

The Molecular Actions of Medroxyprogesterone Acetate on Androgen Receptor Signalling and the Promotion of Breast Cancer

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

by

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Abstract

The Women's Health Initiative (WHI) clinical trial was the first randomised, double blind, placebo-controlled disease prevention trial to demonstrate epidemiological evidence of a casual link between the use of combined hormone replacement therapy (cHRT) comprising conjugated equine estrogens (CEE) and the synthetic progestin medroxyprogesterone acetate (MPA) and increased breast cancer risk in post-menopausal women. Since the first WHI report in 2002, other observational studies have demonstrated that it is the addition of the synthetic progestin in the cHRT that is associated with an increase in breast cancer risk.

The focus of this thesis was to investigate the following hypothesis formulated in the Dame Roma Mitchell Cancer Research Laboratory, which proposed that MPA possesses antagonistic actions on androgen receptor (AR)-signalling and thereby can disrupt the protective effect of androgens in the breast, thus leading to an increased risk of breast cancer. Androgens have been associated with a growth restrictive role in breast tissue in both humans and animals and are now emerging as key hormonal pathways involved in the pathogenesis of breast cancer. The objectives of this thesis were firstly to determine the relationship between the use of cHRT containing MPA and breast cancer incidence in Australian women, and secondly to perform biological studies to investigate the effect of AR-action in breast epithelial cells. Initial findings described in this thesis led to the identification of a positive association between the use of cHRT preparations containing MPA and breast cancer incidence in Australian women. Subsequent biological based studies were undertaken with non-malignant breast tissues samples from pre- and post-

menopausal women in an *ex vivo* breast explant tissue culture experimental model and the oestrogen receptor (ER), progesterone receptor (PR) and AR positive ZR-75-1 breast cancer cell line to investigate the actions of MPA on AR-signalling and cancer-related intracellular signalling pathways. Collectively these studies demonstrated that the actions of MPA can impede the anti-proliferative actions of DHT in both human post-menopausal non-malignant and malignant breast epithelial cells via AR-mediated actions. Furthermore, the combined actions of DHT and MPA were also shown to de-regulate cancer-related intracellular pathways compared to individual hormone treatments. The findings described in this thesis provide novel results indicating that MPA may promote the development of breast cancer in post-menopausal women taking cHRT via AR-mediated actions and that use of this form of hormone therapy remains a major public health concern.

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 Aleksandra Monica Ochnik 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.

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Aleksandra Monica Ochnik

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List of Abbreviations

3β-HSD	3 β -hydroxysteroid dehydrogenase
17β-HSD	17 β -hydroxysteroid dehydrogenase
A	androstenedione
AAT	androgen ablation therapy
AB	alveolar buds
ACTH	adreno-corticotrophic hormone
AGMA	Australian Government Medicare Australia
AIHW	Australian Institute Health and Welfare
AIS	androgen insensitivity syndrome
ANOVA	analysis of variance
AR	androgen receptor
ARE	androgen response element
BALB	albino laboratory-bred strain of the <i>mus musculus</i>
BEK1	fibroblast growth factor receptor 2
Bic	bicalutamide
Bis	bisulphate
BMI	body mass index
Brd-U	bromodeoxyuridine
BRCA1	breast cancer 1, early onset
BRCA2	breast cancer 2, early onset
BSA	bovine serum albumin
C1orf116	chromosome 1 open reading frame 116
CBG	corticosteroid binding protein
CEE	conjugated equine estrogen
ChIA-PET	chromatin interaction analysis by paired-end tag sequencing
ChIP	chromatin immunoprecipitation
CHA	chlormadinone acetate
cHRT	combined hormone replacement therapy
CI	confidence interval

CK	cytokeratin
COC	combined oral contraceptives
CPA	cyproterone acetate
CpG	cytosine and guanine linked by a phosphodiester bond
CT	calibration threshold
CYP11A	gene for cholesterol side chain cleavage cytochrome
CYP17	gene for 17 α -hydroxylase p450c17
CYP19	gene for P450 aromatase
DAB	3, 3'-diaminobenzidine
DBD	DNA binding domain
DCC	dextran coated charcoal-stripped
DCIS	ductal carcinoma <i>in-situ</i>
DD-Ct	delta-delta-Ct
DE	dienogest
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulphate
DHT	5 α - dihydrotestosterone
DMBA	dimethylbez [a]-anthracene
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
DP	drospirenone
DPX	Di-n-butylPhthalate in xylene
DRMCRL	Dame Roma Mitchell Cancer Research Laboratory
DTT	Dithiothreitol
E₁	oestrone
E₂	17 β -oestradiol
E₃	oestriol
ECL	enhanced chemiluminescence
EDTA	ethylenediamine tetra-acetic acid
EG	etonogesterol
EGF	epidermal growth factor

