Characterisation of a Dominant Negative Androgen Receptor in Prostate Cancer Cells

A thesis submitted to the University of Adelaide in total fulfillment of the requirements for the degree of Doctor of Philosophy

by

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April 2008
This thesis is dedicated to my father.

Your prostate cancer was the inspiration for this work.
CHAPTER 3 - OPTIMISATION OF AN AR INHIBITION ASSAY FOR INVESTIGATING DOMINANT NEGATIVE ANDROGEN RECEPTOR ACTIVITY................................................................. 63

3.1-Introduction..............................................................................................................64
3.2-Methods ....................................................................................................................66
  3.2.1-Luciferase Reporter Gene Assay..........................................................................66
  3.2.2-Immunoblot.........................................................................................................66
  3.2.3-Statistical Analysis ..............................................................................................66
3.3-Results.......................................................................................................................67
  3.3.1-Assessment of dominant negative AR inhibition using different AR-regulated reporters ........................................................................................................67
    3.3.1.1-Probasin (ARR3-tk-luciferase) ......................................................................67
    3.3.1.2-Prostate-specific antigen (PSA1540-luciferase) ............................................67
    3.3.1.3-Mouse mammary tumour virus (MMTV-luciferase) .......................................69
  3.3.2-Determination of the optimal amount of AR plasmid DNA to transfet into cells for the AR inhibition assay ...........................................................................................69
  3.3.3-Optimisation of an immunoblot for use in conjunction with the AR inhibition assay ..........................................................................................................................71
  3.3.4-Inhibition of AR by ARi410 using the optimised AR inhibition assay...............71
  3.3.5-Effects of ARi410 on AR protein levels...............................................................73
3.4-Discussion..................................................................................................................76

CHAPTER 4 - MECHANISMS OF DOMINANT NEGATIVE ANDROGEN RECEPTOR ACTION .......................................................... 79

4.1-Introduction..............................................................................................................80
4.2-Methods ....................................................................................................................81
  4.2.1-Luciferase Reporter Gene Assay..........................................................................81
  4.2.2-Transfection and Preparation of Chamber Slides for Confocal Studies .................81
  4.2.3-Confocal Microscopy and Imaging Analysis.......................................................82
  4.2.4-Co-Immunoprecipitation ................................................................................. 83
  4.2.5-Preparation of Nuclear Extracts .......................................................................83
  4.2.6-Electrophoretic Mobility Shift Assay ............................................................... 84
    4.2.6.1-Non-Radioactive Method........................................................................... 84
    4.2.6.2-Radioactive protocol 1 ............................................................................... 86
    4.2.6.3-Radioactive Protocol 2............................................................................... 87
  4.2.7-Statistical Analysis ............................................................................................88
4.3-Results.......................................................................................................................88
Declaration

This work contains no material which has been accepted 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 in the text. I give consent to this copy of my thesis being made available in the University library. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder/s of those works.

Margaret M. Centenera

April 2008
Abstract

Prostate cancer is the second leading cause of death from cancer in Australian men. As prostate cancer cells are reliant on androgens for growth and survival, the standard therapy for metastatic disease is androgen ablation therapy (AAT). AAT inhibits androgen signalling by altering androgen synthesis or prevent binding of androgens to their intracellular mediator, the androgen receptor (AR). Although initially effective, virtually all patients relapse, beyond which there are limited treatment options. The failure of AAT is not necessarily due to a decreased requirement for androgen signalling, but rather the AR is able to maintain signalling and tumour growth in an androgen-depleted environment. Therefore novel strategies that directly target the AR may provide a more effective therapeutic approach.

We have endeavoured to suppress AR activity in prostate cancer cells by utilising a dominant negative AR. The most effective dominant negative construct developed, ARi410, lacks amino acids 39-410 in the AR amino terminal transactivation domain. In studies of transcriptional activity, ARi410 has no intrinsic activity but inhibits the activity of wild type AR (wtAR) and also clinically relevant AR variants, by up to 95%. The objective of this thesis was to characterise the mechanisms of action of ARi410 and assess the functional effects of introducing this dominant negative receptor into prostate cancer cells.

To investigate the mechanism by which ARi410 suppresses AR activity, a robust and sensitive AR inhibition assay was developed. This assay revealed that ARi410 is a potent inhibitor of AR activity on three independent AR-regulated promoters, regardless of the level of AR expression. Furthermore, while ARi410 can inhibit AR activity, it does not alter AR protein levels. By using ARi410 variants with mutations and/or deletions in regions of functional importance, the AR inhibition assay was also used to identify the critical regions of ARi410 required for its dominant negative activity. These studies demonstrate that the
dominant negative activity of ARi410 is ligand-dependent, requires dimerisation through the ligand binding domain (LBD) and an intact DNA-binding domain (DBD).

