

Androgens and Androgen Receptor Signalling in Men

A thesis submitted to the University of Adelaide in the fulfilment of the
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by

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Dedication

To the memory of my wonderful Grandfather, Harold Ernest Langeluddecke who taught me the meaning of hard work, gave me an appreciation of the funny things in life and showed such strength through his battle with prostate cancer. You are missed every day.

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Abstract

Androgens are critical for the development and maintenance of adult male characteristics such as muscle mass and sexual function. Consequently, the established decline with age of serum testosterone (T) in males has major health implications. While the androgen receptor (AR) is the major mediator of genomic androgen action and is required for the development of the male phenotype, reproductive organs and the maintenance of male secondary sexual characteristics, it is the entrance of androgens into the cell that mediates the activation of the AR and the subsequent modulation of expression of androgen regulated genes. Testosterone, biologically the most important androgen in male serum, circulates either free, loosely bound to albumin or tightly bound to sex hormone binding globulin (SHBG). Each of these forms of serum T have different abilities to enter cells, and which proportion of serum T is capable of entering cells and initiating the androgen signalling cascade, thereby leading to the activation of the AR has not been precisely defined. The AR amino terminal domain (NTD) is responsible for the majority of the ability of the AR to activate genes but the relative roles of the two activation functions in the AR NTD (activation functions 1 and 5; AF1 and 5) have not been precisely defined while the role of the AF2 surface which forms in the ligand binding domain upon agonist binding is responsible for interactions with key coregulators and also with the NTD in the amino-carboxyl (N/C) interaction. Our laboratory has recently identified a region within AF5 between amino acids 500-535 to which somatic mutations in castrate resistant prostate tumour samples collocate. Due to the lack of functional information on the AF5 region and the NTD in general, the function of this region and the functional consequences of the mutations remain to be defined.

The objectives of this thesis were to develop a specific mammalian cell based bioassay capable of reliable measuring T in serum and to determine the ability of this bioassay to measure a physiologically relevant fraction of T in serum. Additionally, this thesis aimed to determine the relative contributions and roles of the activation functions of the AR to overall

AR transcriptional activity along with the functional consequences for AR signalling of prostate cancer mutations which have previously been identified in the AF5 region of the AR NTD.

The mammalian-cell based bioassay developed in this thesis is capable of sensitively and reliably measuring serum T. However, evaluation of this bioassay utilising approximately 1000 serum samples from the Florey Adelaide Male Aging Study reveals that this bioassay measures a fraction of T in serum that most closely relates to serum T. Furthermore, this measure does not correlate more strongly with grip strength, sexual function or waist circumference than the existing immunoassay-based measures of serum T, highlighting the limitations of utilising a static mammalian cell-based androgen bioassay to measure physiological levels of serum T in males. The investigation of the roles of the activation functions in the AR in this thesis have revealed that while the AF1 domain is responsible for the majority of the transactivation activity of the AR, AF5 and AF2 govern the sensitivity and cellular response of the AR to androgens by providing protein and interdomain interaction interfaces. Furthermore, the evidence in this thesis demonstrates that the AR requires interdomain communication for sensitive AR signalling. Finally, the findings in this thesis demonstrate that the AF5 surface is required for the N/C interaction and coregulator interactions while advanced prostate cancer mutations identified within this region confer increased transactivation activity of the AR in the presence of high cellular levels of coregulators.

Collectively, the findings in this thesis provide several novel insights into the mechanism of action of serum androgens and challenges several long held assumptions of androgenic action in males. These findings also delineate a mechanism of treatment failure in advanced prostate cancer, provide a novel model for the events leading to sensitive AR transactivation and contribute to the understanding of physiologically relevant levels of serum T.

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 is made in the text. I give consent to this copy of my thesis being made available in the University library.

Eleanor F. Need

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Abbreviations

A	adenine
AAT	androgen ablation therapy
AF	activation function
AF1	activation function 1 (amino acids 38-360)
AF2	activation function 2
AF5	activation function 5 (amino acids 360-535)
AIB1	amplified in breast cancer 1, NCOA3
AIS	androgen insensitivity syndrome
AKR1C3	aldo-keto reductase family 1, member C3
ALU	arbitrary light units
AR	androgen receptor
ARBS	androgen receptor binding site
ARE	androgen response element
ASD	androstenedione
ATP	adenosine triphosphate
bp	base pair
BIC	bicalutamide
BioT	bioactive testosterone
BRET	bioluminescent energy resonance transfer
BSA	bovine serum albumin
cBT	calculated bioavailable testosterone
iBT	immunoassayed bioavailable testosterone
C	cytosine
CAB	combined androgen blockade
CAIS	complete androgen insensitivity syndrome
cAMP	cyclic adenosine monophosphate
cBT	calculated bioavailable testosterone
cFT	calculated free testosterone
cDNA	complementary DNA
cfu	colony forming units
CREB	cAMP-response-element-binding protein
DBD	DNA binding domain
DCC	dextran coated charcoal
DES	diethylstilbestrol
dH ₂ O	distilled water
DHEA	dehydroepiandrosterone
DHEA(S)	dehydroepiandrosterone sulphate
DHT	5 α -dihydrotestosterone
3 α -diol	3 α , 17 β diol
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNase1	deoxyribonuclease 1
dNTPs	deoxyribonucleotide triphosphates
dsDNA	double stranded DNA
DTT	dithiothrietol
E2	17 β -estradiol

