CURCUMIN ACTION IN PROSTATE CANCER CELLS AND FIBROBLASTS

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School of Medicine

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This thesis is dedicated to the people

who never stopped believing in me

INVICTUS

Out of the night that covers me
Black as the pit from pole to pole
I thank whatever gods may be
For my unconquerable soul

In the fell clutch of circumstance
I have not winced nor cried aloud
Under the bludgeoning of chance
My head is bloody, but unbowed

Beyond this place of wrath and tears
Looms but the horror of the shade
And yet the menace of the years
Finds, and shall find, me unafraid

It matters not how strait the gate
How charged with punishments the scroll
I am the master of my fate
I am the captain of my soul

-William Ernest Henley

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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 written by another person, except where due reference has been made in the text.

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SIGNATURE: …….. DATE: ……

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I also wish to respectfully acknowledge the sacrifice of animal life, as without them medical research would not be possible.

ABBREVIATIONS

ABC	ATP binding cassette
ADT	androgen deprivation therapy
amp	ampere
APC	allophycocyanin
AR	androgen receptor
BCR	biochemical relapse
BLI	bioluminescence imaging
BPH	benign prostatic hyperplasia
BSA	bovine serum albumin
$C_6H_5Na_3O_72H_2O$	trisodium citrate dihydrate
CAB	combined androgen blockade
CAF	cancer-associated fibroblast
CAM-DR	cell adhesion-mediated drug resistance
CD	cyclodextrin
CDK	cyclin dependent kinase
cDNA	complementary DNA
ChIP	chromatin immunoprecipitation
cm	centimetre
CMV	cytomegalovirus
CO_2	carbon dioxide
CRPC	castrate resistant prostate cancer
D	day
DAB	3'3'-diaminobenzidine tetrahydrochloride
DCC	dextran-coated charcoal
DcR	decoy receptor
DHT	5α-dihydrotestosterone
DISC	death-inducing signalling complex
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DR	death receptor
DRE	digital rectal examination
DTP	drug-tolerant persister
ECL	enhanced chemiluminescence
ECM	extracellular matrix
EDTA	ethylenediamine tetra-acetic acid
eIF	eukaryote initiation factor
EtOH	ethanol
FACS	fluorescent activated cell sorting
FADD	fas-associated death domain
FAP	fibroblast-activated protein
F_c	fragment crystallisable
FCS	fetal calf serum
FSH	follicle stimulating hormone
g	gram
GFP	green fluorescent protein
GSH	glutathione
h	hour
HCl	hydrochloric acid

HGPIN	high-grade prostatic intraepithelial neoplasia
HPG	hypothalamic-pituitary-gonadal
HRP	horse radish peroxidase
IC_{50}	half maximal inhibitory concentration
IgG	immunoglobulin G
IHC	immunohistochemistry
i.p.	intraperitoneal
IPA	ingenuity pathway analysis
kb	kilo base
kD	kilo dalton
kg	kilogram
L	litre
LB	luria broth
LGPIN	low-grade prostatic intraepithelial neoplasia
LH	luteinising hormone
LiCl	lithium chloride
LNCaP	lymph node carcinoma of the prostate
LTC	long-term curcumin
LTV	long-term vehicle
M	molar
MAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
mg	milligram
$MgSO_4$	magnesium sulphate
min	minute
mL	millilitre
mm	millimetre
mM	millimolar
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
MTT	methylthiazol tetrazolium
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
Na_2HPO_4	sodium phosphate dibasic
NaH_2PO_4	sodium phosphate monobasic
NCBI	national centre for biotechnology information
ng	nanogram
NHS	normal horse serum
nm	nanometre
nM	nanomolar
NPF	normal prostate fibroblast
PARA	pro-apoptotic receptor agonist
PBS	phosphate buffered saline
PC-3	prostate carcinoma 3
pH	potential hydrogen
PI	propidium iodide
PIN	prostatic intraepithelial neoplasia
PRF	phenol red-free
PSA	prostate specific antigen
qRT-PCR	quantitative real-time polymerase chain reaction
RIPA	radioimmunoprecipitation assay
RNA	ribonucleic acid