EGFR	epidermal growth factor receptor
EtOH	ethanol
ER	oestrogen receptor
ERα	oestrogen receptor alpha
ERβ	oestrogen receptor beta
ERE	oestrogen response element
ERT	estrogen replacement therapy
FCS	foetal calf serum
FDA	Food and Drug Administration
FGFR2	fibroblast growth factor receptor 2
FKBP5	FK-506 binding protein 51
FSH	follicle stimulating hormone
GD	gestodene
GFP	green fluorescent protein
GnRH	gonadotrophin releasing hormone
GO	gene ontology
GR	glucocorticoid receptor
GST	glutathione sepharose transferase
GWAS	genome wide association studies
HBECs	human breast epithelial cells
hCG	human chronic gonadotrophin
HC	hormonal contraceptives
H&E	haematoxylin and eosin
HER2	human epidermal growth factor receptor 2
HPG	hypothalamic pituitary gonadal-axis
HRE	hormone response element
HRP	horseradish peroxidase
HRT	hormone replacement therapy
HS	histoscore
hsp	heat shock protein
HT	hormone therapy

HZ	hazard ratio
IDC	invasive ductal carcinoma
ILC	invasive lobular carcinoma
IGF	insulin growth factor
IgG	immunoglobulin
IMVS	Institute of Medical and Veterinary Science
IPA	Ingenuity Pathway Analysis
IUD	intrauterine device
KITL	kit-ligand
LBD	ligand binding domain
LCIS	lobular carcinoma <i>in-situ</i>
LCM	laser capture micro-dissection
LH	luteinising hormone
LHRH	luteinising hormone releasing hormone
LNG	levonorgesterol
LOH	loss of heterozygosity
MG	medrogestone
MIOD	mean integrated optical density
MOD	mean optical density
MPA	medroxyprogesterone acetate
MR	mineralocorticoid receptor
MWS	Millions Women Study
N/C	amino and carboxy terminal domain
NDP	Nanozoomer digital pathology
NETA	norethisterone acetate
NG	norgestimate
NGA	nomegesterol acetate
NLS	nuclear localization sequence
NR	nuclear receptor
NDRG1	n-myc downstream regulated 1
NTD	amino-terminal transactivation domain

OHF	hydroxyflutamide
OLR1	oxidised low density lipoprotein, (lectin-like) receptor 1
Org2058	synthetic progestagenic compound
PAGE	polyacrylamide gel electrophoresis
PBS	Pharmaceutical Benefits Scheme or phosphate buffered saline
PBSS	Pharmaceutical Benefits Scheme Statistics
PCNA	proliferating cell nuclear antigen
PCOS	polycystic ovary syndrome
Pg	progesterone
PG	promegestone
PSA	prostate specific antigen
PR	progesterone receptor
PRA	progesterone receptor isoform A
PRB	progesterone receptor isoform B
PRF	phenol-red free
POC	progestin only contraceptive
P/S	penicillin and streptomycin
qRT-PCR	quantitative real-time polymerase chain reaction
R5020	promegesterone
RAR	retinoic acid receptor
RBA	relative binding affinity
RGS2	regulator of G-protein signalling 2
RIPA	Radio-Immunoprecipitation Assay
RPE	rating of perceived exertion
RPMI	Roswell Park Memorial Institute
RO	reverse osmosis
RNA	ribonucleic acid
RQI	Rice Quantum Institute
RR	relative risk
RT	reverse transcription
RU58668	steroidal anti-oestrogen

RXR	retinoid X receptor
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SERM	selective oestrogen receptor modulator
SHBG	sex hormone binding globulin
siRNA	small interfering RNA
SNPs	single nucleotide polymorphisms
SRD5A1	gene for 5 α -reductase type 1
SRD5A1	gene for 5 α -reductase type 2
STDEV	standard deviation
T	testosterone
TBST	tris-buffered saline and Tween 20
TEB	terminal end buds
TEMED	Tetramethylethylenediamine
TG	trimegestone
TDLU	terminal ductal lobular unit
TIB	tibolone
TM	transcriptional machinery
TPR	tetratricopeptide repeat
TR	thyroid receptor
UDP-GlcDH	uridine diphosphoglucose dehydrogenase
U-PMTVC	under light microscopy
VDR	vitamin D receptor
VIA	video image analysis
WHI	Women's Health Initiative

Units:

°C	degrees Celsius
Da	Daltons(s)
g	grams or relative centrifugal force

kDa	kilo Dalton
kB	kilo bases
L	litre
min	minutes
M	molar (moles per litre)
mA	milliampere
mL	millilitre
nM	nanomolar
μL	microlitre
μM	micromolar
μm	microns
rpm	revolutions per minute
sec	seconds
U	units
V	volts

Chemical Formulas:

Cl	chloride
CO₂	carbon dioxide
H³	hydrogen
HCL	hydrochloride
NaCl	sodium chloride

Country and State:

ADL	Adelaide
AU	Australia
CA	California
DE	Germany
KS	Kansas
JP	Japan
MEL	Melbourne
MO	Missouri

NSW	New South Wales
NY	New York
SA	South Australia
SYD	Sydney
USA	United States of America
UK	United Kingdom
VIC	Victoria
WA	Washington

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