ECL	enhanced chemiluminescence
EDTA	ethylenediamine tetra-acetic acid
ER α	estrogen receptor α
ER β	estrogen receptor β
ERK	extracellular signal related kinase
ERR1	estrogen related receptor 1
ERR2	estrogen related receptor 2
EtOH	ethanol
FBS	foetal bovine serum
FCS	foetal calf serum
FKBP52	FK-506 binding protein 4
FRET	fluorescent resonance energy transfer
FSH	follicle stimulating hormone
g	gram, relative centrifugal force
G	guanine
g	relative centrifugal force
GFP	green fluorescent protein
GnRH	gonadotrophin releasing hormone
GR	glucocorticoid receptor
GRIP1	glucocorticoid receptor interacting protein 1, NCOA2
h	hour
HAT	histone acetyltransferase
HBD	hormone binding domain
HDAC	histone deacetylase
Hip	Hsp interacting protein
Hop	Hsp organising protein
HRE	hormone response element
3 α HSD2	3 α -hydroxysteroid dehydrogenase type 2
3 β HSD1	3 β -hydroxysteroid dehydrogenase type 1
3 β HSD2	3 β -hydroxysteroid dehydrogenase type 2
Hsp	heat shock protein
iBT	immunoassayed bioavailable testosterone
IIEF	international index of erectile function
JNK	c-jun terminal kinase
kb	kilo base
kDa	kilo Dalton
L	litre
LB	Luria Broth
LBD	ligand binding domain
LH	luteinising hormone
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
MKK	MAPK kinase 4
mL	millilitre
mM	millimolar
msec	milliseconds
MPA	medroxyprogesterone acetate
MR	mineralocorticoid receptor
mRNA	messenger RNA
NCOA1	nuclear receptor coactivator 1, SRC-1
NCOA2	nuclear receptor coactivator 2, GRIP1
NCOA3	nuclear receptor coactivator 3, AIB1
NCoR	nuclear corepressor
NES	nuclear export signal
ng	nanogram
nm	nanometer
nM	nanomolar
nmol	nanomolar
NR	nuclear receptor
NTD	amino-terminal domain
OD	optical density
OD ₆₀₀	optical density at 600nm
OHF	hydroxyflutamide
P	progesterone
p300/CBP	CREB-binding protein
p450arom	cytochrome P450 aromatase
P450 _{sc}	cytochrome P450 side chain cleavage
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PKA	protein kinase A
PKC	protein kinase C
pM	picomolar
PR	progesterone receptor
PSA	prostate specific antigen
RT-PCR	quantitative real-time PCR
RFLP	restriction fragment length polymorphism
Rluc	renilla luciferase
RNA	ribonucleic acid
RNase	ribonuclease
RNasin	RNase inhibitor
rpm	revolutions per minute
RT	reverse transcriptase
SA	South Australia
SD	standard deviation
SDI	sexual desire inventory
SDS	sodium dodecyl sulphate
sec	second

SEM	standard error of the mean
SGT α	small glutamine rich TPR containing protein α
SHBG	sex hormone binding globulin
SIRT	sirtuin 4
Slp	sex limited protein
SMRT	silencing mediator of retinoic and thyroid hormone receptors
SRC-1	steroid receptor coactivator 1, NCOA1
SRY	sex-determining region of the Y chromosome gene
SSCP	single stranded conformational polymorphism
ssDNA	single stranded DNA
T	testosterone
TBE	tris borate EDTA
TBL	transducin (β)-like 1
TBS	tris buffered saline
TBST	tris buffered saline-tween 20
TCF/LEF	T-cell factor/lymphoid enhancer factor
TEMED	N,N,N',N'-tetramethylethylenediamine
TIF2	transcriptional intermediary factor2
T _m	melting temperature
TMPRSS2	transmembrane protease, serine 2
TPR	tetratricopeptide repeat
TRAMP	transgenic adenocarcinoma of mouse prostate
TSG101	tumor specific gene product 101
U	uracil
Ubc9	E2 SUMO ubiquitin conjugating enzyme 9
USA	United States of America
UTP	uracil triphosphate
UTR	untranslated region
UV	ultraviolet
V	volt
wtAR	wildtype androgen receptor
x	any amino acid
YFP	yellow fluorescent protein
Other:	
°C	degrees Celsius
α	amin acid
Δ	deletion
μ g	microgram
μ l	microlitre
μ m	micron
μ M	micromolar

Publications arising from this thesis

Articles in scientific journals

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