Further investigation into the mechanism of dominant negative activity revealed that ARi410 does not alter the subcellular localisation of AR, as both receptors are predominantly cytoplasmic in the absence of ligand and rapidly co-localise to the nucleus in response to androgens. Furthermore, an interaction between AR and ARi410 was observed in the presence and absence of ligand, and electrophoretic mobility shift assays demonstrated that AR and ARi410 form heterodimers on DNA. These studies led to the conclusion that the mechanism of dominant negative activity by ARi410 involves the formation of inactive receptor heterodimers that assemble on DNA and suppress AR activity.

To determine the functional consequence of expressing the dominant negative androgen receptor in prostate cancer cells, an adenoviral method of gene delivery was developed. Adenoviral expression of ARi410 in LNCaP prostate cancer cells did not allow assessment of cell viability due to cell-specific toxicity of the viral vectors when expressed long-term. However, short-term expression of ARi410 in LNCaP cells resulted in inhibition of AR signalling, as determined by reduced expression of the androgen regulated genes apolipoprotein D and kallikrein 2. Importantly, this finding is consistent with the inhibitory activity of ARi410 observed using synthetic AR-regulated reporter genes in the AR inhibition assay, and demonstrates that ARi410 can effectively suppress endogenous AR signalling.

The results of this thesis indicate that heterodimerisation between AR and ARi410 is the most likely mechanism of dominant negative inhibition of AR function by ARi410, and that the DBD and dimerisation through the LBD are required for optimal dominant negative activity. Furthermore, this thesis has demonstrated that ARi410 is an effective inhibitor of AR signalling and provides a basis for further functional studies and evaluation of the dominant negative androgen receptor in vitro and in vivo.
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Publications Arising from this Thesis

Articles Published in Scientific Journals


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Abstracts Published in the Proceedings of Scientific Meetings

Centenera MM, Tilley WD, Butler LM. Development of dominant negative androgen receptors as a novel therapeutic strategy for prostate cancer. Australia Society for Medical Research Annual Scientific Meeting, Adelaide, Australia, June 2005

Centenera MM, Tilley WD, Butler LM. Development of dominant negative androgen receptors as a novel therapeutic strategy for prostate cancer. Australia Society for Medical Research Annual Scientific Meeting, Adelaide, Australia, June 2006
Presentations not arising directly from the work in this thesis

Peters AA, Bianco-Miotto T, Centenera MM, O’Loughlin M, Buchanan G, Butler LM and Tilley WD. Inhibition of estrogen receptor alpha activity in breast cancer cells by the androgen receptor. The 3rd PacRim Breast and Prostate Cancer Meeting, Fraser Island, Australia, November 2006

Amelia A. Peters, Tina Bianco-Miotto, Nicole L. Moore, Margaret M. Centenera, Grant Buchanan, Stephen N. Birrell, Lisa M. Butler, Wayne D. Tilley. Inhibition of estrogen receptor alpha activity in breast cancer cells by the androgen receptor. The 98th annual meeting for the American Association for Cancer Research, Anaheim, USA, April 2007


