBC tissues, a third serial section was used for Miralys Photocleavable Mass-Tag Tissue imaging (AmberGen, MALDI-IHC) using a panel of 33 antibodies. MALDI imaging was performed using a MALDI QTOF (timsTOF flex, Bruker).

Results

The purpose of this study was to evaluate the microenvironment within ancestry-defined normal breast and TNBC to understand spatial regulation of collagen stroma that may link to ancestral differences in cancer. Normal breast tissues were obtained from the Susan G. Komen Tissue Bank, African American (AA; n=20) and European American (EA; n=20) samples were annotated with risk scores, age, Body Mass Index (BMI), Breast Imaging-Reporting and Data System (BI-RADS), continental ancestry, and family history. TNBC tissues (n=80) from BW with extensive clinical annotations including age, BMI, stage, metastasis, and survival were evaluated in comparison to 20+ TNBC tissues from WW. In the normal breast cohort, evaluation of BMI (overweight and obese) within ancestry groups showed significant but opposing differences in fiber width measurements. As BMI increased, AA showed statistically thicker fibers while EA showed statistically thinner fibers. At the protein level, two types of collagens were significantly higher in EA and one type was higher in AA. Within the TNBC cohort, collagen fiber length and width measurements by SHG were significantly shorter and thinner in stage II compared to stage I, but no other stage comparisons were significant. Initial analyses showed that collagen peptide intensities vary by TNBC stage (75 peptides, ANOVA test FDR p-value < 0.05). In women who survived, 90 ECM peptides significantly correlated with collagen fiber widths, indicating a relationship between collagen's physical and molecular properties within a defined region. Seven immune markers defined differences in survival when grouped by high vs low expression within tumor regions. Current work focuses on collagen peptide post-translational modifications that may differ in TNBC patients by vital status and are detected in normal breast at risk of cancer. These findings support that molecular composition in breast stroma contributes to aggressive ancestral traits of breast cancer risk and triple-negative breast cancer.

Novel Aspect

This study uses multiplexed, multiomic approaches to comprehensively analyze the ECM in ancestry-defined normal breast and the combined cellular and extracellular microenvironment in TNBC tissues.

Duong, Thao

Alterations in Neuropeptide Distribution in Stomatogastric Nervous System of Blue Crab Callinectes sapidus Under Hypoxia

Thao Duong1, Penghsuan Huang1, Ashley Phetsanthad1, Vu Ngoc Huong Tran2, Lingjun Li1,2

1. Department of Chemistry, University of Wisconsin-Madison, 2. School of Pharmacy, University of Wisconsin-Madison"

Introduction

The arrangement and composition of neuromodulators in the crustacean stomatogastric nervous system (STNS) offer crucial insights into neuropeptide functions in a central pattern generator circuit. However, a comprehensive profiling and mapping of neuropeptides in the esophageal ganglion (OG) and the stomatogastric ganglion (STG) has been constrained by the limitations in the resolving power and sensitivity of the available instrumentation, particularly due to their diminutive sizes and low neuropeptide abundance. Combining tandem mass analysis of analytes in concentrated tissue extract and ion mobility separation can thus improve identification in matrix-assisted laser desorption/ionization (MALDI) imaging. Here, we aim to leverage advanced mass spectrometry techniques to identify and localize the neuropeptidome change in the OG and STG tissues in response to hypoxia.

Methods

Blue crabs C. sapidus were dissected in chilled (10□C) physiological saline to collect intact OG and STG tissues. To facilitate penetration and incorporation of the matrix for higher ionization efficiency, STGs and OGs were de-sheathed, washed, transferred to ITO slides, and applied DHB matrix. For spotting analysis, tissues were homogenized in chilled acidified methanol before neuropeptide extraction and desalted with C18 Ziptips. Crabs were exposed to control (100% oxygen) and hypoxic environments (10%-50% oxygen) by purging nitrogen gas into tanks.

Mass spectrometry imaging was performed on a Bruker TimsTOF Flex. Data were analyzed with SCiLS lab software and neuropeptide identifications were determined using an in-house R script for accurate mass matching (AMM) with 20 ppm error tolerance, and tandem fragmentation.

Results

The washing conditions were examined using Carnoy's solution, 50% ethanol, ammonium acetate, or formalin, employing pairs of pericardial organs (PO) as surrogate neuropeptide-rich tissues. Mass spectrometry characterization of endogenous neuropeptides in the central pattern generator was performed. A preliminary analysis led to a detection of around 40 neuropeptides in each sample, with more than 50% overlap between the 3 studied conditions. Imaging results from the control tissue yielded 35 putative identifications and direct tissue analysis provided 86 matches. Comparison of ion images of ions in the tissues between different conditions was provided qualititavely. In general, accurate mass matching alone is limited in its ability to distinguish coeluting neuropeptide ions. Hence, crustacean tissue extracts containing neuropeptide extracts from 6 pooled tissues were analyzed where the identification of some neuropeptides could be confirmed by in situ fragmentation patterns and presence in the LC-MS/MS spectra. Examples of ubiquitous neuropeptides include the [Ala13]-orcokinin NFDEIDRSGFGFA (m/z 1474.66) the orcokinin NFDEIDRSSFamide (m/z 1228.6), and the RFamide-like myosuppressin pQDLDHVFLRFamide (m/z 1271.66). However, a considerable number of RFamide neuropeptides that are key players in handling hypoxic stress in crabs share the same m/z values with one another. In this case, unambiguous and comprehensive identification of neuropeptides has been shown to benefit from coupling ion mobility spectrometry and multimodal mass spectrometry. In ESI, a simultaneous down regulation of orcokinin neuropeptides and upregulation of allatostatin B (AST-B) neuropeptides in the OG under severe hypoxic stress, while slightly upregulated under moderate hypoxia. Changes in the STG varied among neuropeptides from 8 families. RFamide and pyrokinin neuropeptides were upregulated under both conditions, though there were a few exceptions. Future work aiming at improving spatial resolution and signal intensities while limiting diffusion will benefit the localization of neuropeptides in these thin tissues.

Novel Aspect

Characterizing neuropeptide distribution and abundance changes in crustacean small tissues under hypoxia stress via MALDI ionization and ion mobility separation.

Ebbini, Malik

On-tissue Chemical Derivatization for Mass Spectrometry Imaging of Fatty Acids and Other Carboxyl-Containing Metabolites with Enhanced Detection Sensitivity

Malik Ebbini*1, Hua Zhang1, Kelly Lu2, Lingjun Li 1,2

1. School of Pharmacy, University of Wisconsin-Madison, Madison, WI, 2. Department of Chemistry, University of Wisconsin-Madison, Madison, WI

Introduction

Fatty acids (FAs) perform many biological functions, such as serving as the "building blocks" of numerous lipids, energy fueling, cell signaling, etc. There is evidence that dysregulation of FA metabolism is associated with diseases such as Alzheimer's disease (AD). Clearly, elucidation of the roles of FAs in such diseases is in great demand. While MS imaging can give spatial distribution of FAs in tissue sample and thus provide insight into FA function, FAs are not readily detectable in positive ion mode together with other metabolites like phosphatidylcholines (PCs). To address this limitation, on-tissue chemical derivatization targeting the carboxyl from the FAs could convert the polarity of FAs and make them readily be detected together with other metabolites in positive mode.

Methods

A cryostat (ThermoFisher Scientific, San Jose, CA, USA) was used to section both wild-type (WT) and APP/PS1 mouse brain tissue at the same depth. The resulting tissue sections were mounted onto ITO slides and stored at -80 °C until MALDI analysis. For MALDI MS imaging, Girard's Reagent T (GT, 10 mM) and 2-chloro-1-methylpyridinium iodide (CMPI, 10 mM) were premixed and sprayed onto the WT and APP/PS1 tissue sections. The tissue sections were incubated in triethylamine vapor for 1 hour, with 10% triethylamine in 70% acetonitrile being used for the vapor incubation. Then, CHCA matrix (5 mg/mL) was sprayed onto the tissue sections prior to MALDI MSI using a Bruker rapifleX MALDI Tissuetyper TOF/TOF mass spectrometer (Bruker Scientific, LLC, Bremen, Germany).

Results

It was observed that there was depletion of GT labeled oleic acid in the APP/PS1 corpus callosum, which was consistently seen across 3 technical replicates. This observation has biological relevance, as free oleic acid is known to synthesize myelin phospholipids within the corpus callosum. If free oleic acid is missing in the APP/PS1 corpus callosum, some important myelin phospholipids cannot be synthesized, thus harming myelin function. Literature evidence has shown that myelin function plays a crucial role in memory consolidation and cognition. Consequently, our observation is relevant to AD.

One advantage of the GT method is its ability to detect both FAs and PCs in the same imaging run. Multiple PC images were obtained in the same imaging run, including an image for PC 36:1. PC 36:1 is a myelin phospholipid species that contains oleic acid. Previous literature has found that PC 36:1 is downregulated in the corpus callosum of a multiple sclerosis mouse model. It was of interest to see if that same trend is present in our PC 36:1 data. We did not observe depletion of PC 36:1 in the APP/PS1 corpus callosum, and this was consistently seen across 3 technical replicates.

Our previous study demonstrates that peracetic acid (PAA) can be used for epoxidation of double bonds in positional FA isomers, which would facilitate the elucidation of the position information for double bond. However, that was done in negative mode. With combination of the PAA epoxidation and the GT labeling, both the epoxy-FAs and the epoxy-PCs could be detected under positive ion mode. Then, the isomers of FAs and PCs could be detected simultaneously. This would apply to GT labeled epoxy oleic acid and epoxy PC 36:1. Further, lipid extracts from the mouse brain tissue would subject to LC-MS/MS to confirm the chemical identifications in MALDI-MS imaging.

Novel Aspect

Enhanced sensitivity enabled by on-tissue chemical derivatization for MS imaging of fatty acids and other carboxyl-containing metabolites.

Ferri-Borgogno, Sammy

Using Multiplexed Multi-Omics to Study Spatial Heterogeneity in Ovarian Cancer

Sammy Ferri-Borgogno 1, Jared K. Burks 2, Erin H. Seeley 3, Trevor D. McKee 4, Danielle L. Stolley 2, Akshay V. Basi 2, Javier A. Gomez 2, Basant T. Gamal 1, Shamini Ayyadhury 4, Barrett C. Lawson 5 and Samuel C. Mok 1

1. Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; 2 Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; 3 Department of Chemistry, The University of Texas at Austin, Austin, TX 78712, USA; 4 Pathomics, Inc., Toronto, ON, Canada, M4C3K2; 5 Department of Anatomical Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA.

Introduction

The complex ecosystem of a solid tumor is composed of tumor cells, immune cells, stromal cells, fibroblasts, extracellular matrix, blood vessels, and microbiota, which constitute the tumor–immune microenvironment (TIME). Dynamic and bidirectional interactions occur among various cell types through cell–cell interaction or communication signals such as secreted proteins, metabolites, and microvesicles, to modulate the malignant phenotype of tumor cells so that they can survive, proliferate, and modulate therapeutic efficacy in the oxygen- and nutrient-limiting TIME. Most platforms used for the molecular reconstruction of the TIME of a solid tumor fail to explore the spatial context of the 3D space at a single-cell resolution. To address this issue, a pipeline which integrated multiplex spatially resolved multi-omics platforms was developed.

Methods

In this study, we developed an analytical pipeline which integrated 3D spatially re-solved data generated from non-targeted mass spectrometry im-aging (glycans, metabolites, and peptides) and Stereo-seq (spatial transcriptomics) and targeted seq-IF (IHC proteomics). For each tissue block, seventeen 5 μm serial FFPE sections from an 85 μm-thick tissue block were cut and deposited onto slides specific for each platform. The first and the last sections were stained with hematoxylin and eosin (H&E) for histological evaluation. Every third section was used for non-targeted metabolomics, glycan, and tryptic peptide analysis (by mass spectrometry imaging, MSI), targeted proteomics (by multiplexed seqIF, COMET) or non-targeted Stereo-seq (by STOmics) analyses. To generate a 3D atlas, 3 sections per specimen were evaluated for each of the three platforms.

Results

The dataset we are describing here has many dimensions. A 3D piece of tissue was first sectioned and then split across multiple analytical modalities. Those three dimensions were then indexed on a twenty-plex high resolution seq-IF protein immunostaining platform (COMET), which outputs a high resolution (250 nm) sequence of images, exported in a pyramidal ome.tiff format. Added on to those tens of protein markers, we add thousands of mass spectrometry peaks, corresponding to glycans, metabolites and peptides existing within the tissue. And then added to those thousands of spatial and molecular dimensions, we add the approximately 30,000 human genes and additionally more non-nuclear genetic reads (mitochondrial genomes, microbiome genomes). The spatially resolved imaging data in a two- and three-dimensional space demonstrated various cellular neighborhoods in both samples. The collection of spatially resolved analytes in a voxel (3D pixel) across serial sections of the tissue was also demonstrated. Data collected from this analytical pipeline were used to construct spatial 3D maps with single-cell resolution, which revealed cell identity, activation, and energized status. These maps will provide not only insights into the molecular basis of spatial cell heterogeneity in the TIME, but also novel predictive biomarkers and therapeutic targets, which can improve patient survival rates.

Novel Aspect

To our knowledge, this is the first time that 6 different sets of analytes have been detected from 3 different technologies across the z-plane, merged and used to create 3d reconstructions of these types of data.

Franchina, Davide G.

MnM: Co-spatial tissue imaging using MALDI and MIBI

Davide G. Franchina*1, Marc Bosse1, Sam Kimmey2, Michael Angelo1, Sean Bendall1

1.Stanford School of Medicine, Palo Alto, CA, 2. Ionpath, Menlo Park, CA

Introduction

Multiple technologies allow measurement of structures, cellular organization, and molecular properties across tissues. These spatial mapping tools offer an approach to study tissue complexity and heterogeneity in (patho)physiology.However, due to specific platform-to-platform incompatibility (i.e. slide type, tissue preparation and processing), protocols that maximize output from a single tissue section are lacking. Here, we present MnM, a workflow for the sequential imaging of archival material using matrix assisted laser desorption ionization (MALDI) and multiplexed ion beam imaging (MIBI). MnM enables the acquisition of untargeted molecular composition (MALDI) and targeted single-cell phenotype (MIBI) from a single tissue section. This workflow allows for correlation of ions from MALDI and protein expression from MIBI, as well as multivariate analysis to mine the combined dataset.

Methods

FFPE sections from lymphatic tissues were sectioned onto an organic polymer-coated slide(MIBIblue). The tissue was imaged with a MALDI timsTOFflex using a published protocol for N-glycan imaging. The matrix was washed off and sections were stained with a mix of metal-labelled antibodies targeted to immune phenotypic markers. Data was acquired with a MIBIscope and sections were counterstained with H&E.Bilinear interpolation was used for pixel up-sampling of the MALDI dataset to match the MIBI pixel size. Alignment of the two modalities into a shared coordinate system was achieved by registration between MALDI-H&E and MIBI-H&E using the H&E as ground truth. Registered MnM imaging data were combined into a single dataset where each pixel retains bimodal information from N-glycans and MIBI probes.

Results

Here, we show that dual modality MALDI and MIBI (MnM) imaging is possible on the same tissue section, demonstrating the proof of concept of human lymphatic tissues. We found a general reduction in ion intensities in MIBIblue slides compared to a standard glass slide. The number of ionized species and N-glycan annotations were comparable between the two slides, indicating that MIBIblue can be used for N-glycan MALDI imaging. Then, we used MIBI to measure the expression of metal-tagged immune markers across the tissue area previously rastered with MALDI. MIBI imaging data from MALDI-treated regions were comparable to MALDI-free areas, suggesting that laser ablation by MALDI does not affect MIBI acquisition. Our workflow allows for integration of spatial modalities and analysis of N-glycans that colocalize with specific cell types or microanatomical areas as defined by protein expression acquired by MIBI. We show that MnM phenotypic marker-to-ion correlations can be rapidly achieved from different datasets.

Novel Aspect

We present a bimodal imaging strategy enabling mass spectrometry imaging(MALDI), high-definition spatial proteomics(MIBI), and H&E on the same tissue area.