RNAse	ribonuclease
RO	reverse osmosis
RP	radical prostatectomy
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
RT	room temperature
s.c.	subcutaneous
SCID	severe combined immunodeficiency
SD	standard deviation
SDS	sodium dodecyl sulphate
sec	second
SEM	standard error of the mean
TBS	tris buffered saline
TBST	tris buffered saline-tween 20
TE	tris ethylenediamine tetra-acetic acid
TKR	tyrosine kinase receptor
TNF	tumour necrosis factor
TRAIL	TNF-related apoptosis inducing ligand
Tris	tris(hydroxymethyl)aminomethane
Tris-Cl	tris(hydroxymethyl)aminomethane chloride
tRNA	transfer RNA
UGE	urogenital sinus epithelium
UGM	urogenital sinus mesenchyme
^{o}C	degrees celcius
ΔΔct	delta delta cycle threshold
µg	microgram
µL	microlitre
µM	micromolar
µm	micron

ABSTRACT

Curcumin is a component of the Indian spice turmeric that has shown anti-cancer activity across a range of models. This includes prostate cancer, the most commonly diagnosed cancer in Australia. While much of the current literature relates to epithelial cells, there is no information regarding curcumin activity or resistance in prostate fibroblasts. With curcumin recently entering clinical trials for prostate cancer and being increasingly used as a dietary supplement, it is critical to gain an understanding of curcumin action and potential resistance in these cells, given their reported contribution to cancer progression. Furthermore, with drug resistance being a major setback to cancer therapy, it is also important to investigate curcumin-based combination strategies to enhance efficacy and avoid the development of resistance. The aims of this thesis were therefore to comparatively investigate mechanisms of curcumin action in prostate cancer cells and fibroblasts, to explore the potential for curcumin resistance to occur in prostate fibroblasts and to examine the ability of curcumin to re-sensitise prostate cancers resistant to drozitumab, a monoclonal antibody against death receptor 5 (DR5). Curcumin inhibited prostate cancer cell and fibroblast viability, androgen receptor (AR) activity and androgen-regulated gene expression; effects potentially caused by a decrease in AR residence on DNA. While microarray analysis of curcumin-treated fibroblasts crossed with publically available data from curcumin-treated prostate cancer cells revealed little overlap in genes, both cell lineages underwent cell cycle arrest in response to treatment. However, cell cycle arrest occurred via divergent mechanisms in different prostate cell lines. Long-term culture of prostate fibroblasts in curcumin resulted in curcumin tolerance rather than resistance, characterised by increased cell survival and decreased cell cycle arrest in response to treatment. Curcumin-tolerant fibroblasts were differentiated from sensitive fibroblasts based on a subset of differentially expressed genes, some of which had previously been associated with resistance to cancer therapies, and some of which had lost curcumin-responsiveness in tolerant fibroblasts. Many of the latter genes were androgen-regulated, and tolerant fibroblasts subsequently demonstrated reduced AR function and androgen regulation of genes, concomitant with a decrease in AR residence on DNA. The culture of tolerant fibroblasts in curcumin-free media restored curcumin sensitivity, and partially restored AR function and the ability of androgens to regulate gene expression. Tolerant fibroblasts demonstrated changes in genes responsible for extracellular matrix composition and secretion of growth factors, and when co-cultured with prostate cancer cells, a decrease in cancer cell adhesion and increase in proliferation was observed. Finally, cell line studies confirmed that curcumin re-sensitised drozitumab-resistant prostate cancer cells to drozitumab-induced apoptosis via up-regulation of the drug target DR5. While the same effect was not observed *in vivo*, drozitumab treatment alone demonstrated surprising anti-cancer activity. This thesis provides a greater understanding of curcumin action across multiple prostate cell lines and explores, for the first time, the development and potential implications of curcumin tolerance in prostate fibroblasts. This data provides new insights into how curcumin-based therapies or prevention strategies may affect the whole prostate, and offers considerations for curcumin use in future preclinical and clinical studies.

CONFERENCE PRESENTATIONS

Giorgio. L, Smith. E, Drew. PA & Buchanan. G. Exploring the interaction between fibroblasts and tumour cells in prostate cancer. Basil Hetzel Institute Research Day, Adelaide, October 2011.

