Utilising Quantitative Immunoproteomics To Reveal Differential Autoantibody Biomarker Panels In Serous Ovarian Cancer Patients

A thesis submitted for the degree of

Doctor of Philosophy

as a combination of conventional narrative and portfolio of publications by

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Abstract

Epithelial ovarian cancer accounts for 5% of all cancer deaths and greater than 50% of all gynaecological cancer deaths. It presents at a late clinical stage in more than 60% of patients, and is associated with a 5-year survival of only 30% in this group. In contrast, the 5-year survival for patients with organ-confined stage I ovarian cancer exceeds 90%, and most patients are cured of their disease. Thus, the detection of early stage ovarian cancer is the best way to improve survival. No clinically applicable method exists for the early detection of ovarian cancer. Hence, there is an unmet medical need for an accurate screening test.

Most scientific efforts towards early detection are focused on the discovery of tumour-associated antigens (TAA). Autologous antibodies against TAAs, however, may serve as more sensitive diagnostic markers. They circulate in the blood before TAAs and are usually more abundant than the TAAs themselves as a result of amplification through the humoral immune response. Accumulating evidence also suggests that a humoral response already exists during malignant transformation when aberrant gene expression is translated into premalignant cellular changes.

In this thesis, potential autoantibody biomarkers for ovarian cancer were discovered, verified and validated as an early detection test. A new immunoproteomic strategy was developed to identify novel autoantibodies that were elevated in serous ovarian cancer patients. Lysate extracted from the ovarian tissue of a patient was applied to an immunoaffinity column generated with autologous antibodies and a paired control immunoaffinity column. Relative quantification of captured autoantigens was performed using isotope coded protein label (ICPL) technology coupled with high resolution LC-MS. At a protein ratio cut-off of 1.45-fold, 148 autoantibodies were found to be enriched in ovarian cancer patients compared to the corresponding controls.

Upon bioinformatic prioritisation 50 autoantibody candidates were selected for verification. Protein microarray analysis of 98 samples revealed 9 autoantibody candidates to be significantly different in early stage cancer patients compared to healthy and benign controls. Biomarker candidates anti-ANXA1, anti-SAHH and anti-ARP3 showed the greatest potential where each marker achieved greater than 90% specificity at 83.3% sensitivity. As a 4-biomarker panel with the 'gold standard' for ovarian cancer detection, cancer antigen (CA)125, a sensitivity of 76.5% at 100% specificity was attained. These values of sensitivity and specificity for early stage ovarian cancer surpassed the minimum requirements for an implementable screening test and showed great promise as a diagnostic tool.

Validation of the top three autoantibody candidates using protein microarray revealed anti-ANXA1 to be the most robust and effective biomarker for stage I cancer detection. As a single biomarker anti-ANXA1 had 81.8% sensitivity and 71.9% specificity for stage I cancer compared to healthy and benign controls. Excitingly, in combination with CA125, a sensitivity of 71.4% at 100% specificity was achieved when differentiating stage I cancer patients from healthy individuals. For this level of effectiveness a positive and negative predictive value of 100% and 99.99% was achieved, respectively. Therefore, a biomarker panel containing anti-ANXA1 and CA125 may enable the development of a detection test that can be used to screen for stage I serous ovarian cancer in the general population. This promising discovery demands further investigation where continuing analysis in prospective samples is essential.

The discovery of a screening test is crucial to reduce the morbidity and mortality caused by ovarian cancer. This study investigates the presence of differential autoantibody signatures in serous ovarian cancer patients as potential biomarkers. Additionally, those identified TAAs that are functionally involved in carcinogenesis could also serve as therapeutic targets. Finally, the immunoproteomic approach developed here could be used in studies aiming to discover novel autoantibody biomarkers for other asymptomatic malignancies.

Declaration

I certify that 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 Karina Martin 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Karina Martin

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A successful post-doctoral candidature is measured by the contribution of the work to the body of knowledge for scientists, clinicians and the broader community. However, it could not be achieved without learning, personal growth and support.

To my primary supervisor, Peter Hoffmann, thank you for accepting me into your amazing lab. The confidence you showed in my abilities allowed me to extend my skills beyond that of a typical PhD student. Under your guidance I developed the confidence to take on different challenges, perform experiments that were new to the lab and drive the research that is written on these pages.

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Publications

Directly related to thesis:

Martin, K., Ricciardelli, C., Hoffmann, P., Oehler, M.K. *Exploring the Immunoproteome for Ovarian Cancer Biomarker Discovery.* Int. J. Mol. Sci. 2011, 12, 410-428 - **Chapter 1**

Martin, K., Weiland, F., Oehler, M.K., Hoffmann, P. *Deciphering the Molecular Nature of Ovarian Cancer Biomarker CA125.* Int. J. Mol. Sci. 2012 – **Chapter 1**

Arising from thesis:

Meding, S., Martin, K., Gustafsson, O.J.R., Eddes, J.S., Hack, S., Oehler, M.K., Hoffmann, P. *Tryptic Peptide Reference Data Sets for MALDI Imaging Mass Spectrometry on Formalin-fixed Ovarian Cancer Tissues.* J. Prot. Res. 2013, 12 (1), 308-315

Presentations

Martin, K., Ricciardelli, C., Hack, S., Oehler, M.K., Hoffmann, P. *Identification and relative quantification of autoantibody biomarker candidates for early ovarian cancer*. **Oral** and **poster** presentation delivered at the 18th Lorne Proteomics Symposium, Lorne, VIC, February, 2013

