Analysis of FFPE treated clinical tissue sections obtained from Human Intraocular Malignancy, Uveal Melanoma by Mass Spectrometry Imaging (MSI)

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Introduction

Mass spectrometry (MS) imaging allows the investigation of the spatial distribution of molecules at complex surfaces. The combination of molecular identification and location provides a chemical microscope that can be used for the direct biomolecular characterization of histological tissue surfaces. MS based imaging allows label-free detection and mapping of a wide-range of biological compounds whose presence or absence can be the direct result of disease pathology. Here we describe clinical research into FFPE tissue sections obtained from the human intraocular disease Uveal Melanoma (UM), which remains the most common intraocular malignancy in adults. It is known that over 90% of patients develop UM within the choroid region.

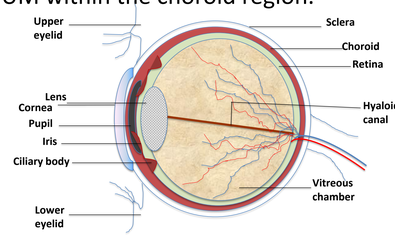

Figure 1. Cross section of the eye

Unlike cutaneous melanoma, UM metastases invade distant sites via the bloodstream. As a result metastases spread to the liver, lungs, skin and bones. Imaging of removed organs and tissues is routinely performed to support clinical patient diagnosis. Two MS imaging techniques, Matrix Assisted Laser Desorption/Ionisation (MALDI) and Desorption Electrospray Ionisation (DESI) (fig. 2) were applied to samples to determine the utility of this imaging modality in this setting. Molecular profiles were obtained and analysed by multivariate statistical approaches, providing insight into the biochemical and biological differences/similarities within the patient sample cohort (n=15).

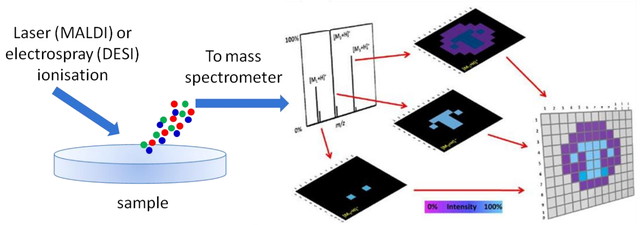

Figure 2. Outline of mass spectrometry Imaging. A laser or electrospray is used to generate a plume from the surface of a sample. The mass spectra at each point is analysed via mass spectrometry and used to generate an image showing the spatial localisation of m/z values and generate a molecular profile of the tissue.

Methods

Enucleations were collected following ocular surgery over a 6 year time period and subjected to standard fixation and paraffin embedding protocols. 5um sections were used for MS Imaging. Tissue sections were prepared for analysis by MALDI MSI by applying a solution of matrix onto the tissue sections to help the ionisation of molecules directly from the tissue. For DESI, no further slide preparation was required. All MS Imaging experiments (MALDI and DESI) were carried out using a version of SYNAPT mass spectrometer (Waters Corporation, Manchester, UK). Multivariate analyses were performed using MATLAB (MathWorks, Inc., MA, US) in conjunction with the Eigenvector PLS_Toolbox.

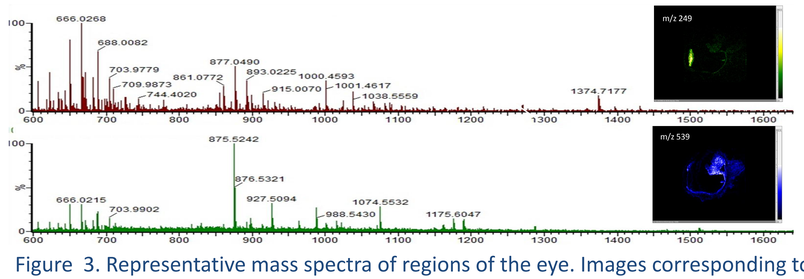

Figure 3. Representative mass spectra of regions of the eye. Images corresponding to selected m/z values are shown inset.

