Molecular characterization of metastatic ovarian cancer by MALDI imaging mass spectrometry

A thesis submitted for the degree of

Doctor of Philosophy

as a combination of research narrative and portfolio of scientific publications by

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Abstract

Imaging mass spectrometry (IMS) is a novel technology which measures the spatial distribution of drugs, lipids, peptides and proteins across tissue sections by application of mass spectrometry (MS) directly to the section surface. Several hundred analytes can be measured across a tissue in a single IMS experiment, without the need for antibodies and without prior knowledge of tissue composition or structure. In the context of human cancers, the molecular information collected by IMS approaches has been used to grade cancers and predict patient survival. IMS is thus a potentially technology capable of providing valuable complementary information to classical histology and immuno-histochemistry.

Ovarian cancers have the highest mortality of any gynaecological cancer. The high mortality results from late diagnosis due to the asymptomatic nature of ovarian malignancies. Advanced stage ovarian tumours will shed cancer cells into the abdominal cavity, where they subsequently implant into the peritoneum and form metastatic tumour nodules. Despite invasive surgery and adjuvant chemotherapy, there is a large increase in patient morbidity following peritoneal metastasis. Compounding this issue further is the absence of reliable grading systems for ovarian cancer and a subsequent lack of individualized treatments for specific cancer sub-types. As a result of the potential ability to grade tumours and provide patient prognoses based on IMS data, the molecular composition of ovarian metastatic tumours was investigated by IMS.

The novelty of IMS required set up of a robust and reproducible workflow. Methods were thus optimized for IMS analysis of both frozen and formalin-fixed paraffin-embedded (FFPE) ovarian tumour tissue. Subsequently it was shown that optimization of available antigen retrieval and tryptic digest methods for accessing FFPE tissues could achieve higher tryptic peptide signal to noise at a better spatial resolution than methods available in the literature. As such, a complete tryptic peptide IMS workflow was developed alongside liquid chromatography (LC) and MS/MS based peptide identification. In conjunction with this workflow, methods for improving the matching of IMS peptides to LC-MS/MS identified peptides using internal calibrants and development of an in-house software tool were described.

As a result of the work presented in this thesis, a complete tryptic peptide IMS workflow which could be applied to virtually any cancer tissue was developed. The application of this workflow, and exploratory k-means clustering, to ovarian peritoneal metastases showed that key tryptic peptides could be found which distinguish cancer tissue from the surrounding peritoneal stroma. This represented the first step in characterizing these metastatic tumours at the molecular level. The results in this thesis are a precursor to future work which will validate these peptide markers and develop a classification system for metastatic ovarian cancers based on patient survival and response to chemotherapy.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Ove Johan Ragnar Gustafsson and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Most acknowledgements finish with a profound statement, something along the lines of…..wise men can't jump…….ok so that's not profound at all but you know what I mean. I've never followed trends so I'm going to start mine with a joke. I don't know the reference but obviously the joke is not mine.

A cation runs into a bar screaming "I've lost my electron, I've lost my electron!". The bartender calms the ion down and asks "Are you sure?" The ion replies, "I'm positive."

Now that the giggles have subsided……When I started my Honors year in 2007, one of my supervisors asked me why I chose science. The answer now, as I write the final parts of my doctoral thesis, is thankfully the same as it was then, which I take to mean that I probably made the right choice. The answer I gave was that I will never be happy as part of the status quo. I want to be a force for change in the world, no matter how small my contribution. I have many people to thank for their support, friendship and help over the past three years. It would take many pages to properly thank everyone who has contributed to my life so I will have to settle for those which have had the greatest impact.

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Publications

Directly related to thesis:

Gustafsson, J. O. R., M. K. Oehler *et al.* (2011). MALDI Imaging Mass Spectrometry (MALDI IMS) - Application of Spatial Proteomics for Ovarian Cancer Classification and Diagnosis. International Journal of Molecular Sciences *12*(1), 773-794 published online 21 January 2011.

Gustafsson, J. O. R., S. R. McColl, *et al.* (2008). "Imaging mass spectrometry and its methodological application to Murine tissue." Journal of Proteomics and Bioinformatics 1(9): 458-463.

Gustafsson, J. O. R., M. K. Oehler, et al. (2010). "Citric Acid Antigen Retrieval (CAAR) for Tryptic Peptide Imaging Directly on Archived Formalin-Fixed Paraffin-Embedded Tissue." J Proteome Res 9(9): 4315-4328.

Arising from thesis:

Condina, M. R., Gustafsson, J. O. R., *et al.* (2010). EZYprep LC-coupled MALDI-TOF/TOF MS: An improved matrix spray application for phosphopeptide characterisation. Proteomics, 10, 2516-2530.

Abbreviations

ACN	Acetonitrile
ANI	Aniline
3-AP	3-acetyl-pyridine
AR	Antigen retrieval
AWM	Abundance weighted mean
CAAR	Citric acid antigen retrieval
CCTV	Close circuit television
CHCA	α-cyano-4-hydroxycinnamic acid
ChIP-1000	Chemical inkjet printer 1000
CID	Collision induced dissociation
Da	Dalton
kDa	Kilo dalton
2-DE	Two dimensional electrophoresis
2,5-DHB	2,5-dihydroxybenzoic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immuno-sorbent assay
EOC	Epithelial ovarian carcinoma
ESI	Electro-spray ionization
EtOH	Ethanol
FA	Formic acid
FFPE	Formalin-fixed paraffin-embedded
FIGO	International federation of gynecology and obstetrics
FWHM	Full width half maximum
H&E	Haematoxylin and eosin
GAPDH	Glyeraldehyde-3-phosphate dehydrogenase
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol

hnRNP	Heterogeneous nuclear ribonucleoprotein
HPLC	High performance liquid chromatography
IHC	Immuno-histochemistry
IMS	Imaging mass spectrometry
IPA	Isopropanol
IT	Ion trap
ITO	Indium tin oxide
LC	Liquid chromatography
LCM	Laser capture micro-dissection
LC-MS	Liquid chromatography – mass spectrometry
LTQ	Linear trap with quadrupole
MALDI	Matrix assisted laser desorption/ionization
MALDI-IMS	Matrix assisted laser desorption/ionization imaging mass spectrometry
MeOH	Methanol
mL	Milli litre
MS	Mass spectrometry
m/z	Mass to charge ratio
nL	Nano litre
NSCLC	Non small cell lung cancer
OCT	Optimal cutting temperature polymer
PCA	Principal component analysis
PEG	Polyethylene glycol
PIC	Percentage intensity contribution
pmol	Pico mole
png	Portable network graphic
POS	Percentage of spectra (within a cluster)
ppm	Parts per million
PTM	Post translational modification

SA	Sinapinic acid
SIMS	Secondary ion mass spectrometry
SLSC	Standard light scatter curve
SNAP	Sophisticated numerical annotation procedure
STS	Soft tissue sarcoma
TOF	Time-of-flight
TFA	Trifluoroacetic acid
TFE	2,2,2-trifluoroethanol