Martin, K., Ricciardelli, C., Oehler, M.K., Hoffmann, P. *Utilising quantitative immunoproteomics to reveal differential autoantibody signatures in serous ovarian cancer patients*. **Poster** presentation delivered at the 11th Annual HUPO World Congress, Boston, Massachusetts, USA, September, 2012

Martin, K., Ricciardelli, C., Oehler, M.K., Hoffmann, P. *Utilising quantitative immunoproteomics to reveal differential autoantibody signatures in serous ovarian cancer patients*. **Oral** presentation delivered at the Adelaide Protein Group Awards Event, Adelaide, SA, June, 2012

Martin, K., Ricciardelli, C., Oehler, M.K., Hoffmann, P. *Mining the serous ovarian cancer immunoproteome for autoantibody biomarkers.* **Poster** presentation delivered at the 17th Lorne Proteomics Symposium, Lorne, VIC, February, 2012.

Martin, K., Ricciardelli, C., Oehler, M.K., Hoffmann, P. *Immunoproteomic approach toward biomarker discovery for ovarian cancer*. **Poster** presentation delivered at the 9th Annual HUPO World Congress, Sydney, NSW, September, 2010.

Abbreviations

µl	Microlitre
1-DE	One-dimensional poly-acrylamide gel electrophoresis
2-DE	Two-dimensional poly-acrylamide gel electrophoresis
AA	Amino acid
Ab	Antibody
ACN	Acetonitrile
ANOVA	Analysis of variance
AU	Absorbance units
BSA	Bovine serum albumin
CHAPS	3[(3-Cholamidopropyl)dimethylammonio]-propanesulfonate
CID	Collision induced dissociation
Da	Dalton
DIGE	Difference gel electrophoresis
DMP	Dimethyl pimelimidate dihydrochloride
DSS	Disuccinimidyl suberate
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
ESI	Electrospray ionisation
ETD	Electron transfer dissociation
FA	Formic acid
Fab	Fragment antigen binding

Fc	Crystallisable fragment
FDA	Food and Drug Association
FIGO	International Federation of Gynecology and Obstetrics
fmol	Femtomole
G-250	Colloidal Coomassie
HCCA	α-cyano-4-hydroxy cinnamic acid
HCl	Hydrochloric acid
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonate
HPLC	High performance liquid chromatography
hr	Hour
HRAM	High resolution, accurate mass
I.D.	Inner diameter
IAA	Iodoacetamide
ICAT	Isotope coded affinity tags
ICPL	Isotope coded protein labels
IEF	Isoelectric focusing
Ig	Immunoglobulin
IHC	Immunohistochemistry
IP	Immunoprecipitation
IPA	Ingenuity pathway analysis
IT	Ion trap
iTRAQ	Isotope tagging for relative and absolute quatitation
LC	Liquid chromatography

LIT	Linear ion trap
m	Mass
m/z	Mass-to-charge
MALDI	Matrix assisted laser desorption/ionization
min	Minute
Mr	Molecular Weight
MS	Mass spectrometry
MS/MS	Tandem MS
Nd:YAG	Neodymium-doped yttrium aluminium garnet
NHS	N-hydroxysuccinimide
nLC	Nano-LC
OCS	Ovarian cancer screening
OVA	Ovalbumin
PA	Phosphoric acid
PAGE	Poly-acrylamide gel electrophoresis
PBS	Phosphate buffered saline
pI	Isoelectric point
pmol	Picomole
PMSF	Phenylmethanesulfonylfluoride
PMT	Photon multiplier tube
PTMs	Post-translational modifications
PVDF	Polyvinylidene fluoride
Q	Quadrupole

RCT	Randomised controlled trial
rf	Radio frequency
roc	Risk of ovarian cancer
ROC	Receiver operating characteristic
RP	Reverse-phase
SD	Standard deviation
SDS	Sodium dodecyl sulphate
sec	second
SILAC	Stable isotope labeling by amino acids in cell culture
S/N	Signal-to-noise
t	Time
TAA	Tumour associated antigen
TBST	Tris-buffered saline Tween-20
TFA	Trifluoroacetic acid
TIC	Total ion chromatogram
TOF	Time-of-flight
T_R	Retention time
Tris	Tris(hydroxymethyl)-aminomethane
VSN	Variance stabilisation and normalisation
XIC	Extracted ion chromatogram
z	charge

Chapter 1 Manuscript Context

A deep understanding of ovarian cancer, the immune system, biomarkers as well as proteomics and mass spectrometry was essential to perform the quality of research presented in this thesis. To this end a comprehensive literature review on ovarian cancer autoantibody biomarkers and discovery techniques was performed. Furthermore, an appreciation of the current 'gold standard' biomarker for ovarian cancer, cancer antigen (CA)125 was critical in order to understand the current challenges, requirements and need for a screening test for ovarian cancer. In joint authorship, mining of the literature was performed to produce an extensive review on the structure and function of CA125. These two reviews, which form parts of chapter 1, summarise the current knowledge and challenges faced in the field of ovarian cancer detection and screening.

Chapter 1 Manuscript 1

Exploring the Immunoproteome for Ovarian Cancer Biomarker Discovery

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Review

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Chapter 1 Manuscript 2

Deciphering the Molecular Nature of Ovarian Cancer Biomarker CA125

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Review

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