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

Karen Chiam HuiQin B.Sc (Biomed), B.Hsc (Hons)

Discipline of Medicine,
The University of Adelaide and Hanson Institute
Adelaide. South Australia

June 2010
Table of contents

Declaration......................................................................................................................... 7
Abstract.............................................................................................................................. 8
Acknowledgments ........................................................................................................... 11
Publications Arising from this Thesis........................................................................... 13
Abbreviations .................................................................................................................. 16

CHAPTER 1 - INTRODUCTION

1.1 Introduction............................................................................................................... 20
  1.1.1 Biology and treatment of prostate cancer. ........................................................... 20
  1.1.2 Basic mechanisms of epigenetic modifications. .................................................. 23
    1.1.2.1 DNA methylation........................................................................................... 24
    1.1.2.1 Histone modifications. .................................................................................... 29
    1.1.2.3 MicroRNAs (miRNAs). .................................................................................. 29
    1.1.2.4 Cross-talk between epigenetic modifications................................................ 32
  1.2 Epigenetics: mechanisms involved in prostate cancer development .................... 33
    1.2.1 Epigenetic alterations associated with prostate tumourigenesis. ......................... 33
    1.2.1.1 Global hypomethylation and gene-specific hypermethylation............................ 34
    1.2.1.2 Global changes of specific histone modifications......................................... 36
    1.2.1.3 Potential epigenetic therapy. ......................................................................... 37
  1.3 Epigenetics: early origin of disease ......................................................................... 38
    1.3.1 Dynamic epigenetic reprogramming during development. ................................. 38
    1.3.2 Developmental plasticity and influence on phenotypes...................................... 42
      1.3.2.1 Effects of maternal diet and care.................................................................... 43
      1.3.2.2 Early hormonal perturbations. ..................................................................... 46
    1.3.3 Early origins of prostate cancer: a role for maternal nutrition............................. 48
      1.3.3.1 Epigenetics and obesity............................................................................... 48
      1.3.3.2 Link between obesity and prostate cancer.................................................. 52
      1.3.3.3 Maternal high fat diet: a risk factor of prostate cancer? ............................. 54
  1.4 Objectives of this thesis .......................................................................................... 55

CHAPTER 2 - MATERIALS AND METHODS

2.1 Materials .................................................................................................................... 59
  2.1.1 Chemicals, general reagents and equipments. ..................................................... 59
  2.1.2 Antibodies............................................................................................................ 62
    2.1.2.1 Antibody working dilutions............................................................................. 63
  2.1.3 Drugs.................................................................................................................... 64
  2.1.4 Primers................................................................................................................ 64
  2.2 Buffers and Solutions............................................................................................... 68
  2.3 Methods..................................................................................................................... 72
    2.3.1 Cell culture........................................................................................................... 72
    2.3.1.1 Maintenance of cell lines................................................................................. 72
2.3.1.2 Freezing of cell lines ................................................................. 73
2.3.1.3 Thawing of cell lines .............................................................. 73
2.3.2 Drug treatments and proliferation assays .............................. 74
2.3.3 Western blot analysis .............................................................. 75
2.3.3.1 Protein harvest from treated cell lines .............................. 75
2.3.3.2 Bradford Protein Assay .......................................................... 75
2.3.3.3 Immunoblotting ................................................................. 75
2.3.4 Gene expression (mRNA) analysis of treated cell lines ....... 76
2.3.4.1 RNA extraction of cell lines with Trizol ......................... 76
2.3.4.2 DNAse treatment ............................................................... 77
2.3.4.3 First strand cDNA synthesis ............................................... 77
2.3.4.4 Quantitative polymerase chain reaction (qPCR) ............... 77
2.3.4.5 Agarose gel electrophoresis ............................................... 78
2.3.5 DNA methylation analyses .................................................... 78
2.3.5.1 DNA extraction from cell lines ......................................... 78
2.3.5.2 Bisulphite modification of DNA ................................. 79
2.3.5.3 Methylation-specific PCR (MSP) .................................... 80
2.3.5.4 Combined bisulphite restriction analysis (COBRA) ........ 80
2.3.6 Immunohistochemistry .......................................................... 81
2.3.6.1 Paraffin embedding tissues .............................................. 81
2.3.6.2 Haematoxylin and eosin staining .................................... 81
2.3.6.3 Immunostaining (citrate acid antigen retrieval) ............... 81
2.3.6.5 Quantification of immunostaining (VIA) ......................... 82

CHAPTER 3: EARLY ORIGINS OF PROSTATE CANCER:
EFFECTS OF A MATERNAL HIGH FAT DIET ON RAT ADULT
OFFSPRING PROSTATE DEVELOPMENT