Hapuarachchige, Pubudu Nuwan Perera

Enhanced Reproducibility and Resolution in Mass Spectrometry Imaging: Introducing a Home-Built Pneumatic Press in Plant Tissue Imprinting onto Porous PTFE Sheets

Pubudu Nuwan Perera Hapuarachchige*1, Young-Jin Lee1

1. Iowa State University

Introduction

For over a decade, imprinting plant tissue onto a porous polytetrafluoroethylene (PTFE) sheet has been used as a sample preparation method for mass spectrometry (MS) imaging. The plant tissue is placed on the porous PTFE sheet and then sandwiched between two metal plates to apply uniform pressure. Various devices, such as pliers, a vice, and a hydraulic press, have been used to apply pressure to the metal plates. However, the reproducibility of this technique and the effectiveness of the pressure application methods have not been thoroughly evaluated. One of the primary challenges is the limited control over pressure application parameters. Here, we present a custom-built pneumatic press that addresses these issues and generates high-quality imprinted plant tissue samples.

Methods

The pneumatic press utilized a twin rod double-acting pneumatic cylinder, with a 10 mm bore and 25 mm stroke, operating at 60 psi. The pneumatic cylinder was mounted on an adjustable stage, which allowed fine control of the impact on the PTFE sheet. The performance of the pneumatic press compared with pliers and a vice. Lemna minor fronds were used as the model plant tissue. The porous PTFE sheet was cut to 25 mm × 25 mm. The abaxial side of the L. minor fronds were placed on it and secured by sticking packing tape pressure was applied. The imprinted samples were analyzed using MALDI-MS imaging on a QExactive HF (Thermo) with a MALDI source (Spectroglyph) in negative mode.

Results

One significant limitation of the imprinting techniques reported so far is low spatial resolution due to analyte smearing during the pressure application. The new pneumatic press method we have developed shows almost no analyte smearing according to the microscope and MS images, with clean and high-quality analyte localizations on the frond boundary line and various cellular areas of L. minor, including daughter fronds and mother frond. In contrast, using pliers and a vice results in analyte delocalization with blurry MS images. Furthermore, cross-sections through the porous PTFE imprinted samples reveal that plant metabolites penetrate more than 1 mm in pliers and vice-used samples but less than 0.4 mm in pneumatic press-used samples, keeping most metabolites near the analyzing surface. This is because the pneumatic press is applying pressure for a short period in a controlled manner, limiting the metabolite movement to a short distance in both lateral and depth dimensions, resulting in high metabolite signals with no apparent smearing. In contrast, pressure is applied slowly in the other methods compared to the pneumatic press, often with excessive force to ensure imprinting occurs, allowing the metabolites to spread further than necessary. Several plant metabolite classes were identified, including sugar, sugar derivatives, alkaloids, and flavonoids on imprinted L. minor samples. Notably, there was lower sample-to-sample signal variation in pneumatic press-used samples than in the other two methods. This suggests that the pneumatic press used porous PTFE imprinting sample preparation method has higher reproducibility in MS imaging.

Novel Aspect

For the first time, we demonstrated that the pneumatic press is a better pressure application method for plant tissue imprinting onto a porous PTFE sheet.

Hardesty, William

Current State of Quantitative Drug Imaging Mass Spectrometry in Combination with Orthogonal Imaging Modalities at GSK

William M Hardesty, Fang Xie, Reid Groseclose

Cellular Systems Imaging, GSK, Collegeville, PA

Introduction

The unique ability of IMS to measure the spatial distribution of both unlabeled exogenous and endogenous molecular species simultaneously has driven a growing interest in the pharmaceutical industry to implement this approach as a platform technology for providing unique insight into drug tissue distribution and pharmacological response. Two key factors in the successful implementation of IMS into the pharmaceutical industry have been 1) the development of robust quantitative IMS workflows and 2) integration with other imaging modalities. Here, we provide an overview of the current state of quantitative MALDI IMS drug imaging and its integration with other technologies at GSK and show examples of how this technology is helping to accelerate drug discovery and development.

Methods

Quantitative drug imaging relies on measuring tissues containing a range of precisely known drug concentrations. To achieve this, bulk tissue homogenates of rat liver were aliquoted into 7 or more vials, spiked with drug standards, refrozen, and stacked to generate a mimetic tissue model. The mimetic tissue models were sectioned and prepared for MALDI IMS concurrently and under identical parameters as dosed animal tissues. Post-processing of peaks for alignment, S/N thresholds and LoD/LoB values was performed using an in-house software platform. For Imaging Mass Cytometry (IMC) images, a panel of metal-conjugated antibodies were optimized for compatibility in both frozen and FFPE tissues and applied to serial sections to measure cellular composition and cell type ratios.

Results

In pharmaceutical development, DMPK studies answer fundamental safety and efficacy questions. MALDI imaging of drug and drug related material is routinely utilized to provide robust spatial distribution information within tissues. The general relative drug distribution is, however, often insufficient to answer key mechanistic questions. Absolute quantification of drug is needed to understand drug at target concentrations, develop in silico models, and understand dose-toxicity profiles. In quantitative imaging mass spectrometry, the complex tissue environment of lipids, salts, and numerous proteins and metabolite species, can influence the measured abundance of a target ion. Within GSK, we have developed and implemented a spiked mimetic tissue model to account for these complex parameters on drug ionization within tissues. These mimetic models, spiked with and across a wide variety of drug classes and indications, are routinely utilized to provide a robust, absolute quantitation regression curve. With the need for more specificity and sensitivity for target compounds in our pipeline, our raw quantitative data sets are further aligned, calibrated, and denoised in house to push lower LoB, LoD, and LoQ values, with sub-1 µg/g drug/tissue concentrations routinely performed in small molecule drug analyses. While liver and kidney tissues are often key organs in metabolism, excretion, and toxicity studies, we describe here a variety of target organs with key drug at target questions, including retina, lymph nodes, dorsal root ganglions, skin, and vertebrae. Finally, we present developmental work in parallel imaging using IMC to distinguish and count specific cell populations within the pixels of our MALDI imaging datasets. All studies conducted according to GSK's Policy on the Care, Welfare and Treatment of Lab Animals and reviewed by the Institutional Animal Care and Use Committee at GSK or by the ethical review process at the institution where the work was performed.

Novel Aspect

The current state of robust, routine quantitative imaging mass spectrometry in drug development at GSK

Holbrook, Joseph

Quantitative Mass Spectrometry Imaging of Liposomal Doxorubicin Delivery and Bilayer Fate in Three-Dimensional Tumor Models

Joseph H. Holbrook*1, Arbil Lopez2, Gabrielle E. Kemper2, Jessica K. Lukowski2, William T. Andrews2, Amanda B. Hummon1,2

1. Ohio State Biochemistry Program, The Ohio State University, Columbus, OH, USA, 2. Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, USA

Introduction

Extended release medications rely upon diverse mechanisms to control drug release in the body. Liposomes are a popular drug delivery vehicle. While most studies of liposomal drug delivery focus on drug distribution in tissue, there is a dearth of information on drug metabolism and the fate of the liposomal bilayer. In this work, a fluorescent tag is chemically cross-linked to the outer lipid bilayer of a liposome encapsulating the chemotherapeutic doxorubicin. Upon administration to multicellular tumor mimics, drug penetration, distribution, and metabolism is tracked with matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI). Quantitative MALDI MSI determines the concentration of doxorubicin in treated spheroids, while the fluorescent tag allows for tracking the distribution and degradation of the liposomal bilayer.

Methods

Liposomes were synthesized via the thin-layer method from the lipids 16:0-18:1 PC, 16:0-18:1 PE, 16:0 hexynoyl PE, and cholesterol. The lipid film was dissolved in a warmed solution of doxorubicin in deionized water and sonicated to form nanoscale liposomal bilayers. Click chemistry was performed to link the fluorophore Alexa Fluor 647 to the alkyne group of hexynoyl PE. Spheroids were grown from the HCT 116 human colon carcinoma cell line and dosed with doxorubicin-containing liposomes on day 12 of growth, then harvested at 12, 24, 48, and 72 hours post-dosing. Spheroids were embedded in gelatin or (hydroxypropyl)methyl cellulose-polyvinylpyrrolidone, sectioned, sprayed with 2,5-dihydroxybenzoic acid and imaged on a Bruker timsTOF fleX MALDI-2 with spatial resolution of 20 μm.

Results

Liposomes are among the most popular and successful drug delivery vehicles, and many studies show their promise for extended or delayed drug release. Three-dimensional, cell aggregate “spheroids” recapitulate the nutrient, oxygen, and pH gradients of solid tumors. These tumor mimics offer a promising model with which to investigate liposomal drug penetration, distribution, and metabolism within an avascular tumor. Preliminary results reveal that liposomes can be tagged on the outer bilayer with a fluorophore through copper-based click chemistry. In this work, a novel fluorescent liposome encapsulating doxorubicin is tracked in spheroids via fluorescence imaging. While fluorescence microscopy imaging is used to visualize the penetration and fate of the tagged liposome, doxorubicin and its metabolites are detected at the same wavelength due to their structural similarity. MALDI MSI provides a powerful complementary imaging approach to fluorescence because doxorubicin and its metabolites can be distinguished by their m/z. Preliminary data shows that liposomal doxorubicin exhibits delayed penetration into spheroids. Free doxorubicin fully penetrates spheroids 24 hours after dosing, while spheroids dosed with liposomes containing the same concentration of doxorubicin do not exhibit full drug penetration until 72 hours post-dosing. Doxorubicin metabolites 7-deoxydoxorubicinolone and 7-deoxydoxorubicinone are also detected by MALDI MSI after 48 hours in both free doxorubicin and liposomal doxorubicin conditions. These exciting results, in agreement with overwhelming literature evidence that liposomes can provide a delayed or extended release of encapsulated drug, indicate that MALDI MSI is a powerful tool for examining the penetration, distribution, and metabolism of liposome-encapsulated drugs. Fluorescent imaging and MALDI MSI serve as orthogonal and complementary methods, as MALDI MSI allows for the detection of distinct metabolites of doxorubicin that are detected at the same wavelength in fluorescent imaging.

Novel Aspect

We propose a novel workflow combining complementary MALDI MSI and fluorescence imaging to track liposomal bilayer fate and drug metabolism.

Joignant, Alena

Quantitative sampling by IR-MALDESI indifferent to tissue heterogeneity in multi-organ model

Alena N. Joignant*1, Emily C. Hector2, Morgan M. Ritter Barnes3, Seth W. Kullman3, David C. Muddiman1

1. FTMS Laboratory for Human Health Research, Department of Chemistry, North Carolina State University; 2. Department of Statistics, North Carolina State University; 3. Toxicology Program, Department of Biological Sciences, North Carolina State University

Introduction

Quantitative mass spectrometry imaging (qMSI) provides the spatial localization of known analyte quantities in a biological specimen. While incredibly useful, the chemical and physical complexity of biological samples requires the careful account of matrix effects in qMSI platforms. Infrared matrix assisted laser desorption electrospray ionization (IR-MALDESI) samples completely through a volume of biological sample, enabling the use of a normalization standard sprayed underneath a tissue for qMSI applications. It is not mechanistically understood whether this sampling is quantitative, or whether its quantitative nature is affected by high tissue heterogeneity. Additionally, the mechanism of IR-MALDESI sampling on sucrose-embedded tissue is critical to elucidate for qMSI studies as the method moves away from the standard ice matrix.

Methods

To investigate this, two standards were pneumatically sprayed below and above whole-body zebrafish sections prepared either with an ice matrix or sucrose embedding. The ratios between the two standards were monitored across the fish and underwent statistical analysis. Given quantitative sampling, the ratios should not be affected by tissue heterogeneity, as the two standards would be both sampled and ionized with equal efficiency.

Results

The zebrafish results were benchmarked against a pseudo-homogenous liver sample. The ratios of the standards were comparable across the whole-body zebrafish sample despite high endogenous heterogeneity. There were observable differences between the ice matrix and sucrose-embedded samples, and the ratios of the standards also differed slightly. There is also an influence of tissue thickness to consider as a result of the study. Further, these considerations will inform future qMSI studies by IR-MALDESI and other laser-based sampling mechanisms.

Novel Aspect

These results provide a mechanistic understanding of quantitative sampling by IR-MALDESI, and shows for the first time that IR-MALDESI sampling is indifferent to tissue heterogeneity.

Kanchustambham, Vijaya Lakshmi

Spatially-resolved top-down proteomics of human skin using proteoform imaging mass spectrometry (PiMS)

Vijaya Lakshmi Kanchustambham1, Pei Su1, Tian Xu1, Michael A. Hollas1, Korvell Russel2, Bethany E Perez White2, Michael Caldwell1, Jared O. Kafader1, Neil L. Kelleher1

1The Proteomics Center of Excellence, Chemistry of Life Processes Institute, Northwestern University, 2170 Campus Drive, Evanston, IL 60208, United States of America, 2Dermatology, Feinberg School of Medicine, Northwestern University, 303 E Chicago Avenue, Chicago, IL 60611, United States of America

Introduction

Human skin is a complex organ that plays a vital role in the immune system and serves as the first line of defense against the external environment. Human skin contains a multilayer architecture comprised of many synergizing cell types, primarily keratinocytes and fibroblasts, and various immune cells, melanocytes, adipocytes, and endothelial cells. Proteoform imaging mass spectrometry (PiMS) has enabled the detection of intact proteoforms up to 70 kDa from various types of tissues. Herein, we applied PiMS to map spatially encoded proteoforms from human skin, and skin-derived models, including biopsies, organoids, and isolated primary cells, advancing our understanding of human skin proteoform landscape in deciphering the mechanisms of regenerative capability and immunologic function of human skin.

Methods

Fresh frozen tissue sections of human skin were obtained from human neonatal foreskin. Human epidermal primary single cells were isolated from the foreskin according to established enzymatic digestion protocols. 10 µm cryosections of human neonatal foreskin were used for PiMS imaging, and isolated single cells are plated onto a poly-lysine functionalized glass slide for PiMS analysis. Followed by cold ethanol fixation, PiMS utilizes nano-DESI, a liquid extraction ionization technique coupled to I2MS to obtain mass profiles of tissues and single cells. Data was acquired on Q Exactive Plus Orbitrap (Thermo Fisher Scientific) MS using custom built nano-DESI source and analyzed using I2MS processing software (Proteinaceous) and an in-house developed MATLAB script. Proteoforms were annotated against existing bottom-up proteomics databases.

Results

PiMS imaging experiments were performed on human skin tissue sections with optimized solvent conditions maximizing the extraction and detection of skin proteoforms. The aqueous fraction of the extraction solvent and pre-wash solvents with increasing non-polarity were evaluated to minimize the lipid content of the skin sections. We detected a total of ~1300 proteoforms and annotated over 300 proteoforms at intact mass level. We successfully detected skin-specific keratin family proteins that exist as multiple proteoforms. In addition, we observed histones, apolipoproteins, and S100 family proteins with proposed roles in proinflammatory and keratinocyte differentiation. Next, we performed single-cell PiMS (scPiMS) analysis on human epidermal primary single cells isolated from neonatal foreskin. The scPiMS experiment was performed in a high throughput manner by raster scanning the probe across the glass slide similar to PiMS on tissue sections. We developed a working protocol to drop cast cells transferred in a spatially separated manner from M154 complete media to phosphate buffer saline (PBS). We were able to obtain proteoform profiles of ~1000 cells per day with in-house developed code for single-cell feature identification. Our initial and resulting scPiMS analysis on different layers of human skin is to facilitate high-throughput single-cell proteomic analysis over ~5000 cells at a depth of 1,500–2,500 proteoforms across all cells, in differentiating dominant keratinocytes and fibroblast cell, from minor population of rare, yet functionally important cell types present in skin tissue that have been reported previously, which is potentially of great interest in inflammatory diseases, aging, and differentiation of skin cells for regenerative therapy.

Novel Aspect

Spatial and single-cell proteoform analysis advancing our understanding of skin architectures and the diverse and rare cell types.

Khan, Shazia

Unveiling the Neurosteroids Landscape: 3D Mass Spectrometry Imaging Atlas of the Mouse Brain

Shazia Khan*1, Eylan Yutuc2, Joyce L.W. Yau1, Yuqin Wang2, William J Griffiths2 and Ruth Andrew1

1Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, EH16 4TJ, UK., 2Swansea University Medical School, Swansea, SA2 8PP, UK.

