Characterisation of the Co-chaperone Small Glutamine-rich Tetratricopeptide Repeat containing protein alpha as a Regulator of Androgen Receptor Activity in Prostate Cancer Cells

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

by

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This thesis is dedicated to my mum and dad. Thank you for all your love and support.

Declaration

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Abbreviations

3C	chromatin conformation capture
17-AAG	17-allylamino-demethoxygeldanamycin
Aβ	intracellular beta amyloid
ADT	androgen depriation 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)
ATCC	American Type Culture Collection
AR	androgen receptor
ARE	androgen response element
ADP	adenosine diphosphate
ATP	adenosine triphosphate
APS	ammonium persulphate
bp	base pair
BPH	benign prostatic hyperplasia
BSA	bovine serum albumin
$CaCl_2$	calcium chloride
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
ChIP	chromatin immunopreicpitation
ChIP-chip	chromatin immunopreicpitation microarray
ChIP-seq	chromatin immunoprecipitation sequencing
cm	centimetre
CoIP	co-immunoprecipitation
CREB	cAMP-response-element-binding protein
CsA	cyclosporine A
CSP	cysteine string receptor
Cyp40	cyclophilin 40
DAB	3’3’-diaminobenzidine tetrahydrochloride
DBD	DNA binding domain
DCC	dextran coated charcoal
dH_2O	distilled water
Dex	dexamethasone
DHEA	dehydroepiandrosterone

DHT	5α-dihydrotestosterone
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DRE	digital rectal examination
dsDNA	double stranded DNA
DTT	dithiothrietol
E2	17β-estradiol
EC_{50}	half maximal effective concentration
ECL	enhanced chemiluminescence
EDTA	ethylenediamine tetra-acetic acid
ER	estrogen receptor
EtOH	ethanol
FBS	foetal bovine serum
FKBP	fk506 binding protein
g	gram
g	relative centrifugal force
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GFP	green fluorescent protein
GTPase	small gunosine triphosphatase
GR	glucocorticoid receptor
hr	hour
H	hinge region
HAT	histone acetyltransferase
HCl	hydrochloric acid
HD	homodimerisation domain
HDAC	histone deacetylase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hGHR	human growth hormone receptor
HGPIN	high-grade prostatic intraepithelial neoplasia
HIV	human immunodeficiency virus
HRE	hormone response element
HRP	horse radish peroxidise
Hsc	constitutively expressed heat shock protein
Hsp	heat shock protein
Hip	heat shock interacting protein
Hop	heat shock organising protein
IgG	immunoglobulin class G
IHC	immunohistochemistry

IMVS	Institute of medical and veterinary sciences
IPTG	isopropyl-thio-b-D-galactoside
Jak	Janus kinase
kB	kilo base
KCl	potassium chloride
kd	dissociation constant
kDa	kilo Dalton
kg	kilogram
l	litre
LB	Luria Broth
LBD	ligand binding domain
LGPIN	low grade prostatic intraepithelial neoplasia
LHRH	luteinizing hormone releasing hormone
LiCl	lithium chloride
LNCaP	lymph node carcinoma of the prostate
LR	linker region
M	molar
mA	milliampere
MAPK	mitogen activated protein kinase
MCS	multiple cloning site
mg	milligram
min	minute
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
mL	millilitre
mM	millimolar
mm	millimetre
MOPS	morpholinopropane sulphonic acid
MR	mineralocorticoid receptor
mRNA	messenger RNA
msec	millisecond
NaCl	sodium chloride
Na_2Mo_4	sodium molybdate
N/C	amino terminal/carboxyl terminal
NCOR	nuclear co-repressor
ng	nanogram
$(NH_4)SO_4$	ammonium sulphate
NLS	nuclear localisation sequence
nm	nanometer

nM	nanomolar
NS	non specific siRNA
NTD	amino-terminal transactivation domain
p300/CBP	CREB-binding protein
PAGE	polyacrylamide gel electrophoresis
PB	probasin
PBS	phosphate buffered saline
PC3	prostate carcinoma 3
PCR	polymerase chain reaction
PDGFRα	platelet derived growth factor receptor alpha
PIN	prostatic intraepithelial neoplasia
PI3K	phosphatidylinsoitiol-3-kinase
pM	picomolar
PP5	serine/theronine protein phosphatase 5
PPIase	peptidylprolyl isomerase
PR	progesterone receptor
Prog	progesterone
PSA	prostate specific antigen
QRD	glutamine-rich domain
QRT-PCR	quantitative real-time PCR
RFLP	restriction fragment length polymorphism
RGH	Repatriation General Hospital
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	reverse transcriptase
SA	South Australia
SD	standard deviation
SDS	sodium dodecyl sulphate
sec	second
SEM	standard error of the mean
SGTA	small glutamine rich TPR containing protein alpha
siRNA	small interfering RNA
SM	sodium molybdite lysis buffer
SMRT	silencing mediator of retinoic and thyroid hormone receptors
SRC-1	steroid receptor coactivator 1
STAT	signal transducer and activator of transcription
T	testosterone
TBE	tris borate EDTA

