

**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.

<b>DECLARATION</b>	<b>I</b>
<b>ACKNOWLEDGEMENTS</b>	<b>II</b>
<b>ABBREVIATIONS</b>	<b>V</b>
<b>ABSTRACT</b>	<b>X</b>
<b>CHAPTER 1: INTRODUCTION</b>	<b>2</b>
<b>1.1 Overview</b>	<b>2</b>
<b>1.2 Development of the prostate</b>	<b>4</b>
1.2.1 Androgen physiology	4
1.2.2 Development of the normal prostate	5
<b>1.3 Prostate cancer and progression</b>	<b>9</b>
1.3.1 Pathogenesis	9
<b>1.4 Diagnosis</b>	<b>10</b>
1.4.1 Clinically localized and advanced disease	11
<b>1.5 Treatment</b>	<b>12</b>
1.5.1 Localised Disease	12
1.5.2 Metastatic Disease	12
<b>1.6 The androgen signalling axis</b>	<b>14</b>
1.6.1 The androgen receptor	15
1.6.2 The androgen receptor gene	16
1.6.3 The androgen receptor protein and domains	19
<b>1.7 Androgen receptor co-regulators</b>	<b>24</b>
1.7.1 Co-activators	24
1.7.2 Co-repressors	25
1.7.3 Chaperones	26
<b>1.8 The molecular chaperone complex and androgen receptor maturation</b>	<b>27</b>
1.8.1 Chaperones involved in ligand binding and nuclear translocation	32
1.8.2 Chaperones and transcriptional activation	37
<b>1.9 Chaperones in prostate cancer</b>	<b>37</b>

<b>1.10 Chaperones as therapeutic targets</b>	<b>39</b>
<b>1.11 Tetratricopeptide repeat containing co-chaperones</b>	<b>40</b>
1.11.1 Structure of TPR domain	43
1.11.2 Hsp90 and Hsp70 TPR binding sites	43
1.11.3 Regulation of Hsp70 and Hsp90 ATPase activity by TPR co-chaperones	45
1.11.4 The role of TPR co-chaperones in steroid receptor signalling	46
1.11.5 Small glutamine-rich tetratricopeptide repeat containing protein alpha	54
<b>1.12 Conclusions</b>	<b>59</b>
<b>1.13 Objectives of this thesis</b>	<b>61</b>
<b>CHAPTER 2: MATERIALS AND METHODS</b>	<b>63</b>
<b>2.1 Materials</b>	<b>63</b>
<b>2.2 Buffers and solutions</b>	<b>68</b>
<b>2.3 General Methods</b>	<b>71</b>
2.3.1 Cloning procedures	71
2.3.2 Mammalian cell culture	76
2.3.3 General transfection methods	78
2.3.4 Mammalian two-hybrid interaction assays	80
2.3.5 Western blotting procedures	81
2.3.6 Real time PCR	83
2.3.7 Statistical analysis	84
<b>CHAPTER 3: CHARACTERISATION OF THE SGTA HOMODIMERISATION INTERFACE</b>	<b>86</b>
<b>3.1 Introduction</b>	<b>86</b>
<b>3.2 Materials and Methods</b>	<b>90</b>
3.2.1 Plasmids	90
3.2.2 Creation of SGTA substitution and deletion variants	92
3.2.3 SGTA phylogeny tree	93

3.2.4 Secondary structure prediction, residue composition and logo creation	94
3.2.5 Co-Immunoprecipitation	94
3.2.6 <i>In vitro</i> luciferase assays	98
<b>3.3 Results</b>	<b>99</b>
3.3.1 Characterisation of SGTA functional domains	99
3.3.2 Amino acid analysis of the SGTA homodimerisation domain	107
3.3.3 SGTA homodimerisation cannot be captured by co-immunoprecipitation.	111
3.3.4 Segmented deletions within the first 80 amino acids of the amino terminal domain ablate SGTA homodimerisation.	116
3.3.5 Inhibition of SGTA homodimerisation by expression of a peptide sequence encompassing SGTA amino acids 1-80	122
3.3.6 Deletion of SGTA intra-molecular domains does not alter the capacity for SGTA to form a homodimer.	125
3.3.7 Disruption of SGTA homodimerisation does not alter its ability to inhibit AR activity	128
<b>3.4 Discussion</b>	<b>130</b>
<b>CHAPTER 4: SGTA ACTS TO RESTRICT THE SENSITIVITY OF ANDROGEN RECEPTOR ACTIVITY</b>	<b>138</b>
<b>4.1 Introduction</b>	<b>138</b>
<b>4.2 Materials and Methods</b>	<b>142</b>
4.2.1 Plasmids	142
4.2.2 Quantitative real time PCR and Immunoblot	142
4.2.3 Transient luciferase assay	143
4.2.4 Chromatin integrated reporter luciferase assay	143
4.2.5 Statistical analyses	144
<b>4.3 Results</b>	<b>145</b>
4.3.1 Tetratricopeptide repeat containing co-chaperones are expressed in prostate cancer cell lines	145

4.3.2	Optimisation of AR and SGTA transactivation assays	149
4.3.3	SGTA decreases AR sensitivity to ligand	152
4.3.4	SGTA affects GR and PR transactivation activity	157
4.3.5	Deletions within the SGTA homodimerisation domain variably alter AR transactivation activity	160
4.3.6	The SGTA linker region is necessary for an effect on AR activity	160
<b>4.4</b>	<b>Discussion</b>	<b>164</b>

