

The functional role of PPP2R2A in luminal breast cancer

Abdul Mannan

DVM, MSc Biology

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

October 2019

STATEMENT OF ORIGINALITY

*I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution 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. I give consent to the final version of my thesis being made available worldwide** when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo*

****Unless an Embargo has been approved for a determined period.**

**Abdul Mannan
October 2019**

TABLE OF CONTENTS

ABSTRACT	VI
ACKNOWLEDGEMENT	IX
Publications	XI
Professional award and scholarship	XIII
ABBREVIATIONS	XIV

Contents

1	INTRODUCTION	1
1.1	Breast cancer epidemiology	1
1.2	Breast cancer development and progression	3
1.2.1	Pre-malignant carcinoma	3
1.2.2	Malignant carcinoma.....	3
1.3	Classification of breast carcinoma	5
1.4	Molecular classification of breast carcinoma.....	6
1.4.1	Luminal A breast cancer	6
1.4.2	Luminal B breast cancer	6
1.4.3	HER2 amplified breast cancer.....	7
1.4.4	Triple negative breast cancer	7
1.5	Signalling pathways in breast carcinoma.....	9
1.5.1	Steroid hormone receptor signalling	9
1.5.2	Receptor tyrosine kinase (RTK) signalling pathways.....	10
1.5.3	1.4.3 Cyclin D/CDK4/6-Rb pathway	14
1.5.4	DNA damage repair pathways	16
1.6	Current breast cancer therapies	21
1.7	Protein phosphatase 2A (PP2A).....	25
1.7.1	Structural/Scaffold subunit of PP2A.....	25
1.7.2	Catalytic subunit of PP2A.....	26
1.7.3	Regulatory B subunit of PP2A.....	28
1.8	Regulation of PP2A activity	31
1.9	The tumour suppressive role of PP2A	31

1.10	Mechanisms of PP2A dysregulation in cancer.....	34
1.11	Mechanisms of PP2A dysregulation in breast cancer.....	34
1.11.1	.Mutation and LOH of PP2A in breast cancer	34
1.11.2	PP2A subunits DNA hypermethylation	37
1.11.3	Overexpression of miRNA targeting PP2A subunits.....	37
1.1.1	Post-translational modifications (PTM).....	38
1.12	The modulation of PP2A binding and/or endogenous inhibiting proteins.....	42
1.12.1	SET	42
1.12.2	SET binding protein.....	42
1.12.3	Cancerous inhibitor of PP2A (CIP2A).....	43
1.12.4	Alpha 4.....	43
1.12.5	Other PP2A regulators	44
1.13	The tumour suppressive role of PPP2R2A in breast cancer	45
1.14	The role of PP2A-B55 α in cellular signaling.....	48
1.15	Strategies to activate PP2A	54
1.16	Rationale of the study	60
1.17	Hypothesis.....	61
1.	Low PP2A-B55 α expression is a biomarker for poor outcome in luminal breast cancer.	61
2.	Molecular inhibition of PP2A-B55 α augments luminal breast cancer cell proliferation, migration, invasion and <i>in vivo</i> tumour formation, and inhibits DNA damage repair.	61
3.	Molecular inhibition of PP2A-B55 α induces resistance to targeted breast cancer therapies.	61
4.	Molecular inhibition of PP2A-B55 α sensitizes breast cancer cells to DNA damaging agents and PP2A activating drugs.....	61
2	Materials and Methods.....	62
2.1	Chemicals and Reagents	62
2.2	Molecular Biology	62
2.2.1	Cell culture.....	62
2.2.2	Cryopreservation of cells	65
2.2.3	BT474 cell luciferase transfection	65
2.2.4	PP2A B55 α Knockdown.....	66
2.3	Analysis of cellular functions	71
2.3.2	2.2.6.4 Cell migration and Invasion.....	71
2.4	Testing of <i>in vitro</i> drug sensitivity.....	72
2.4.1	Cytotoxicity.....	72
2.4.2	Clonogenic colony formation assay.....	72
2.5	DNA damage experiments.....	73
2.6	Flow cytometry	74
2.6.1	Cell cycle analysis.....	74

