Mass Spectrometry Imaging for Protein Localization and Characterization in Thermoembolized Hepatic Porcine Tissue

Jacob E. Porter[1]; Danielle Stolley[2]; Erik N.K. Cressman[2]; Erin H. Seeley[1]

[1]University of Texas at Austin, Austin, TX, [2]University of Texas MD Anderson Cancer Center, Houston, TX,

OVERVIEW

Purpose:

- To determine the spatial distribution of proteins in porcine liver tissue treated with thermoembolization reagents, and determine biomolecular differences in regions of thermoembolized tissue by correlation with histologically-stained serial sections.

Methods:

- Transarterial *in vivo* delivery of thermoembolization reagents
- MS imaging of proteins
- H&E staining

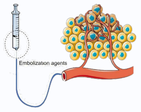
Results:

- Several protein signals were localized to areas of tissue damage and nonviable tissue
- Comparison with *ex vivo* treatment showed several differences from transarterial delivery

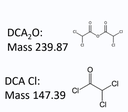
INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, with a 5-year survival rate of <10%. Embolotherapy is a standard treatment for primary liver cancer, but is often unsuccessful at completely eradicating treated tumors. Thermoembolization, involving simultaneous exothermic reactions in parallel to embolization, has shown recent promise. However, the mechanism of action between reagents and tissue upon injection is unclear. Here, mass spectrometry imaging (MSI) is used to determine the spatial distribution of proteins in thermoembolized tissue and protein changes in comparison to both untreated control tissue and control tissue treated directly *ex vivo* with thermoembolization reagents.

METHODS



- Transarterial delivery of thermoembolization reagents
- Reagents delivered in segmental hepatic artery branch using ethiodized oil as a vehicle



- 2M Dichloroacetic anhydride (DCA_2O) or DCA chloride (DCA Cl)+ lipiodol, + Evans blue/caffeine + benzothonium chloride

- Outbred swine treated, housed overnight, scanned by CT and euthanized
- Hepatic tissue snap frozen and sent for MSI

- Thermo NX50 Cryostat
- Tissue sectioned at 12 µm thickness
- Collected on ITO slides
- Serial sections for H&E staining

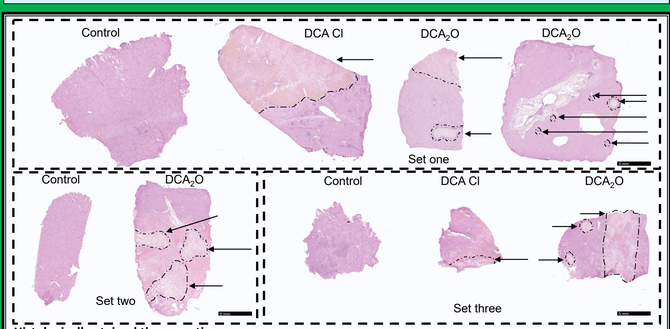
- Hamamatsu NanoZoomerSQ
- Digital microscopy images acquired at 40X magnification
- Images extracted at 10x magnification

- HTX M5 Sprayer
- Sections washed with Carnoy's fluid
- Sections spotted with 1 µL DCA Cl or DCA_2O for *ex vivo* comparison
- Sprayed with 10 mg/mL SA in 90% ACN, 0.1% TFA, followed by rehydration in 22% HOAc at 85°C for 3.5 min

- Bruker rapifleX MALDI TOF/TOF
- Positive linear ion mode, *m/z* 2,000-20,000
- Images acquired at 100 µm spatial resolution using FlexImaging

- Images combined for normalization and visualization in SCiLS Lab Pro 2021b
- Mass spectra extracted from SCiLS Lab Pro
- All intensities normalized to root mean square
- All images extracted with a ± 8 Da window

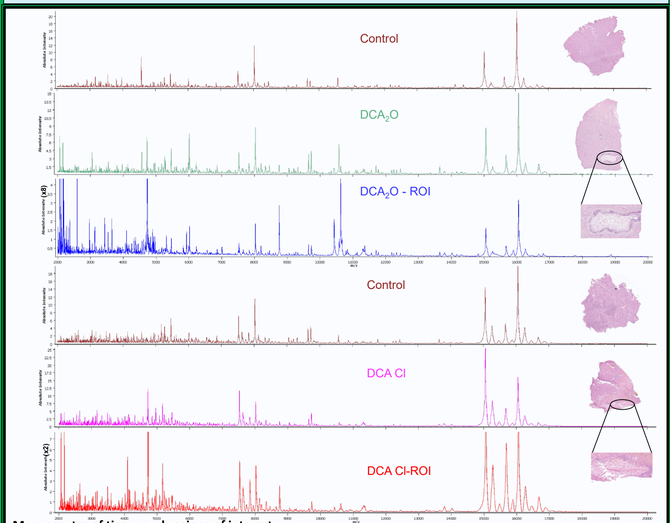
Histological Staining



Histologically-stained tissue sections

Three sets of tissue stained with Hematoxylin & Eosin are shown above, including control tissue and tissue from DCA Chloride/DCA Anhydride *in vivo* treatment. Necrotic regions are delineated by dashed lines and arrows.