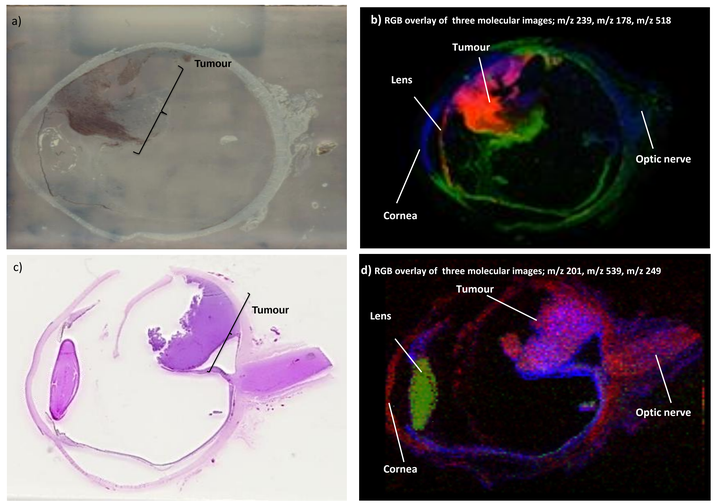

Figure 4. Mass spectrometry images compared to standard techniques. a) photographic image of eye section, b) MALDI image of three overlayed m/z values showing distinct regions identified by MS imaging, c) H&E stained section of eye, d) DESI image of 3 overlayed m/z values showing distinct regions identified by MS imaging.

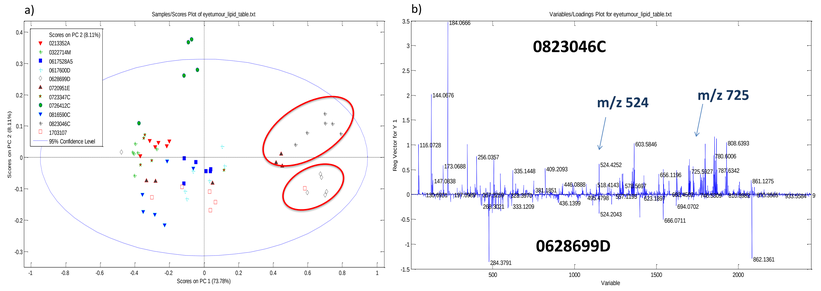

Figure 5 Multivariate statistical analysis of the patient sample cohort in the lipid and metabolite mass range m/z 100-1000 using PCA (a) and PLS-DA (b)

Results

Initial MALDI MSI images acquired with a spatial resolution of 100 µm x 100 µm in positive ion mode showed the distinct spatial distribution of many molecular species throughout the various regions of the eye; choroid, cornea, retina, lens and UM tumour regions. Further MALDI MS imaging experiments using consecutive tissue sections at 50 µm x 50 µm spatial resolution showed substantial variation in the spatial distribution of species within the low molecular mass range (Figure 4b). DESI MS imaging experiments were carried out at 200 µm x 200 µm in negative ionization mode. Deprotonated molecular ions could be detected, from a variety of lipid related species, localized to specific regions within the eye including the tumour region (fig. 4d). Multivariate statistical analysis was used to classify between UM samples (good vs. poor prognosis). Using unsupervised PCA, an unbiased representation of the data was generated, with clear sample grouping and differentiation observed based upon tumour status (fig. 5a). Use of the supervised PLS-DA (PLS-discriminate analysis) technique provided even clearer separation between the selected samples (fig. 5b), with the tumour profiles displaying dominant discriminatory peaks. These peaks could be identified as sphingolipids and Lyso-phosphocholine, a phosphatidylcholine degradation product.

Conclusions

The use of MS imaging allowed for the profiling of specific small molecular species localized to certain tissue types, including Uveal Melanoma. Distinct regions relating to numerous species could be readily determined within the eye without the need for additional labelling techniques, and differences between certain lipids/metabolites were readily observable via statistical modelling. These studies show the potential value of mass spectrometry imaging as a complementary pathological technique, with the ability to identify both healthy and tumour tissue.