

**The Role of Epigenetic Modifications
in Prostate Tumourigenesis**

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

by

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Declaration

This work contains no material which has been accepted for the award of any other degree or other diploma in any university or other tertiary institution to Karen Chiam HuiQin 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. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Karen Chiam HuiQin

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Abstract

Prostate cancer is the second-leading cause of cancer death in Australian men. Current therapies for advanced prostate cancer are not curative and most patients eventually develop castrate-resistant prostate cancer. Epigenetic modifications are heritable and reversible biochemical changes of the chromatin that regulate gene expression and are important in prostate tumourigenesis. There is also evidence that excess foetal nutrition is associated with increased risk of developing prostate cancer. Hence, the aims of this thesis were to determine the involvement of epigenetic modifications in: the early origin of prostate cancer, prostate cancer progression, as prognostic and therapeutic targets in prostate cancer.

The first aim of this thesis was to use a rodent model to determine if a maternal high fat diet (MHFD) is associated with increased risk of prostate cancer in offspring. Offspring exposed to a MHFD had increased incidence of prostate abnormalities compared to offspring exposed to a maternal control diet. *GSTP1* is hypermethylated and silenced in human prostate cancer and was decreased in these offspring prostates. The MHFD altered the male offspring prostates microRNA expression and provided insights of possible underlying mechanisms that support a link between MHFD and risk of prostate cancer in adult offspring.

The second aim was to investigate if specific histone modifications H3K18Ac and H3K4diMe were prognostic markers for prostate cancer. High levels of H3K18Ac and H3K4diMe were associated with increased risk of prostate cancer relapse respectively.

To further investigate the underlying mechanisms, epigenetic genes were mined in microarray data, and an epigenetic gene signature was identified which distinguished non-malignant from tumour prostate tissues in an independent prostate cancer cohort.

To investigate if the DNA methyltransferase inhibitor (DNMTi) 5-aza-CdR was a potential treatment agent for prostate cancer, proliferation assays were performed in prostate cancer cells. A daily low-dose and prolonged 5-aza-CdR treatment regime was the most effective treatment in prostate cancer cells compared to high doses administered less frequently. Furthermore, *GSTP1* DNA methylation and protein status were good indicators of DNMTi efficacy *in vitro*, where demethylation indicated growth suppression and protein re-expression indicated cell death induction.

To investigate if the Kruppel-like-factor 6 (*KLF6*) prostate cancer susceptibility gene is epigenetically altered during prostate cancer progression, DNA methylation analyses were performed in human and mouse (TRAMP) prostate cancers. Our results suggest that DNA hypermethylation is not responsible for decreased *KLF6* expression in human and TRAMP prostate cancers in our study.

Collectively, the findings of this thesis further support the importance of epigenetic modifications in prostate tumourigenesis. We demonstrated the potential of using epigenetic modifications as prognostic markers, therapeutic targets and as a marker of treatment efficacy. Lastly, we provide evidence, for the first time, that MHFD is a risk factor for prostate cancer and that miRNAs are involved. This finding is important and

suggests the potential of early prevention/ intervention of prostate cancer by targeting epigenetic modifications and diet intervention.

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Publications Arising from this Thesis

Articles Published in Scientific Journals

The Dynamic and static modification of the epigenome by hormones: A role in the developmental origin of hormone-related cancers. **Karen Chiam**, Wayne D. Tilley, Lisa M. Butler, Tina Bianco-Miotto. *Biochimica et Biophysica Acta*, 2009 1795(2): p104-9

Articles Submitted to Scientific Journals

Global levels of histone modifications and an epigenetic gene signature predict prostate cancer progression and development. Tina Bianco-Miotto*, **Karen Chiam***, Grant Buchanan, Shalini Jindal, Tanya K Day, Mervyn Thomas, Marie A Pickering, Melissa O'Loughlin, Natalie K Ryan, Wendy A Raymond, Lisa G Horvath, James G Kench, Phillip D Stricker, Villis R Marshall, Robert L Sutherland, Susan M Henshall, William L Gerald, Howard I Scher, Gail P. Risbridger, Judith A Clements, Lisa M Butler, David J Horsfall, Wayne D Tilley, Carmela Ricciardelli and the Australian Prostate Cancer BioResource. *Clinical Cancer Research*, 2010.