3.1 Introduction ................................................................................. 84
3.2 Materials and methods ............................................................ 86
3.2.1 Animals and diets ................................................................. 86
3.2.2 Body weights, body composition, glucose and insulin tolerance tests .... 89
3.2.3 Collection of the rat prostate tissues .................................... 91
3.2.3 Histology assessment of the rat prostates and seminal vesicles .... 91
3.2.4 RNA extraction with Precellys 24 ....................................... 91
3.2.5 Quantitative real-time PCR in rat ventral and dorso-lateral prostates ... 92
3.2.6 miRNA microarray analysis ................................................ 94
3.2.7 Statistical analysis ............................................................... 95
3.3 Results ...................................................................................... 95
3.3.1- Effect of maternal high fat diet on the body weights and body compositions of the male progeny ................................. 95
3.3.2- Effect of a maternal high fat diet on the metabolism of the male progeny: Glucose and insulin tolerance tests ................. 99
3.3.3- Effect of a maternal high fat diet on the development of male progeny prostates. ......................................................... 101
3.3.3.1 Structural abnormalities of the male progeny prostates .... 101
3.3.3.2 Prostate weights and associated fat ................................................................. 103
3.3.3.3 Incidence of inflammation and hyperplasia .................................................... 106
3.3.4- Analysis of prostate cancer susceptibility genes Ar, Klf6, Cdh1 and Gsp1 .... 110
3.3.5- Analysis of epigenetic enzymes Dnmt1, Dnmt3a, Dnmt3b and the imprinted
  gene Igf2. .................................................................................................................... 113
3.3.6- MicroRNA (miRNA) array analysis of the male progeny ventral and dorso-
  lateral prostates. ....................................................................................................... 113
3.5 Discussion ............................................................................................................. 128

CHAPTER 4 - GLOBAL LEVELS OF SPECIFIC HISTONE
  MODIFICATIONS AS PROGNOSTIC MARKERS FOR PROSTATE
  CANCER

4.1 Introduction ........................................................................................................... 140
4.2 Materials and methods ....................................................................................... 141
  4.2.1 Patients cohort ................................................................................................ 141
  4.2.2 Immunohistochemistry for H3K18Ac, H3K4diMe and Ki67 expression .......... 142
  4.2.3 Quantitation of immunostaining ................................................................. 143
  4.2.4 Statistical analyses. ...................................................................................... 143
4.3 Results .................................................................................................................. 145
  4.3.1- H3K4diMe, H3K18Ac and Ki67 immunostaining in prostate cancer .......... 145
  4.3.2- Quantitation of H3K18Ac, H3K4diMe and Ki67 ....................................... 145
  4.3.2.1 Levels of positive nuclear staining for H3K18Ac, H3K4diMe and Ki67 .... 146
  4.3.2.2 Staining intensities MIOD and MOD for H3K18Ac and H3K4diMe. ......... 154
  4.3.3- H3K18Ac, H3K4diMe and Ki67 associations with prostate cancer relapse... 154
  4.3.3.1 Association of H3K18Ac and H3K4diMe immunostaining levels with clinico-
  pathological parameters. ....................................................................................... 154
  4.3.3.2 Global levels of H3K18Ac and H3K4diMe independently predict prostate cancer
  survival in the patient cohort. ................................................................................ 159
  4.3.3.3 Combination of H3K18Ac and H3K4diMe levels better predict prostate tumour
  recurrence. ........................................................................................................... 166
4.4 Discussion ............................................................................................................. 166

CHAPTER 5 - THE IMPORTANCE OF EPIGENETIC GENES IN
  PROSTATE TUMOURIGENESIS

5.1 Introduction ........................................................................................................... 176
5.2 Materials and methods ....................................................................................... 177
  5.2.1 Microarray analysis. ..................................................................................... 177
  5.2.2 Patients cohort ............................................................................................. 177
  5.2.3 Epigenetic gene expression analysis by quantitative real-time PCR (qPCR) ... 178
  5.2.4 Statistical analysis ....................................................................................... 178
5.3 Results .................................................................................................................. 179
  5.3.1- Epigenetic gene expression during prostate cancer progression. ............... 179
  5.3.2- The candidate epigenetic gene signature discriminates non-malignant from
  tumour prostate tissues. ...................................................................................... 186
5.3.2.1 EZH2 and MLL3 mRNA expression was significantly altered between matched non-malignant and tumour prostate tissues. 186
5.3.2.2 The epigenetic gene signature with and without EZH2 distinguishes tumour from non-malignant prostate samples. 186
5.3.3 The functions of the individual epigenetic genes identified in our microarray analysis. 189
5.3.3.1 Classification of the epigenetic genes. 189
5.3.3.2 Association with prostate cancer, AR, H3K4diMe and H3K18Ac histone modifications. 191
5.4 Discussion. 201

