Role of Annexin A2 in Ovarian Cancer Metastasis

A Thesis Submitted for the Degree of Doctor of Philosophy by

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February 2014
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 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.

I give consent to the copy of my thesis to be deposited in the University Library to be made available for loan and photocopying, subject to embargoes, and the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the ADTP, and the library catalogue, subject to embargoes.

………………………………
Noor Alia Lokman
February 2014
Acknowledgements

I am grateful to a lot of people for their help and support throughout my PhD experience. First of all, I would like thank my supervisor, Dr Carmela Ricciardelli that has given me the best supervision over the last four years. Thank you for being patience, supportive, helping me out with my data analysis and a great mentor to me. I would like to thank my co-supervisor, Prof Martin Oehler for your supervision, guidance, ideas and discussion throughout my PhD. Thank you Carmela and Martin for evaluating my manuscripts and being committed to my research project.

I would like to thank previous and present members of Reproductive Cancer group that assist me in my PhD studies, Dr Miranda Ween, Dr Carmen Pyragius, Wendy Bonner, Dr Alison Elder and Dr Izza Tan for their friendship, technical assistance in the lab and manuscript evaluations.
I would like to thank A/Prof Peter Hoffmann and Karina Martin from Adelaide Proteomics Center for your assistance throughout my PhD and also Prof Ray Rodgers, Dr Katja Hummitzsch and Nick Hatzirodos for their help throughout my PhD.
Previous and present PhD students for their friendship and assistance throughout my studies: Nok, Lisa, Adrian, Jacky, Siew, Macarena, Zahied, Siti Mariam, Jess, Dulama, David, Vincent and Sally. Thank you all.

I am fortunate to receive scholarships from Adelaide Graduate Fee Scholarship, Florey Foundation, Faculty of Health Sciences and Robinson Institute for supporting my PhD journey and opportunity to present my research in conferences. Thank you also to the staff of Discipline of Obstetrics and Gynaecology for excellent resources throughout my studies.
I’m also grateful to the patients at Royal Adelaide Hospital and Burnside Hospital that have given consent for their samples to be used in this study.

Thank you to my friends Ayuni, Suhana, Affah and Solehah for your friendship and support.
To my best friend, Nur Hezrin Shahrin, for her continuous support in good and hard times and being a good listener in our coffee catch-up sessions in these past few years, all the best with your baby boy, Adyan Hamzah.
Finally, to my loving parents, Norma Muhammad and Lokman Abdul Hamid, for everything that they have done for me and made me who I am today. There’s no way that I can thank you enough for your endless love, support and encouragement. Mak and ayah, I’ve been blessed to have both of you in my life, I love you so much. To my one and only sister, Dr Noor Dayana for her love and strong character, I’m so proud of you. To my brothers, Adi Ikmal, Dani Aizat and Dhiya Aiman, who always had been there for me. And to my family, Cik Yah, Cik Nin, Azwa and everyone for their love and support.
Publications Arising During Ph D Candidature


Publications Contributing to This Thesis


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)

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, Postgraduate Research Expo, Faculty of Health Science, University of Adelaide, 29th August 2013, National Wine Centre, Adelaide, South Australia, Australia. (poster)


2012

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, Robinson Institute Research Symposium, 12th December 2012, National Wine Centre, Adelaide, South Australia, Australia. (poster)

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

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, Matrix Biology Society of Australia and New Zealand (MBSANZ) 2012, 5th to 8th September 2012, Mantra Legends, Gold Coast, Queensland, Australia. (oral and poster)


2011

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 Convention Center, 7th June 2011, South Australian Division (poster)

2010

Lokman NA, Ween MP, Hoffmann P, Oehler MK and Ricciardelli C, Role of Annexin A2 in ovarian cancer metastasis, Postgraduate Research Expo, Faculty of Health Science, University of Adelaide, 1st September 2010, The National Wine Centre, Adelaide, South Australia, Australia. (poster)

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, 4\textsuperscript{th} November 2013, National Wine Centre, Adelaide, South Australia, Australia.**

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

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

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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Ê-ACA</td>
<td>6-Aminocaproic Acid</td>
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<tr>
<td>Ab</td>
<td>Antibody</td>
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<tr>
<td>ABC</td>
<td>ATP binding cassette</td>
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<tr>
<td>ANXA2</td>
<td>Annexin A2</td>
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<tr>
<td>CA125</td>
<td>Cancer Antigen 125</td>
</tr>
<tr>
<td>CAM</td>
<td>Chick Chorioallantoic Membrane</td>
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<tr>
<td>CD44</td>
<td>Cluster of Differentiation 44</td>
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<tr>
<td>CM</td>
<td>Conditioned Media</td>
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<tr>
<td>CMI</td>
<td>Cancer Cells and Matrigel Implant</td>
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<tr>
<td>DAB</td>
<td>Diaminobenzidine Tetrahydrochloride</td>
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<tr>
<td>ECT</td>
<td>Ectoderm</td>
</tr>
<tr>
<td>ECL</td>
<td>Enhanced chemiluminescence</td>
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<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
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<tr>
<td>EDTA</td>
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<tr>
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<td>Endoderm</td>
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<td>ELISA</td>
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<td>ERK</td>
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<td>FBS</td>
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<td>International Federation of Gynaecologist and Obstetricians</td>
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<td>GFP</td>
<td>Green Fluorescent Protein</td>
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<td>GM6001</td>
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<td>Matrix Metalloproteinases</td>
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<td>Abbreviation</td>
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<tr>
<td>N-terminal domain</td>
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