# 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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**Table of contents**

## CHAPTER 8 - GENERAL DISCUSSION

## Declaration

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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 DNMTis 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'-deoxycitidine/ 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 | 5α-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 |
| ERα | 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 | primodial 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 polyphorphism |
| 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 |