ANDROGEN SIGNALLING IN NORMAL AND MALIGNANT BREAST EPITHELIAL CELLS

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by

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CHAPTER 7 - GENERAL DISCUSSION

Summary

The growth and survival of normal breast epithelial cells and breast cancer cells is promoted by estrogens. In contrast, androgens inhibit the proliferation of normal and malignant breast epithelial cells. While this effect of androgens on breast cells appears to be androgen receptor (AR) dependent, the precise mechanism of inhibition and its functional significance are unknown. The aims of this thesis were to investigate the effect of androgen signalling on growth of normal and malignant breast epithelial cells, and to assess the interactions between androgen and estrogen signalling in the breast.

To investigate the role of androgen signalling in the growth and development of the normal mammary gland, female mice were treated with either the native androgen 5α-dihydrotestosterone (DHT) or the antiandrogen, flutamide. Analysis of the mammary glands at the end of the treatment period demonstrated that DHT reduced ductal branching and mammary epithelial cell proliferation when treatment commenced mid-puberty. Conversely, flutamide treatment that commenced post-puberty significantly increased ductal branching and proliferation of mammary epithelial cells. This data demonstrates that androgen signalling inhibits proliferation in the normal mammary gland, and may therefore oppose to the growth stimulatory effects of estrogen signalling to regulate breast growth and development.

The antiproliferative effects of androgens on breast epithelial cells may be due in part to direct AR-mediated activation of androgen regulated genes, or alternatively, androgens could act indirectly through AR to inhibit estrogen receptor alpha (ERα) activity. Expression of full-length AR or a truncated, constitutively active AR (AR-T707) significantly inhibited the activity of ectopically expressed ERα in MDA-MB-231 breast cancer cells (ERα- and AR-negative), in a dose-dependent manner. The functional consequences of inhibition of estrogen signalling by overexpressing AR were investigated in the T-47D breast cancer cell line (ERα- and AR-positive). Expression of AR-T707 in T-47D cells resulted in inhibition of both basal

and estradiol-induced cell proliferation and a marked reduction in the steady-state protein levels of the estrogen regulated gene, PR.

The final chapter investigated the mechanism by which AR inhibits ERα activity. A co-immunoprecipitation assay demonstrated an interaction between ectopically expressed AR and ERα in COS-1 cells, but not endogenous AR and ERα in a breast cancer cell line. To delineate the regions of AR required for inhibition of ERα signalling, various functional domains of the AR were mutated or deleted. Reporter gene assays showed that the inhibitory effects of AR were abrogated by deletion or mutation of the DNA binding domain (DBD). Furthermore, overexpression of the AR-DBD alone was sufficient to inhibit ERα activity. Consistent with a requirement for the DBD of AR to inhibit ERα activity, mobility shift assays demonstrated binding of AR to the *Xenopus* vitellogenin A2 consensus estrogen response element (cERE); however AR/ERα heterodimers were not detected on a cERE. Consistent with these findings, molecular modelling demonstrated that it is feasible for the DBD of AR to bind to a cERE and that it is unlikely that AR/ERα heterodimers could bind. Chromatin immunoprecipitation demonstrated recruitment of AR to the promoters of endogenous estrogen regulated genes. The findings suggest that the inhibitory effect of AR on ERα activity may occur either via formation of non-functional AR/ERα heterodimers that are unable to bind to EREs, or AR homodimers competing effectively for binding to EREs, in ERα target genes.

The results in this thesis demonstrate an inhibitory effect of androgen signalling on growth of normal and malignant breast epithelial cells. Additionally, the inhibition of breast epithelial cell proliferation by androgen signalling can be attributed, at least in part, to inhibition of ERα activity. These studies have provided insight into androgen action in the breast, and support a model whereby androgens balance the stimulatory effects of estrogen signalling in normal and malignant breast epithelial cells.

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 institute 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.

I give my consent to this copy of my thesis, when deposited in the University library, being made available for loan and photocopy.

Amelia A. Peters

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Publications arising from this Thesis

Articles published in scientific journals:

Decreased androgen receptor levels and receptor function in breast cancer contribute to the failure of response to medroxyprogesterone acetate, Buchanan G, Birrell SN, **Peters AA**, Bianco-Miotto T, Ramsay K, Cops EJ, Yang M, Harris JM, Simila HA, Moore NL, Bentel JM, Ricciardelli C, Horsfall DJ, Butler LM and Tilley WD Cancer Research (2005) 65: 8487-8496

Articles in preparation:

The androgen receptor inhibits estrogen receptor alpha signaling in breast cancer cells by binding to estrogen response elements, **Peters AA**, Bianco-Miotto T, Murphy N, Ricciardelli C, Centenera MM, Buchanan G, Segara D, Henshall SM, Birrell SN, Jia L, Harris JM, Sutherland RL, Butler LM and Tilley WD

Androgen signaling inhibits the proliferation of the mouse mammary gland, **Peters AA,** Bianco-Miotto T, Butler LM and Tilley WD

Abstracts published in the proceedings of scientific meetings:

Androgen receptor status predicts outcome in estrogen receptor positive breast cancers, Tilley WD, Ricciardelli C, Bianco-Miotto T, **Peters AA**, Centenera MM, Segara D, McNeil CM, Henshall SM, Sutherland RL, Sakko AJ, Buchanan G, Birrell SN and Butler LM, The Endocrine Society's 90th annual meeting, San Francisco, California, USA, June 2008

The androgen receptor inhibits estrogen receptor alpha (ER) signaling and predicts outcome in ER positive breast cancers, Tilley WD, Ricciardelli C, Bianco-Miotto T, **Peters AA**, Centenera MM, Moore NL, Murphy N, Segara D, McNeil C, Henshall SM, Sutherland RL, Sakko AJ, Buchanan G, Birrell SN and Butler LM, Era of Hope 2008 Meeting, Baltimore, MD, USA, June 2008

