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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This thesis is dedicated to my father.

Your prostate cancer was the inspiration for this work.

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.

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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

Butler LM*, Centenera MM*, Neufing PJ*, Buchanan G, Choong CS, Ricciardelli C, Saint K, Lee MA, Ochnik A, Yang M, Brown MP and Tilley WD. Suppression of androgen receptor signalling in prostate cancer cells by an inhibitory receptor variant. Molecular Endocrinology 20(5): 1009-1024 (2006). *Equal first authors.

Margaret Centenera*, Eleanor Need*, Lisa Butler and Wayne Tilley. The Androgen Receptor in Prostate Cancer. Australian Biochemist 38(1): 12-15 (2007). *Equal first authors

Margaret M. Centenera, Jonathan M. Harris, Wayne D. Tilley and Lisa M. Butler. The Contribution of Different Androgen Receptor Domains to Receptor Dimerization and Signalling. Molecular Endocrinology (In press 2008)

Grant Buchanan, Eleanor F. Need, Tina Bianco-Miotto, Norman M. Greenberg, Margaret M. Centenera, Lisa M. Butler, Wayne D. Tilley. Insights from AR gene mutations. Chapter for book titled 'Androgen action in Prostate Cancer'. Springer (In press 2008)

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

Centenera MM, Tilley WD, Butler LM. Development of dominant negative androgen receptors as a therapeutic strategy for prostate cancer. The Endocrine Society of Australia Annual Scientific Meeting, Gold Coast, Australia, August 2006

Centenera MM, Tilley WD, Butler LM. Characterisation of a dominant negative androgen receptor. The 3rd Pacific Rim Breast and Prostate Cancer Meeting, Fraser Island, Australia, November 2006

WD Tilley, MM Centenera, G Buchanan, C Ricciardelli, MP Brown and LM Butler. Characterisation of a dominant negative androgen receptor. The American Association for Cancer Research special conference; Innovations in Prostate Cancer Research, San Francisco CA USA, December 2006

MM Centenera, WD Tilley, G Buchanan, C Ricciardelli, MP Brown and LM Butler. Characterisation of a dominant negative androgen receptor. Faculty of Health Sciences Postgraduate Research Expo, Adelaide SA, October 2007

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

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Peters AA, Bianco-Miotto T, Murphy N, Ricciardelli C, Centenera MM, Buchanan G, Segara D, Henshall SM, Birrell SN, Butler LM, Sutherland RL, Tilley WD. The protective role of AR in breast cancer is in part due to its ability to inhibit ERα signaling. The Endocrine Society's 89th Annual Meeting, Toronto, Canada, June 2007

Peters AA, Bianco-Miotto T, Murphy N, Ricciardelli C, Centenera MM, Buchanan G, Segara D, Henshall SM, Birrell SN, Sutherland RL, Butler LM and Tilley WD. The androgen receptor attenuates estrogen signaling in breast cancer cells. Gordon Mammary Gland Biology Conference, Newport, USA, June 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

Abbreviations

0-T	no treatment/mock transduced
17-AAG	17-allylamino-demethoxygeldanamycin
AAT	androgen ablation therapy
AF	activation function
AF-1	activation function 1 (amino acids 38-360)
AF-2	activation function 2
AF-5	activation function 5 (amino acids 360-535)
AIB1	amplified in breast cancer 1
AIS	androgen insensitivity syndrome
APOD	apolipoprotein D
AR	androgen receptor
ARA54	androgen receptor coactivator 54
ARA55	androgen receptor coactivator 55
ARA70	androgen receptor coactivator 70
ARE	androgen response element
ATP	adenosine triphosphate
bp	base pair
BSA	bovine serum albumin
C	cytoplasmic
CAB	combined androgen blockade
CAIS	complete androgen insensitivity syndrome
cAMP	cyclic adenosine monophosphate
CAR	coxsackie adenovirus receptor
CAT	chloramphenicol acetyl transferase
cDNA	complementary DNA
cfu	colony forming units
CPE	cytopathic effect
ChIP	chromatin immunoprecipitation
CREB	cAMP-response-element-binding protein
CBP	CREB-binding protein
CsCl	Caesium Chloride
CTE	carboxyl-terminal extension
DAB	3,3'-Diaminobenzidine
DBD	DNA binding domain
DCC	dextran coated charcoal
DCC-FCS	dextran coated charcoal-fetal calf serum
DHT	5α-dihydrotestosterone
DIG	digoxigenin-11-ddUTP
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNase1	deoxyribonuclease 1
dNTPs	deoxyribonucleotide triphosphates
dsDNA	double stranded DNA
DTT	dithiothrietol
ECL	enhanced chemiluminescence
EDTA	ethylenediamine tetra-acetic acid
EMSA	electrophoretic mobility shift assay
ER	estrogen receptor

EtOH	ethanol
FCS	fetal calf serum
FKBP5	FK506 binding protein 5
g	gram
GOI	gene of interest
GFP	green fluorescent protein
GR	glucocorticoid receptor
GRIP1	glucocorticoid receptor interacting protein 1
h	hour
H	hinge
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDAC1	histone deacetylase 1
HI-DBS	heat inactivated-donor bovine serum
HGPIN	high-grade prostate intraepithelial neoplasia
HRE	hormone response element
Hsp	heat shock protein
IgG	immunoglobulin
kb	kilo base
kDa	kilo Dalton
KLK2	Kalikrein 2
KRAB	Kruppel-associated box
L	litre
LATS2	large tumour suppressor 2
LB	Luria Broth
LBD	ligand binding domain
LHRH	luteinising hormone releasing hormone
LNCaP	lymph node carcinoma of the prostate
M	molar
mA	milliampere
MAGE11	melanoma gene product 11
MAPK	mitogen activated protein kinase
MCS	multiple cloning site
mg	milligram
min	minute
mL	millilitre
mM	millimolar
msec	milliseconds
MMTV	mouse-mammary tumour virus
MPA	medroxyprogesterone acetate
MR	mineralocorticoid receptor
mRNA	messenger RNA
N	nuclear
N/C	amino-terminal/carboxyl-terminal
NCoR	nuclear corepressor
NE	nuclear extract
NES	nuclear export signal
ng	nanogram
nm	nanometer
nM	nanomolar
nmol	nanomolar
NLS	nuclear localisation signal

NR	nuclear receptor
NTD	amino-terminal domain
OD	optical density
OD_{260}	optical density at 260nm
OD_{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
SGTα	small glutamine rich TPR containing protein α
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’-tetramethylethylenediamine
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	utraviolet

V	volt
WC	whole cell
wtAR	wild type androgen receptor
x	any amino acid

Other:

^{o}C	degrees Celsius
αα	amin acid
Δ	deletion
µCi	microcurie
µg	microgram
µl	microlitre
µm	micron
µM	micromolar