Introduction

Neurosteroids are synthesized locally within the central nervous system and play essential roles in modulating neuronal activity and various brain functions. They can have a wide range of effects, including anxiolytic, sedative, analgesic, and neuroprotective actions. Understanding their distribution in the brain and how they interact with neurotransmitter systems is of significant interest to researchers studying brain function and related disorders. We aim to construct a 3D atlas of a panel of neurosteroids to uncover the complex network of functions of neurosteroids in the mouse brain.

Methods

Mass spectrometry imaging (MSI) is a powerful bioimaging tool that combines mass spectrometry with spatial information to three-dimensional maps of the distribution of molecules within a sample with direct histopathological correlation. Matrix-assisted laser desorption ionisation (MALDI)-MSI was used to create distribution maps of neurosteroids, with 100 and 150 µm spatial resolution, from brains of 2-year-old and 56-days-old male and female C57BL/6 mice. Serial sagittal 10µm cryostat brain sections were collected at around 200 µm intervals across the right hemisphere from cortex to midline. On-tissue chemical derivatisation with Girard-T reagent was applied to enhance the signal sensitivity of detection of neurosteroids containing keto functional groups. MSI data were collected on Bruker-12T-SolariX-Fourier-transform–ion-cyclotron-resonance (FT-ICR)-MS.

Results

Our global aim is to create a web-based 3-D Neurosterol Atlas of postnatal, adult and aged mice of both sexes utilising our developed on tissue derivatisation method and mass spectrometry imaging technology to uncover the complex network of neurosteroids. We selected a panel of 13 neurosteroids which contain ketonic functional group for 3D brain imaging. Our results show endogenous distribution of estrone, androstenedione, 7aOH-DHEA, progesterone, 17aOH-Progesterone/11-deoxycorticosterone, 11-dehydrocorticosterone and corticosterone in 2 years old (male and female at 100um) and 56 days male and female mouse brain discrete regions mainly localised in cortex, hippocampus and cerebellums at 100um and 150um spatial resolution respectively. Z-Stacking of sequential MSI plates allow generation of 3D models. Future work includes MSI data alignment and co-registration with the 20 Allen Mouse Brain Reference Atlas. This will be made publicly available via interactive webpages to allow precise anatomical annotations to search and visualise concentrations of individual neurosteroids in different areas of the mouse brain.

Novel Aspect

This represents the first-ever comprehensive 3D atlas of neurosteroids constructed using MSI. This groundbreaking work will significantly influence the field of neuroscience, particularly in the areas of neurodegeneration, stress response, and cognition etc.

Korte, Claire

Complementary Quantitative and Qualitative Mass Spectrometry Analysis for Environmental Samples

Claire Korte*1, Kendra G. Selby1, Stephanie Shan1, Summy Shrestha1, and Kevin R. Tucker1

1- Southern Illinois University Edwardsville

Introduction

Most mass spectrometry-based environmental analyses are performed via bulk analysis methods including LC-MS and GC-MS. These techniques are vital for identification and quantitation of environmental contaminants; however, they eliminate the heterogeneity of environmental samples through homogenization. Mass spectrometry imaging (MSI) enables the ability to visualize contaminant distribution within a model organism. A disadvantage of MSI is that it typically is unable to perform strict quantitation, which is exacerbated by high sample preparation and instrumentation costs. If employed in a complementary approach, these two techniques can provide a holistic analysis for environmental samples. This study explores the complementary application of quantitative LC-MS/MS and qualitative MSI as an asset to environmental research, highlighting lower-cost sample preparation alternatives.

Methods

Mung beans and earthworms were exposed to herbicides and pharmaceuticals, respectively, under modeled environmental conditions. Each sample was individually flash-frozen and stored at -40°C. LC-MS/MS samples were homogenized, and the contaminants were extracted via QuEChERS. MALDI MSI samples were cryosectioned at -20°C with Type I water mounting media. The 10-20 µm sections were thaw-mounted to ITO-coated glass slides, and matrix was applied using an artist airbrush powered by an air compressor. Qualitative MALDI MSI data was acquired on the Shimadzu MALDI-8020, and images were processed to display detected analytes. Quantitative data was collected via LC-MS/MS on the Shimadzu LCMS-8060NX (or 8050) in MRM mode. Instruments produced complementary datasets that provided a well-rounded understanding of environmental contamination.

Results

Mung beans were grown in the lab while being exposed to environmentally relevant concentrations of the herbicide diuron. Diuron was detected and quantified in homogenized tissue using LC-MS/MS, and small molecules related to herbicide metabolism were imaged within the bean using MALDI MSI from 10 µm sections. In addition to analysis of the whole mature bean, specific anatomical regions at different germination stages were independently quantified and imaged to determine localization and relative quantities of the herbicide. The cotyledon, or embryonic leaf, demonstrated the highest quantity of diuron by LC-MS/MS. Earthworms were kept in the lab exposed to soil that was contaminated with environmentally relevant concentrations of various statins, a class of antilipidemic. In addition to quantitation and imaging of drug and metabolite distribution, this project sought to determine the mechanism of uptake for statins by this non-target organism. Using the complementary approach of LC-MS/MS and MALDI MSI, the biological concentration of the statins, their location, and distribution of various metabolites were determined. MALDI MSI was used to identify dermal absorption and ingestion as different uptake mechanisms, which correlated with structural of the statins. These studies demonstrate a holistic approach to environmental analysis using plant and invertebrate model systems respectively exposed to pharmaceuticals and herbicides as environmental contaminants. The overarching goal of was to demonstrate the utility of complementary MS while minimizing costs in sample preparation to expand its applicability within environmental science. For LC-MS/MS, a quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction was performed, which is a well-established cost-effective technique. For MALDI MSI, several cost-saving measures were taken, including cryosectioning in Type I water and matrix application via artist airbrush. Nonetheless, the analytes of interest were still successfully quantified by LC-MS/MS and related metabolites were imaged via MALDI MSI."

Novel Aspect

LC-MS/MS and MALDI MSI offer a novel, complementary method for model environmental toxicology studies, enhancing data dimensionality and analysis.

Lakkimsetty, Sai Srikanth (Oral)
Unsupervised co-registration of H&E microscopic images and mass spectrometry images (MSI) with neural networks

Sai Srikanth Lakkimsetty*1, Andreas Weber2, Kylie Bemis1, Melanie Föll2,3, Olga Vitek1

1. Northeastern University, 2. University of Freiburg, 3. German Cancer Consortium and German Cancer Research Center

Introduction
Joint analysis of Mass Spectrometry Imaging (MSI) and H&E images has the capabilities to improve performance of clinical applications using MSI alone. However, integrating the two modalities should be prefaced by co-registration to eliminate histological deformities arising from proximal tissue sections, and tissue handling. While there are supervised frameworks such as VoxelMorph, Deep Learning Image Registration framework that capitalize on neural network frameworks, they need prior training and their performance on multi-modal registration is untested. Unsupervised registration techniques do exist but they are not comprehensive enough to handle idiosyncratic attributes of the image pairs such as differing aspect ratios, positioning of the tissue in the image, and the presence of background; and appropriate feature filtering prior to dimensionality reduction.

Methods
We propose MSIregNN, a python package consisting of an improved approach to multi-modal registration using neural networks to perform coregistration. We address the multivariate nature of the MSI by using custom layers and processing pipeline for MSI. The proposed framework imports MSI and optical images into separate processing pipelines and extracts spatial features separately using a combination of convolutional and fully connected layers. The resulting features are then used by the spatial transformers to extract transformation parameters. The usage of neural networks enable us to leverage state-of-the-art optimizers for efficiently arriving at the correct alignment. The approach also provides tools to remove background using masks, visualize features and resample transformed images. Finally, we demonstrate the coregistration performance using various performance metrics (Dice coefficient, Jacobian matrix and image gradient distance) and visual inspection.

Results
The proposed approach consists of separate processing pipelines for MSI and H&E images, thus separately handling the different natures of the images. While conventional coregistration methods require the two modalities to share the spatial dimension size and number of features (channels), the proposed method does not impose such restrictions. Furthermore, the method does not require summarization of MS images using dimensionality reduction methods which can remove rare morphological features in MSI. The spatial transformer layers are implemented for differing numbers of features between MSI and optical images. Overall, the proposed approach is much suited and designed for MSI and optical image modalities and the flexible platform of neural networks enable usage of efficient optimization methods for coregistration. Finally, the package is much easier to install (within a minute) and bypasses the complex multi-hour installation of the current state-of-the-art packages such as MSIreg. (This is because of a dependency of MSIreg, SimpleITK, which needs to be built on the machine.)

Novel Aspect
Contrary to the traditional iterative optimization models, the layers of the neural network encode the coregistration parameters for achieving spatial alignment.

Lakkimsetty, Sai Srikanth (Poster)
What's new in Cardinal v3 for MSI processing and analysis?

Kylie Bemis1, Sai Srikanth Lakkimsetty*1, Olga Vitek1

1. Northeastern University

Introduction
Cardinal v3 is an open-source software package for scalable and reproducible analysis of mass spectrometry imaging (MSI) experiments. Over the past decade, the increasing size and complexity of MSI datasets have posed challenges for computational analysis. In the next decade, new practices such as integration with ion mobility and other imaging modalities such as magnetic resonance imaging (MRI) and microscopy introduce new levels of complexity. In this major update, Cardinal v3 streamlines and simplifies existing workflows, while establishing new infrastructure for analyzing the next generation of MSI experiments.

Methods
As a free and open-source R package, Cardinal v3 provides a powerful yet accessible platform for the full pipeline of MSI analysis, including data import, pre-processing, visualization, machine learning, and statistical inference. Cardinal v3 is hosted by the Bioconductor project for open-source bioinformatics software, enabling easy installation on all major systems (Windows, macOS, and Linux) with minimal external dependencies. Out-of-memory computation enables Cardinal v3 to process and analyze datasets much larger than computer memory using efficient native (C/C++) code, with the option to use parallel processing if the user's system supports it. We provide a collection of case studies and tutorials to show the user how to perform common workflows.

Results
The poster discusses the specific improvements made available in Cardinal v3, specifically to preprocessing methods such as peak picking and alignment. Cardinal v3 updated the existing data structures and introduced new ones for better representing MSI data. The poster also discusses the new spectral processing methods for smoothing images, baseline reduction, alignment, and peak picking. We also present improvements to handling and combining different datasets for analyzing multi-tissue experiments.

Novel Aspect
Cardinal v3 is a major update to a versatile open-source software for MSI analysis that streamlines existing workflows and sets groundwork for supporting new technologies.

Li, Feifei

Comprehensive study of Alzheimer's disease mouse brain regional metabolite and lipid patterns based on desorption electrospray ionization with ion mobility mass spectrometry imaging

Feifei Li1,2*, Sze Ching Lee3, Xueting Zhang2, Xia Min2, Kaiwen He2, Min Lei2 Zonglun Li5

1Department of Biochemistry and molecular biology, Mayo clinic, Rochester,55902, Minnesota, USA. , 2University of Chinese Academy of Sciences, Beijing 100049, China, 3Department of Neurology & Neurosurgery, Mayo clinic, Rochester,55902, Minnesota, USA, 4School of Pharmaceutical Sciences, Tsinghua University, Beijing, 100084, China, 5School of Chemistry and Chemical Engineering, Henan Normal university, Xinxiang, 453007, China.

Introduction

Desorption electrospray ionization (DESI) mass spectrometry imaging (MSI) is a technique that enables imaging at the molecular level without destroying the morphology of the sample. Using this technique, the distribution, tissue structure and physiological processes of chemical components can be analyzed in cells, organs or whole organisms in a manner without special labeling or staining. For example, it can also be used for brain tissue analysis in the field of neuroscience.

Methods

To address the problem that DESI-MSI quadrupole time-of-flight mass spectrometry (Q-TOF) is difficult to obtain secondary mass spectra directly and the data identification accuracy, a combined UHPLC-TWIMS-QTOF MS and DESI-TWIMS-QTOF MS method was developed for the multidimensional lipid composition analysis of 14 different brain sagittal regions olfactory bulb (OB), frontal cortex (FC), occipital cortex (OC), hippocampus (HIP), striatum (ST), preoptic area (PA), thalamus (TM), hypothalamus (HTM), midbrain (MB), cerebellar cortex (CBC), pons (PO), medulla (MD), corpus callosum (CC), cerebellar medulla (CM).

Results

A study of spatial-temporal metabolic differences in the brains of 5- month-old and 10-month-old 5xFAD Alzheimer's disease (AD) mice was carried out based on the DESI-MSI analysis established in both positive and negative ion modes, which revealed complex and regional brain tissue molecular alterations associated with AD. There is accumulating evidence suggesting alterations in various lipid classes, including glycerophospholipids, neutral lipids (such as sterols) and sphingolipids. Moreover, investigation of lipid alterations in the brains of 5xFAD mice have reported decreased sulfatide levels, particularly in brain regions vulnerable to AD pathology, such as the hippocampus and cortex. In addition, ion trickle separation effectively reduces the interference of solvent peaks, overlapping isotope peaks of endogenous molecules extracted from tissues, and isomers, which is essential for obtaining accurate concentration gradients in samples using mass spectrometry imaging. The introduction of CCS (Collision cross section) values for isomer differentiation and multi-charge ion resolution is illustrated with ganglioside components. The results provide a more comprehensive and in-depth understanding of the location of the diverse metabolite and lipid profiles in 5xFAD mouse brain and of their relationship to disease progress.

Novel Aspect

This approach enables the identification of molecular changes in specific brain regions, providing insights into the progression of AD and the potential mechanisms underlying neurodegeneration.

Lu, Kelly

Deciphering the Three-Dimensional Biomolecular Distribution in the Alzheimer's Disease Brain: A Multiomic Approach Integrating Immunohistochemistry with MALDI MS Imaging

Kelly H. Lu*1, Hua Zhang2, Gargey B. Yagnik3, Mark J. Lim3, Kenneth J. Rothschild3,4, Andrew J. Schneider,5,6, Luigi Puglielli5,6, and Lingjun Li1,2

1. Department of Chemistry, University of Wisconsin-Madison, WI, 53706, USA, 2. School of Pharmacy, University of Wisconsin-Madison, WI, 53705, USA, 3. AmberGen Inc., MA 01821, USA, 4. Department of Physics and Photonics Center, Boston University, Boston, MA 02215, USA, 5. Department of Medicine, University of Wisconsin-Madison, Madison, WI 53705, USA, 6. Waisman Center, University of Wisconsin-Madison, Madison, WI 53705, USA

Introduction

Alzheimer's Disease (AD) is the most prevalent form of dementia, affecting over fifty-five million people globally. Using mouse brain as a model, we present a three-dimensional multiomic approach to decipher the spatial localization of biomolecules by integrating immunohistochemistry and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-IHC MSI). Serial imaging experiments were performed on the same tissue slice where the small molecules were imaged in a label-free manner. Subsequently, proteins were labeled with novel photocleavable mass tag (PC-MT) antibody probes for targeted MALDI-IHC, utilizing a unique polypeptide sequence as a reporter ion to spatially image each protein target. The collective information provides comprehensive insights into potential biomarkers for AD progression.

Methods

Frozen, 10 µm coronal sections of C57BL6/J mouse brain tissue were acquired from five coronal planes, each separated by 2000 µm. Untargeted small molecule MSI was performed on a Bruker TimsTOF flex MS under positive mode at a resolution of 30 µm. The ions were identified via MALDI-MS/MS and LC-MS data matching. The slide was washed, fixed with formaldehyde, rehydrated prior to antigen retrieval, and subsequently incubated overnight with PC-MT probes (2 µg/mL). After excess probe removal and vacuum drying of the slide, remaining probes were photocleaved (365 nm). CHCA matrix was applied and subjected to targeted protein MSI under positive mode with 30 µm resolution. The MS images from different depths were aligned to illustrate an overall molecular distribution.