TBP	TATA binding protein
TBS	tris buffered saline
TBST	tris buffered saline-tween 20
TE	tris EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
Tm	melting temperature
TPR	tetratricopeptide repeat
tRNA	transfer RNA
TSS	transcription start site
TURP	transurethral resection of the prostate
USA	United States of America
UTR	untranslated region
UV	ultraviolet
V	volt
v	volume
W	watt

Other:

^{0}C	degrees Celsius
Δ	deletion
μg	microgram
μL	microlitre
μm	micron
μM	micromolar

Abstract

Prostate cancer remains one of the leading causes of cancer related morbidity and mortality in Australian men. The androgen receptor (AR) is an intracellular transcription factor that mediates the biological actions of circulating androgens to drive the growth and survival of prostate cancer cells. However, current treatment options for non-localised, advanced stage prostate cancer invariably fail, which is a consequence of continued AR signalling during all stages of disease progression. Therefore, understanding the regulatory mechanisms of AR action is essential for the development of more effective therapies.

Molecular regulation of the AR can occur during the process of protein maturation. This includes the incorporation of tetratricopeptide repeat (TPR) containing co-chaperones into the heat shock protein 90 (Hsp90) molecular chaperone complex, which collectively acts to generate AR proteins capable of high affinity ligand binding, nuclear translocation and gene regulation. The co-chaperone small glutamine-rich TPR containing protein alpha (SGTA) acts to restrict AR nuclear translocation and thereby regulate AR transcriptional activity. The clinical implications of SGTA are evident by a decline in protein levels with prostate cancer progression. The loss of SGTA may therefore disrupt the regulatory process of AR cytoplasmic retention, and thus contribute to continued AR activity. However the precise regions of SGTA that mediate these effects or how SGTA may contribute to prostate cancer proliferation have yet to be elucidated. The aims of this thesis were to further characterise the mechanisms of SGTA action on AR signalling, to further

define the biological actions of SGTA in prostate cancer cells and to determine the functional consequences these actions have on prostate cancer growth.

SGTA is unique amongst the steroid receptor associated TPR co-chaperones through its ability to form homodimers. Therefore, the requirement of the SGTA homodimerisation domain may be essential in deciphering how SGTA affects AR activity. Deletion mapping combined with structural prediction analysis revealed that the first 80 amino acids of the amino terminus are required to form SGTA homodimers, as well as maintain SGTA protein steady state levels. Further studies also confirmed that SGTA homodimerisation occurs independently of the Hsp90 interacting TPR domain or glutamine-rich carboxyl terminus, implying that interactions between SGTA and the Hsp90 molecular chaperone complex do not contribute to the formation of SGTA homodimers. Moreover, inhibition of SGTA homodimerisation did not alter the ability of SGTA to inhibit AR activity. These studies demonstrate that while the SGTA homodimerisation is characterised by conserved structural elements within the first 80 amino acids, SGTA can sufficiently act as a monomer to inhibit AR activity.

Further investigations into the mechanisms of how SGTA affects AR transactivation activity demonstrated that SGTA restricts the ligand sensitivity of AR activity across multiple androgen responsive luciferase reporters in a low hormone environment. These studies also revealed that the strongest inhibitory effect by SGTA was observed on those loci that exhibited the greatest fold change to androgen treatment. Additionally, the effects of SGTA on AR are not mediated through the homodimerisation, TPR or glutamine-rich domains. These results suggest that SGTA has the ability to constrain the sensitivity of AR

activation to ligand in a castrate environment and therefore SGTA may act as a buffer against aberrant AR responses.

To determine the functional consequences of modulating SGTA levels in prostate cancer cells, both SGTA over expression and knockdown models were developed. Modulation of SGTA levels in prostate cancer cell lines decreased proliferation. This effect was not a result of altered AR activity, as determined by expression of the androgen regulated gene prostate specific antigen (PSA). Collectively, these results suggest that the impact of SGTA on prostate cancer cell proliferation is independent of AR signalling. Microarray analysis was used to determine the effects of SGTA knockdown on the prostate genome, which demonstrated that cells depleted of SGTA differentially regulated the expression of genes involved in cell cycle regulation. Specifically, genes with decreased expression included several within the Class I phophoinositide-3 kinase (PI3 kinase) pathway, a critical pro-proliferation signalling pathway in prostate cancer. Consequently, knockdown of SGTA resulted in decreased Akt phosphorylation at the threonine 308 (T308) and serine 473 (S473) residues, which are key indicators of active PI3 kinase signalling. Collectively, these studies demonstrated that SGTA is able to modulate the expression and/or activity of multiple signalling pathways within prostate cancer cells to influence cell proliferation.

The findings from this thesis provide novel insights into the structural and functional complexity of SGTA action on AR activity in prostate cancer cells. Importantly, this highlights the potential mechanistic consequences that SGTA may have for AR and other signalling pathways that occur during prostate cancer pathogenesis and provides the basis for further investigations evaluating SGTA as a novel therapeutic target in prostate cancer.