2.6.2	Apoptosis assay.....	74
2.7	Immunofluorescence.....	74
2.8	Confocal microscopy	75
2.9	Western blotting.....	75
2.9.1	RIPA cell lysis	75
2.9.1	Protein quantitation.....	77
2.9.2	Gel electrophoresis and immunoblotting	77
2.10	Animal Experiments	81
2.10.1	Tumour Xenografts	81
2.10.2	Processing of collected mouse xenografts and collected organs.....	81
2.11	Analysis of PP2A subunits and endogenous inhibitor expression in breast tumours	83
2.11.1	Online data analysis	83
2.11.2	Breast tumour specimens	83
2.11.3	Immunohistochemistry and scoring	84
2.11.4	Statistical analysis.....	84
3	PP2A subunit expression and association with breast cancer subtypes and patient outcome.....	85
3.1	INTRODUCTION	85
3.1	RESULTS	86
3.1.1	Dysregulation of PP2A subunits and inhibitors is frequent in breast tumours	86
3.1.2	The gene expression of PP2A subunits and endogenous inhibitors varies between different subtypes of breast cancer.....	89
3.1.3	Expression of PP2A subunits and associated genes varies across breast tumour grade.....	97
3.1.4	Copy number variations and mRNA alterations are involved in the dysregulation of PP2A subunits and inhibitory/binding protein gene expression in breast tumours.....	106
3.1.5	Low <i>PPP2R2A</i> mRNA expression predicts poor outcome in breast cancer patients ..	108
3.1.6	Altered PP2A protein expression is associated with breast tumour grade and subtype	114
3.1.7	PP2A-C protein expression is negatively associated with aggressive breast tumours	117
3.1.8	Low PPP2R2A (B55 α) is associated with aggressive breast tumours	117
3.1.9	High CIP2A expression is associated with TNBC.....	121
3.2	DISCUSSION	121
4	Generation of PP2A-B55 α KD cell lines and characterizing its functional role in luminal breast cancer	128
4.1	INTRODUCTION	128
4.2	RESULTS	129
4.2.1	Generation of PP2A-B55 α KD cell lines	129
4.2.2	Functional role of PP2A-B55 α in luminal breast cancer cells	131
4.2.3	Molecular KD of PP2A-B55 α increases <i>in vivo</i> tumour growth and reduces overall survival in a mouse model of breast cancer	142

4.2.4	Impact of PP2A-B55 α knockdown (KD) on DNA damage repair.....	146
4.1.1	Impact of reduced expression of PP2A-B55 α on apoptosis after IR induced DNA damage	152
4.3	DISCUSSION	154
5	The sensitivity of B55 α knockdown luminal breast cancer cells to targeted therapies and PP2A activators	160
5.1	INTRODUCTION	160
5.2	RESULTS	162
5.2.1	Molecular knockdown of PP2A-B55 α does not impact the sensitivity of BT474 cells to DNA damaging agents or PARP inhibitors	162
5.2.2	Reduced expression of PP2A-B55 α increased resistance to tamoxifen.....	164
5.2.3	Reduced expression of PP2A-B55 α increased resistance to Lapatinib in BT474 cells	169
5.2.4	Reduced expression of PP2A-B55 α did not affect the sensitivity of BT474 cells to mTOR inhibitors	171
5.2.5	Reduced expression of PP2A-B55 α did not impact the sensitivity of BT474 cells to the CDK4/6 inhibitor (Palbociclib).....	171
5.2.6	PP2A-B55 α knockdown cells are sensitive to PP2A activators.....	173
5.3	DISCUSSION	181
6	FTY720 and its derivatives elicit synergy with targeted therapies and small molecule activators of PP2A (SMAPs).....	189
6.1	INTRODUCTION	189
6.2	RESULTS	191
6.2.1	PP2A activating drugs increased the sensitivity of MCF7 cells to 4 hydroxy-tamoxifen	191
6.2.2	6.2.2 PP2A activating drugs increased the sensitivity of BT474 cells to lapatinib.....	192
6.2.3	Palbociclib elicits synergy in combination with PP2A activators.....	195
6.2.4	A combination of FTY720 or AAL(S) with small molecule activators of PP2A (SMAPs) is synergistic in MCF7 and BT474 breast cancer cells.....	195
6.2.5	A combination of FTY720 or AAL(S) with DBK1154 is synergistic at low doses in MCF7 and BT474 cells	199
6.2.6	A combination of FTY720 or AAL(S) with DBK382 is synergistic at low doses in MCF7 and BT474 cells	199
6.2.7	Low doses of DT061 in combination with FTY720 and AAL(s) elicit synergism in MCF7 and BT474 cells	204
6.2.8	Combination of inactive DBK1310 and 766 compounds with the DBK1154 elicit synergism at lower concentrations, and antagonism at higher doses	207
6.2.9	The inactive compounds DBK1310 and 766, are synergistic with the active DBK382 compound at low concentrations, and antagonistic at higher doses.....	207
6.2.10	Combination of inactive DBK1310 and 766 compounds with FTY720 showed additive to synergistic effects in MCF7 and BT474 cells	212