CHAPTER 6 - EPGENETIC ANALYSIS OF THE PROSTATE CANCER SUSCEPTIBILITY GENE, KLF6

6.1 Introduction. 208
6.1.1 KLF6 as a candidate tumour suppressor gene in prostate cancer. 210
6.2 Materials and methods. 213
6.2.1 Microarray analysis. 213
6.2.2 5-aza-CdR treatment in LNCaP cells for KLF6 expression and DNA methylation analysis. 213
6.2.3 KLF6 expression analysis in human prostate cancer tissues. 215
6.2.5 Human prostate cancer tissue microarray. 216
6.2.6 TRAMP prostate cancer progression tissue microarray. 216
6.2.7 TRAMP Klf6 methylation-specific PCR. 217
6.2.8 Statistical analysis. 217
6.3 Results. 218
6.3.1 Alterations of KLF6 gene expression during prostate cancer progression. 218
6.3.2 DNA methylation analysis of KLF6 promoter in human prostate cancer cells. 218
6.3.2.1 5-aza-CdR treatment induced KLF6 mRNA expression in LNCaP cells. 218
6.3.2.2 DNA demethylation of KLF6 promoter upon 5-aza-CdR treatment. 221
6.3.3 Validation of KLF6 expression changes in matched human non-malignant and tumour prostate samples. 224
6.3.4 KLF6 protein expression in human prostate tissues. 229
6.3.5 KLF6 protein expression in human endogenous cell lines and mouse prostate tissues. 233
6.3.6 Decreased Klf6 protein expression in the transgenic adenocarcinoma mouse prostate cancer model (TRAMP) during prostate cancer progression. 235
6.3.7 DNA methylation analysis of Klf6 in the TRAMP prostates. 236
6.4 Discussion. 238

CHAPTER 7 - EPGENETIC THERAPIES FOR PROSTATE CANCER: OPTIMISATION OF DNMTi 5-AZA-CdR TREATMENT AND GSTP1 AS A MARKER OF DNMTi EFFICACY

7.1 Introduction. 250
7.2 Materials and methods

7.2.1 Drug treatments and proliferation assays................................. 254
7.2.2 Western blot analysis............................................................... 255
7.2.3 GSTP1 methylation analysis by MSP and COBRA.................... 255
7.2.4 Statistical analysis................................................................. 256

7.3 Results

7.3.1- Effects of 5-aza-CdR on prostate cancer cell growth.............. 256
7.3.2- Effects of 5-aza-CdR on DNMT1, AR and HDAC1 protein expression.... 261
7.3.3- Demethylation and re-expression of GSTP1 protein by 5-aza-CdR in LNCaP prostate cancer cells. 263
7.3.3.1 GSTP1 methylation-specific polymerase chain reaction (MSP). ....................... 265
7.3.3.2 GSTP1 combined bisulphite restriction analysis (COBRA). ...................... 269
7.3.4- Effects of Zebularine on prostate cancer cell growth................... 272
7.3.6- Zebularine resulted in GSTP1 demethylation but not GSTP1 protein re-expression. ......................................................... 274
7.3.7- Combination and sequential treatments of 5-aza-CdR with SAHA and Zebularine were not as effective as a daily 5-aza-CdR low dose treatment regime... 277
7.3.7.1 Combination treatments of 5-aza-CdR and SAHA. ....................... 278
7.3.7.2 Sequential treatments of SAHA and 5-aza-CdR............................... 281
7.3.7.3 Sequential treatments of Zebularine and 5-aza-CdR......................... 283

7.4 Discussion

CHAPTER 8 - GENERAL DISCUSSION

8.1 Role of epigenetic modifications in prostate tumourigenesis ......... 299
8.2 Major findings and future directions of this thesis......................... 300
8.2.1 Maternal high fat diet increased the incidence of prostate abnormalities in rat adult offspring and altered miRNA expression. 300
8.2.2 Clinical importance of epigenetic modifications in prostate tumourigenesis.... 305
8.2.2.1 Histone modifications and epigenetic genes as prognostic markers and therapeutic targets in prostate cancer. 305
8.2.2.2 Establishment of an effective 5-aza-CdR treatment and GSTP1 as a marker of DNMTi efficacy in prostate cancer cells. 306
8.2.3 Hypermethylation of KLF6 is unlikely to be the cause of the loss of KLF6 in prostate cancer. 308