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Abstracts Published in the proceedings of Scientific Meetings

GSTP1 is a marker of treatment response to epigenetic therapy in prostate cancer. **Karen Chiam**, Margaret M Centenera, Carmela Ricciardelli, Wayne D Tilley, Lisa M Butler, Tina Bianco-Miotto. *Epigenetics 2009 Australian Scientific Conference*, Melbourne, Australia, December 2009.

Changes in DNA methylation and expression of GSTP1 is a marker of treatment response to epigenetic therapy in prostate cancer. **Karen Chiam**, Margaret M Centenera, Carmela Ricciardelli, Wayne D Tilley, Lisa M Butler, Tina Bianco-Miotto. Endocrine Society of Australia, SA, Australia, August 2009.

Maternal Obesogenic diet may induce prostate abnormalities characteristic of an increased risk of prostate cancer in rat offspring. **Karen Chiam**, Shalini Jindal, Karen Kind, Wayne Tilley, Julie Owens and Tina Bianco-Miotto. The Australian Society for Medical Research, SA, Australia, June 2009.

Identification of a putative epigenetic gene signature associated with prostate cancer progression. **K. Chiam**, T. Bianco-Miotto, G. Buchanan, S. Jindal, M. Pickering, M. O'Loughlin, W. A. Raymond, L. G. Horvath, J. G. Kench, P. D. Stricker, V. R. Marshall, W. L. Gerald, H. I. Scher, R. L. Sutherland, S. M. Henshall, L. M. Butler, D. J. Horsfall, W. D. Tilley, C. Ricciardelli. Chromatin Conference: Histones, Nucleosomes, Chromosomes and Genomes, Singapore, February 2009.

Histone modifications are independent predictors of relapse free survival in prostate cancer. T Bianco-Miotto, C Ricciardelli, S Jindal, **H Chiam**, LG Horvath, JG Kench, PD Stricker, VR Marshall, DJ Horsfall, RL Sutherland, SM Henshall, LM Butler and WD Tilley. The Endocrine Society's Annual Meeting, San Francisco, USA, June 2008.

Global level of specific histone modification, acetylated Histone H3 Lysine 18, is an independent predictor of relapse free survival in prostate cancer. **Karen Chiam**, T Bianco-Miotto, C Ricciardelli, S Jindal, LG Horvath, JG Kench, PD Stricker, VR Marshall, DJ Horsfall, RL Sutherland, SM Henshall, LM Butler and WD Tilley. The Australian Society for Medical Research, SA, Australia, June 2008.

Global level of specific histone modification, acetylated Histone H3 Lysine 18, is an independent predictor of relapse free survival in prostate cancer. **Karen Chiam**, T Bianco-Miotto, C Ricciardelli, S Jindal, LG Horvath, JG Kench, PD Stricker, VR Marshall, DJ Horsfall, RL Sutherland, SM Henshall, LM Butler and WD Tilley. Faculty of Health Sciences Postgraduate Research Expo, SA, Australia, July 2008.

The role and mechanisms of epigenetic modifications during prostate cancer progression. **Karen Chiam**, Tina Bianco-Miotto, Wayne Tilley, Lisa Butler. Faculty of Health Sciences Postgraduate Research Expo, SA, Australia, October 2007.

