APP NOTE: 137

DESI 2D

PROSOLIA

Molecular Imaging of lymph node metastasis using DESI MS

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INTRODUCTION

Desorption electrospray ionization (DESI) is the most commonly used ambient ionization technique. Unlike MALDI or SIMS, DESI is performed under ambient conditions, does not require high vacuum or matrix applied uniformly on the sample prior to analysis. This provides a relative inexpensive and direct technique for examination of tissues. Since the DESI experiment is like a solvent extraction, spray solvents can be tailored to selectively monitor desired species of interest. Because of the ease of obtaining data, many types of carcinoma have been investigated using DESI-MS. In most of cases, different lipid species were observed but distributions were different in normal versus carcinoma tissue. The non destructive nature of DESI-MSI allows post analysis H&E staining of tissue as confirmation of MSI data when morphologically friendly spray solvents are utilized.

In this study, DESI was coupled with an Agilent 6545 Q-TOF to study thyroid tumor that has metastasized to the lymph node.

EXPERIMENTAL

Frozen tissue sections were cut to 16 micron thickness and thaw mounted onto microscope glass slides for interrogation by DESI-MSI. The DESI spray solvent was 2:1 Ethanol / DMF delivered using a syringe pump at a flow rate of 2 µL/min. The nitrogen gas pressure was set to 180 psi. Alcohol / DMF mixtures are advantageous for imaging experiments as they tend to be morphologically friendly and are efficient for extractions of lipids from most tissue types. Raw data files from Agilent Mass Hunter software were converted to imzML format using Firefly® 3 (v3.1) software (Prosolia, Inc). Images were generated using SCiLS Lab MVS (scils.de/).

RESULTS

The lipids in the lymph node tissue have been characterized previously (1). In thyroid cancer, increases abundance of several ceramides and glycophosphoinositols were observed. Mass spectra from thyroid cancer metastasis and the adjacent lymph node tissue are illustrated in **Figure 1.** PI(36:4) (*m/z* 857.5322) was noticeably higher abundance than other lipids in the tumor region of the tissue. Other differences in relative abundance for *m/z* 834.5402, 835.5429, 848.6104, and 858.5338 were also observed but subsequent images did not provide significant visual evidence of tumor or healthy tissue.