6.3	DISCUSSION	217
7	CONCLUSION AND FUTURE DIRECTIONS	222
	APPENDIX.....	243
8	REFERENCES	271

ABSTRACT

Breast cancer is a leading cause of morbidity and mortality worldwide, and despite advancements made in early diagnosis and new treatments over the past few decades, disease progression and therapy resistance remains an unmet challenge. A better understanding of the mechanisms involved in disease progression and resistance to therapies is essential in order to develop improved treatment strategies.

Reversible phosphorylation is controlled by the balanced activities of protein kinases and phosphatases, and is a central regulator of the signal transduction pathways required for cell proliferation, differentiation and survival. As such, dysregulation of this balance can result in loss of cellular differentiation and sustained proliferation and survival – key characteristics of cell transformation. While the role of protein kinases in tumourigenesis has been extensively studied, the role protein phosphatases is less well understood.

Protein phosphatase 2A (PP2A), is a family of serine/threonine phosphatases that is inactivated in many cancers, including breast cancer, and as such is considered a tumour suppressor. PP2A controls over 50% of serine/threonine phosphatase activities in cells, and regulates numerous growth and survival signalling pathways including the PI3K-AKT and MAPK pathways. PP2A is a trimeric protein complex consisting of a structural subunit (PP2A-A), a catalytic subunit (PP2Ac) and a regulatory subunit (PP2A-B), of which there are at least 4 families each with multiple isoforms. While the dimeric complex of PP2A-AC can dephosphorylate multiple proteins, it is the PP2A-B regulatory subunit that provides substrate specificity and subcellular localization of PP2A. Recent large scale genomic analyses have identified recurrent loss of heterozygosity (LOH) at the *PPP2R2A* gene locus, which encodes the PP2A-B55 α regulatory subunit. *PPP2R2A* LOH was most common in estrogen receptor positive (ER⁺) luminal breast tumours, and in particular in the aggressive Luminal B subtype. However, the functional role of *PPP2R2A* loss in breast cancer is not known. Therefore, the overall goal of this thesis was to characterise the gene and protein expression of PP2A subunits, including *PPP2R2A* (PP2A-B55 α), in human breast tumours, and to identify the specific functional role of reduced PP2A-B55 α in luminal breast cancer.

The first aim of this thesis was to use *in silico* analysis of publically available gene expression databases, and immunohistochemistry (IHC) of human breast tumours, to compare the gene and protein expression of PP2A subunits and associating proteins between normal breast

tissues and breast tumours, and to examine the association of PP2A subunit expression with breast tumour subtypes and disease outcome. This analysis, presented in chapter 3, revealed that low gene and protein expression of the PP2A-B55 α and PP2Ac subunits was associated with high grade tumours, and with Luminal B, Human epidermal growth factor positive (HER2⁺), and triple negative breast (ER⁻, progesterone receptor (PR)⁻, HER2⁻; TNBC) molecular subtypes, which are more aggressive than the Luminal A breast tumour subtype. Thus, low PP2A-B55 α and PP2Ac protein expression is associated with aggressive breast tumours, and this is likely regulated at the gene expression level. Low *PPP2R2A* (PP2A-B55 α) was further shown to predict for poor relapse-free and overall survival in breast cancer patients, most notably for patients with luminal (ER⁺) tumours. Reduced gene expression of the PP2A regulators SETBP1 and alpha-4, was also associated with aggressive subtypes and worse outcome, suggesting that they may function as tumour suppressors. In contrast, high expression of a number of PP2A-B56 family subunits, and the PP2A inhibitors SET and CIP2A, were associated with more aggressive breast tumours, and thus may play an oncogenic role and be targets for breast cancer therapy.