Trotta. AP, **Giorgio. L**, Leach. DA, Taylor. RA & Buchanan. G. Androgen receptor function in prostate fibroblasts. Australian Science and Medical Research Conference, Adelaide, June 2012.

Giorgio. L, Leach. DA**,** Smith. E, Drew. PA, Trotta. AP & Buchanan. G. Curcumin inhibits cell growth and androgen receptor function in prostate tumour and fibroblast cell lines. University of Adelaide Faculty of Health Science Postgraduate Research Conference, Adelaide, August 2012.

Giorgio. L, Leach. DA**,** Smith. E, Drew. PA, Trotta. AP & Buchanan. G. Curcumin inhibits cell growth and androgen receptor function in prostate tumour and fibroblast cell lines. Basil Hetzel Institute Research Day, Adelaide, October 2012.

Giorgio. L, Trotta. AP, Need. EF & Buchanan. G. Curcumin targets unique transcriptional programmes in prostate epithelial and fibroblast cell lines. Australian Society for Medical Research, Adelaide, June 2013.

Giorgio. L, Leach. DA**,** Trotta. AP, Wang. Q, Holst. J, Need. EF & Buchanan. G. The anti-proliferative effects of curcumin are mediated by p53-independent BRCA1 signalling in prostate fibroblasts. Prostate Cancer World Congress, Melbourne, August 2013.

Giorgio. L, Leach. DA**,** Trotta. AP, Wang. Q, Holst. J, Need. EF & Buchanan. G. The anti-proliferative effects of curcumin are mediated by p53-independent BRCA1 signalling in prostate fibroblasts. Australian-Canadian Prostate Cancer Research Alliance Symposium, Port Douglas, August 2013.

Giorgio. L, Leach. DA**,** Trotta. AP, Wang. Q, Holst. J, Need. EF & Buchanan. G. The anti-proliferative effects of curcumin are mediated by p53-independent BRCA1 signalling in prostate fibroblasts. University of Adelaide Faculty of Health Science Postgraduate Research Conference, Adelaide, August 2013.

Giorgio. L, Leach. DA, Trotta. AP, Need. EF & Buchanan. G. Curcumin induces divergent mechanisms of cell cycle arrest in prostate fibroblasts and cancer cells. Basil Hetzel Institute Research Day, Adelaide, October 2013.

Giorgio. L, Zinonos. I, Liapis. V, Trotta. AP, Buchanan. G & Evdokiou. A. Drozitumab, a fully humanised agonistic antibody against Apo2L/TRAIL death receptor DR5, exhibits anti-tumour activity in a PC-3 prostate cancer xenograft model. Ninth International Conference of Anticancer Research, Sithonia Greece, October 2014.

PRIZES

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ENGAGEMENT IN THE SCIENTIFIC COMMUNITY

Radio interview with David Hearn for Coast FM: Research update. June 2014

Invited presentation at the Walkerville Rotary Club for The Hospital Research Foundation Community Awareness Program titled '*Secret men's business: discovering new ways to treat prostate cancer'*. April 2014

Presentation at the Basil Hetzel Institute of Translational Health Research titled *'Curcumin and prostate cancer: hope or hype?'* March 2014

Invited presentation at the Freemason's Foundation for Men's Health titled '*Curcumin and prostate cancer: understanding the mechanisms of action in cancer cells and fibroblasts'*. September 2013

Invited presentation at the Holdfast Bay Rotary Club for The Hospital Research Foundation Community Awareness Program titled '*Secret men's business: discovering new ways to treat prostate cancer'*. August 2013

Radio interview with Belinda Heggen for 5AA: Research update. April 2013

Presentation at the Disciple of Surgery Surgical Science Journal Club titled '*Sipuleucel-T immunotherapy for castrate resistant prostate cancer*'. March 2013

Presentation at the Basil Hetzel Institute of Translational Health Research titled *'Curcumin and prostate cancer: mechanisms of resistance and improving delivery'*. November 2012

University of Adelaide Three Minute Thesis Competition '*Prostate fibroblasts: putting Jeckyll and Hyde to better use'*. August 2012

Radio interview with David Hearn for Coast FM: Research update. June 2012

Presentation at the Basil Hetzel Institute of Translational Health Research titled *'Exploring the interaction between fibroblasts and cancer cells in prostate cancer*'. August 2011