Role of Annexin A2 in Ovarian Cancer Metastasis

A Thesis Submitted for the Degree of Doctor of Philosophy by

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Mak and Ayah, this is for you, I love you so much.

This thesis is dedicated to my loving parents, Norma Muhammad and Lokman Abdul Hamid.

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Summary

Ovarian cancer is the most lethal gynaecological cancer. We identified annexin A2 to be modulated in the co-culture of ovarian cancer and peritoneal cells *in vitro*. Annexin A2, a calcium phospholipid binding protein, has been characterized in many malignancies and mediates various cellular functions such as cell motility, invasion, proliferation, angiogenesis and cell adhesion. Annexin A2 heterotetramer consists of annexin A2 and S100A10 monomers on the cell surface and plays an important role in the plasminogen activator system that enhances cancer cell invasion and metastasis. The aim of this Ph D thesis was to investigate the role of annexin A2 in ovarian cancer metastasis using *in vitro* and *in vivo* ovarian cancer models.

Annexin A2 expression was characterized in serous ovarian cancer cell lines and human serous ovarian cancer tissues. Annexin A2 inhibitors were used to evaluate the effects of annexin A2 on ovarian cancer cell motility, invasion and adhesion to the peritoneal cells. Furthermore, annexin A2 neutralizing antibodies were used to examine the role of annexin A2 in tumour invasion and metastasis using a chick chorioallantoic membrane (CAM) assay and an intraperitoneal xenograft mouse model. We evaluated whether annexin A2 can be used as a diagnostic marker by measuring blood annexin A2 levels in serous ovarian cancer patients. Moreover, annexin A2 and its binding protein, S100A10 expression were assessed using immunohistochemistry to determine their relationship with clinical outcome in a cohort of stage III serous ovarian cancers.

We showed that annexin A2 immunostaining was significantly increased in cancer-associated stromal cells compared with non-malignant ovarian tissues. Annexin A2 siRNAs significantly inhibited ovarian cancer cell motility, invasion and adhesion to peritoneal cells. Moreover, annexin A2 neutralizing antibodies significantly inhibited OV-90 cell motility and invasion *in vitro* and in the *in vivo* CAM assay. Furthermore, we also demonstrated that annexin A2 neutralizing antibodies significantly inhibited the invasion of primary ovarian cancer cell lines in the CAM assay. The growth of SKOV-3/GFP Luc cells and peritoneal dissemination in nude mice was significantly inhibited by annexin A2 neutralizing antibodies. Our findings suggested that reduced tumour burden and metastatic spread was a result of reduced cell survival.

Blood annexin A2 levels were increased in early stage and advanced stage ovarian cancer patients compared with women without malignancy (normal ovaries and benign ovarian tumours). We showed an improved sensitivity for detecting early stage ovarian cancer by combining annexin A2 and CA125 at the 95% and 98% specificity level. Kaplan-Meier analyses of stage III serous ovarian cancers showed that high stromal annexin A2 expression was significantly associated with

reduced progression-free survival and overall survival. Moreover, we also showed high cytoplasmic S100A10 in the cancer cells to be associated with reduced overall survival. Both, high stromal annexin A2 and high cytoplasmic S100A10, were independent predictors of overall survival in a multivariate analysis which included positive residual disease.

In conclusion, our findings indicate that annexin A2 plays an important role in ovarian cancer tumourigenesis and metastasis is therefore a potential novel therapeutic target against ovarian cancer. We also demonstrated that annexin A2 has both diagnostic and prognostic significance and may be useful for serous ovarian cancer diagnosis and patient management.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for the award of any degree or diploma in any university; and that to the best of my knowledge and belief, this work does not contain any material previously published or written by any other person except where due reference is made in the text.

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Noor Alia Lokman

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Publications Arising During Ph D Candidature

1. Ricciardelli C, Ween MP, **Lokman NA**, Tan IA, Pyragius CE and Oehler MK, Chemotherapy-induced hyaluronan production: A novel chemoresistance mechanism in ovarian cancer. *BioMed Central Cancer*, 2013, 13:476. (Impact factor: 3.33)

2. **Lokman NA**, Elder ASF, Ween MP, Pyragius CE, Hoffmann P, Oehler MK and Ricciardelli C, Annexin A2 is regulated by ovarian cancer-peritoneal cell interactions and promotes metastasis. *Oncotarget*, 2013, 4(8):1129-1211. (Impact factor: 6.636)

3. **Lokman NA**, Elder ASF, Ricciardelli C and Oehler MK, Chick chorioallantoic membrane (CAM) assay as an *in vivo* model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. *Int.J.Mol.Sci.,* 2012,13, 9959-9970. (Impact factor: 2.464)

4. **Lokman NA**, Ween MP, Oehler MK and Ricciardelli C, The role of annexin A2 in tumourigenesis and cancer progression. *Cancer Microenvironment*, 2011, 4, 199-208.

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2. **Lokman NA**, Elder ASF, Ricciardelli C and Oehler MK, Chick chorioallantoic membrane (CAM) assay as an *in vivo* model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. *Int.J.Mol.Sci.,* 2012,13, 9959-9970. (Impact factor: 2.464)

3. **Lokman NA**, Ween MP, Oehler MK and Ricciardelli C, The role of annexin A2 in tumourigenesis and cancer progression. *Cancer Microenvironment*, 2011, 4, 199-208.

