ANDROGEN SIGNALLING IN THE PROSTATE CANCER MICROENVIRONMENT

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ABSTRACT

Prostate cancer remains the second leading cause of cancer related death in Australian males, with approximately 28000 Australian men being diagnosed in 2007 and more than 3000 deaths as a result of this disease. The probability of patient survival from prostate cancer is greatly diminished when the disease has spread outside of the confines of the prostate. Disease spread, or the potential for spread, also determines the therapy received by the patient, be it surgical removal, chemotherapy, radiotherapy, or hormonal therapy, or any of these in combination. The advent of serum PSA testing has allowed for both earlier and increased detection of prostate cancer over the past 20 years. Despite this, the mortality rate for prostate cancer has remained relatively constant. Moreover, it is thought that increased detection may lead to over diagnosis and over treatment of indolent disease in up to 50% of cases, burdening patients who would not actually die from prostate cancer. Central to this issue is that there is no accurate means of predicting, at the time of diagnosis, the likelihood of prostate cancer becoming aggressive and metastasising, and thus identifying the patient population that would benefit most from more aggressive treatment.

The prostate is a glandular structure composed of secretory epithelial cells embedded in a stroma containing smooth muscle cells, fibroblasts, and extracellular matrix (ECM) proteins, as well as immune cells and vasculature. Prostate cancer arises from the epithelial cells, which can subsequently evolve the capacity to breach the basement membrane, navigate through the surrounding stroma and ultimately enter the bloodstream allowing metastasis to distant sites such as bone. Recent studies suggest that each of these processes depends on a bi-directional interaction between the cancer cells and the prostate microenvironment, and moreover that

structural or functional changes in that microenvironment are a necessary component of progressive tumourigenesis. As an example, activated cancer associated fibroblasts (CAFs) are thought to secrete paracrine factors that provide a more supportive environment, increasing cancer cell initiation and subsequent tumour growth.

Another key aspect of prostate cancer development and maintenance are androgens, which act through their cognate receptor, the androgen receptor (AR). Current therapy for prostate cancer includes androgen deprivation therapy (ADT), which aims to ablate AR action. This therapy is most commonly used for advanced metastatic disease, however, in the USA between 1998 and 2005, ADT alone or in conjunction with radiotherapy was used as a primary treatment for advanced but organ confined prostate cancer in 70% of men. Studies have since shown an increased likelihood of disease progression and re-emergence in such cases. An explanation for the increased risk of progression may reside in understanding the role of AR in CAFs. Previously, CAF AR has been shown to be required for cancer initiation and tumour development. However, the specific cellular and molecular effect of androgens on fibroblasts is not well known, and the role of fibroblast AR in mediating progressive tumourigenesis of the cancer epithelia is unclear.

The AR is a transcription factor which, upon activation by cellular internalized androgen, binds to DNA and mediates biological functions such as proliferation. Our understanding of the molecular actions of the AR are, however, almost entirely based on studies of its action in isolated prostate cancer epithelial cells, and inferences from the studies of other members of the nuclear receptor superfamily of ligand activated transcription factors. In general terms, the AR mediates the biological actions of androgens by becoming activated, translocating to the

nucleus, and acting in concert with accessory proteins and coregulators to control chromatin structure and transcription at specific genomic sites. Whether these same general principles apply in fibroblasts is not known. In this thesis, human prostatic myofibroblast cell lines expressing AR (PShTert-AR) were compared to AR negative fibroblasts (PShTert-ctrl) and epithelial cancer cell lines (C4-2B), with view to defining the molecular and physiological roles of fibroblast AR. Microarray profiling revealed that only 10% of genes regulated by androgens in fibroblasts were also regulated in epithelial cells. Genes regulated by AR in fibroblasts were involved in pathways such as proliferation, ECM production, and adhesion. Conversely, in epithelial cells, AR regulated genes were involved in apoptosis, mitosis, and signal transduction. Functional studies showed that AR has an intrinsic anti-proliferative action in fibroblasts, but also promotes the release of paracrine factors that enhance growth of prostate cancer cells, both *in vitro* and *in vivo*. Androgens also induced fibroblast-mediated deposition of ECM proteins, such as collagen, whilst reducing the expression of enzymes involved in ECM degradation such MMP1 and MMP3. Further *in vitro* testing, demonstrated that ECM produced under androgenic stimulation increased attachment of the fibroblasts themselves by 60% in under 30 minutes ($p<0.05$), as well as attachment of prostate cancer epithelial cells by up to 35% ($p<0.05$). In addition, androgens increased fibroblast cell adhesion and suppressed motility and invasion over a four hour period by 30% ($p<0.05$).