To determine if the reduced *PPP2R2A* expression observed in aggressive poor outcome tumours, is functionally important, I next investigated the effects of molecular inhibition of *PPP2R2A* in the human luminal breast cancer cell lines, ZR751, MCF7 and BT474. The data in chapter 4 shows that short-hairpin RNA (shRNA) mediated inhibition of *PPP2R2A*, resulting in reduced PP2A-B55 α protein expression, increased breast cancer cell proliferation, migration and invasion, and increased tumour growth in an orthotopic xenograft mouse model. This was associated with increased activation of ER and AKT signalling, and evidence of an epithelial-to-mesenchymal transition (EMT) phenotype. These results suggest that functional inactivation of PP2A-B55 α complexes is important for breast cancer progression, and supports a tumour suppressive role for *PPP2R2A*.

The introduction of anti-estrogen therapies, such as Tamoxifen, to ER⁺ breast cancer patients has led to remarkable improvements in survival. However, many patients are either intrinsically resistant to therapy, or develop resistance and later relapse with therapy-resistant disease. Given that low *PPP2R2A* expression was associated with poor outcome in ER⁺ patients, in chapter 5 I explored whether inhibition of PP2A-B55 α mediated tamoxifen resistance. Analysis of publically available datasets showed that the loss of *PPP2R2A* was a strong predictor of earlier relapse and distant metastasis in tamoxifen treated breast cancer patients. Furthermore,

molecular knockdown of PP2A-B55 α induced resistance to tamoxifen in ER⁺ breast cancer cells. In contrast, ER⁺ breast cancer cells selected for resistance to tamoxifen, expressed reduced PP2A-B55 α compared to parental drug sensitive cells, demonstrating a functional role for PP2A-B55 α in ER signalling and therapy resistance. In addition, PP2A-B55 α knockdown in the HER2⁺ breast cancer cell line, BT474, induced resistance to the anti-HER2 therapeutics, Trastuzumab and Lapatinib. Therefore, the poor outcome observed in patients with low *PPP2R2A* expression may be mediated by intrinsic resistance to standard therapies.

Importantly however, breast cancer cells with low PP2A-B55 α were highly sensitive to pharmacological activators of PP2A. Clonogenic and cytotoxicity assays showed that PP2A-B55 α knockdown cells were just as sensitive, and in some cases were more sensitive, than control cells, to sphingolipid PP2A agonists (FTY720 and derivatives) and small molecule activators of PP2A (SMAPs). This suggests that PP2A activities can still be enhanced in tumours with low *PPP2R2A*, and thus is a potential therapeutic strategy for poor outcome breast cancer patients.

In chapter 6 I further showed that treating breast cancer cells with PP2A activating drugs can increase the sensitivity of breast cancer cells to targeted therapies, including tamoxifen, Lapatinib and the CDK4/6 inhibitor, Palbociclib. Importantly, the addition of a PP2A activating drug sensitized tamoxifen-resistant breast cancer cells to tamoxifen, providing a strong rationale to combine PP2A activating drugs with standard therapies for the treatment of therapy sensitive and resistant breast tumours. Finally, given that sphingolipid PP2A activators and SMAPs are distinct classes of drugs with different mechanisms of PP2A activation, I examined the effects of combining these two classes of drugs. Intriguingly, I found that the combination displayed highly synergistic cytotoxicity in breast cancer cells with or without PP2A-B55 α knockdown.

Collectively, the body of work presented in this thesis enhances our understanding of the function of PP2A-B55 α in breast cancer signalling and therapy resistance, and suggests that PP2A-B55 α expression may be a useful biomarker for predicting disease outcome in luminal breast cancer. Furthermore, these data support the clinical testing of PP2A activating drugs alone and/or in combination, in relapsed/resistant ER⁺ breast cancer patients, with the ultimate goal of improving the survival of breast cancer patients..

ACKNOWLEDGEMENT

First of all I thanks to Allah, the almighty, for giving me this opportunity, and mental and physical health to do my PhD work and put forth thesis.