8.4 Summary and conclusions.......................................................... 309
Appendix 1............................................................................................ 310
Bibliography .......................................................................................... 317
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

June 2010
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.
Acknowledgments

This thesis would not have been possible without the support and help from all my dear family, friends, colleagues and mentors. First of all, I would like to say a big THANK YOU to my most wonderful supervisor, Dr Tina Bianco-Miotto, who has patiently guided and encouraged me throughout my honours and PhD years in Dame Roma Mitchell Cancer Research Laboratories. I would not have done it without you and thank you for putting up with me as your student for 5 long years!! I would also like to thank my other great supervisors, who are always there whenever I needed help. Dr Carmella Ricciardelli, thank you for all your guidance and help with the immunohistochemistry and scary statistics work; Dr Lisa Butler, thank you for all the useful advises and help with the growth curves and xenograft studies; Professor Wayne Tilley, thank you for giving me the opportunity and a chance to continue my study in the Dame Roma Mitchell Cancer Research Laboratories. You have taught me the passion and responsibility that one should have in research.

I would also like to acknowledge my colleagues in the Dame Roma Mitchell Cancer Research Laboratories for giving me all the help and support. A big thank you to Natalie Ryan, who has helped and assisted me when I most needed them while writing my thesis. And thank you Maggie Centenera, for reading through my thesis and all the advice and help throughout my study. I would also like to show my appreciation for the following people whom I have received help and assistance; Jo Treloar and Lee Sook Ching for the xenograft work, Michelle Newman and Ean Phing Lee who were the PCR queens, Elisa Cops, Marie Pickering, Silke and Amelia Peters who have helped me with the
immunohistochemistry. A special thanks to Dr Shalini Jindal for providing her valuable expertise. I would also like to thank my fellow PhD students, Astrud Tuck, Andrew Trotta, Ali Ochnick, Miriam Butler and Sarah Carter for making the PhD room always bright and fun, even during the stressful PhD days. I would also like to specially thank Professor Julie Owens, Dr Karen Kind and fellow mates in the Discipline of Paediatrics and Reproductive Health (University of Adelaide) for the wonderful collaboration work.

To my dear family and friends, I would not have achieved anything without your love and support. I would like to say the greatest thanks to my family; to my coolest mum and dad who are always encouraging, understanding and supportive. I love you mummy and daddy! To my best friend and best sister, Grace, who has to listen to all my complaints and nonsense almost everyday of her life. My little brother Alex, thank you for all the fun times we have spent together, especially when it comes to your favourite TV and games. To my dear Sarah, I love you and I missed you. Thank you to my bestest friend, Ling Eng, you are like a family to me and I would not have done it without you love and support all these years. I would also like to specially thank the following people, Uncle Larry, Aunt Sally, Peter, Uncle John, Aunt Terry and Jane, who are family to me in Adelaide. Thank you for keeping me sane and happy all these years! A special thanks to my best mates that I have known in Adelaide, Seryik, Zhiyu and Kee. What do I do without you guys, and thanks for putting up with all my nonsense too! And of course, I would not have forgotten my dearest ACJC mates who have never failed to brighten my days whenever I think of them. Thank you Grace, Qiyue, Charlene, Deying and Yilin!
Publications Arising from this Thesis

Articles Published in Scientific Journals


Articles Submitted to Scientific Journals


* Equal first authors.