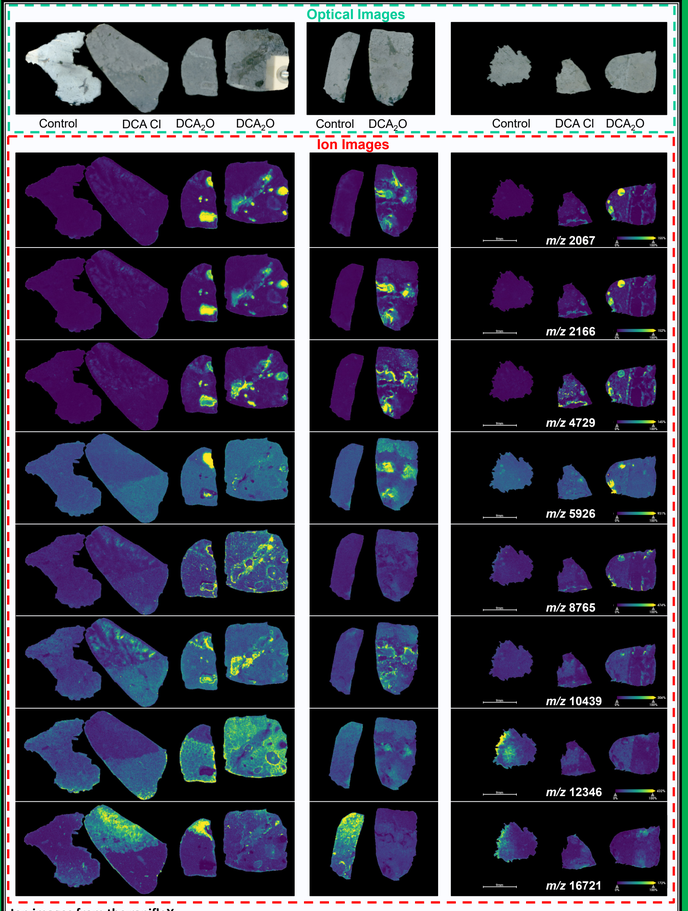
Mass Spectra



Mass spectra of tissue and regions of interest

Mass spectra are shown from set one (top) and set three (bottom), including control tissue, tissue from DCA Chloride *in vivo* treatment and tissue from DCA Anhydride *in vivo* treatment, with circled regions of interest. While overall spectra from control and treated tissue are similar, many *m/z* values are increased or decreased in regions corresponding to tissue damage.

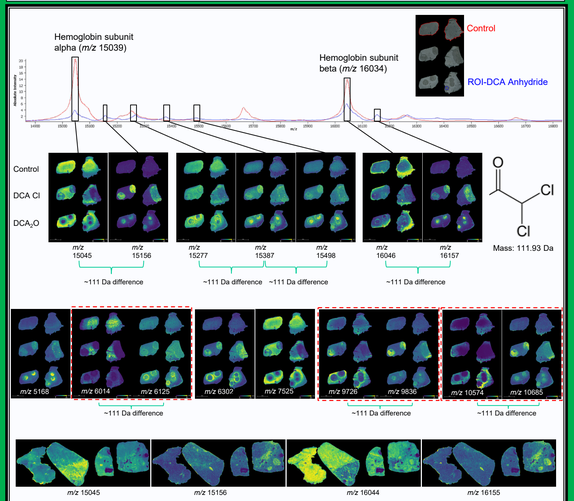
Comparison of Control and *in vivo* Treated Tissue



Ion images from the rapifleX

Several signals were exclusively localized to areas of necrotic tissue identified from histologically-stained sections. Certain signals (e.g. those at *m/z* 2072 and 2176) are localized in areas of coagulative necrosis, while others (*m/z* 4726, 5926, 8754 and 10431) are present in the small ring of neutrophils around damaged tissue. Signals such as those at *m/z* 12334 highlight the system of connective tissue, and are only present in healthy tissue.

Comparison of *ex vivo* and *in vivo* Treated Tissue



Analysis of modified proteins from direct treatment with DCA Anhydride and DCA Chloride.

MSI of directly-treated tissue sections (top) showed modified hemoglobin alpha and beta subunits, with additional peaks at +111 Da in treated tissue corresponding to dichloroacetylated peaks. Other peaks through the spectrum showed similar patterns matching dichloroacetylation (highlighted in red, and additional *m/z* values enriched or depleted in treated tissue (middle). There were low-intensity signals matching the *m/z* of modified hemoglobin in select areas of *in vivo*-treated tissue (bottom), but these were also present in control samples and localized to holes in the tissue, and likely an artifact of slide conductance.

CONCLUSIONS AND FUTURE DIRECTIONS

- Distinct proteomic differences were observed between control and *in vivo*-treated tissue
- Regions of damage from thermoembolized tissue were identified from H&E-stained serial sections, and correlated to protein signals at specific and replicable *m/z* values.
- Direct *ex vivo* treatment with both DCA chloride and DCA anhydride showed modified protein signals correlating to dichloroacetylated hemoglobin.
- The present work has found substantial, reproducible alterations in the proteomic profile of targeted treatment with thermoembolization agents in mammals, as well as *ex vivo* evidence of direct reactions between proteins and thermoembolization agents, giving insight into the mechanism of action of chemotherapeutic agents in mammalian biological systems.
- Further treatment with non-exothermic embolization reagents may help to elucidate the mechanism of tissue damage in embolic processes, by diffusing further into tissue and avoiding the extensive tissue damage associated with DCA reagents.

ACKNOWLEDGEMENTS

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