Abbreviations

5-aza-CdR	5-aza-2'-deoxycytidine/ Decitabine
5-aza-CR	5-aza-cytidine
AAT	Androgen ablation therapy
AR	Androgen receptor
AP	Anterior prostate
APCB	Australian Prostate Cancer BioResource
BMI	Body mass index
BSA	Bovine serum albumin
BPA	Bisphenol A
BPH	Benign prostate hyperplasia
BR	Biochemical recurrence
C	Cytosine
Cdh1	Cadherin 1
cDNA	Complementary DNA
COBRA	Combined bisulphite restriction analysis
CpG	Cytosine and guanine dinucleotides
DAB	3,3'-Diaminobenzidine
DCC	Dextran coated charcoal
DES	Diethylstilbestrol
DHT	5 α -dihydrotestosterone
DLP	Dorso-lateral prostate
DMBA	7,12-dimethylbenz(a)anthracene
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DNMT1	DNA methyltransferase 1
DNMT3A	DNA methyltransferase 3A
DNMT3B	DNA methyltransferase 3B
DNMTi	DNA methyltransferase inhibitor
DRE	Digital rectal examination
ER α	Estrogen receptor alpha
EtOh	Ethanol
FCS	Foetal calf serum
FD	False discovery
FDA	Food and Drug Administration
G	Guanine
GR	Glucocorticoid receptor
GSTP1	Gluthatione-S-transferase P1
GTT	Glucose tolerance test
H3K4diMe	Dimethylated Histone 3 Lysine-4 residue
H3K18Ac	Acetylated Histone 3 Lysine-18 residue
HAT	Histone acetyltransferase
HDAC	Histone deacetylase

HDAC1	Histone deacetylase 1
HDACi	Histone deacetylase inhibitor
HDMT	Histone demethylase
HGPIN	High-grade prostate intraepithelial neoplasia
HMT	Histone methyltransferase
HMTi	Histone methyltransferase inhibitor
HPA	Hypothalamic-pituitary-adrenal
IGF2	Insulin growth like factor 2
IHC	Immunohistochemistry
ITT	Insulin tolerance test
KLF6	Kruppel-like factor 6
LG	Licking and Grooming
LNCaP	Lymph node carcinoma of the prostate
LOH	Loss of heterozygosity
LOI	Loss of imprinting
MBD	Methyl-DNA binding
miRNA	Micro ribonucleic acid
MSP	Methylation-specific polymerase chain reaction
mRNA	Messenger ribonucleic acid
MDS	myelodysplastic syndromes
MIOD	Mean integrated optical density out of total field area
MOD	Mean integrated optical density out of the positive nuclear area
NM	Non-malignant prostate
PBS	Phosphate buffered saline
PCa	Prostate cancer
PGC	primordial germ cells
PIN	Prostate intraepithelial neoplasia
PSA	Prostate specific antigen
QMSP	Quantitative methylation-specific polymerase chain reaction
qPCR	Quantitative real time polymerase chain reaction
Rb	Retinoblastoma
RIN	RNA Integrity Number
RT-PCR	Real time polymerase chain reaction
SAHA	Suberoylanilide hydroxamic acid acid/ Vorinostat
SAM	S-adenosylmethionine
SDS	Sodium dodecyl sulphate
SNP	Single-nucleotide polymorphism
SV	Splice variants
SV	Seminal vesicles
TBS	Tris buffered saline
TBST	Tris buffered saline-tween 20
TEMED	N,N,N', N'-tetramethylethylenediamine
TMA	Tissue microarray
TRAMP	Transgenic adenocarcinoma of mouse prostate

VIA	Video Image analysis
VP	Ventral prostate
WHR	Waist hip ratio
wt	Wild-type
Xi	X chromosome inactivation

Units

°C	Degree Celsius
μl	Microlitre
μg	Microgram
μM	Micromolar
μm	Micron
bp	Base pairs
Da	Dalton
g	Gram
kDa	Kilodalton
h	Hour
M	Molar
mA	Milliampere
min	Minute
ml	Millitre
n	Number
ng	Nanogram
Rpm	Revolutions per minute
s	second
t	Time