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

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B. Biomed Sci (Hons)

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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.
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PRESENTATIONS

Oral Conference Presentations:


Watt, LF. Altered protein 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.

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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>2D</td>
<td>Two dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>5-FU</td>
<td>Fluorouracil</td>
</tr>
<tr>
<td>ADH</td>
<td>Atypical ductal hyperplasia</td>
</tr>
<tr>
<td>BAD</td>
<td>Bcl2-agonist of death</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>BME</td>
<td>Basement membrane extract</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CAMKII</td>
<td>Calcium/calmodulin-dependent protein kinase II</td>
</tr>
<tr>
<td>CHO cells</td>
<td>Chinese hamster ovary cells</td>
</tr>
<tr>
<td>CIP2A</td>
<td>Cancerous inhibitor of PP2A</td>
</tr>
<tr>
<td>CSF-1R</td>
<td>Colony-stimulating factor receptor</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’,6 Diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DCIS</td>
<td>Ductal carcinoma in situ</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified eagle’s medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DUSP</td>
<td>Dual specificity phosphatase</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>EMT</td>
<td>Epithelial to mesenchymal transition</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>ERE</td>
<td>Estrogen response element</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence activated cell sorting</td>
</tr>
<tr>
<td>FAK</td>
<td>Focal adhesion kinase</td>
</tr>
<tr>
<td>FCS</td>
<td>Foetal calf serum</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>Glycogen synthase kinase-3β</td>
</tr>
<tr>
<td>HEAT (repeat)</td>
<td>Huntington-elongation-PP2A-A subunit-TOR</td>
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<tr>
<td>HEK-TER</td>
<td>Human embryonic kidney cells immortalised by addition of hTERT, SV40 LT and active Ras</td>
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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)P2
PIP3  Phosphatidylinositol (3,4,5)P3
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