Results

In untargeted MSI, over 100 small molecules, including lipids and metabolites, were identified, revealing distinctive localization patterns at different coronal depths (D) between the wildtype (WT) and AD mice. Notably, the AD sample exhibited higher intensities of phosphatidylcholine (PC 18:0_18:0) (m/z 790.6264) from D3 to D5, particularly in the fiber tracts, midbrain, hypothalamus, and striatum-like amygdala nuclei. The cortex, thalamus, and retro-splenial areas showed elevated signals of PC (18:2_20:4) (m/z 806.5739) at D2 and D3. An increased signal was specifically found in AD D1 for PC (36:3) (m/z 784.5695) and PC (16:1_20:3) (m/z 782.5715). However, other lipids such as PC (40:6) (m/z 834.6003), PC (40:7) (m/z 832.5893), and PC (36:1) (m/z 788.6208) revealed no significant differences between the WT and AD sample across different depths. Interestingly, species like Ceramide (Cer 40:2;2O) (m/z 620.5995) and PC (PC O-34:1) (m/z 746.6038) were exclusively found in the WT sample, while PC (32:0) (m/z 757.5584), PC (32:1) (m/z 771.5146), and PC (36.6) (m/z 796.5266) were only found in the AD sample. Ten neurology-related proteins including phospho-tau and amyloid-beta 42, etc. were targeted by MALDI-IHC. In AD samples, the phospho-tau observed across the hippocampal to the cerebral cortex and brain stem at D4, while the amyloid-beta 42 distribution appeared concentrated in the hippocampal, midbrain, and the hindbrain region at D5. Other prominent changes including the localization of glial fibrillary acidic protein (GFAP) (reporter ion: m/z 1011.55) at D5 shifted from the center of the hippocampal region in WT to its boundary in AD, and the neuronal nuclear protein (NeuN) (reporter ion: m/z 1308.71) exhibited no brain region-specific pattern in WT but showed a clear outline of the pyramidal layer of the hippocampus in AD D5. These findings suggest that dysregulated proteins in the posterior brain regions may be relevant with AD progression.

Novel Aspect

Integration of lipid and targeted protein imaging using MALDI, capturing a three-dimensional multiomic molecular distribution within the AD brain.

Lu, Wenyun

Acidic Methanol Treatment Facilitates MALDI-Mass Spectrometry Imaging of Energy Metabolism

Wenyun Lu*, Noel R. Park, Tara TeSlaa, Connor S.R. Jankowski, Joshua D. Rabinowitz, and Shawn M. Davidson

Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey 08544, United States

Introduction

Detection of small molecule metabolites (SMM), particularly those involved in energy metabolism using MALDI-mass spectrometry imaging (MSI), is challenging due to factors including ion suppression. Ion suppression during MALDI occurs from a combination of other analytes, including proteins, matrix compounds, salts, and analytes with high ionization efficiency (IE) or are present in high abundance. One potential solution to enhance SMM detection is to remove analytes that cause ion suppression from tissue sections before matrix deposition through solvent washes. Here, we systematically investigated solvent treatment conditions to improve SMM signal and preserve metabolite localization.

Methods

Serial sections of mouse tissues at a thickness of 10 µm were acquired using a cryostat and thaw-mounted on indium tin oxide (ITO)-coated glass slides. Tissue slides were desiccated under vacuum for 10 min and underwent solvent wash before matrix application, which involves placing the tissue slide on an incline and pipetting solvents (5 ml) over the tissue surface. The slide was desiccated again for 5 min before matrix application. MALDI image runs were performed on a solariX XR FT-ICR mass spectrometer with a 9.4 T magnet. Data were analyzed using IsoScope. The metabolites were identified using high-resolution accurate mass with a ppm window of 10 ppm compared to an in-house metabolite list established on liquid chromatography-mass spectrometry (LC–MS) using authenticated standards.

Results

Washing with acidic methanol significantly enhances the detection of phosphate-containing metabolites involved in energy metabolism. The improved detection is due to removing lipids and highly polar metabolites that cause ion suppression and denaturing proteins that release bound phosphate-containing metabolites. Stable isotope infusions of [13C6]nicotinamide coupled to MALDI-MSI (“Iso-imaging”) in the kidney reveal patterns that indicate blood vessels, medulla, outer stripe, and cortex. We also observed different ATP:ADP raw signals across mouse kidney regions, consistent with regional differences in glucose metabolism favoring either gluconeogenesis or glycolysis. In mouse muscle, Iso-imaging using [13C6]glucose shows high glycolytic flux from infused circulating glucose in type 1 and 2a fibers (soleus) and relatively lower glycolytic flux in type 2b fiber type (gastrocnemius). Thus, improved detection of phosphate-containing metabolites due to acidic methanol treatment combined with isotope tracing provides an improved way to probe energy metabolism with spatial resolution in vivo.

Novel Aspect

We developed a washing strategy to significantly improve the detection of phosphate metabolites for MALDI-MSI

Luu, Gordon

prmImaging: an integrated workflow for the analysis and interpretation of spatial on-tissue tandem mass spectrometry of lipids

Nannan Tao1, Bram Heijs2, Tobias Boskamp2, Soeren-Oliver Deininger2, Nikolas Kessler2, Arne Fütterer2, Arne Behrens2, Corinna Henkel2, Nadine T. Smit2, Katherine Stumpo3, Gordon T. Luu3

1. Bruker Daltonics Inc., Billerica, MA, 2. Bruker Daltonics GmbH & Co. KG, Bremen, Germany, 3. Bruker Scientific, LLC, Billerica, MA

Introduction

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MS imaging) offers highly detailed spatial characterization of tissue samples and can cover a various analytes at single-cell resolution. However, assigning molecular identities to features (i.e. m/z or combinations of m/z and collisional cross section; CCS) with high confidence remains a challenging task. While several automated data acquisition and annotation approaches exist, they all rely on "profiling" tandem MS data in which the spatial relationship between precursor ions and their fragments is severely reduced or non-existent. Here, we provide a prototypical workflow that integrates CCS-enabled MALDI-MS imaging (MS1 data) of lipids with prmImaging (MS/MS data) via computation tools to reveal meaningful results from the data.

Methods

CCS-aware MALDI imaging was performed on a 10µm thick cryosection from a fresh-frozen mouse kidney on the Bruker timsTOF fleX. Post acquisition, the MS1 data was imported into a prototypical version of SCiLS Lab. T-Rex^3 Feature Finding and automated CCS feature extraction were performed during the import. A list of up to 25 features was curated based on their abundance in key morphological areas, and then exported as a precursor list for import into timsControl for prmImaging (MS/MS) acquisition. The MS/MS data were imported into the prototypical version of SCiLS Lab and merged with the MS1 precursor ion images. Using SCiLS Lab and MetaboScape Lipid Species annotation, MS/MS spectra were elucidated, and various fragment images were studied.

Results

CCS-aware spatial lipidomics data were obtained by MALDI imaging on a Bruker timsTOF fleX MALDI-2 instrument with microGRID. The data was imported into SCiLS Lab, and a digital scan of the hematoxylin and eosin (H&E) stained consecutive tissue was acquired. Based on the H&E image and several CCS features, regions of interest for key morphological features of the kidney were created, including glomeruli and the proximal tubuli (S3 segment). Using the colocalization tool in SCiLS Lab, the features that were most strongly correlated with these regions were selected and automatically exported as a precursor list for prmImaging acquisition. The MS/MS acquisition was performed over the same tissue area as the MS1 analysis, resulting in a shared coordinate system. Following the prmImaging acquisition, the data were imported into SCiLS Lab and the MS1 ion images of the precursor features were merged with the dataset using the SCiLS Lab Ion Image Mapper. For every precursor ion, an MS/MS spectrum was generated ans submitted to the Molecular Annotation workflow making use of the MetaboScape Lipid Species annotation tool, which enables us to identify the majority of the selected precursor features with high confidence based on mass tolerance, isotope pattern quality, CCS tolerance and fragmentation pattern. Using the computational tools in SCiLS Lab, the correlations between different fragment ions were also calculated, and the fractional contribution images were generated to assess the spatial correlation between the different fragments and the precursor ions. This prototypical workflow allows for a complete and comprehensive analysis of spatial tandem mass spectrometry data in an intuitive and integrated manner to enable users to turn their raw mass spectrometry data into meaningful (bio)chemical insights.

Novel Aspect

This work presents a fully integrated solution for tandem mass spectrometry imaging of lipids, including: precursor selection, data acquisition, and data interrogation.

Macdonald, Jade

Optimizing Liquid Chromatography Trapped Ion Mobility Spectrometry Tandem Mass Spectrometry Reference Libraries for Improved MALDI-MSI Collagenase Peptide Identification

Jade K Macdonald*1, Stephen C Zambrzycki1, Montana Quick2, Richard R Drake1, Peggi M Angel1

1. Medical University of South Carolina, Charleston, SC, 29425, 2. Bruker Scientific, Billerica, MA, 01821

Introduction

The extracellular matrix (ECM) is a heavily post-translationally modified, collagenous, insoluble scaffold that plays a major role in cell signaling as well as disease progression. The very properties that give the ECM structure and function make it difficult to target analytically and are the cause of undersampling in MS imaging studies. Digestion by collagenase, a protease that recognizes ECM structures, is a recent advancement in targeting the ECM in tissues for MALDI mass spectrometry imaging (MSI). Like all proteases, identification of collagenase-generated peptides for MALDI-MSI remains a challenge. Here, we examine differences between tryptic and collagenase peptides to optimize trapped ion mobility spectrometry (TIMS) for increased detection of collagenase-generated peptides and improved MALDI-MSI peptide identification.

Methods

Experiments were performed on a nanoElute2 UHPLC with timsTOF fleX (Bruker Daltonics). Two hundred nanograms of Pierce™ HeLa Protein Digest Standard or commercial rat tail collagen solution digested with collagenase or trypsin was subjected to reverse-phase liquid chromatography (PepSep™ Xtreme C18 column, 41-minute 3-35% ACN gradient) followed by a standard TIMS-MS/MS workflow. Molecular weight, charge, and retention time of peptide readouts were compared across sample type. Mobility parameters were tested to optimize mobility ranges and to construct a mobility exclusion polygon that maximizes detection of unique peptides without compromising overall sensitivity.

Results

Collagen peptides have altered biophysical properties when compared to tryptic cellular protein digests. Specialized workflows are needed to better improve identifications in imaging experiments. Here we compare biophysical properties of tryptic cellular protein digest (t-CPD) peptides, trypsin-generated rat tail collagen solution peptides (t-RTCS), and collagenase-generated rat tail collagen solution (c-RTCS) peptides. t-CPD peptides had longer retention times (1419 ± 510 s) and lower average molecular weight (1460 ± 467 Da), when compared to t-RTCS (931 ± 314 s, 1681 ± 477 Da) or c-RTCS (924 ± 353 s, 1640 ± 450 Da). The charge distribution of t-CPD peptides was +1 (0.01%), +2 (72.9%), +3 (25.6%), +4 (1.5%) compared to t-RTCS (0.2%, 45.5%, 41.9%, 12.3%) or c-RTCS (0.7%, 49.1%, 39.3%, 10.9%). Trapped ion mobility spectrometry (TIMS) range and exclusion polygons were tested by evaluating t-CPD peptide readouts. A narrow (0.85-1.45 1/K0) TIMS range (nTMS) detected greater total peptides (45,280 ± 320 peptides) with fewer low molecular weight peptides (1,000 ± 150 peptides < 1000 Da) and a lower proportion of singly charged peptides (1.6%) compared to broad (0.45-1.85 1/K0) TIMS range (bTIMS) (36,140 ± 290, 3,740 ± 120, 12.4%). A high mobility polygon (HMP) resulted in more detectable peptides (35,400 ± 430 peptides) with more low molecular weight species (4,020 ± 150 peptides) and a greater proportion of singly charged species (15.6%) compared to a low mobility polygon (LMP) species (33,280 ± 390, 1,820 ± 110, 0.02%). c-RTCS reference libraries were generated from mobility experiments and evaluated for c-RTCS MALDI-MSI peptide identifications (mass error < 15 ppm). bTIMS+HMP was able to identify the most MALDI-MSI peptides (149) followed by bTIMS (146), bTIMS+LMP (124), and lastly nTIMS (96). This study establishes workflows for unconventional enzymes accessing analytes from the tissue microenvironment. Current work focuses on applying these workflows to FFPE patient tissues.

Novel Aspect

Trapped ion mobility spectrometry tandem mass spectrometry is optimized to boost peptide identification for collagen mass spectrometry imaging proteomics.

Martin, Roy

Multiplexed MALDI-immunohistochemistry Imaging of Intact Proteins in Tissue by Multi Reflecting Time of Flight (MRT)

Mark Towers1; Roy Martin*2; Gabriel Stefan Horkovics-Kovats3; Steven Pringle1; Joanne Ballantyne1; Lisa Reid1 ; Bindesh Shrestha2

1Waters Corporation, Wilmslow, United Kingdom; 2Waters Corporation, Milford, MA; 3Hevesy György PhD School, Eötvös Loránd University, Budapest, Hungary

Introduction

Multiplexed MALDI-immunohistochemistry (IHC) is an advanced mass spectrometry imaging technique used to efficiently map protein localization on tissue. In MALDI-IHC, photo-cleavable peptide tags are attached to antibodies that bind to the target proteins on the tissue. Before MALDI imaging, these tags are released, providing localization information for the original proteins. This approach is particularly beneficial for proteins that are not easily ionized or have a molecular weight beyond the instrument's mass range, which would traditionally require on-tissue digestion. MALDI-IHC has been shown to yield results comparable to traditional fluorescence immunohistochemistry, with the added advantage of superior multiplexing capabilities in a single sample. Here, we demonstrate the compatibility and benefits of this technique using the SELECT SERIES™ MRT mass spectrometer.

Methods

Sections of human tonsil (Ambergen) and human kidney (ccRCC ISUP grade 3) FFPE tissues were prepared following the AmberGen MALDI-HiPLEX-IHC Miralys™ Imaging Laboratory Workflow user guide (Control number v173(J)). The tonsil control sections were purchased pre-stained, while the kidney sections were stained in-house. The samples were analyzed using a SELECT SERIES MRT mass spectrometer with MALDI in positive ionization mode, covering a mass range of 50-2400 Da. Due to the small mass distribution of the tags, a fixed quadrupole setting of 1000 Da was used. The laser repetition rate was set at 2 kHz with a scan speed of 10 scans/sec. Images were acquired with pixel sizes of 50 µm and 20 µm, using laser-focus settings of 4.0 mm and 5.8 mm, respectively.

Results

Here, we demonstrate the full compatibility of MALDI-IHC analysis on a SELECT SERIES MRT mass spectrometer and highlight its potential advantages over a standard orthogonal acceleration MALDI-TOF. The high mass accuracy (>500 ppb) of the SELECT SERIES MRT allows for the unambiguous identification of released tags, which can be easily distinguished from endogenous signals. Additionally, the high mass resolution (>200,000 FWHM) significantly reduces potential signal overlap with endogenous signals of similar mass, providing higher confidence in protein localization compared to traditional immunofluorescence. Traditional immunofluorescence is often limited to 2-5 markers per tissue section due to spectral overlap in excitation and emission wavelengths. In contrast, MALDI-IHC can image more than 40 markers per tissue section, limited only by the number of available masses and tags for all proteins of interest. However, MALDI-IHC detection does take longer than traditional fluorescence methods, and once a tag is consumed, the data cannot be re-acquired. In our analysis, the bio-localization of the tags was consistent with the expected biology of the tissue. Excellent signal strength was achieved with both 50 µm and 20 µm pixel sizes, suggesting that good data could be obtained with even smaller pixel sizes or faster analysis times if desired. This demonstrates the robustness and high resolution of MALDI-IHC, making it a powerful tool for protein imaging in tissue.

Novel Aspect

Multiplexed MALDI IHC performed on a multi-reflecting time of flight mass spectrometer.

Miles, Hannah

Integrating MALDI-MSI and LC-MS/MS for Spatial Localization of Disease Markers in a Model of Benign Prostatic Hyperplasia

Hannah N. Miles1,2,3, Lauren Fields4, William A. Ricke1,2,3,5, Lingjun Li1,4

1School of Pharmacy, 2Department of Urology, 3George M. O'Brien Urology Research Center, 4Department of Chemistry, 5School of Medicine and Public Health, University of Wisconsin-Madison

Introduction

Mass spectrometry imaging (MSI) allows for spatial mapping of multiple analytes, ranging from peptides, metabolites and lipids to post-translational modifications. However, MS/MS confirmation can only be targeted with MALDI-MSI, unlike DDA- and DIA-based approaches utilized in LC-MS/MS, forcing users to do additional LC-MS/MS extractions to obtain MS/MS confirmation. While software exists that allows for data integration of MSI and LC-MS/MS datasets, many are not open-source, requiring researchers to manually merge results from both datasets. This hinders analyses of disease states, such as benign prostatic hyperplasia (BPH), where cellular heterogeneity makes it difficult to determine cell populations contributing to disease progression. Here, we focused on integrating MALDI-MSI and LC-MS/MS data for on-tissue localization of markers contributing to BPH disease progression.