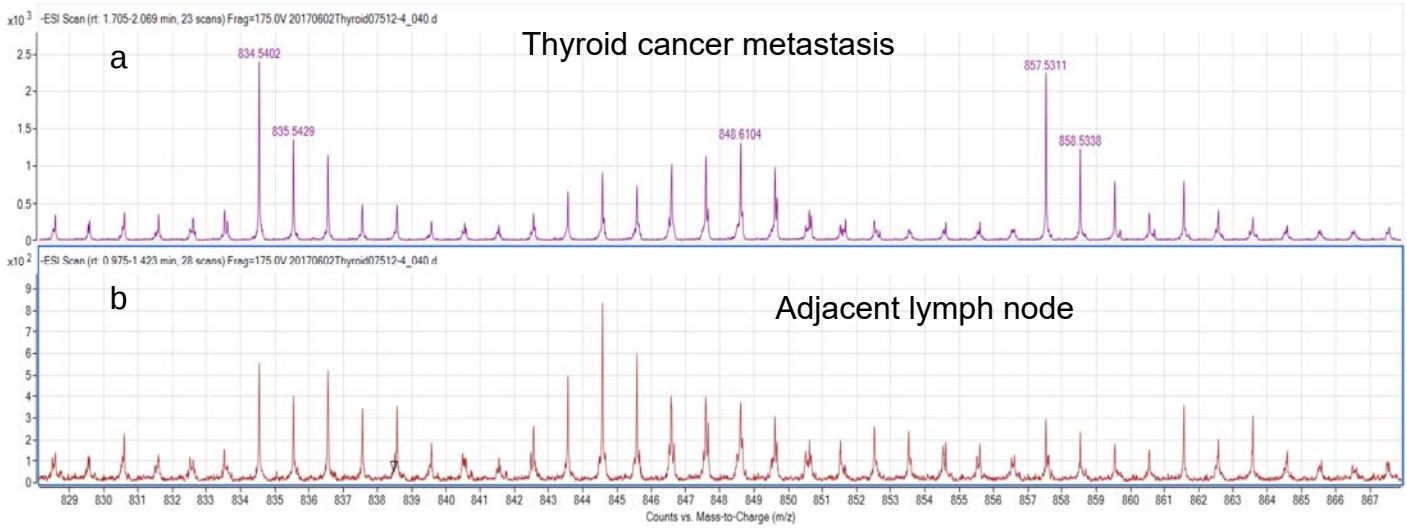

Figure 1: Mass spectra from (a) metastasis tumor region and (b) adjacent lymph node tissue indicating higher abundance of *m/z* 857.5322 in tumor section.

Using a statistical feature within SCiLS laboratory software called segmentation pipeline, two regions of interest were identified within the lymph node tissue samples. These regions of interest (ROI) are illustrated in **Figure 2** as red and black areas. MS image of *m/z* 572.492 (Cer(43:1)) from the red ROI is illustrated in (b) and primary lipid m/z 857.5322 (PI(36:4) from the black ROI is illustrated in (c). An optical image of the tissue (d) is included for reference. The relative abundance of ascorbic acid (*m/z* 175.0276) and glutamic acid (*m/z* 146.049) were also higher in the tumor versus normal tissue. These molecular species proved to be good markers for the tumor and using these molecular ions, tumor and normal lymph node tissue margins can easily be distinguished.

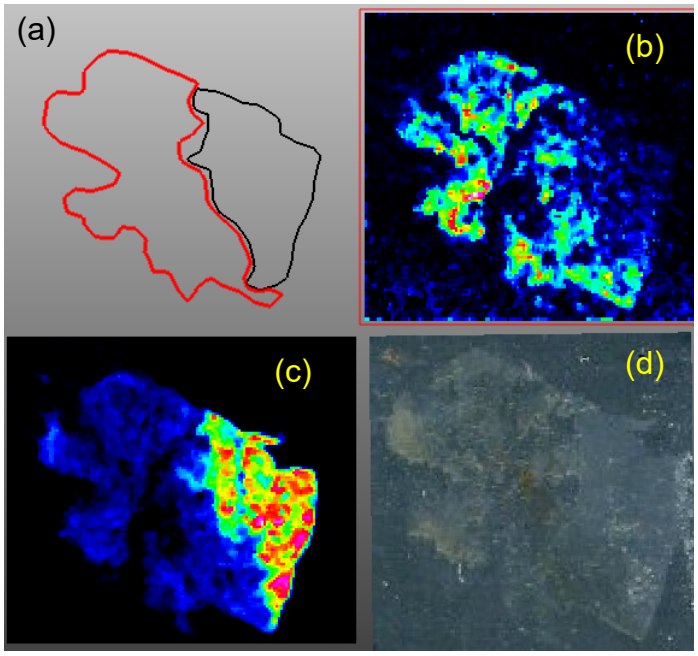

Figure 2: (a) Segmentation image of tissue indicating two regions of interest. (b) MS image of *m/z* 572.492 (Cer(34:1) from Red ROI , and (c) *m/z* 857.5322 (PI(36:4) from Black ROI (d) Optical image of lymph node tissue.

The primary lipids observed in this study were identified based on accurate mass and the Human Metabolome Data Base (*www.hmdb.ca*) and summarized in **Table 1**. Images from SCiLS laboratory software for each lipid are presented in **Figure 3.** These images illustrate the ability of DESI-MS Imaging to detect tumors in tissue and provide real time analysis to aid possible clinical procedures.