Wayne Tilley, Carmela Ricciardelli, Tina Bianco-Miotto, Amelia A Peters, Margaret M Centenera, Nicole L Moore, Niamh Murphy, Dave Segara, Catriona McNeil, Susan M Henshall, Rob L Sutherland, Andrew J Sakko, Grant Buchanan, Stephen N Birrell, Lisa M Butler. The androgen receptor inhibits estrogen receptor alpha (ER) signaling and predicts outcome in ER positive breast cancer. Department of Defence Era of Hope Meeting, Baltimore, USA, June 2008
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>0-T</td>
<td>no treatment/mock transduced</td>
</tr>
<tr>
<td>17-AAG</td>
<td>17-allylamino-demethoxygeldanamycin</td>
</tr>
<tr>
<td>AAT</td>
<td>androgen ablation therapy</td>
</tr>
<tr>
<td>AF</td>
<td>activation function</td>
</tr>
<tr>
<td>AF-1</td>
<td>activation function 1 (amino acids 38-360)</td>
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<td>AF-2</td>
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<tr>
<td>AF-5</td>
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<tr>
<td>AIB1</td>
<td>amplified in breast cancer 1</td>
</tr>
<tr>
<td>AIS</td>
<td>androgen insensitivity syndrome</td>
</tr>
<tr>
<td>APOD</td>
<td>apolipoprotein D</td>
</tr>
<tr>
<td>AR</td>
<td>androgen receptor</td>
</tr>
<tr>
<td>ARA54</td>
<td>androgen receptor coactivator 54</td>
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<tr>
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</tr>
<tr>
<td>ARE</td>
<td>androgen response element</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>C</td>
<td>cytoplasmic</td>
</tr>
<tr>
<td>CAB</td>
<td>combined androgen blockade</td>
</tr>
<tr>
<td>CAIS</td>
<td>complete androgen insensitivity syndrome</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CAR</td>
<td>coxsackie adenovirus receptor</td>
</tr>
<tr>
<td>CAT</td>
<td>chloramphenicol acetyl transferase</td>
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<tr>
<td>cDNA</td>
<td>complementary DNA</td>
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<tr>
<td>cfu</td>
<td>colony forming units</td>
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<tr>
<td>CPE</td>
<td>cytopathic effect</td>
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<tr>
<td>ChIP</td>
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</tr>
<tr>
<td>CREB</td>
<td>cAMP-response-element-binding protein</td>
</tr>
<tr>
<td>CBP</td>
<td>CREB-binding protein</td>
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<tr>
<td>CsCl</td>
<td>Caesium Chloride</td>
</tr>
<tr>
<td>CTE</td>
<td>carboxyl-terminal extension</td>
</tr>
<tr>
<td>DAB</td>
<td>3,3’-Diaminobenzidine</td>
</tr>
<tr>
<td>DBD</td>
<td>DNA binding domain</td>
</tr>
<tr>
<td>DCC</td>
<td>dextran coated charcoal</td>
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<tr>
<td>DCC-FCS</td>
<td>dextran coated charcoal-fetal calf serum</td>
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<tr>
<td>DHT</td>
<td>5α-dihydrotestosterone</td>
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<tr>
<td>DIG</td>
<td>digoxigenin-11-ddUTP</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulphoxide</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>DNase1</td>
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</tr>
<tr>
<td>dNTPs</td>
<td>deoxyribonucleotide triphosphates</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double stranded DNA</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothriotel</td>
</tr>
<tr>
<td>ECL</td>
<td>enhanced chemiluminescence</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>EMSA</td>
<td>electrophoretic mobility shift assay</td>
</tr>
<tr>
<td>ER</td>
<td>estrogen receptor</td>
</tr>
</tbody>
</table>
NR | nuclear receptor  
NTD | amino-terminal domain  
OD | optical density  
OD\textsubscript{260} | optical density at 260nm  
OD\textsubscript{280} | optical density at 280nm  
OHF | hydroxyflutamide  
PAGE | polyacrylamide gel electrophoresis  
PB | probasin  
PBS | phosphate buffered saline  
PCR | polymerase chain reaction  
PIN | prostate intraepithelial neoplasia  
PKC | protein kinase C  
pM | picomolar  
PMT | photomultiplier tube  
PR | progesterone receptor  
PSA | prostate specific antigen  
Rb | retinoblastoma  
RAC3 | nuclear receptor coactivator 3  
RT-PCR | quantitative real-time PCR  
RLU | relative light units  
RNA | ribonucleic acid  
RNase | ribonuclease  
RNasin | RNase inhibitor  
rpm | revolutions per minute  
RT | reverse transcriptase  
SA | South Australia  
SAHA | suberoylanilide hydroxamic acid; vorinistat  
SDS | sodium dodecyl sulphate  
sec | second  
SEM | standard error of the mean  
SGT\textalpha | small glutamine rich TPR containing protein \alpha  
SMRT | silencing mediator of retinoic and thyroid hormone receptors  
SRC-1 | steroid receptor coactivator 1  
ssDNA | single stranded DNA  
t707 | truncated at amino acid 707  
T | testosterone  
TBE | tris borate EDTA  
TBS | tris buffered saline  
TBST | tris buffered saline-tween 20  
TEMED | N,N,N',N'-tetramethylthylethylenediamine  
TIF2 | transcriptional intermediary factor 2  
Tm | melting temperature  
TMPRSS2 | transmembrane protease, serine 2  
TPR | tetratricopeptide repeat  
TRAMP | transgenic adenocarcinoma of mouse prostate  
U | uracil  
UGE | urogenital sinus epithelium  
UGM | urogenital sinus mesenchyme  
USA | United States of America  
UTP | uracil triphosphate  
UTR | untranslated region  
UV | ultraviolet
V         volt
WC        whole cell
wtAR      wild type androgen receptor
x         any amino acid

Other:
°C         degrees Celsius
αα         amino acid
Δ          deletion
μCi        microcurie
μg         microgram
μl         microlitre
μm         micron
μM         micromolar