Utilizing several sets of microarray data, qualitative and quantitative differences in expression of AR coregulators in fibroblasts and epithelial cells were also determined. Intriguingly, it was observed that a number of previously described epithelial-cell AR coregulators were expressed almost exclusively in fibroblasts, including several proteins such as the coactivator Hic-5 (TGFB1I1), known to interact with focal adhesions. Despite evidence in previous

studies for expression predominantly in fibroblasts, Hic-5 has been investigated almost exclusively in a small number of epithelial cell lines. Here, the biological roles of this AR coregulator were investigated using isogenic myofibroblasts with or without Hic-5, and in a transient siRNA model of Hic-5 depletion. Microarray and RT-qPCR investigation revealed that the level of Hic-5 affects the expression of over 50% of androgen mediated genes, and in the majority of cases (85%) works to amplify (coactivate) the natural androgenic response. Supporting this finding, depletion of Hic-5 decreased the androgenic response from 34 of the 69 tested AR-responsive luciferase reporter constructs tested ($p<0.01$). Finally, in a novel and important finding, it was demonstrated that androgens result in a dynamic cellular localisation of Hic-5 in fibroblasts, and that this process is a key determinant of the observed androgen-mediated responses, adhesion and motility. Collectively, these data demonstrate that Hic-5 is an important modulator of both androgen mediated gene transcription and androgen driven cell movement in fibroblasts. Furthermore, these studies demonstrate that the cell specificity of AR signalling may be mediated by unique interactions with pools of coregulators specific to fibroblasts, and that changes in these pools could significantly alter androgen signalling within the cancer environment.

In order to assess the physiological relevance of fibroblast AR, a tissue microarray was constructed as part of this thesis that contained both benign and malignant prostate samples from 64 patients, on which quantitative immunohistochemical assessment of AR expression was performed in both epithelial and stromal compartments. Changes in epithelial AR expression did not affect patient outcome, but were significantly related to Gleason grade ($p<0.001$) and serum PSA ($p<0.001$). In contrast, expression of AR in stroma were significantly associated with prostate cancer related death ($p<0.05$). This relationship was

found to be unique to the cancer associated stroma, as AR expression in patient-matched benign tissue did not associate with any of the clinical parameters. Combined with the *in vitro* findings in this thesis, those data strongly suggest that although stromal AR is required for cancer initiation and growth, a subsequent loss of stromal AR produces a cancer more likely to be associated with a detrimental patient outcome.

The findings from this thesis identify two mechanisms by which AR signalling in fibroblasts may contribute to prostate cancer progression. First, a loss of stromal AR results in increased fibroblast movement and proliferation, and the secretion of a permissive ECM for cancer cell detachment and movement. Second, AR activation by androgen results in redistribution of fibroblast-specific cofactors such as Hic-5 from the cytoplasm to the nucleus, leading to enhanced AR transcriptional activity. The movement of Hic-5 from focal adhesion sites concomitantly increases adhesion and reduces motility. By increasing the understanding of the molecular pathways and function of AR and androgens in the stroma, the research undertaken in this thesis has been able to establish new models of AR action in the cancerous environment. Significantly, these data provide a mechanism for the increased likelihood of disease progression and re-emergence upon treatment of advanced organ confined prostate cancer with ADT, as a loss of fibroblast AR activity and altered ECM production is predicted to enable cancer metastasis. This thesis concludes that dysregulation of AR action in the stroma potentiates prostate cancer progression, and may thus be used as an indicator of cancer aggressiveness and metastasis.

DECLARATION

I certify that 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 published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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ACKNOWLEDGMENTS

I would like to thank my supervisors, Dr Grant Buchanan, Dr Eleanor Need, Dr Andrew Trotta, and Prof Villis Marshall, for guiding me through my PhD. I've learnt so much from you all, and couldn't think of better to have as my teachers. Thank you for the great guidance, unwavering support, and effort you've put in over the past few years.

Grant, thank you for your continuous, and apparently limitless, enthusiasm. It made doing my PhD much easier and enjoyable. A big thanks for all the time you've put in given your full-on schedule, very much appreciated. And thanks for being a head of lab who is not only dedicated to the science and hard work, but can also actually have a laugh. Oh, and dragging me to the pub.

Elle, thank you for your continued support, and humour, and the caring, and being the organised one. Thank you for putting up with my continued poor spelling and bad grammar. A massive thank you for actually coming back, I don't know what I would have done if you didn't, you've been a great mentor.

Andrew, thank you for being such a good example for me. When I first started I quickly tried to follow your example of hard work and dedication, and I'm grateful that you showed the same efforts when coming back to the lab as a supervisor.