Methods

A hormone-induced mouse model of lower urinary tract dysfunction (LUTD) was used as previously described. At experiment end, mice were euthanized and lower urinary tracts (urethra, prostate lobes and bladder) were collected. Bladder tissues collected from mice were formalin fixed and paraffin embedded (FFPE) prior to sectioning onto ITO-coated slides. Tissues were dewaxed and underwent antigen retrieval followed by tryptic digestion and CHCA matrix application for MALDI-MS analysis using a Bruker RapifleX at a spatial resolution of 25 μm. A serial section was prepared similarly until matrix application, at which point digestion was quenched and peptides were extracted, desalted, and resuspended for LC-MS/MS confirmation using a Thermo Q-Exactive HF mass spectrometer.

Results

For preliminary work, accurate mass matching of MALDI-MSI and MS2 confirmation via LC-MS/MS showed increases in Col1a1 versus control mice, which has shown to be significantly increased in our mouse model and contributes to prostatic fibrosis. We then developed IMSight, an open-source platform designed to automate the merging of MALDI-MSI and LC-MS/MS datasets for confident localization of peptides in an untargeted manner. IMSight further increases the confidence and throughput of MSI workflows through application of automated tissue segmentation and merging with IHC images, providing a detailed understanding of regulatory underpinnings involved in BPH.

Novel Aspect

Integrated MALDI-MSI and LC-MS/MS data processing via IMSight allows further probing into the cellular heterogeneity of understudied benign prostate disease.

Moreno-Pedraza, Abigail

Plasma-based ionization sources for coupled techniques in ambient imaging

Abigail Moreno-Pedraza*1, Robert Winkler 2

1Leibniz Institute of Vegetable and Ornamental Crops (IGZ) e.V., Großbeeren, Germany, 2 CINVESTAV UGA-Langebio, Irapuato, Mexico

Introduction

Plasma-based sources are novel ambient ionization techniques that provide unique insights into biological samples in natural conditions. These sources, which operate externally from the mass spectrometer (MS), have several advantages: they require minimal sample preparation, allow the analysis of a wide range of substances, and can be coupled to additional desorption/ionization sources. Their ability to maintain the integrity of the samples while providing spatial and molecular information highlights their importance. Ambient plasma-based sources are, therefore, advantageous in various fields, including biological and environmental studies.

Methods

Here, we summarize significant developments and applications of plasma ionization for ambient MS imaging experiments. Plasma sources have evolved since their first report, when they were used as the main desorption/ionization probe. Today, plasma sources are frequently coupled to additional desorption systems to increase sensitivity and enhance lateral resolution in imaging analysis. These innovations have proven beneficial across different research fields, offering molecular information under natural conditions. However, despite their advantages, plasma-based ionization sources still need to be represented in the MS imaging community.

Results

The use of plasma-based ionization sources for imaging is limited. Only some groups worldwide have worked with plasma sources for imaging. Therefore, we provide an overview of the notable advances in imaging using plasma-based sources. In 2005, Chip Cody presented 'direct analysis in real-time (DART),' one of the first ambient ionization sources that initiated a revolution in mass spectrometry 1. Several plasma-based sources were published shortly after. In 2010, an imaging system using a low-temperature plasma (LTP) source demonstrated its possibilities in analyzing the composition of colors in delicate pieces of artwork. In 2014, we presented a plug-and-play setup for directly analyzing and imaging macroscopic biological samples, demonstrating the spatial distribution of capsaicin in a Jalapeño chili slice3. In 2016, a proof of principle technique for imaging called nanotip ambient ionization (NAIMS) was presented4. In 2017, DART was coupled to an ultraviolet (UV) laser to generate images; the setup included an ablation chamber that increased the setup's complexity and cost of the setup5. In 2019, we improved our imaging setup, including a continuous UV laser and improving the later resolution with an Open LabBot6,7, achieving images with a lateral resolution of 50 µm. Depending on the biological question, the lateral resolution can be adjusted by tuning the laser desorption. Other popular ionization techniques use plasma-based sources as an extra ionization source, for instance, the atmospheric pressure matrix-assisted laser desorption ionization (AP-MALDI) coupled to an in-line plasma for post-ionization8. The setup increased the ionization efficiency. We want to highlight these examples during the talk to bring interest to plasma-based sources and suggest ideas for future collaborations and developments.

1) https://doi.org/10.1021/ac050162j
2) https://doi.org/10.1002/anie.200906975
3) https://doi.org/10.1016/j.jprot.2014.03.003
4) https://doi.org/10.1021/acs.analchem.6b01212
5) https://doi.org/10.1021/acs.analchem.6b04137
6) https://doi.org/10.1021/acs.analchem.8b04406
7) https://doi.org/10.1016/j.microc.2019.104343
8) https://doi.org/10.1021/acs.analchem.0c03524"

Novel Aspect

We review the use of plasma-based ionization sources for imaging, illustrating their versatility and broad applications.

Na, Sumin

Mass spectrometry imaging of Arabidopsis thaliana with in vivo D2O labeling

Sumin Na1, Young Jin Lee*1

1. Department of Chemistry, Iowa State University, Ames, IA, United States

Introduction

Metabolomics is a critical 'omics' technology that bridges the gap between phenotype and genotype. Current mass spectrometry (MS)-based metabolomics analysis faces two significant challenges. First, the sample preparation process, which involves extracting metabolites from homogenized tissue samples, often results in the loss of spatial metabolite distribution among cells. Second, this approach provides information about metabolite concentrations, but not actual metabolic activities. Mass spectrometry imaging (MSI) and isotope labeling technique have addressed these issues, respectively. However, there has been limited research on integrating MSI with in vivo isotope labeling, referred as MSIi. In this study, we investigated D2O labeling of Arabidopsis thaliana to explore the utility of MSIi.

Methods

Arabidopsis were hydroponically grown in 0.5x Hoagland medium (HM)for day-after-sowing (DAS) 14 or 28, then transferred to 35% D2O with 0.5x HM, and harvested at different time points. For ESI-MS, lipids were extracted using IPA with 0.01% Butylated Hydroxytoluene (BHT), followed by chloroform and H2O (2.5:1, v/v), and then chloroform/methanol (2:1, v/v). Leaves were fractured and attached to a glass slide for MALDI-MSI, and Au was sputtered for measuring chloroplast lipids. The flower and stem were attached to a stainless steel plate using carbon tape, and colloidal silver was sprayed. All mass spectrometry analysis was performed using a Q Exactive HF Orbitrap with a MALDI/ESI dual source (Spectroglyph) in positive mode. Data were processed using XCalibur, MSiReader, MATLAB, and ElemCor.

Results

We explored MSIi of Arabidopsis thaliana with D2O labeling to study and visualize D-labeling in three classes of lipids: arabidopsides, chloroplast lipids, and epicuticular wax. The changes in arabidopsides were monitored over incubation times by harvesting the plants at four intervals (30, 60, 180, and 540 minutes). Similar to other stress responses, D2O-induced stress increased arabidopsides within an hour, but this effect was relatively minor in matured plants and returned to normal levels within a few hours for both arabidopside A and B. Additionally, the D-labeling isotopologue patterns of arabidopsides matched those of galactolipid precursors, supporting the currently accepted biosynthesis mechanism. Matrix-assisted laser desorption/ionization (MALDI)-MSI was used to visualize the spatiotemporal distribution of deuterated chloroplast lipids, including pheophytin a, MGDGs, and DGDGs, in DAS 28 plants grown in D2O for 3-12 days. In the leaf base, pheophytin a showed a D-labeling efficiency of 14% on day 3, increasing to 31% on day 6 and further to 52% on day 12. In contrast, galactolipids had a much lower D-labeling efficiency of 7%-10% on day 3, but this increased to levels similar to pheophytin a by day 6 and beyond. Finally, deuterium incorporation in epicuticular wax was visualized on the surfaces of stems and flowers. The conversion efficiency of newly synthesized C30 aldehyde to C29 ketone was very low in the lower stem but very high at the top of the stem near the flower or on the flower carpel. The conversion ratio of C30 aldehyde to C29 ketone was approximately 20% or less in the lower stem but ranged from 60% to 90% at the top of the stem and on the flower.

Novel Aspect

For the first time, D2O labeling was successfully applied to the MSIi of Arabidopsis thaliana using a hydroponic culture

OKyem, Samuel

Spatial Mapping of D-Amino Acid Containing Peptides via MALDI timsTOF Mass Spectrometry Imaging

Samuel Okyem* ,1, Timothy J. Trinklein, 1, Seth W. Croslow ,1, Stanislav S. Rubakhin ,1, Jonathan V. Sweedler ,1

1. Department of Chemistry and Beckman Institute for Advanced Science and Technology,
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Introduction

Post-translational modifications (PTMs) of neuropeptides play important roles in their activity and bioavailability. One of the understudied PTMs is endogenous isomerization of L amino acid residues to its D form generates D-amino acid-containing peptides (DAACPs). Compared to their all L-amino acid-containing peptide (LAACP) counterparts, DAACPs exhibit distinct resistance to protease digestion and receptor binding. A challenge involves DAACP spatial localization compared to their all L counterparts in tissues and organs. To bridge this gap, we use matrix-assisted laser desorption
ionization trapped ion mobility mass spectrometry imaging (MALDI timsTOF MSI) to map the localization of several endogenous isomers in the central nervous system (CNS) of Aplysia Californica.

Methods

Adult Sea slugs Aplysia californica were anesthetized via an injection of isotonic $MgCl_2$ and the animal's CNS surgically isolated. The CNS was flash frozen, cryosectioned to 16 µm thickness at -20 °C, and thaw mounted onto a conductive ITO glass slide. After drying in a vacuum desiccator, 25 mg/ml 2,5-dihydroxybenzoic acid was applied with 8 passes using a HTX M5 robotic sprayer (HTX technologies). Tissue sections were analyzed in positive mode with TIMS on using a Bruker timsTOF Flex MALDI-2 mass spectrometer. MS imaging was performed with a 50 µm pixel size and 50 µm pitch, 1000 laser shots and a TIMS ramp time of 1.2 s.

Results

Here, we utilize MALDI timsTOF MSI to separate, detect, characterize, and determine the spatial distribution of DAACPs in the CNS of classic neurobiological model Aplysia californica. MS method optimization was performed using a buccal ganglion extract targeting pleurin-1 isomers. Pleurin-1 (MFYTKGSDSDYPR1-NH_2) has two main epimers with isomerization at D2-Phe. TIMS method optimization allowed separation of the epimers. Following this, MALDI timsTOF MSI was performed on sectioned A. californica CNS to determine the spatial distribution of the pleurin-1 epimers. Investigation of serial sections of the CNS revealed broader than previously reported localization of the Pleurin-1 DAACP. This epimers was detected in buccal, pleural, cerebral ganglia and several connective nerves. Intriguingly, Pleurin-1 LAACP and DAACP distinct localization is observed within the CNS. This indicates differences in neuropeptide packaging and transport and/or specifically localized enzymatic racemization of affected residues. Our methodological work establishes an approach for discovery of DAACPs using combination of ion mobility and imaging mass spectrometry

Novel Aspect

Mapping of the spatial distribution of endogenous D-amino acid containing peptides in Aplysia californica's central nervous system using MALDI with gas-phase isomer separation.

Parise, Rachel

MALDI Mass Spectrometry Imaging of Verapamil Parent and Metabolite Drug Distribution along a Rat Liver Sinusoid

Rachel Parise*1, Bingming Chen2, Christopher Gibson2, Swati Nagar1, and Kenneth Korzekwa1

1. Department of Pharmaceutical Science, Temple University School of Pharmacy, Philadelphia, PA 19140, 2. Department of Pharmacokinetics, Pharmacodynamics & Drug Metabolism (PPDM), Merck & Co., Inc., West Point, PA 19486

The well-stirred hepatic model assumes the liver is a single compartment with instantaneous mixing of blood, resulting in uniform unbound drug concentration throughout the liver. Physiologically, however, the liver is made up of discrete hepatic lobules. The hexagonal lobules have multiple portal triads delivering unbound drug to travel the lengths of hepatocyte cell-lined sinusoids which empty into a single central vein. In the liver, drugs can be sequentially metabolized via cytochrome p450 enzymes (CYPs) to primary and secondary metabolites. Verapamil, a calcium channel blocker, is a high-permeability drug that undergoes extensive metabolism via CYPs. Here we show a gradient in the spatial distribution of verapamil concentrations as well as sequential metabolism occurring along the length of a sinusoid with matrix-assisted laser desorption/ ionization mass spectrometry imaging (MALDI-IMS). Rats were dosed with verapamil (5 mg/kg IV) and liver lobes were excised at 4 minutes post dose for MALDI-MS imaging. The imaging experiments were performed using a Bruker timsTOF using a 30 µm diameter laser spot rastered over an 8 um thick liver section. Drug and relevant biological molecules identified were spatially mapped and compared to corresponding optical images of the liver sample using Bruker SCiLS Lab software. Additionally, we have developed partial differential equation based models for drug concentration as a function of sinusoidal length as well as time. The verapamil spatial concentrations along the sinusoid collected experimentally with MALDI-IMS will be used to develop and test mathematical models to predict spatio-temporal verapamil and metabolite concentrations.

Pyatkivskyy, Yuriy

Targeted DESI Imaging MS of Drug Distribution and Drug-Induced Liver Injury (DILI) Metabolites from Methapyrilene in the Wistar Rat

Anthony Midey,1 Yuriy Pyatkivskyy, 1* Steven K Lai,1 Ian D Wilson,2 Robert S Plumb,1 Roy Martin1, and Bindesh Shrestha1

1 Waters Corporation, Milford, MA; 2 Imperial College, London, UK

Introduction

Methapyrilene is known to cause liver damage, evident by the lipidomic changes of methapyrilene-dosed Wistar rats. Understanding the metabolic spatial compartmentalization between liver zones—periportal near the hepatic arteries (zone 1), mid-lobular hepatocytes (zone 2), and pericentral adjacent to the central vein (zone 3)—is crucial for assessing the drug's physiochemical effects. Desorption electrospray ionization (DESI) imaging mass spectrometry (MS) offers high sensitivity for small molecules readily ionized by electrospray, providing spatial molecular differences in specific liver zones. DESI with MS/MS on a tandem quadrupole further improves sensitivity and increases metabolite identification specificity compared to full scan imaging. Here, we employ targeted DESI imaging MS to show spatial differences between control and dosed liver tissue.

Methods

Male Wistar rats were orally dosed with 50 or 150 mg/kg methapyrilene for 5 days. Liver tissues were harvested from the vehicle (control) and dosed rats. Fresh frozen cryosections were thaw mounted onto specimen slides stored at -80C until thawing for analysis. A panel of MSMS transitions for key disregulated lipids and metabolites was optimized on a tandem quadrupole MS (Waters Xevo™ TQ-Absolute) for DESI targeted imaging. Commercial DESI-XS source (Waters) was used to map the distribution differences of these lipids between the vehicle and dosed liver tissues. DESI solvent of 98:2 methanol:water with 0.01%(v/v) formic acid desorbed and ionized the lipids. High Definition Imaging (HDI™) software (Waters v1.7) processed and created the spatial maps from the tandem quad MSMS data.

