

Targeting of Hypoxia-related Proteins in AQ4N treated solid Tumour Xenografts by MALDI-Ion Mobility Separation- Mass Spectrometry Imaging

Marie-Claude Djidja¹, Emmanuelle Claude², Paul M. Loadman³, Chris W. Sutton³, Vikki Carolan¹ and Malcolm R. Clench¹.

¹Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield UK S1 1WB, ²Waters Corporation, Manchester, UK, ³ Institute of Cancer Therapeutics, University of Bradford, Bradford, UK

Overview

- *In situ* investigation and characterisation of hypoxia-related protein markers in AQ4N treated tumour xenografts.
- MALDI-IMS-MSI enabled the visualisation of the distribution of AQ4N and AQ4 within the tissue sections, hence the selective localisation hypoxic tissue regions.
- Protein distribution and identification were obtained directly from AQ4N treated and non treated tumour tissue sections after *in situ* digestion.

Introduction

Hypoxia is a common feature in most solid human tumours. It results from an inadequate supply of oxygen within the tumour due to creation of an abnormal tumour vasculature system [1]. Tumour hypoxia has been found to be closely associated with tumour progression and aggressiveness and confers resistance to chemotherapy as well as radiotherapy. AQ4N (banoxatrone) is a bioreductive prodrug currently under clinical investigations which is selectively activated in hypoxic regions in tumour [2,3]. MALDI-mass spectrometry imaging (MALDI-MSI) is a powerful technique that allows the study of the distribution and identification of proteins directly from tissue section. 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 [4]. In the work presented here the localisation and *in situ* identification of hypoxia-related proteins in correlation with the distribution of AQ4N in tumour xenografts are studied using MALDI-IMS-MSI.

Methods

- SW620 human tumour-bearing mice were treated with AQ4N (60 mg/kg, intraperitoneally (i.p)). 12 µm sections of treated and non-treated xenografts were obtained using a cryostat at -20°C.
- **AQ4N investigation:** α-CHCA was used as matrix and deposited using a gravity fed pneumatic air spray gun set at 40 psi.
- **Protein investigation:** *In situ* digestion was performed using trypsin. Trypsin solution containing 0.1% octylglucoside was deposited using a SunCollect™ (SunChrom, Germany) automatic sprayer. α-CHCA/anilin 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 [5]. Statistical analysis was performed using MarkerView™ software (Applied Biosystems/MDSSciex).

Results

Investigation of the distribution of AQ4N and its active metabolite AQ4 within tumour xenografts with MALDI-IMS-MSI

Figure 1: Schematic representation of the reductive metabolism of AQ4N in tissue [6].

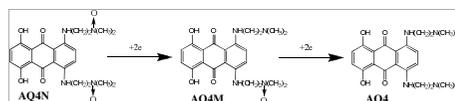
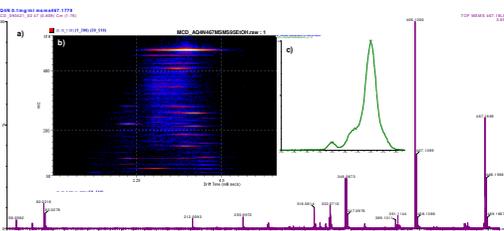


Figure 3: MALDI-IMS-MS/MS of AQ4N sodium adducts (m/z 467.2). a) MALDI-IMS-MS spectrum of AQ4N m/z 467.2. When spiked on tissue, 3 isobaric ions are observed: b and c display the ion mobility separation of these ions.



In situ investigation of the expression of proteins within non-treated and AQ4N dosed xenografts with MALDI-IMS-MSI

Figure 5: MALDI-IMS-MS images of the distribution of peptides within non-treated and AQ4N dosed tumour xenografts after on-tissue digestion. Differences in protein intensities were observed

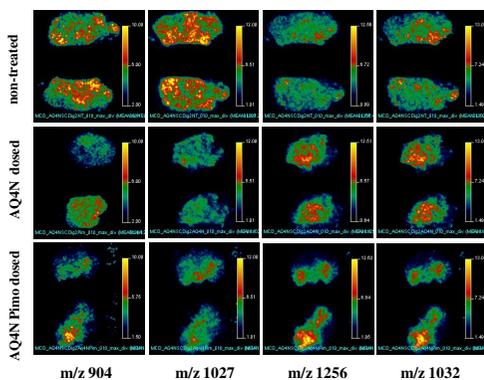


Figure 2: Localisation of the tumour region within non-treated and AQ4N dosed xenografts using MALDI-IMS-MS images of lipids at m/z 184, 478 and 725.