Then, of course there are many people who have helped me during this amazing journey without which it would have been extremely difficult to make it. Special thanks to my supervisors, who have been there all the time for their feedbacks on my research work, and taught me new skills that are pivotal both in laboratory and life outside. First to A/Prof Nikki Verrills, who have been wonderful mentor during this very important 4 years of my life. Dear Nikki thank you very much for providing me this opportunity of PhD in your lab. For your support and being patience, allowing me to make mistakes and to learn from them, and being available all the time throughs emails, text messages and even on phone calls whenever I needed help. For listening to me and providing me the best advices during the times of stress and desperation, both related to lab and personal. For always encouraging me to do best, providing me guidance and focus when required, and sometime pushing me beyond to accomplish tasks that I thought I was competent of. You are and will always be an inspiration to me in and out of the lab. To Dr Matt Dun, for your support in the lab to solve problems, and all those discussions and suggestions to improve the quality of my thesis. I am really inspired of you for your passion for science and to do something different and meaningful. To Dr Kathryn Skelding, for always keeping your office doors open for me. For discussing and providing me feedback on my progress and to plan future experiments, and above all helping me with the animal work.

I am also highly thankful to Dr Liz Caldon, from the Garvan Institute, for gifting me some of the cell lines that they developed in their lab. Also thanks to the Dr Simon King for his help in IHC scoring and Ms Megan Clarke in optimization and staining of tumour tissue mirco-arrays, both from Hunter Medical Research Institute, Newcastle. A very big thanks to Dr Severine Roselli for reading and giving feedback on my thesis, to write all those ethics and safety applications and variations, helping me with the animal and IHC experiments

To all of my lab friends who I have been working with during these four year in providing me wonderful and fun lab environment, good ideas and extending your hands for help to share work load. Special thanks to Mr Richard Kahl for being such a nice and welcoming person and also being the first person to seek for help in the lab. Also special thanks to Ms Heather Murray, Ms Nikita Panicker, Ms Yanfang Chen, Mr David Skerret-Byrne, Dr Trisha Al Mazi.

A big thanks to my Muslim and Pakistani friends for their love, prayers, guidance and support. Special thanks to Brother Sameer Ahamad for cooking all those delicious meal for last two year. Especial thanks to Dr Shahol Mujahid, Brother Sameer Ahamad, Brother Kamal, Brother Muhammad Tanko, Muhammad Umar and Brother Muhammad Fairoze for their advices and support to settle in Newcastle. Especial thanks to my Arabic teacher, Brother Muhammad Farooq, who provided an opportunity for me to learn another language. To, University of Newcastle Islamic Society for arranging all those events with delicious meals especially Ramdhan feast and Masjid open day.

Finally, endless thanks to my family, especially my mother, my father and my elder brother without their support it would have been almost impossible for me to continue my journey of gaining knowledge starting from a small village in Pakistan and ending up doing PhD in one of the best institution in the world. My mother, a very caring person, who always advised me in doing good, being a caring and good person, getting best and highest possible education, and supported me through her prayers especially during the time of stress. She is an amazing women, a best mentor, and a teacher during my early years of education and a friend, whom I can never thank does not matter how hard I try to serve her. To my father, a math teacher, who always believed in me, listened me whenever I needed and advised me accordingly, and supported me both financially and emotionally. Also to my Sisters, younger brother, and offcourse my cute nieces for telling me all those fun stories from back home to help me to relax..

Publication:

Watt LF, Panicker, Mannan A, Copeland B, Kahl RGS, Dun MD, Young B, Roselli S, Verrills NM (2017). Functional importance of PP2A regulatory subunit loss in breast cancer. *Breast Cancer Res Treat.* 2017;166(1):117-131. doi: 10.1007/s10549-017-4403-5

Oral Conference Presentations (presenter underlined):

Full length oral presentations:

Mannan A, Panicker N, Kahl R, Dun M, Skelding K, Verrills (2016) Reduced expression of the protein phosphatase 2a regulatory subunit B55 α : impact on luminal b breast cancer cells progression and DNA damage repair. *Hunter Cancer Research Symposium*, Newcastle, Australia. *Published abstract:* Asia-Pacific Journal of Clinical Oncology

Mannan A, Panicker N, Chem Y, Kahl RG, Dun MD, Roselli S, Verrills NM (2018). The functional role of PP2A-B55 α in breast cancer. *FASEB Protein Phosphatases Meeting*, Snowmass, CO, USA.