Results

Drug Induced Liver Injury (DILI) is a major cause of failures during preclinical/clinical drug development. Methapyrilene acts as an antihistamine and anticholinergic. It was developed in the 1950s for the treatment of insomnia, but it was removed from the market in 1970 as it caused cancer in rats following chronic administration. To gauge the hepatotoxicity of the drug, plasma metabolites were monitored for vehicle (control), 50 mg/kg, and 150 mg/kg dosed rats at the end of 1 day, 3 days, and 6 days after oral dosing. Multivariate statistical analyses of the previous LC-MS and LC-MSMS data comparing the three conditions showed a select set of lipids including glusosyl ceramides (GlcCer), ceramides (Cer), sphingomyelins (SM), phosphotidylcholines (PC), PC plasmologens, and lysophosphotidylcholines (LPC) that were up-regulated in the dosed rats. Those lipids were identified to lipid class and acyl chain lengths from the MSMS. Thus, the targeted DESI imaging experiments used the acyl chain MSMS transitions to link the spatial distributions to the known lipid, along with the known drug and drug metabolite MRM.

The MSMS transitions for the key biomarker lipids from the targeted LC-MSMS experiments were subsequently used to create a targeted MSMS method to map them in rat liver tissue sections with targeted DESI imaging MS. Vehicle (control), 50 and 150 mg/kg dosed livers were imaged at 50 µm pixel size using the acyl-chain specific MSMS transitions, and also imaged using drug and drug-related metabolites MSMS transitions. The DESI images illustrated the differences in lipid, drug, and metabolite localization induced by methapyrilene metabolism as a function of dosage and time from administration. The spatial lipidomics and metabolomic information co-registered with tissue section morphology provided an additional understanding of the biological mechanism in the liver.

Novel Aspect

DESI targeted MSMS imaging of drugs and lipids on a tandem quadrupole mass spectrometer

Rashford, Kameisha

Glycomic Analysis of Immune Cell Clusters in Prostatic and Pancreatic Adenocarcinoma Tissues

Kameisha Rashford*1, Caroline Kittrell*1, Lyndsay EA Young*1, Joseph Ippolito*2, Jennifer Wu*3, Robin Leach*4, Richard R. Drake*1

1. Medical University of South Carolina, Charleston SC, 2. Washington University, St. Louis MO, 3. Northwestern University, Chicago IL, 4. Robin University of Texas Health Science Center at San Antonio, San Antonio TX

Introduction

Immune cells can have both pro- and anti-tumorigenic roles in cancers, with their activation or deactivation often influenced by glycosylation. This study focuses on N-linked glycosylation, where glycans are added to asparagines on carrier proteins, crucial for protein folding, stability, and quality control. In cancer, altered N-glycosylation can produce tumor-associated glycans (TAGs), leading to different glycosylation patterns that can impair immune surveillance by interacting with immune cells and masking antigen presentation. While several studies on prostatic and pancreatic cancers report differences in glycosylation patterns between tumor and normal tissues, few have fully explored TAGs and their role in the tumor microenvironment (TME). This project aims to characterize glycans that colocalize with immune cells to develop new biomarker and immunotherapeutic targets.

Methods

We utilized a combination of immunohistochemistry (IHC) and mass spectrometry to characterize N-glycans that co-localize with immune cells in formalin-fixed paraffin-embedded prostate and pancreatic adenocarcinoma tissues. Immune clusters were identified by staining the tissues with Mayer's hematoxylin and visualizing them with a Hamamatsu NanoZoomer. Next, the tissues were treated with peptide N-glycosidase F, an enzyme that cleaves N-glycans from their carrier proteins and analyzed using matrix-assisted laser desorption and ionization mass spectrometry imaging (MALDI-MSI). Select tissues were analyzed by MALDI-IHC (Ambergen) to identify immune cell subtypes. Images from MALDI-IHC, MALDI-MSI and H&E were co-registered and analyzed using SCiLS Lab software.

Results

Immunotherapy for prostatic and pancreatic cancers has shown poor results due to limited immune cell infiltration and an immunosuppressive microenvironment. The metabolic state of effector immune cells in these ""immune cold"" cancers is not well understood. As changes in N-glycosylation is a hallmark of cancer and Warburg glucose and glutamine metabolism, we hypothesize that metabolically active immune cell clusters within prostate and pancreas tumor tissue will exhibit more intense N-glycan signal than immune suppressed clusters. Utilizing 43 FFPE tissue samples collected from patients with varying stages of prostatic (n=34) and pancreatic adenocarcinoma (n=9), our lab has characterized the N-linked glycan profile of 207 immune cell clusters within and surrounding these tumors via Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging (MALDI-MSI). The N-glycans most commonly detected in the immune clusters were noted across both cancer types. We found that high mannose and a diverse set of core fucosylated N-linked glycans are typical of immune cell clusters within and surrounding pancreas and prostate tumors. These immune glycan signatures are independent of the prostate and pancreas-specific N-glycan tissue distributions. Additionally, N-glycan signal intensity was lower within intra and peri tumoral immune cell clusters versus immune cell clusters more distal from the primary tumor, and some clusters had no detectable glycan signal. Additionally, we utilized MALDI-IHC with photocleavable mass tags to analyze the composition (CD3, CD8, CD11b, CD20, and CD68) of each immune cluster and extracellular matrix components (E-cadherin, Collagen 1A1, and actin αSmooth Muscle). We established that CD20+ B cells account for the majority of signal colocalized within these immune cell clusters with additional signal from CD3+ T cells. Future studies will determine a link between immune cell glycan profiles, progressive stages of these “immune cold” cancers, and immunotherapy response.

Novel Aspect

N-glycosylation signatures of immune cell clusters can be identified in tissues using MALDI-MSI and MALDI-IHC. Detected N-glycans may reflect the metabolic state of each cluster.

Rensner, Josiah

In-Source Ozonolysis for Efficient MALDI-MS Imaging of Lipid Double-Bond Isomers

Josiah Rensner, Anna Uhlmansiek, Young Jin Lee

Iowa State University

Introduction

Lipid mass spectrometry imaging is an important area of research, but one major challenge is the common presence of isomers, especially isomers that differ by double bond position. Various techniques exist to resolve these isomers, including ozonolysis, epoxidation, and photochemical reactions. We developed an ozonolysis method by introducing ozone directly into the MALDI source, dubbed ozMALDI, resulting in simultaneous gas-phase ozonide formation from all unsaturated lipids. This allows us to generate ozonides without increasing sample preparation time and allows in-parallel fragmentation of multiple ozonides. This technique has successfully been applied to rat brain samples and we will also present studies of lipid localization changes in cardiac-arrested rat brain tissue and an investigation of omega-3 lipids in genetically modified soybean seeds.

Methods

Experiments are performed on an Orbitrap Q-Exactive HF MS (Thermo Scientific) with an intermediate pressure MALDI source (Spectroglyph). Ozone is generated using a TG-40 ozone generator (Ozone Solutions) with air or O2 as the inlet gas, resulting in ~1% or ~5% ozone respectively. Ozone is introduced directly into the MALDI source and reacts with ionized metabolites in the gas phase. A continuous flow of ozone is maintained and the source pressure is held at 7 Torr. Rat brain samples are washed with 4°C 50 mM ammonium acetate to remove excess cations, and then analyzed in positive ion mode using 2,5-dihydroxyacetophenone matrix. Ozonides are selected and fragmented using tandem MS and images of diagnostic fragments are generated.

Results

Ozone-induced dissociation (OzID) is a well-established tandem MS method for determining double bond localization in unsaturated lipids. This has previously been done in MSI and has been applied to a variety of biological systems. Existing techniques involve introducing ozone into a collision cell but this requires instrument modification and careful tuning. Alternatively, tissue samples can be subjected to ozonization before MALDI-MS but this requires additional experimental set up and reaction time. We have developed OzMALDI that introduces ozone directly into an intermediate pressure MALDI source. This process is similar to an in-source hydrogen-deuterium exchange protocol we recently developed with ozone being introduced to the same 7 Torr MALDI source. This allows for an in-source ozonolysis reaction that simultaneously reacts with all unsaturated lipids and does not require any instrument modification or additional sample preparation time while producing mass spectrometry images that are comparable to other methods. In preliminary data using rat brain tissue sections, we achieved reaction efficiencies of ~18% for monounsaturated phospholipids and up to ~50% for polyunsaturated lipids. Though we do see some minor in-source fragmentation, this reaction generates high abundances of intact ozonides that can be isolated and fragmented. This allows for MS images with high mass and spatial resolution, and has potential to fragment multiple ozonides simultaneously, generating isomer resolved images of different lipids within the same acquisition without sacrificing spatial resolution. This new method is being tested for unique biological systems including cardiac-arrested rat brains samples and genetically modified soybean strains that are rich in omega-3 lipids. These collaborations are ideal model systems to demonstrate ozonolysis and will also provide novel biological information for medical and oil seed production applications. We plan to collect data related to both these systems over the next two months and will present it at the upcoming workshop.

Novel Aspect

High-efficiency gas-phase ozonolysis reaction in the MALDI source and novel applications to cardiac-arrested rat and omega-3 rich soybean samples.

Scott, Alison

Neutrophil-driven impacts on lung lipid homeostasis in a Cystic Fibrosis-like mouse model of sodium hyperabsorption

Janette M. Harro1, Tialfi Bergamin de Castro1, Courtney Chandler1,2, Kristen J. Brao1,3, Robert K. Ernst1,4, and Alison J. Scott1,4

1 Department of Microbial Pathogenesis, University of Maryland School of Dentistry, Baltimore, Maryland, 2 Food and Drug Administration, Silver Spring, Maryland, 3 Brigham and Women's Hospital, Boston, Massachusetts, 4 Department of Microbiology & Immunology, University of Maryland School of Medicine, Baltimore, Maryland

Introduction

We recently reported dysregulation of lung lipid homeostasis linked to neutrophil immunometabolism as a possible therapeutic target during Pseudomonas aeruginosa (Pa) infection in wild type mice. We sought to extend these findings into a relevant model of Cystic Fibrosis (CF)-like lung conditions including sodium hyperabsorption, mucus thickening, and defective mucociliary clearance. These CF-like lung conditions are present in βENaC transgenic mice (PMID: 32631918). Neutrophil lipids play an important role in the host response to bacterial infection, but are poorly understood in the context of a tissue response under CF-like conditions. Here, we investigated lung lipid homeostasis as a function of neutrophils in a CF-like mouse model.

Methods

Adult, female ENaC mice on a BALB/c background were rendered neutropenic with cyclophosphamide (CYP) given intraperitoneally (i.p.) at 150 mg/kg on day -3 and 100 mg/kg on day -1, or treated with vehicle (control groups). On study day 0, lungs were prepared and assayed for phospholipids and cardiolipins using our previously established methods (PMIDs: 31882724, 32924474). Norharmane matrix (7 mg/mL in 2:1 chloroform:methanol) applied with an HTX M5 sprayer followed by data collection on a Bruker timsTOF Flex at 10 and 50 μm resolution.

Results

In the βENaC mice treated with CYP, no significant clinical observations were observed at necropsy. As we previously observed in wild type mice, phosphatidylserine (PS) and phosphatidylinositiol (PI) lipids were major features of the differential lipid populations of the lung parenchyma in the resting lungs compared to neutropenic CF-like model. Further, in the baseline (resting) lungs in the transgenic animals, the shift in head group usage suggested a compensatory change to accommodate the change in salt concentrations in the mucus. Studies are ongoing to determine the impact of neutropenia in the transgenic model on susceptibility patterns for pathogens commonly found in the lungs of patients with CF, including Staphylococcus aureus (Sa) and co-infections with Sa and Pa.

Novel Aspect

This study is the first to determine neutrophil-driven changes in the lung lipid profile in a CF-like mouse model.

Sekera, Emily

Strategies for Utilizing MALDI Imaging in the Drug Discovery Pipeline

Emily R. Sekera*1, Nathaniel R. Twarog1, Rebekah DeVries1, Anup Aggarwal1, P. Jake Slavish1, Gisele Nishiguchi1, Elizabeth D. Barker2, Anang A. Shelat1, John J. Bowling1

1.St. Jude Children's Research Hospital, Department of Chemical Biology and Therapeutics, Memphis, TN, USA, 2.The University of Tennessee Knoxville, Department of Mechanical, Aerospace and Biomedical Engineering, Knoxville, TN, USA

Introduction

To fully evaluate drug candidates for costly clinical trials, it is imperative to validate its efficacy, and then characterize its penetration to model achievable clinical concentrations. Quantitation by matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has been an ongoing challenge of reproducibility. Calibrated images have been generally produced in situ by including standards in tissue homogenates or directly spotted solutions using pipettes. Herein, we discuss the development of methods to impact early drug discovery processes. We intend to apply these technologies to assess drug candidates for therapeutic efficacy and improve our quantitative imaging by directly depositing consistent quantities of standards discreetly to tissues for our workflows in MALDI MSI.

Methods

Testing of drug analysis workflows were completed using JQ1 and doxorubicin within murine tissues. JQ1 was dosed intravenously at 10 mg/kg and brain and liver tissues were frozen for subsequent analyses. Doxorubicin was dosed intratumorally at 500 μg/10 μL and flank tumors were collected. Samples were sectioned and thaw-mounted to ITO slides for MALDI analyses. Drug quantitation workflows utilized an in-house script for ImageJ to define locations to dispense drug standards and were deposited onto tissues using an Echo 650. MSI data was collected on either a 7T SolariX FT-ICR or timsTOF fleX mass spectrometer. Data was analyzed using the Bruker SCiLS lab software suite.

Results

In this talk, we detail our current methods to evaluate drugs and their formulations by MALDI MSI and move them into quantitative assessments. In one case study, the evaluation of the extended release of doxorubicin from its formulation, Amygel, is interrogated. Rhabdomyosarcoma flank tumors were dosed intratumorally, and sample sections were taken across multiple locations throughout the tumor to aid in assessing the pharmacodynamics. Confirmation of drug and Amygel signals were ascertained through utilization of trapped ion mobility spectrometry (TIMS) to obtain collisional cross section values from drug and formulation standards. In our second case study, we analyzed intravenously (IV) administered bromodomain inhibitor, JQ1, to create pipelines for future pre-clinical studies. While IV dosing allows most of the drug to bypass the first pass metabolism, low levels of JQ1 make it to the intended organ (brain). To increase low intensity signals in imaging experiments of brain and liver, we utilized a combination of deuterated standards and isolation windows, to allow us to evaluate drug distribution within these tissues. Beyond distribution within the aforementioned studies, we aim to create routine workflows for absolute quantitation in MSI. To improve the reproducibility of calibration, we leveraged echo acoustic dispensing of nanoliters of standards. By utilizing low volumes, we expected to mitigate difficulties that arose from the solvents used to dissolve drug standards. Additionally, a script implemented in ImageJ was developed to enable researchers to choose discrete positions for standard deposition within samples. We will discuss general approaches of utilizing the script to quantitate drug signals. Finally, we will briefly discuss the implementation of imaging strategies to validate cytotoxicity of drug candidates by analyzing markers of cell death or stress within multi-cell systems in early stages of the pipeline.

Novel Aspect

We evaluate the utilization of acoustic liquid handlers for the deposition of small volumes of drug solutions for quantitation workflows in MALDI-MSI.

Shedlock, Cameron

MetaVision3D: Automated Framework for the Generation of Spatial Metabolome Atlas in 3D

Cameron J. Shedlock*1,2, Xin Ma,1,2,3, Terrymar Medina1,2, Roberto A. Ribas1,2, Harrison A. Clarke1,2, Tara R. Hawkinson1,2, Praveen K. Dande1,2, Lei Wu1,2, Sara N. Burke4,6,10, Matthew E. Merritt1, Craig W. Vander Kooi1, Matthew S. Gentry1,2, Nirbhay N. Yadav8,9, Li Chen2,3, and Ramon C. Sun1,2,

1 Department of Biochemistry & Molecular Biology, College of Medicine, University of Florida, Gainesville, FL, USA, 2 Center for Advanced Spatial Biomolecule Research, University of Florida, Gainesville, FL, USA, 3 Department of Biostatistics College of Public Health and Health Professions & College of Medicine, University of Florida, Gainesville, FL, USA, 4 Department of Neuroscience, University of Florida, Gainesville, FL, USA, 5 McKnight Brain Institute, University of Florida, Gainesville, FL, USA, 6 Center for Translational Research in Neurodegenerative Disease (CTRND), University of Florida, Gainesville, FL, USA, 7 Institute on Aging, University of Florida, Gainesville, FL, USA, 8 F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA, 9Russell H. Morgan Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction

Current methodologies in mass spectrometry imaging are predominantly two-dimensional. To bridge this gap, we introduce MetaVision3D, an automated framework for transforming serial 2D MALDI MSI sections into a high-resolution 3D spatial metabolome. MetaVision3D employs advanced algorithms for image registration, normalization, and interpolation, enabling comprehensive 3D models of metabolite distributions across tissues. As a proof of principle, MetaVision3D was used to generate the mouse brain 3D metabolome atlas, accessible online for further research.