Mel, thanks for being very patient with me when teaching me in the lab, and forgiving me when I melted the electrophoresis apparatus, oops.

Thanks to Lauren, Erin, Bill, Irene, and all the other students at the BHI for stumbling through this PhD with me and providing laughs along the way.

Coffee and beer, you know you’re great.

Thanks to all my friends, for providing much needed exercises in sanity, and reminding me that life does exist outside of the lab.

Thanks to Eb and Holy for always wagging their tails when I would come home from the lab, no matter how late that would be.

Thanks to Mum and Dad, Kir and Jeremy, Meg and Rhett, for being heaps supportive and understanding, putting up with lateness and occasional grumpiness. Thanks to lil Oscar and Arley for cheering me up with photogenic-ness.

ABBREVIATIONS

αSMA	alpha Smooth Muscle Actin
ADT	Androgen Deprivation Therapy
AF	Activation Function
AKT	v-Akt murine Thymoma viral oncogene homolog 1
AR	Androgen Receptor
ARA70	Androgen Receptor Associated protein
ARE	Androgen Response Element
BIC	Bicalutamide
BLM	Bladder Mesenchyme
BPH	Benign Prostate Hyperplasia
CAF	Cancer-Associated Fibroblast
CBP	CREB Binding Protein
ChIP	Chromatin Immunoprecipitation
COL	Collagen
CREB	cAMP-Response Element Binding Protein
D	Aspartate
DBD	DNA Binding Domain

DCC	Dextran Coated Charcoal
DHEA	Dihydroepialdosterone
DHT	Dihydrotestosterone
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DRE	Digital Rectal Exam
E2	17β-Oestradiol
EBRT	External Beam Radiation Therapy
ECM	Extracellular Matrix
EDTA	Ethylene-diamine-tetra Acetic Acid
EGF	Epidermal Growth Factor
ELISA	Enzyme Linked ImmunoSorbent Assay
EMT	Epithelial Mesenchymal Transition/Transformation
ERK	Extracellular Regulated Kinase
FA	Focal Adhesion
FAK	Focal Adhesion Kinase
FAP	Fibroblast Activation Protein
FBS	Foetal Bovine Serum
FBN	Fibronectin
FBXO32	F-Box Only Protein 32

FCS	Foetal Calf Serum
FGF	Fibroblast Growth Factor
FKBP	FK506 Binding Protein
FSH	Follicle Stimulating Hormone
GR	Glucocorticoid Receptor
GTP	Guanine Triphosphate
H	Hinge region
HAT	Histone Acetyl Transferase
HDAC	Histone Deacetylase
HRE	Hormone Response Element
HSP	Heat Shock Protein
IGF	Insulin-like Growth Factor
KLK	Kallikrein
L	Leucine
LBD	Ligand Binding Domain
LD	Leucine Aspartate
LDB	Low Dose Brachytherapy

LH	Leutenising Hormone
LHRH	Leutenising Hormone Releasing Hormone
MAPK	Mitogen Activated Protein Kinase
MMP	Matrix Metalloproteinase
N/C interaction	Amino Terminal Domain/Carboxy Terminal Domain interaction
NCOA	Nuclear Receptor Coactivator
NES	Nuclear Export Signal
NLS	Nuclear Locating Domain
NPC	Nuclear Pore Complex
NPF	Normal Prostatic Fibroblast
NTD	Amino Terminal Domain
NTMS	Nuclear Matrix Transport Signal
OS	Oxidative Stress
PCa	Prostate Cancer
PIN	Prostatic Intraepithelial Neoplasia
PKA	Protein Kinase A
PKC	Protein Kinase C
PRF	Phenol Red Free

PSA	Prostate Specific Antigen
PTEN	Phosphatase and Tensin homolog
PXN	Paxillin
Raf1	V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1
RNA	Ribonucleic Acid
RPMI	Roswell Park Memorial Institute
RT-qPCR	Real Time Quantitative Polymerase Chain Reaction
SFRP1	Secreted Fizzled Related Protein 1
SHBG	Sex Hormone Binding Globulin
shRNA	Short Hairpin Ribonucleic Acid
siRNA	Small Interfering Ribonucleic Acid
SMRT	Silencing Mediator for Retinoic acid and Thyroid receptor
SRC1	Steroid Receptor Coactivator 1
T	Testosterone
Tfm	Testicular Feminized Mouse
TGFB	Transforming Growth Factor Beta
TGFBR	Transforming Growth Factor Beta Receptor
TNM	Tumour, Lymph Node, Metastasis
TURP	Transurethral Resection of the Prostate