<b>CHAPTER 5: GLOBAL ANALYSIS OF SGTA BIOLOGICAL PATHWAYS IDENTIFIED PI3 KINASE AS A POTENTIAL TARGET IN PROSTATE CANCER CELLS</b>		<b>171</b>
<b>5.1</b>	<b>Introduction</b>	<b>171</b>
<b>5.2</b>	<b>Materials and Methods</b>	<b>173</b>
5.2.1	Small Interfering RNA (siRNA) transfections and Immunoblot	173
5.2.2	RNA Isolation and QRT-PCR	173
5.2.3	Microarray and Gene Ontology Analysis	173
5.2.4	Statistics	174
<b>5.3</b>	<b>Results</b>	<b>176</b>
5.3.1	Knockdown of SGTA in C42B cells variably alters AR regulated genes	176
5.3.2	SGTA knockdown does not alter AR transcriptional activity at low ligand concentrations	182
5.3.3	SGTA knockdown alters the genome-wide expression profile of C42B cells.	187
5.3.4	Validation of SGTA knockdown microarray	191
5.3.5	SGTA knockdown affects cell proliferation gene ontology categories	196
5.3.6	SGTA down-regulates Class I phosphoinositide 3-kinase genes	200
5.3.7	SGTA knockdown modulates PI3 kinase-Akt activity	205
<b>5.4</b>	<b>Discussion</b>	<b>207</b>

<b>CHAPTER 6: THE EFFECT OF SGTA ON PROSTATE CANCER</b>	
<b>CELL PROLIFERATION</b>	<b>216</b>
<b>6.1 Introduction</b>	<b>216</b>
<b>6.2 Materials and Methods</b>	<b>219</b>
6.2.1 Cell proliferation assays, siRNA knockdown and immunoblot	219
6.2.2 Cloning of SGTA into two independent stable integration mammalian expression vectors	220
6.2.3 Creation of stable transformants	221
6.2.4 Selection of stable transformants	221
6.2.5 Clinical cohort and creation of tissue microarrays	222
6.2.6 Immunohistochemistry	223
6.2.7 Statistics	224
<b>6.3 Results</b>	<b>225</b>
6.3.1 Knockdown of SGTA in C42B cells reduces cell proliferation	225
6.3.2 Stable SGTA over expression in LNCaP cells does not alter AR activity	231
6.3.3 Stably transfected LNCaP cells display reduced cell proliferation	231
6.3.4 Expression of AR and SGTA does not change in patient matched BPH and prostate cancer samples.	236
<b>6.4 Discussion</b>	<b>240</b>
<b>CHAPTER 7: GENERAL DISCUSSION</b>	<b>247</b>
<b>7.1 Major Findings of this thesis</b>	<b>247</b>
7.1.1 The characterisation of the SGTA homodimerisation domain and its effects on AR activity.	247
7.1.2 The linker region of SGTA is required for its inhibitory effects on AR activity	248
7.1.3 SGTA acts to restrain the sensitivity of AR activity in prostate cancer cells.	249
7.1.4 SGTA acts as a regulator of intracellular signalling pathways critical for prostate cancer cell proliferation	251
<b>7.2 Future Directions</b>	<b>252</b>

7.2.1 Identifying the regions of SGTA that determine AR cytoplasmic retention	252
7.2.2 Identification of SGTA client proteins in prostate cancer	253
7.2.3 Further investigations into the effects of SGTA on PI3 kinase signalling in prostate cancer	254
<b>7.3 Summary &amp; Conclusions</b>	<b>255</b>
<b>APPENDIX A: PRIMERS AND PCR CONDITIONS</b>	<b>257</b>
<b>APPENDIX B: PLASMIDS</b>	<b>263</b>
<b>APPENDIX C: MICROARRAY DATASET</b>	<b>273</b>
<b>APPENDIX D: IPA CANONICAL PATHWAY ANALYSIS</b>	<b>302</b>
<b>BIBLIOGRAPHY</b>	<b>307</b>

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## **Declaration**

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## Abbreviations

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3C	chromatin conformation capture
17-AAG	17-allylamino-demethoxygeldanamycin
A $\beta$	intracellular beta amyloid
ADT	androgen deprivation 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 <sub>2</sub>	calcium chloride
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
ChIP	chromatin immunoprecipitation
ChIP-chip	chromatin immunoprecipitation 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 <sub>2</sub> O	distilled water
Dex	dexamethasone
DHEA	dehydroepiandrosterone

DHT	5 $\alpha$ -dihydrotestosterone
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DRE	digital rectal examination
dsDNA	double stranded DNA
DTT	dithiothrietol
E2	17 $\beta$ -estradiol
EC <sub>50</sub>	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 <sub>2</sub>	magnesium chloride
MgSO <sub>4</sub>	magnesium sulphate
mL	millilitre
mM	millimolar
mm	millimetre
MOPS	morpholinopropane sulphonic acid
MR	mineralocorticoid receptor
mRNA	messenger RNA
msec	millisecond
NaCl	sodium chloride
Na <sub>2</sub> Mo <sub>4</sub>	sodium molybdate
N/C	amino terminal/carboxyl terminal
NCOR	nuclear co-repressor
ng	nanogram
(NH <sub>4</sub> )SO <sub>4</sub>	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
PDGFR $\alpha$	platelet derived growth factor receptor alpha
PIN	prostatic intraepithelial neoplasia
PI3K	phosphatidylinositol-3-kinase
pM	picomolar
PP5	serine/threonine protein phosphatase 5
PPlase	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 molybdate 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
T <sub>m</sub>	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
<b>Other:</b>	
°C	degrees Celsius
Δ	deletion
μg	microgram
μL	microlitre
μm	micron
μM	micromolar

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## Abstract

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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 phosphoinositide-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.