Methods

MetaVision3D converts 2D MALDI MSI sections into 3D models. This starts with MetaAlign3D which aligns sections based on enhanced correlation coefficient maximization, ensuring precise alignment of serial tissue sections. Next, MetaNorm3D normalizes metabolite intensities across sections to account for intra-slide variabilities. MetaImpute3D then addresses gaps in tissue sections by using adjacent sections for imputation, creating consistent pixel values for missing regions. Finally, MetaInterp3D interpolates additional sections to enhance z-axis continuity, facilitating optimal 3D reconstruction. The framework's efficacy was demonstrated through the creation of a mesoscale 3D metabolome atlas of the brain in 4 mice, visualized using ImageJ.

Results

MetaVision3D is a pipeline transforming serial 2D MALDI MSI sections into high-resolution 3D spatial metabolomes. This framework includes MetaAlign3D for image registration, MetaNorm3D for normalization, MetaImpute3D for imputation, and MetaInterp3D for interpolation. As a proof of concept, we generated a 3D metabolome atlas of the brain in 2 WT, 1 5XFAD, and 1 GAA genetically engineered mouse models. MetaVision3D's precision in spatial data and ability to mitigate technical variabilities inherent to MALDI imaging marks a significant advancement in spatial metabolomics. The 3D atlas and pipeline simplicity in generating future atlas provides unprecedented insights into the spatial heterogeneity of metabolic activities within the brain, offering a new standard for neurological research and potential therapeutic interventions. MetaVision3D's integration of computational techniques bridges the gap between 2D and 3D metabolomics, providing a deeper understanding of metabolic distributions in tissues and opening new options for research in other organs and disease models.

Novel Aspect

Ease and utility of automated 3D spatial metabolome for 3D MSI.

Shrestha, Bindesh

Enhancing Spatial Resolution Towards Cellular Imaging with Low-Flow DESI

Mark Towers, 1 Emrys Jones, Joanne Ballantyne, 1 Sheba Jarvis,2 Anthony Midey, 3 Bindesh Shrestha 3*

1 Waters Corporation, Wilmslow, UK, 2 Imperial College London, Hammersmith Hospital, London, 3 Waters Corporation, Milford MA

Introduction

Desorption electrospray ionization (DESI) has become an established imaging technique. As with all imaging methods, there is an ongoing drive toward smaller pixel sizes and higher image resolution. In this study, we demonstrate a low-flow DESI technique to enhance spatial resolution toward cellular imaging using a commercially available DESI system. The time required to acquire a higher resolution image increases exponentially as pixel size decreases - even with a fast acquisition rate of more than 30 scans a second. We employed a time-saving data-driven reacquisition technique to address this issue. This method involves acquiring a low-resolution initial scan image followed by a high-resolution image in a specific region of interest.

Methods

Experiments were conducted using a Waters mass spectrometer equipped with a DESI XS source. A µBinary solvent manager (M-class Acquity™ BSM, Waters) was used to supply the spray solvent since the low-flow DESI relies on stable solvent delivery. A 1.7 µm (300 µm x 150 mm) ACQUITY C18 column was fitted to the solvent line and a post-grounding union tubing was replaced with 25 µm ZenFit™ tubing to improve flow stability by increasing back pressure. The initial experiments focused on porcine liver tissue. We acquired images at various pixel sizes: 100 µm, 50 µm, 20 µm, 10 µm, and 5 µm. The flow rates used were 500 nL/min and 250 nL/min.

Results

DESI images of the liver acquired at 500 and 250 nL/min were assessed for image fidelity and resolution - if there was an increase in image quality with smaller pixel sizes. When using 500 nL/min flow rate, the image details did not visually increase beyond a 20 µm pixel size. This suggests spray diameter obtained at 500 nL/min flow rate was a limiting factor for images acquired at 10 and 5 µm pixel sizes. When using 250 nL/min, there is a clear improvement in the image fidelity. Additional details in the image can be observed between 10 µm and 5 µm pixel sizes, which suggests the sampling area was >10 µm. When comparing 5 µm data to optical images at a suitable magnification, a 1:1 mapping of optically observed anatomical features could be matched. Furthermore, we introduced a time-saving, data-driven microscopy mode in the Waters HDI software. Researchers can draw regions of interest (ROIs) directly from the HDI software's visualization of previously acquired data. The ROI can then be exported for reanalysis of the same tissue at a smaller pixel size. A comparison between reanalyzed and fresh tissue acquisition showed no significant differences, due to the very soft nature of the low-flow DESI technique. The improvements in a low-flow DESI technique have been able to increase the spatial resolution in DESI images - moving us closer toward routine cellular imaging.

Novel Aspect

The enhancement in a low-flow DESI technique can improve the spatial resolution of DESI images.

Stumpo, Kate

A spatial multiomics workflow on the neofleX benchtop MALDI-TOF instrument

Janina Oetjen1, Corinna Henkel1, Ulrike Schweiger Hufnagel1, Sebastian Boehm1, Christoph Nordmann1, Andree Schuster1, Katherine Stumpo2, Michael Easterling2, Andrea Tannapfel3, Jens Christmann3

1 Bruker Daltonics GmbH & Co. KG, Fahrenheitstr. 4, 28359 Bremen, Bremen, Germany, 2 Bruker Scientific, 40 Manning Road, Billerica, Massachusetts 01821, 3 Institute for Pathology, Ruhr-University Bochum, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

Introduction

Spatial biology enables the visualization of different chemical landscapes in biological tissue making it an important technology in clinical research. MALDI mass spectrometry imaging (MALDI Imaging) is well established for the spatial analysis of biomolecules in tissue. Additionaly, multiomic spatial localization of lipids with expressed protein distributions can be done using MALDI HiPLEX-IHC, thus increasing our understanding of localized cellular processes in tissue. Automation is a fundamentally desired component of the technology, making it accessible to a range of researchers. Here, we demonstrate an automated workflow on a new benchtop axial TOF that starts with human colorectal cancer FFPE tissue sections and ends with simultaneous visualization of lipids and expressed proteins in a clinically relevant sample.

Methods

FFPE sections of a human colorectal cancer sample were prepared for lipid imaging on a benchtop MALDI axial TOF instrument at 20 µm pixel size in the mass range 500-1100 Da. Red phosphorous was used for external mass calibration and endogenous compounds for an online calibration. Lipid annotations were confirmed by a measurement on a timsTOF fleX instrument (Bruker). To assess the spatial protein landscape, the same slide was processed by MALDI HiPLEX-IHC using 14 photocleavable mass-tagged antibodies (AmberGen). DHB matrix was applied by sublimation (HTX Technologies) and the data acquisition performed in the mass range 800-2200 Da using the software FlexControl 2025 and FlexImaging 7.4. Visualization of the data was done in SCiLS Lab 2024b and SCiLS Scope 1.0.

Results

Our spatial multiomics workflow was performed on a new benchtop axial MALDI-TOF instrument at high speed (more than 20 pixel/s) and high spatial resolution (20 µm pixel size), where it allowed for automated instrument setup and acquisition, as well as visualization of the differential lipid distributions and expressed protein data. The expressed protein distribution images of the mass tags from all of the 14 antibodies were obtained by automatic generation of ome.tiff files from a target list of the antibody mass tags as [M+H]+ ion species and were visualized in the software SCiLS Scope. SCiLS Scope is a new tool for fast browsing through the color-coded ion images of the MALDI HiPLEX-IHC data, capable of visualizing both individual and composition ion images. Fast access to the data was additionally managed by automatic creation of SCiLS Lab files from both measurements. The online calibration tool implemented in FlexControl 2025 allowed the increase in mass stability up to 40 ppm during imaging runs. Fifty-eight lipids, mainly phosphatidylcholine and sphingomyelin lipid species were annotated in positive ion mode from the FFPE sample, and their annotations were confirmed by a collisional cross section (CCS) aware analysis on a timsTOF fleX. Protein expression and lipid imaging data were combined and mapped for examination of feature colocalization. Lipids colocalizing with individual tumor or immune markers from the HiPLEX-IHC experiment were thereby obtained using the mapped images. For example, PC_32:0 was detected with higher abundance in the same region, where also cytokeratin marker PanCK was binding. Our workflow provides a simple method to visualize the targeted spatial protein expression profiles from FFPE tissue. As an add on, our method can be combined with lipid imaging from the same tissue section to receive deeper insights into the cellular mechanisms of disease. "

Novel Aspect

A new automated workflow from acquisition to visualization of spatial protein expression data developed on a new benchtop mass spectrometer.

Tat, Vy

Mass Spectrometry Imaging with in vivo Isotope Labeling for Spatio-Temporal Study of Galactolipid Biosynthesis

Vy T. Tat*1, Young Jin Lee*1

1. Department of Chemistry, Iowa State University, Ames, IA 50011

Introduction

Mass spectrometry imaging (MSI) allows visualizing metabolite localization on tissues, while isotope labeling reveals metabolic pathway activities. Yet, the combination of these two techniques has remained largely unexplored in plant systems. Here, spatio-temporal changes in galactolipid biosynthesis are unraveled by coupling MSI and in vivo D2O labeling in an aquatic plant, Lemna minor. During deuterium labeling progress (forward labeling), three distinct isotopologue distributions appeared, which corresponded to partial labeling of structural moieties and localized in different parts of the plant. Additionally, 13CO2-labeling and backward labeling experiments were conducted to confirm observations above. The newly emerging tool, MSI coupled with isotope labeling, is expected to provide more details in lipid biosynthesis under different conditions in various plant systems.

Methods

In forward labeling, duckweeds were transferred from 0.5x H2O Schenk and Hilderbrandt to 50% D2O media over 15 days, while duckweeds propagated in 50% D2O for 3 months were transferred back to H2O media in backward labeling. For 13CO2-labeling, 13CO2 was generated by the neutralization between lactic acid and barium 13C-carbonate for 3 days. Three plants from forward and 13CO2-labeling experiment at each time point were subjected to MALDI-MSI analysis after fracturing. Samples were sputter coated with gold and then sprayed with 2,5-dihydroxybenzoic acid matrix with TM sprayer (HTX Technologies). MALDI-MSI was performed on a QExactive HF (Thermo) with a MALDI source (Spectroglyph) in positive-ion mode. Additionally, lipid extract from forward and backward labeling samples was analyzed with ESI.

Results

Galactolipid biosynthesis mostly occurs in chloroplast, including photosynthesis, fatty acid (FA) synthesis, galactosylation. FAs are utilized for phospholipids like phosphatidylcholine before forming galactolipids in eukaryotic pathway, while they are directly used to synthesize galactolipid in prokaryotic pathway. Though well-established, the spatio-temporal dynamics of galactolipid biosynthesis remained unexplored. The fractured plant spectrum was dominated by galactolipids, monogalactosyldiacylglycerol (MGDG 36:6) and digalactosyldiacylglycerol (DGDG 36:6 and DGDG 34:3), and pheophytin a. MGDG 36:6 showed three distinct binomial isotopologue groups centered at 3.5, 18, 35 deuterations corresponding to galactose moiety, 7 C-H (Group 1), galactose and a fatty acyl chain 18:3, 36 C-H (Group 2), entire molecule, 70 C-H (Group 3), respectively. DGDG had a similar trend but mostly groups 1 and 3, with Group 1 extended to one more galactose. Group 2 had very low abundance in MALDI but was seen in lipid extract data, confirming its presence in the synthesis pathway. Lastly, pheophytin a had one single isotopologue distribution of the whole molecule over 15 days. Interestingly, three labeling distribution groups had different localizations across the tissue morphology in MS images. The unlabeled monoisotopic peak and Group 1 co-localized in the parent frond, while Group 3 appeared only in the newly grown daughter fronds. Group 2 distributed in the newer sections of parent frond and older regions of daughter fronds. The spatio-temporal pattern could be explained with newly formed isotope-labeled building blocks being attached to unlabeled precursors.Both backward labeling and 13CO2-labeling distributions showed three isotopologue groups. The labeled glycerol backbone, previously hidden in forward labeling, emerged in both supporting experiments. Furthermore, the average mass of galactolipids served as quantitative measure, with half-way change average mass occurring 1.5-2 days later in forward labeling than backward labeling due to D2O-stress-induced growth delay. This work is published in Plant and Cell Physiology, 2024;pcae032, https://doi.org/10.1093/pcp/pcae032.

Novel Aspect

Mass spectrometry imaging coupled with isotope labeling elucidates the spatio-temporal details of galactolipid biosynthesis in plant development.

Verhaert, Peter

MSHC: digestion- and antibody-free top-down IMS/MSI analysis of histological samples from FFPE biobanks; more than expected...

Peter Verhaert*1,2, Raf Sciot1, Sooraj Baijnath3, Aletta Millen3, Dick Swaab4, Marthe Verhaert2,5, Gilles Frache6

1. University of Leuven (Leuven, Belgium), 2. ProteoFormiX (Vorselaar, Belgium), 3. University of the Witwatersrand (Johannesburg, South Africa), 4. Netherlands Institute for Neuroscience (Amsterdam, Netherlands), 5. University of Brussels Faculty of Medicine (Jette, Belgium), 6 Luxembourg Institute for Science and Technology (Belvaux, Luxembourg)

Introduction

Mass spectrometry histochemistry (MSHC) is an IMS/MSI variant which analyzes histological sections using (top-down) mass spectrometry for (untargeted) detection of (bio)molecular ions.

In the last years we have focused on optimizing MSHC for formaldehyde-fixed paraffin-embedded (FFPE) samples, such as those archived and documented in the infinite tissue biobanks hosted in hospitals all over the world.

Methods

We here will elaborate on our latest efforts to demonstrate that not only neuropeptides and a selection of metabolites, but also neurotransmitters can be imaged by FFPE MSHC using atmospheric pressure (AP)/MALDI (MassTech) connected to Orbitrap (ThermoFisher Scientific) HRMS analyzers. Validation experiments include (MS)IHC as well as comparative MSHC analyses of selected brain areas rich in specific neurotransmitters. In addition, we are evaluating on-tissue chemical derivatization (OTCD) with reactive MALDI matrices like FMP-10 which have been described to boost the ionization efficiency of neurotransmitters.

Results

Whereas IMS/MSI datasets of histological sections of fresh/frozen material are dominated by signals from lipids and other highly ionizable and/or high abundant (bio)molecules, top-down MSHC of FFPE tissues typically detects relatively small molecules/ions which are not tightly crosslinked through formaldehyde fixation and are not washed-out by the paraffin embedding. Since the latter protocol effectively removes lipids from a biological sample, MSHC of FFPE tissues has an increased likelihood to detect and histologically localize less-ionizable and lower-abundant biomolecules, including neuropeptides and small (non-hydrophobic) metabolites, especially when post-deparaffinization sample washings are maximally reduced. By avoiding conventional antigen-retrieval steps which are critical to allow antibodies to bind their respective epitopes in the firmly crosslinked network of formalin-fixed molecules, and by leaving out trypsin or Lys/C digestion which is essential for bottom-up protein identifications, members of biologically very interesting families of biomolecules including endogenous signaling peptides and neurotransmitters, including histamine, acetylcholine, noradrenaline, serotonin, dopamine and some of their metabolites, become accessible for MSHC spatial analyses. With a lateral resolution of <10 micrometer achievable, we can rightfully state that MSHC is a spatial multi-omics technology with single cell resolution.

Novel Aspect

Top-down, enzyme- and antibody-free AP/MALDI mass spectrometry histochemistry (MSHC) of FFPE biobanked tissues stored for prolonged times reveals, besides (neuro)peptides and metabolites, also neurotransmitters.