Table I: Summary of identified lipids present in lymph node tissue and tumor

Compound	Formula	Measured Mass $[M-H]^-$	Theoretical Mass	Delta ppm
Glutamic Acid	$C_5H_9NO_4$	146.0490	147.0531	16
Ascorbic Acid	$C_6H_8O_6$	175.0286	176.032	22
Cer(34:1)	$C_{34}H_{67}NO_3$	572.4920	537.5121	18
PS(36:1)	$C_{42}H_{80}NO_{10}P$	788.5554	789.5520	14
PE(42:5)	$C_{47}H_{84}NO_8P$	820.5740	821.5935	15
PI(36:4)	$C_{45}H_{79}O_{13}P$	857.5322	858.5258	16
PI(36:2)	$C_{45}H_{83}O_{13}P$	861.5635	862.5571	16
PI(38:4)	$C_{47}H_{83}O_{13}P$	885.5640	886.5571	16
PI(38:4)	$C_{47}H_{85}O_{13}P$	887.5738	888.5728	9

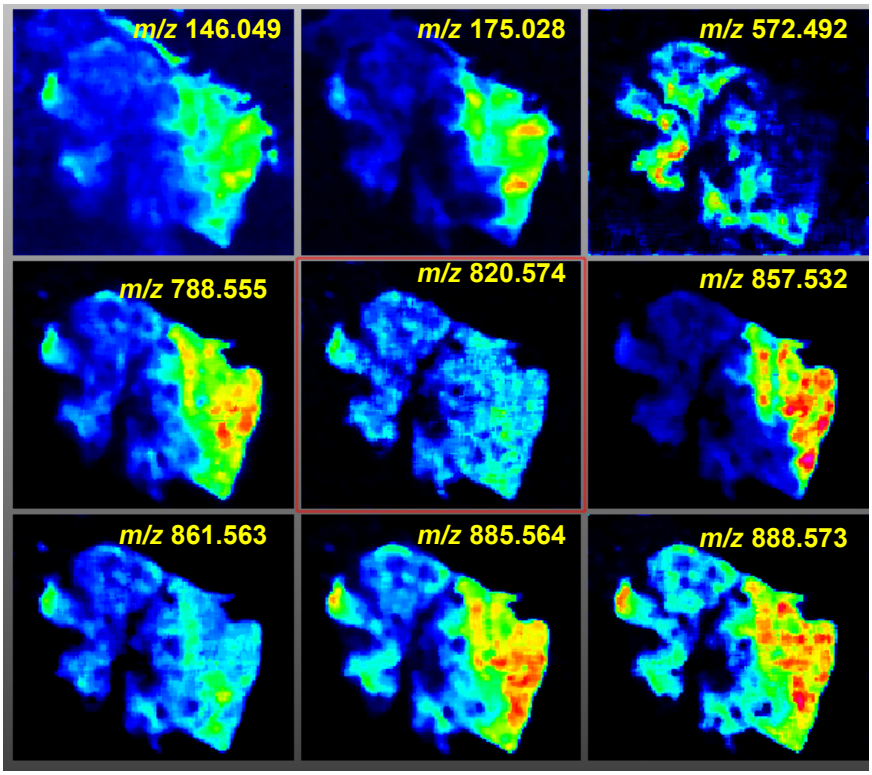

Figure 3: DESI-MS images from lymph node tissue with tumor. Images represent nine different molecular ions commonly present in lymph node tissue samples. Note ascorbic acid (*m/z* 175.0286) and PI(36:4) (*m/z* 857.5322) were noticeably higher intensity in the tumor section of tissue.

CONCLUSION

These results illustrate that DESI-MS provides a rapid and quantitative approach to Mass Spectrometry Imaging without the addition of chemical matrices to the sample. The combination of DESI with the Agilent 6545 Q-ToF yields powerful phenotyping data on tissue samples revealing key lipid and metabolite signatures.

REFERENCES

1) Jialing Zhang, Clara Feider, Chandandeep Nagi, Wendong Yu, Stacey A.Carter, James Suliburk, Hop S. Tran Cao, Livia S. Eberlin **J Am Soc Mass Spectrom**. 2017 28:1166-1174

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