MALDI-Ion Mobility Separation- Mass Spectrometry Imaging for direct Biomarker Targeting in Human Breast Adenocarcinoma

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Overview

- *In situ* investigation and characterisation of tumour markers and metastasis-associated proteins in formalin fixed paraffin embedded (FFPE) human breast cancer tissue sections.
- MALDI-IMS-MSI enabled the visualisation of the distribution of biomarkers including heat shock proteins (Hsps) directly within early stage and metastatic lymph node breast cancer tissue sections.

Introduction

Metastasis is a complex molecular process which occurs often in cancers. It involves a multistep molecular mechanism in which tumour cells escape from the primary tumour site to other distant organs, hence creating new neoplasm environments [1]. Tumour growth and progression also results from defects in apoptotic pathways during cell cycles. Protein markers of tumour progression and metastasis formation are required in order to improve therapies and hence target appropriate genes and/or proteins. [2]. MALDI-mass spectrometry imaging (MALDI-MSI) is a powerful technique that allows the study of the distribution and identification of proteins directly from tissue sections. Recently the use of the ion mobility separation (IMS) in combination with MALDI-MSI has been found to improve the selectivity and specificity of the technique for the investigation of peptide distribution in tissue sections as well as the direct identification of proteins after performing on-tissue digestion [3]. In the work presented here, FFPE early stage (ES) and metastatic lymph node (MLN) breast tumour tissue sections were analysed using MALDI-IMS-MS Imaging in order to perform a comparison study and highlight potential protein markers that can be used as indicators of tumour progression and metastasis-associated proteins.

Methods

- Following fully informed patient consent and full ethical committee approval anonymised **5 µm *ex vivo* human FFPE breast tumour tissue sections** were obtained.
- **Tissue preparation**: paraffin wax was removed from tissue sections using xylene followed by tissue section rehydratation in series of ethanol solutions. Antigen retrieval was performed in a microwave oven for 15 minutes.
- **Protein investigation**: *In situ* digestion was performed using trypsin. Trypsin solution containing 0.1% octyl glucoside was deposited using a SunCollect™ (SunChrom,Germany) automatic sprayer. α-CHCA/aniline was used as matrix and deposited onto the tissue sections using a SunCollect™ automatic sprayer.
- **Data acquisitions** were carried out using a MALDI SYNAPT™HDMS system (Waters Corporation, UK) operating with a 200 Hz Nd:YAG laser in the V-mode and positive mode with ion mobility separation.
- **Images** were acquired at 150 µm spatial resolution and generated using Biomap 3.7.5.5 software [4]. Statistical analysis was performed using MarkerView™ software (Applied Biosystems/MDSciex).

Results

Study of the distribution and *in situ* identification of tumour markers in FFPE human early stage breast cancer tissue sections

Figure 1: Identification of proline oxidation as protein modification via *de novo* sequencing. The peptide signal at m/z 1286 was identifed as arising from histone-lysine N-methyltransferase protein.

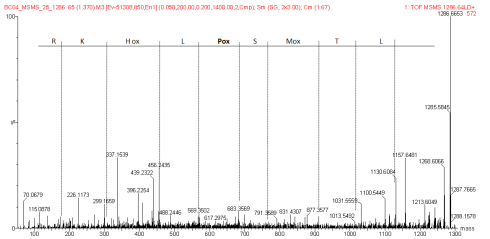


Figure 2: Peptides identified after *in situ* digestion and MALDI-IMS-MS/MS analysis of FFPE early stage breast cancer tissue sections were classified into molecular functions using the PANTHER classification system [5].

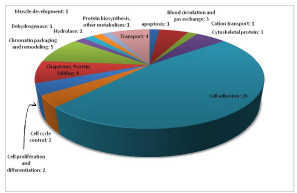
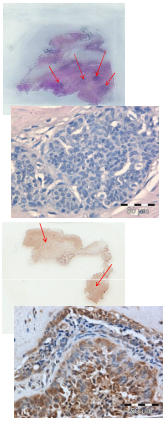
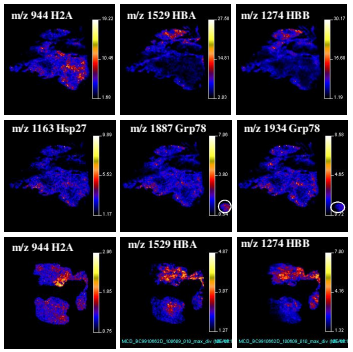


Figure 3: HE and immunohistochemistry staining images of FFPE early stage breast tumour tissue sections where tumour regions are indicated with red arrows.