Presentations at Scientific Meeting

2013

Lokman NA, Elder ASF, Ween MP, Pyragius CE, Hoffmann P, Oehler MK and Ricciardelli C, Annexin A2 is regulated by ovarian cancer-peritoneal cell interactions and promotes metastasis, Robinson Institute Research Symposium, 4th November 2013, National Wine Centre, Adelaide, South Australia, Australia. **(poster)**

Lokman NA, Elder ASF, Ween MP, Pyragius CE, Hoffmann P, Oehler MK and Ricciardelli C, Annexin A2 is regulated by ovarian cancer-peritoneal cell interactions and promotes metastasis, Matrix Biology Society of Australia and New Zealand (MBSANZ), 20-23rd October 2013, McCracken Country Club, South Australia, Australia. **(oral and poster)**

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Lokman NA, Elder ASF, Ween MP, Pyragius CE, Hoffmann P, Oehler MK and Ricciardelli C, Annexin A2 is regulated by ovarian cancer-peritoneal cell interactions and promotes metastasis, The Centre for Personalised Cancer Medicine Symposium 'Driving personalised cancer medicine through next generation sequencing', 25th July 2013, National Wine Centre, Adelaide, South Australia, Australia. **(poster)**

2012

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Lokman NA, Ween MP, Hoffmann P, Oehler MK and Ricciardelli C, Annexin A2 released during ovarian cancer-peritoneal cell interaction promotes a pro-metastatic cancer cell behaviour in ovarian cancer metastasis, Cold Spring Harbor Asia/International Cancer Microenvironment

Society Joint Conference on Tumour Microenvironment, 13th to 17th November 2012, Suzhou Dushu Lake Conference Center, Suzhou, China. **(poster)**

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2011

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2010

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meeting with the 5th Australian Health & Medical Research Congress 2010, 14th-18th November 2010, Melbourne Convention and Exhibition Centre, Victoria, Australia. **(oral and poster)**

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Lokman NA, Ween MP, Hoffmann P, Oehler MK. and Ricciardelli C, Role of Annexin A2 in ovarian cancer metastasis. Australian Society for Medical Research (ASMR), SA Scientific Meeting, Adelaide Entertainment Centre, 9th June 2010, South Australian Division. **(oral)**

Awards Arising Out of This Thesis

1. High Commended - Student Poster Award, 2013 Robinson Institute Research Symposium, 4th November 2013, National Wine Centre, Adelaide, South Australia, Australia.

2. Vice-Chancellor's Prize for Best Poster, Faculty of Health Sciences Postgraduate Research Conference, 29th August 2013, National Wine Centre, Adelaide, South Australia, Australia.

3. Best Student Poster Award, 2012 Robinson Institute Research Symposium, 12th December 2012, National Wine Centre, Adelaide, South Australia, Australia.

4. Best Student Poster Award 2012, Matrix Biology Society of Australia and New Zealand (MBSANZ), 5th to 8th September 2012, Mantra Legends, Gold Coast, Queensland, Australia.

Abbreviations

Є-ACA	6-Aminocaproic Acid
Ab	Antibody
ABC	ATP binding cassette
ANXA2	Annexin A2
CA125	Cancer Antigen 125
CAM	Chick Chorioallantoic Membrane
CD44	Cluster of Differentiation 44
CM	Conditioned Media
CMI	Cancer Cells and Matrigel Implant
DAB	Diaminobenzidine Tetrahydrochloride
ECT	Ectoderm
ECL	Enhanced chemiluminescence
ECM	Extracellular Matrix
EDTA	Ethylenediaminetetraacetic Acid
EMT	Epithelium-Mesenchymal Transition
END	Endoderm
ELISA	Enzyme Linked Immunosorbent Assay
ERK	Extracellular Signal Regulated Kinase
FBS	Fetal Bovine Serum
FIGO	International Federation of Gynaecologist and Obstetricians
GFP	Green Fluorescent Protein
GM6001	Galardin
H_2O_2	Hydrogen Peroxide
HE4	Human Epididymis Protein 4
HCC	Hepatocellular Carcinoma
HCl	Hydrochloric Acid
Ig	Immunoglobulin
MAPK	Mitogen Activated Protein Kinase
MES	Mesoderm
MMPs	Matrix Metalloproteinases

N-terminal domain	Amino Terminus Domain
NPV	Negative Predictive Values
pI	Isoelectric Point
P11	Protein P11
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline with Tween-20
PPV	Positive Predictive Values
RMI	Risk Malignancy Index
ROC	Receiver Operating Curve
ROMA	Risk of Ovarian Malignancy Algorithm
RR	Relative Risk
RT-PCR	Real-time Polymerase Chain Reaction
u-PA	Urokinase-Type Plasminogen Activator
SDS	Sodium Dodecyl Sulphate
siRNAs	Small Interfering RNAs
SKOV-3/GFP Luc	SKOV-3 cells labeled with GFP and Luciferase
t-PA	Tissue-Type Plasminogen Activator
TGFBIp	Transforming Growth Factor Inducible Protein
Tris	Tris(hydroxymethyl)-aminomethane
TMA	Tissue Microarray
LC-MS/MS	Liquid Chromatography-Mass Spectrometry