Mannan A, Panicker N, Kahl R, , King S, Clarke M, , Roselli S, Skelding K, Dun MD Verrills NM (2018) Reduced expression of the tumour suppressor PP2A-B55 α associates with poor outcome and induces an aggressive, tamoxifen-resistant phenotype. *Australian Society for Medical Research (ASMR) Satellite Scientific Meeting*, Hunter Medical Research Institute, Newcastle, Australia.

Fast forward oral presentations:

Mannan A, Panicker N, Kahl R, Dun MD, Skelding K, Verrills NM (2016). Impact of reduced expression of the protein phosphatase 2A subunit, B55 α , in luminal B breast cancer cells and in DNA damage repair. Australian Society for Medical Research (ASMR) Satellite Scientific Meeting, Hunter Medical Research Institute, Newcastle, Australia.

Mannan A, Panicker N, Kahl R, Dun MD, Skelding K, Verrills N (2017) Role of the tumour suppressor, PP2A-B55 α , in breast cancer. Australian Society of Medical Research Satellite Scientific Meeting, Newcastle, Australia.

Mannan A, Panicker N, Kahl R, Skelding K, Dun MD, Verrills N (2017) Reduced Expression of the Protein Phosphatase 2A Regulatory Subunit B55 α in Luminal Breast Cancer: Effect on Tumor Progression and Antihormonal Therapy. *Hunter Cancer Research Symposium*, Newcastle, Australia. *Published abstract:* Asia Pacific J Clin Oncol 13: 11.

Poster Presentations:

Mannan A, Panicker N, Watt L, Kahl R, Dun M, Skelding K, Verrills N (2015), Role of reduced protein phosphatase 2a subunit, B55 α , expression in luminal b breast cancer cell line

DNA damage repair Pathway'. *Hunter Cancer Research Symposium*, Newcastle, Australia.
Published abstract: Asia-Pacific Journal of Clinical Oncology

Mannan A, Panicker N, Kahl R, Dun MD, Skelding K, Verrills NM (2016). Reduced expression of the protein phosphatase 2A regulatory subunit B55 α : Impact on luminal B breast cancer cells and DNA damage repair. *Australian Society for Medical Research (ASMR) National Scientific Meeting*, Powerhouse Museum, Darling Harbour, Sydney, Australia.

Mannan A, Panicker N, Kahl R, Dun MD, Skelding K, Verrills N (2017) Role of the tumour suppressor, PP2A-B55 α , in breast cancer and DNA damage response. *The 17th Australian Cell Cycle Meeting* 27-29th March 2017, Sydney, Australia

Mannan A, Roselli S, Kahl R, Skelding K, Dun MD and Verrills N (2018) Loss of protein phosphatase 2A regulatory subunit B55 α enhances tumour progression and tamoxifen resistance in luminal breast cancer. *Hunter Cancer Research Symposium*, Newcastle, Australia

Mannan A, Panicker N, Kahl R, King S, Clarke M, Skelding K, Dun MD, Roselli S, Verrills N (2018). Inhibition of the tumour suppressor PP2A-B55 α induces aggressive and therapy resistant phenotype in breast cancer. *6th Sydney Cancer Conference (SCC2018)*, Sydney Australia.

Mannan A, Panicker N, Kahl R, King S, Clarke M, Skelding K, Dun MD, Roselli S, Verrills N. (2018), Reduced expression of the tumour suppressor PP2A-B55 α associates with poor outcome and induces an aggressive, tamoxifen-resistant phenotype in human breast cancer cells. *9th Garvan Signaling Symposium*, Sydney, Australia.

Panicker N, Mannan A, Watt LF, Copeland B, Dun MD, King S, Clarke M, Skelding K, Roselli S, and Verrills NM. 2017. Functional role of the tumor suppressor protein phosphatase, PP2A-B55 α , in breast cancer ' *American Association for Cancer Research Annual Meeting*, Washington DC, USA. *Published abstract: Cancer Research* 77: 2375.

