Rapid Detection of Necrosis in Breast Cancer with Desorption ElectroSpray Ionization Mass Spectrometry

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Necrosis in tumors is often associated with aggressive forms of cancer and is of prognostic value in treatment planning. Intraoperative pathological is the most common method of detecting necrosis. This takes 10-30 min, and only permits analysis of a limited number of samples.

Mass Spectrometry (MS) can rapidly identify necrosis in a tumor sample but the technique requires known and validated MS profile of necrotic breast cancer.

Our Solution:

• Determine MS lipid profile of necrotic breast cancer and validate the profile using blind tests and independent samples.

Materials & Methods:

Murine xenografts of human breast cancer from LM2-4 cell line were subjected to slicing, optical imaging, polarimetric imaging, Desorption ElectroSpray Ionization Mass Spectrometry (DESI-MS) Imaging, Pathology and statistical analysis of the DESI-MS spectra.

Mass Spectrometry Analysis and Imaging:

Spatially resolved molecular content of tissue slices, interpreted using pathology, allow the MS profile (i.e. biomarker ion library) of pathohistological heterogeneities to be determined.

Polarimetry Guided Mass Spectrometry:

Areas of polarimetric heterogeneity suspected of pathology are determined and subjected to target MS analysis. Regions of interest (ROI) are defined based on depolarization ratio and imaged with DESI-MS to reduce the data collection time.

Optical Imaging:

1. OPTICAL IMAGING (10 SECONDS)

2. POLARIMETRIC IMAGING (60-120 SECONDS)

Validation of DESI-MS Imaging Results:

1. ACQUIRE MS data
2. REPEAT on another slice
3. CONSTRUCT ion images based on intensities

Polarimetry-based Mass Spectrometry:

Figure 1. DESI-MS imaging of necrotic breast cancer tumor. (A) The average DESI-MS spectrum of the viable cancer site. (B) The same area as in (A) but with the ROI boundaries marked in red (C) The ROI boundaries are shown in red (D) The histogram shows that the ROI is less than 1% of the total area.

Figure 2. The DESI-MS ion images of the consecutive slices of the "training" tumor. The ion images were created with the known and validated MS profiles of necrotic breast cancer.

Figure 3. Principal Component Analysis (PCA) of the necrotic breast cancer: (A) PCA scores plot shows the statistical discrimination between MS profiles of the necrotic and viable breast cancer regions within the same tumor. The PCA scores for components 1,2,3 are determined by the statistical heterogeneity of the sections. (B) The box plot showing changes in the absolute intensity of biomarker ions selected by the PCA method to contribute most significantly to the statistical separation (loading plots not shown).

Figure 4. The DESI-MS ion images of the consecutive slices of tumor 1. The ion image of the same slice is also given. The ion images show the prominent peak of marker ion and the different MS profile of the necrotic tumor region.

Figure 5. The DESI-MS ion images of the consecutive slices of tumor 2. The ion images showing the prominent peak of the marker ions and the different MS profile of the necrotic tumor region.

Figure 6. The DESI-MS analysis of tissue smears. The representative ion images including histograms of the smear using Known Ions (KI). (B) Single MS scans of the necrotic tumor region on porous PTFE. (C) The H&E stained image of tumor 1. The staining of the necrotic regions is highlighted with yellow arrows. (D) The H&E stained image of tumor 2. The staining of the necrotic regions is highlighted with yellow arrows.

Figure 7. Pathology assessment of all tumors used in this study using H&E staining and microscopy. (A) The viable region of the "training" tumor. (B) The necrotic region of the "training" tumor. (C) The H&E image of tumor 1 shown in Fig. 4 that contains a uniform distribution of the necrotic regions highlighted with yellow arrows. (D) The H&E image of tumor 2 shown in Fig. 5 that contains a uniform distribution of the necrotic regions highlighted with yellow arrows.

In Situ Detection

Figure 8. In Situ laser ablation MS. (A) Laser output and MS settings. (B) Laser spot location. (C) Mass spectrum from laser-ablated section. (D) Mass spectrum from laser-ablated section with a 3 mm Tygon tube and heated to provide desorption and soft ionization of aliphatic material. The laser was then scanned across the tissue with a 200 μm spot size. (E) Mass spectrum from laser-ablated section with a 200 μm spot size and a 3 mm Tygon tube and heated to provide desorption and soft ionization of aliphatic material. The laser was then scanned across the tissue with a 200 μm spot size.

Figure 9. Schematic of S hallmark laser probe based on Picoscanert (integrated Laser (PILS) ablation soft ionization mass spectrometry (MS)). The MS spectra were acquired from a 2.5 mm sample within a 2 x 2 mm area using a 200 μm spot size. The laser was then scanned across the tissue with a 200 μm spot size.

Conclusions:

• Rapid detection of necrosis in breast cancer in less than 1 min of overall sample preparation is possible with ex vivo and in situ mass spectrometry.
• Tissue depolarization ratio is affected by necrosis and can guide DESI-MS to viable/necrotic cancer border.
• The ceramide ion of m/z 572.48 is a marker for necrotic breast cancer.

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