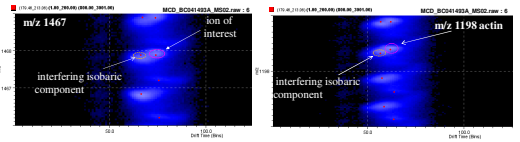


MALDI-IMS-MS images of the distribution of proteins within FFPE early stage breast tumour tissue sections. Hsps were found to be mainly located in tumour regions.



Study of the distribution of metastasis-associated proteins in FFPE human breast cancer metastatic lymph node tissue sections

Figure 4: Driftscope™ plots of peptide signals at m/z 1467 and actin (m/z 1198). Separation of isobaric ions allowed the obtaining of improved statistical analysis and the generation of the exact distribution of peptides within FFPE breast tumour tissue sections.



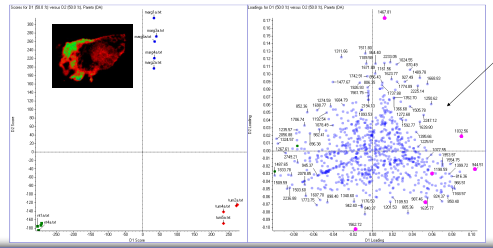
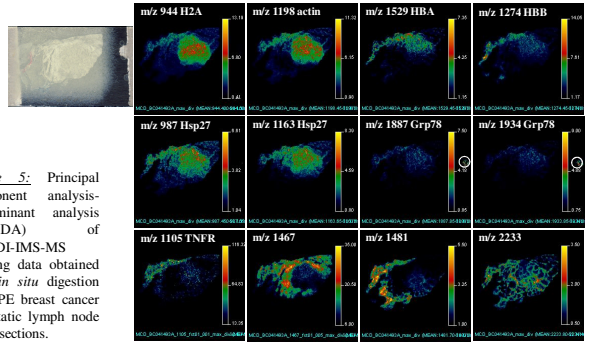


Figure 5: Principal component analysis-discriminant analysis (PCA-DA) of MALDI-IMS-MS imaging data obtained after *in situ* digestion of FFPE breast cancer metastatic lymph node tissue sections.

Figure 6: MALDI-IMS-MS images of the distribution of peptides after *in situ* digestion of FFPE breast cancer metastatic lymph node tissue sections. The identification of proteins is included when confirmed with on-tissue MALDI-IMS- MS/MS analysis.



Discussion

i) Protein modification: The identification of proline oxidation, which may result from the sample preparation, facilitated and improved the MASCOT search and *de novo* sequencing for protein identification.

ii) In situ identification: Several peptides were detected and identified within FFPE early stage breast cancer tissue sections, including tumour markers such as Hsp27 and Grp78, using both bottom-up and top-down proteomic approaches with MALDI-IMS-MSI. Identified proteins were classified into molecular functions using the PANTHER classification system.

iii) Statistical analysis: Using the identified proteins from FFPE early stage breast cancer tissue sections and PCA-DA allowed the localisation of tumour cells within FFPE metastatic lymph node (MLN) breast tumour tissue sections, hence the targeting of metastasis-associated proteins. Tumour regions were localised in FFPE MLN breast cancer tissue sections using tumour protein markers identified within FFPE early stage breast cancer. Additional proteins, including tumour necrosis factor receptor and interleukin 1, were also identified.

iv) MALDI-IMS-MSI: The high efficiency of IMS enabled the generation of peptide/protein localisation within both FFPE early stage and metastatic lymph node breast cancer tissue sections while minimising peak interferences.

Conclusion

- The successful identification of tumour biomarkers, including heat shock proteins such as Hsp27 and Grp78 is described here. The study of their distribution across the tissue sections revealed that these proteins were mainly located and highly intense in tumour regions of FFPE early stage and MLN breast cancer tissue sections.
- This demonstrated the ability of the technique to identify tumour markers as well as potential metastasis-associated proteins with no requirement for pre-defined targets.
- The identification of proline modification improved protein identification following direct MALDI-IMS-MS/MS analysis.

Further Work

- Method development is still required to overcome on-tissue protein identification as well as improved top-down MALDI-MSI for biomarker discovery.

References

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Acknowledgements

✓ BMRC/Clinical Research Fellow scheme for project funding.
✓ The International Mass Spectrometry Foundation is gratefully acknowledged for their financial assistance.

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