THE ROLE OF PROTEIN PHOSPHATASE 2A AS A TUMOUR SUPPRESSOR IN BREAST CANCER

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B. Biomed Sci (Hons)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

August 2012

STATEMENT OF ORIGINALITY

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ABSTRACT

Breast cancer is a worldwide health issue, and while many advances have been made in recent years, continued understanding of the development and progression of breast cancer is required to produce novel therapies to improve patient survival. Breast cancer is characterised by disruption in signalling pathways that control key cellular processes such as growth, proliferation and survival. Protein Phosphatase 2A (PP2A) is a key cellular signalling molecule that regulates numerous signalling pathways involved in breast cancer. PP2A is a trimeric protein complex, consisting of a structural subunit (PP2A-A), to which a catalytic subunit (PP2A-C) and a regulatory B subunit bind. PP2A is a proposed tumour suppressor, yet the role of PP2A in breast cancer has not been examined in detail to date. This thesis firstly examines PP2A expression in breast cancer cell lines and human breast cancer tissue. Dramatic reductions in expression of the PP2A-A and also a number of regulatory B subunits were observed in a panel of breast cancer cell lines compared to normal human mammary epithelial cells. In addition, a significant reduction in PP2A-A expression was identified in human breast tumours compared to normal mammary tissue. These results suggest that PP2A is important for the development or progression of breast cancer. In order to determine the functional role of PP2A in breast cancer, PP2A subunit expression was altered in a mammary breast epithelial cell line, MCF10A. A number of MCF10A cell lines were generated by transduction of shRNA directed to the PP2A-A or regulatory B subunits, or by expression of cancer-associated PP2A-A mutant genes. Functional analyses showed that shRNA knockdown or PP2A-A mutant expression had very little effect on MCF10A cells when grown using traditional two-dimensional cell culture techniques. However, in a more physiologically relevant three-dimensional culture method that maintains cellular polarisation and signalling with the basement membrane, a number of phenotypes indicative of cellular transformation were observed. MCF10A cells with reduced expression of regulatory B subunits, or PP2A-A mutations unable to bind regulatory B subunits, demonstrated increased cellular proliferation, MCF10A PP2A-A mutants that cannot interact with either the catalytic or regulatory B subunits displayed invasive properties. The results presented in this thesis provide clear evidence that PP2A is involved in breast cancer and presents a number of avenues for future investigation and potential novel therapies.

ACKNOWLEDGEMENTS

To all the people who have supported me throughout the production of this thesis I extend my sincere thanks and praise.

To my supervisors who have trained me, I thank you for the hard work you have put in to teaching me many new skills both in the laboratory and also for life. Alistair, thanks for being there in the beginning and modelling what it means to give your life to the most important things. To Severine, thank you for reading this thesis and providing helpful feedback. Nikki, thank you for being so patient with me, allowing me to make mistakes, have other priorities in my life and still be there always at the end of an email or phone call when I needed help. Thank you for not only teaching me laboratory skills, but also how to present well and most importantly logical flow. No matter where I end up in my life and career these are skills which I will continue to be grateful for. Thank you for being not only my supervisor, but also my friend. I hope you will remember me fondly and that we keep in touch as much as possible in this busy life.

To all of the lab friends I have made during the number of years I have been with you I thank you for your friendship, good ideas and willingness to share the load. I would especially like to thank Helen Carpenter for teaching me how to perform many lab tasks and for being so willing to lend a hand when I needed it. To people who have provided laboratory tools that make my life easier I am also thankful. Prof. Stephan Strack provided the mutant constructs I have utalised to produce this thesis and Prof. Rodger Daly, Prof. Leonie Ashman and Dr Rick Thorne provided breast cell lines. Thanks to Amanda Smith for just being around for a chat. Ben Copland and Fiona McDougall for optimising all the IHC so I could just come along and run my samples. Also pathologist Barbara Young for making sure my results were accurate. I pray that you might all have a blessed life and that is completely fulfilled.

To my church family, who have loved and supported me, prayed for me, encouraged me, challenged me and helped me grow over the last few years I thank you all. It is such a joy that there are far too many people to mention by name. It is so wonderful to know that one day my brothers and sisters will one day be all over Australia and the world, and yet we will still have a common life in Jesus.

Finally to my family, who are my joy and delight, my love and my comfort, I cannot thank you enough for supporting me through the journey that has been this thesis. Thank you Mum for staying up late, making me buckets of tea and lunch and dinner and...everything, for driving me around, giving me hugs and always being there for me no matter what. Thank you Dad for always supporting me whatever I am doing, I have always felt safe knowing you are there right behind me if ever I need you. John and Jenny you will always be so special to me, thank you Jenny for keeping me up to date and making me laugh so much. John I pray we will always be there to love and support each other no matter where life takes us. My darling Steve, thank you for marrying me during this crazy time, for not waiting, but giving me the true joy of being your wife. Thank you for the constant love, encouragement, teaching and leadership. As this chapter of our life closes, I look forward to what next God has in store for us with great joy.

PRESENTATIONS

Oral Conference Presentations:

Cottrell LF, Sim AT, Verrills NM. (2007) Role of Protein Phosphatase 2A in Breast Tissue Remodelling and Tumourigenesis. Australian Society for Medical Research National Scientific Conference, Katoomba, NSW.

Cottrell LF, Roselli S, Sim AT, Verrills NM. (2008) Protein Phosphatase 2A as a tumour suppressor in breast cancer. Australian Society for Medical Research NSW Scientific Meeting, Sydney, NSW.