The androgen receptor attenuates estrogen signaling in breast cancer cells, **Peters AA**, Bianco-Miotto T, Murphy N, Ricciardelli C, Centenera MM, Buchanan G, Segara D, Henshall SM, Birrell SN, Sutherland RL, Butler LM and Tilley WD, Gordon Mammary Gland Biology Conference, Newport, RI, USA, June 2007

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Inhibition of estrogen receptor alpha activity in breast cancer cells by the androgen receptor, **Peters AA**, Bianco-Miotto T, Centenera MM, O'Loughlin M, Buchanan G, Butler LM and Tilley WD, 3rd PacRim Breast and Prostate Cancer Meeting, Fraser Island, Queensland, Australia, November 2006

Inhibition of estrogen signalling in breast cancer cells by the androgen receptor, **Peters AA**, Buchanan G, Bianco-Miotto T, Butler LM and Tilley WD, National meeting for the Australian Society for Medical Research, Couran Cove, Queensland, Australia, November 2005

Inhibition of estrogen signalling in breast cancer cells by the androgen receptor, **Peters AA**, Buchanan G, Bianco-Miotto T, Butler LM, Tilley WD, Annual meeting for the Australian Society for Medical Research, Adelaide, SA, Australia, June 2005

Inhibition of estrogen signalling in breast cancer cells by the androgen receptor, **Peters AA**, Buchanan G, Butler LM, Tilley WD, Annual meeting for the Australian Society for Medical Research, Adelaide, SA, Australia, June 2004

Abbreviations

17β-HSD	17β-hydroxysteroid dehydrogenase
3α-HSD	3α-hydroxysteroid dehydrogenase
3β-HSD	3β-hydroxysteroid dehydrogenase
A	adenine
Ab	antibody
Ad-ART707	adenovirus expressing AR-T707
Ad-LacZ	adenovirus expressing LacZ
AF	activation function
AI	aromatase inhibitor
ALU	arbitrary light units
APS	ammonium persulphate
AR	androgen receptor
ARA 70	androgen receptor coactivator 70
ARE	androgen response element
AR-T707	AR construct truncated at amino acid 707
ATAC	Arimidex, Tamoxifen Alone or in Combination trial
BRCA1	breast cancer susceptibility gene 1
BRCA2	breast cancer susceptibility gene 2
BrdU	5-bromo-2'deoxyuridine
BSA	bovine serum albumin
C	cytosine
cARE	consensus androgen response element
cDNA	complementary DNA
cERE	consensus estrogen response element
ChIP	chromatin immunoprecipitation
cHRT	combined estradiol and progestin hormonal replacement therapy
CoIP	co-immunoprecipitation
COUP-TF	orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor
CPE	cytopathic effect
CTSD	cathepsin D gene
DAB	3,3'-Diaminobenzidine
DBD	DNA binding domain
DCC	dextran coated charcoal
DHEA	dehydroepiandrosterone

DHEA-S	dehydroepiandrosterone sulphate
DHT	5α-dihydrotestosterone
DMEM	Dulbecco's modified eagle medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
dox	doxycycline
DTT	dithiothreitol
E2	17β-estradiol
ECL	enhanced chemiluminescence
EDTA	ethylenediamine tetra-acetic acid
EGFP	enhanced green fluorescent protein
ER	estrogen receptor alpha and estrogen receptor beta
ERα	estrogen receptor alpha
ERE	estrogen response element
ERβ	estrogen receptor beta
FBS	fetal bovine serum
FSH	follicle-stimulating hormone
G	guanine
GR	glucocorticoid receptor
GU	genitourinary
H	hinge region
HRP	horseradish peroxidase
HRT	hormone replacement therapy
Hsp	heat shock protein
IgG	immunoglobulin
IMPACT	IMmediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen
KO	knockout
LB	luria broth
LBD	ligand binding domain
LH	luteinising hormone
Luc	luciferase
MMTV	mouse-mammary tumour virus
MOIs	multiplicity of infections
MPA	medroxyprogesterone acetate
MR	mineralocorticoid receptor
n	any nucleotide

NTD	amino-terminal transactivation domain
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PGR	progesterone receptor gene
PMSF	phenylmethylsulphonyl fluoride
PR	progesterone receptor
PRF	phenol red free
PROACT	Preoperative Arimidex Compared with Tamoxifen
PSA	prostate-specific antigen
RAR	retinoic acid receptor
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
rtTA	reverse tetracycline controlled transactivator
RXR	retinoid X receptor
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SERM	selective estrogen receptor modulator
SHBG	sex hormone-binding globulin
T	thymine
TAE	tris acetate EDTA
Tam	tamoxifen
TBE	tris borate EDTA
TBS	tris buffered saline
TBST	tris buffered saline tween 20
TE	tris-EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
tk	thymidine kinase
TRα	thyroid hormone receptor alpha
TR4	testicular orphan receptor 4
TRE	tetracycline response element
UV	ultra violet light
WHI	Women's Health Initiative
wt	wild-type
x	any amino acid

X-gal	5-bromo-4-chloro-3-indolyl -D-galactopyranoside
Δ	deletion

Units:

bp	base pairs
cpm	counts per minute
Ci	curie(s)
°C	degrees Celsius
Da	Dalton(s)
g	gram(s)
g	relative centrifugal force
kDa	kilo Dalton
kb	kilobases
L	litre
min	minute(s)
M	molar (moles per litre)
mA	milliampere
rpm	revolutions per minute
sec	seconds(s)
U	units
V	volts