UGE	Urogenital Epithelium
UGM	Urogenital Mesenchyme
UGS	Urogenital Sinus
VEGF	Vascular Endothelial Growth Factor
WT	Wild Type
Y	Tyrosine

CONFERENCE PRESENTATIONS

- SA ASMR Scientific Meeting, 2010, Androgen signalling in prostate cancer stroma**,** Adelaide SA, **DA Leach,** AP Trotta, EF Need, DB DeFranco, RA Taylor, GP Risbridger, & G Buchanan. POSTER
- Faculty of Health Sciences Postgraduate Research Conference, Adelaide SA, August 2010. Androgen signalling in prostate cancer stroma, **DA Leach**, AP Trotta, EF Need, DB DeFranco, RA Taylor, GP Risbridger, & G Buchanan. POSTER
- Faculty of Health Sciences Postgraduate Research Conference, Adelaide SA, August 2011. Androgen signalling in prostate cancer stroma, **DA Leach**, EF Need, S Chopra, RA Taylor, GP Risbridger, CB Pinnock, DB DeFranco & G Buchanan. POSTER
- TQEH Research Day, Adelaide SA, October 2011. Androgen signalling in prostate cancer stroma, **DA Leach**, PA Drew, E Smith, EF Need, & G Buchanan. ORAL PRESENTATION
- 33rd Annual Lorne Genome Conference, Lorne VIC, February 2011. Androgen signalling in prostate cancer stroma, **DA Leach**, EF Need, S Chopra, RA Taylor, GP Risbridger, CB Pinnock, DB DeFranco & G Buchanan. POSTER
- 12th Australasian Prostate Cancer Conference, Melbourne VIC, August 2011, Stromal Androgen Receptor is inversely associated with prostate cancer related death, **DA Leach**, EF Need, S Chopra, RA Taylor, GP Risbridger, CB Pinnock, DB DeFranco & G Buchanan. POSTER
- 15th International Congress of Endocrinology, Florence, Italy, May 2012, Immunohistochemical, ChIP, and microarray analysis reveals how stromal AR controls prostate cancer outcome through fibroblast specific action of androgen signalling on ECM production and DNA licensing, **DA Leach**, EF Need, S Chopra,

RA Taylor, GP Risbridger, CB Pinnock, P Lee, DB DeFranco & G Buchanan. POSTER

- Faculty of Health Sciences Postgraduate Research Conference, Adelaide SA, August 2012. Androgen signalling in prostate cancer stroma, **DA Leach**, EF Need, S Chopra, RA Taylor, GP Risbridger, CB Pinnock, DB DeFranco & G Buchanan. POSTER
- TQEH Research Day, Adelaide SA, October 2012. Androgen signalling in prostate cancer stroma, **DA Leach**, PA Drew, E Smith, EF Need, & G Buchanan. POSTER
- SA ASMR Scientific Meeting, 2013, Fibroblast Specific Androgen Signalling and Implications for Prostate Cancer, **DA Leach**. ORAL PRESENTATION.
- Aus-Can PCRA Symposium, 2013, Stromal androgen receptor regulates a protective microenvironment and promotes poor outcome in prostate cancer, **DA Leach**, EF Need, R Toivanen, AP Trotta, S Chopra, DJ Tamblyn, T Kopsaftis, GM England, CB Pinnock, G Risbridger, DB DeFranco, P Lee, RA Taylor, & G Buchanan. POSTER

AWARDS

- Discipline of Medicine Prize for Best Presentation. 2012 Faculty of Health Sciences Postgraduate Conference.
- International Congress of Endocrinology Travel grant, 2012
- Faculty of Health Sciences Postgraduate Travel Scholarship, 2012
- South Australian Heath and Medical Research Institute Beat Cancer Project Travel Grant 2012

CO-AUTHORED PUBLICATIONS

Trotta AP, Need EF, Selth LA, Chopra S, Pinnock CB, **Leach DA**, Coetzee GA, Butler LM, Tilley WT, and Buchanan G, 2013, Knockdown of the co-chaperone SGTA results in suppression 1 of androgen 2 and PI3K/AKT signalling and inhibition of prostate cancer cell proliferation. International Journal of Cancer.

Bianco-Miotto T, Trotta AP, Need EF, Lee AMC, Ochnik AM, Giorgio L, **Leach DA**, Swinstead EE, O'Loughlin MA, Newman MR, Birrell SN, Butler LM, Harris JM, and Buchanan G, 2013, Molecular and structural basis of androgen receptor responses to dihydrotestosterone, medroxyprogesterone acetate and Δ^4-tibolone, Molecular and Cellular Endocrinology.