Watt, LF. Altered proetin phosphatase expression in breast cancer. 10 Best research showcase - University of Newcastle Faculty of Health.

Watt, LF. Protein phosphatase 2A in breast cancer. Hunter Medical Research Cancer Research Program seminar day, Newcastle.

Poster Conference Presentations:

Cottrell LF, Carpenter HC, Sim AT, Verrills NM (2007) Role of Protein Phosphatase 2A in Breast Cancer. Australian Breast Cancer Conference, Melbourne, VIC.

Cottrell LF, Roselli S, Sim AT, Verrills NM (2008) Protein Phosphatase 2A in Tumourigenesis. FASEB protein phosphatases conference, Colorado, USA.

Cottrell LF, Roselli S, Verrills NM (2009) Protein Phosphatase 2A In Breast Cancer. Hunter Medical Research Institute Cancer Research Program, Newcastle, NSW.

Cottrell LF, Roselli S, Verrills NM (2010) Alterations in Protein Phosphatase 2A expression suggest a tumour suppressive role in breast cancer. Lowry Cancer symposium, Sydney, NSW.

Watt LF, Roselli S, Verrills NM (2010) Altered Expression Of Protein Phosphatase 2A Subunits In Breast Cancer. Australian Society for Medical Research (ASMR) week, Sydney, NSW.

Professional Awards and scholarships:

2007 Cancer Institute of NSW Research Scholar Award

2007 Honourable mention for the Campion Ma Playhoust award (Best oral presentation or poster by a student or any member under 30 year of age at Australian Society for Medical Research National Scientific Conference, Katoomba, NSW)

2010 Winner Faculty of Health 10 of the Best Research Showcase, University of Newcastle.

ABBREVIATIONS

2D	Two dimensional
3D	Three dimensional
5-FU	Fluorouracil
ADH	Atypical ductal hyperplasia
BAD	Bcl2-agonist of death
bp	Base pair
BME	Basement membrane extract
BSA	Bovine serum albumin
CAMKII	Calcium/calmodulin-dependent protein kinase II
CHO cells	Chinese hamster ovary cells
CIP2A	Cancerous inhibitor of PP2A
CSF-1R	Colony-stimulating factor receptor
DAPI	4',6 Diamidino-2-phenylindole
DCIS	Ductal carcinoma in situ
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
DUSP	Dual specificity phosphatase
E. coli	*Escherichia coli*
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial to mesenchymal transition
ER	Estrogen receptor
ERE	Estrogen response element
FACS	Fluorescence activated cell sorting
FAK	Focal adhesion kinase
FCS	Foetal calf serum
GFP	Green fluorescent protein
GSK-3β	Glycogen synthase kinase-3β
HEAT (repeat)	Huntington-elongation-PP2A-A subunit-TOR
HEK-TER	Human embryonic kidney cells immortalised by addition of hTERT, SV40 LT and active Ras

HMEC	Human mammary epithelial cell
HRT	Hormone replacement therapy
hTERT	Human catalytic subunit of telomerase
IEX-1	Immediate early response gene X-1
kDa	Kilodalton
KSR1	Kinase suppressor of Ras
LCIS	Lobular carcinoma in situ
LCMT-1	Leucine Carboxyl Methyltransferase
LT	SV40 Large T antigen
MAPK	Mitogen activated protein kinase
MCF10A ecoR	MCF10A cell line expressing the mouse ecotropic retroviral receptor
Mdm-2	Mdouble minute homologue 2
M-Leu309	Methylated PP2A-C at Leucine 309
MMP	Matrix metalloproteinase
M-PP2A-C	Methylated PP2A-C
mTOR	Mammalian target of rapamycin
Mut3	SV40 Small T mutant unable to bind PP2A
NF-κB	Nuclear factor of κB
NHMRC	Nation health and medical research council
OA	Okadaic acid
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDK1	3-phosphoinositide-dependent protein kinase 1
PH	Plecstrin homology (domain)
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol (4,5)P_2
PIP3	Phosphatidylinositol (3,4,5)P_3
PKA	Protein kinase A
PKR	Protein kinase R
PME-1	Phosphatase methylesterase (specific for PP2A)
PP	Ser/Thr protein phosphatase

PP2A	Protein Phosphatase 2A
PP2A-A	Structural subunit of PP2A
PP2A-C	Catalytic subunit of PP2A
P-PP2A-C	Phosphorylated PP2A-C
PPM	Metallo-protein dependent phosphatase
PTEN	Phosphatase and tensin homologue
PTP	Protein Tyrosine Phosphatase
PTPA	Phosphotyrosyl phosphatase activator
PyMT	Polyoma virus middle T antigen
PyST	Polyoma virus small T antigen
RPMI	Roswell park memorial institute media
RTK	Receptor tyrosine kinase
SAP	Shrimp Alkaline Phosphatase
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
Ser	Serine
SERM	Selective estrogen receptor modulator
shRNA	Short hairpin RNA
siRNA	Small interfering RNA
SMP	Skim milk powder
ST	SV40 Small T antigen
SV40	Simian virus 40
TBST	Tris buffered saline-Tween 20 buffer
TH	Tyrosine hydroxylase
Thr	Threonine
Tyr	Tyrosine
UICC	Union for International Cancer Control
UTD	Untrasduced (MCF10A) cells
WT	Wildtype