The Role of Estrogen Receptor α and the Androgen Receptor in Human Breast Cancer

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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ॐ भूर्भुवः स्वः
ॐ तत्सवितुर्वरेण्यं भर्गो देवस्य
धीमहि धियो यो नः प्रचोदयात्।

'We meditate on the splendor of the Creator;
Who has manifested the Worlds;
Who alone is worthy of Worship;
Who is the embodiment of Knowledge and Light;
Who is the remover of all duality and ignorance;
May He awaken us.'

Dedicated to the anchor of my life, my dear husband

Sudeep

And my adorable children

Aadil & Advika

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Abstract

The androgenic signalling axis interacts with other major growth pathways in breast cancer, such as estrogen receptor (ER) signalling, and is of renewed interest due to the promise of exploiting the pathway for therapeutic benefit. The effects of signalling via the androgen receptor (AR) are pleiotropic, and there is evidence *in vitro* and *in vivo* that it can both promote and inhibit proliferation of breast epithelia, largely depending on ER expression and activation. Given this complexity, the effect of pathway modulation in individual women with breast cancer remains unclear.

Therefore, the purpose of the studies undertaken in this thesis was to establish baseline parameters in terms of tissue expression of AR and apply them to meaningful clinical scenarios to better establish which population of patients might benefit from androgen pathway-targeting therapies. In the first part of the study, dual-labelling immunofluorescence was performed on a tissue microarray (TMA) containing normal breast and an array of malignant tissues representing tumour progression. AR was expressed more frequently than ER, and AR+ER- cells comprised one third of the total epithelial cell population. 26.6% of the total epithelial population were AR+ER+, 37.5% AR-ER-, and a minor proportion AR-ER+ (2.8%). There were no significant differences in AR expression (either alone or co-localised) between primary and nodal metastasis lesions, and expression remained constant in *in situ*, invasive, and metastatic disease. AR and ER expression therefore show remarkable but stable intratumoural heterogeneity, with implications for how individual cells might respond to therapy within the tumour population as a whole.

The second part of this thesis aimed to firmly establish: a) the prognostic value of AR in two independent cohorts of patients with primary breast cancer and with long-term follow-up, and b) criteria for measurement of the biomarker to pave the way for biomarker measurement in androgen-therapy trials. AR was an independent prognostic factor in two independent cohorts of primary breast cancers tested with different antibodies, and ROC analysis established that the optimal cut-point of AR positivity was 78%. Patients with high AR expression had approximately two-fold reduced risk of cancer-related death in both cohorts, and AR expression was significantly associated with ER expression. Patients with equal or high AR:ER ratios had the best 10-year overall survival of over 80%. Although unlikely to add much to existing prognostic algorithms and approaches, establishing a simple and robust diagnostic test with an appropriate cut-point will expedite studies using androgen pathway-targeting

therapies.

Finally, the third part of this thesis explored the hypothesis that some of the risk of breast cancer associated with increased breast density might be associated with AR expression. Although AR expression was higher in malignant than benign disease, it was not associated with breast density; breast density is likely to be more related to cumulative exposure to estrogen and drive the underlying pathogenesis.

The data presented in this thesis open up several further avenues for investigation, including a robust immunohistochemical assay that can be used in prospective clinical trials and a quantitative immunofluorescence double-staining methodology that can be applied to large clinical cohorts with documented clinical outcomes to help reveal the significance and relative contributions of the co-expressing AR/ER subpopulations to breast cancer pathogenesis and progression. AR expression needs to be investigated in suitable dynamic models of disease progression in order to establish exactly how different populations of cells within the tumour interact and change over time and in response to therapy. These data provide the starting point for these more advanced studies.

THESIS 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 to Shalini Jindal 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 consent to this copy of my thesis when deposited in the University Library being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Date:

ABBREVIATIONS

17-OHP	17 alpha-hydroxyprogesterone
ACTH	Adrenocorticotropic hormone
ADH	Atypical ductal hyperplasia
ALH	Atypical Lobular hyperplasia
AR	Androgen receptor
BSSA	BreastScreen South Australia
CDK	Cyclin-dependent kinase
DBD	Deoxyribonucleic acid binding domain
DCIS	Ductal carcinoma *in situ*
DFS	Disease free survival
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone-sulphate
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
FFPE	Formalin-fixed paraffin-embedded
FSH	Follicle stimulating hormone
HER2	Human epidermal growth factor receptor-2
HREs	Hormone response elements
HRT	Hormone response therapy
HSPs	Heat shock proteins
IDC	Invasive ductal carcinoma
IGFR-1	Insulin-like growth factor receptor-1
KO	Knockout
LBD	Ligand binding domain
LCIS	Lobular carcinoma *in situ*
LDL	Low density lipoprotein
LH	Luteinising hormone

MAPK	Mitogen-activated protein kinase
MBD	Mammographic breast density
NR	Nuclear receptor
NTD	Amino-terminal transactivation domain
OCP	Oral contraceptive pill
OS	Overall survival
PBS	Phosphate buffer solution
PR	Progesterone receptor
RNA	Ribonucleic acid
TDLU	Terminal duct-lobular unit
TMA	Tissue microarray
WHI	Women's Health Initiative

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