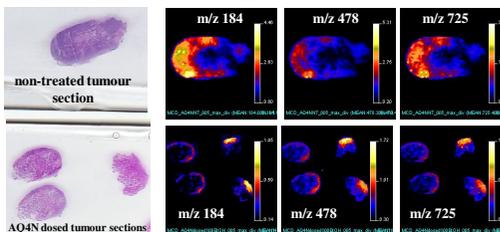


Figure 4: MALDI-IMS-MS images of the localisation of AQ4N within the tissue sections. A spot of AQ4N standard was used as positive control. Interferences between peaks are minimised using their ion mobility.

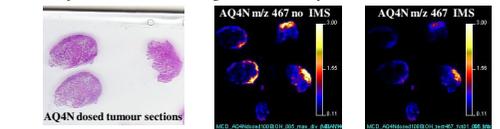
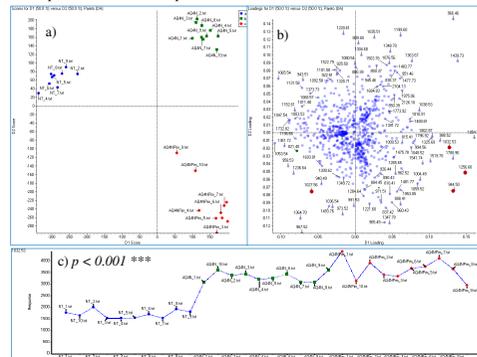


Figure 6: (a, b) Principal component analysis-discriminant (PCA-DA) analysis of MALDI-IMS-MS imaging data obtained after *in situ* digestion of non-treated and AQ4N treated xenograft tissue sections. Non-treated tumour samples are separated from AQ4N dosed xenografts. (c) The distribution of peptides ions, here m/z 1032, can be plotted across the samples.



Discussion

- MALDI-IMS-MS Imaging of AQ4N:** Imaging AQ4N within the tissue section allowed the localisation of the tumour region and hypoxic tumour area. Using the IMS aimed to reduce peak interferences, hence improved the specificity and selectivity of the method used.
- In situ digestion:** Numerous signals were obtained after on-tissue digestion of non-treated and AQ4N treated tumour xenograft tissue sections. Differences in protein intensities were noticed when comparing peptide profiles obtained from non-treated and AQ4N dosed xenograft section, as well as spectra resulting from tumour regions and necrotic regions.
- In situ identification:** The use of the IMS improved the specificity of direct MALDI-MS/MS for the database search, hence the peptide identification. Several proteins including actin, histone H3, tumour necrosis factor and Retinoic acid early transcript 1G protein were identified.
- Statistical analysis:** PCA-DA analysis allowed the discrimination between MALDI-IMS-MS images obtained from non-treated tumour and AQ4N dosed tumour. The highlighted peptide ions from the loading plots are in agreement with the obtained images. Additionally, PCA-DA analysis distinguished between treatments administrated to the animals.

Conclusion

- The ability to use MALDI-MSI to discriminate protein localisation and expression in response to a chemotherapy treatment is described here.
- The use of the ion mobility has been found powerful for both small molecule and peptide analysis after on-tissue digestion. Additionally the discriminations between non-treated and AQ4N dosed xenografts were obtained using both MALDI-IMS-MSI and statistical analysis.

Further Work

- Further experiments are required for the study of the distribution and the *in situ* identification of glucose transporter proteins, such as Glut-1 which have been found closely associated with tumour hypoxia.
- The results generated by PCA-DA analysis will be used to build a tumour classification model in response to chemotherapy treatment.

References

- [1] Kunz M. *et al.*, *Mol. Cancer* 2003, 2:23.
- [2] Atkinson S. *et al.*, *Rapid Commun. Mass Spectrom.* 2007, 21:1271-1276.
- [3] Albertella M.R. *et al.*, *Clin. Cancer Res.* 2008, 14:1096-1104.
- [4] Djidja M.C. *et al.*, *Proteomics* 2009, 9: 2750-2763.
- [5] <http://www.maldi-msi.org>
- [6] Loadman P.M. *et al.*, *DMD* 2001, 29:422-426.

Acknowledgements

- ✓ BMRC/Clinical Research Fellow scheme for project funding.
- ✓ British Mass Spectrometry Society is gratefully acknowledged for their financial assistance.