Abstracts Published in the proceedings of Scientific Meetings


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-aza-CdR</td>
<td>5-aza-2’-deoxycytidine/ Decitabine</td>
</tr>
<tr>
<td>5-aza-CR</td>
<td>5-aza-cytidine</td>
</tr>
<tr>
<td>AAT</td>
<td>Androgen ablation therapy</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>AP</td>
<td>Anterior prostate</td>
</tr>
<tr>
<td>APCB</td>
<td>Australian Prostate Cancer BioResource</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>BPA</td>
<td>Bisphenol A</td>
</tr>
<tr>
<td>BPH</td>
<td>Benign prostate hyperplasia</td>
</tr>
<tr>
<td>BR</td>
<td>Biochemical recurrence</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>Cdh1</td>
<td>Cadherin 1</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>COBRA</td>
<td>Combined bisulphite restriction analysis</td>
</tr>
<tr>
<td>CpG</td>
<td>Cytosine and guanine dinucleotides</td>
</tr>
<tr>
<td>DAB</td>
<td>3,3’-Diaminobenzidine</td>
</tr>
<tr>
<td>DCC</td>
<td>Dextran coated charcoal</td>
</tr>
<tr>
<td>DES</td>
<td>Diethylstilbestrol</td>
</tr>
<tr>
<td>DHT</td>
<td>5α-dihydrotestosterone</td>
</tr>
<tr>
<td>DLP</td>
<td>Dorso-lateral prostate</td>
</tr>
<tr>
<td>DMBA</td>
<td>7,12-dimethylbenz(a)anthracene</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNMT</td>
<td>DNA methyltransferase</td>
</tr>
<tr>
<td>DNMT1</td>
<td>DNA methyltransferase 1</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>DNA methyltransferase 3A</td>
</tr>
<tr>
<td>DNMT3B</td>
<td>DNA methyltransferase 3B</td>
</tr>
<tr>
<td>DNMTi</td>
<td>DNA methyltransferase inhibitor</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital rectal examination</td>
</tr>
<tr>
<td>ERα</td>
<td>Estrogen receptor alpha</td>
</tr>
<tr>
<td>EtOh</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FCS</td>
<td>Foetal calf serum</td>
</tr>
<tr>
<td>FD</td>
<td>False discovery</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>G</td>
<td>Guanine</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Gluthatione-S-transferase P1</td>
</tr>
<tr>
<td>GTT</td>
<td>Glucose tolerance test</td>
</tr>
<tr>
<td>H3K4diMe</td>
<td>Dimethylated Histone 3 Lysine-4 residue</td>
</tr>
<tr>
<td>H3K18Ac</td>
<td>Acetylated Histone 3 Lysine-18 residue</td>
</tr>
<tr>
<td>HAT</td>
<td>Histone acetyltransferase</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone deacetylase</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>HDAC1</td>
<td>Histone deacetylase 1</td>
</tr>
<tr>
<td>HDACi</td>
<td>Histone deacetylase inhibitor</td>
</tr>
<tr>
<td>HDMT</td>
<td>Histone demethylase</td>
</tr>
<tr>
<td>HGPIIN</td>
<td>High-grade prostate intraepithelial neoplasia</td>
</tr>
<tr>
<td>HMT</td>
<td>Histone methyltransferase</td>
</tr>
<tr>
<td>HMTi</td>
<td>Histone methyltransferase inhibitor</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>IGF2</td>
<td>Insulin growth like factor 2</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>ITT</td>
<td>Insulin tolerance test</td>
</tr>
<tr>
<td>KLF6</td>
<td>Kruppel-like factor 6</td>
</tr>
<tr>
<td>LG</td>
<td>Licking and Grooming</td>
</tr>
<tr>
<td>LNCaP</td>
<td>Lymph node carcinoma of the prostate</td>
</tr>
<tr>
<td>LOH</td>
<td>Loss of heterozygosity</td>
</tr>
<tr>
<td>LOI</td>
<td>Loss of imprinting</td>
</tr>
<tr>
<td>MBD</td>
<td>Methyl-DNA binding</td>
</tr>
<tr>
<td>miRNA</td>
<td>Micro ribonucleic acid</td>
</tr>
<tr>
<td>MSP</td>
<td>Methylation-specific polymerase chain reaction</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td>MIOD</td>
<td>Mean integrated optical density out of total field area</td>
</tr>
<tr>
<td>MOD</td>
<td>Mean integrated optical density out of the positive nuclear area</td>
</tr>
<tr>
<td>NM</td>
<td>Non-malignant prostate</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCa</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>PGC</td>
<td>Primodial germ cells</td>
</tr>
<tr>
<td>PIN</td>
<td>Prostate intraepithelial neoplasia</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>QMSP</td>
<td>Quantitative methylation-specific polymerase chain reaction</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative real time polymerase chain reaction</td>
</tr>
<tr>
<td>Rb</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>RIN</td>
<td>RNA Integrity Number</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real time polymerase chain reaction</td>
</tr>
<tr>
<td>SAHA</td>
<td>Suberoylanilide hydroxamic acid acid/ Vorinostat</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosylmethionine</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-nucleotide polyphorphism</td>
</tr>
<tr>
<td>SV</td>
<td>Splice variants</td>
</tr>
<tr>
<td>SV</td>
<td>Seminal vesicles</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris buffered saline</td>
</tr>
<tr>
<td>TBST</td>
<td>Tris buffered saline-tween 20</td>
</tr>
<tr>
<td>TEMED</td>
<td>N,N,N’, N’-tetramethylethylenediamine</td>
</tr>
<tr>
<td>TMA</td>
<td>Tissue microarray</td>
</tr>
<tr>
<td>TRAMP</td>
<td>Transgenic adenocarcinoma of mouse prostate</td>
</tr>
</tbody>
</table>
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