Professional awards and scholarship:

- 1- University of Newcastle International Postgraduate Research Scholarships (UNIPRS)
- 2- University of Newcastle Research Scholarship Central 50:50 (UNRSC 50:50)
- 3- Winner of 2017 HCRA RHD Award from the Biomarkers and Targeted Therapies Flagship funds (5000 A\$)

ABBREVIATIONS

53BP1	p53 binding protein 1
CIP2A	Cancerous inhibitor of PP2A
3D	Three dimensional
4OH-tam	4 hydroxytamoxifen
5-FU	Fluorouracil
ADH	Atypical ductal hyperplasia
AI	Aromatase inhibitor
ATM	Ataxia telangiectasia mutated
B55 α	PP2A regulatory subunit B55 isoform α
BME	Basement membrane extract
BRCA	Breast cancer associated
BSA	Bovine serum albumin
CHK	Checkpoint kinase
CI	Combination index
CtIP	C-terminal Binding Protein Interacting Protein
ERK	Extracellular signal-regulated kinase
DCIS	Ductal carcinoma in situ
DMEM	Dulbecco's modified Eagle's medium
DMFS	Distant metastasis free survival
DMSO	Dimethyl sulphoxide
DNA-PKcs	DNA-dependent protein kinase
DSB	Double strand DNA break
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial to mesenchymal transition
ER	Estrogen receptor
ERE	Estrogen response element
FAK	Focal adhesion kinase
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate

g	gram
g	gravity
GOBO	Gene expression-based Outcome for Breast cancer Online
GSK-3 β	Glycogen synthase kinase-3 β
GWL	Greatwall kinase
HEAT (repeat)	Huntington-elongation-PP2A-A subunit-TOR
HER2	Human epidermal growth factor receptor
HR	Homologous recombination
HR	Hazard ratio
HRP	Horse radish peroxidase
ID50	concentration of drug that inhibits cell viability by 50%
JAK	Janus kinases
kDa	Kilodalton
KSR1	Kinase suppressor of Ras
LCMT-1	Leucine Carboxyl Methyltransferase
LOH	Loss of heterozygosity
Luc	Luciferase
MAPK	Mitogen activated protein kinase
Mdm-2	Mdouble minute homologue 2
MEFs	Mouse embryonic fibroblasts
mg	Milligram
ml	Millilitre
mM	Millimolar
mRNA	messenger RNA
mTOR	Mammalian target of rapamycin
NHEJ	Non-homologous end-joining
nM	Nanomolar
OS	Overall survival
PARP	Poly ADP ribose polymerase
PBS	phosphate buffered saline
PDK1	3-phosphoinositide-dependent protein kinase 1

PH	Plestrin homology (domain)
PI	Propidium iodide
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol (4,5)P2
PIP3	Phosphatidylinositol (3,4,5) phosphate
PKA	Protein kinase A
PME-1	Phosphatase methylesterase (specific for PP2A)
PP2A	Protein Phosphatase 2A
PP2A-A	Structural subunit of PP2A
PP2A-C	Catalytic subunit of PP2A
PPP2R2A	PP2A B55 α regulatory subunit
PTEN	Phosphatase and tensin homologue
Rb	Retinoblastoma
RFS	Relapse free survival
RIPA	Radio-immunoprecipitation assay
RPMI	Roswell park memorial institute media
RTK	Receptor tyrosine kinase
SBDS	Shwachman-bodian-diamond syndrome
SDS	sodium dodecyl sulphate
SEM	Standard error of the mean
Ser	Serine
SERM	Selective estrogen receptor modulator
shB55 α	Short hairpin targeting mRNA encoding for PP2A-B55 α
shCont	short hairpin control
shRNA	Short hairpin RNA
SMAP	Small molecule activator of PP2A
STAT	Signal transducer and activator of transcription
TamR	Tamoxifen resistant
TBST	Tris buffered saline with 0.1% Tween 20
TCGA	The cancer genome atlas
Thr	Threonine

TNBC	Triple negative breast cancer
Tyr	Tyrosine
Wnt	wingless
WT	Wild-type
μg	Microgram
μl	Microlitre
μM	Micromolar