Program Development for MALDI-MSI Data Processing

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Introduction

High throughput biomarker identification using Matrix Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) can increase diagnostic accuracy and efficiency at a lower cost, leading to better patient outcomes. One challenge in MALDI-MSI is the extensive manual data processing. This project aims to develop computer programming templates through R scripts for streamlined data processing based on putative peptides associated with diseases of interest. For the purposes of this study, we examined proteins of interest associated with mitochondrial dysfunction.

Methods

Tissues were collected then formalin-fixed, and paraffin-embedded (FFPE). Tissues were sectioned (5 μM) and mounted onto either ITO-coated or glass slides. Slides were rehydrated prior to antigen retrieval in a 10 mM citrate buffer. For mass spectrometry analysis, a robotic TM sprayer system was used for on-tissue tryptic digestion and subsequently quenched with the α-Cyano-4-hydroxycinnamic acid (CHCA) matrix application. Slides were imaged using a Bruker RapifleX MALDI Tissuetyper TOF Mass Spectrometer.

Results

This project utilizes data from MALDI-MSI of rat prostate. Optimization has demonstrated that 5 μM sections are sufficient for adequate putative peptide identification, and using a decloaker at 95°C for 15 minutes, provided a robust signal for MALDI-MSI. Going forward, further adjustments will be made to improve spatial resolution and adjust matrix types for targets of interest.

Using more than proteins related to mitochondrial function MALDI peak data points were processed through R v. 4.3.1 programs developed in our labs to sort and quantify the putative peptide images of interest for each tissue. Initial data supports MALDI-MSI as a robust method for identifying spatial and quantitative data about proteins of interest in a higher throughput manner than traditional methods. Continued exploration will show that MALDI-MSI is an excellent tool for examining protein expression while accounting for the heterogeneity associated with disease states in the prostate.

Conclusions

Development and optimization of MALDI-MSI data analysis methods for prostate disease models using FFPE tissues will increase the efficiency of protein identification data analysis and make the method more accessible.

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