Winkler, Robert

Prototyping ambient ionization mass spectrometry imaging (AIMSI) systems

Robert Winkler*1, Ignacio Rosas Román2, Abigail Moreno Pedraza3, Nancy Shyrley García Rojas1, Héctor Guillén Alonso4, Cesaré Ovando Vázquez5

1. Cinvestav UGA-Langebio, Irapuato, Mexico, 2. University of Guanajuato, Campus León, Mexico, 3. Leibniz Institute of Vegetable and Ornamental Crops (IGZ) e.V., Großbeeren, Germany, 4. Technological Institute of Celaya, Celaya, Mexico, 5. CONAHCYT CNS-IPICYT San Luis Potosí, Mexico

Introduction

Building an ambient ionization mass spectrometry imaging (AIMSI) system requires an ion source, sampling stage, hardware control, and data processing software. The AIMSI assembly must also be coupled to a mass analyzer.

Provider MS imaging (MSI) solutions are user-friendly and optimized for specific applications, such as medical tissue analysis. However, due to technical and legal restrictions, integrating novel hardware components and software functions takes time and effort. In addition, the costs of commercial MSI systems are prohibitive for many research groups.

Therefore, we designed an open modular platform for developing and testing novel AIMSI sources.

Methods

We constructed the Open LabBot for custom mass spectrometry imaging and high-throughput sampling [1]. Ion sources and other components, such as a desorption laser and its focusing lens, can be mounted on the robot's frame using 3D-printed adapters. For robot control, data processing, and image analysis, we built the RmsiGUI web interface, which is based on R and, therefore, easy to extend with additional functions for statistics and visualization. Further, we created LABI-Imaging to synchronize the sampling with data acquisition on a Thermo mass analyzer.

We recently published libraries for the high-performance programming language Julia to analyze massive MSI datasets."

Results

Using 3D-printed adapters, we mounted different ion sources to the Open LabBot, such as a NovionX PlasmaChip and a 3D-printed low-temperature plasma (LTP) probe [2]. Combining a continuous ultraviolet laser with a lens for desorption and an LTP ionization probe enabled the fast AIMSI of native plant tissues with a lateral resolution of 50 µm [3]. Depending on the analytical and biological question, higher spatial resolution is possible.

The LABI-imaging and RmsiGUI software provides the complete informatics workflow for instrument control, creation, and analysis of MSI datasets. Since we implemented the community data format imzML, the generated results are compatible with most MSI data analysis programs. Our Julia libraries allow for about 100 times faster imaging data processing than R, which enables the execution of computationally intensive projects, such as mining MSI Big Data collections [4]. Altogether, we built a modular and open platform for the agile development of multimodal AIMSI systems and advanced MSI data analysis.

Funding: CONACyT Fronteras project 2015-2/814 and the bilateral grant CONACyT-DFG 2016/277850.

References

1. Rosas-Román, I. _et al._: Open LabBot and RmsiGUI: Community Development Kit for Sampling Automation and Ambient Imaging. Microchemical Journal 2020, 152, 104343. https://doi.org/10.1016/j.microc.2019.104343.

2. Martínez-Jarquín, S. _et al.: Template for 3D Printing a Low-Temperature Plasma Probe. Analytical Chemistry 2016, 88 (14), 6976–6980. https://doi.org/10.1021/acs.analchem.6b01019.

3. Moreno-Pedraza, A. _et al._: Elucidating the Distribution of Plant Metabolites from Native Tissues with Laser Desorption Low-Temperature Plasma Mass Spectrometry Imaging. Anal. Chem. 2019, 91 (4), 2734–2743. https://doi.org/10.1021/acs.analchem.8b04406.

4. Rosas-Román, I. _et al._: Technical Note: mzML and imzML Libraries for Processing Mass Spectrometry Data with the High-Performance Programming Language Julia. Anal. Chem. 2024, 96 (10), 3999–4004. https://doi.org/10.1021/acs.analchem.3c05853."

Novel Aspect The Open LabBot and our software use provider-independent community data formats and enable the rapid prototyping and testing of multimodal AIMSI systems.

Wu, Wenxin

MS Imaging for Differential Neuropeptides and Neurotransmitters Colocalization in Cancer borealis Brain

Wenxin Wu1; Penghsuan Huang1; Haoran Zhang1; Angel E. Ibarra1; Lingjun Li1

1University of Wisconsin Madison, Madison, WI

Introduction

Most biological processes are largely modulated by neuromodulators like neuropeptides and neurotransmitters. Cancer borealis is a prime model for neuromodulatory studies due to its relatively simple nervous systems comparing to mammal. Investigating variations in neuromodulator expression, co-localization and spatial distribution within the brain of Cancer borealis offers a profound understanding of the associated neural circuits. However, studying neurotransmitters with MSI are especially hard due to their poor ionization efficiency, low in vivo concentration, and complex matrix background interference. Through the application of Trapped Ion Mobility Spectrometry (TIMS) coupled to matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and combining with liquid chromatography-electrospray ionization mass spectrometry (LC-ESI MS), the identity of these neurotransmitters can be validated through ccs value, retention time, and MSMS. This research aims to profile, localize, and validate the differential colocalization patterns of neuropeptides and neurotransmitters in the Cancer borealis brain.

Methods

Cancer borealis were acclimated in artificial sea water for at least one week before the experiment. Immediately following dissection, the brain was rinsed briefly in deionized water embedded with aqueous gelatin. The embedded tissue was then sliced to 12 µm serial sections and thaw mounted to the ITO slide. Matrix were applied to the slide via HTX TM-Sprayer. timsTOF MALDI2 Flex instrument (Bruker) was used for obtaining imaging data for both small molecule and peptide mass range. Accurate mass matching (AMM) was performed for peak identification against an in-house built crustacean neuropeptides and neurotransmitters database. The rest of the brain sample was retrieved and subjected to extraction for neurotransmitter and neuropeptide validation via LC-MS.

Results

Serial section showed similar localization. Protonated and sodiated neuropeptides were identified. 55 full-length neuropeptides were identified from various neuropeptide families, for example Allatostatin type A, Allatostatin type B, Pyrokinin, RFamide, Orcokinin, and SIFamide. Neurotransmitters such as choline, glutamate derivatives, aniline and adenine were successfully characterized with high mass accuracy. In total, there were 117 different phospholipids identified, including phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), and lysophosphatidylethanolamine (LPE). All spectra were checked manually to eliminate matrix peaks. Although phospholipids are not the targeting molecule here, their intensities are quite high comparing to the neuromodulators'. Chloroform rinsing will be performed in the future to get rid off the lipids and enhance the signal of the desired neuromodulators. Many peaks that were not matched to the database could be degradants of the neuropeptides or other ion adducts. MS/MS were performed to validate the identified neuropeptide peaks. However, due to the low analyte concentration, good MS/MS spectra was difficult to obtain for most of the target peaks. Further LC-MS validation is necessary for confident neuropeptide assignment. Neurotransmitter mapping and validation will also be performed. The stomatogastric nervous system (STNS) of crustacean, specifically innervating the foregut and midgut, would be a good model for feeding as well. Future steps include feeding experiments and looking at brain and STNS tissues.

Novel Aspect

This research couples timsTOF MALDI MSI and LC-MS together for high-accuracy neuromodulator localization in the crustacean nervous system.

Young, Lyndsay

Multi-Omic Application of MALDI-MSI and MALDI-IHC for Biomolecular Profiling of Immune Cells in Tissues and Single Cells

Lyndsay E.A. Young1,2, James W. Dressman1, M. Furkan Bayram1, Richard R. Drake1,2, Anand S. Mehta1,2

1Department of Cell and Molecular Pharmacology and Experimental Therapeutics, College of Medicine, Medical University of South Carolina, 68 President Street, Charleston, SC, 29425, USA, 2Hollings Cancer Center, Medical University of South Carolina, 86 Jonathan Lucas Street, Charleston, SC, 29425, USA

Introduction

Immune desert tumors, characterized by their lack of immune cell infiltration and response, pose significant challenges in the treatment. These tumor types exhibit resistance to immunotherapy, underscoring the necessity for advanced diagnostic techniques to better understand their microenvironment and cellular composition. N-glycosylation and glycogen reservoirs are indispensable features of both innate and adaptive immune cells for proper development, activation, and trafficking throughout the body.

Methods

To gain a better understanding of immune cell metabolic status and cellular composition, we employed Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) and MALDI-immunohistochemistry (IHC) for the spatially resolved analysis of biomolecular and cellular distributions at both the tumor tissue section and single-cell levels. We obtained serial clinical tissues sections of the immune cold tumors, colon, prostates and pancreatic cancers, and employed the high-throughput single cell array-based platform with antibodies for CD4, CD8, CD19, and CD14 to profile human PBMC isolates. For both tissues sections and single cells, N-glycans were released by PNGase F PRIME, glycogen accumulations were digested by isoamylase and cellular compositions were determined by established photocleavable-tagged antibodies from Ambergen Inc.

Results

We hypothesize that metabolically active immune cell clusters within prostate and pancreatic tumor tissues will exhibit a more intense N-glycan signal compared to immune-suppressed clusters. Using tissue samples from patients with various stages of thirty colorectal carcinoma, thirty-four prostatic and nine pancreatic adenocarcinoma, we have characterized the N-linked glycan profiles of ~250 immune cell clusters within and around these tumors through MALDI-MSI. N-glycan clusters exhibited signatures of ~25 high mannose, bisecting and branched N-glycans across all immune clusters. We also demonstrated distinct glycogen exhaustion in the majority of these immune clusters. Further, using the established photocleavable-tagged antibody suite (CD20, CD3, CD44, CD11b, CD68) we probed for an active immune microenvironment in these immune desert tumors. We identified unique cellular compositions of immune clusters of CD20+ B cells and CD3/CD44+ T cells. Finally, bulk and single-cell PBMC analysis revealed over 40 N-glycans of distinct N-glycan structural classes present in each immune cell subtype that cleanly differentiate CD4 and CD8 T-cells, and CD19 B-cells, each also with variable glycogen levels.

Novel Aspect

This multimodal interrogation enhances our knowledge of tumor biology and offers potential biomarkers for identifying patients who might benefit from specific therapeutic interventions. These insights pave the way for novel therapeutic strategies aimed at converting immune desert tumors to immune-responsive states, potentially improving the efficacy of immunotherapies and patient outcomes.

Zemaitis, Kevin

Combination of microfluidic models, passive imprinting, chemical derivatization, and in-silico predictive strategies for comprehensive spatial metabolomics in the rhizosphere

Kevin J. Zemaitis*1, Christopher R. Anderton1, Gregory W. Vandergrift1, Jayde Aufrecht1, Daisy Herrera1, Tanya Winkler1, Aiko Barsch2, Arne Behrens2, Nannan Tao3, Nikolas Kessler², Sofie Weinkouff², Sharon L. Doty4, Amir H. Ahkami1, Dušan Veličković1

1Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA , 2Bruker Daltonics GmbH & Co. KG, Fahrenheitstr. 4, 28359 Bremen, Germany, 3Bruker Daltonics LLC, 40 Manning Road, Billerica, MS, USA, 4University of Washington, Seattle, WA, USA"

Introduction

Mass spectrometry imaging (MSI) is a powerful tool for visualizing molecular distributions, with rapid implementation within spatial metabolomics. Nevertheless, numerous challenges remain related to molecular coverage, and annotation of isomers. Recently, on-tissue/on-target chemical derivatization (OTCD) has become popularized, enhancing the sensitivity and expanding applications of MSI for spatial metabolomics. Additionally, pre-mass analysis trapped ion mobility spectrometry (TIMS) provides orthogonal measurements of molecules, increasing confidence in high resolution accurate mass molecular measurements. Here, we explore advanced MSI with unique apparatus to investigate the poplar rhizosphere in tandem with new computational tools that enable in-silico prediction of derivatized molecules. Using unique microfluidic soil models, we capture gradients in controlled environments and complement this with passive imprinting of plants grown in native soil.

Methods

Direct analyses of thin sections of poplar tissue, and passive imprints of real and microfluidic soil models were analyzed by MALDI using either solariX FTICR or timsTOF fleX (Bruker Daltonics), or nanoDESI using hybrid 21T Ion trap-FITCR. MSI was processed in SCiLS Lab 2024b (Bruker Daltonics) or Mozaic (Spectroswiss). Annotations were performed using a preliminary version of MetaboScape 2025 (Bruker Daltonics) and/or with METASPACE. For MALDI, a two-step OTCD approach spraying 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) followed by 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide (4-APEBA) was used with a M5 Sprayer (HTX Technologies). Subsequently, derivatized samples were covered with 2,5-dihydroxybenzoic acid (DHB). Various standards (1 mg/mL) were derivatized in solution with the same reagents and analyzed by LC-MS with a timsTOF HT (Bruker Daltonics) with PASEF.

Results

Our work enabled confident annotation of over 200 primary metabolites within, and directly related to, the energy metabolism of poplar root with solariX FTICR MS and METASPACE annotation. Utilizing both synthetic soil models, termed Rhizochips, and mycorrhizal soil within Rhizobox apparatus housing mature poplar our developed OTCD and RhizoMAP protocols for passive imprinting of native soil captured the spatial dependencies of metabolites in broad field-of-views. This complemented nanoDESI and MALDI-MSI findings of the poplar rhizosphere with and without endophyte in Rhizochips. We present on these unique findings of metabolite distributions, and high-resolution MSI of bacterial hotspots within soil channels which may be indicative of exometabolomic processing. When 4-APEBA OTCD was combined with timsTOF fleX MS, not only did we detect the same carbonyl containing metabolites, including various aliphatic and aromatic carboxylic acids, aldehydes, and ketones. But with and without laser post-ionization (e.g., MALDI-2) we multiplexed detection of nearly every class of phospholipid, that remain underivatized by 4-APEBA. This included hard to detect TGs, which are enhanced with MALDI-2, and a variety of PC, PE, PG, and SM species. With TIMS enabled, we separated both isomeric and isobaric metabolites with MSI down to 5 µm lateral resolution on tissue sections. To provide accurate molecular annotations, datasets were imported into MetaboScape. Here, annotations were generated using in-house and open-source databases, as well as a newly developed in-silico workflow that generates CCS values of derivatized metabolites based on input structures for matching to these to extracted features. In practice, these efforts applying 4-APEBA OTCD chemistry within the rhizosphere with various modes of passive imprinting and advanced analytics translated to the ability to probe metabolites within distinct anatomical regions, as well as exuded metabolites within root hairs and root tip exudates. Providing the most comprehensive look into spatial dependence of the rhizosphere to date.

Novel Aspect

MSI is enhanced with multiple modes, OTCD chemistry, TIMS separations, and in-silico derivatization predictions for high confidence spatial metabolomics in poplar tissue and the rhizosphere.

Zhu, Yinyue

TIMSImaging: a Python package for trapped-ion mobility mass spectrometry imaging data visualization and preprocessing

Yinyue Zhu*1, Kylie Bemis1, Sai Lakkimsetty1, Andreas Weber2, Melanie Föll2, Olga Vitek1

1. Khoury College of Computer Sciences, Northeastern University, Boston, MA, USA, 2. Institute of Surgical Pathology, Medical Center, University of Freiburg, Faculty of Medicine, Freiburg, Germany

Mass spectrometry imaging(MSI) is a powerful tool to study biomolecules spatially. However, there is no separation in traditional MSI workflow, limiting peak capacity, dynamic range and specificity. Ion mobility is a fast separation technology compatible with MSI, which provides new data dimensionality to address these challenges, but the extra dimension makes the data difficult to process and store. There is no standard workflow to analysis MSI data with ion mobility in the community, and the lack of open-source software is a bottleneck. Here we developed a open-source Python package, TIMSImaging, to visualize and process MALDI(Matrix Assisted Laser Desorption/Ionization)-TIMS(Trapped Ion Mobility Spectrometry)-MS(Mass Spectrometry) data from Bruker's TIMS TOF instrument. The package provides functionality to interactively visualize data slices along different dimensions (image, 2D spectrogram, 1D spectrum and 1D mobilogram), which enables users to explore over the whole dataset easily. A two-dimensional peak-picking algorithm was implemented to find peaks in sparse data efficiently, the package visualized the peak-picking results as well to show the ability of ion mobility, which successfully distinguished ions with the same mass-to-charge ratio. The peak list could be exported for downstream statistical analysis like image classification and segmentation. All of these are integrated in a GUI that doesn't require Python expertise. We demonstrated the performance of TIMSImaging on a human kidney imaging dataset. Our method covered all 11 peaks detected by conventional 1D peak picking workflow, and further distinguished the signal of biological informative ions and the matrix.